MEDICINSKI GLASNIK

Official publication of the Medical Association of Zenica-Doboj Canton, Bosnia and Herzegovina
Volume 14, Number 2, August 2017.
ISSN 1840-0132

MEDICINSKI GLASNIK

Published and copyright by: Medical Assotiation of Zenica-Doboj Canton; Address: Zenica, 72000, Bulevar kralja Tvrtka I 4, Bosnia and Herzegovina;

tel./fax: +387 32 444 270; Email: ljkozedo@bih.net.ba, web site: http//www.ljkzedo.ba

For ordering information please contact: Tatjana Žilo, ljkozedo@bih.net.ba; Access to this journal is available free online trough: www.ljkzedo.com.ba

The Journal is indexed by MEDLINE, EMBASE (Exerpta Medica), Scopus, EBSCO; ISSN 1840-0132

DTP by: Graphic and web design studio "B Panel" Zenica, Zmaja od Bosne bb, www.bpanel.ba, e-mail: info@bpanel.ba, tel. +387 32 441 290, 441 291;

Printed by: Graforad d.o.o. Zenica, Zmaja od Bosne bb, Zenica
Tel.: 032 201-821; e-mail: graforad@bih.net.ba

Medicinski Glasnik

Official Publication of the Medical Association of
Zenica-Doboj Canton
Bosnia and Herzegovina

MEDICINSKI GLASNIK

Official Publication of the Medical Association of Zenica-Doboj Canton, Bosnia and Herzegovina
Volume 14, Number 2, August 2017

Free full-text online at: www.ljkzedo.com.ba, and www.doaj.org (DOAJ, Directory of Open Access Journals)

Original article

Medicinski Glasnik is indexed by MEDLINE, EMBASE (Exerpta Medica), EBSCO and Scopus

ORIGINAL ARTICLE

Cytotoxic and genotoxic studies of essential oil from Rosa damascene Mill., Kashan, Iran

Mohammad Shokrzadeh[1], Emran Habibi[2], Mona Modanloo[3]

[1]Department of Toxicology-Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, [2]Department of Pharmacognosy, Faculty of Pharmacy, Mazandaran University of Medical Sciences; Sari, Iran , [3]Pharmaceutical Sciences Research Centre, Student research committe Mazandaran University of Medical Sciences, Sari, Iran

ABSTRACT

Aim *Rosa damascene* Mill. belongs to the family of *Roseaceae* and its essential oil is produced in large amounts in Iran. The wide application of rose oil has raised questions about potential adverse health effects. We have investigated cytotoxic activity and genotoxic effects of Rosa oil from Kashan, Iran.

Methods The cytotoxic effect and IC50 of the essential oil on the cell lines was studied followed by MTT assay. In this assay mitochondrial oxidoreductase enzymes with reducing the tetrazolium dye MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) reflect the number of viable cells. Genotoxic effect of the oil was evaluated by micronucleus assay by evaluating produced micronuclei due to cytogenetic damage in binucleated lymphocytes.

Results The results showed that essential oil significantly had cytotoxic and genotoxic effects at doses over 10μg/mL ($p<0.05$). Also, essential oil of Rose showed lower IC50 in cancer cell line (A549) in comparison with the normal cell line (NIH3T3).

Conclusion Cytotoxic and genotoxic properties of essential oil of Rose in Kashan, Iran, are safe at a dose of 10μg/mL. Also, a good cytotoxic effect was shown and could be introduced as an anticancer compound. Further studies are needed with regard to anti-cancer effects of Rose essential oil.

Key words: micronucleus assay, MTT, Rose oil

Corresponding author:
Mona Modanloo
Pharmaceutical Sciences Research Center, Student research committee, Mazandaran University of Medical Sciences,
Khazarabad Road, 48175-861 Sari, Iran;
Phone: +98 911 1520 410;
Fax: +981133543084;
Email: dr_modanloo@yahoo.com
Mohammad Shokrzadeh ORCID ID: http://www.orcid.org/0000-0002-0071-6530

Original submission:
28 February 2017;
Revised submission:
15 March 2017;
Accepted:
09 May 2017.
doi: 10.17392/901-17

Med Glas (Zenica) 2017; 14(2): 152-157

INTRODUCTION

Iran has a long history in cultivation and consumption of *Rosa damascena*, and it is known as an important producer of rose oil in the world (1). *Rosa damascena* Mill. belongs to the family of *Roseaceae* (2). This ornamental is not only known as one of the most valuable sources of flavours and fragrances in the world, but also it has some applications in medicine and food industry (3).

Some evidence showed that Rose oil has some beneficial effects in the treatment of various diseases like premenstrual breast tenderness, inflammatory reactions, gall care and spasms (4).

It also seems to have antidepressant and relaxing effects. It is helpful for long-lasting cough, wound healing, allergies and severe headache (3).

Some evidence reported various potential adverse effects of *Rosa damascene* (5-7). On the other hand, a new study showed that while it is cytotoxic in high doses, it did not show genotoxic effects (8).

The MTT test is an appropriate assay that has often been used to investigate cytotoxicity caused by medical plants (6). It is a rapid, low-cost method based on the reduction of yellow tetrazolium salt, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), to dark blue formazan in mitochondria of active cells (9). Thus, the amount of produced formazan has a direct relation with viable cells (10).

The micronucleus assay in binucleate human lymphocytes is an effective tool to measure cytogenetic damage of agents with different mechanisms of genotoxicity in vitro (11). Recent studies have shown that genomic instability is an early event that occurs in some malignancies and it can be detected by examination of the peripheral blood lymphocytes as a sample of precursor cells (12).

Different studies reported some differences in the composition of Rose oil in various regions (8, 13, 14).

Due to high application of Rose essential oil and with regard to the fact that Kashan is one of the biggest producers of Rose oil in Iran, this study aimed at assessing cytotoxicity of Rosa damascene Mill.'s essential oil on both normal and cancer cell lines using MTT assay and also evaluating its genotoxicity on human blood lymphocytes using micronuclei assay.

MATERIALS AND METHODS

Essential oil distillation

Flowers were picked by hand before sunrise in May 2015 in the city of Kashan (Vidorj region), Iran. Botanical identification was confirmed by morphologic characteristics at the Department of Pharmacognosy, Sari Faculty of Pharmacy. The flowers were subjected to steam distillation within the same day (400gr fresh flowers in 2.0 L water). After 3h hydrodistillation the obtained oil was dried using anhydrous sodium sulphate. Pure essential oil was stored at 4°C in a dark place (1).

Dimethyl sulphate (DMSO) was used to dissolve essential oil (the final concentration of DMSO was not over 1%) (14).

Cell culture

Experiments were carried out with cell lines NIH3T3 (non-tumour fibroblast) and A549 (human NSCLC cell line) (Pasteur Institute of Iran, Tehran, Iran).

Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco-BRL, Germany) with 10% fetal bovine serum (Gibco-BRL, Germany) and 100µg/mL streptomycin (Gibco-BRL, Germany) and 100IU/mL penicillin (Gibco-BRL, Germany). Cell cultures were adjusted to allow for exponential growth.

MTT assay

The protocol was adapted from the method described by Shokrzadeh et al. (15).

Cells (10^4 cells) were cultured with 200 µL DMEM/F12 medium containing bovine serum in 96 wells plate and incubated in 37 °C for 24 hours.

Stock solutions of Rose oil and cisplatin (a platinum coordination complex with potent anti-neoplastic activity induces apoptosis in cancer cells, possibly via caspase-3 activation) (16) were prepared in DMSO (1%) and phosphate buffered saline (PBS), respectively.

After 48 hours of cell incubation with different doses of essential oil (1, 10, 50, 100, 150 and 200 µl), 20µL MTT solution (5 mg/mL) was added to each well. After 4 hours incubation at 37°C, the formazan was dissolved in DMSO. Finally the optical density (OD) of wells was measured on a micro plate ELISA reader at 570 nm. All expe-

riments were performed twice and each experiment was run in triplicate, and mean values were recorded.

A linear relation between cell viability and OD of each well is an exact determination of cell proliferation (17). The percentage of cell viability was calculated using the equation (18): [Mean (OD) of treated cells/mean OD of control cells (1% DMSO)]×100

Micronucleus assay

Fresh blood was collected from 10 healthy, no smoking, no alcoholic, male donors aged between 25-35 years by venepuncture in heparinized falcons; 0.5 mL of whole blood was added to 4.5mL of Roswell Park Memorial Institute (RPMI) culture medium 1640 supplemented with fetal bovine serum containing L-glutamin, antibiotics and phytohemagglutinin (PHA), and different doses of Rose oil (1, 10, 50, 100, 150 and 200 µL) were added. Cytochalasin B (Cyt-B) (Sigma, MO, USA) at the final concentration of 6µg/mL was added at 44h post PHA stimulation.

Cyt B prevents complete cytokinesis in mitosis, thus causing an appearance of multi-nuclear cells (19).

The binucleated lymphocytes were harvested 28 hour after adding Cyt-B, they were treated by hypotonic KCl (0.075M) to red blood cell (RBC) lysis. Then fixative solution (methanol: acetic acid= 6:1) was added to the cells prior to slide preparation and staining. For slide preparation 2-3 drops of cell suspension were thrown on a clean slide. The slides were stained with Giemsa solution (4%) for 7-10 mins. They were observed at 40× and 100× magnifications using a light microscope to estimate mitotic index (the cells with 2 or more nuclei per 1000 observed cells) and micronuclei frequency (the number of micronuclei in at least 1000 binucleated cells) (8,20) binucleated cells are lymphocytes that were once divided by mitosis (21). The experiment was performed twice. Mitotic Index has a direct relation with cells' proliferative activity (8,22).

Statistical analysis

One way analysis of variance and tukey's honestly significant differences (HSD) test were used for multiple comparisons of data. A p value less than 0.05 was considered as significant. The IC50 (half maximal inhibitory concentration) values were calculated by PRISM software using nonlinear regression. Standard deviations represent average results of double experiments. The IC50 values were compared using the student's T-test measuring the effectiveness of a substance to cause cell death or inhibit cell growth. So the lower amount of IC50 represents a higher toxicity of a compound, which leads to death or inhibition of cell growth (23).

RESULTS

During this study 0.2 mL essential oil of 1200 gr Rose flowers was produced. Comparing the results in both MTT and micronucleus tests, data showed no significant difference between DMSO control group and the control without DMSO (Figures 1-4).

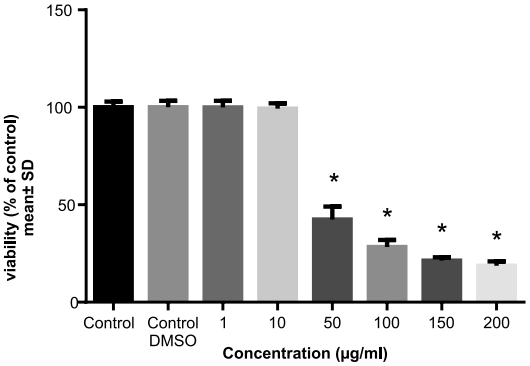

Figure 1. Effect of *Rosa damascene* essential oil on A549 cell viability *significant difference compared to the control group ($p<0.05$)

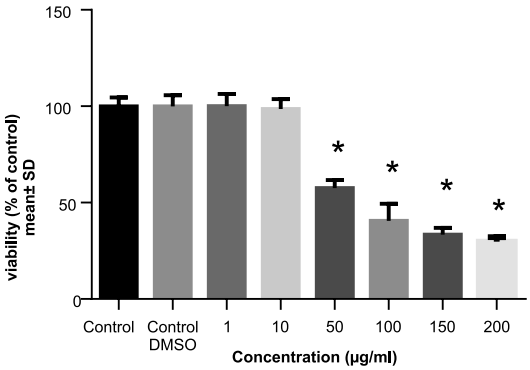

Figure 2. Effect of *Rosa damascene* essential oil on NIH3T3 cell viability *significant difference compared to the control group ($p<0.05$)

The MTT test with increasing doses of oil showed a decrease in viability in both normal and cancer cells, A549 and NIH3T3, respectively (Figures 1, 2). It seems that the dose of 1 and 10 µg/mL in both

Table 1. Micronuclei frequency in different doses of rose essential oil, normal control and colchicine treated cultures

Micronuclei frequency	Control	Control (1% DMSO)	Concentration (µg/mL) 1	10	50	100	150	200	Colchicine 0.1µM
Mean±SD (% of control)	0.70±0.80	0.60±0.69	0.80±0.62	1.00±0.79	5.30±1.22	5.80±1.15	6.40±1.31	8.20±1.80	10.30±2.25

cell lines did not observe toxic effects (the absence of a significant difference with the DMSO control group). However, at higher doses there was a significant difference between the group affected by rose oil and the control group ($p<0.05$) (Figures 1, 2).

The IC50 values were significantly different in the A549 and NIH3T3 cell lines between rose oil (36.43±3.373 and 42.93±0.502, respectively) and cisplatin (8.068±2.670 and 16.67±2.212, respectively) groups ($p=0.0010$ and $p=0.0014$, respectively).

The effect of different doses of rose oil on the frequency of micronuclei in binucleated lymphocytes is shown in Table 1. While the frequency of micronuclei in concentrations of 1 and 10 µg/mL was not much different from the control group, the amount in higher concentrations, of 50-200 µg/mL, was significantly more increased than in the control group ($p<0.05$) (Figure 3).

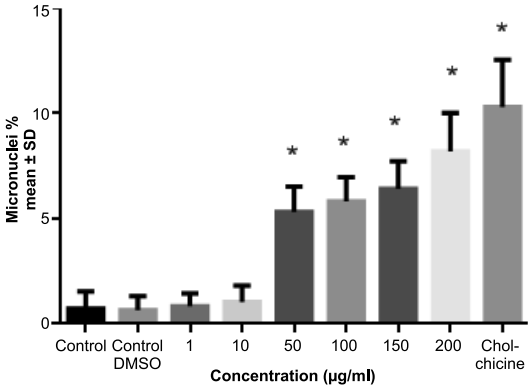

Figure 3. Micronuclei frequency in different doses of rose essential oil, normal control and cholchicin treated cultures
*significant difference compared to the control group ($p<0.05$)

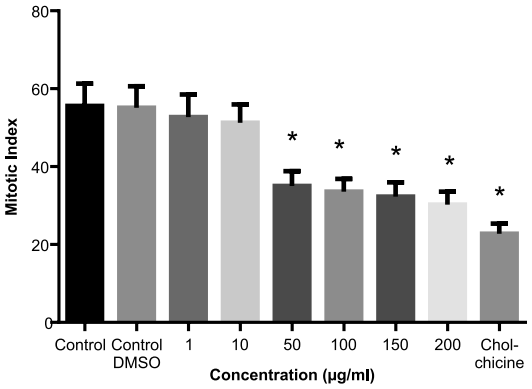

Figure 4. Mitotic index of peripheral blood lymphocytes in different doses of rose essential oil, normal control and colchicine treated cultures *significant difference compared to the control group ($p<0.05$)

The mitotic activity in the cells affected by the rose oil represents an obvious toxic effect at concentrations higher than 10 µg/mL ($p<0.05$ compared to control DMSO group) (Figure 4).

DISCUSSION

The results of this study indicate that the Rose essential oil of Kashan, Iran, at doses higher than10 µg/mL had obvious cytotoxic and genotoxic effects. The material in both normal and cancerous cell lines caused damage and cell death. Also, our findings showed that DMSO in the concentration of 1% had no significant effect on the cells. These findings are similar to previous studies (14).

Based on the results of the MTT test, the sensitivity of cancer cells to rose oil was significantly higher than that of normal cells. This may be due to cancerous cells malfunction, impairment disorders in immune cells process or increased permeability and absorption by them due to the high proliferation rate (24). In a recent study conducted in 2014 by Heba et al., phenyl ethanol blend in Rose essence is reported to have an anticancer activity (8). Rose oil is a matter of gross and includes various pharmaceutical compounds, with each of them having distinct effects (24).

Recent surveys showed that the presence of terpenes in essential oils is able to change the nature of the cell membrane (25). This disturbs the equilibrium concentration of intracellular electrolytes and ultimately causes cell death (26).

In the study of Loghmani-Khouzani et al. (14) carried out on Rose in Kashan, using gas chromatography/mass spectroscopy (GC/MS) of flower essential oil, more than 95 different compounds were identified; the most frequently identified components were β-citronellol (32.49%), nonadecane (23.99%), geraniol (18.12%) and henicosane (9.64%), followed by eicosane (1.29%), linalool (0.29%), methyl eugenol (0.55%) and many other compounds.

It seems that phenolic compounds in this oil through one of two mechanisms of interaction with energy-generating enzymes or protein denaturation leading to cell death (27). Geraniol is the main

ingredient in Rose and is a monoterpene alcohol which causes an increase in cell sensitivity to certain toxic substances by reducing the amount of thymidilatesyntase (TS) and thymidine kinase (TK) enzymes in colon cancer cells (8). Previous research has shown the essential oils by internal and external changes in mitochondrial membrane fluidity thus increasing their permeability; induced cell death by both apoptosis and necrosis (24). Also the inhibitory effects of methylated eugenol on some cancer cells have been reported (28).

The other results of our study included the ability of Rose essence in micronuclei creation induction in peripheral blood lymphocytes. Micronuclei induction is generally recognized as a factor for chromosome damage (19, 29).

In previous studies, the sensitivity of lymphocytes isolated from peripheral blood has been reported into chemicals more than the lymphocytes of the blood as whole blood. This is due to the presence of some protective factors in the blood and also other targets rather than lymphocytes are known for the chemical (19). Since all of these targets and protective factors are in the human body's peripheral blood, it was decided in this study to use whole blood cultures for further similarity of test conditions with the human body (19).

High rate of hydrocarbons and the presence of a minor amount of monoterpenes (linalool 0-0.29%) can prevent the activity of DNA gyrase enzyme thereby causing the genetic damage (30).

Gene toxicity is a very important factor in the safety of a substance because tumours can be caused by mutagenic substances (31). Genetic instability is not the only factor in many malignancies and genetic syndromes including Fanconi anemia, ataxia-telangiectasia and Warner syndrome (31).

In conclusion, since roses have very abroad application in the cosmetics, perfume, food and pharmaceutical industries from the past and considering the fact that this is the first time the cytogenetic safety of rose oil of Kashan as one of the most important producers of oil in the world was evaluated, based on the results of this study, a dose of 10 μg/mL and less is considered as the dose of confidence. On the other hand, despite the Rose oil's IC50 in cancer cell line was higher than the cisplatin's IC50, its value is considerably low because the lower IC50 the more power of sample in killing cell or inhibiting their growth (24). Accordingly, we can suggest that the Rose essential oil can be used as a complementary therapy in cancer.

ACKNOWLEDGMENT

The authors thank the Vice Chancellor for Research of the Mazandaran University of Medical Sciences for his cooperation and also the volunteers who participated in this study.

FUNDING

No specific funding was received for this study.

REFERENCES

1. Jalali-Heravi M, Parastar H, Sereshti H. Development of a method for analysis of Iranian damask rose oil: Combination of gas chromatography–mass spectrometry with Chemometric techniques. Anal Chim Acta 2008; 623:11-21.
2. Pellati F, Orlandini G, Leeuwen KA, Anesin G, Bertelli D, Paolini M, Benvenuti S, Camin F. Gas chromatography combined with mass spectrometry, flame ionization detection and elemental analyzer/isotope ratio mass spectrometry for characterizing and detecting the authenticity of commercial essential oils of Rosa damascena Mill. Rapid Commun Mass Spectrom 2013; 27:591-602.
3. Shafei MN, Saberi Z, Amini S. Pharmacological effects of Rosa damascena. Iran J Basic Med Sci 2011; 14:295-307.
4. Kaul VK, Singh V, Singh B. Damask rose and marigold: prospective industrial crops. Int J Med Arom Plants 2000; 313-8.
5. Rezaie-Tavirani M, Fayazfar S, Heydari-Keshel S, Rezaee MB, Zamanian-Azodi M, Rezaei-Tavirani M, Khodarahmi R. Effect of essential oil of Rosa Damascena on human colon cancer cell line SW742. Gastroenterol Hepatol Bed Bench 2013; 6.
6. Talib WH, Mahasneh AM. Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. Molecules 2010; 15:1811-24.
7. Khatib H, Rezaei-Tavirani M, Keshel SH, Azodi MZ, Omidi R, Biglarian M, Sobhi S. Flow cytometry analysis of Rosa damascena effects on gastric cancer cell line (MKN45). Iran J Cancer Prev 2013; 6:30-6.
8. Hagag HA, Bazaid SA, Abdel-Hameed E-SS, Salman M. Cytogenetic, cytotoxic and GC–MS studies on concrete and absolute oils from Taif rose, Saudi Arabia. Cytotechnology 2014; 66:913-23.
9. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65:55-63.

10. Kiani M, Zamani Z, Khalighi A, Fatahi R, Byrne DH. A unique germplasm of Damask roses in Iran. Proceedings of the Vth International Symposium on Rose Research and Cultivation, Gifu/Japan, May 24-28 2009. Acta Hort (ISHS) 2010; 870:131-6.
11. Kirsch-Volders M, Decordier I, Elhajouji A, Plas G, Aardema MJ, Fenech M. In vitro genotoxicity testing using the micronucleus assay in cell lines, human lymphocytes and 3D human skin models. Mutagenesis 2011; 26:177-84.
12. Maffei F, Moraga JM, Angelini S, Zenesini C, Musti M, Festi D, Cantelli-Forti G, Hrelia P. Micronucleus frequency in human peripheral blood lymphocytes as a biomarker for the early detection of colorectal cancer risk. Mutagenesis 2014; 29:221-5.
13. Yassa N, Masoomi F, Rankouhi SR, Hadjiakhoondi A. Chemical composition and antioxidant activity of the extract and essential oil of Rosa damascena from Iran, population of Guilan. DARU 2015; 17:175-80.
14. Loghmani-Khouzani H, Sabzi Fini O, Safari J. Essential oil composition of Rosa damascena mill cultivated in central Iran. Scientia Iranica 2007; 14:316-9.
15. Shokrzadeh M, Saravi SS, Mirzayi M. Cytotoxic effects of ethyl acetate extract of Sambucus ebulus compared with etoposide on normal and cancer cell lines. Pharmacogn Mag 2009; 5:316.
16. Fuertes MA, Alonso C, Pérez JM. Biochemical modulation of cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance. Chem Rev 2003; 103:645-62.
17. Greim H, Bury D, Klimisch H-J, Oeben-Negele M, Ziegler-Skylakakis K. Toxicity of aliphatic amines: Structure-activity relationship. Chemosphere 1998; 36:271-95.
18. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, IL: Allured publishing corporation, 2007.
19. Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. Mutat Res 1985; 147:29-36.
20. Shokrzadeh M, Ahangar N, Abdollahi M, Shadboorestan A, Omidi M, Payam SH. Potential chemoprotective effects of selenium on diazinon-induced DNA damage in rat peripheral blood lymphocyte. Hum Exp Toxicol 2013; 32:759-65.
21. Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 2004; 94:223-53.
22. Shokrzadeh M, Chabra A, Naghshvar F, Ahmadi A. The mitigating effect of Citrullus colocynthis (L.) fruit extract against genotoxicity induced by cyclophosphamide in mice bone marrow cells. Scientific World J 2013; 2013.
23. Saravi SS, Shokrzadeh M, Shirazi FH. Cytotoxicity of Sambucus ebulus on cancer cell lines and protective effects of vitamins C and E against its cytotoxicity on normal cell lines. Afr J Biotechnol 2013; 12.
24. Hussain AI. Characterization and biological activities of essential oils of some species of Lamiaceae. University of Agriculture, Faisalabad, 2009; Ph. D. Thesis.
25. Oussalah M, Caillet S, Lacroix M. Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of Escherichia coli O157: H7 and Listeria monocytogenes. J Food Prot 2006; 69:1046-55.
26. Marrufo T, Nazzaro F, Mancini E, Fratianni F, Coppola R, De Martino L, Agostinho AB, De Feo V. Chemical composition and biological activity of the essential oil from leaves of Moringa oleifera Lam. cultivated in Mozambique. Molecules 2013; 18:10989-1000.
27. Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen P. Application of natural antimicrobials for food preservation. J Agric Food Chem 2009; 57:5987-6000.
28. Pisano M, Pagnan G, Loi M, Mura ME, Tilocca MG, Palmieri G, Fabbri D, Dettori MA, Delogu G, Ponzoni M, Rozzo C. Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. Mol Cancer 2007; 6:1.
29. Titenko-Holland N, Windham G, Kolachana P, Reinisch F, Parvatham S, Osorio AM, Smith MT. Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay in vitro and in vivo: a study of malathion-exposed workers. Mutat Res 1997; 388:85-95.
30. Cushnie TT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005; 26:343-56.
31. Bonassi S, El-Zein R, Bolognesi C, Fenech M. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis 2011; 26:93-100.

ORIGINAL ARTICLE

Significance of parathyroid scintigraphy and correlation of findings with parathyroid hormone values in patients undergoing hemodialysis

Nermana Ahmetbegović[1], Nadira Suljagić[2], Vahidin Katica[3]

[1]Department of Nuclear Medicine, Cantonal Hospital Zenica, [2]Department of Family Medicine, Public Health Institution Brčko,[3] Clinic for Gynecology and Obstetrics, University Clinical Center Sarajevo; Bosnia and Herzegovina

ABSTRACT

Aim To compare finding of thyroid gland scintigraphy with serum concentration of parathyroid hormone in patients undergoing hemodialysis.

Methods This retrospective-prospective study included 50 patients undergoing hemodialysis with established hyperparathyroidism who were treated at Cantonal Hospital Zenica in the period 2009 – 2014. Besides anthropological data, concentration of parathyroid hormone in serum of patients was monitored too. Scintigraphy was performed at the Department of Nuclear Medicine using two-headed gamma camera Prism 2000xp.

Results Of the total number of 50 patients, 24 (48%) were males and 26 (52%) were females. The average age was 49.34±11.82 years. A total of 17 (34%) patients had normal findings of scintigraphy, 11 (22%) had mildly pronounced uptake of radiopharmaceuticals (score of 1), 14 (28%) had moderately pronounced uptake (score of 2), and eight (16%) had intensive uptake of radiopharmaceuticals (score of 3). A statistically significant difference was established in the length of hemodialysis treatment and scintigraphy finding (p=0.041).

Conclusion Scintigraphy of parathyroid glands in patients undergoing hemodialysis allows us to select them for parathyroidectomy. Scintigraphy of parathyroid glands and a value of parathormone in serum should be incorporated into the test algorithm for patients with secondary hyperparathyroidism caused by chronic kidney disease.

Key words: chronic kidney disease, hyperparathyroidism, parathyroidectomy

Corresponding author:
Nermana Ahmetbegović
Department of Nuclear Medicine,
Cantonal Hospital Zenica
Crkvice 67, 72 000 Zenica,
Bosnia and Herzegovina
Phone: +387 32 405 133;
Fax: +387 32 405 534;
E-mail: nermana.ahmetbegovic@hotmail.com
ORCID ID: http://www. orcid.org/0000-0001-5740-9171

Original submission:
10 June 2016;
Revised submission:
10 July 2016;
Accepted:
27 January 2017.
doi: 10.17392/864-17

Med Glas (Zenica) 2017; 14(2): 158-163

INTRODUCTION

The term "chronic kidney insufficiency" is used to denote terminal (fifth) stage of chronic kidney disease (1). With progression of kidney damage, chronic kidney weakness is developed and leads to progressive disruption of homeostasis in bone mineral turnover, which is manifested by an abnormal concentration of phosphorus and calcium in serum and tissues, as well as changes in concentrations of individual circulating hormones, parathyroid hormone (PTH) (2,3). Due to reduced phosphorus excretion by kidneys, hyperfunction of parathyroid glands occurs (4). Secondary hyperparathyroidism is developed and it influences the metabolism of calcium and balance of bone degradation and formation (5).

Parathyroid glands constantly stimulate creation of PTH and with time they acquire an increasing volume (6).

Hypercalcemia occurs as elevated levels of parathyroid hormone concentration in serum, which results in increased mobilization of calcium from bones mediated by osteoclasts, increased reabsorption of calcium in kidney glomeruli, increased gastrointestinal reabsorption of calcium and 1,25 dihydroxyvitamin D and increased secretion of phosphate in urine, hence hypophosphatemia appears in serum (7-9).

Secondary hyperparathyroidism represents one of the leading complications in patients undergoing hemodialysis, which occurs due to disorder of level regulation of serum phosphorus (PO4), calcium (Ca), PTH and (tissue) vitamin D. The disease is characterized by disorder of parathyroid glands, which are responsible for secretion of parathyroid hormone. Long-lasting secondary hyperparathyroidism is associated with cardiovascular complications and kidney osteodystrophy (10).

When the function of parathyroid glands is increased, it cannot be reduced in any way, and the disease that causes it has advanced, it is recommended to undergo an operation which implies extracting glands (11-13).

Parathyroid gland scintigraphy is an imaging method that shows, by means of radiopharmaceutical Tc-99 MIBI, abnormal tissue of parathyroid glands (14). Successful parathyroidectomy depends on recognition and surgical removal of all hyperfunctional parathyroid glands. Screening is not used to establish the diagnosis (15). Increased Ca in plasma and PTH are sufficient. Scintigraphy does not identify normal parathyroid glands which are small to be seen (20-50mg). Scintigraphy detects abnormal parathyroid glands, it determines size and precisely defines a relation with the thyroid gland. Also, scintigraphy detects ectopic glands (16).

With PTH concentration in patient's serum, scintigraphy is used as a screening method for the assessment of functional status of parathyroid glands and as an important diagnostic procedure in preoperative evaluation of patients with hyperparathyroidism (17-21).

The control of parathyroid glands has not been automatic so far, therefore, the patient and his/her doctor have to get actively involved into solving the problem that has arisen.

The world prevalence of chronic kidney disease (CKD) from the third to fifth stage is 5% or 50,000 (cases) per one million inhabitants, while the prevalence of the end-stage of kidney insufficiencies is 0.1%, or more than 100 new dialysis patients per one million of the population annually (18). Due to unregulated value of phosphorus in hemodialysis patients, kidney osteodystrophy occurs (18). In this research we wish to highlight the importance of regular monitoring of calcium, phosphorus and parathyroid hormone and to compare it with scintigraphic finding, which facilitates access to the surgeons and saves their time, especially in detecting ectopic parathyroid glands.

The aim of this study was to compare findings of parathyroid gland scintigraphy with the serum concentration of parathyroid hormone, calcium and phosphorus in patients undergoing hemodialysis.

PATIENTS AND METHODS

Patients and study design

The retrospective-prospective study included all patients undergoing hemodialysis with established hyperparathyroidism who were treated at Cantonal Hospital Zenica (covering 12 municipalities with a total of 400,000 inhabitants), Bosnia and Herzegovina (B&H), in the period 2009 – 2014 (retrospectively from 2009 to 2012, and prospectively from 2012 to 2014). A total of 50 patients was included. Besides anthropological

data (age, gender), concentration of parathyroid hormone in serum of patients as well as of calcium and phosphorus was monitored.

The research was approved by the Ethics Committee of the Cantonal Hospital Zenica.

Methods

A concentration of PTH in plasma of patients was determined by chemiluminescent enzyme immunoassay test (intact PTH) (Immulite 2000 immunoassay analyzer, Berlin/Germany Siemens). Reference value for parathormone of 9.25-7.5 pg/m was used. Serum levels of calcium and phosphorus were routinely determined in the clinical laboratory with automated equipment by using colorimetric method and quality control of standard. Reference values for calcium were 2.14-2.65 mmol/L and for phosphorus 0.87-1.45 mmol/L.

Scintigraphy was performed at the Department of Nuclear Medicine of the Cantonal Hospital Zenica by using two-headed gamma camera Prism 2000xp, according to the standard protocol with the application of 99m Tc-MIBI. Model PRISM 2000 XP is gamma camera, with computer console Philips and software version Odyssey (Cleveland, U.S.A., 1997). A degree of scintigraphy was determined as per intensity of radiopharmaceuticals accumulation (22): normal (score 1), moderately pronounced (score 2), intensive accumulation of radiopharmaceuticals (score 3).

Statistical analysis

For the description of the sample depending on nature of data, adequate methods of classical descriptive statistics were used: arithmetic mean (AM), standard deviation (SD), median (Med.), interquartile range (25.perc. and 75.perc.), absolute frequency (N) and relative frequency (%). The level of significance was <0.05.

RESULTS

Of the total number of 50 patients included in this research 24 (48%) were males and 26 (52%) were females.

The age of males was 48.25±11.62 and 50.34± 12.14 (p=0.537) of females.

The average length of hemodialysis treatment was 8.45±3.94 years in males and 9.00±5.16 years in females (p=0.537).

Of the total number of 50 patients 17 (34%) had normal finding of scintigraphy, 11 (22%) had mildly pronounced accumulation of radiopharmaceuticals (score 1) (average age of 46.45 years), 14 (28%) had moderately pronounced accumulation of radiopharmaceuticals (score 2) (average age of 47.71 years), while eight (16%) patients had intensive accumulation of radiopharmaceuticals (score 3) (53.00 years). No statistically significant difference was established in gender and age of patients in relation to degree of scintigraphic finding (p=0.595) (Table 1).

A statistically significant difference was established in the duration of hemodialysis treatment and scintigraphy finding: patients who had longer hemodialysis treatment had worse scintigraphy finding (p=0.016).

The average value of PTH in patients who had a regular scintigraphy was 605.17pg/m, in patients with mild accumulation of radiopharmaceuticals 1068.90 pg/m, in patients with moderate accumulation of radiopharmaceuticals 1575.00pg/m, while in patients with intensive accumulation of radiopharmaceuticals it was 1830.12pg/m (p=0.001). The average value of calcium in patients who had a regular scintigraphy was 2.23 mmol/L, in patients with mild accumulation 2.12 mmol/L, in patients with moderate accumulation 2.23 mmol/L, while in patients with intensive accumulation of

Table 1. Average age of patients and duration of hemodialysis treatment in relation to scintigraphy finding

Parameter	Scintigraphy finding	No. of patients	Value	SD	SEM	95% CI	Min.	Max.
Age (years)	Normal	17	50.82	13.56	3.29	43.84-57.79	20.00	67.00
	Mild	11	46.45	11.57	3.49	38.67-54.23	31.00	64.00
	Moderate	14	47.71	10.35	2.76	41.73-53.69	25.00	66.00
	Intensive	8	53.00	11.32	4.00	43.53-62.46	41.00	74.00
				p=0.595				
Duration of hemodialysis treatment (years)	Normal	17	6.45	4.65	1.12	5.07-9.86	2.00	23.00
	Mild	11	7.47	3.11	0.93	4.36-8.54	3.00	12.00
	Moderate	14	10.14	3.52	0.94	8.10-12.17	5.00	18.00
	Intensive	8	12.12	5.59	1.97	7.45-16.79	2.00	19.00
				p=0.016				

SD, standard deviation; SEM, Standard Error of Mean; Min, minimum; Max, maximum;

Table 2. Average values of parathyroid hormone (PTH), calcium and phosphate in relation to scintigraphy finding

Parameter	Scintigraphy finding	No of patients	Value	SD	SEM	Min.	Max.
PTH (pg/mL)	Normal	17	605.17	342.84	83.15	79.00	1032.00
	Mild	11	1068.90	411.84	124.17	378.00	1643.00
	Moderate	14	1575.00	598.29	159.90	535.00	2473.00
	Intensive	8	1830.12	733.47	259.32	1369.00	3519.00
				p=0.001			
Calcium (mmol/L)	Normal	17	2.23	0.21	0.05	1.71	2.64
	Mild	11	2.12	0.31	0.09	1.54	2.51
	Moderate	14	2.33	0.24	0.06	1.94	2.68
	Intensive	8	2.42	0.26	0.09	2.08	2.66
				p=0.069			
Phosphate (mmol/L)	Normal	17	2.1224	0.59	0.14	1.00	3.25
	Mild	11	1.8427	0.96	0.29	0.59	3.04
	Moderate	14	2.0921	0.78	0.20	0.86	4.08
	Intensive	8	2.0938	0.68	0.24	0.86	2.93
				p=0.788			

SD, standard deviation; SEM, Standard Error of Mean; Min, minimum; Max, maximum;

radiopharmaceuticals the average value of calcium was 2.42 mmol/L. The average value of phosphorus in patients who had a regular scintigraphy was 2.1224 mmol/L, in patients with mild accumulation of radiopharmaceuticals it was 1.8472 mmol/L, in patients with moderate accumulation 2.0921 mmol/L, and in patients with intensive accumulation of radiopharmaceuticals the average value of phosphorus was 2.0938 mmol/L (p=0.069 and p=0.788, respectively) (Table 2).

DISCUSSION

Demographic data of the patients included in this research correlate with the results of other researchers. Study released by Resić et al. (23) included 100 patients from Bosnia and Herzegovina (55 males and 45 females) who were on chronic hemodialysis program, with average age of 52.6 years, which is in accordance with our research. Hadžibulić et al. (24) noticed 59.26% males and 40.74% females with average age of 53.16 and 51.72 years, respectively, and found that length of time spent on dialysis for females was statistically significantly longer, 5.8 years on average, comparing to males, 3.4 years. It is not case in our research, where the average duration of hemodialysis treatment was 8.74 years minimum and maximum 23 years. No statistically significant difference was established in the average duration of hemodialysis treatment in relation to gender (8.45and 9.00 years in males and females, respectively) in our research. Kudamija et al. (25) found that the average time spent on hemodialysis treatment was 8.7 years, which is in correlation with our research.

Resić et al. (23) noticed a statistically significant difference in the duration of hemodialysis, the average duration of dialysis was 6 years, and the largest number of patients (62%) was up to 5 years on hemodialysis, which is different from the results we obtained in the present research.

The results of this study showed that parathyroid hormone values were statistically significantly higher in the patients who had worse scintigraphy finding than the patients with normal scintigraphy finding; average values of calcium and phosphate did not statistically significantly differentiate in relation to scintigraphic finding. According to the literature (17), the percentage of patients on dialysis who, according to parathyroid hormone values, have an inclination to secondary hyperparathyroidism is about 32.7%. Secondary hyperparathyroidism in chronic kidney disease is characterized by high level of parathyroid hormone which is the key link in the development of bone and cardiovascular complications (19).

Reihel et al. (26) suggested that their patients with chronic kidney disease, who had been on hemodialysis treatment, had parathyroid hormone values five times higher than referential values with the average value of 625.17 pq/mL. According to KDIGO Guidelines, targeted values of parathyroid hormone in dialysis patients are 3 to 9 times higher than referential values (27). Resić et al. (23) found average parathyroid hormone value of 493.7 pg/dL, which is three times higher than the reference values and it is in accordance with the result of our research. They also found that 60.5% of patients had hypophosphatemia and 49% had

the elevated product CaxP>4.4 mmol2/L2, which represents a risk factor of vascular and soft tissue calcifications and development of coronary and peripheral arterial diseases. Positive correlations between scintigraphy finding and increased parathyroid hormone value were reported by Akim et al. (28) in their research.

Chronic kidney disease is a worldwide public health problem with serious adverse health consequences for affected individuals. Secondary hyperparathyroidism is a frequent complication of chronic kidney disease and a leading cause of clinically significant bone disease. The consequences of insufficiently controlled secondary hyperparathyroidism and negative effects of selected therapeutic interventions lead to high rates of morbidity and mortality in patients with chronic kidney disease (29).

Parathyroid gland scintigraphy in patients on hemodialysis allows us to select the same patients for parathyroidectomy. In our research, it was shown that parathyroid hormone values are statistically significantly higher in subjects who had worse scintigraphy finding than the patients with normal scintigraphy finding; statistically significant, positive correlation was established between the duration of hemodialysis treatment and parathyroid hormone values. In conclusion, scintigraphy of parathyroid glands and parathyroid hormone value in serum as a golden standard should be incorporated into the algorithm of tests in patients with secondary hyperparathyroidism caused by chronic kidney diseases.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATIONS

Competing interest: none to declare.

REFERENCES

1. Ostojić L. Mineralno koštani poremećaji u hroničnoj bubrežnoj bolesti. Medicina Fluminensis 2010; 46:463-70.
2. Andress DL, Coyne DW, Kalantar-Zadeh K, Molitch ME, Zangeneh F, Sprague SM. Management of secondary hyperparathyroidism in stages 3 and 4 chronic kidney disease. Endocr Pract 2008; 14:18-27.
3. Mejia N, Roman-Garcia P, Miar AB, Tavira B, Cannata-Andia JB. Chronic kidney disease-mineral and bone disorder: a complex scenario. Nefrologia 2011; 31:514-9.
4. Orellana JM, Esteban RJ, Castilla YA, Fernández-Castillo R, Nozal-Fernández G, Esteban MA, García-Valverde M, Bravo J. Use of cinacalcet for the management of hyperparathyroidism in patients with different degrees of renal failure. Nefrologia 2016; 36:121-125.
5. Egido J, Martínez-Castelao A, Bover J, Praga M, Torregrosa JV, Fernández-Giráldez E, Solozábal C. The pleiotropic effects of paricalcitol: Beyond bone-mineral metabolism**.** Nefrologia 2016; 36:10-8.
6. Martínez Díaz-Guerra G, Guadalix Iglesias S, Hawkins Carranza F. Normocalcemic primary hyperparathyroidism: a growing problem. Med Clin (Barc) 2013; 141:125-9.
7. Joy MS, Karagiannis PC, Peyerl FW. Outcomes of secondary hyperparathyroidism in chronic kidney disease and the direct costs of treatment. J Manag Care Pharm 2007; 13:397-411.
8. Slinin Y, Foley RN, Collins AJ. Calcium, phosphorus, parathyroid hormone, and cardiovascular disease in hemodialysis patients: the USRDS waves 1.3, and 4 study. J Am Soc Nephrol 2005; 16:1788-93.
9. Orlić L. Mineral and bone disorder in chronic kidney disease. Medicina Fluminensis 2010; 46:463-70.
10. Cordellat IM. Hyperparathyroidism: primary or secondary disease? Reumatol Clin. 2012; 8:287-91.
11. Moe S, Drüeke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G.Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2006; 69:1945-53.
12. Nishitani H, Miki T, Morii H, Nishizawa Y, Ishimura E, Hagiwara S, Nakatsuka K, Yamakawa M . Decreased bone mineral density in diabetic patients on hemodialysis. Contrib. Nephrol 1991; 90:223-7.
13. Pei Y, Hercz G, Greenwood C, Segre G, Manuel A, Saiphoo C, Fenton S, Sherrard D.Renal osteodystrophy in diabetic patients. Kidney Int 1993; 44:159-64.
14. Eknoyan G, Lameire N, Barsoum R, Eckardt KU, Levin A, Levin N, Locatelli F, MacLeod A, Vanholder R, Walker R, Wang H. The burden of kidney disease: improving global outcomes. Kidney Int 2004; 66:1310-4.
15. Palestro CJ, Tomas MB, Tronco GG. Radionuclide imaging of the parathyroid glands. Semin Nucl Med 2005; 35:266-76.
16. Díaz-Expósito R, Casáns-Tormo I, Cassinello-Fernández N, Ortega-Serrano J, Mut-Dólera T. Contribution of intraoperative scintigraphy to the detection of intrathyroidal parathyroid adenoma. Rev Esp Med Nucl Imagen Mol 2014; 33:296-8.
17. Dukanovid Lj, Alcsio-Milidevid B, Antic M. Rezultati liječenja dijalizama u Srbiji: 10 godina kasnije. Monografije naučnih skupova. AMN SLD 2010; 1:3.
18. Chauhan V, Kelepouris E, Chauhan N, Vaid M. Current concepts and management strategies in chronic kidney disease-mineral and bone disorder. South Med J 2012; 105:479:85.

19. Martin KJ, Gonzales EA. Metabolic bone disease in chronic kidney disease. J Am Soc Nephrol 2007; 18:875-85.
20. Russell CF, Edis AJ. Surgery for primary hyperparathyroidism: experience with 500 consecutive cases and evaluation of the role of surgery in the asymptomatic patient. Br J Surg 1982; 69:244-7.
21. Echenique-Elizondo M, Díaz-Aguirregoitia FJ, Amondarain JA, Vidaur F. Parathyroid graft function after presternal subcutaneous autotransplantation for renal hyperparathyroidism. Arch Surg 2006; 141:33-8.
22. Clark PB. Parathyroid scintigraphy: optimizing preoperative localization. Appl Radiol 2005; 34:24-8.
23. Resić H, Kučukalić Selimović E, Kapidžić A, KukavicaN, Šahović V, Mašnić F. Renalna osteodistrofija kod pacijenata na hemodijalizi. Medicinski **žurn**al 2009; 15:106-10.
24. Hadžibulić E, Birđozlić Š.F. Poremećaji mineral kostiju kod bolesnika na dijalizi u zdravstvenom centru Novi Pazar. Sanamed 2010; 5:17-20.
25. Kudamija M, Dits S, Hršak PI, Mihovilović K, Pavlović D. Liječenje sekundarnog hiperparatireoidizma malim dozama sinakalceta i parikalcitolom. Acta Med Croatica 2014; 68:34-38.
26. ReihelM, Detelić D, ŠeferS, Pavlović D. Parikalcitrol u liječenju sekundarnog hiperparatireoidizma u starijih osoba. Acta Med Croatica 2014; 68:30-3.
27. KidneyDisease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO Clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl 2009; 113:S1-130.
28. Akin M, Atasever T, Kurukahvecioglu O, Dogan M, Gokaslan D, Poyraz A, Koksal H, Taneri F. Preoperative detection of parathyroid adenomas with Tc-99m MIBI and Tc-99m pertechnetate scintigraphy: histopathological and biochemical correlation with Tc-99m MIBI uptake. Bratisl Lek Listy 2009; 110:166-9.
29. Goodman WG. The consequences of uncontrolled secondary hyperparathyroidism and its treatment in chronic kidney disease. Semin Dial 2004; 17:209-16.

Značaj scintigrafije paratireoidnih žlijezda i korelacija nalaza s vrijednostima paratireoidnih hormona kod pacijenata na hemodijalizi

Nermana Ahmetbegović[1], Nadira Suljagić[2] Vahidin Katica[3]

[1]Odjel za nuklearnu medicinu, Kantonalna bolnica Zenica, [2]Dom zdravlja Brčko,[3]Klinika za ginekologiju i akušerstvo, Univerzitetski klinički centar Sarajevo; Bosna i Hercegovina

SAŽETAK

Cilj Komparirati nalaze scintigrafije paratireoidnih žlijezda sa serumskom koncentracijom parathormona kod pacijenata na hemodijalizi.

Metode U retrospektivno-prospektivno istraživanje, u periodu od 2009. do 2014. godine, bili su uključeni pacijenti na hemodijalizi s utvrđenim hiperparatireoidizmom koji su klinički obrađivani u Kantonalnoj bolnici Zenica. U istraživanje je uključeno ukupno 50 pacijenata. Uz antropološke podatke, praćena je koncentracija parathormona u serumu pacijenata. Scintigrafija je rađena na Odjelu za nuklearnu medicinu Kantonalne bolnice Zenica na dvoglavoj gama-kameri *Prism* 2000xp.

Rezultati Od 50 ispitanika 24 (48%) je bilo muškog, a 26 (52%) ženskog spola. Prosječna starosna dob je iznosila 49.34±11.82 godine. Od 50 ispitanika 17 (34%) je imalo uredan nalaz scintigrafije, blago naglašen unos radiofarmaka (scor 1) imalo je 11 (22%), umjereno naglašen (scor 2) 14 (28%), a intenzivan unos radiofarmaka (scor 3) 8 (16%) ispitanika. Ustanovljena je statistički značajna razlika u dužini trajanja hemodijaliznog tretmana i nalaza scintigrafije.

Zaključak Scintigrafija paratireoidnih žlijezda kod pacijenata na hemodijalizi omogućava njihovu selekciju za paratireoidektomiju. Scintigrafiju paratireoidnih žlijezda i vrijednost parathormona u serumu kao zlatni standard, treba uvrstiti u algoritam pretraga kod pacijenata sa sekundarnim hiperperatireoidizmom uzrokovanim hroničnim bubrežnim oboljenjima.

Ključne riječi: hronična bubrežna bolest, hiperparatireoidizam, paratireoidektomija

ORIGINAL ARTICLE

The effect of functional insulin therapy on glycaemic parameters in children with diabetes mellitus type 1

Diana Štimjanin-Koldžo[1], Salem Alajbegović[2], Ena Štimjanin[2], Jasmina Mehinagić[1]

[1]Department of Paediatrics, [2]Department for Endocrinology, Diabetes and Metabolic Diseases; Cantonal Hospital Zenica, Zenica, Bosnia and Herzegovina

ABSTRACT

Aim The aim of the study is to evaluate the effect of a prospectively conducted interactive 5-day education programme based on Düsseldorf model on glycated haemoglobin (A1C), and total daily dose of insulin in type 1 diabetes patients**.**

Methods A total of 67 type 1 diabetes patients was analysed; mean age of 11±0.68 years, 43 females and 24 males. The programme was led by a trained team of diabetes specialist doctors and nurses. All subjects and their parents completed a knowledge test about diabetes at beginning, and at the end of education, and after 12 months (30 questions). Subjects were evaluated for total daily insulin, and HbA1c at baseline, as well as 3, 6, 9 and 12 months after the end of the education programme.

Results Results of the knowledge test after the education have shown higher knowledge at baseline. At the end of the education programme an average of total daily insulin dose was significantly lower. There was a 3.17% reduction in HbA1c values over 9 months, and 1.8% over 12 months in the comparison to the baseline values ($p<0.001$).

Conclusions Structured education programme of functional insulin therapy was associated with improved glycaemic control in type 1 diabetes patients and their parents. It motivated patients and parents to improve glycaemic control. One year after the follow up, glycaemic control was worsening, due to lack of patients' motivation, therefore, there is a need for yearly re-education.

Key words: child, parents, education of diabetes, functional insulin therapy, glycaemic control

Corresponding author:
Diana Štimjanin-Koldžo
Department of Paediatrics,
Cantonal Hospital Zenica
Crkvice 67, 72000 Zenica,
Bosnia and Herzegovina
Phone: +387 32 447 336;
Fax: +387 32 226 576;
E-mail: dstimjanin@gmail.com
ORCID ID: http://www.orcid.org/0000-0002-7596-6630

Original submission:
29 March 2017;
Revised submission:
07 April 2017;
Accepted: 07 June 2017.
doi: 10.17392/907-17

Med Glas (Zenica) 2017; 14(2): 164-168

INTRODUCTION

Diabetes mellitus (DM) is the most common, chronic metabolic endocrine disease in children and adolescents (1). Optimal treatment for diabetes mellitus, except insulin therapy, is dietary measurement, self-control, adequate physical activity and education (2,3). Every young diabetic patient and their parents/carers have the right to accessible, planned diabetes self-management education (4). Diabetes education should be delivered by an interdisciplinary paediatric diabetes team (as a minimum a doctor, nurse and dietician), understanding of the special and changing needs of young people and their families as they grow through different stages of life; it needs to be patient-centred and thus adaptable to suit individual needs. Successful treatment of DM incorporates behavioural changes in patients and their parents/carers. Education is the keystone for diabetes care and structured diabetes self-management education (DSME) is the key to a successful outcome (5). Therapeutic education programme needs to be structured taking into account therapeutic scheme and a need to show its effectiveness. Interactive methods and materials are used taking into account patients' daily routine. In Diabetes Control and Complication Trials study (DCCT) this was one of four education programmes, as well as in DAFNE-Dose Adjustment For Normal Eating in Great Britain, Düsseldorf model of structured therapy and education, and functional intensified insulin therapy-FIT (Vienna, Ljubljana, Zagreb). Prospective studies based on carbohydrate counting evaluated a 5-days education programme in type 1 diabetic patients (6,7). Functional insulin therapy (FIT) knowledge include: functional insulin replacements, all about insulin, insulin dose adjustment, tests of basal insulin, carb factor, correction factor; functional insulin replacement under special conditions (exercise, illness). The effects are greater for children than adults, and are most effective when integrated into routine care, when education is learner-centred, parents are involved, empowerment principles are utilised, and self-management, problem-solving, goal setting and self-efficacy are promoted (2,3,6).

The aim of the study was to evaluate the effect on glycated haemoglobin (A1C) of a structured intervention in type 1 diabetes patients.

Functional insulin therapy is implemented at the Department of Paediatrics Endocrinology and Diabetes in Cantonal Hospital Zenica, Bosnia and Herzegovina (B&H) as a routine way of education and re-education of children with diabetes type 1and their parents. The purpose of monitoring the group of children is to show the importance of such access to education as well as the need for re-education after a certain period of motivation to both children and parents. Similar research was not carried out in other paediatric departments in the B&H. The results of our work should serve as a motivation for other B&H centres to organize education on this model, with the aim of improving the glycaemic control of children with diabetes type 1.

PATIENTS AND METHODS

Patients and study design

This prospective study evaluated routine and usual 5-day education programme for type 1 diabetic patients and their parents at the Department of Paediatrics Endocrinology and Diabetes, Cantonal Hospital Zenica, B&H. The study took place in the period between January 2012 to March 2013. The education programme was adapted from the Düsseldorf "Diabetes Training and Treatment Programme" (8) and based on carbohydrate counting for persons with type 1 diabetes. Intensive group education course took place over 5 consecutive days with skills based focus led by a diabetes specialist and a nurse (normally 12 participants per group, 6 children and 6 parents). Each group was followed for one year. A total of 67 type 1 diabetes patients was analysed, mean age of 11±0.68 years, 43 females and 24 males.

Methods

All subjects and their parents completed a knowledge test about diabetes at beginning and at the end of education, after five days (30 questions) and after 12 months. Total daily dose of insulin (TDD) and HbA1c were evaluated at the baseline, and 3, 6, 9 and 12 months after the education programme. Time table of education: 1st day: What is diabetes, hypoglycaemia and hyperglycaemia; 2nd day: Which food is best for me? 3rd day: Self-management of diabetes; 4th day: Therapy for me; 5th day: Chronic complications of diabetes and foot care.

Statistical analysis

Arithmetic mean value and standard deviation, Wilcoxon test for average 6-point glycaemic profile was used. For HbA1c baseline and 3, 6, 9, 12 months checks Freidman test was used.

RESULTS

In this study, 67 type 1 diabetes patients on intensive insulin therapy were analysed; mean age 11±0.68 years, 43 (64.18%) females and 24 (35.82%) males.

At the end of the education programme, the results showed significant increase of correct answers average compared to the number of correct answers before the beginning of the educational intervention (18.02:2.19; p<0.001). After 13 months the results showed a decrease of correct answers average comparing to the results at the end of the five-day education (2.19:23.85; p<0.001) (Table 1).

The percentage of pronounced results of the knowledge test before and after education as well as after 13 months was significantly increased, 60.6%, 88.17%, and 79.5%, respectively (Table 1).

Table 1. Results of knowledge test before and after education (1st day, 5th day, 12 months)

Number (%) of right answers of 30 questions							
1st day(before education)		5th day after education			12 months after education		
MV	SD	MV	SD	p	MV	SD	p
18.02 (60.06)	20.99	2.19 (88.17)	10.12	<0.001	23.85 (79.5)	12.13	<0.001

MV, middle value; SD, standard deviation

There was a statistically significant decrease in the total daily dose of insulin (TDD) at the end of education. The average decrease in insulin TDD was 2.90 IU after 5 days, but the average decrease after 12 months was 1.97 IU (Figure 1).

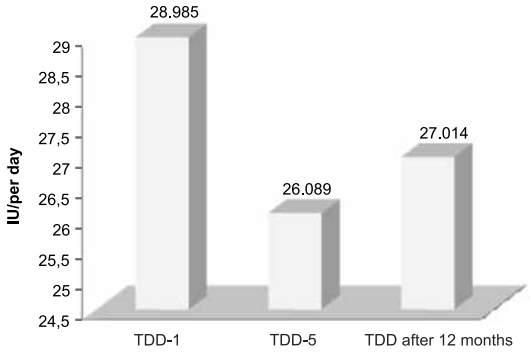

Figure 1. Average total daily insulin dosage, TDD-1, total daily dose first day; TDD-5, total 5 days daily dose

There was a 3.17% reduction in HbA1c average value over 9 months (10.60% and 7.47%, respectively; p<0.001) and 1.8% over 12 months (10.60% and 8.24%, respectively; p<0.001) in the comparison to the baseline values. HbA1C values increased by 0.77% in the period between 9 and 12 months (7.46% and 8.24%, respectively; p<0.001) (Table 2, Figure 2).

Table 2. Clinical approvement of functional intensified insulin therapy in 67 patients with type 1 diabetes mellitus

HbA1c (%)									
1st day (beginning)		6 months		Changes	p	12 months		Changes	p
MV	SD	MV	SD	MV		MV	SD	MV	
10.60	2.19	8.07	1.40	-2.53	<0.001	8.25	0.99	-2.35	<0.001

MV, middle value; SD, standard deviation

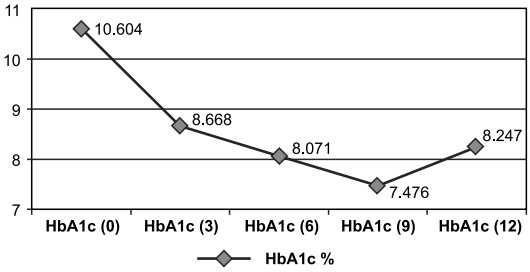

Figure 2. Results of average value of HbA1c at the beginning and the end of the study. Control checks of HbA1c at 1st day (0), after 3 days (3), 6 days (6), 9 days (9) and 12 months (12)

DISCUSSION

Structured DM education improves behavioural changes and consequently leads to better control of the disease with lower doses of insulin needed to achieve adequate glycaemia. Effects of structured DM education weaken after 13 months (18).

The rationale for pre- and post-prandial self-monitoring is to help patients understand the effects of food choices, physical activity, and medications on blood glucose concentrations, and to guide therapeutic adjustments (9) without increasing the hypoglycaemic risk. The results of this study have shown that educational intervention improved patients' knowledge of DM, e. g. results of knowledge test before and after education showed a significant increase of average correct answers to the knowledge test. Unfortunately, effects of education faded after 12 months and showed a decrease in average correct answers in relation to the test after education. Improvements in patients' knowledge resulted in improvement of their disease showing lower HbA1C values as

well as lower doses of insulin needed to achieve satisfactory glycaemic control. Observed effects weakened as time from the educational intervention passed and there was 3.17% reduction in HbA1c over 9 months, and 1.8% over 12 months compared to the baseline values; HbA1C values have increased by 0.77% between 9 months and 12 months. Effects of educational intervention were stable for 9 months and after that time effects they begun fading, but not reaching the baseline after 12 months. Average doses of insulin needed to achieve glycaemic values decreased 2.90 IU after 5 days of education, and 1.97 IU after 13 months. This finding could be explained by frequent therapeutic modifications with the intensified self-monitoring blood glucose (SMBG) and making insulin adjustments related to the additional blood glucose testing and food intake (10). The SMBG and some psychosocial factors are believed to be associated with active self-management in type 1 diabetic patients after the education programme (11). In many studies, there is evidence of an increased risk for hypoglycaemia as the HbA1c decreases (11-13), but this is not always the case (14), particularly in recent years with the increasing use of insulin analogues (15,16).

The disadvantages of the study include insubstantial analysis of newly discovered and previously discovered diabetics as well as the absence of a control group of patients who did not undergo this type of education.

The strength of this study includes selection of children with type 1 diabetes and their parents who were already on SMBG to avoid the potential impact of SMBG in ongoing and new patients and the fact that all participants had been educated and followed-up by the same diabetes team with the same education and treatment policies and clinical feedback. Educators (paediatric endocrinologist or physician trained for the care of children and adolescents with diabetes, diabetes educators, dieticians, psychologists, social workers, and other health care providers) should have access to continuing specialized training in current principles of insulin therapy, new diabetes technologies, advances in diabetes education, and educational methods (17). Diabetes education needs to be a continuous process and repeated in order to be effective (18).

In conclusion, one year after our education programme intensive management of diabetes, enabled by various and sustained changes in patient skills, was associated with improved HbA1c. Every child with type 1 diabetes with basal bolus therapy and their parents should be offered a structured functional intensive therapy (FIT) education course. Carbohydrate counting is an important skill for everyone involved, offered to all newly diagnosed patients, and to anyone who wants to improve self-management skills. Profit of FIT is a significantly better glycaemic control, raised and refreshed level of knowledge, self-satisfaction and better self-control, reduction of possible acute and chronic complications of the disease.

AKNOWLEDGEMENTS

The author would like to thank Mujezinović Alisa, bacc. nurse for participation in intensive diabetes education.

FUNDING

No specific funding was received for this study.

TRANPARENCY DECLARATIONS

Competing interests: none to declare.

REFERENCES

1. Watkins RA, Evans-Molina C, Blum JS, Dimeglio LA. Established and emerging biomarkers for the prediction of type 1 diabetes: a systematic review. Transl Res 2014; 164:110–21.
2. Dunbar JA. The RCPE UK Consensus Statement on Diabetes. J R Coll Physicians Edinb. 2010; 40:283.
3. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman S, Lernmark A, Metzger BE, Nathan DM. Guidelines and recommendation for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2011; 34:1419-1423.
4. Haas L, Maryniuk M, Beck J, Cox CE, Duker P, Edwards L, Fisher EB, Hanson L, Sklaroff S, Tomky D, Youssef G. National standards for diabetes self management education and support. Diabetes Care 2012; 37:144–53.
6. Funnell MM, Brown TL, Childs BP, Haas LB, Hosey GM, Jensen B, Maryniuk M, Peyrot M, Piette JD, Reader D, Siminerio LM, Weinger K, Weiss MA. National standards for diabetes self- management education. Diabetes Care 2007; 30:1630-7.
7. Gerich JE, Odawara M, Terauchi Y. The rationale for paired pre- and postprandial self-monitoring of blood glucose: the role of glycemic variability in

micro- and macrovascular risk. Curr Med Res Opin 2007; 23:1791-8.
8. Franklin VL, Waller A, Pagliari C, Greene SA. A randomized controlled trial of Sweet Talk, a text-messaging system to support young people with diabetes. Diabetic Med 2006; 23:1332-13.
9. Svoren BM, Volkening LK, Butler DA, Moreland. EC, Anderson BJ, Laffel LM. Temporal trends in the treatment of pediatric type 1 diabetes and impact on acute outcomes. J Pediatr 2007; 144:660–1.
10. American Diabetes Association. Clinical practice recommendations. Diabetes Care 2014:37(Suppl.1):S14-S80.
11. Murphy HR, Rayman G, Skinner TC. Psycho-educational interventions for children and young people with Type 1 diabetes. Diabetic Med 2006; 23:935-43.
12. DCCT Research Group (Diabetes Control and Complications Trial Research Group). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus. N Engl J Med 1993; 329: 977–86. 13.
13. DCCT Research Group (Diabetes Control and Complications Trial Research Group). Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. J Pediatr 1994; 125:177–88.
14. de Beaufort CE(1), Swift PG, Skinner CT, Aanstoot HJ, Aman J, Cameron F, Martul P, Chiarelli F, Daneman D, Danne T, Dorchy H, Hoey H. Continuing stability of center differences in pediatric diabetes care: do advances in diabetes treatment improve outcome? The Hvidore Study Group on childhood diabetes. Diabetes Care 2007; 30:2245–50.
15. Johnson SR, Cooper MN, Jones TW, Davis EA. Long-term outcome of insulin pump therapy in children with type 1 diabetes assessed in a large population-based case-control study. Diabetologia 2013; 56:2392–400
16. Downie E, Craig ME, Hing S, Cusumano J, Chan AK, Donaghue KC. Continued reduction in the prevalence of retinopathy in adolescents with type 1 diabetes: role of insulin therapy and glycemic control. Diabetes Care 2011; 34:2368–73.
17. Haller MJ, Stalvey MS, Silverstein JH. Predictors of control of diabetes: monitoring may be the key. J Pediatr 2004; 144:660–1.
18. Cameron FJ, de Beaufort C, Aanstoot HJ, Hoey H, Lange K, Castano L, Mortensen HB. International Study Group. Lessons from the Hvidoere International Study Group on childhood diabetes: be dogmatic about outcome and flexible in approach. Pediatr Diabetes 2013; 14:473–80.

Efekti funkcionalne inzulinske terapije na parametre glikemijske kontrole kod djece s dijabetesom tipa 1

Diana Štimjanin-Koldžo[1], Salem Alajbegović[2], Ena Štimjanin[2], Jasmina Mehinagić[1]

[1]Služba za dječije bolesti, [2]Odjeljenje za endokrinologiju, dijabetes i bolesti metabolizma; Kantonalna bolnica Zenica, Zenica, Bosna i Hercegovina

SAŽETAK

Cilj Cilj studije je evaluirati efekte interaktivnog petodnevnog edukacijskog programa po Dizeldorfskom modelu, provedenog prospektivno, na nivo HbA1c i ukupne dnevne doze inzulina kod pacijenata s dijabetesom tipa 1.

Metode Praćeno je 67 djece s dijabetesom tipa 1, prosječne dobne starosti 11±0.68, odnosno 43 djevojčice i 24 dječaka. Edukacijski program je proveo educirani tim subspecijalista (endokrinolog, dijabetolog i medicinska sestra). Svi praćeni pacijenti i njihovi roditelji pristupili su testu znanja od 30 pitanja na početku programa, na kraju petodnevne edukacije te na kraju praćenja tokom 12 mjeseci. Pacijenti i njihovi roditelji pratili su ukupnu dnevnu dozu inzulina, HbA1c na početku edukacije te nakon 3, 6, 9 i 12 mjeseci po edukativnom programu.

Rezultati Rezultati testa znanja pokazali su znatno bolje rezultate nakon edukacije i opadanje nivoa znanja nakon 12 mjeseci. Na kraju edukacijskog rada ukupna dnevna doza inzulina bila je značajno niža. HbA1c je reduciran za 3.17% tokom 9 mjeseci praćenja, a zabilježen je ukupni pad HbA1c nakon 12 mjeseci praćenja od 1.8%.

Zaključak Strukturirani edukacijski program funkcionalne inzulinske terapije ukazao je na poboljšanje kontrole dijabetesa tipa 1 kod djece. On je motivirajući i za djecu i za njihove roditelje u održavanju glikemične kontrole. S obzirom da nakon godinu dana praćenja motivacija opada, što je registrirano pogoršanjem glikoregulacije, potrebna je reedukacija.

Ključne riječi: dijete, roditelji, edukacija, funkcionalna inzulinska terapija, glikemijska kontrola.

ORIGINAL ARTICLE

Importance of inflammatory markers and IL-6 for diagnosis and follow up of patients with type 2 diabetes mellitus

Maja Malenica[1], Mira Šilar[2], Tanja Dujić[1], Tamer Bego[1], Sabina Semiz[1,3], Selma Škrbo[4], Besim Prnjavorac[5,6], Adlija Čaušević[1]

[1]Department for Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina, [2]University Clinic of Pulmonary and Allergic Diseases, Golnik, Slovenia, [3]Faculty of Engineering and Natural Sciences, International University of Sarajevo, [4]Department for Clinical Pharmacy, Faculty of Pharmacy, University of Sarajevo, [5]General Hospital, Tešanj, [6]Department for Pathophysiology, Faculty of Pharmacy, University of Sarajevo; Bosnia and Herzegovina

ABSTRACT

Aim To analyse the long-term impact of altered metabolism on the level of mediators of inflammatory response in female patients with type 2 diabetes.

Methods This study included 97 female patients with type 2 diabetes and 107 female, nondiabetic control subjects, who were recruited at the Clinical Centre University of Sarajevo and the General Hospital Tešanj. The effects of glycaemic control on markers of inflammatory response represented by C-reactive protein (CRP), fibrinogen, leukocytes, sedimentation rate, and cytokine IL-6 were tested. All subjects were free of evidence of infections, surgery, thyroid disease, polycystic ovarian syndrome, active liver and kidney damage. All biochemical analyses were performed according to standard International Federation of Clinical Chemistry (IFCC) protocols.

Results A significant increase of fibrinogen ($p<0.001$), CRP ($p=0.001$), interleukin-6 ($p=0.013$), leukocytes ($p<0.001$) and sedimentation rate ($p=0.008$) in diabetic female population compared to control subjects was found. A significant correlation between CRP and haemoglobin A_{1c} ($p=0.035$), interleukin-6 and glucose ($p=0.032$), IL-6 and body mass index ($p=0.007$) was found.

Conclusion Our data suggest that inflammation plays an important role in the pathogenesis of diabetes in female diabetic population. A more detailed study on a far larger number of subjects is needed if they were to be used effectively as biomarkers in the primary prevention of type 2 diabetes in this population.

Keywords: inflammation, cytokine, hyperglycaemia

Corresponding author:
Maja Malenica
Faculty of Pharmacy, University of Sarajevo
Zmaja od Bosne 8, 71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 33 586188;
Fax: +387 33 586356
E-mail: malenica_maja@yahoo.com
ORCID ID: http://www.orcid.org/0000-0002-3392-9778

Original submission:
05 July 2017;
Accepted:
09 July 2017.
doi: 10.17392/920-17

Med Glas (Zenica) 2017; 14(2): 169-175

INTRODUCTION

Type 2 diabetes (T2D) is a major global health problem affecting 415 million people (215 million of men and 199 million of women). It is considered that this number will rise to 642 million in 2040. Of these, 90-95% of cases are T2D (1,2). The tendency of increase of T2D epidemic is present throughout the world, especially in Europe, Southern America, Africa and East Asia. According to the International Diabetes Federation in Bosnia and Herzegovina estimated prevalence of diabetes is 10% with a tendency of continuous increase (3).

As the rates of diabetes increase it is important to study factors associated with late diagnosis of diabetes and whether these determinants differ for males and females. In this sense, it is very important to recognize risk factors for diabetic complications as soon as possible. This is especially the case for female diabetic population since, according to newest data, even though more males have diabetes, females with diabetes have a greater risk of mortality and hospitalizations (4).

Recent research suggests that inflammation could be a crucial factor in the development of a disease that has reached epidemic proportions worldwide. Due to metabolically provoked changes in diabetes, primarily at the cellular level, altered functionality of cells and tissues is to be expected, with proper manifestations at the level of inflammatory reaction and all its components, including inflammatory parameters (high sensitivity C reactive protein (hsCRP) and fibrinogen), mediators, and inflammation regulators (cytokines) (5).

Differences between sexes in the diabetes risk associated with inflammatory markers have been reported in various studies (6-8). Studies on woman population have revealed that CRP was more strongly associated with type 2 diabetes when compared to interleukin-6 (IL-6) (9,10). However, other authors have published contradictory results. Findings in non-obese subjects prove that body fat content is the main predictor of fibrinogen levels as well of hsCRP levels thus supporting the speculation of direct mechanism by which adipose tissue regulates the levels of circulating acute phase reactants (11).

The aim of the study was to analyse the level of inflammatory markers hsCRP, IL-6 and leukocyte count, sedimentation rate as well as antropometric parameters in a group of female patients with diabetes type 2 and a group of healthy controls, and to correlate their values with the parameters of glycoregulation such as haemoglobin A_{1c}(HbA_{1c}), and fasting glycaemia.

PATIENTS AND METHODS

Patients and study design

A total of 204 subjects were enrolled in the study: 97 patients with T2D, recruited from the Clinical Centre University in Sarajevo and General Hospital Tešanj, and 107 female, control nondiabetic patients.

Subjects included in this study were free of evidence of active liver and kidney disease, chronic pancreatitis, gastrointestinal disease, inflammatory bowl disease, endocrine disorders, infection, and were not using hormonal therapy. Non-diabetic controls were of approximately same age (40-60 years old) having normal glucose tolerance (fasting plasma glucose less than 6.2 mmol/L, and two hours postprandial glycaemia less than 7.8 mmol/L). They also had no abdominal obesity as a criteria for insulin resistance.

Each participant gave a written informed consent. The study was performed in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Cantonal Hospital Zenica and International University of Sarajevo.

Methods

In all the subjects arterial blood pressure and body parameters as height, weight and waist circumference were measured. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest (10). Blood samples were drawn after an overnight fast, at least for 8 hours. HbA_{1c} was measured in the whole blood by immunoturbidimetry method using the autoanalyser Dimension X Pand (Siemens, München, Germany). hsCRP was measured at the same autoanalyser with nephelometric method. Fibrinogen was determined using nephelometric method, modified Clauss principle in Behring Coagulation Systems (12). For determination of IL-6, flow cytometry was used. For the assay for IL-6, particles with defined fluorescence intensity were used for the detection of soluble cytokine at very low concentrations (10-2500 pg/mL) (Human IL-6 Flex Set, BDTM Cytometric Bead Array)(13).

Statistical analysis

Before statistical analysis, normal distribution and homogeneity of the variances were tested using Kolmogorov-Smirnov test respectively. Groups were compared using Student's unpaired t test for parameters with normal distribution or Mann–Whitney test for parameters with non-normal distribution. Correlations between parameters were analysed using the Pearson R test for variables with normal distribution and the Spearman test for variables with non-normal distribution. Data are expressed as mean ± standard deviation or medians (interquartile range). $p < 0.05$ was considered significant.

RESULTS

A group of 97 patients with type 2 diabetes mellitus (age>40 years) and 107 healthy controls (age>40 years) were studied (Table 1).

Table 1. Anthropometric, clinical and biochemical characteristic of female patients with type 2 diabetes mellitus (T2D) and control subjects*

Characteristic	Healthy controls (n=107)	T2D patients (n=97)	p
Age (years)	55 (53-58)	57 (53-61)	0.282
BMI (kg/m^2)	26.30 (23.90-27.90)	29.80 (26.80-33.00)	< 0.001†
Waist circumference (cm)	0.88 ± 0.05	0.93 ± 0.07	< 0.05*
Fasting glucose (mmol/L)	4.95 (4.67-5.30)	7.90 (6.95-9.07)	< 0.001†
HbA1c (%)	5.05 (2.76-5.40)	7.30 (6.80-8.50)	< 0.001†
hsCRP (mg/L)	2.20 (1.02-3.78)	3.10 (1.92-5.00)	0.001†
Fibrinogen (g/L)	3.28 (2.90-3.50)	3.70 (3.20-4.30)	< 0.001†
IL-6 (pmol/L)	1.23 (0.41-1.90)	1.75 (1.22-3.29)	0.013†
Leukocytes (10^9 L)	6.00 (4.73-7.23)	7.00 (5.72-8.60)	< 0.001†
Sedimentation rate	10 (5-16)	15 (9-24)	0.008†

*Data are presented as means ± SD or medians (interquartile range);
†Significance of difference in Mann-Whitney test for data following non-normal distributed and t-test for normal distributed date. T2D-Type 2 diabetes mellitus;
BMI, body mass index; HbA1c, haemoglobin A1c; IL6, interleukin-6; hsCRP, high-sensitivity C-reactive protein

The criteria for the selection of subjects were based on levels of fasting glucose and HbA_{1c}. The levels of hsCRP, fibrinogen, IL-6, white blood cell count and sedimentation were determined for all the study participants.

Subjects with T2D were older than subjects without diabetes. The mean age of the subjects in the study group (cases) was 57 years and the subjects in the control group (controls) were aged 55 years. It was observed that among diabetics the mean of body mass index (BMI) (29.80) and waist–hip ratio (WHR) (0.93) were significantly higher compared to non-diabetics ($p<0.05$), whereas no significant difference was noted in relation to age ($p=0.282$) among diabetics and non-diabetics. Subjects with T2D had significantly higher fasting plasma glucose and HbA_{1c} than those without it (mean value 7.90 versus 4.95 and 7.30 versus 5.05, respectively).

Median levels of IL-6, fibrinogen and hsCRP were significantly higher in diabetic patients: 1.75 versus 1.23 pmol/L for IL-6, 3.70 versus 3.28 g/L for fibrinogen and 3.10 versus 2.20 mg/L for hsCRP ($p=0.013$ for IL-6, $p<0.001$ for fibrinogen and $p=0.001$ for hsCRP).

T2D subjects showed a significant increase in white blood cell count and sedimentation rate (7.00×10^9/L versus 6.00×10^9/L for leukocytes ($p<0.001$) and 15 versus 10 for sedimentation rate ($p=0.008$), respectively)

HbA_{1c} was positively correlated with hsCRP with a significant p of 0.035 (Figure 1) and BMI was positively and significantly correlated with IL-6 ($p=0.007$) and fibrinogen ($p=0.029$) (Figure 2, 3).

The glucose levels of the patients was significantly correlated with IL-6 ($p=0.032$) (Figure 4).

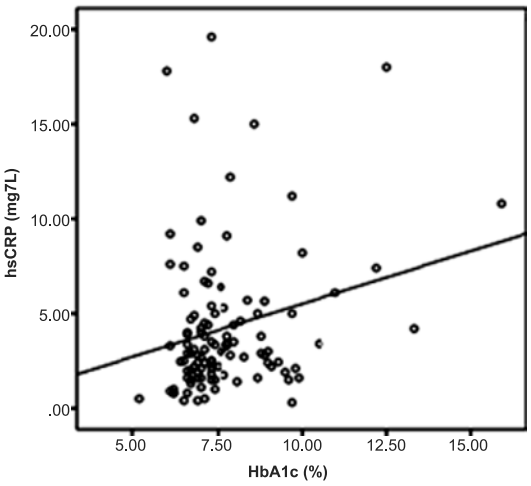

Figure 1. Correlation between serum levels HbA_{1c} and hsCRP in female patients with type 2 diabetes mellitus (T2D)

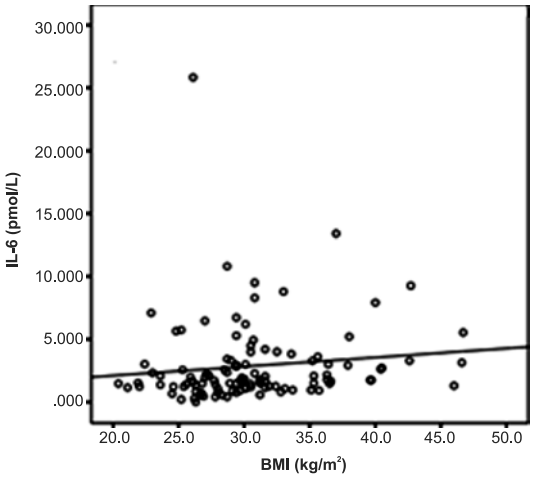

Figure 2. Correlation between serum levels IL-6 and body mass index (BMI) in female patients with type 2 diabetes mellitus (T2D)

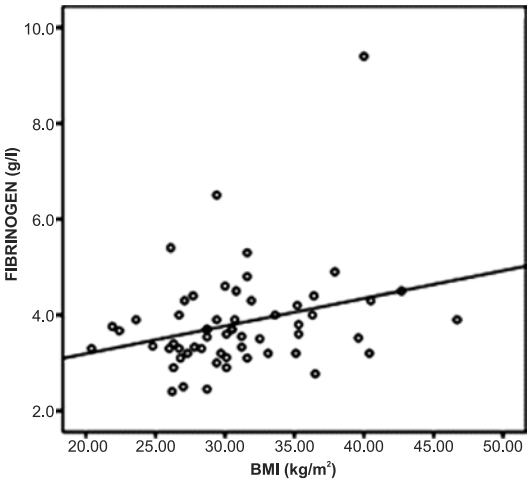

Figure 3. Correlation between serum levels fibrinogen and body mass index (BMI) in female patients with type 2 diabetes mellitus (T2D)

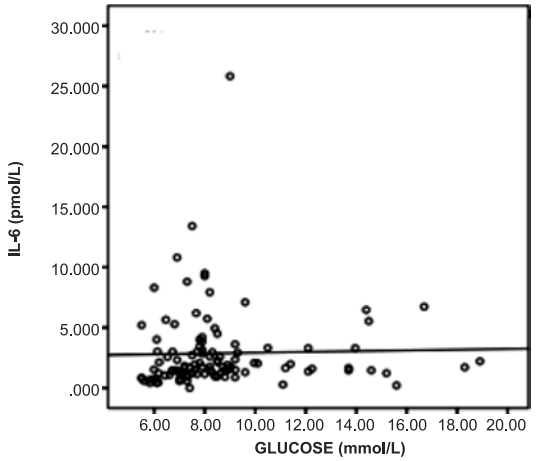

Figure 4. Correlation between serum levels IL-6 and glucose in female patients with type 2 diabetes mellitus (T2D)

DISCUSSION

It is known for T2D that the concentration of many proteins of the acute phase (CRP, fibrinogen, α_1-acid glycoprotein, plasminogen activator inhibitor-1, IL-1, IL-6 and tumour necrosis factor-alpha (TNFα) is increased and that this increase correlates with the development of characteristics associated with this condition (obesity, insulin resistance, impaired glucose tolerance and/or diabetic complications) (14,15), which has been proved in our study.

This research tackled dynamic changes in concentrations of proinflammatory and antiinflammatory cytokines, concerning change and dynamic course of parameters of inflammation in female patients with T2D. The levels of all of the measured inflammatory parameters (hsCRP, fibrinogen, IL-6, sedimentation, and the white blood cell count) were statistically higher in female T2D patients compared to healthy female population. Already in the 1990s, research on this subjects demonstrated that levels of markers of inflammatory reactions increased with the decrease in insulin sensitivity depending on the severity of T2D and metabolic syndrome (16).

Studies conducted for both sexes have demonstrated the importance of inflammatory mediators in the pathogenesis of T2D, and also that levels of IL-6 rise significantly in both sexes compared to control group even after adjustment for BMI, WHR and smoking, being considered an independent predictor of risk of developing diabetes (17,18). Studies conducted on female population have associated elevated levels of IL-6 and CRP with T2D independent of BMI, physical activity and other risk factors for T2D, provided that CRP is considered to be the most robust predictor (9). However, the results of a Mexico City study group suggest that CRP is an important predictor for the development of T2D and the metabolic syndrome for female, but not male population (19). Magnitude of association between CRP and diabetes seems comparable and even stronger than the association between CRP with coronary heart disease (20,21). These data support the hypothesis that systemic inflammation is a common precursor for T2D (14,22), which is in line with our results. It is believed that IL-6 contributes to the pathology and physiology of T2D through its interactions with insulin-signalling pathways and β-cell

function by stimulating production of CRP in liver (23). In accordance with studies conducted by Pradhan et al. (24) and Mirza et al. (15), it is considered that the predictive role of IL-6 in diabetes is less consistent than the role of CRP, because of greater stability of CRP as an inflammation marker in comparison to IL-6 whose effects are modulated by TNFα production (9). However, in our study, IL-6 concentrations were significantly associated with the levels of glucose, suggesting a role of IL-6 in the process of insulin secretion under certain conditions such as high glucose concentrations, high IL-6 levels or being in synergy with other inflammatory mediators (23). An activation of the immune system and inflammation leads to elevated levels of inflammatory markers, not just cytokines but also leukocytes, as well as to accelerated erythrocyte sedimentation rate (25).

Based on the above, one might support the hypothesis that inflammation markers, such as leukocytes and fibrinogen, as well as the erythrocyte sedimentation rate, plasminogen activator inhibitor-1, gamma globulins, and albumin concentration have an extremely important role in the pathogenesis of T2D (26). Ever since Vazarova et al. discovered a positive relationship between the white blood cell count and onset of insulin resistance, their potential mechanisms have been a matter of debate (27). The only possible explanation for elevated white blood cell count and increased insulin resistance lies in the potential activation of the immune system, through IL-6, which is considered to be a potent factor in differentiation of leukocytes, thus associating them with insulin resistance (28,29). In recent years, it has been shown that insulin resistance is associated with CRP levels and the amount of visceral fat, but the relationship is very complex (30). Excessive metabolic activity of visceral fat is associated with insulin resistance, hypercoagulability, dyslipidaemia, hypertension and cardiovascular risk (31). Fat appears to be the most active tissue metabolically that secretes many hormones and cytokines (adipokines, TNFα, IL-6, monocyte chemoattractant protein-1), which participate in inflammatory reactions. Fat tissue is also considered as an important source of inflammation in obese diabetic patients not only because of cytokines secretion from adipocytes, but also because of the infiltration of proinflammatory macrophages (32). Our results seem to support these findings.

Interestingly, results of our study showed a high degree of correlation between inflammatory markers (CRP, fibrinogen and IL-6) and BMI. In previous studies, fibrinogen levels were positively correlated with BMI as well as with other components of the metabolic syndrome and a history of diabetes or hypertension in general (33). This could potentially justify the link between BMI and T2D is the presence of insulin resistance, which is considered to be responsible for the elevated levels of inflammatory parameters (34). Strikingly, the association of BMI with fibrinogen levels in women was proven to be approximately two times stronger than in men (35).

Our study pointed out the likelihood of elevated CRP concentrations increasing with increase in HbA_{1c} levels in female population, thus, further supporting the association demonstrated in previous studies between glycaemic control and systemic inflammation in people with established diabetes (36-38).

In conclusion, our results support the hypothesis that systemic inflammation in moderate, high or subclinical intensity is always present in clinical course of T2D, and the positive correlation between IL-6 and glucose level as well as other inflammatory markers was shown.

ACKNOWLEDGEMENTS

The authors thank all the subjects who participated in the study, as well as to the physicians and paramedical staff from the Department of Endocrinology, Clinical Centre University of Sarajevo and General Hospital Tešanj, who assisted in the study.

FUNDING

The study was supported by grant for EU-FP7 project preparation by the Council of Ministry Bosnia and Herzegovina, in the area of Characterization of Genetic Variation in European Populations for Safe and Efficient Treatment of Diabetes and Prevention from Cardiovascular Disease, 2009-2010.

TRANSPARENCY DECLARATION

Conflict of interests: None to declare.

REFERENCES

1. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet 2011; 378: 169-81.
2. Dall TM, Narayan KM, Gillespie KB, Gallo PD, Blanchard TD, Solcan M, O'Grady M, Quick WW. Detecting type 2 diabetes and prediabetes among asymptomatic adults in the United States: modeling American Diabetes Association versus US Preventive Services Task Force diabetes screening guidelines. Popul Health Metr 2014; 12:12.
3. World Health Organization. Global report on diabetes. Diabetes country profiles. 2016. Geneva: WHO, 2016.
4. Roche MM, Wang PP. Factors associated with a diabetes diagnosis and late diabetes diagnosis for males and females. J Clin Transl Endocrinol 2014; 1:77-84.
5. Herder C, Carstensen M, Ouwens DM. Anti-inflammatory cytokines and risk of type 2 diabetes. Diabetes Obes Metab 2013; 15:39-50.
6. Thorand B, Baumert J, Kolb H, Meisinger C, Chambless L, Koenig W, Herder C. Sex differences in the prediction of type 2 diabetes by inflammatory markers: results fromthe MONICA/KORA Augsburg case-cohort study, 1984-2002. Diabetes Care 2007; 30:854-60.
7. Meisinger C, Thorand B, Schneider A, Stieber J, Doring A, Lowel H. Sex differences in risk factors for incident type 2 diabetes mellitus: the MONICA Augsburg cohort study. Arch Intern Med 2002; 162:82-9.
8. Thorand B, Baumert J, Doring A, Herder C, Kolb H, Rathmann W, Giani G, Koenig W; KORA Group. Sex differencesin the relation of body composition to markers of inflammation. Atherosclerosis 2006; 184:216-24.
9. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 2004; 53:693-700.
10. Qi L, Rifai N, Hu FB. Interleukin-6 receptor gene, plasma C-reactive protein, and diabetes risk in women. Diabetes 2009; 58:275-8.
11. Bo M, Raspo S, Morra F, Cassader M, Isaia G, Poli L. Body fat is the main predictor of fibrinogen levels in healthy non-obese men. Metabolism 2004; 53:984-8.
12. Dessert J, Bocquet P, Belleville J. Later R, Quincy C. An automated immuno-nephelometric method for the quantitative determination of plasma fibrinogen. Pathophysiol Haemos Thromb 1972–73; 1:215–22.
13. Human IL-6 Flex Set http://www.bdbiosciences.com/us/applications/research/bead-based-immunoassays/bd-cba-flex-sets/soluble-proteins/human/human-il-6-flex-set/p/558276. (02 May 2017)
14. Grossmann V, Schmitt VH, Zeller T, Panova-Noeva M, Schulz A, Laubert-Reh D, Juenger C, Schnabel RB, Abt TG, Laskowski R, Wiltink J, Schulz E, Blankenberg S, Lackner KJ, Münzel T, Wild PS. Profile of the immune and inflammatory response in individuals with prediabetes and type 2 diabetes. Diabetes Care 2015; 38: 1356-64.
15. Mirza S, Hossain M, Mathews C, Martinez P, Pino P, Gay JL, Rentfro A, McCormick JB, Fisher-Hoch SP. Type 2-diabetes is associated with elevated levels of TNF-alpha, IL-6 and adiponectin and low levels of leptin in a population of Mexican Americans: a cross-sectional study. Cytokine 2012; 57:136-42.
16. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004; 27:813-23.
17. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 2011; 11:98-107.
18. Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P, Liu LG. Inflammatory markers andrisk of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care 2013; 36:166-75.
19. Han TS, Sattar N, Williams K, Gonzalez-Villalpando C, Lean ME, Haffner SM. Prospective study of C-reactive protein in relation to the development of diabetes and metabolic syndrome in the Mexico City Diabetes Study. Diabetes Care 2002; 25:2016-21.
20. Dehghan A, Kardys I, de Maat MP, Uitterlinden AG, Sijbrands EJ, Bootsma AH, Stijnen T, Hofman A, Schram MT, Witteman JC.Genetic variation, C-reactive protein levels, and incidence of diabetes. Diabetes 2007; 56:872-8.
21. Lee CC, Adler AI, Sandhu MS, Sharp SJ, Forouhi NG, Erqou S, Luben R, Bingham S, Khaw KT, Wareham NJ. Association of C-reactive protein with type 2 diabetes: prospective analysis and meta-analysis. Diabetologia 2009; 52:1040-7.
22. Donath MY. Inflammation as a sensor of metabolic stress in obesity and type 2 diabetes. Endocrinology 2011; 152:4005-6.
23. Kristiansen OP, Mandrup-Poulsen T. Interleukin-6 and diabetes: the good, the bad, or the indifferent? Diabetes 2005; 54:S114-24.
24. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001; 286:327-34.
25. Ford ES. Leukocyte count, erythrocyte sedimentation rate, and diabetes incidence in a national sample of US adults. Am J Epidemiol 2002; 155:57-64.
26. Lindsay RS, Krakoff J, Hanson RL, Bennett PH, Knowler WC. Gamma globulin levels predict type 2 diabetes in the Pima Indian population. Diabetes 2001; 50:1598-603.
27. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002; 51:455-61.
28. Twig G, Afek A, Shamiss A, Derazne E, Tzur D, Gordon B, Tirosh A. White blood cells count and incidence of type 2 diabetes in young men. Diabetes Care 2013; 36:276-82.
29. Popko K, Gorska E, Stelmaszczyk-Emmel A, Plywaczewski R, Stoklosa A, Gorecka D, Pyrzak B, Demkow U. Proinflammatory cytokines Il-6 and TNF-alpha and the development of inflammation in obese subjects. Eur J Med Res 2010; 15:120-2.
30. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004; 25:4-7.
31. Klein S. Is visceral fat responsible for the metabolic abnormalities associated with obesity?: implications of omentectomy. Diabetes Care 2010; 33:1693-4.
32. Jung UJ, Choi MS. Obesity and its metabolic com-

plications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci 2014; 15:6184-223.
33. Bahceci M, Gokalp D, Bahceci S, Tuzcu A, Atmaca S, Arikan S. The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? J Endocrinol Invest 2007; 30:210-4.
34. Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. The metabolic syndrome and insulin resistance: relationship to haemostatic and inflammatory markers in older non-diabetic men. Atherosclerosis 2005; 181:101-8.
35. Bembde AS. A study of plasma fibrinogen level in type-2 diabetes mellitus and its relation to glycemic control. Indian J Hematol Blood Transfus 2012; 28:105-8.
36. Gustavsson CG, Agardh CD. Inflammatory activity increases with haemoglobin A1c in patients with acute coronary syndrome. Scand Cardiovasc J 2009; 43:380-5.
37. King DE, Mainous 3rd AG, Buchanan TA, Pearson WS. C-reactive protein and glycemic control in adults with diabetes. Diabetes Care 2003; 26:1535-9.
38. Liu S, Hempe JM, McCarter RJ, Li S, Fonseca VA. Association between inflammation and biological variation in hemoglobin A1c in U.S. nondiabetic dults. J Clin Endocrinol Metab 2015; 100:2364-71.

Značaj inflamatornih markera i IL-6 u dijagnozi i praćenju pacijenata s dijabetes melitusom tipa 2

Maja Malenica[1], Mira Šilar[2], Tanja Dujić[1], Tamer Bego[1], Sabina Semiz[1,3], Selma Škrbo[4], Besim Prnjavorac[5,6], Adlija Čauševic[1]

[1]Katedra za biohemiju i kliničke analize, Farmaceutski fakultet, Univerzitet u Sarajevu, Bosna i Hercegovina, [2]Univerzitetska klinika za plućne i alergijske bolesti Golnik, Slovenija, [3]Fakultet inžinjeringa i prirodnih nauka, Internacionalni univerzitet, Sarajevo, [4]Katedra za kliničku farmaciju, Farmaceutski fakultet, Univerzitet u Sarajevu, [5]Opća bolnica Tešanj, Tešanj, [6]Katedra za patofiziologiju, Farmaceutski fakultet, Univerzitet u Sarajevu; Bosna i Hercegovina

SAŽETAK

Cilj Analizirati dugoročni utjecaj izmijenjenog metabolizma na nivoe medijatora upalnog odgovora kod žena s dijabetesom tipa 2.

Metode Istraživanje je obuhvatilo 97 pacijentica s dijabetesom tipa 2 i 107 žena bez dijabetesa (kontrola) koji su „regrutovani" na Kliničkom centru Univerziteta u Sarajevu i Općoj bolnici Tešanj. Ispitivani su učinci kontrole glikemije na markere upalnog odgovora mjerenjem nivoa C-reaktivnog proteina (CRP), fibrinogena, leukocita, brzine sedimentacije i citokina interleukina-6. Svi ispitanici su bili bez dokaza o infekcijama, ranijim kirurškim procedurama, bolestima štitnjače, sindromu policističnih jajnika, aktivnom oštećenju jetre i bubrega. Sve biohemijske analize su provedene korištenjem standardnih protokola Internacionalne federacije kliničke hemije (IFCC).

Rezultati Utvrđeno je značajno povećanje fibrinogena ($p<0.001$), CRP ($p=0.001$), interleukina-6 ($p=0.013$), leukocita ($p<0.001$) i brzine sedimentacije ($p=0.008$) u pacijentica s dijabetesom u usporedbi s kontrolnim ispitanicima. Ustanovljena je signifikantna korelacija između CRP i hemoglobina A1c ($p=0.035$), interleukina-6 i glukoze ($p=0.032$), te interleukina-6 i indeksa tjelesne mase ($p=0.007$).

Zaključak Rezultati ovog istraživanja sugeriraju da upala igra važnu ulogu u patogenezi dijabetesa kod dijabetičara ženskog spola. Da bi se ispitivani markeri mogli učinkovito koristiti kao biomarkeri u primarnoj prevenciji dijabetesa tipa 2 u ovoj populaciji, potrebna je detaljnija studija s daleko većim brojem ispitanika.

Ključne riječi: inflamacija, citokin, hiperglikemija

ORIGINAL ARTICLE

Metformin use associated with lower risk of cancer in patients with diabetes mellitus type 2

Jasna Kusturica[1], Aida Kulo Ćesić[1], Edis Gušić[2], Sanita Maleškić[1], Maida Rakanović-Todić[1], Damir Šečić[3]

[1]Department of Pharmacology and Toxicology, School of Medicine, University of Sarajevo, [2]Department of Health Care, Ministry of Internal Affairs of Canton Sarajevo, [3]Department of Pathophysiology, School of Medicine, University of Sarajevo; Sarajevo, Bosnia and Herzegovina

ABSTRACT

Aim In order to increase the database related to the antineoplastic potential of metformin, association between the use of metformin and risk of cancer occurence in patients with diabetes mellitus type 2 (DM2) was investigated.

Methods In this cross-sectional study, medical records of patients with DM2 were reviewed for cancer occurence. Data on age, body mass index (BMI), alcohol and nicotine consumption, glucose and HbA1c levels, duration of DM2, medication used in the treatment of DM2 and cancer occurence were collected and analyzed. Unpaired Student's t-test or Mann-Whitney U test were used for comparisons between treatment groups, and logistic regression to asses how well our set of predictor variables predicts occurence of carcinoma. P-value less than 0.05 was considered statistically significant.

Results The mean age of 234 included patients was 66.8±11.5 years, and DM2 duration was 7± 6.49 years. Mean glucose value was 8.51±4.17mmol/L, and HbA1c 7.74±1.53. Metformin therapy was prescribed in 190 (81%) patients. Cancer was diagnosed in 16 (6.8%) patients: prostate cancer in eight (3.4%), breast cancer in four (1.7%), rectal cancer in two (0.9%) and cancer of the uterus and cervix in one patient. Age, duration of DM2 and BMI did not contribute significantly to the model, while metformin use was shown to be a significant independent predictor (OR=0.049; 95% CI=0.013–0.181; p=0.001).

Conclusion Our findings support the hypothesis that the use of metformin compared to the use of other oral antidiabetic drugs is associated with a lower risk of cancer in patients with DM2.

Key words: hypoglycemic agents, type 2 diabetes, neoplasms

Corresponding author:
Jasna Kusturica
Department of Pharmacology and Toxicology, School of Medicine, University of Sarajevo
Čekaluša 90, 71000 Sarajevo,
Bosnia and Herzegovina
E-mail: jasna.kusturica@mf.unsa.ba
ORCID ID: http://www. orcid.org/: 0000-0002-4120-7230

Original submission:
07 April 2017;
Revised submission:
24 May 2017;
Accepted:
01 June 2017.
doi: 10.17392/910-17

Med Glas (Zenica) 2017; 14(2): 176-181

INTRODUCTION

The search for effective and well-tolerated antineoplastic agent is permanent. Antineoplastic activity is being investigated not only for new substances but for drugs that are already in use in the treatment of other diseases (1-5). One of them is metformin. Metformin is a drug of a biguanide group. Since the fifties of the last century, it has been used in the treatment of diabetes mellitus type 2 (DM2) (6).

In addition to glucose regulation, from 2002. epidemiological studies have shown many favourable effect of metformin. Those effects include lower mortality rate (7), protective effects against cardiovascular diseases (8) as well as reduced risk of cancer in patients with DM2 (9).

Soon after Evans et al. (2005) and Bowker et al. (2006) hypothesized metformin antineoplastic activity, its antineoplastic properties have become the focus of interest (9,10). It has been found that the main mechanism takes place primarily in the liver by decreasing hepatic glucose production through a mostly mild and transient inhibition of mitochondrial respiratory-chain complex 1 (11,12). So far adenosine monophosphate protein kinase (AMPK) has been suggested as a key target point for this action (13). Decrease in hepatic energy status activates the AMPK. Activated AMPK responds to decreased cellular energy level by changing the rate of metabolism of carbohydrate, protein and fat (13). Further research has identified liver kinase B1 (LKB-1), a well recognised tumour suppressor gene, to be the essential factor for AMPK activation by metformin (13-15). Activated AMPK reprograms cellular metabolism by acting on mammalian target of rapamycin (mTOR), fatty acid synthase and p53, the molecules which are involved in regulating cell growth and metabolism (15). Accordingly, AMPK has been suggested to affect cell growth and replication, it slows it down or, as it seems, excludes the growth of aberrant cells (13,16).

Bearing in mind that metformin treatment is inexpensive and relatively safe, its potential antineoplastic activity might be of great importance for future cancer pharmacotherapy.

However, the results of preclinical and clinical studies of antineoplastic activity of metformin are contradictory (17-20). Preclinical study results range from no effect to strong inhibition in carcinogenesis models. Because of the inconsistency of the results of different clinical trials and in order to reach the best possible evidence, more than 200 clinical trials are currently investigating the effect of metformin on tumours in both diabetic and non-diabetic patients (21).

The aim of this study was to investigate association between the risk of cancer and metformin use in patients with DM2 at the Public Health Centre of Sarajevo, Bosnia and Herzegovina.

PATIENTS AND METHODS

Patients and study design

This was a cross-sectional study. Patients with DM2 whose medical records had been available at the Public Health Centre of Sarajevo were screened for the inclusion in this study. The patients who met the inclusion criteria with the confirmed diagnosis of DM2 and a treatment with oral antidiabetic medications were included in the study. Patients treated with insulin, patients without complete medical records and patients whose cancer was diagnosed prior to DM2 were not included in the study.

The study was approved as an academic project by the Ethics Committee at School of Medicine, University of Sarajevo.

Methods

Data on age, body mass index (BMI), alcohol and nicotine consumption, last measured blood glucose and HbA1c levels, duration of DM2, medication used in the treatment of DM2 and cancer occurrence were collected.

Included patients were divided into two groups: patients with or without cancer occurrence.

Statistical analysis

Continuous numerical variables with normal distribution were expressed as mean±standard deviation, while those not normally distributed were expressed as median and interquartile range. Depending on the type of distribution of continuous numerical variables, a comparison between two treatment groups was made either by using unpaired Student's t-test or Mann-Whitney U test as appropriate. Chi-square test (χ^2) was used to

determine the relationship between categorical variables. Logistic regression was used to assess how well our set of predictor variables (type of medicine use, age, duration of DM2, BMI) predicts the occurrence of cancer. Omnibus Tests of Model Coefficients and Hosmer and Lemeshow Test were performed to assess how well the set of our predictor variables is able to predict the occurrence of cancer. The p-values less than 0.05 were considered statistically significant.

RESULTS

The final analysis was performed based on data obtained from 234 patients. The mean age was 66.8±11.5 years and DM2 duration was 7±6.49 years. Mean glucose value was 8.51±4.17mmol/L, and HbA1c was 7.74±1.53. Metformin therapy was prescribed in 190 patients, representing 81% of patients, while other oral antidiabetics were prescribed in 44 patients. Cancer was diagnosed in 16 (6.8%) patients: prostate cancer in eight (3.4%), breast cancer in four (1.7%), rectal cancer in two patients (0.9%) and cancer of the uterus and cervix in one patient. The differences in demographic and clinical characteristics of the patients with and without cancer are shown in Table 1.

Table 1. The differences in demographic and clinical characteristics of patients with and without cancer

Variable	Cancer occurrence*		p
	Absent (n=218)	Present (n=16)	
Age (years)	66 (59.8; 73)	75 (64.5; 79.8)	0.04
Body mass index (kg/m²)	29 (26; 32)	30.5 (28;34,7)	0.229
Nicotine consumption	102 (46.8%)	5 (31.3%)	0.228
Alcohol consumption	33 (15.1%)	1 (6.25%)	0.479
Duration of DM2 (years)	5 (2; 10.3)	9.5 (4.25;13.7)	0.05
Non regulated glucose	67 (30.7%)	5(31.3%)	0.966
HbA1c level (%)	7.2 (6.7;8.45)	7.42 (6.92; 8.43)	0.516

*Data are expressed as median (25; 75 percentile) or relative count (%)

From a total of 190 participants treated with metformin, four (4/190; 2.1%) had cancer, whereas in the group treated with other oral antidiabetics 12 (12/44; 27.3%) had cancer (Figure 1). Proportion of metformin-treated patients who had a diagnosis of carcinoma was significantly lower (p<0.001).

Logistic regression was used to assess how well our set of predictor variables (type of medicine use, age, duration of DM2, BMI) predicted the occurrence of carcinoma. Omnibus Tests of Model Coefficients (χ^2 (4)=31.188; p<0.001), Hosmer and Lemeshow Test (χ^2=13.002; p=0.112), Cox-Snell (R^2=0.125) and Nagelkerke (R^2=0.318) supported the test model (Table 2). The overall accuracy of classification was 93.2%.

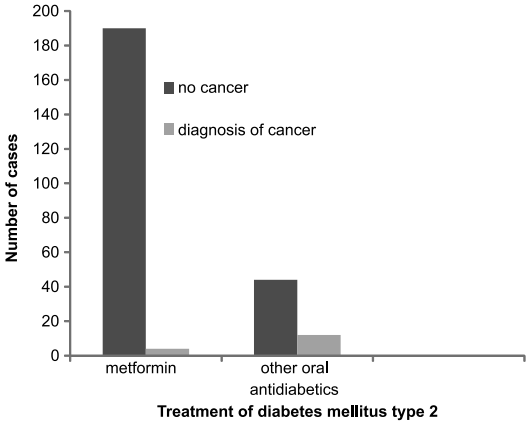

Figure 1. Prevalence of cancer in metformin-treated vs other oral antidiabetic-treated patients with DM2

Variables age, duration of DM2 and BMI did not contribute significantly to the model, while metformin use was shown to be a significant independent predictor (OR=0.049; 95% CI 0.013–0.181; p=0.001), indicating evidence of negative association between metfomin use and the diagnosis of carcinoma in the population of patients with DM2 (Table 2).

Table 2. The logistic regression model assessing independent predictors of carcinoma in patients with DM2*

	B coefficient	p	Exp(B)	95% CI for EXP(B)	
				Lower	Upper
Age (years)	0.018	0.533	1.018	0.963	1.076
Duration of DM2 (years)	0.52	0.296	1.053	0.956	1.161
Body mass index	0.117	0.052	1.124	0.999	1.265
Type of medicine use	-3.015	0.000	0.049	0.013	0.181
Constant	-6.113	0.040	0.002		

*Omnibus Tests of Model Coefficients χ2 (4)=31.188, p<0.001; Hosmer; and Lemeshow Test χ2=13.002; p=0.112; Cox & Snell R2=0.125; Nagelkerke R2=0.318; Exp(B), odds ratio ; 95% CI, 95% confidence interval;

DISCUSSION

Our results showed that compared to other oral antidiabetics, metformin use was significantly associated with decreased risk of cancer disease in patients with DM2. Although well known risk factors such as patients' age (22-24), BMI (23), nicotine and alcohol consumption (24), duration of DM2, regulation of glucose and HbA1c value were modelled, in the presented study only metformin use was found as a significant independent predictor of cancer occurrence.

Prevalence and type of cancer recorded in this study were in accordance with literature data on general cancer statistics, where the probability of developing cancer is the largest for the following five cancer types: prostate, breast, lung and bronchus, colorectal, uterine corpus and cervix (25). Extensive research has shown that DM2 itself is associated with an increased risk of cancer (26-33), as well as with increased rate of mortality in patients with cancer (34). The mechanisms of such an increased cancer risk in the patients with DM2 may be related to insulin resistance, hyperinsulinemia, proinflammatory status and increased oxidative stress (35). On the contrary but in accordance with our results, the use of metformin was proposed to be an associated protective factor against cancer (1-3).

Anticancer potential of metformin has been shown in Libby et al. (2009) observational cohort study among people with DM2. In this study metformin users had significantly lower risk of overall cancer incidence (7.3% vs. 11.6%) (2), like in our study (2.1 vs. 27.3%). In addition, our results, even in a small sample, have shown that metformin contibutes significantly to the predictive abilitiy of the model, which indicates that an increase of metfomin use will result in decreased probability of the cancer occurrence (B=-3.015; OR=0.049; 95% CI 0.013–0.181). Accordingly, in systematic review and meta-analysis of 47 independent studies with 65540 cancer cases in patients with DM2, Gandini et al. (2014) reported overall cancer incidence reduction in 31% with summary relative risk (SRR) 0.69 (95% CI: 0.52–0.90) and cancer mortality in 34% patients with SRR 0.66 (95%CI: 0.54–0.81) (1).

Our findings refer to the use of metfomin in monotherapy, while a population-based cohort study showed the magnitude of cancer risk reduction and prolonged cancer onset time produced by metformin in patients with DM2 do not depend on whether metformin was used alone or combined with other antidiabetic drugs, but depend on a metformin dose (3). We did not investigate the association between the metfomin dose and cancer risk, which is one of the limitations of our study. But in any case the doses of metfomin that were used in our study were within the recommended dosage for DM2, which would mean that potential anticancer effects may not require higher doses. Additionally, limitations of this study are related to the retrospective data collection and a lack of stratification of the sample.

Presented results are related to the general occurrence of cancer, but according to other studies, metformin efficacy may be limited to just several cancer types (4,5). These results are contradictory (36,37), which indicates the necessity of further research related to this topic.

If encouraging results arise, metformin will be an attractive candidate adjuvant in the management of human neoplasias, due to its safety, tolerability and low-cost, expected to mitigate adverse effects and no-response parameters of current anti-cancer therapeutics, thus improving the quality of life and survival of cancer patients. Further long-term prospective clinical trials are needed to focus on specific types of cancer, the use in patients without DM2 as well as its use in adjuvant cancer therapy.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Competing interests: None to declare.

REFERENCES

1. Gandini S, Puntoni M, Heckman-Stoddard BM, Dunn BK, Ford L, De Censi A, Szabo E. Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. Cancer Prev Res (Phila) 2014; 7:867–85.
2. Libby G, Donnelly LA, Donnan PT. New users of metformin area at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care 2009; 32:1620–5.
3. Lin HC, Kachingwe BH, Lin HL, Cheng HW, Uang YS, Wang LH. Effects of metformin dose on cancer risk reduction in patients with type 2 diabetes mellitus: A 6-year follow-up study. Pharmacotherapy 2014; 34:36-45.
4. Donghui Li. Diabetes and pancreatic cancer. Mol Carcinog 2012; 51:64–74.
5. Li D, Yeung SJ, Hassan MM, Konopleva M, Abbruzzese JL. Anti-diabetic therapies affect risk of pancreatic cancer. Gastroenterology 2009; 137:482–8.

6. Godarzi MO, Brier-Ash M. Metformin revisited: re-evaluation of its properties and role in the pharmacopoeia of modern antidiabetic agents. Diabetes Obes Metab 2005; 5:654–65.
7. Gosmanova EO, Canada RB, Mangold TA, Rawls WN, Wall BM. Effect of metformin-containing antidiabetic regimens on all-cause mortality in veterans with type 2 diabetes mellitus. Am J Med Sci 2008; 336:241-7.
8. Evans JM, Ogston SA, Emslie-Smith A, Morris AD. Risk of mortality and adverse cardiovascular outcomes in type 2 diabetes: a comparison of patients treated with sulfonylureas and metformin. Diabetologia 2006; 49:930-6.
9. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ 2005; 330:1304-5.
10. Bowker SL, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. Diabetes Care 2006; 29:254-8.
11. Lemke TL, Williams DA, Roche VF, Zito SW. Foye's principles of medicinal chemistry. 6th ed. Philadelphia: Wolters Kluwer, Lippincott Williams & Willkins, 2008.
12. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 2012; 122:253-70.
13. Mičić D, Cvijović G, Trajković V, Duntas LH, Polovina S. Metformin: its emerging role in oncology. Hormones 2011; 10:5-15.
14. Sun G, Kashyap SR. Cancer risk in type 2 diabetes mellitus: metabolic Llnks and therapeutic considerations. J Nutr Metab 2011; 2011:708183.
15. Li W, Saud SM, Young MR, Chen G, Hua B: Targeting AMPK for cancer prevention and treatment. Oncotarget 2015; 6:7365-78.
16. Luo Z, Zang M, Guo W. AMPK as a metabolic tumor suppressor: control of metabolism and cell growth. Future Oncol 2010; 6:457-70.
17. Thompson MD, Grubbs CJ, Bode AM, Reid JM, McGovern R, Bernard PS, Stijleman IJ, Green JE, Bennett C, Juliana MM, Moeinpour F, SteeleVE, Lubet RA. Lack of effect of metformin on mammary carcinogenesis in nondiabetic rat and mouse models. Cancer Prev Res 2015; 8:231-9.
18. Anisimov VN, Berstein LM, Popovich IG, Zabezhinski MA, Egormin PA, Piskunova TS, Semenchenko AV, Tyndyk ML, Yurova MN, Kovalenko IG, Poroshina TE. If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. Aging (Albany NY) 2011; 3:148–57.
19. Noto H, Goto A, Tsujimoto T, Noda M. Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. PLoS One 2012; 7:e33411.
20. Franciosi M, Lucisano G, Lapice E, Strippoli GF, Pellegrini F, Nicolucci A. Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. PLoS One 2013; 8:e71583.
21. Li K-L, Li L, Zhang P, Kang J, Wang Y-B, Chen H-Y, He Y. A multicenter double-blind phase II study of metformin with gefitinib as first-line therapy of locally advanced non–small-cell lung cancer. Clin Lung Cancer 2017; 18:340-3.
22. Cancer Research UK. Cancer incidence by age. December 2016. http://www.cancerresearchuk.org/health-professional/cancer-statistics/incidence/age#heading-Zero (06 January 2017).
23. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003; 348:1625–38.
24. Togashi Y, Hayashi H, Okamoto K, Fumita S, Terashima M, de Velasco MA, Sakai K, Fujita Y, Tomida S, Nakagawa K, Nishio K. Chronic nicotine exposure mediates resistance to EGFR-TKI in EGFR-mutated lung cancer via an EGFR signal. Lung Cancer 2015; 88:16-23.
25. American Cancer Society. Cancer facts and figures 2017. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2017.html (29 May 2017)
26. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. Int J Cancer 2007; 121:856-62.
27. Friberg E, Orsini N, Mantzoros CS, Wolk A. Diabetes mellitus and risk of endometrial cancer: a meta-analysis. Diabetologia 2007; 50:1365-74.
28. Wang P, Kang D, Cao W, Wang Y, Liu Z. Diabetes mellitus and risk of hepatocellular carcinoma: a systematic review and meta-analysis. Diabetes Metab Res Rev 2012; 28:109-22.
29. El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol 2006; 4:369–80.
30. Huxley R, Ansary-Moghaddam A, de González AG, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. Br J Cancer 2005; 92:2076–83.
31. Zhu Z, Wang X, Shen Z, Lu Y, Zhong S, Xu C. Risk of bladder cancer in patients with diabetes mellitus: an updated meta-analysis of 36 observational studies. BMC Cancer 2013; 13:310.
32. Mitri J, Castillo J. and Pittas AG. Diabetes and risk of non-Hodgkin's lymphoma: A meta-analysis of observational studies. Diabetes Care 2008; 31:2391-7.
33. Deng L, Gui Z, Zhao L, Wang J, Shen L. Diabetes mellitus and the incidence of colorectal cancer: an updated systematic review and meta-analysis. Dig Dis Sci 2012; 57:1576-85.
34. Barone BB, Yeh HC, Snyder CF, Peairs KS, Stein KB, Derr RL, Wolff AC, Brancati FL: Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis. JAMA 2008; 300:2754–64.
35. Garg SK, Maurer H, Reed K, Selagamsetty R. Diabetes and cancer: two diseases with obesity as a common risk factor. Diabetes Obes Metab 2014; 16:97-110.
36. Onitilo AA, Stankowski RV, Berg RL, Engel JM, Glurich I, Williams GM, Doi SA. Type 2 diabetes mellitus, glycemic control, and cancer risk. Eur J Cancer Prev 2014; 23:134-40.
37. Margel D, Urbach D, Lipscombe LL, Bell CM, Kulkarni G, Austin PC, Fleshner N. Association between metformin use and risk of prostate cancer and its grade. J Natl Cancer Inst 2013; 105:1123-31.

Primjena metfomina povezana s manjim rizikom karcinoma kod pacijenata s dijabetesom melitusom tipa 2

Jasna Kusturica[1], Aida Kulo Ćesić[1], Edis Gušić[2], Sanita Maleškić[1], Maida Rakanović-Todić[1], Damir Šečić[3]

[1]Katedra za farmakologiju i toksikologiju, Medicinski fakultet, Univerzitet u Sarajevu, [2]Zavod za zdravstvenu zaštitu, Ministarstvo unutrašnjih poslova Kantona Sarajevo, [3]Katedra za patofiziologiju, Medicinski fakultet, Univerzitet u Sarajevu; Sarajevo, Bosna i Hercegovina

SAŽETAK

Cilj U cilju povećanja baze podataka vezane za antikancerski potencijal metformina, istražili smo povezanost rizika pojave karcinoma i primjene metformina u pacijenata s dijabetesom melitusom tipa 2 (DM2).

Metode U prikazanoj presječnoj studiji obrađeni su medicinski kartoni pacijenata s DM2 na pojavu karcinoma. Analizirani su sljedeći parametri: starost pacijenta, indeks tjelesne mase (BMI), konzumacija alkohola i nikotina, vrijednost glukoze i HbA1c, dužina trajanja DM2, lijekovi koji se primjenjuju i pojava karcinoma. U statističkoj analizi za poređenje tretmanskih grupa korišten je nezavisni Studentov t-test ili Mann-Whitneyev U-test, a logistička regresija za analizu varijabli kao prediktora pojave karcinoma. Statistički značajna smatrana je vrijednost $p< 0.05$.

Rezultati U istraživanje su bila uključena 234 pacijenta, prosječne starosti 66,8±11,5 godina i prosječnim trajanjem DM2 7± 6,49 godina. Prosječna vrijednost glukoze iznosila je 8,51±4,17mmol/L i HbA1c 7,74±1,53. Metformin je primijenjen kod 190 (81%) pacijenata. Karcinom je dijagnosticiran kod 16 (6,8%) pacijenata, odnosno karcinom prostate kod osam (3,4%), karcinom dojke kod četiri (1,7%), karcinom debelog crijeva kod dva (0,9%) te karcinom uterusa i cerviksa kod po jednog pacijenta. Starost, dužina trajanja DM2 i BMI nisu značajno utjecali na model, dok se metfomin pokazao kao signifikantan neovisni prediktor smanjenog rizika pojave karcinoma (OR=0,049; 95% CI=0,013–0,181; p=0,001).

Zaključak Rezultati našeg istraživanja potvrdili su hipotezu da je primjena metfomina u odnosu na druge oralne antidijabetike povezana s manjim rizikom pojave karcinoma u pacijenata s DM2.

Ključne riječi: hipoglikemici, dijabetes tipa 2, neoplazme

ORIGINAL ARTICLE

Efficacy of carbocisteine in the treatment of chronic obstructive pulmonary disease and impact on the quality of life

Esad Alibašić[1], Amira Skopljak[2], Aida Čengić[2], Gorana Krstović[2], Nataša Trifunović[2], Tarik Čatić[3], Belma Kapo[3], Meliha Mehić[3], Amila Hadžimuratović[4]

[1]Department of Family Medicine, Primary Health Care Center Kalesija, Kalesija, [2]Health Care Centre of Canton Sarajevo, Sarajevo, [3]''Bosnalijek'' d.d., Sarajevo, [4]Faculty of Medicine, University of Sarajevo, Sarajevo; Bosnia and Herzegovina

ABSTRACT

Aim To investigate the effects of carbocisteine treatment in the reduction of frequency of productive cough episodes, preventing disease progression and improving the quality of life as well as the tolerability of the administered treatment and patient compliance during the study.

Methods This observational, non-interventional, multicenter, cohort study included 501 patients with chronic obstructive pulmonary disease (COPD) who were administrated carbocisteine capsules 375 mg and followed up during the next 15 days. The patients were observed at 3 points, baseline and two additional assessments. General clinical condition of patients, along with the spirometry testing at all three points were examined. Thr quality of life was assessed on the 1st and 3rd observation with Leicester Cough Questionnaire. Tolerability and patient compliance were measured throughout the study.

Results There was a significant change of forced expiratory volume in 1 second (FEV1) status between the second and third observation (p=0.002). Examination of general symptoms showed a statistically significant reduction in cough by 74.9%, in sputum production by 48.5%, in dyspnea by 29% and in fatigue by 50%. After the administration of carbocisteine the median value of overall quality of life was 3.79 (3.63 – 3.89).

Conclusion 375mg carbocisteine capsules were found to be effective and well-tolerated in the treatment of COPD, with a small percentage of reported mild adverse reactions and with a significant improvement of quality of life.

Keywords: lung disease, cough, expectorants

Corresponding author:
Belma Kapo
Bosnalijek d.d.
Jukićeva 53, 71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 33 560600;
Fax: +387 33 814250;
E-mail: belma.kapo@bosnalijek.com
Esad Alibašić ORCID ID: http://www.orcid.org/0000-0001-5779-0769

Original submission:
29 March 2017;
Revised submission:
04 July 2017;
Accepted:
10 July 2017.
doi: 10.17392/906-17

Med Glas (Zenica) 2017; 14(2): 182-188

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a leading cause of mortality and morbidity both in developed and developing countries that is mainly characterized by progressive and not fully reversible airflow limitation (1). There are wide variations in the prevalence of COPD across countries (2). The COPD is common in older population and is highly prevalent in those aged over 75 years. The Burden of Obstructive Lung Disease (BOLD) study from 12 sites involving 9425 subjects, who had completed post bronchodilator spirometry testing, found that the overall prevalence of COPD of GOLD stage II or higher was 10.1 per cent and the prevalence was 11.8 per cent for men and 8.5 per cent for women (2).

According to the Health institute of the Federation of Bosnia and Herzegovina, a leading cause of morbidity of patients in the Federation of Bosnia and Herzegovina are the diseases of the respiratory system with 30% of share. They are also the largest burden for the primary care units in the Federation of Bosnia and Herzegovina. In the structure of morbidity, the most common respiratory system diseases are acute respiratory infections (70.7%) and COPD (6.4%). For most patients, the diagnosis of COPD is made in the mid – fifties. 45-65% of COPD patients are not diagnosed because dyspnea and fatigue are commonly associated with normal process of aging, and the smoker's cough is considered normal (3).

COPD is characterized by a decline in lung function over time and accompanied by respiratory symptoms, primarily dyspnea, cough, and sputum production (4).Active smoking remains the main risk factor, but other factors are becoming better known, such as occupational factors, infections and the role of air pollution (5).Current data suggest that COPD mortality is increasing, and by 2020, COPD is predicted to be the third-leading cause of death worldwide (4).

Guidelines from the Czech Republic, England and Wales, France, Germany, Poland, Portugal, Russia and Sweden stratified patients on the basis of the degree of airflow limitation into the four stages set forth in the Global Initiative for Chronic Obstructive Lung Disease (GOLD) strategy, as follows. Stage 1 (mild): FEV1 (forced expiratory volume) ≥80% predicted; stage 2 (moderate): FEV1 50–79% predicted; stage 3 (severe): FEV1 30–49% predicted; and stage 4 (very severe): FEV1 <30% predicted (6).

Health-related quality of life (HRQOL) is a multi-dimensional concept that includes domains related to physical, mental, emotional and social functioning (8). The Leicester Cough Questionnaire (LCQ) is a 19-item questionnaire that assesses cough-related QOL and takes 5 to 10 minutes to complete. Scores in three domains (physical, psychological and social) are calculated as a mean for each domain (range 1 to 7). A total score (range 3 to 21) is also calculated by adding the domain scores together. Higher scores indicate better quality of life (8). In this study, the LCQ used was in the Bosnian language. Because the LCQ is a measure originally developed in the English language, it should be translated to the target language, in our case Bosnian, and adapted to the social and cultural circumstances of the target country (8).

Over the years evidence has accumulated that mucus hypersecretion is an important manifestation of chronic obstructive pulmonary disease (COPD). In the classical phenotype of chronic bronchitis, mucus hypersecretion is the key presenting symptom that appears independent of airflow obstruction (9). A large number of studies have been performed on the use of mucolytic drugs in the treatment of chronic bronchitis and COPD (9). These studies clearly demonstrate that treatment with mucoactive drugs reduces exacerbations in patients with COPD with virtually no side-effects, though the mechanisms by which these effects might be observed are still unclear (9).

The aim of this study was to examine the effects of carbocisteine treatment in the reduction of the frequency of productive cough episodes, preventing disease progression and improving the quality of life as well as the tolerability of the administered treatment and patient compliance during the study.

PATIENTS AND METHODS

Patients and study design

An observational, non-interventional, multicenter, cohort study in 15 medical centers from major cities of Bosnia and Herzegovina including Sarajevo, Tuzla, Zenica, Žepče, Gradačac, Ljubače, Bukinje, Devetak, Kalesija, Tešanj, Hadžići and

Ilijaš was conducted. The study included a total of 501 patients with COPD, who were admitted to a family medicine practice at the primary healthcare level in the period from February 2016 to April 2016. The patients were administered carbocisteine capsules 375 mg manufactured by Bosnalijek Sarajevo and followed up 15 days after the start of carbocisteine administration. Criteria for inclusion in the study included patients with stage I COPD (FEV1/FVC <70 %; FEV >80%), patients with stage II COPD (FEV1/FVC < 70%; 50% < FEV1< 80%) and patients with productive cough for a period longer than three months a year. The clasification is based on the GOLD Classification of severity of airflow obstruction (6).

Patients who showed hypersensitivity to carbocisteine or had an active peptic ulcer were not included in the study, and the patients who showed deterioration of the underlying disease, development of serious adverse events that require discontinuation of the therapy or developed a disease that affected the flow of research were excluded from the study.

Methods

Patients were observed at 3 time points, e.g. the first baseline and two additional control assessments. For assessments of HRQOL on COPD the cough-specific quality-of-life questionnaire, Leicester cough questionnaire (LCQ) was used (8), since the questionnaire addresses some of the main symptoms of COPD.

The FEV1 in the first second obtained by spirometry, before and after the treatment with carbocisteine capsules 375 mg, was measured. For result comparison general guidelines were used as the criteria for defining airflow obstruction in the diagnosis of COPD. Blood examination including C - reactive protein (CRP), leukocytes (LKC) and sedimentation (SE) as well as the patient's general symptoms were examined.

First observation (day 0) included spirometry and an assessment of whether the inclusion criteria were met. Patients were further divided into two groups based on the spirometry: Group A - patients with stage I COPD and Group B – patients with stage II COPD. The first observation also includes an assessment of the quality of life based on the validated LCQ. The second observation was performed on the 5th day and included only an assessment of the general clinical condition of the patient along with spirometry. Third observation was performed on the 15th day (from the start of the study) and included a final assessment of the effectiveness of the treatment based on the improvement of the patient's general condition, spirometry as well as the assessment of the quality of life based on the LCQ. After 15 days, effectiveness, tolerability and patient compliance were also recorded. The measurements of the study outcomes was independent from the study investigators.

The drug effectiveness was assessed on the basis of physician's examination of the patients. The safety of the product administration was observed by monitoring the incidence of adverse reactions of drugs with the assessment of the relation between drug administration and adverse reaction occurrence (certain, probable, possible, not probable, unclassified relation and non-classifiable).

Statistical analysis

The study results were presented as the mean value (X) and standard deviation (SD) for variables that followed a normal distribution, or as a median and interquartile range for variables that did not follow a normal distribution and as absolute and relative numbers. Evaluation of categorical variables was performed by the $\chi 2$- test for independent variables or by the McNemar's test for dependent categorical data. Fridman test was used for dependent variables that are ordinal. The accepted statistical significance was set at the level $p<0.05$.

RESULTS

The study included a total of 501 patients with COPD, who met the inclusion criteria. Out of the total of 501 patients, 273 (54%) were males and 228 (46%) were females with no significant difference in gender distribution. The average age of the patients was 52±17.3 years. Out of the total of 501 patients, 222 (44%) were smokers and 279 (56%) were non-smokers. The patients were given carbocisteine during 15 days. Primary dosage was two capsules of carbocisteine 375 mg three times a day for the first five days. Maintenance dosage was one capsule of carbocisteine 375 mg four times a day for the next ten days.

The FEV1 in the first second did not show a significant change in the status of patients between the first and second observation (p=0.092). There

was a significant change of FEV1 status between the second and third observation (p=0.002) (Table 1). During the first observation 2.5% of patients had FEV1 < 30%. During the second observation 2.27% of patients had FEV1 < 30% and during the third observation a significant change was noticed, 0.62% patients had FEV1 < 30%. During the first observation 23.29% patients had FEV1 between 30-50%, during the second observation the percentage of patients with FEV1 between 30-50% was 19.32% and during the third observation it was 18.63%. During the first observation 62.33% of patients had FEV1 between 50-80% from expected. During the second observation the percentage of patients with FEV1 between 50-80% increased to 65.91% and during the third observation the percentage of patients was lowered to 52.18%. During the first observation 12.33% of patients had FEV1>80 % than expected. During the second observation the percentage of patients was 12.5% and during the third observation the percentage of patients with FEV1>80% was 28.5%.

Table 1. Results of forced expiratory volume (FEV) 1spirometry measurements in patients with chronic obstructive pulmonary disease (COPD) before and after the administration of carbocisteine

	FEV1 (%)				FEV1 (%)		
Obstruction level (%)	Observation I	Observation II	Change from observation I to II (%)	p	Observation III	Change from observation II to III (%)	p
<30.00	2.05	2.27	0.22	0.092	0.62	-1.65	0.002
30.01 – 50.00	23.29	19.32	-3.97	0.092	18.63	-0.69	0.002
50.01 – 80.00	62.33	65.91	3.58	0.092	52.17	-13.74	0.002
80.01 +	12.33	12.50	0.17	0.092	28.57	16.07	0.002

Examining general symptoms in patients with COPD before and after the treatment with carbocisteine, a significant reduction in the number of patients with cough, sputum production, dyspnea and fatigue was found (Table 2). There was also a significant reduction of CRP, LKC and SE before and after the treatment with carbocisteine. Blood examination showed a significant improvement after the treatment with carbocisteine in the number of patients involved in the study. The median value of CRP before the treatment was 5.75 (2.08 - 9.85) and after the treatment the median value was 4.0 (2.0 – 5.5). The median value for leukocytes before the treatment was 9.10 (7.10 – 11.19) and the median value after the administration of carbocisteine was 7.88 (6.30 – 9.10). Also, the median value for sedimentation before the treatment was 16.00 (10.00 – 24.00) and the median value after the treatment was 11.00 (7.50 – 17.00). These results show a significant reduction in all three points of blood examination (p=0.001).

Table 2. Presence of general symptoms in patients with chronic obstructive pulmonary disease (COPD) before and after the administration of carbocisteine

General symptoms	Observation I (%)	Observation II (%)	Observation III (%)	Change from observation I to III (%)	p
Cough	485 (96.9)	346 (69.1)	110 (22)	-74.9	0.0001
Sputum production	396 (79)	326 (65)	153 (30.5)	-48.5	0.0001
Dyspnea	194 (39)	131 (26)	48 (10)	-29	0.0001
Fatigue	323 (65)	167 (33)	77 (15)	-50	0.0001

Patients' quality of life (Leicester Cough Questionnaire)

The median value for physical domain was 3.63 (3.25 – 4.0) before the treatment. After the administration of carbocisteine the median value for physical domain was 3.88 (3.64 – 4.0). These results showed a significant improvement of physical domain (p=0.0001).

The median value for psychological domain before the treatment with carbocisteine was 4.14 (3.71 – 4.4) and the median value after the treatment was 3.57 (3.43 – 3.86), which shows a significant deterioration of psychological domain (p=0.0001).

When it comes to social domain there was no significant improvement (p=0.722). Before the treatment, the median value for social domain was 3.75 (3.5 – 4.25). After the treatment, the median value for social domain was 4.0 (3.5 – 4.0) (Figure 1).

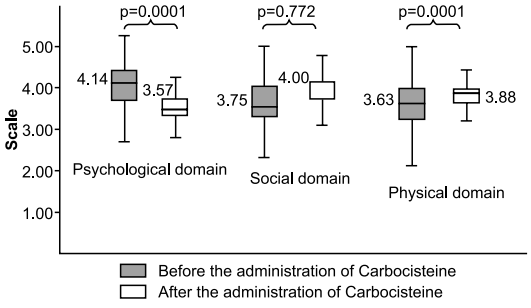

Figure 1. Quality of life in all three domains before and after the administration of carbocisteine

Examining the results of overall assessment of quality of life obtained by the LCQ before and after the treatment, we found no significant improvement of the quality of life in patients involved in the study (p=0.065).

The median value of overall quality of life before the treatment was 3.84 (3.53 – 4.16). After the administration of carbocisteine the median value of overall quality of life was 3.79 (3.63 – 3.89).

DISCUSSION

This study was conducted to show the effectiveness of carbocisteine in patients with COPD that was measured as an improvement in the general clinical features as well as the quality of life conditions. Examination of general symptoms in patients before and after the treatment with carbocisteine showed a statistically significant reduction in cough by 74.9%, in sputum production by 48.5%, in dyspnea by 29% and in fatigue by 50%. The overall results of spirometry testing showed a reduction in airflow obstruction in patients with all stages of COPD (I, II, III and IV).

S-Carboxymethylcysteine (carbocysteine or SCMC) is a mucoactive drug with antioxidant and anti-inflammatory properties, and is commonly used for the treatment of COPD. Pre-clinical and clinical studies on the pharmacological properties of SCMC have demonstrated that this cysteine derivative has the ability to increase the synthesis of sialomucins, important structural components of mucus. In effect, SCMC resets the balance between sialomucins and fucomucins, possibly by intracellular stimulation of sialyl transferase activity, restoring the viscoelastic properties of mucus (10).

Carbocisteine is available as an oral preparation both as SCMC and its lysine salt (SCMC-Lys). The structure and mechanism of action of carbocisteine differs from other commonly available mucolytic drugs such as N-acetylcysteine (NAC) and erdosteine that bear free sulfhydryl (thiol) groups via which they split glycoprotein bonds in mucus (11). Carbocisteine is well absorbed when taken orally. Peak serum concentrations are achieved at 1 to 1.7 hours and the plasma half-life is 1.33 hours. It achieves good penetration into lung tissue and bronchial secretions. Approximately 30% to 60% of the drug is excreted unchanged in urine (11). Thanks to its unique pharmacological properties, it has an excellent safety profile and is suitable for special groups of patients such as children, adults and people with impaired liver and/or kidney function.

This study shows that COPD exacerbation may have important effects on health status and is a useful outcome measure in clinical studies of the disease. Reduction of exacerbation frequency would be expected to improve well-being, though this has not been formally tested in an interventional study. Health status measures show a strong relationship with exacerbation frequency and thus may be useful in determining which patients are at risk of exacerbation and associated disability (12).

A study conducted in 1998 has shown the impact of chronic cough on the quality of life. First, chronic cough was significantly associated with meaningful adverse psychological and physical effects on the quality of life. Compared with individuals with no health-related dysfunction, baseline Sickness Impact Profile (SIP) scores revealed that cough was associated with dysfunction in patients' usual daily activities, particularly in the categories of ambulation, social interaction, sleep and rest, work, home management and recreation and pastimes. Secondly, successful treatment of chronic cough was associated with the resolution of patients' deterioration in the quality of life (13).

Carbocisteine reduces intercellular adhesion molecule-1 expression and blocks entry of rhinovirus ribonucleic acid into the endosomes. Therefore, carbocisteine may be useful in the prevention of common colds and exacerbations in COPD patients (7). The preventive effects of carbocisteine on acute exacerbations of COPD were investigated in a multicenter (n523), parallel-group, randomized study (7). The study showed a significant reduction in the number of common colds and exacerbations in the study group than in the control group.

St George's Respiratory Questionnaire (SGRQ) total score and components (impacts, symptoms, and activities) total score decreased in the study group but not in the control group, indicating that carbocisteine improved patients' quality of life (QOL). In addition, all three component scores decreased in the study group, suggesting that carbocisteine administration improved both physical and mental aspects of QOL (7).

Most recently, PEACE study supported previous findings that long-term use of carbocisteine re-

duced the rate of exacerbations of COPD. The advantage of carbocisteine over placebo in prevention of exacerbations was note-worthy even after the adjustment for COPD severity and concomitant therapy (14). In addition to preventing COPD exacerbations, carbocisteine was shown to improve the patients' quality of life (14).This finding differed from our study in which significant improvement of the quality of life by carbocisteine was shown, which may indicate that only long-term studies may evaluate the effect of carbocisteine on exacerbation rate.

Future randomized controlled trials should examine the value of mucolytic drugs in patients who have repeated, prolonged or severe exacerbations or who are repeatedly admitted to hospital with exacerbations of chronic obstructive pulmonary disease. The use of mucolytic in acute exacerbations of chronic obstructive pulmonary disease should also be studied. All of these studies should include a measure of the use of healthcare resources (15).

While a great deal is known about the diagnosis and treatment of cough, there are methodological challenges that need to be solved in order to achieve further advances in our understanding of how to best manage patients with this common symptom. One of the most basic challenges is the development of a valid and reliable method by which to assess the impact of cough on the health-related quality of life of patients. Such a method would provide an important measure of the efficacy of cough therapies (16).

The other part of our study was focused on examining the quality of life in patients with COPD. The examination of physical domain showed a significant improvement.

The main limitation of this study is the short duration as well as the limited follow up with the patients in the study. However, this study is the first in our region that has been conducted to show the importance of measurement of the quality of life in patients with COPD. Also, it is the first application of the Leicester Cough Questionnaire in monitoring the quality of life in patients with COPD in Bosnia and Herzegovina.

Available medical evidence supports the conclusion that mucolytic drugs containing carbocisteine in the dose of 375 mg xx daily in the treatment of chronic obstructive pulmonary disease contribute to the improvement of the general condition of the patient and reduce cough, decrease sputum production, reduce dyspnea and fatigue. In addition, the therapy with carbocisteine affects the reduction of C – reactive protein, leukocytosis and sedimentation, which confirms carbocisteine anti-inflammatory properties. The results of our study show that cabocisteine has good effectiveness and tolerability.

FUNDING

No specific funding was received for this study.

DECLARATION OF INTEREST

Competing interests: None to declare.

REFERENCES

1. Antó JM, Vermeire P, Vestbo J, Sunyer J. Epidemiology of chronic obstructive pulmonary disease. Eur Resp J 2001; 17:982-94.
2. Vijayan VK. Chronic obstructive pulmonary disease. Indian J Med Res 2013; 137:251–69.
3. Institute of Public health of Federation of Bosnia and Herzegovina. Health statistics annual Federation of Bosnia and Herzegovina 2014. Sarajevo: Maligraf, 2014.
4. Rycroft C, Heyes A, Lanza L, and Becker E. Epidemiology of chronic obstructive pulmonary disease: a literature review. Int J Chron Obstruct Pulmon Dis 2012; 7:457–94.
5. Raherison C, Girodet PO. Epidemiology of COPD. Eur Respir Rev 2009; 18:213-21.
6. Miravitlles M, Vogelmeier C, Roche N, Halpin D, Cardoso J, Chuchalin AG, Kankaanranta H, Sandström T, Śliwiński P, Zatloukal J, Blasi F. A review of national guidelines for management of COPD in Europe. Eur Respir J 2016; 47:625-37.
7. Tatsumi K, Fukuchi Y. Carbocisteine improves quality of life in patients with chronic obstructive pulmonary disease. J Am Geriatr Soc 2007; 55:1884–6.
8. Čatić T, Kapo B, Duranović LR, Krstović G, Mehić M. Translation and adaptation of Leichester Cough Questionnaire (LCQ) for use in Bosnia and Herzegovina as quality of life measure instrument in acute and chronic cough. Pharmacia 2016; 19:5-10.
9. Decramer M, Janssens W. Mucoactive therapy in COPD. Eur Respir Rev 2010; 19:134-40.
10. Balsamo R, Lanata L, Egan CG. Mucoactive drugs. Eur Respir Rev 2010; 19:127-33.
11. Hooper C, Calvert J. The role for S-carboxymethylcysteine (carbocisteine) in the management of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2008; 3:659–69.

12. Seemungal TAR, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of Exacerbation on Quality of Life in Patients with Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 1998; 157:1418–22.
13. French CL, Irwin RS, Curley FJ, Krikorian CJ. Impact of chronic cough on quality of life. Arch Intern Med 1998; 158:1657–61.
14. Zheng JP, Kang J, Huang SG, Chen P, Yao WZ, Yang L, Bai CX, Wang CZ, Wang C, Chen BY, Shi Y, Liu CT, Chen P, Li Q, Wang ZS, Huang YJ, Luo ZY, Chen FP, Yuan JZ, Yuan BT, Qian HP, Zhi RC, Zhong NS. Effect of carbocisteine on acute exacerbations of chronic obstructive pulmonary disease (PEACE study): a randomized placebo-controlled study. Lancet 2008; 371:2013–8.
15. Poole PJ, Black PN. Oral mucolytic drugs for exacerbations of chronic obstructive pulmonary disease: systematic review. BMJ 2001; 322:1271.
16. French CT, Irwin RS, Fletcher KE, Adams TM. Evaluation of a cough-specific quality-of-life questionnaire. Chest 2002; 121:1123–31.

Efikasnost karbocistina u tretmanu hronične opstruktivne plućne bolesti i njegov utjecaj na kvalitet života

Esad Alibašić[1], Amira Skopljak[2], Aida Čengić[2], Gorana Krstović[2], Nataša Trifunović[2], Tarik Čatić[3], Belma Kapo[3], Meliha Mehić[3], Amila Hadžimuratović[4]

[1]Centar za porodičnu medicinu, Dom zdravlja Kalesija, Kalesija, [2]Dom zdravlja Kantona Sarajevo, Sarajevo, [3]Bosnalijek d.d., Sarajevo, [4]Medicinski fakultet Univerziteta u Sarajevu, Sarajevo

SAŽETAK

Cilj Ispitati efekte terapije s karbocisteinom u smanjenju učestalosti epizoda produktivnog kašlja, sprečavanju progresije bolesti i poboljšanju kvalitete života, te podnošljivost primijenjene terapije i suradljivost ispitanika tokom istraživanja.

Metode Ova opservacijska, neintervencijska, multicentrična, kohortna studija uključivala je 501 pacijenta s hroničnom opstruktivnom plućnom bolesti (HOPB), kojima su ordinirane kapsule karbocisteina od 375 mg, te su praćeni narednih 15 dana. Pacijenti su se pratili kroz tri mjerenja: nulto i dva kontrolna. Opće kliničko stanje i spirometrija su praćeni tokom sva tri mjerenja. Kvalitet života je procjenjivan na prvom i trećem mjerenju, koristeći *Leicester Cough Questionnaire*. Podnošljivost terapije i suradljivost ispitanika su praćeni tokom čitave studije.

Rezultati Postojala je značajna promjena FEV1 statusa između drugog i trećeg mjerenja (p=0.002). Ispitivanje općih simptoma pokazalo je statistički značajno smanjenje kašlja za 74.9%, iskašljaja za 48.5%, dispneje za 29% i zamora za 50%. Nakon terapije karbocisteinom srednja vrijednost kvalitete života iznosila je 3.79 (3.63 – 3.89).

Zaključak Primjena karbocistein kapsula od 375 mg pokazala se efikasnom, i dobro podnošljivom u tretmanu HOPB-a, sa rijetkim neželjenim efektima te značajnim poboljšanjem kvalitete života pacijenata.

Ključne riječi: plućne bolesti, kašalj, ekspektoranti

ORIGINAL ARTICLE

Attainment of gross motor milestones in children with Down syndrome in Kosovo - developmental perspective

Samire Beqaj[1,2], Njomza Jusaj[1], Vujica Živković[2]

[1]Department of Physiotherapy, School of Medicine, University of Prishtina, Prishtina, Republic of Kosovo, [2]School of Physical Education, Sport and Health, Ss. Cyril and Methodius University in Skopje, Skopje, Republic of Macedonia

ABSTRACT

Aim To investigate the age (in months) at which motor skills are developed in children with Down syndrome (DS), and compare it to the age of the development of the same skills in both, children with typical development (TD), and children with DS reported by four other studies.

Methods Sixteen children (7 girls and 9 boys) were monthly assessed for the development of nineteen motor skills between 2008 and 2011. The mean ages when the skills were accomplished were presented using descriptive statistics. Independent T-samples test (significance < 0.05) was used to compare the mean developmental ages from our study with those seen in children with TD (Comparison 1) and also in children with DS reported by four other authors (Comparison 2a-2d).

Results Children with DS developed at a significantly slower pace compared to children with TD (p=0.005). Generally, delay and variance of developmental age in children with DS increased chronologically with the complexity of the skills. No significant difference was found between developmental age in children from the present study and children with DS from other studies.

Conclusion The rate of attainment of motor skills is delayed in children with DS in comparison to children with TD, however, the developmental sequence is the same. The delayed development is more prominent in more complex skills.

Keywords: motor skills, physical therapy, early intervention

Corresponding author:
Samire Beqaj
School of Medicine,
University of Prishtina
St. Bulevardi i Dëshmorve, nn,
10 000 Prishtina, Republic of Kosovo
Phone & fax: +381 38 512 223;
E-mail: samirebeqaj@gmail.com
ORCID ID: http://www.orcid.org/0000-0001-5239-7263

Original submission:
11 June 2017;
Revised submission:
04 July 2017;
Accepted:
09 July 2017.
doi: 10.17392/917-17

Med Glas (Zenica) 2017; 14(2): 189-198

INTRODUCTION

Down syndrome is the most frequent genetic intellectual disability (1). From 2005 until now 878 cases with Down syndrome (DS) have been recorded in the Republic of Kosovo, while approximately 30-35 children with this syndrome are born annually (S. Beqiri, personal communication, 2017). The prevalence of live births of children with DS is 11.2 in 10,000 births in Europe (2), while 9.0 to 11.8 in 10,000 births in 10 regions of the United States (3).

One of the challenges faced by children with DS are the difficulties in motor development. According to some authors, motor development of children with intellectual disabilities, and those with DS in particular, is delayed compared to typically developing (TD) children (4,5). On the other hand, other authors claim that motor development of children with intellectual disabilities differs from that of children without disability, especially applying to children with DS, arguing that their central nervous system has different physical construction due to chromosomal deviations and that they also have a unique learning style (6).

Children with DS have dominance of tracks of primitive muscle response controlled by the spinal cord, compared to tracks of more coordinated movements (7). This happens due to poor myelination of descending brain and stem neurons and a reduced number of connections of neurons in higher nerve centres, such as the motor cortex, basal ganglia, cerebellum, and brain stem. These pathophysiologic processes in the brain change in size at maturity, and disorders in the central nervous system are observed especially after the 6th month of life (8,9).

Characteristic motor disturbances which appear in children with DS and which seem to influence their motor development are reduced postural tone as a typical neuromuscular symptom, inadequate postural control and reactions, insufficient stabilizing myogenic contractions around joints, disturbed proprioception and joint hypermobility (7,10). It is considered that cerebellum hypoplasia is responsible for low muscle tone, problems of trunk control, balance, coordination and speech disorders. Furthermore, commonly accompanying health problems such as heart defect, decreased thyroid gland function, difficulties with vision and hearing, and obesity can impact the motor function of a person with DS (11).

There is a limited number of studies that evaluated the effect of early motor development intervention in children with DS. Gross motor and fine motor skills of children with DS who participated in Early Intervention Programs had shown to improve over time (12). However, interventions in accordance with the principles of Neurodevelopmental Treatment or Vojta (techniques that facilitate movement with the assistance of a therapist using passive handling), or functional skills training, did not show to accelerate development or improve quality of movement, and interventions that aim to stimulate the child's exploration of active motor behaviour was seen as a potential method to positively affect motor development (13,14).

Even fewer studies, such as the one carried out by Malak et al. (15) assessed the effect of physical therapy (PT) on gross motor function in children with DS. They found that standing and walking skills, among other motor skills, were significantly delayed despite PT treatment.

The aim of this study was to prospectively follow children with DS who are treated with PT and identify their age at the time of development of 19 motor skills, and to compare the motor skill developmental age of the same children with DS to that of TD children, and to that of children with DS as reported by four other authors.

EXAMINEES AND METHODS

Examinees and study design

Sixteen children with DS (7 girls and 9 boys) participated in this study. All of them are of Kosovar nationality and registered members of the Down Syndrome Kosova (DSK) organization (a non-governmental organization representing the community of people with DS in the Republic of Kosovo). The participants were receiving PT sessions as part of the Early Intervention and Education (EIE) program offered by the DSK between 2008-2011. Their mean age at the start of treatment was 10.56 months ± 6.28 months. Five of these children presented congenital medical problems: heart disease, umbilical hernia, epigastric hernia, congenital cataract, right hemiparesis, and strabismus.

The permission for the development of this study was granted by the Oversight Board for Professional Ethics of the Ministry of Health in the Republic of Kosovo. An informed consent was obtained from all children's parents/guardians.

Methods

Sixteen (16) children participated in this prospective study. They underwent PT sessions once a week and each session lasted for 45 minutes. Sessions were offered by two physical therapists recruited by the DSK, and were performed only within the premises of this organization in the city of Prishtina. The mean duration of the treatment was 9.5 months.

The approach used focused on strategically teaching the chronologically sequenced motor skills, and targeting the skills of the next higher level. The equipment used were mats, pillows, sofa cushions, a table, an exercise ball, and other items usually found at home. Toys were a very important component and played a crucial role in motivating a child to perform desired moves needed to practice a new skill. The therapists, using holding and moving techniques, guided the child in performing the new skills, while embracing all components needed to learn those particular skills. During the mastering of a new skill, the tendency to use compensatory patterns was avoided. Parents/guardians were present during each session and were instructed to practice the skill/s at home daily, several times a day.

Nineteen motor skills were observed and recorded. The list of observed milestones was derived from already well-established data on motor milestones of TD children (16,17). The description, testing method and performance criteria for all motor skills observed in this study are found in Table 1. During the course of the treatment, each child was assessed once a month for identification of a newly accomplished skill/s. Achievement of milestones did not have to follow an exact sequence. Each of the skills was recorded as accomplished if the child was able to perform it three times. Only those milestones witnessed by the therapists were taken into consideration and the exact age of their accomplishment recorded (skills 0-16). After the children ended the PT program (could walk with support), respective parents/guardians were asked to report the age of achievement of remaining motor milestones (skills 16-19).

The motor milestones were in most cases presented in a successive pattern coinciding with what is generally seen in the literature (18). Sometimes it happens that general sequential presentation is reversed between two or more motor milestones, or already observed milestones might be inhibited later (19). In certain cases, development of a milestone was skipped, or did not occur at all, such as crawling. Hence, the age of achievement of those particular milestones is left blank.

Statistical analysis

Using descriptive statistics, the following was presented for each examined motor skill: number of children for whom the exact age of the skill accomplishment is recorded, range, minimal and maximal values, mean, standard deviation, standard error, variance, skewness, and kurtosis.

In order to compare the means of motor skill developmental age found in children with DS who participated in our study to that of TD children (comparison 1), and also to that of children with DS as reported by four other authors, Cunningham (20) (comparison 2a), Berry, Andrews & Gunn as seen in Sacks & Buckley (5) (comparison 2b), Winders (21) (comparison 2c) and Melyn&White (22) (comparison 2d), an Independent T-samples test was used. Values $p< 0.05$ were considered statistically significant. A Cohen's d, as an effect size measure, was also calculated. For comparison 1, means of 19 motor skills, were used. For comparisons 2a-2d, means of the skills which were commonly assessed in both comparison groups were used: comparison 2a - 8 skills, comparison 2b – 5 skills, comparison 2c – 11 skills, and comparison 2d – 6 skills.

RESULTS

In table 2 descriptive statistics were used to present the participants' mean age (in months) of development of motor skills.

In general, all motor skills were delayed. The delay was more prominent in the development of a fine motor skill 'grasps using thumb and index finger' (18.3 months), and in skills which also incorporate the trunk and lower limb muscles such as ''stands with support'' (16.9 months), ''gets to

Table 1. Testing method and performance criteria of 19 motor skills

	Motor skills	Testing method	Performance criteria
1	Holds head straight without support	Support the child in sitting, holding him/her firmly around his/her chest or shoulders.	Child holds her/his head steady for five seconds, the chin should not bob forward or to the side.
2	Grasps small objects and puts them into mouth	Place a toy or object in a child's hand, wait for him/her to grasp it, and observe. Choose a light, well-balanced object.	The child grasps the toy and brings his/her hand to his/her mouth while still holding the toy.
3	Routinely rolls from stomach to back and back to stomach	Observe the child when he/she is playing on his/her tummy/back, with the toys to the side out of reach.	The child deliberately rolls to his/her back/tummy to reach the toys.
4	Pushes down against a surface when in vertical position	Place the child in standing, supporting him/her around his/her upper trunk.	The child maintains the standing position while taking most of his/her own weight.
5	Sits unsupported	The child is placed in a sitting position. Then he/she is given a toy to play using both hands so that he/she does not use arms for support.	The child sits up straight with the head erect at least for 10 seconds. The child does not use arms or hands to balance body or support position.
6	Grasps objects with one hand and puts into the other	Place a toy (e.g. a rattle) in one hand.	The child transfers a rattle to the other hand.
7	Grasps using thumb and index finger	Place a small object on the table, e.g. a raisin or similar sized-object. Draw the child's attention to it and direct him to 'take'.	The child picks up the object with thumb and fingers in opposition.
8	Stands with support	Place the child standing and holding onto a table or another stable object, with toys to play with.	The child can maintain the position, supporting himself/herself with the hands. The child no longer leans his/her chest or arms on the support.
9	Gets to sitting position from tummy without assistance	Place the child on tummy and encourage him/her to sit up or observe him/her during play.	The child gets from tummy to sitting without assistance.
10	Pulls self-up to standing position at furniture	Place the child in a crawl or sitting position, in front of the table with a toy on it. Show the child the toy and encourage him/her to take it.	The child pulls up to stand by pushing down strongly on his/her arms, at the same time straightening both legs.
11	Crawls	Place the child on tummy or in the crawl position with toys two meters in front.	The child crawls for two meters without stomach touching the supporting surface.
12	Walks holding onto furniture	Place the child so that he/she is standing at a low table. Use toys to encourage him/her to cruise around the table.	The child travels around the table, half-turned in the direction of his/her travel.
13	Stands without support	Stand the child in the middle of the floor, or lean the child against the wall and then gently pull him/her forward away from the wall. Or lean the child forward against a couch and encourage him/her to stand by himself/herself.	The child stands unsupported for 10 seconds.
14	May walk two or three steps without support	Place a child standing against the first support. Put the toys on the other support at a distance two to three steps away. Encourage the child to reach to the second support by walking.	The child can get himself/herself from one support to the next.
15	Walks independently	Place your child standing in the middle of the floor or leaning back against a wall or supported by furniture. Encourage him/her to walk to you.	The child walks alone for four or five steps.
16	May climb stairs	Ask the child to walk up the stairs.	The child steps up one step at a time, holding the rail with one hand, and with the other hand free. He/she may go up two feet to a step.
17	May run gently	Encourage the child to run by either chasing him/her, or getting him/her to chase you or a ball or another child.	The child runs for at least 2 meters; a stiff, upright run on the whole foot, rather than the toes.
18	Climbs onto and down from furniture unsupported	Ask the child to climb up a table, a chair or another piece of furniture.	The child can climb up and down a piece of furniture without assistance.
19	Walks on tiptoes	Ask the child to walk on tiptoes or demonstrate.	The child can walk on tiptoes without his/her heels touching the ground for 3 meters.

Adapted from Pieterse & Treloar (43)

sitting position from tummy without assistance'' (16.8 months), and so on. The skills that developed the slowest were ''pulls himself/herself up to the standing position at furniture'' (21.8

Table 2. Descriptive statistics for developmental age (months) of 19 motor skills in children with Down syndrome (N=16)

	Motor skills	N (16)	Range	Min.	Max.	Mean	Std. err	Std. Deviation	Variance	Skewness	Kurtosis
1	Holds head straight without support	9	7.0	4.0	11.0	6.4	0.8	2.74	7.5	0.6	1.5
2	Grasps small objects and puts them into mouth	8	4.0	4.0	8.0	5.4	0.4	1.5	2.3	0.7	2.0
3	Routinely rolls from stomach to back and back to stomach	13	12.0	5.0	17.0	8.9	0.8	3.0	8.9	1.6	4.1
4	Pushes down against a surface when in vertical position	14	20.0	4.0	24.0	9.0	1.4	5.1	25.8	2.3	5.9
5	Sits unsupported	15	7.0	5.0	12.0	8.2	0.5	2.0	4.0	0.5	0.1
6	Grasps objects with one hand and puts into the other	14	24.0	6.0	30.0	11.9	1.6	6.0	36.0	2.4	6.7
7	Grasps using thumb and index finger	12	24.0	12.0	36.0	18.3	1.9	6.7	45.5	1.7	3.9
8	Stands with support	14	37.0	11.0	48.0	16.9	2.5	9.4	88.7	3.1	10.7
9	Gets to sitting position from tummy without assistance	13	25.0	11.0	36.0	16.8	1.7	6.3	39.3	2.6	8.3
10	Pulls self-up to standing position at furniture	13	39.0	14.0	53.0	21.8	2.9	10.5	110.6	2.5	6.9
11	Crawls (quadruped)	12	16.0	11.0	27.0	18.2	1.4	4.9	24.0	0.4	-0.5
12	Walks holding onto furniture	15	34.0	14.0	48.0	20.8	2.3	9.0	81.7	2.2	5.6
13	Stands without support	14	35.0	13.0	48.0	21.3	2.3	8.6	73.6	2.7	7.9
14	May walk two or three steps without support	12	42.0	18.0	60.0	29.0	3.3	11.5	131.3	1.9	4.8
15	Walks independently	10	44.0	19.0	63.0	32.5	3.9	12.3	152.1	1.7	4.4
16	May climb stairs	9	37.0	19.0	56.0	30.2	4.1	12.2	149.9	1.3	1.3
17	May run gently	8	52.0	20.0	72.0	37.9	5.4	15.3	235.6	1.7	4.2
18	Climbs onto and down from furniture unsupported	10	36.0	17.0	53.0	28.2	3.5	11.0	122.0	1.4	1.9
19	Walks on tiptoes	5	35.0	25.0	60.0	44.2	5.8	12.9	166.2	-0.6	1.0

months), ‘’may walk two or three steps without support’’ (29 months), and ‘’may run gently’’ (37.9 months). On the contrary, the delay in the age of development of the skill ‘’climbs onto and down from furniture unsupported’’ was not that large (28.2 months).

The values of standard deviation (SD) mainly showed an increase with chronological development of skills: they were lowest in skills that developed first, such as ‘’grasps small objects and puts them into mouth’’ (SD=1.5), whereas largest values were seen in the latter skills such as ‘’may run gently’’ (SD=15.3). The range and variance values followed the same trend as SD.

Five different comparisons of means of motor skill developmental age were done. Comparison 1: Children with DS from the present study vs. children with TD (p=0.005, Cohen’s d=1.6). Comparisons 2a-2d: Children with DS from the present study vs. children with DS reported by Cunningham (20) (p=0.86); Berry, Andrews & Gunn as seen in Sacks & Buckley (5) (p=0.72); Winders (21) (p=0.29); Melyn&White (22) (p=0.63). These results show that a statistically significant difference, with a very high Cohen’s d, was found only in Comparison 1 (Table 3).

In table 4, the mean developmental ages of the same motor skills as seen in TD children, and as seen in children with DS were descriptively presented in order to contribute to better interpretation of Table 3.

Table 3. Overview of comparisons of mean motor skill developmental age

	Comparison 1*	Comparison 2a†	Comparison 2b‡	Comparison 2c§	Comparison 2d¶
p	0.005	0.86	0.72	0.29	0.63
Cohen’s d	1.6	0.02	0.23	0.46	0.3

*Children with DS (our study sample) vs. children with TD; †Children with DS (our study sample) vs children with DS according to Cunningham (20); ‡Children with DS (our study sample) vs. children with DS according to Berry, Andrews & Gunn as seen in Sacks & Buckley (5); §Children with DS (our study sample) vs children with DS according to Winders (21); ¶Children with DS (our study sample) vs. children with DS according to Melyn&White (22)

DISCUSSION

In this study children with DS underwent PT sessions once a week and during this period the developmental age of motor skills was recorded. Descriptive statistics were used to present the sequence and age of attainment of 19 observed motor skills. These data were further compared to existing data on motor development in children with TD, and to that of children with DS who participated in four other studies. A significant difference was only found in the first comparison, whereas the developmental age of motor skills of children with DS observed in our study matched quite well with the data on developmental age of motor milestones in the same population reported by other studies.

From the motor development presented in table 4 we understand that the sequence of development of motor skills in this study matches the one observed in TD children. No big difference was seen in the age of attainment of first two motor skills

Table 4. The mean age (in months) of development of motor skills in typically developing children, in our study sample of children with Down syndrome, as well as in four other studies involving children with Down syndrome

	Motor skills	N (16)	Typical development (16,17)	Our study	Cunningham (20)	Berry, Andrews & Gunn as seen in Sacks & Buckley (5)	Winders (21)	Melyn& White (22)
1	Holds head straight without support	12	4.0	6.4	5	-	-	4
2	Grasps small objects and puts them into mouth	12	4.0	5.4	-	-	-	-
3	Routinely rolls from stomach to back and back to stomach	13	6.0	8.9	8.0	6 to 7	6 to 7	6.4
4	Pushes down against a surface when in vertical position	14	6.0	9.0	-	-	-	-
5	Sits unsupported	15	8.0	8.2	9.0	11	11	11.8
6	Grasps objects with one hand and puts into the other	14	9.0	11.9	-	-	-	-
7	Grasps using thumb and index finger	12	9.0	18.3	20.0	-	-	-
8	Stands with support	14	9.0	16.9	-	-	-	-
9	Gets to sitting position from tummy without assistance	13	9.0	16.8	-	-	17	-
10	Pulls self-up to standing position at furniture	13	9.0	21.8	15.0	17	15-17	-
11	Crawls (quadruped)	12	9.0	18.2	-	-	17	12.2
12	Walks holding onto furniture	15	12.0	20.8	-	-	18	-
13	Stands without support	14	12.0	21.3	18.0	21	21	20.9
14	May walk two or three steps without support	12	12.0	29.0	-	24.0	26	-
15	Walks independently	10	18.0	32.5	23	-	26	24.4
16	May climb stairs	9	18.0	30.2	-	-	20	-
17	May run gently	8	18.0	37.9	48	-	-	-
18	Climbs onto and down from furniture unsupported	10	24.0	28.2	-	-	20-22	-
19	Walks on tiptoes	5	24.0	44.2	-	-	-	-

('holds head straight without support', and 'grasps small objects and puts them into mouth') between children from our study and TD children. From the 3rd skill onwards ('routinely rolls from stomach to back and back to stomach') the difference in age increases, with the exception of the 5th skill ('sits unsupported'), where hardly any difference is seen. The skill 'routinely rolls from stomach to back and back to stomach' was achieved at the mean age 8.9 months in the present study (range 5-17 months old), somewhat later than what Cunningham (8 months) (20) and Berry, Andrews & Gun (6-7 months) (5), Winders (6-7 months) (21), and Melyn&White (6.38 months) (22) reported. Palisano et al. (7) predicted the probability for achieving the rolling skill by 6 months of age to be 51%, and by 18 month 74%.

The unsupported sitting was achieved at around 11-15 months of age according to Berry, Andrews & Gun (5), Winders (21), Melyn&White (22), and Vasques (23). However, the participants of our study showed to have achieved the same skill at a mean age of 8.2 months, which is similar to the finding of Cunningham (20) that children with DS sit unsupported at the mean age of 9 months.

When compared to children with TD, a more pronounced difference is found in the 7th skill ("grasps using thumb and index finger"), where mean age of its achievement in children with DS is 18 months, whereas in TD children it is 9 months. When compared to other studies involving children with DS, according to Cunningham (20), the same skill was attained at a mean age of 20 months, similar to what was noted in this study.

The mean developmental age of the 13th skill ("stands without support"), which is one of the most reported milestones, was achieved by the participants of this study at a mean age of 22.3 months. This corresponds to the mean developmental age for the same skill reported in several other studies: 21 months (5,21), 21.2 months (20), and 18.97-22.17 months (22). The maximal age for the unsupported standing seen in this study is 48 months, which coincides with the estimated probability of Palisano et al. (4) according to whom all children with DS are expected to achieve this skill by 48 months of age.

In comparison to TD children, the trend of increasing difference is seen in all skills, particularly in the 14th and 17th ("may walk two or three steps without support" and "may run gently"). The age of attaining these two skills is more than twofold in children with DS compared to those with TD. The 15th skill ("walks independently") was attained at the mean age of 32.5 months in this study. When other studies involving children with DS were consulted, a variability in the mean age of attainment of the same skill was found. It appeared to had been acquired sooner according to a few authors, such as at 26 months (5) and 22.72-26.09 months

(22). However, our mean age for the walking skill compares favourably with the mean age reported by Vasques (23), which is 30.2 months and Hall B (24), who stated that the debut of walking usually occurred at the age of 30 months. Similarly, according to Palisano et al. (4) the estimated probability of walking by 30 months is 74%, while by 36 months 92%. Also, Centerwall (25) and Carr (26) reported that 78-80 % of children with DS were able to walk by the age of 36 months. According to Malak et al. (15), only 10% of children with DS under three years of age walked at expected age, while 95% of those 3-6 years old.

The data for the last skill ('walks on tiptoes') was reported for only 5 children in the present study, and the difference in age is almost doubled compared to children with TD (TD-24 months, DS-44.2 months). On the other hand, the difference in the mean age of attainment of the 18th skill ("climbs onto and down from furniture unsupported") is only 4 months of age (TD-24 months, DS-28.2 months). The probable reason for expressed and increased difference in age of attainment of motor skills after the 6th month of life are the pathophysiological processes in cerebrum, change in its size, and disorders in central nervous system maturation, observed in children with DS notably after the 6th month of life (9). The slower motor development of children with DS seen in this study coincides with the existing data on delayed motor and postural control development in this population (4, 27-29). However, it is still ambiguous whether the motor development is just delayed or if it is a result of differently constructed central nervous system and unique learning style (5,10).

High values of standard deviation (DS) indicate a large variability within the sample, showing that 16 children developed same skills at quite different ages. Standard error (SE) values are high as well, meaning that mean ages of the development of skills in this sample cannot be very representative for the whole community of children with DS. The difference in mean age of skill attainment between the observed sample and reference values for TD children increases with the complexity of the skill. The values of SD and SE follow the similar trend, letting us understand that the more complex the skill, the larger the variability in age of its development in children with DS. The lowest values of SD and SE were found in the 2nd skill ('grasps small objects and puts them into mouth'), 1.5 and 0.4, respectively, whereas the largest range value, 52, was seen in the 17th skill ('may run gently').

Consistent with the findings of this study, a large variability in the age of the development of motor skills in children with DS is also reported by other authors (5, 20-22). When comparing the results of these authors with each other, we note that according to Melyn&White (22) and Winders (21) motor skills were acquired earlier than what is seen in the studies of Cunningham (20), and Berry, Andrews & Gun (5).

As expected, in this study we found a statistically significant difference ($p=0.005$) with a very high Cohen's d (1.6) between the developmental age of motor skills between the study sample of children with DS and the reference developmental age of the same skills in children with TD. This finding agrees with the previous studies that claim that motor development in children with DS is delayed compared to children with TD (4,5). Children with DS often present with health problems (30). Participants included in the present study had noticeable health difficulties. Ill health negatively impacts motor performance in TD population (31-33), and it is expected that the same applies to persons with DS. The slower motor development seen in the participants of our study was probably affected by the above mentioned health implications.

Our results also show that children with DS who participated in this study presented with a motor developmental sequence, which did not differ from that of children with TD, supporting the stands of Cunningham (20) and Winders (21), while contrasting the stands of Haley (32) who claims the opposite.

Another important finding was a lack of statistically significant difference between the results from our study in comparison to the results of four authors described in the statistical analysis section, which possibly tells us that children with DS from our study, who were treated with PT as part of early intervention program, did not physically develop differently from what is generally reported for the same population. Differently from the children in our study who were being treated with PT, some of the studies which our results were compared to (5,22) were carried out before the spread of early intervention programs, whereas others did not provide any information regarding whether their par-

ticipants took part in early intervention programs. Perhaps PT, being widely offered within the framework of early information programs, does not affect sooner development of motor milestones in children with DS as commonly assumed. The role of PT might instead be a promotion of more efficient motor skills and reduction or avoidance of compensatory movements, which might lead to orthopaedic problems if left untreated (35). This issue has not been discussed in the present study, but should be explored in the future knowing from current studies that impaired posture and walking can lead to changes in step characteristics in adolescence and adulthood such as slower walking, wider strides, longer stance and double support (36,37), greater lower limb muscles' co-contraction during swing phase of gait (38), and lesser stability (39). Accordingly, it has been suggested that physical therapists continue to address impairments in children with DS enhancing their participation in sport and leisure activities with their peers by focusing on coordination and balance problems, and strengthening of the trunk and the legs (40).

The mean age of participants in our study at the time of dismissal from PT was 20.5 months, whereas according to Winders (41), PT should follow the child's development until the age of six. This stand is very much acceptable having in mind that more complex skills, as seen in this study, develop notably later in comparison to TD children. This is especially evident for the skills requiring body vertical position. Consistent with our results, the study of Pereira (42) showed that only 40% of children with DS managed to pull themselves to stand with support by 12 months of age. In our study this skill was achieved at a mean age of 21 months. In addition, as previously mentioned, the study of Malak (15) showed that walking was achieved by 95% of children with DS only at the age of six. In compliance with the aforementioned author, our results show that maximal age at which the 15th skill ('Walks independently') was developed was 63 months or slightly more than 5 years. Also, the maximal age for the 17th skill ('May run gently') was 72 months or 6 years. Seeing the large variability and delay in the development of motor skills in children with DS, which is especially emphasized in subjects dealing with accompanying health problems, in order for PT to have greater impact, we believe the treatment should last until a toddler with DS achieves all motor skills needed for efficient motor function, including those developing after independent walking, such as running and using stairs independently.

Limitations of this study are a relatively small sample size of participants and a short duration of PT treatment (9.5 months). There was also a variability in the age of start of the treatment (4-29 months). Input from parents regarding the amount of time weekly dedicated to the application of home exercises prescribed by physical therapists was not taken into consideration. For future studies, we recommend that the possible effect of PT on acceleration of mastering of motor skills should be more thoroughly investigated in a larger sample size and randomly assigned participants into the treated and untreated groups who do not have accompanying health problems. Also, standardized tests, validated for children with DS should be used. We consider that active involvement of parents in carrying out stimulating daily exercises as instructed by a physical therapist is mandatory and a key factor in optimizing motor function and efficiency in children with DS. The effect of parent engagement should be comprehensively examined in future studies, together with effects of PT on efficiency of motor skills and prevalence of compensatory movements in this population.

This longitudinal study showed a significantly delayed development of 19 motor skills in children with DS when compared to the reference developmental age for the same skills in children with TD. As much as this was expected, it was interesting to find that, despite the treatment with PT, no significant discrepancy was found between the motor developmental age of our study sample and other four studies involving children with DS. A large variability in the age of attainment of the skills was observed, which increased chronologically with the complexity of the skill.

ACKNOWLEDGEMENT

Mr. Driton Bajraktari (former) and Mrs. Sebahate Beqiri (present), Director of the Down Syndrome Kosova Organization who supported the study.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Competing interests: None to declare.

REFERENCES

1. Sherman SL, Allen EG, Bean LH, Freeman SB. Epidemiology of Down syndrome. Ment Retard Dev Disabil Res Rev 2007; 13:221–7.
2. Loane M, Morris JK, Addor MC, Arriola L, Budd J, Doray B, Garne E. Twenty-year trends in the prevalence of Down syndrome and other trisomies in Europe: impact of maternal age and prenatal screening. Eur J Hum Genet 2013; 21:27–33.
3. Shin M, Besser LM, Kucik JE, Lu C, Siffel C, Correa A. Prevalence of Down syndrome among children and adolescents in 10 regions of the United States. Pediatrics 2009; 124: 1565-71.
4. Palisano RJ, Walter SD, Russell DJ, Rosenbaum PL, Gémus M, Galuppi BE, Cunningham L. Gross motor function in children with Down syndrome: Creation of motor growth curves. Arch Phys Med Rehab 2001; 82:494–500.
5. Sacks B, Buckley S. What do we know about the movement abilities of children with Down syndrome? Down Syndrome News and Update 2001; 2:131-41.
6. Wishart JG. Taking the initiative in learning: a developmental investigation of children with Down syndrome. Int J Disabil Dev Ed 1991; 38:27-44.
7. Cook AS, Woollacott MH. Dynamics of postural control in the child with Down syndrome. Phys Ther 1985; 65:1315–21.
8. Teipel SJ, Alexander GE, Schapiro MB. Age related cortical grey matter reduction in non demented Down's syndrome adults determined by MRI with voxel – based morphometry. Brain 2004; 127:811–24.
9. Pinter JD. Neuroanatomy of Down's syndrome: a high-resolution MRI study. Am J Psychiatry 2001; 158:1659–65.
10. Chen HL, Yeh CF, Howe TH. Postural control during standing reach in children with Down syndrome. Res Dev Disabil 2015; 38:345–351.
11. Block ME. Motor development in children with Down's syndrome: a review of the literature. Adapt Phys Act Q 1991;8:179-20.
12. Connolly BH, Morgan SB, Russell FF, Fulliton WL. A Longitudinal Study of Children with Down Syndrome Who Experienced Early Intervention Programming. Phys Ther 1993; 73:170-9.
13. Mahoney G, Robinson C, Fewell RR. The effects of early motor intervention on children with Down syndrome or cerebral palsy: a field-based study. J Dev BehavPediatr 2001; 22:153-62.
14. Blauw-Hospers CH, Hadders-Algra M. A systematic review of the effects of early intervention on motor development. Dev Med Child Neurol 2005; 47:421-32.
15. Malak R, Kostiukow A, Krawczyk-Wasielewska A, Mojs E, Samborski W. Delays in motor development in children with Down syndrome. Med Sci Monit 2015; 21:1904–10.
16. WHOMulticentre Growth Reference Study Group. WHO Motor Development Study: Windows of achievement for six gross motor development milestones. Acta Pediatr Suppl 2006; 450:86-95.
17. Albers CA, Grieve AJ. Test Review: Bayley, N. (2006). Bayley Scales of Infant and Toddler Development. 3rd ed. San Antonio (TX): Harcourt Assessment. J Psychoeduc Assess 2007; 25:180-90.
18. Capute AJ, Accardo PJ. The infant neurodevelopmental assessment: a clinical interpretive manual for CAT-CLAMS in the first two years of life, part 1. Curr Probl Pediatr 1996; 26:238–57.
19. Bergenn VW, Dalton TC, Lipsitt LP, Myrtle B. McGraw: a growth scientist. Dev Psychol 1992; 28:381-95.
20. Cunningham C. Down syndrome – An introduction for parents and carers. 3rd ed. Bodmin, England: MPG Books Ltd, 2006.
21. Winders PC. Gross Motor Skills for Children with Down Syndrome: A Guide for Parents and Professionals (Topics in Down Syndrome). Bethesda, USA: Woodbine House, 1997.
22. Melyn MA, White DT. Mental and developmental milestones of non-institutionalized Down's syndrome children. Pediatrics 1973; 52:542-5.
23. Ferreira-Vasques AT. Motor, linguistic, personal and social aspects of children with Down syndrome. J Appl Oral Sci 2015; 23:424–30.
24. Hall B. Somatic deviations in newborn and older mongoloid children. Acta Paediatr Scand 1970; 59:199-204.
25. Centerwall SA, Centerwall WR. A study of children with mongolism reared in the home compared to those reared away from the home. Pediatrics 1960; 25:678-85.
26. Carr J. Mental and motor development in young mongol children. J Ment Defic Res 1970; 14:205-20.
27. Lopes VB, Lima CD, Tudella E. Motor acquisition rate in Brazilian infants. Infant Child Dev 2009; 18:122-32.
28. Tudella E, Pereira K, Basso RP, Savelsbergh GJ. Description of the motor development of 3–12 month old infants with Down syndrome: the influence of the postural body position. Res Dev Disabil 2011; 32:1514–20.
29. Cardoso AC, Campos AC, Santos MM, Santos DC, Rocha NA. Motor performance of children with Down syndrome and typical development at 2 to 4 and 26 Months. Pediatr Phys Ther 2015; 27:135-41.
30. Roizen NJ, Magyar CI, Kuschner ES, Sulkes SB[2], Druschel C[4], van Wijngaarden E[5], Rodgers L[2], Diehl A[2], Lowry R[6], Hyman SL[2]. A community cross-sectional survey of medical problems in 440 children with own syndrome in New York State. J Pediatr 2014; 164:871-5.
31. Haibach PS, Wagner MO, Lieberman LJ. Determinants of gross motor skill performance in children with visual impairments. Res Dev Disabil 2014; 35:2577–84.
32. Sluijs VL, Wiedijk BM, Last BF, Grootenhuis MA, Vulsma T. Evaluation of cognitive and motor development in toddlers with congenital hypothyroidism diagnosed by neonatal screening. J Dev Behav Pediatr 2012; 33:633-40.

33. Bjarnason-Wehrens B, Dordel S, Schickendantz S, Krumm C, Bott D, Sreeram N, Brockmeier K. Motor development in children with congenital cardiac diseases compared to their healthy peers. Cardiol Young 2007; 17:487-98.
34. Haley SM. Sequence of development of postural reactions by infants with Down's syndrome. Dev Med Child Neurol 1987; 29:674-9.
35. Winders PC. The goal and opportunity of physical therapy for children with Down syndrome. Down Syndrome Quarterly 2001; 6:1-5.
36. Elshemy SA. Comparative study: Parameters of gait in Down syndrome versus matched obese and healthy children. Egypt J Med Hum Genet 2013; 14:285–91.
37. Smith BA, Ulrich BD. Early onset of stabilizing strategies for gait and obstacles: Older adults with Down syndrome. Gait Posture 2008; 28:448–55.
38. Gontijoa APB, Mancinib MC, Silvac PLP, Chagasd PSC, Sampaioa RF, Luze RE, Fonseca ST. Changes in lower limb co-contraction and stiffness by toddlers with Down syndrome and toddlers with typical development during the acquisition of independent gait. Hum Movement Sci 2008; 27:610–21.
39. Agiovlasitis S, McCubbin JA, Yun J, Mpitsos G, Pavol MJ. Effects of Down syndrome on three-dimensional motion during walking at different speeds. Gait Posture 2009; 30:345–50.
40. Capio CM, Rotor ER. Fundamental movement skills among Filipino children with Down syndrome. J Exerc Sci Fit 2010; 8:17-24.
41. Winders PC. Gross Motor Skills for Children with Down Syndrome: A Guide for Parents and Professionals (Topics in Down Syndrome). 2nd ed. Bethesda, MD: Woodbine House, 2014.
42. Pereira K, Basso RP, Lindquist AR, da Silva LG, Tudella E. Infants with Down syndrome: percentage and age for acquisition of gross motor skills. Res Dev Disabil 2013; 34:894-901.
43. Pieterse M, Treloar R. Small Steps - An Early Intervention Program for children with Developmental Delays. Booklet 4. Sidney (Australia): Macquarie University, Special Education Centre, 1989.

ORIGINAL ARTICLE

Positron emission tomography/computed tomography (PET/CT) and CT for N staging of non-small cell lung cancer

Sandra Vegar Zubović, Spomenka Kristić, Besima Hadžihasanović

Clinic of Radiology, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina

ABSTRACT

Aim The aim of this study is to investigate the possibilities of non-invasive diagnostic imaging methods, positron emission tomography/computed tomography (PET/CT) and CT, in clinical N staging of non-small cell lung cancer (NSCLC).

Methods Retrospective clinical study included 50 patients with diagnosed NSCLC who have undergone PET/CT for the purpose of disease staging. The International association for the study of lung cancer (IASLC) nodal mapping system was used for analysis of nodal disease. Data regarding CT N-staging and PET/CT N-staging were recorded. Two methods were compared using $\chi 2$ test and Spearman rank correlation coefficient.

Results Statistical analysis showed that although there were some differences in determining the N stage between CT and PET/CT, these methods were in significant correlation. CT and PET/CT findings established the same N stage in 74% of the patients. In five patients based on PET/CT findings the staging was changed from operable to inoperable, while in four patients staging was changed from inoperable to operable.

Conclusion PET/CT and CT are noninvasive methods that can be reliably used for N staging of NSCLC.

Key words: pulmonary cancer, multimodal imaging, lymph nodes

Corresponding author:
Spomenka Kristić
Radiology Clinic,
University Clinical Center Sarajevo
Bolnička 25, 71000 Sarajevo,
Bosnia and Herzegovina
Phone +387 33 298 156;
Fax : +387 33 297 811;
E-mail: spomenka79@net.hr
Sandra Vegar Zubović ORCID ID: http://www.orcid.org/0000-0002-6276-2348

Original submission:
17 February 2017;
Revised submission:
08 May 2017;
Accepted:
13 June 2017.
doi: 10.17392/899-17

Med Glas (Zenica) 2017; 14(2): 199-203

INTRODUCTION

Lung cancer is a malignant disease with the highest mortality rate despite advances in diagnosis, staging and treatment (1). Of the total number of diagnosed lung cancers, 75-85% are classified as non-small cell lung cancer (NSCLC) (2).

Best results in the treatment and long-term survival are achieved when surgical resection of the cancer is possible. Accurate staging of NSCLC is obligatory (3) in order to properly select the patients who will have the benefits of surgical treatment and to mitigate unnecessary surgical procedures in the advanced stages of the disease (4).

The NSCLC staging is based on the TNM system, using the tumour (T), node (N) and metastasis (M) evaluations provided by non-invasive and invasive procedures (5). Traditionally, computed tomography (CT) of thorax and upper abdomen is used for the staging of NSCLC, while recently there is a tendency to use positron emission tomography/computed tomography (PET/CT). This method combines anatomic information of CT and metabolic information of the PET (6).

Accurate N staging is an important prognostic factor and is critical in deciding the best treatment option. Stage N1 and N2 of the disease may be resectable, while N3 is considered to be inoperable and could treated with concurrent chemo-radiation therapy (7).

Mediastinal nodal disease is evaluated using CT scan and more recently PET/CT has been used for the evaluation of metastatic spread in the mediastinal lymph nodes (8). Size of the lymph node is the criterion used on CT scan to distinguish benign from malignant nodes: a node with short axis diameter of more than 1 cm is generally considered to be malignant (9-11).

The use of size cut-off can be problematic because inflammatory lymph nodes larger than 1 cm can be considered as malignant and metastatic lymph nodes smaller than 1 cm can be considered as benign (12). On the other hand, hybrid imaging method PET/CT enables obtaining anatomical data regarding the presence and size of lymph nodes as well as functional information regarding eventual pathological metabolic activity in these lymph nodes (7,13).

The aim of this study was to investigate the possibilities of CT and PET/CT non-invasive diagnostic imaging methods in clinical N staging of NSCLC. Results of this study should provide additional information that should be considerd when deciding on a diagnostic method to be used for staging of NSCLC, e. g. whether to give the preference to traditional method - CT or insist on the newer method - PET /CT.

PATIENTS AND METHODS

Patients and study design

The retrospective clinical study conducted at the Radiology Clinic of the University Clinical Centre of Sarajevo, Bosnia and Herzegovina, during the period June 2015 – October 2016 included 50 patients with diagnosed NSCLC who have undergone PET/CT for the purpose of disease staging.

Before the realisation of PET/CT examination for the purpose of staging, diagnosis of NSCLC was confirmed based on histopathological analysis of tumour tissue samples obtained during bronchoscopy or by biopsy under CT control for all patients included in the study.

Methods

The PET/CT was performed using a scanner GE Discovery (GE Healthcare, General Electric, Milwakee, Viskonsin, USA). Multislice CT and PET emission data were acquired from the skull to the mid-thigh in all the patients. Image acquisition started 60 min after intravenous injection of 370–550 MBq of 18F-FDG. CT (120 kV; auto mA: 15-200 mAs; slice thickness for CTAC – 3.75 mm and for interpretation – 1.25 mm; pitch – 1.75; rotation time, 0.8 s) was performed. CT scan was acquired in a middle respiratory position. Directly after CT, the PET acquisition was started. The acquisition time was 1.45 min per bed position (5–8 bed positions per patient). During imaging of the chest, patients were instructed to breathe shallowly. Each position had 35 scanning planes with a 14.6-cm longitudinal field of view and a 1-slice overlap between scanning positions. PET images were reconstructed using CT attenuation correction and an ordered-subset expectation maximization algorithm. Scans were evaluated separately for CT alone and combined PET/CT using standard criteria for the evaluation of CT and PET/CT.

The International Association for the Study of

Lung Cancer (IASLC) nodal mapping system was used for an analysis of nodal disease (14).

CT findings were analysed and the study was interpreted as positive based on shape and size of mediastinal lymph nodes. Lymph nodes were measured on the short-axis diameter and lymph nodes were considered abnormal if they were 1 cm or greater in short axis diameter on CT (15,16).

PET images were analysed qualitatively for regions of focally increased glucose metabolism as well as quantitatively by determining standardized uptake values. An increase in the glucose uptake to a level greater than that in the surrounding tissue at qualitative analysis and a standard glucose uptake value of more than 2.5 were considered to characterize malignancy. For fused PET/CT data sets the same criteria used to determine malignancy among the individual CT and PET data sets were applied. However, lymph nodes with increased glucose uptake were deemed positive for metastatic spread even when they were smaller than 1 cm in short-axis diameter. PET-negative lymph nodes were characterized as benign even when they were larger than 1 cm in short-axis diameter (17).

Data regarding CT N-staging, PET/CT N-staging and sex were also recorded.

Statistical analysis

The test results were analysed using descriptive statistics, which included determining the absolute values (N) and percentage (%). Two methods were compared using $\chi 2$ test and Spearman rank correlation coefficient. Accepted statistical significance was $p<0.05$.

RESULTS

Of the total of 50 patients, 25 (50%) were males and 25 (50%) were females.

Statistical analysis showed that although some differences in determining the N stage between CT and PET/CT were found there was significant correlation between these two methods (rho=0.723; p = 0.0001). According to both methods the majority of the cases were staged as N2 both according to CT (21; 42.0%) and PET/CT (17; 34.0%) while the least number of cases were staged as N3 both according to CT (5; 10.0%) and PET/CT (10; 20.0%) (Table 1).

Table 1. N staging of patients with diagnosed non-small cell lung cancer (NSCLC) based on positron emission tomography/computed tomography (PET/CT) and CT imaging

	No (%) of patients	
N stage	**CT**	**PET-CT**
N0	8 (16.0)	11 (22.0)
N1	16 (32.0)	12 (24.0)
N2	21 (42.0)	17 (34.0)
N3	5 (10.0)	10 (20.0)
Total	50 (100.0)	50 (100.0)

CT and PET/CT findings established the same N stage in 37 (74%), while the staging was not analogous in 13 (26%) patients.

In four (6%) patients PET/CT determined lower N stage of NSCLC comparing to CT, while in nine (18%) patients −PET/CT determined higher N stage comparing to CT (Table 2).

Table 2. Changes of CT based N staging compared to positron emission tomography/computed tomography (PET/CT) findings

N stage CT	No (%) of patients	**PET-CT change**
Up-staged		
N0	0	
N1	4 (8.0)	in N2
N1	1 (2.0)	in N3
N2	4 (8.0)	in N3
N3	0	
Total	**9 (18.0)**	
Down staged		
N0	0	
N1	0	
N2	3 (6.0)	in N0
N2	1 (2.0)	in N1
N3	0	
Total	**4 (8.0)**	

Changes of N stage based on PET/CT findings were presented in 13 patients. In five patients based on PET/CT findings the N stage changed from operable to inoperable (N1 to N2 in four and to N3 in one case), while in four patients staging changed from inoperable to operable (N2 to N0 in three cases and to N1 in one case) (Table 2). Due to small counts it was impossible to conduct valid statistical analysis ($\chi 2=58.182$; $p=0.0000000001$).

DISCUSSION

Considering the increasing number of diagnosed cases of NSCLC and all advances in the therapy of this disease, the correct staging is essential in order to avoid unnecessary surgery (3).

The gold standard for the evaluation of metastatic involvement of hilar and mediastinal lymph nodes is mediastinoscopy. Since the mediastinos-

copy is an invasive method, the tendency is to replace it with non-invasive methods such as PET/CT and CT (6). Numerous studies have demonstrated higher sensitivity and specificity of PET/CT compared to CT in N staging of NSCLC. The conclusion of those studies is that when it comes to N staging NSCLC the preference should be given to PET/CT as a non-invasive method of choice (17,18).

In our study we did not observe a significant difference in N staging based on PET/CT and CT examinations. Such result can be explained by the fact that the CT scans were analysed by a radiologist with extensive experience in thoracic radiology who deals with NSCLC staging on a daily basis. Also, the limited number of patients should be taken into account and the fact that patients did not undergo mediastinoscopy, which is the gold standard for determining the N staging.

However, in our study out of the total number of discrepant findings regarding N staging, based on PET/CT N staging five patients were reclassified from operable stage of the disease to inoperable stage which assumes chemo-radiotherapy and the postponing of surgical procedures, while four patients were under-staged from inoperable to operable stage of the disease. An additional advantage of PET/CT certainly is that this method allows analysis of larger regions with the consequent possibility of detecting distant metastases which influence the staging of disease, all conducted simultaneously without requiring the patient to be exposed to multiple and different examinations (18). On the other hand CT is, especially in the South East European countries, far more accessible and more widespread method.

In conclusion, both PET/CT and CT are noninvasive methods that can be reliably used for N staging of NSCLC.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATIONS

Competing interests: none to declare.

REFERENCES

1. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008; 83:584-94.
2. Janssen-Heijnen ML, Coebergh JW. Trends in incidence and prognosis of the histological subtypes of lung cancer in North America, Australia. New Zealand and Europe. Lung Cancer 2001; 31:123-37.
3. Detterbeck FC,Boffa DJ, Kim AW, Tanoue LT. The Eighth Edition Lung Cancer Stage Classification. Chest 2017; 151:193–203.
4. Kung BT, Yong TK, Tong CM. The pearl of FDG PET/CT in preoperative assessment of patients with potentially operable non-small cell lung cancer and its clinical impact. World J Nucl Med 2017; 16:21–5.
5. Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, Postmus PE, Rusch V, Sobin L, International Association for the Study of Lung Cancer International Staging Committee, Participating Institutions. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007; 2:706–14.
6. Chao F, Zhang H. PET/CT in the staging of the non-small-cell lung cancer. J Biomed Biotechnol 2012; 2012:783739.
7. Purandare NC, Rangarajan V. Imaging of lung cancer: implications on staging and management. Indian J Radiol Imaging 2015; 25:109–20.
8. Gao SJ, Kim AW, Puchalski JT, Bramley K, Detterbeck FC, Boffa DJ, Decker RH. Indications for invasive mediastinal staging in patients with early non-small cell lung cancer staged with PET-CT. Lung Cancer 2017; 109:36-41.
9. Beigelman-Aubry C, Dunet V, Brun AL. CT imaging in pre-therapeutic assessment of lung cancer. Diagn Interv Imaging 2016; 97:973-89.
10. Dwamena BA, Sonnad SS, Angobaldo JO, Wahl RL. Metastases from non-small cell lung cancer: mediastinal staging in the 1990s – meta-analytic comparison of PET and CT. Radiology 1999; 213:530–6.
11. Ikezoe J, Kadowaki K, Morimoto S, Takashima S, Kozuka T, Nakahara K. Mediastinal lymph node metastases from non-small cell bronchogenic carcinoma: reevaluation with CT. J Comput Assist Tomogr 1990; 14:340–4.
12. Mattes MD, Weber WA, Foster A, Moshchinsky AB, Ahsanuddin S, Zhang Z, Shi W, Rizk NP, Wu AJ, Ashamalla H, Rimner A. A predictive model for lymph node involvement with malignancy on PET/CT in non-small cell lung cancer. J Thorac Oncol 2015; 10:1207-12.
13. Vaz AP, Fernandes G, Souto Moura C, Bastos P, Queiroga H, Hespanhol V. Integrated PET/CT in non-small cell lung cancer staging – clinical and pathological agreement. Rev Port Pneumol 2012; 18:109-14.

14. Asamura H, Chansky K, Crowley J, Goldstraw P, Rusch VW, Vansteenkiste JF, Watanabe H, Wu YL, Zielinski M, Ball D, Rami-Porta R. International association for the study of lung cancer staging and prognostic factors committee, advisory board members and participating institutions. The International association for the study of lung cancer lung cancer staging project: proposals for the revision of the N descriptors in the forthcoming 8th edition of the TNM classification for lung cancer. J Thorac Oncol 2015; 10:1675-84.
15. Moon Y, Lee KY, Kim KS, Park JK. Clinicopathologic correlates of postoperative N1 or N2 nodal upstaging in non-small cell lung cancer. J Thorac Dis 2016; 8:79-85.
16. Verschakelen JA, De Wever W, Bogaert J. Role of computed tomography in lung cancer staging. Curr Opin Pulm Med 2004; 10:248-55.
17. García de Castro AB, Domínguez JF, Bolton RD, Pérez CF, Martínez BC, García-Esquinas MG, Carreras Delgado JL. PET-CT in presurgical lymph node staging in non-small cell lung cancer: the importance of false-negative and false-positive findings. Radiologia 2017; 59:147-58.
18. Schreyögg J, Weller J, Stargardt T, Herrmann K, Bluemel C, Dechow T, Glatting G, Krause BJ, Mottaghy F, Reske SN, Buck AK. Cost-effectivness of hybrid PET/CT for staging of non-small cell lung cancer. J Nucl Med 2010; 51:1668-75.

Pozitronska emisiona tomografija/kompjuterizirana tomografija (PET/CT) i CT u N-*stagingu* nemikrocelularnog karcinoma pluća

Sandra Vegar Zubović, Spomenka Kristić, Besima Hadžihasanović

Klinika za radiologiju, Klinički centar Univerziteta u Sarajevu, Sarajevo, Bosna i Hercegovina

SAŽETAK

Cilj Istražiti mogućnosti neinvazivnih dijagnostičkih slikovnih metoda, pozitronske emisione tomografije/kompjuterizirane tomografije (PET/CT) i CT-a, u kliničkom N-*stagingu* nemikrocelularnog karcinoma pluća.

Metode Retrospektivna klinička studija obuhvatila je 50 pacijenata s dijagnosticiranim nemikrocelularnim karcinomom pluća, kojima je urađen PET/CT u svrhu *staginga* bolesti. Za analizu zahvaćenosti limfnih čvorova bolešću korišten je sistem za mapiranje predložen od strane Međunarodnog udruženja za proučavanje karcinoma pluća. Zabilježeni su podaci koji se odnose na CT N-*staging* i PET/CT N-*staging*. Ove dvije metode su komparirane korištenjem $\chi 2$-testa i Spearmanovog koeficijenta korelacije ranga.

Rezultati Statistička analiza je pokazala, iako su ustanovljene određene razlike u određivanju N-stadija korištenjem CT-a i PET/CT-a, da ove metode pokazuju signifikantnu korelaciju. Nalaz CT-a i PET/CT-a ukazao je na isti N-stadij kod 74% pacijenata. Kod pet pacijenata, na osnovu nalaza PET/CT-a, stadij bolesti je promijenjen iz operabilnog u inoperabilni, dok je kod četiri pacijenta stadij promijenjen iz inoperabilnog u operabilni.

Zaključak PET/CT i CT su neinvazivne metode koje se mogu pouzdano koristiti za N-*staging* nemikrocelularnog karcinoma pluća.

Ključne riječi: karcinom pluća, multimodalni *imaging,* limfni čvorovi

ORIGINAL ARTICLE

Is post-treatment standardized uptake value a prognostic factor in unresectable non-small cell lung carcinoma?

Ufuk Yılmaz[1], Zehra Yasar[2], Esra Korkmaz[3], Burcu Yalcın[1], Hakan Koparal[4], Engin Ozbilek[4]

[1]Department of Chest Diseases, Suat Seren Chest Disease and Surgery Training and Research Hospital, İzmir, [2]Chest Diseases, Medistate Kavacık Hospital, İstanbul [3]Department of Radiation Oncology, Suat Seren Chest Disease and Surgery Training and Research Hospital, İzmir, [4]Department of Nuclear Medicine, Suat Seren Chest Disease and Surgery Training and Research Hospital, İzmir; Turkey.

ABSTRACT

Aim Concurrent chemoradiotherapy (CRT) is the standard of care for locally advanced, unresectable non-small cell lung carcinoma (NSCLC). The aim of this study was to assess the prognostic value of maximum standardized uptake values (SUV_{max}) in patients with unresectable stage III NSCLC treated with concurrent CRT.

Method ^{18}F-FDG PET-CT scans were obtained before and after treatment in patients with unresectable stage III NSCLC treated with concurrent CRT. To determine the prognostic value of SUV_{max} of the primary tumor (PT), univariate and multivariate Cox regression model were carried out.

Results Between January 2008 and December 2013, this study included 43 patients (median age 56 years, 95% male). Univariate analysis showed that having a high post-treatment PT-SUV_{max} was associated with a higher risk of death and having a high post-treatment PT-SUV_{max} with a higher risk of disease recurrence. Multivariate analysis showed that having a low post-treatment PT-SUV_{max} (cut off 3.9) was associated with longer overall and progression free survival (HR 8.55, 95% CI; 2.56-28.55, p=0.000 and HR 2.854, 95% CI; 1.43-5.67, p=0.003, respectively).

Conclusion Post-treatment PT-SUV_{max} may be an independent prognostic factor in patients with unresectable stage III NSCLC treated with concurrent chemoradiotherapy.

Key words: lung cancer, prognosis, positron emission tomography

Corresponding author:
Zehra Yasar
Chest Diseases,
Medistate Kavacık Hospital
Rüzgarlıbahçe Mah. Cumhuriyet Cad.
No 24 Beykoz, 34805 İstanbul
Phone: +90 21 633 140 40;
Fax: +90 21 641 375 15;
E-mail: zehraasuk@hotmail.com
Ufuk Yilmaz ORCID ID: http://www.orcid.org/0000-0003-3676-4355

Original submission:
09 February 2017;
Accepted:
12 May 2017;
doi: 10.17392/898-17

Med Glas (Zenica) 2017; 14(2): 204-210

INTRODUCTION

About one third of patients with non-small cell lung cancer (NSCLC) present locally advanced disease at the time of the diagnosis (1). The standard of care in patients with unresectable, stage III, NSCLC and good performance status is concurrent chemoradiotherapy (CRT) (2). Despite curative treatment with concurrent chemoradiotherapy (CRT), only a low rate of patients become long-term survivors and 15-40% develop recurrence (3,4). Several prognostic factors have been studied, but the two most prominent are performance status and the distinction between stage IIIA and IIIB in stage III NSCLC. Other prognostic factors have been suggested such as age, weight loss, response to treatment and some characteristics describing the locoregional extension of the tumour (4).

Positron emission tomography (PET) is an imaging method based on the metabolic activity of viable tumor cells. The PET-CT (computer tomography) is superior over conventional staging methods in the initial staging of NSCLC (5). A decrease in fludeoxyglucose (FDG) uptake in the primary tumor and/or lymph nodes is expected in patients who responded well to treatment (6, 7). Standardized uptake value (SUV) is a semi-quantitative index of radiolabeled glucose uptake in tumor tissue and has been demonstrated to be correlated with some prognostic factors, including tumor differentiation and aggressiveness (8-10). Prior studies reported that pretreatment maximum SUV (SUV_{max}) of primary tumor predicted treatment response, poor prognosis and especially recurrence and survival (11-19). Nearly most of them included patients with different stages of NSCLC and treated by different therapy modalities. Data evaluating the predictive value SUV_{max} in prognosis, treatment response, clinical outcome and survival in unresectable stage III NSCLC treated with CRT are still limited (4, 19-23).

The aim of this prospective cohort study was to investigate the prognostic value of PET-CT obtained after concurrent CRT in patients with stage III, unresectable NSCLC.

PATIENTS AND METHODS

Patients and study design

Patients treated with CRT for unresectable stage III NSCLC (according to the 6th edition of the TNM staging system) (24) in Suat Seren Chest Disease and Surgery Training and Research Hospital between January 2008 and December 2013 enrolled in the study.

Patients who met the following criteria were included in the study: the FDG uptake by PET-CT before and after concurrent CRT, unresectable stage III disease defined by multidisiplinary team, and those with histological diagnosis.

Methods

All patients received cisplatin 50 mg/m^2 intravenously (IV) on days 1, 8, 29, and 36 with etoposid 50 mg/m^2 IV on days 1-5 and 29-33. Radiotherapy (RT) was delivered using conventional fractionation (1.8-2.0 Gy per day, 5 days per week) with a total dose of 60-66 Gy using 6-10 MV photon beams. All patients received 3D conformal radiotherapy. The gross tumor volume (GTV) consisted of the primary tumor and the regional lymph nodes considered positive (SUV_{max} >2.5) on PET scan even if not involved by CT scan. Any intrathoracic lymph nodes with a diameter greater than 10 mm in the short axis were included in GTV regardless of the PET scan. Adjuvant chemotherapy was not allowed.

Initial (pre-treatment) ^{18}F-FDG PET/CT scans were obtained within 30 days before the treatment. Post-treatment ^{18}F-FDG PET/CT scans were performed at 90 days after CRT. Post-treatment PET-CT were not performed in patients with clear evidence of progression with systemic disease or who died before undergoing post-treatment PET/CT scan or who had reimbursment problem for post-treatment PET-CT scans.

All patients involved in the study underwent whole-body ^{18}F-FDG PET-CT scaning (Biograph 16 HR, by Siemens Medical Solutions, Illinois, United States). The PET component was a high resolution scanner with a spatial resolution of 4.7mm and had no interval, thus allowing 3-dimensional-only acquisitions. The CT portion of the scanner was the Somatom Sensation 16-slices. All patients were advised to fast for at least six hours before PET-CT scan. All patient's were required a blood sugar level of less than 180 mg/dL before ^{18}F-FDG (0.10 mCi/kg) intavenous injection. After injection, the patients rested for a period of about 60±10 minutes in a comfortable room and chair. The patients were positioned

supine, with both arms positioned over the head. Next tomography images were acquired in order to attenuate correction and anatomic localization, PET images were acquired in axial planes from the proximal femur until the vertex, 5-7 bed positions in a 2-minutes per bed postion. Processed images were displayed in coronal, transverse, and sagittal planes.

The PET-CT scans were interpreted semiquantitatively by two nuclear medicine physicians with experience in lung cancer and reported the SUV_{max} values in the primary tumor and in each regional lymph nodes. The final PET interpretation was based on a consensus of the two observers. The SUV for the region of interest (ROI) was decided using SUV_{max} which indicates the highest single voxel SUV within ROI. The lesions with SUV_{max}>2.5 were considered as pathological.

Patients also underwent response evaluation with CT of chest through the upper abdomen in 4 weeks of completing treatment and follow-up continued every 3 months for the first 2 years, every 6 months for third year, and yearly thereafter, with repeated CT of chest through the adrenals on each visit.

Statistical analysis

Overall and progresion free survival (OS and PFS, respectively) were estimated by the Kaplan–Meier method. Univariate and multivariate Cox regression (Backward Stepwise) model were carried out to determine prognostic factors for OS and PFS. Multivariate analysis was performed to the variables that were 2> WALD values with the univariate analysis. Results of this model were presented as Hazard Ratio (HR) and 95% confidence intervals (95% CIs) for OR and PFS. The $p \leq 0.05$ was considered statistically significant. To analyze the impact of SUV on the study end points, the median values were used to divide patients into groups of equal numbers. The variables associated with PET scaning were defined as follows; ΔPT SUV_{max}; Pre-treatment Primary Tumor SUV_{max} - Post-treatment Primary Tumor SUV_{max}, ΔPT % SUV_{max}; Pre-treatment Primary Tumor SUV_{max} - Post-treatment Primary Tumor SUV_{max} / Pre-treatment Primary Tumor SUV_{max} X 100, Post-PT SUV_{max}; Post-treatment Primary Tumor $SUV_{max.}$

RESULTS

Between January 2008 and December 2013, 67 patients were treated with CRT for unresectable stage III NSCLC, of whom 43 met all inclusion criteria for this analysis (Figure 1).

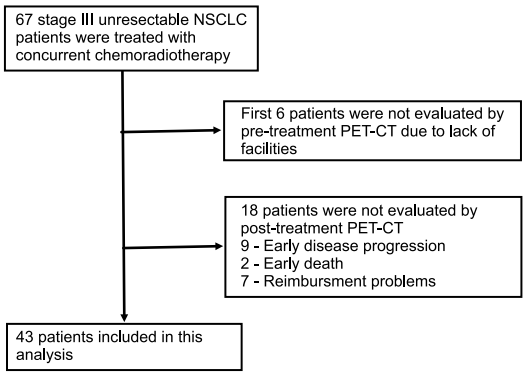

Figure 1. Patient selection

The median age of patients was 56 years (40-71) with 41 (95.3%) males. Squamous cell carcinoma (79.1%) was the most common histologic type. All patients were designated as stage III (including 28 patients who were in stage IIIb, (65.1 %), and all patients had good performance status. The median follow-up time was 20.4 months (8.3–84.0). The median OS and 4-year OS were 25.1 (95%, CI: 20.0-30.1) months and 21.7%, respectively. The median PFS and 3-year PFS were 12.5 (95%, CI: 9.0-15.9) months and 17.5%, respectively (Table 1).

Table 1. Patient characteristics

	No (%) of patients
Age median (range) (years)	56 (40-71)
Gender	
Males	41 (95.3)
Females	2 (4.7)
TNM stage	
T3N1	1 (2.3)
T3N2	2 (4.7)
T3N3	1 (2.3)
T4N0	8 (18.6)
T4N2	21 (48.8)
T4N3	4 (9.3)
T2N3	1 (2.3)
T2N2	3 (7.0)
T1N2	1 (2.3)
T1N3	1 (2.3)
Disease stage	
IIIa	15 (34.9)
IIIb	28 (65.1)
Histology	
Squamous Cell	34 (79.1)
Non-squamous Cell	9 (20.9)
Performance status	
ECOG 0	32 (74.4)
ECOG 1	11 (25.6)

ECOG, Eastern Cooperative Oncology Group

Among the 6 variables of univariate analysis, one was significantly associated with overall survival: post-PT SUV_{max} (hazard ratio [HR], 3.227; p=0.002) (Table 2). Multivariate analysis for OS included the 4 variables (Wald>2) of univariate analysis (Table 2). The results displayed that post-PT SUV_{max} was an independent prognostic factor (HR, 8.558, 95% CI; 2,565-28,552, p=0.000) for overall survival. ΔPT % SUV_{max} showed a trend for overall survival (p=0.051).

Table 2. Univariate analyses of pre and post-treatment variables for overall survival after concurrent chemoradiotherapy

Variable	Wald test	p	HR	95.0% CI for HR	
				Lower	Upper
Stage IIIa vs Stage IIIb	2.070*	0.150	0.585	0.282	1.214
Squamous vs Non-squamous	0.672	0.412	0.669	0.256	1.749
ECOG 0 vs 1	3.537*	0.060	2.098	0.969	4.541
Δ PT %SUVmax ≤ vs >72.6	2.742*	0.098	1.859	0.892	3.873
ΔPT SUVmax ≤ vs >12.1	1.282	0.258	1.520	0.736	3.138
Post-PT SUVmax ≤3 vs >3.9	9.245*	0.002†	3.227	1.517	6.889

*Wald test >2, †p<0.05; HR, hazard ratio; Sig, significance; ECOG, Eastern Cooperative Oncology Group; PT; primary tumor; $SUV_{max,}$ maximum standard uptake value

One variable was significantly associated with PFS in univariate analysis; post-PT SUV_{max} (HR; 2.721; p=0.004) (Table 3). Multivariate analysis for PFS included the 4 variables (Wald>2) of univariate analysis (Table 3). The results displayed that post-PT SUV_{max} was an independent prognostic factor (HR, 2.854, 95% CI; 1,437-5,670, p=0.003) for PFS.

Table 3. Univariate analyses of pre and post-treatment variables for progression free survival after concurrent chemoradiotherapy

Variable	Wald tes t	p	HR	95.0% CI for HR	
				Lower	Upper
Stage IIIa vs Stage IIIb	2.278*	0.131	0.591	0.299	1.170
Squamous vs Non-squamous	0.729	0.393	0.681	0.282	1.645
ECOG 0 vs 1	2.437*	0.118	1.787	0.862	3.705
Δ PT %SUVmax ≤ vs >72.6	2.912*	0.088	1.813	0.915	3.592
ΔPT SUVmax ≤ vs >12.1	0.061	0.804	1.088	0.557	2.127
Post-PT SUVmax ≤ vs >3.9	8.237*	0.004†	2.721	1.374	5.392

*Wald>2, †p<0.05; PT, primary tumor; HR, hazard ratio; Sig, significance; ECOG, Eastern Cooperative Oncology Group; PT; primary tumor; $SUV_{max,}$ maximum standard uptake value

DISCUSSION

This study showed that post-treatment primary tumor SUV_{max} can be an independent prognostic factor for PFS and OS. Cut off level of post-treatment primary tumor SUV_{max} was determined as 3.9. We found no further significant prognostic factors associated with survival in the current study.

An assessment of tumor response to therapy using PET-CT has been proposed and supported in various malignancies (25-28). Higher FDG uptake has been suggested by clinicians to be a useful prognostic indicator as a noninvasive method in a routine clinical setting (29-32). Eschmann et al. reported FDG uptake as an independent prognostic factor in patients with stage III NSCLC (33). Also, it has been used to predict response to chemotherapy and clinical outcome in stage III NSCLC treated with conventional radiotherapy (34, 35). As contrary, Ikushima et al. reported that FDG uptake has no prognostic significance for predicting survival and Vesselle et al pointed that the predictive value of FDG uptake disappears after considering tumor size (36, 37). Also Machtay et al. exuded that neither pretreatment SUV_{max} nor SUV_{peak} could predict long term prognosis (22). Similar to these studies we found no further prognostic significance of pre-treatment SUV_{max} after multivariate analysis.

In previous studies, FDG PET/CT after definitive chemoradiation therapy was shown to predict survival in patients with NSCLC (20, 37). Mc Manus et al. demonstrated that in patients with NSCLC who were treated with concurrent chemoradiotherapy, post-treatment PET scan was a better predictor than CT (20).

Xiang et al. reported that post treatment SUV predicted local recurrence free survival, PFS and OS (23). Lopez Guerra et al. showed that the post RT SUV_{max} in both the primary tumor and the lymph node was a predictor of survival, -specifically the higher residuel SUV_{max} after RT, the poorer for OS and PFS (21). Similar to these studies, Machtay reported that post treatment tumor SUV_{max} is associated with worse survival in stage III NSCLC (22). Consistent with these studies, we found post-treatment primary tumor SUV_{max} was an independent prognostic factors for PFS and OS. Predicting survival and identifying patients who have high risk for progression seems to be important for deciding further management strategies such as new targeting therapies, consolidation or maintenance treatments. Similar to Xiang et al. (23) there was no correlation between ΔSUV and survival in our study, although previous studies have shown such an association among patients with stage III/IV NSCLC treated with chemotherapy. And, patients demonstrating

an absence of metabolic response on post-treatment PET had a shorter time to disease progression and decreased overall survival (38, 40).

The cut-off values used for survival have varied across all previous studies. The best cut-off value that could be used universally remains unknown. Lopez Guerra et al. reported the median post-treatment PT SUV_{max} was 3.7 and patients with SUV_{max} less than the median had a 2 year survival rate 50% when compared with 20% for patients greater than the median (21). Machtay et al. studied various cut off ranges but they could not identify a clinically sufficent cut off value (22). Ryu et al. reported FDG uptake by residual tumor masses 2 weeks after induction chemoradiotherapy predicted pathologic response with 88% sensitivity when an SUV cutoff of 3.0 was used (40). In Xiang et al.'s findings, cut off value 3.6 predicts local relaps free survival (23). In consistence with this study, cutt of value of 3.9 was found in our study population. Variability of these values may be because of the different prevalence of NSCLC subtype and whether or not carcinoids have been studied as well.

The optimal timing of post-treatment PET scan has also been questioned by previos studies, especially in light of potential alteration of the SUV_{max} reading due to the inflammatory response associated with chemoradiotherapy in various malignancies (40, 41). Lopez Guerra et al. suggested that scans done sooner than 2.5 mo after RT may not reflect much of the effect of the RT (21). In our study, all post-treatment PET scans were invariably performed at 90 days after the treatment.

These results are limited by the modest sample size. Also, post-treatment biopsies were not performed. So, we could not exactly confirm whether high post-treatment SUV_{max} represented tumor versus radiation induced inflamation.

İn conclusion, post-treatment primary tumor SUV_{max} may be an independent prognostic factor for unresectable stage III NSCLC. High values than cut off point 3.9 predicts a worse prognosis in this patient group. This study can provide a basis for further trials for evaluating PET scanning as a prognostic indicator in this group of patients.

ACKNOWLEDGMENTS

We thank Funda Cansun and Atilla Atasever for their language and statistic analysis contributions on this report.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATIONS

Competing interest: none to declare.

REFERENCES

1. Rami-Porta R, Crowley JJ, P Goldstraw P. The revised TNM staging system for lung cancer. Ann Thorac Cardiovasc Surg 2009; 15:4-9.
2. Vokes EE. Optimal therapy for unresectable stage III non-smallcell lung cancer. J Clin Oncol 2005; 23:5853-5.
3. Furuse K, Fukuoka M, Kawahara M, Nishikawa H, Takada Y, Kudoh S, Katagami N, Ariyoshi Y. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small cell lung cancer. J Clin Oncol 1999; 17:2692-9.
4. Berghmans T, Paesmans M, Sculier JP. Prognostic factors in stage III non-small cell lung cancer: a review of conventional, metabolic and new biological variables. Ther Adv Med Oncol 2011; 3:127-38.
5. Kligerman S, Digumarthy S. Staging of non-small cell lung cancer using integrated PET/CT. Am J Roentgenol. 2009;193:1203-11.
6. Pieterman RM, van Putten JW, Meuzelaar JJ, Mooyaart EL, Vaalburg W, Koeter GH. Preoperative staging of nonsmall- cell lung cancer with positron-emission tomography. N Engl J Med 2000; 43:254-61.
7. Toloza EM, Harpole L, McCrory DC. Noninvasive staging of non-small cell lung cancer: a review of the current evidence. Chest 2003; 23:137-46S.
8. Chen JC, Huang TW, Cheng YL, Chang H, Tzao C, Huang WS, Lee SC. Prognostic value of 18-FDG uptake in early stage NSCLC. Thorac Cardiovasc Surg 2009; 7:413-6.
9. Vesselle H, Grierson J, Muzi M, Pugsley JM, Schmidt RA, Rabinowitz P, Peterson L.M., Vallieres E, Wood DE. In vivo validation of 3'deoxy-3'-[(18)F]fluorothymidine ([(18)F]FLT) as a proliferation imaging tracer in humans: correlation of [(18)F]FLT uptake by positron emission tomography with Ki-67 immunohistochemistry and flow cytometry in human lung tumors. Clin Cancer Res 2002; 8:3315-23.
10. Usuda K, Saito Y, Sagawa M, Sato M, Kanma K, Takahash S, Endo C, Chen Y, Sakurada A, Fujimura S. Tumor doubling time and prognostic assessment of patients with primary lung cancer. Cancer 1994; 74:2239-44.
11. Higashi K, Ueda Y, Arisaka Y, Sakuma T, Nambu Y, Oguchi M, Seki H, Taki S, Tonami H, Yamamoto I.18F-FDG uptake as a biologic prognostic factor for recurrence in patients with surgically resected non-small cell lung cancer. J Nucl Med 2002; 43:39-45.

12. Hoang JK, Hoagland LF, Coleman RE, Coan AD, Herndon JE 2nd, Patz EF Jr. Prognostic value of fluorine-18 fluorodeoxyglucose positron emission tomography imaging in patients with advanced-stage non-small-cell lung carcinoma. J Clin Oncol 2008; 26:1459-64.
13. Lee HY, Lee KS, Park J, Han J, Kim BT, Kwon OJ, Ahn YC, Ahn MJ, Park K, Kim J, Shim YM. Baseline SUVmax at PET-CT in stage IIIA non-small-cell lung cancer patients undergoing surgery after neoadjuvant therapy: prognostic implication focused on histopathologic subtypes. Acad Radiol 2012; 19:440-5.
14. Jeong HJ, Min JJ, Park JM, Chung JK, Kim BT, Jeong JM, Lee DS, Lee MC, Han SK, Shim YS. Determination of the prognostic value of [18F] fluorodeoxyglucose uptake by using positron emission tomography in patients with non-small cell lung cancer. Nucl Med Commun 2002; 23:865-70.
15. Paesmans M, Berghmans T, Dusart M, Garcia C, Hossein-Foucher C, Lafitte JJ, Mascaux C, Meert AP, Roelandts M, Scherpereel A, Terrones Munoz V, Sculier JP. Primary tumor standardized uptake value measured on fluorodeoxyglucose positron emission tomography is of prognostic value for survival in non-small cell lung cancer. Update of a systematic review and meta analysis by the European Lung Cancer Working Party for the International Association for the Study of Lung Cancer Staging Project. J Thorac Oncol 2010; 5:612-9.
16. Kaira K, Endo M, Asakura K, Tsuya A, Nakamura Y, Naito T, Murakami H, Takahashi T, Yamamoto N. Ratio of standardized uptake value on PET helps predict response and outcome after chemotherapy in advanced non-small cell lung cancer. Ann Nucl Med 2010; 24:697-705.
17. Usmanij EA, de Geus-Oei LF, Troost EG, Peters-Bax L, van der Heijden EH, Kaanders JH, Oyen WJ, Schuurbiers OC, Bussink J. 18F-FDG PET early response evaluation of locally advanced non-small cell lung cancer treated with concomitant chemoradiotherapy. J Nucl Med 2013; 54:1528-34.
18. Kim S, Oh SW, Kim JS, Kim KH, Kim YK. Prediction of response by FDG PET early during concurrent chemoradiotherapy for locally advanced non-small cell lung cancer. Radiat Oncol J 2014; 32:231-7.
19. Paesmans M, Garcia C, Oliver Wong CY, Patz EF Jr, Komaki R, Eschmann S, Govindan R, Vansteenkiste J, Meert AP, de Jong WK, Altorki NK, Higashi K, Van Baardwijk A, Borst GR, Ameye L, Lafitte JJ, Berghmans T, Flamen P, Rami-Porta R, Sculier JP. Primary tumour standardised uptake value is prognostic in nonsmall cell lung cancer: a multivariatepooled analysis of individual data. Eur Respir J 2015; 46:1751-61.
20. MacManus MP, Hicks RJ, Matthews JPP, McKenzie A, Rischin D, Salminen EK, Ball DL. Positron emission tomography is superior to computed tomography scanning for response-assessment after radical radiotherapy or chemoradiotherapy in patients with non-small-cell lung cancer. J Clin Oncol 2003; 21:1285-92.
21. Lopez Guerra JL, Gladish G, Komaki R, Gomez D, Zhuang Y, Liao Z. Large decreases in standardized uptake values after definitive radiation are associated with better survival of patients with locally advanced non-small cell lung cancer. J Nucl Med 2012; 53:225-33.
22. Machtay M, Duan F, Siegel BA, Snyder BS, Gorelick JJ, Reddin JS, Munden R, Johnson DW, Wilf LH, DeNittis A, Sherwin N, Cho KH, Kim SK, Videtic G, Neumann DR, Komaki R, Macapinlac H, Bradley JD, Alavi A. Prediction of survival by [18F] fluorodeoxyglucose positron emission tomography in patients with locally advanced non-small-cell lung cancer undergoing definitive chemoradiation therapy: results of the ACRIN 6668/RTOG 0235 trial. J Clin Oncol 2013; 31:3823-30.
23. Xiang ZL, Erasmus J, Komaki R, Cox JD, Chang JY. FDG uptake correlates with recurrence and survival after treatment of unresectable stage III non-small cell lung cancer with high-dose proton therapy and chemotherapy. Radiat Oncol 2012; 7:144-52.
24. Greene FL, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG, Morrow M, eds. American Joint Committee on Cancer: AJCC Cancer Staging Manual. 6th Ed. New York, USA: Springer, 2002.
25. Decoster L, Schallier D, Everaert H, Nieboer K, Meysman M, Neyns B, De Mey J, De Grève J. Complete metabolic tumour response, assessed by 18-fluorodeoxyglucose positron emission tomography ((18) FDG-PET), after induction chemotherapy predicts a favourable outcome in patients with locally advanced non-small cell lung cancer (NSCLC). Lung Cancer 2008; 62:55-61.
26. Dehdashti F, Mortimer JE, Trinkaus K, Naughton MJ, Ellis M, Katzenellenbogen JA, et al. PET-based estradiol challenge as a predictive biomarker of response to endocrine therapy in women with estrogen-receptor-positive breast cancer. Breast Cancer Res Treat 2008; 113:509-17.
27. Connell CA, Corry J, Milner AD, Hogg A, Hicks RJ, Rischin D, Peters LJ. Clinical impact of, and prognostic stratification by, F-18FDG PET/CT in head and neck mucosal squamous cell carcinoma. Head Neck 2007; 29:986-95.
28. Schwarz JK, Siegel BA, Dehdashti F, Myerson RJ, Fleshman JM, Grigsby PW. Tumor response and survival predicted by post-therapy FDG PET/ CT in anal cancer. Int J Rad Oncol Biol Phys 2008; 71:180-6.
29. Atkins CD. Overestimation of the prognostic significance of SUV measurement by positron emission tomography for non-small-cell lung cancer. J Clin Oncol 2005; 23:6799-800.
30. Brundage MD, Davies D, Mackillop WJ. Prognostic factors in non-small cell lung cancer-a decade of progress. Chest 2002; 122:1037-57.
31. Dhital K, Saunders CA, Seed PT, O'Doherty MJ, Dussek J. [F-18]fluorodeoxyglucose positron emission tomography and its prognostic value in lung cancer. Eur J Cardio Thoracic Surg 2000; 18:425-8.
32. Eschmann SM, Friedel G, Paulsen F, Reimold M, Hehr T, Budach W, Scheiderbauer J, Machulla HJ, Dittmann H, Vonthein R, Bares R. Is standardised F-18- FDG uptake value an outcome predictor in patients with stage III non-small cell lung cancer? Eur J Nucl Med Mol Imaging 2006; 33:263-9.
33. Weber WA, Petersen V, Schmidt B, Tyndale-Hines L, Link T, Peschel C, Schwaiger M. Positron emission tomography in non-small-cell lung cancer: prediction of response to chemotherapy by quantitative assessment of glucose use. J Clin Oncol 2003; 21:2651-7.

34. Sasaki R, Komaki R, Macapinlac H, Erasmus J, Allen P, Forster K, Putnam JB, Herbst RS, Moran CA, Podoloff DA, Roth JA, Cox JD. [18 F] fluorodeoxyglucose uptake by positron emission tomography predicts outcome of non-small-cell lung cancer. J Clin Oncol 2005; 23:1136-43.
35. Ikushima H, Dong L, Erasmus J, Allen P, McAleer MF, Zhuang Y, Sasaki R, Komaki R. Predictive value of 18F-fluorodeoxyglucose uptake by positron emission tomography for non-small cell lung cancer patients treated with radical radiotherapy. J Radiat Res 2010; 51:465-471.
36. Vesselle H, Freeman JD, Wiens L, Stern J, Nguyen HQ, Hawes SE, Bastian P, Salskov A, Vallières E, Wood DE. Fluorodeoxyglucose uptake of primary non-small cell lung cancer at positron emission tomography: new contrary data on prognostic role. Clin Cancer Res 2007; 13:3255-63.
37. Ichiya Y, Kuwabara Y, Sasaki M, Yoshida T, Omagari J, Akashi Y, Kawashima A, Fukumura T, Masuda K. A clinical evaluation of FDG-PET to assess the response in radiation therapy for bronchogenic carcinoma. Ann Nucl Med 1996; 10:193-200.
38. Kim YS, Lee MK, Kim SJ, Kim IJ, Kim YK, Jo WS, Park SK. Prognostic stratification using F-18 FDG PET/CT in patients with advanced stage (stage III and IV) non-small cell lung cancer. Neoplasma 2010; 57:241-6.
39. Dooms C, Verbeken E, Stroobants S, Nackaerts K, De Leyn P, Vansteenkiste J. Prognostic stratification of stage IIIAN2 non-small-cell lung cancer after induction chemotherapy: a model based on the combination of morphometric-pathologic response in mediastinal nodes and primary tumor response on serial 18-fluoro-2-deoxy-glucose positron emission tomography. J Clin Oncol 2008; 26:1128-34.
40. Ryu JS, Choi NC, Fischman AJ, Lynch TJ, Mathisen DJ. FDG-PET in staging and restaging non-small cell lung cancer after neoadjuvant chemoradiotherapy: correlation with histopathology. Lung Cancer 2002; 35:179-187.
41. Song SY, Kim JH, Ryu JS, Lee GH, Kim SB, Park SI, Song HY, Cho KJ, Ahn SD, Lee SW, Shin SS, Choi EK. FDG-PET in the prediction of pathologic response after neoadjuvant chemoradiotherapy in locally advanced, resectable esophageal cancer. Int J Rad Oncol Biol Phys 2005; 63:1053-9.

ORIGINAL ARTICLE

Nitric oxide biosynthesis during normal pregnancy and pregnancy complicated by preeclampsia

Jasmin Hodžić[1], Sebija Izetbegović[2], Bedrana Muračević[1], Rasim Iriškić[1], Hana Štimjanin Jović[1]

[1]Department of Women's Health, Neonatology and Perinatology, Cantonal Hospital Zenica, [2]Univerity Clinical Center Sarajevo; Bosnia and Herzegovina

ABSTRACT

Aim To investigate biosynthesis in nitric oxide (NO) during normal pregnancy and in pregnancies complicated by preeclampsia.

Methods This prospective cross-sectional study included 80 patients hospitalized at the Department of Women's Health, Neonatology and Perinatology Cantonal Hospital in Zenica. Serum NO concentration in 20 non-pregnant women, 40 healthy pregnant women and 20 pregnant women with preeclampsia aged 17-40 years were measured. The group of healthy pregnant women were divided into 4 subgroups by gestational age. For each woman with preeclampsia, a healthy pregnant control was matched for age, parity and gestational age. Serum NO concentrations were determined after reduction of nitrates to nitrites using the Griess reaction.

Results NO concentrations during second trimester of pregnancy (37.2±1.7μM; $p<0.05$) and third trimester of pregnancy (40.9±2.8μM; $p<0.05$) were significantly higher in healthy pregnant women than in non-pregnant women (29.3±1.7μM). Serum NO concentrations were lower in preeclamptic women (30.7±1.8μM) compared to matched healthy pregnant women of the third and the late third trimester (35.1±2.2μM), without significant differences. Mean NO concentrations in pre-eclamptic women was positively correlated with systolic blood pressure ($r=0.58$; $p<0.01$), diastolic blood pressure ($r=0.45$; $p<0.05$), creatinine clearance ($r=0.48$; $p<0.05$), uric acid ($r=0.49$; $p<0.05$), and negatively correlated with platelet count ($r=-0.57$; $p<0.05$).

Conclusion NO production was increased with gestational age during normal pregnancy and slightly decreased in preeclampsia suggesting that NO may modulate the cardiovascular changes during normal pregnancy and pregnancy complicated by preeclampsia.

Key words: pathogenesis, vasoconstriction, hypertension, pregnancy complications

Corresponding author:
Jasmin Hodžić
Department of Women's Health, Neonatology and Perinatology,
Cantonal Hospital Zenica
Crkvice 67, 72000 Zenica,
Bosnia and Herzegovina
Phone: +387 32 447 316;
Fax: +387 32 226 576;
E-mail: drjassmin@hotmail.com
ORCID ID: http://www.orcid.org/0000-0002-3740-2113

Original submission:
23 May 2017;
Revised submission:
21 June 2017;
Accepted:
04 July 2017.
doi: 10.17392/915-17

Med Glas (Zenica) 2017; 14(2): 211-217

INTRODUCTION

Normal pregnancy is associated with intensive changes in the maternal cardiovascular system that enables adequate oxygen delivery and nutritive ingredients to the fetus. Physiological vascular adaptation (increased blood volume, increased cardiac minute volume and reduced vascular resistance) is followed by increased endogenous production of nitric oxide (NO) and improved response of smooth muscles on the reaction of NO (1,2). Impaired response of the blood vessels on vasoconstrictor agonists during the pregnancy could be partly regulated by NO (3). Recently, in several *in vitro* studies the role of NO in vascular reactivity in pregnancy and preeclampsia has been estimated (4). Choi et al. and Shaamash et al. (5,6) studies showed that the biosynthesis of NO has increased in normal pregnancy, especially in the second trimester, with its peak in the third trimester of pregnancy. However, Hata et al. (7) obtained the results about reduced production of NO, while Brown et al. and Smarason et al. (8,9) showed that there were no changes in the biosynthesis of NO in normal pregnancy compared with non-pregnant women suggesting that the biosynthesis of NO during normal pregnancy still remains controversial. Preeclampsia is considered to be one of the most significant health problems in pregnancy, complicating 6-10% of all gestation over 20 weeks (10). However, World Health Organisation estimates that frequency of preeclampsia is seven times higher in developing countries than in developed countries (11). This disease is one of the leading causes of fetal growth disorders, fetal morbidity and mortality, premature labour and mother's death. Despite numerous findings and surveys, pathophysiology, treatment and early prediction of preeclampsia, it still represents a major challenge in modern medicine (12). Reliable evidence suggests that general vascular endothelial dysfunction, which occurs during preeclampsia can explain mechanism responsible for its pathogenesis. Endothelial cell dysfunction can cause hypertension with its increased production of vasoconstrictor agents such as plasma endothelin or reduced release of vasodilator agents such as prostacyclin and NO (13). NO biosynthesis data are also controversial in preeclampsia. Therefore, some authors obtained different results and they showed that the biosynthesis of NO in preeclampsia was decreased (14,15), unchanged (16,17) and increased (18,9).

The aim of this research was to examine the biosynthesis of NO during normal pregnancy and pregnancy complicated by preeclampsia, as well as factors that influence its production (foods high in nitrates, consuming cigarettes, anaemia, low serum iron and renal function).

PATIENTS AND METHODS

Patient and study design

Eighty females aged 17 to 40 who were hospitalized at the Department of Women's Health, Neonatology and Perinatology, Cantonal Hospital Zenica during the period January 2011 to January 2013, were included in this study. The study was approved by the Ethics Committee of the School of Medicine, University of Sarajevo, and all subjects gave informed, written consents. Investigations were carried out in accordance with the Declaration of Helsinki as revised in 2000.

In order to clarify the biosynthesis of NO in normal pregnancy the two groups of were formed: a control group of non-pregnant women (n=20) and the group of healthy pregnant women (n=40). The group of healthy pregnant women was divided into 4 subgroups according to gestational age: the first trimester (1.0-12.0 weeks, n=10), the second trimester (12.1-24.0 weeks, n=10), the third trimester (24.1-32.0 weeks, n=10) and late third trimester (32.1-40.0 weeks, n=10). In order to clarify the biosynthesis of NO in pregnancy complicated by preeclampsia the two groups were formed: a group of pre-eclamptic women (n=20) and the control group of healthy pregnant women in the third and the late third trimester (n=20). The control group of healthy pregnant women were matched to the pre-eclamptic women by age (±4 years old), parity (0,1,2,3≥4) and gestational age (±13 days). Patients were allowed to take only these medications: antihypertensive (methyldopa), corticosteroids (dexamethasone) and benzodiazepine (diazepam) for which it has been proven not to affect serum NO concentration (9). The patients suffering from chronic diseases such as renal diseases and diabetes mellitus were not included in the research.

Methods

Body weight, blood pressure and the haematological and biochemical parameters (complete blood count, creatinine clearance, uric acid

level) were measured in all patients using the standard diagnostic methods. Gestational age was calculated from the first day of the mother's last menstrual period. Diagnosis of preeclampsia was made by strict criteria: appearance of blood pressure >140/90 mmHg on at least two consecutive measurements and proteinuria over 300 mg/24 hours which appeared for the first time after 24 weeks of gestation, and disappeared the six months after labour (19).

Serum was prepared from peripheral vein blood, collected between 7 and 10 AM, 24 hours after prescription of diets low in nitrite and nitrate (no spinach, beetroot, cured meats, fish and cheese) followed by overnight fasts. Blood samples for the determination of NO concentrations were diluted 1:1 (vol/vol) with 0.9% saline, protein-precipitated (30% $ZnSO_4$, 0.05 mL per mL of blood), centrifuged at 2000 g for 10 minutes and frozen at -20°C until the determination of NO concentrations.

The measurement of NO concentrations in serum was carried out at the Institute of Physiology and Biochemistry, School of Medicine, University of Sarajevo. Conversion of NO_3- into NO_2- was done with elementary zinc. NO_2- concentrations in serum was determined by classic colorimetric Griess reaction (20). Briefly, equal volumes of samples and Griess reagent (N-(1-Naphthyl) ethylenediamine) were mixed at room temperature. After 5 min, the absorbance was measured at 570 nm using Perkin Elmer 550 S spectrophotometer. The concentrations of nitrite were determined by a standard curve prepared with sodium nitrite ($NaNO_2$) (1-200 μM).

Statistical analysis

Results were expressed as a mean ± SEM. In order to compare the results between comparative groups, one-way analysis of variants (ANOVA) was carried out, followed by post hoc multiple comparisons with Bonferroni adjustment. To assess correlation between serum NO concentrations in pre-eclampsia and various laboratory and clinical parameters the Pearson's coefficient of correlation was calculated as well as Spearman's rank correlation since some of these parameters may not have a normal distribution. The Student's t-test was used to compare NO concentrations of women with pre-eclampsia and pregnant controls. The value of $p<0.05$ were taken as significant.

RESULTS

Systolic and diastolic blood pressure of the preeclamptic women showed more values compared with healthy pregnant women in the third and the late third trimester ($p<0.05$). There was no statistically significant difference in age, body weight and parity between the four studied groups (Table 1).

Table 1. Clinical characteristics of the women included in the study

	Clinical characteristics (X±SEM)			
	Non-pregnant women	Healthy pregnant women	Healthy pregnant women in third and late third trimester	Preeclamptic women
Age (years)	36.2±1.2	25.7±0.9	25±1.2	26.6±1.6
Week of gestation	/	23.9±1.9	34±1.3	33.9±1.4
Body weight (kg)	68.3±2.3	70.1±1.6	76.7±1.6	85.6±2.3
Systolic blood pressure (mmHg)	119±1.7	114±1.1	111±1	171±1.8*
Diastolic blood pressure (mmHg)	79.5±1.2	78.6±0.9	77±1.1	107±1*
Parity	1±0.2	1.3±0.1	1.4±0.2	1.6±0.2

* $p<0.05$

There were no significant differences in serum NO concentration between healthy pregnant women in the first trimester (27.3±1.3μM) and control group of non-pregnant women (29.3±1.7μM). The values of serum NO concentrations in the second trimester (37.2±1.7μM; $p<0.05$) and the third trimester (40.9±2.8μM; $p<0.05$) were significantly higher compared to control group of non-pregnant women. In the late third trimester the values of serum NO concentrations in healthy pregnant women (29.2±2.1μM) were not significantly different comparing to control group of

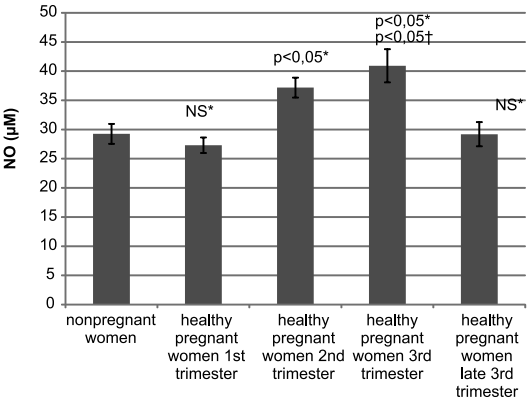

Figure 1. Serum NO concentrations of nonpregnant (controls) and healthy pregnant women

*healthy pregnant women 1st, 2nd, 3rd and late 3rd trimester vs non-pregnant women; †healthy pregnant women 3rd trimester vs healthy pregnant women 1st trimester

NS, non-significant

nonpregnant women (Figure 1). There was not statistically significant difference in serum NO concentrations between the preeclamptic women (30.7±1.8μM) and nonpregnant women (29.3±1.7μM). Serum NO concentrations in the preeclamptic women were lower than in healthy pregnant women of the third and the late third trimester (35.1±2.2μM). However, this difference was not statistically significant (Figure 2).

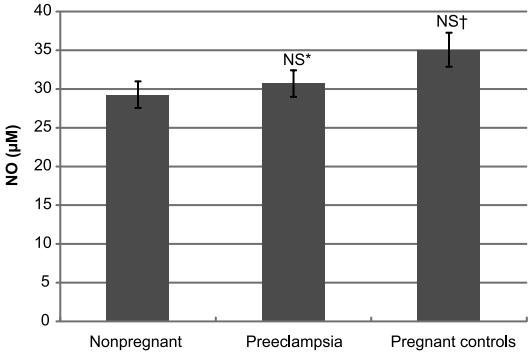

Figure 2. Serum NO concentration of non-pregnant, preeclamptic and healthy pregnant women in third and late third trimester

*preeclamptic vs non-pregnant women; †healthy pregnant women in third and late third trimester vs preeclamptic women
NS, non-significant

There was a positive correlation between serum NO concentrations and systolic blood pressure (r=0.58; p<0.01), diastolic blood pressure (r=0.45; p<0.05), creatinine clearance (r=0.48; p<0.05), uric acid (r=0.49; p<0.05), and a negative correlation of platelet count (r=0.57; p<0.05) of the preeclamptic women (Figure 3). Gestational age, as well as the level of serum iron, were not related to serum NO concentrations.

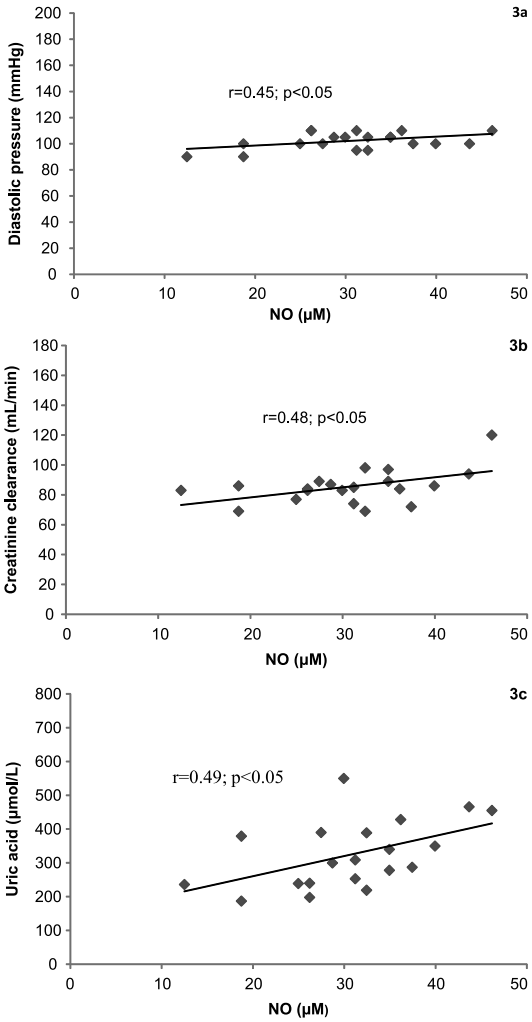

Figure 3. Correlation of NO level with diastolic blood pressure, creatinine clearance, uric acid level and platelet count in serum samples from preeclamptic women. A) positive correlation (r=0.45; p<0.05) between serum NO concentration and diastolic blood pressure; B) positive correlation (r=0.48; p<0.05) between serum NO concentration and creatinine clearance; C) positive correlation (r=0.49; p<0.05) between serum NO concentration and uric acid level; D) negative correlation (r=-0.57; p<0.05) between serum NO concentration and platel

DISCUSSION

In our study we found that NO production has increased with gestational age during normal pregnancy and slightly decreased in preeclampsia. These results are in accordance with the results of the authors who also established that the biosynthesis of NO increases in normal pregnancy, especially in the second trimester, with its peak in the third trimester of pregnancy (5,6). However, the results of NO biosynthesis vary in different studies. Hata et al. (7) reported that the level of circulating NO in mother's blood decreases during pregnancy. Brown et al. (8) and Smarason et al. (9) have proved that there is no change in NO production during normal pregnancy compared to the nonpregnant healthy subjects. From the above examples, we can conclude that the biosynthesis of NO during pregnancy still remains unclear. These discrepancies could be explained by using different methods for the determination of NO in different types of biological tissue (serum, plasma, urine etc.) (21).

Several studies have shown that iron-deficiency anaemia increases production of NO at humans, and that the increased concentrations of NO return to the normal level after supplemental therapy with iron (22). Ni et al. (23) have found that

the level of NO in plasma grows significantly in animal models of iron-deficiency anaemia. Mabott et al. (24) have proved that the production of NO correlates directly with development of anaemia and that systemic inhibition of NO synthesis leads to significant increase of haemoglobin concertation in the animal experiments. In order to exclude the effects of iron-deficiency anaemia on the results of serum NO concentrations during normal pregnancy, we analysed serum NO concentrations only in those healthy pregnant women in whom serum iron concentrations did not statistically significantly differ in relation to control group of nonpregnant subjects.

Serum NO concentration of the healthy pregnant women was significantly higher during the second and the third trimester of pregnancy in relation to control nonpregnant subjects, although there was no statistically significant difference in the concentration of serum iron between these groups. Also, there was no statistically significant difference in serum NO concentration between the women with preeclampsia and healthy pregnant women. Our results are in accordance with previous results of Lyall et al. (25) and Davidge et al. (16) who showed no significant difference in serum NO concentrations between the control group of healthy pregnant women and the group of women with preeclampsia. On the other hand, Brennecke et al. (26) and Zeng et al. (15) obtained the results which show the reduced production of NO in the group of women with preeclampsia. Reduced NO serum levels of preeclamptic women could be caused by increased binding and reduced releasing of nitrates from red blood cells (27). However, there are some groups of authors who showed that the biosynthesis of NO became increased at preeclamptic subjects (25), as well as that serum NO concentration in patients with preeclampsia is significantly higher in comparison with the control group of healthy pregnant women (18). These results could be explained in many ways. Food rich in nitrates such as cured meats or vegetables are sources of exogenous nitrates. In these studies, the subjects were not subjected to the long-lasting specific diet which is poor in nitrates, before collecting the serum samples. In our research, all patients with diagnosis of preeclampsia were hospitalized at our department and at a certain time they were at identical dietary regimen such as the control group of healthy pregnant women of the third and late third trimester. We can ascertain that in our study we were measuring the level of the main metabolite of NO (nitrates and nitrites) in serum, but only at subjects who were subjected to diet poor in nitrates and nitrites.

The severity of preeclampsia is another factor that could explain a disagreement in results of these studies. Cameron et al. (17) found no difference between hypertensive pregnant women and control normotensive groups even though the systolic pressure of hypertensive pregnant women correlated positively with excretion of nitrates and nitrites, similarly as in our study, suggesting that this could be compensatory response in achieving homeostasis. In addition, our research showed a positive correlation between serum NO concentrations and creatine clearance as well as uric acid concentration of the preeclamptic group, which is in accordance with the research of authors who observed the correlation between serum NO concentrations and creatine clearance of patients at intensive care (28). Davidge et al. (16) found no difference in the concentration of nitrates in plasma of healthy and preeclamptic pregnant women, but they proved decreased fractional excretion of nitrates and reduced urinary relation of nitrates and creatine in preeclamptic pregnant women; in non-pregnant healthy women clearance of nitrate was about 20 mL/min suggesting a component of tubular reabsorption. Paşaoğlu et al. (29) showed that serum NO concentrations correlates positively with plasma urate concentration of preeclamptic patients, what is in accordance with our results. Our results showed a negative correlation between serum NO concentrations and platelet count of the preeclamptic group. Beside direct effect on cardiovascular system, NO inhibits the adhesion of monocytes on layers of blood vessels and prevents aggregation of thrombocytes. Under conditions which activate thrombocytes and stimulate the production of thrombin, thrombocytes and endothelial cells release NO, so in that way they restrict thrombus formation and maintain mobility of vascular system. Therefore. NO inhibits mobility and flow of calcium ions in thrombocytes. We can say that our results are in accordance with above-mentioned facts.

In our study, we found that the biosynthesis of NO increases gradually in normal pregnancy and slightly decreases in preeclampsia. Although significant difference of serum NO concentrations between the preeclamptic and healthy pregnant women was not proven, our research shows that NO affects the cardiovascular changes during normal pregnancy and pregnancy complicated by preeclampsia.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Competing interests: none to declare.

REFERENCES

1. Wedel JC, Mandala M, Barron C, Bernstein I, Osol G. Mechanisms underlying maternal venous adaptation in pregnancy. Reprod Sci 2009; 16:596–604.
2. Leo CH, Jelinic M, Ng HH, Marshall SA, Novak J, Tare M, Conrad KP, Parry LJ. Vascular actions of relaxin: nitric oxide and beyond. Br J Pharmacol 2017; 174:1002–14.
3. Valdes G, Kaufmann P, Corthorn J, Erices R, Brosnihan KB, Joyner-Grantham J. Vasodilator factors in the systemic and local adaptations to pregnancy. Reprod Biol Endocrinol 2009; 7:79.
4. Poniedziałek-Czajkowska E, Marciniak B, Kimber-Trojnar Z, Leszczyńska-Gorzelak B, Oleszczuk J. Nitric oxide in normal and preeclamptic pregnancy. Curr Pharm Biotechnol 2011; 12:743–9.
5. Choi JW, Im MW, Pai SH. Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. Ann Clin Lab Sci 2002; 32:257–63.
6. Shaamash AH, Elsnosy ED, Makhlouf AM, Zakhari MM, Ibrahim OA, EL-dien HM. Maternal and fetal serum nitric oxide (NO) concentrations in normal pregnancy, pre-eclampsia and eclampsia. Int J Gynaecol Obstet 2000; 68:207–14.
7. Hata T, Hashimoto M, Kanenishi K, Akiyama M, Yanagihara T, Masumura S. Maternal circulation nitrite levels are decreased in both normal normotensive pregnancies and pregnancies with preeclampsia. Gynecol Obstet Invest 1999; 48:93–7.
8. Brown MA, Tibben E, Zammit VC, Cario GM, Carlton MA. Nitric oxide excretion in normal and hypertensive pregnancies. Hypertens Pregnancy 1995; 14:319–26.
9. Smarason AK, Allman KG, Young D, Redman CW. Elevated levels of serum nitrate, a stable end product of nitric oxide, in women with pre-eclampsia. Br J Obstet Gynaecol 1997; 104:538–43.
10. Carty DM, Delles C, Dominiczak AF. Preeclampsia and future maternal health. J Hypertens 2010; 28:1349–55.
11. World Health Organization. Make every mother and child count. The world health report 2005. Geneva, Switzerland: World Health Organization, 2005. http://www.who.int/whr/2005/whr2005_en.pdf (30 June 2017)
12. Poon LC, Kametas NA, Chelemen T, Leal A, Nicolaides KH. Maternal risk factors for hypertensive disorders in pregnancy: a multivariate approach. J Hum Hypertens 2010; 24:104–10.
13. Su JB. Vascular endothelial dysfunction and pharmacological treatment. World J Cardiol 2015; 26:719-41.
14. Matsubara K, Matsubara Y, Hyodo S, Katayama T, Ito M. Role of nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. J Obstet Gynaecol Res 2010; 36:239–47.
15. Zeng Y, Li M, Chen Y, Wang S. Homocysteine, endothelin-1 and nitric oxide in patients with hypertensive disorders complicating pregnancy. Int J Clin Exp Pathol 2015; 8:15275–9.
16. Davidge ST, Stranko CP, Roberts JM. Urine but not plasma nitric oxide metabolites are decreased in women with preeclampsia. Am J Obstet Gynecol 1996; 174:1008–13.
17. Cameron IT, van Papendorf CL, Palmer RMJ, Smith SK, Moncada S. Relationship between nitric oxide synthesis and increase in systolic blood pressure in women with hypertension in pregnancy. Hypertens Pregnancy 1993; 12:85–92.
18. Acauan Filho BJ, Pinheiro da Costa BE, Ogando PB, Vieira MC, Antonello IC, Poli-de-Figueiredo CE. Serum nitrate and NOx levels in preeclampsia are higher than in normal pregnancy. Hypertens Pregnancy 2016; 35:226–33.
19. National High Blood Pressure Education Program Working Group. Report on high blood pressure in pregnancy. Am J Obstet Gynecol 2000; 183(Suppl):S1–22.
20. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and 15N nitrate in biological fluids. Anal Biochem 1982; 126:131–38.
21. Bryan NS, Grisham MB. Methods to detect nitric oxide and its metabolites in biological samples. Free Radic Biol Med 2007; 43:645-57.
22. Choi JW, Pai SH, Kim SK, Ito M, Park CS, Cha YN. Iron deficiency anemia increases nitric oxide production in healthy adolescents. Ann Hematol 2002; 81:1–6.
23. Ni Z, Morcos S, Vaziri ND. Up-regulation of renal and vascular nitric oxide synthase in iron deficiency anemia. Kidney Int 1997; 52:195–201.
24. Mabbott N, Sternberg J. Bone marrow nitric oxide production and development of anemia in Trypanosoma brucei-infected mice. Infect Immun 1995; 63:1563–6.

25. Lyall F, Young A, Greer IA. Nitric oxide concentrations are increased in the fetoplacental circulation in preeclampsia. Am J Obstet Gynecol 1995; 173:714–8.
26. Brennecke SP, Gude NM, Di Iulio JL, King RG. Reduction of placental nitric oxide synthase activity in pre-eclampsia. Clin Sci 1997; 93:51–5.
27. Owusu BY, Stapley R, Honavar J, Patel RP. Effects of erythrocyte aging on nitric oxide and nitrite metabolism. Antioxid Redox Signal 2013; 19:1198-208.
28. MacKenzie IMJ, Ekangaki A, Young D, Garrard CS. Effect of renal function on serum nitrogen oxide concentrations. Clin Chem 1996; 42:440–4.
29. Paşaoğlu H, Bulduk G, Oğüş E, Paşaoğlu A, Onalan G. Nitric oxide, lipid peroxides, and uric acid levels in pre-eclampsia and eclampsia. Tohoku J Exp Med 2004; 202:87–92.

Biosinteza nitričnog oksida u normalnoj trudnoći i trudnoći kompliciranoj preeklampsijom

Jasmin Hodžić[1], Sebija Izetbegović[2], Bedrana Muračević[1], Rasim Iriškić[1], Hana Štimjanin Jović[1]

[1]Služba za ženske bolesti, perinatologiju i neonatologiju, Kantonalna bolnica Zenica, [2]Klinički centar Univerziteta u Sarajevu; Bosna i Hercegovina

SAŽETAK

Cilj Ispitati biosintezu nitričnog oksida (NO) u normalnoj trudnoći i trudnoći kompliciranoj preeklampsijom.

Metode Ovo prospektivno presječno istraživanje obuhvatilo je 80 pacijentica, starosne dobi između 17 i 40 godina, koje su bile hospitalizirane na Službi za ženske bolesti, perinatologiju i neonatologiju Kantonalne bolnice Zenica. Ispitivana je koncentracija NO u serumu 20 negravidnih žena, 40 zdravih trudnica i 20 trudnica s preeklampsijom. Grupa zdravih trudnica bila je podijeljena u 4 podgrupe prema gestacijskoj dobi. Za svaku pacijenticu s preeklampsijom uzeli smo kontrolnu zdravu trudnicu koja je odgovarala po starosnoj dobi, paritetu i gestacijskoj dobi. Nivo NO u serumu određivali smo mjerenjem koncentracije nitrita korištenjem klasične kolorimetrijske Griessove metode.

Rezultati Vrijednosti serumske koncentracije NO zdravih trudnica u drugom (37.2±1.7μM; $p<0.05$) i trećem trimestru (40.9±2.8μM; $p<0.05$) bile su statistički signifikantno više u odnosu na kontrolnu grupu negravidnih ispitanica (29.3±1.7μM). Serumska koncentracija NO grupe ispitanica s preeklampsijom (30.7±1.8μM) bila je niža u poređenju s kontrolnom grupom zdravih trudnica trećeg i kasnog trećeg trimestra (35.1±2.2μM). Međutim, ova razlika nije bila statistički signifikantna. Utvrđena je pozitivna korelacija između serumske koncentracije NO i sistolnog pritiska ($r=0.58$; $p<0.01$), dijastolnog pritiska ($r=0.45$; $p<0.05$), klirensa kreatinina ($r=0.48$; $p<0.05$), urične kiseline ($r=0.49$; $p<0.05$), te negativna korelacija nivoa trombocita ($r=-0.57$; $p<0.05$) grupe ispitanica s preeklampsijom.

Zaključak Biosinteza nitričnog oksida se postepeno povećavala u normalnoj trudnoći, a blago smanjivala u preeklampsiji. Rezultati ukazuju da nitrični oksid utječe na kardiovaskularne promjene tokom normalne trudnoće i trudnoće komplicirane preeklampsijom.

Ključne riječi: patogeneza, vazokonstrikcija, hipertenzija, komplikacije u trudnoći

ORIGINAL ARTICLE

Socioeconomic and demographic factors associated with abdominal obesity in women of childbearing age

Amira Kurspahić-Mujčić[1], Emir Zećo[2]

[1]Department of Social Medicine, School of Medicine, University of Sarajevo, [2]Health Care Centre of Sarajevo Canton; Sarajevo, Bosnia and Herzegovina

ABSTRACT

Aim To determine socioeconomic and demographic factors associated with abdominal obesity in women of childbearing age.

Methods This cross-sectional study was carried out in family medicine outpatient departments of the Primary Health Care Centre of Canton Sarajevo, Bosnia and Herzegovina. The study included 300 respondents who were divided into two groups: non-abdominal obesity (n=150) and abdominal obesity (n=150). Abdominal obesity was estimated measuring waist circumference. Data concerning socioeconomic and demographic factors (age, marital status, "live alone", place of residence, formal education level, self-perceived financial status) were collected using a designed questionnaire. Logistic regression analysis was used to estimate the association between socioeconomic, demographic factors and abdominal obesity.

Results Abdominal obesity was significantly associated with the life in the urban area (OR=2.174, 95%CI=1.362-3.471), university education (OR=9.881, 95%CI=3.222-30.301) and slightly better financial status than average (OR=2.412, 95%CI=1.302-4.470). Marital status (OR=0.190, 95%CI=0.104-0.347) and no living alone (OR=0.357, 95%CI=0.165-0.773) protect from abdominal obesity. Respondents aged between 20-29 years represent a particularly vulnerable group in terms of abdominal obesity (OR=1.030, 95%CI=0.097-10.946).

Conclusion The strongest associations have been found between abdominal obesity and education. Public health programs that aim to reduce abdominal obesity in women of childbearing age should mainly focus on women with university education.

Key words: abdomen, adipose tissue, female, social-class

Corresponding author:
Amira Kurspahić-Mujčić
School of Medicine, University of Sarajevo
Čekaluša 90, 71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 33 202 051;
Fax: +387 33 202 051;
Email: amira.kurspahic@mf.unsa.ba
ORCID ID: http://www.orcid.org/0000-0002-0741-5490

Original submission:
07 February 2017;
Revised submission:
09 March 2017;
Accepted:
17 April 2017.
doi: 10.17392/897-17

Med Glas (Zenica) 2017; 14(2): 218-223

INTRODUCTION

Due to the epidemiological and nutrition transition, low and middle-income countries have experienced increase in the prevalence of abdominal and overall obesity (1). Especially, abdominal obesity among women of childbearing age has become one of the main public health problems, which needs an instant action to prevent and control (2).

Untreated abdominal obesity negatively impacts the reproductive health of women in many ways (3). Abdominal obesity negatively affects both contraception and fertility as well (4,5). Maternal obesity is linked with higher rates of cesarean section as well as higher rates of high risk obstetrical conditions such as diabetes and hypertension (6,7). Visceral fat appears to be associated with insulin resistance which leads to type 2 diabetes (8,9). Visceral fat is also associated with adverse lipid profiles which in turn predispose to cardiovascular disease (10,11).

There are various risk factors, which are responsible for abdominal obesity (12). Some studies showed a significant correlation between socioeconomic, demographic factors and abdominal obesity (13,14). Previous studies have shown that educational level (15,16), occupational status (17), and income level (18) are associated with abdominal obesity, especially among women. Musaiger carried out a systematic review of published papers which discuss possible factors that are associated with obesity in the Eastern Mediterranean region between 1990 and 2011. The review has shown that abdominal obesity in women in this region was significantly associated with socioeconomic and demographic factors such as marital status and urbanization (19).

In Bosnia and Herzegovina risk factors for abdominal obesity have been poorly explored, particularly with regard to women of childbearing age.

The aim of this study was to determine socioeconomic and demographic factors associated with abdominal obesity in women of childbearing age in the area of Sarajevo Canton.

EXAMINEES AND METHODS

Study design

This cross-sectional study was carried out in family medicine outpatient departments of the Public Institution Primary Health Care Centre of Canton Sarajevo, Bosnia and Herzegovina (B&H) in the period 15 February– 31 March 2015.

The study was approved by the Ethics Committee of the School of Medicine, University of Sarajevo. For this investigation a written consent of the General Director of the Primary Health Care Centre of the Sarajevo Canton was obtained. An informed consent for participation in the study was taken from all respondents.

The respondents were patients who used health care services at the Primary Health Care Centre during the course of the study period. The study included 300 respondents on the principle of systematic random sampling. The respondents were divided into two groups: non- abdominal obesity (n=150) and abdominal obesity (n=150). The inclusion criteria were females aged 18-49 years who had a medical record in the Primary Health Care Centre of the Sarajevo Canton. The exclusion criteria were male gender, persons younger than 18 or older than 49 years, persons who did not have medical records at the Primary Health Care Centre of the Sarajevo Canton, pregnant women and women who had given birth within twelve months before the date of inclusion in the study.

Methods

The study used a specially designed and structured questionnaire which was first piloted on a small intentional sample of respondents selected from the sampling population. The questionnaire consisted of two parts: the part that was completed by a nurse and a part that was completed by a respondent.

The part that was completed by nurse contained: general information about the respondent (initials, date of birth) and data on anthropometric measurement (waist circumference).

Waist circumference was measured at midpoint between the lower rib and the iliac crest using a flexible tape measure. During the measurement, participants stood in an upright position, with arms relaxed at the side, feet evenly spread apart, and body weight evenly distributed in accordance with the World Health Organization (WHO) expert consultation report on waist circumference and waist-to-hip ratio (20).

The part that was completed by the respondent contained information on marital status, “live

alone", place of residence, formal education level and self-perceived financial status. Marital status was categorized as living with a spouse (including married and co-habiting subject), single, divorced and widowed. The respondents were supposed to respond with "yes" or "no" to the question whether she lives alone. Place of residence was categorized as urban and suburban. Formal education level was categorized as incomplete elementary school, completed elementary school, completed secondary school, high school diploma and completed high school/college. Self- perceived financial status was categorized as a lot worse than average, slightly worse than average, average, slightly better than average and much better than average.

Abdominal obesity was defined as waist circumference ≥80 cm (the criteria of abdominal obesity given by the International Diabetes Federation) (21). Respondents with measured waist circumference less than 80 cm were considered as those with no abdominal obesity. Respondents with measured waist circumference ≥80 cm were considered as those with abdominal obesity.

Statistical analysis

Testing of differences in the age distribution of respondents between non-abdominal obesity group and abdominal obesity group was performed by Mann-Whitney test. Testing of the difference in socioeconomic and demographic characteristics between non-abdominal obesity group and abdominal obesity group was performed by $\chi 2$ test. The individual effects of categorical predictors variables, socioeconomic and demographic characteristics of the respondents, on the presence of abdominal obesity were obtained by logistic univariate regression analysis through the calculation of the odds ratio (OR). The level of significance was set at $p<0.05$, and the confidence level of 95%.

RESULTS

The study evaluated 300 respondents in two groups of 150 each (i.e., non-abdominal obesity and abdominal obesity).

Age of the respondents in the abdominal obesity group and non-abdominal obesity group was significantly different (p=0.000). As many as three times more respondents aged 20-29 were in the abdominal obesity group, 34 (22.7%), than in non-abdominal obesity group, 11 (7.3%) (Table 1). Respondents aged between 20-29 years were represented a particularly vulnerable group in terms of abdominal obesity (OR=1.030, 95%CI=0.097-10.946; 0.000).

Table 1. Socioeconomic and demographic characteristics of the respondents according to the presence of abdominal obesity

Socioeconomic and demographic characteristics	No (%) of respondents		p
	Abdominal obesity	Non-abdominal obesity	
Age group (years)			
<20	3 (2.0)	1 (0.7)	
20-29	34 (22.7)	11 (7.3)	0.000
30-39	68 (45.3)	53 (35.3)	
40-49	45 (30,0)	85 (56.7)	
Marital status			
Single	63 (42.0)	18 (12.0)	
Divorced	10 (6.7)	14 (9.3)	0.000
Living with a spouse	74 (49.3)	111 (74.0)	
Widowed	3 (2.0)	7 (4.7)	
Lives alone			
Yes	25 (16.7)	10 (6.7)	0.007
No	125 (83.3)	140 (93.3)	
Place of residence			
Urban	101 (67.3)	73 (48.6)	0.001
Suburban	49 (32.7)	77 (51.4)	
Formal education level			
Incomplete elementary school	0 (0.0)	7 (4.7)	
Completed elementary school	4 (2.7)	24 (16.0)	0.000
Completed secondary school	80 (53.3)	64 (42.6)	
Completed high school/college	15 (10.0)	15 (10.0)	
High school diploma	51 (34.0)	40 (26.7)	
Self-perceived financial status			
Lot worse than average	4 (2.7)	16 (10.7)	
Slightly worse than average	13 (8.7)	17 (11.3)	0.000
Average	74 (49.3)	85 (56.7)	
Slightly better than average	42 (28.0)	20 (13.3)	
Much better than average vs average	17 (11.3)	12 (8.0)	

Marital status in the abdominal obesity group and non-abdominal obesity group was significantly different (p=0.000). There were three and a half times as many unmarried respondents in the abdominal obesity group, 63 (42.0%), than in non-abdominal obesity group, 18 (12.0%) (Table 1). There was interdependence that existed between abdominal obesity and marital status, e.g. marital status in total protects from abdominal obesity (OR=0.190, 95%CI=0.104-0.347; 0.000) (Table 2).

The abdominal obesity group and non-abdominal obesity group had significant difference regarding the variables whether respondents were living or not living alone (p=0.007). Even two and a half times more respondents who lived alone were in the abdominal obesity group, 25 (16.7%), than in the non-abdominal obesity group 10 (6.7%) (Table 1). There was an interdependence between abdomi-

Table 2. Individual effects of categorical predictor variables for the presence of abdominal obesity obtained by logistic regression analysis

Categorical predictor	p	OR (95% CI)
Age group (years)		
20-29 vs <20	0.000	1.030 (0.097-10.946)
30-39 vs <20		0.428 (0.043-4.230)
40-49 vs <20		0.176 (0.018-1.746)
Marital status		
Divorced vs single	0.000	0.204 (0.078-0.536)
Living with a spouse vs single		0.190 (0.104-0.347)
Widowed vs single		0.122 (0.029-0.522)
Lives alone	0.006	
No vs Yes		0.357 (0.165-0.773)
Place of residence	0.001	
Urban vs suburban		2.174 (1.362-3.471)
Formal education level		
Completed high school/college vs incomplete/ completed elementary school	0.000	9.881 (3.222-30.301)
Completed secondary school / high school diploma vs incomplete/ completed elementary school		9.320 (3.155-27.532)
Self-perceived financial status		
Much better than average vs average		1.627 (0.730-3.629)
Slightly better than average vs average	0.001	2.412 (1.302-4.470)
Lot worse than average vs average		0.287 (0.092-0.897)
Slightly worse than average vs average		0.878 (0.400-1.929)

OR, Odds Ratio; CI, confidence interval

nal obesity and the variables whether respondents were living or not living alone. Not living alone in total protect from abdominal obesity (OR=0.357, 95%CI=0.165-0.773; p=0.006) (Table 2).

Place of residence in the abdominal obesity group and non-abdominal obesity group was significantly different (p=0.001). More respondents with the residence in urban areas were in the abdominal obesity group than in the non- abdominal obesity, 101 (67.3%) and 73 (48.6%) (Table 1). There was an interdependence that existed between abdominal obesity and the place of residence, e.g. living in an urban area was a risk for abdominal obesity (2.174, 95%CI=1.362-3.471; p=0.001) (Table 2).

Formal education level in the abdominal obesity group and non-abdominal obesity group was significantly different (p=0.000). A slightly larger number of respondents with the university degree were in the abdominal obesity group, 51 (34%), than in the non-abdominal obesity group, 40 (26.7%). In the same group, there were no respondents with incomplete primary education, while those respondents were present in the non-abdominal obesity group in 4.7% (Table 1). There was an interdependence that existed between abdominal obesity and formal education level, e.g. university degree has a risk for abdominal obesity (9.320, 95%CI=3.155-27.532; p=0.000) (Table 2).

Self-perceived financial status in the abdominal obesity group and non-abdominal obesity group was significantly different (p=0.000). Twice as many respondents with the financial status, which was slightly better than average, was in the abdominal obesity group, 13 (8.7%), than in the non-abdominal obesity group, 17 (11.3%) (Table 1). There was an interdependence between abdominal obesity and self-perceived financial status. Financial status better than an average was a risk for abdominal obesity (OR=2.412, 95%CI=1.302-4.470; p=0.001) (Table 2).

DISCUSSION

This study evaluated socioeconomic and demographic factors associated with abdominal obesity in women of childbearing age. The results revealed that university education, slightly better financial status than the average and life in the urban area are positively associated with abdominal obesity.

The respondents with a university degree compared to those with complete or incomplete primary education had almost ten times higher risk of having abdominal obesity. Respondents who evaluated their financial status as somewhat better-than average compared to those who evaluated their financial status as average had two and a half times higher risk of having abdominal obesity. These findings are consistent with the results of studies conducted in the middle-income and developing countries where abdominal obesity was mainly prevalent among women of higher economic status and women with higher education (22,23). In highly developed countries greater economic, educational status was generally negatively associated with abdominal obesity (24). Studies carried out in countries with the GDP (gross domestic product) of more than 12 275 US$ per capita demonstrated that the GDP increase leads to abdominal obesity as a growing problem of the poor, population with lower education, especially poor women (25).

In this study marital status and not living alone protect from abdominal obesity. This finding is the opposite to the results of a study conducted by Veghari et al. They found that abdominal obesity was approximately four times more prevalent in married subjects than in single ones. According to them it is possible that marriage increases cues and opportunities for eating because they tend to

eat together and thus reinforce each other's increased intake (26).

In the majority of studies abdominal obesity is more often prevalent in urban than in rural areas (27). In this study, respondents with residence in an urban area were twice more likely to have abdominal obesity than respondents who lived in a suburban area. In a research conducted in Iran urban area was the main determinant of abdominal obesity. Logistic regression analysis also included other socioeconomic and demographic factors (28). Even in developing countries obesity is significantly more common among the urban population than rural (29). Urbanization means decreased levels of physical activity and increased availability of food, as well as exposure to fast foods (27).

In this study, younger respondents represent a particularly vulnerable group in terms of abdominal obesity. Several recent studies reported that respondents aged between 20-29 years had highest increase rates of abdominal obesity (30). This high increase in prevalence of abdominal obesity among young people may be due to changes in lifestyle and a shift in nutritional patterns. Research conducted in the area of Sarajevo Canton, in the period 2011-2012 has shown greater representation of inadequate nutrition among young people aged 15-27 years than people over 27 years (32).

Therefore, the future studies are needed to explore lifestyle factors that are influenced or modified by socioeconomic and demographic factors in women of childbearing age.

In conclusion, the study findings underscore the importance of socioeconomic and demographic factors of abdominal obesity among the women of childbearing age. Identification of socioeconomic and demographic factors that are associated with abdominal obesity is critical for the development of effective public health programs. Recommended programs may include improvement of socioeconomic standard as well as public health measures aimed at reducing the prevalence of sedentary lifestyle, regulation of the food industry, the media and parenting courses. The implementation of programs designed in this manner can contribute to the prevention and control of abdominal obesity in women of childbearing age.

FUNDING

No funding was received for this study.

TRANSPARENCY DECLARATION

Competing interests: None to declare.

REFERENCES

1. Popkin BM, Adair LS, Nig SW. Global nutrition transition and the pandemic of obesity in developing countries. Nutr Rev 2012; 70:3–21.
2. Kanter R, Caballero B. Global gender disparities in obesity: a review. Adv Nutr 2012; 3:491–8.
3. Ramsay J, Greer I, Sattar N. Obesity and reproduction. BMJ 2006; 333:1159–62.
4. Robker R. Evidence that obesity alters the quality of oocytes and embryos. Pathophysiology 2008; 15:115–21.
5. Yilmaz N, Kilic S, Kanat-Pektas M, Gulerman C, Mollamahmutoglu L. The relationship between obesity and fecundity. J Womens Health 2009; 18:633–6.
6. Chu SY, Bachman DJ, Callaghan WM, Whitlock EP, Dietz PM, Berg CJ, O'Keeffe-Rosetti M, Bruce FC, Hornbrook MC. Association between obesity during pregnancy and increased use of health care. N Engl J Med 2008; 358:1444–53.
7. Chu SY, Callaghan WM, Kim SY, Schmid CH, Lau J, England LJ, Dietz PM. Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care 2007; 30:2070–6.
8. Wahrenberg H, Hertel K, Leijonhufvud BM, Persson LG, Toft E, Arner P. Use of waist circumference to predict insulin resistance: retrospective study. BMJ 2005; 330:1363-4.
9. Gallagher EJ, LeRoith D, Karnieli E. The metabolic syndrome–from insulin resistance to obesity and diabetes. Endocrinol Metab Clin North Am 2008; 37:559-79.
10. Lofgren I, Herron K, Zern T, West K, Patalay M, Shachter NS, Koo SI, Fernandez ML. Waist circumference is a better predictor than body mass indeks of coronary heart disease risk in overweight premenopausal women. J Nutr 2004; 134:1071-6.
11. Weiss AM. Cardiovascular disease in women. Prim Care 2009; 36:73–102.
12. Demerath EW. Causes and consequences of human variation in visceral adiposity. Am J Clin Nutr 2010; 91:1–2.
13. McLaren L. Socioeconomic status and obesity. Epidemiol Rev 2007; 29:29–48.
14. Liese AD, Doring A, Hense HW, Keil U. Five year changes in waist circumference, body mass index and obesity in Augsburg, Germany. Eur J Nutr 2001; 40:282-8.
15. Boissonnet C, Schargrodsky H, Pellegrini F, Macchia A, Marcet CB, Wilson E, Tognoni G. Educational inequalities in obesity, abdominal obesity, and metabolic syndrome in seven Latin American cities: the CARMELA Study. Eur J Cardiovasc Prev Rehabil 2011; 18:550-6.
16. Lahmann PH, Lissner L, Gullberg B, Berglund G. Sociodemographic factors associated with long-term weight gain, current body fatness and central adiposity in Swedish women. Int J Obes Relat Metab Disord 2000; 24:685–94.

17. Langenberg C, Hardy R, Kuh D, Brunner E,Wadsworth M. Central and total obesity in middle aged men and women in relation to lifetime socioeconomic status: evidence from a national birth cohort. J Epidemiol Commun Health 2003; 57:816–22.
18. Monteiro CA, Moura EC, Conde WL, Popkin BM. Socio-economic status and obesity in adult populations of developing countries: A review. Bull WHO 82:960-6.
19. Musaiger AO. Overweight and obesity in Eastern Mediterranean region: Prevalence and possible causes. J Obes 2011; 1-17.
20. World Health Organization. Waist circumference and waist–hip ratio: report of a WHO expert consultation. Geneva: WHO, 2008.
21. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome – a new world-wide definition. A consensus statement from the International Diabetes Federation. Diabet Med 2006; 23:469-80.
22. Aekplakorn W, Hogan MC, Chongsuvivatwong V, Tatsanavivat P, Chariyalertsak S, Boonthum A, Tiptaradel S, Lim SS. Trends in obesity and associations with education and urban or rural residence in Thailand. Obesity 2007; 15:3113–21.
23. Maddah M, Eshraghian MR, Djazayery A, Mirdamadi R. Association of body mass index with educational level in Iranian men and women. Eur J Clin Nutr 2003; 57:819–23.
24. Marques-Vidal P, Bochud M, Mooser V, Paccaud F, Waeber G, Vollenweider P. Prevalence of obesity and abdominal obesity in the Lausanne population. BMC Public Health 2008; 8:330.
25. Wang Y, Beydoun MA. The obesity epidemic in the United States – gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis. Epidemiol Rev 2007; 29:6–28.
26. Veghari G, Sedaghat M, Joshaghani H, Hoseini A, Niknajad F, Angizeh A,Tazik E, Moharloei P. The prevalence and associated factors of central obesity in Northern Iran. Iranian Cardiovascular Research Journal 2010; 4:164-8.
27. Galal OM. The nutrition transition in Egypt: obesity, undernutrition and the food consumption context. Public Health Nutr 2002; 141–8.
28. Esmaeily H, Azimi-Nezhad M, Ghayour-Mobarhan M, Mohamed-Reza P. Association between socioeconomic factors and obesity in Iran. Pakistan Journal of Nutrition 2009; 8:53–6.
29. Ziraba AK, Fotso JC, Ochako R. Overweight and obesity in urban Africa: a problem of the rich or the poor? BMC Public Health 2009; 9:465–73.
30. Hosseinpanah F, Barzin M, Eskandary PS, Mirmiran P, Azizi F. Trends of obesity and abdominal obesity in Tehranian adults: a cohort study. BMC Public Health 2009; 9:426.
31. Suliga E, Wronka I, Pawlińska-Chmara R. The prevalence and correlates of abdominal obesity in female students. Pediatr Endocrinol Diabetes Metab 2011; 17:201-5.
32. Kurspahić-Mujčić A, Hadžagić-Ćatibušić F, Sivić S, Hadžović E. Association between high levels of stress and risky health behavior. Med Glas (Zenica) 2014; 11:367-71.

Socioekonomski i demografski faktori povezani s abdominalnom pretilošću u žena fertilne dobi

Amira Kurspahić-Mujčić[1], Emir Zećo[2]

[1]Katedra za socijalnu medicinu, Medicinski fakultet, Univerzitet u Sarajevu, [2]Dom zdravlja Kantona Sarajevo; Sarajevo, Bosna i Hercegovina

SAŽETAK

Cilj Utvrditi socioekonomske i demografske faktore povezane s abdominalnom pretilošću u žena fertilne dobi.

Metode Rad predstavlja studiju presjeka provedenu u ambulantama porodične medicine javne ustanove Dom zdravlja Kantona Sarajevo, Bosna i Hercegovina. U istraživanje je bilo uključeno 300 ispitanika koji su bili podijeljeni u dvije grupe: bez abdominalne pretilosti (n=150) i s abdominalnom pretilošću (n=150). Mjerenje obima struka je korišteno za određivanje prisustva abdominalne pretilosti. Podaci o socioekonomskim i demografskim karakteristikama ispitanika (starost, bračni status, „živi sama“, mjesto stanovanja, formalno obrazovanje, samoprocijenjeni materijalni status) dobiveni su korištenjem posebno dizajniranog anketnog upitnika. Logistička regresiona analiza je korištena za određivanje postojanja povezanosti između socioekonomskih, demografskih faktora i abdominalne pretilosti.

Rezultati Abdominalna pretilost je statistički značajno povezana sa životom u gradu (OR=2.174, 95%CI=1.362-3.471), fakultetskim obrazovanjem (OR=9.881, 95%CI= 3.222-30.301) i materijalnim statusom koji je nešto bolji od prosječnog (OR=2.412, 95%CI=1.302-4.470). Brak (OR=0.190, 95%CI=0.104-0.347) i život u zajednici (OR=0.357, 95%CI=0.165-0.773) štite od nastanka abdominalne pretilosti. Posebno osjetljiva grupa za nastanak abdominalne pretilosti su ispitanice u dobi od 20 do 29 godina (OR=1.030, 95%CI=0.097-10.946).

Zaključak Utvrđeno je postojanje najjače povezanosti abdominalne pretilosti s obrazovanjem. Javnozdravstveni programi koji imaju za cilj smanjenje broja abdominalno pretilih žena fertilne dobi trebali bi se fokusirati na univerzitetski obrazovane žene.

Ključne riječi: abdomen, masno tkivo, žena, društvena klasa

ORIGINAL ARTICLE

Revision of the demographic and clinical data of patients with ulcerative colitis in Turkey

Hatice Karagoz[1], Abdulsamet Erden[2], Hatice Karaman[3], Ahmet Karaman[4]

[1]Internal Medicine Department, Acıbadem Kayseri Hospital, [2]Internal Medicine Department, [3]Clinical Pathology Department; Kayseri Training and Research Hospital; Kayseri, [4]Internal Medicine Department, Acıbadem University School of Medicine, Istanbul; Turkey

ABSTRACT

Aim To evaluate the demographic and clinical data of fifty two patients with the diagnosis of ulcerative colitis.

Methods A total of 52 patients diagnosed with ulcerative colitis by clinical, endoscopical and histopathological evaluations were included the study. Demographic data, colon involvement site of the disease and disease severity were examined from the patients' records. The patients were divided into groups according to the Baron grading system.

Results Distribution of patients according to the colon involvement site was: 10 (9.2%) rectal, 16 (30.8%) rectosigmoid, 13 (25%) left sided, 10 (19.2%) extensive colitis and 3 (5.8%) pancolitis. According to colon involvement sites and Baron classification the results were: five (9.6 %), two (3.8%) and three (5.7%) patients with ulcerative proctitis were at grade 1, 2 and 3, respectively. Two (3.8%), six (11.5 %), six (11.5 %) and two (3.8%) patients with rectosigmoid ulcerative colitis were at grade 0, 1, 2 and 3, respectively. Four (7.6 %), five (9.6%) and four (7.6%) patients with left sided ulcerative colitis were at grade 1, 2, and 3, respectively. One (1.9 %), three (5.7%), and six (11.5 %) patients with extensive ulcerative colitis were at grade 1, 2 and 3, respectively. All three (5.7%) patients with pancolitis were at grade 3 (p=0.11)

Conclusion The ratio of pancolitis is found to be lower in our series. There was no statistically significant difference among the genders according to the age, involvement site and the severity of the disease as well as according to the colon involvement sites and grading.

Key words: disease severity, inflammatory bowel diseases, colonoscopy, classification

Corresponding author:
Abdulsamet Erden
Kayseri Training and Research Hospital, Internal Medicine Department, Kayseri, Turkey
Kayseri Eğitim Araştırma Hastanesi, İç Hastalıkları Kliniği, 9. Blok 3. Kat, 38050 Kocasinan/Kayseri, Turkey
Phone: +90 532 780 3805;
fax: +90 352 207 4349;
Email: drsameterden@gmail.com
ORCID ID: http://www.orcid.org/0000-0002-9891-5211

Original submission:
05 December 2016;
Revised submission:
04 January 2017;
Accepted:
20 June 2017.
doi: 10.17392/891-17

Med Glas (Zenica) 2017; 14(2): 224-228

INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders that affect the gastrointestinal tract and are characterised by the chronic and repetitive activation of the immune system and inflammation (1). There are two clinical forms of IBD: ulcerative colitis (UC) and Crohn's disease. CD affects both the colon and small intestine, whereas UC affects the colon and rectum, but does not involve the small intestine (2). It is a relapsing and remitting disorder with disease free intervals and the inflammation limited to the mucosal layer of the colon (3). The number of new cases of UC each year in North America is 10-12/100,000 individuals/year and the disease is most often seen between the ages of 15-25 (3). A family history is considered a risk for the development of UC because several studies have demonstrated an increase in the prevalence of UC among the relatives of patients with UC (4-6). The UC is mainly presented with diarrhoea mixed with blood and other symptoms such as abdominal pain, mucous stool, weight loss and tenesmus also can be seen (7).

The UC presents variable demographic and clinical characteristics in different regions of the world and the environmental and genetic factors seem to be related to this phenotypic heterogeneity (8). Some studies have shown that UC usually shows a less severe form in Asian countries than in developed Western countries (i.e. a decreased need for surgery, a lower incidence of colorectal cancer and fewer intestinal manifestations) (9,10).

The aim of this study was to describe the demographic and clinical characteristics of patients with UC in Turkey as well as to evaluate the relationship between the disease severity and the colon involvement site in UC patients.

PATIENTS AND METHODS

Study design

Fifty two patients with the diagnosis of ulcerative colitis from the Gastroenterology Clinic of Kayseri Training and Research Hospital, Kayseri, Turkey, were included this study. The patients were diagnosed according to clinical, histopathological and endoscopical evaluations of the disease. Demographic data, colon involvement site and disease severity were retrospectively examined from the patients' records for the periods 2010-2012. The patients who were admitted with weight loss, chronic diarrhoea, bloody diarrhoea, abdominal pain and anaemia were diagnosed as UC after colonoscopy and biopsy.

Methods

The patients were classified in five groups after colonoscopy: ulcerative proctitis (if only rectum is affected), rectosigmoid ulcerative colitis (if rectum and sigmoid colon are affected), left sided ulcerative colitis (if the colon up to the splenic flexura is affected), extensive ulcerative colitis (if the colon above the splenic flexura up to the hepatic flexura is affected), and pancolitis (if the entire colon from rectum to caecum is affected).

Severity of the disease was defined according to Baron classification (11). Baron score includes four grades (0-3) according to the severity of macroscopic inflammation of the rectal mucosa appearances at rigid sigmoidoscopy: grade 0 - normal matt mucosa, no spontaneous bleeding or no bleeding to light touch; grade 1- abnormal but non-haemorrhagic (appearances between 0 and 2); grade 2 - moderately haemorrhagic, bleeding to light touch but no spontaneous bleeding on initial inspection; grade 3 - severely haemorrhagic, spontaneous bleeding on initial inspection. After colonoscopic and pathologic studies the patients were divided into 4 groups according to Baron classification: grade 0 (normal), grade 1 (mild), grade 2 (moderate) and grade 3 (severe).

Statistical analysis

Continuous variables were tested for normal distribution by the Kolmogorov–Smirnov test. The data of descriptive analysis for normally distributed variables were expressed as mean and standard deviations whereas the minimum, maximum and median values were defined for the variables that do not distribute normally. Mann-Whitney U test was used for the comparison of groups that do not distribute normally. For the comparison of normally distributed groups, Student's t-test was used. Categorical variables were summarized as percentages and compared with the chi-square test. The $p<0.05$ was considered as statistically significant.

RESULTS

Among 52 patients included in the study, 32 (61.5%) were males and 20 (38.5%) were fe-

males. The mean age among male patients was 47.15± 13.06 while the mean age among female patients was 40.90±12.98 (p=0.09).

Ten (19.2%) patients were presented with proctitis, 16 (30.8%) with rectosigmoid UC, 13 (25%) with left sided UC, 10 (19.2%) with extensive UC and 3 (5.8%) with pancolitis. The distribution of the colon involvement sites of the disease among genders were: one (5%) proctitis, eight (40%) rectosigmoid ulcerative colitis, four (20%) left sided ulcerative colitis, five (25%) extensive ulcerative colitis and two (10%) were pancolitis for females; nine (28.1%) proctitis, eight (25%) rectosigmoid ulcerative colitis, nine (28.1%) left sided ulcerative colitis, five (15.6%) extensive ulcerative colitis and one (3.1%) were pancolitis for males (p=0.18).

According to Baron classification two (3.8%) patients were in remission (normal) at grade 0, 16 (30.8%) patients were at grade 1, 16 (30.8%) patients were at grade 2, and 18 (34.6%) patients were at grade 3. The distribution of the patients according to gender was: for males - 10 (31.2%) patients were at grade 1, 10 (31.2%) patients were at grade 2 and 12 (37.5 %) patients were at grade 3; for females - two (10%) patients were in remission (normal) at grade 0, six (30%) patients were at grade 1, six (30%) patients were at grade 2 and six (30%) patients were at grade 3 (p=0.33).

The relation between disease severity and the involvement places is shown in Table 1. Five (9.6 %) patients with ulcerative proctitis were at grade 1, two (3.8%) patients were at grade 2 and three (5.7%) patients were at grade 3. Two (3.8%) patients with rectosigmoid ulcerative colitis were at grade 0, six (11.5%) patients were at grade 1, six (11.5%) patients were at grade 2 and two (3.8%) patients were at grade 3. Four (7.6%) patients with left sided ulcerative colitis were at grade 1, five (9.6%) patients were at grade 2 and four (7.6%) patients were at grade 3. One (1.9%) patient with extensive ulcerative colitis were at grade 1, three (5.7%) patients were at grade 2 and six (11.5 %) patients were at grade 3. All three (5.7%) patients with pancolitis were at grade 3. There was no statistically significant difference among the patients according to the colon involvement sites and grading (p=0.11)

Table 1. Number of patients according to Baron classification and colon involvement site

Colon involvement site	Number (%) of patients according to Baron classification				
	Grade 0	Grade 1	Grade 2	Grade 3	p
Ulcerative proctitis	0	5 (9.6)	2 (3.8)	3 (5.7)	
Rectosigmoid ulcerative colitis	2 (3.8)	6 (11.5)	6 (11.5)	2 (3.8)	
Left sided ulcerative colitis	0	4 (7.6)	5 (9.6)	4 (7.6)	0.11
Extensive ulcerative colitis	0	1 (1.9)	3 (5.7)	6 (11.5)	
Pancolitis	0	0	0	3 (5.7)	

DISCUSSION

The UC is a chronic inflammatory condition causing continuous mucosal inflammation of the colon which is characterised by a relapsing and remitting course (3). Although the etiology is not clear yet, genetic, immunologic and environmental factors have been confirmed to contribute to the etiopathogenesis of ulcerative colitis (12). It appears more frequently in developed countries (13). Colonoscopy is an important diagnostic tool and it enables seeing the whole colon segments directly and performing a biopsy (14). In our study, rectosigmoid was the most commonly involved area among all patients. The previous studies showed the involvement from rectum to splenic flexura in 80% of patients and pancolitis in 20% of patients (15). Kühbacher et al. demonstrated that pancolitis was present in 11-30 % of patients (16). The results of a study including 116 cases from Istanbul were as follows: 60.3 % of pancolitis, 25% of left sided ulcerative colitis, 13.8% of proctitis and 0.9% of backwash ileitis (15). Su and Lichtenstein revealed that 37% of the patients had pancolitis, 36-41% proctitis and 44-49% proctosigmoiditis in their study (17). Regueiro showed proctosigmoiditis in 25-75% of patients with UC (11). Ozin et al investigated 507 UC patients in their study and they found that 161 of them (31.7%) had proctitis, 150 (29.6%) had left-sided colitis and 196 (38.7%) had pancolitis and extensive colitis (18). The distribution of the disease in the colon showed 45% pancolitis, 14% left-sided colitis, 21% proctosigmoiditis and 20 % proctititis in a study from Kuwait (19). Compared to the studies above (both from Turkey and other countries), our data showed significantly less pancolitis. When we analysed our results according to gender, rectosigmoid was the most commonly affected area in females with the

percentage of 40%. On the other hand, there were two most affected places for males, both rectum and left side colon with 28.1%. The studies showed the risk of developing colorectal cancer as 1.7% for proctitis, 2.8 for left sided ulcerative colitis and 14% for extensive colitis and also it has been shown that the risk of developing colorectal cancer is higher in patients with pancolitis than the left-sided ulcerative colitis patients (20).

In our study, ulcerative colitis is more often seen in male patients with a ratio of 61.5% and the mean age of males was higher than women. Karlén et al. followed 1547 UC patients in the years between 1955-1989 and they found that the risk of developing cancer was higher in the population under 29 than the population over the age of 29 (20). Ekbom et al. compared the patients in their study by dividing them into three groups: patients under the age of 14 -high risk group-, patients in the age 14-29 -medium risk group- and patients aged over 30 - low risk group (21). Also in our study young people were the majority so we must be careful at this point.

In our study, most patients were in stage 3 according to the Baron classification. When the female patients were examined, 10% of them were in remission. The distribution of patients was equal for each stage among the women (30% for stage 1, 2 and 3). A high number of male patients was in stage 3 and all female patients in remission suggest that the disease progresses more severely in males.

Severe inflammation disrupts the homeostasis and increases the risk of developing neoplasias by contributing the formation of genetic mutations (22). Microsatellites are the simple and short nucleotide repeats situated along the human genome. It was shown that chronic inflammation causes insufficiency in the mechanism of DNA repair because of increased cell destruction and proliferation and microsatellite instability occurs as a sign of this insufficiency (22,23). Microsatellite instability has been detected in colorectal cancers associated with UC. The attention is drawn to the point that the microsatellite instability is significantly higher in the lesions showing severe inflammation (23). Microsatellite instability and the risk of developing malignancy in the neoplastic and non-neoplastic colonic mucosa of the patients with UC have been found to be related with continuous and severe inflammation (22). Among UC patients, those with pancolitis and high disease activity are under a higher risk of malignity (20). Patients with widespread and severe involvement have to be closely followed up for malignity.

In conclusion, the ratio of pancolitis was found to be lower in our series. There was no statistically significant difference among the genders according to the age, involvement site and the severity of the disease as well as according to the colon involvement sites and grading.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATIONS

Competing interest: none to declare.

REFERENCES

1. Danese S, Fiocchi C. Ulcerative colitis. N Engl J Med 2011; 365:1713-25.
2. Torpy JM, Lynm C, Golub RM. Ulcerative Colitis. JAMA 2012; 307:104.
3. Thoreson R, Cullen JJ. Pathophysiology of inflammatory bowel disease: an overview. Surg Clin North Am 2007; 87:575-85.
4. Moller FT, Andersen V, Wohlfahrt J, Jess T. Familial risk of inflammatory bowel disease: a population-based cohort study 1977-2011. Am J Gastroenterol 2015; 110:564–71.
5. Andreu M, Márquez L, Domènech E, Gisbert JP, García V, Marín-Jiménez I, Peñalva M, Gomollón F, Calvet X, Merino O, Garcia-Planella E, Vázquez-Romero N, Esteve M, Nos P, Gutiérrez A, Vera I, Cabriada JL, Martín MD, Cañas-Ventura A, Panés J; Spanish GETECCU group (ENEIDA project). Disease severity in familial cases of IBD. J Crohns Colitis 2014; 8:234–9.
6. Orholm M, Munkholm P, Langholz E, Nielsen OH, Sørensen TI, Binder V. Familial occurrence of inflammatory bowel disease. N Engl J Med 1991; 324:84-8.
7. Li J, Wang F, Zhang HJ, Sheng JQ, Yan WF, Ma MX, Fan RY, Gu F, Li CF, Chen DF, Zheng P, Gu YP, Cao Q, Yang H, Qian JM, Hu PJ, Xia B. Coticosteroid therapy in ulcerative colitis: clinical response and predictors. World J Gastroenterol 2015; 21:3005-15.
8. Wong SH, Ng SC. What can we learn from inflammatory bowel disease in developing countries? Current Gastroenterol Rep. 2015; 15:313.
9. Jiang XL, Cui HF. An analysis of 10218 ulcerative colitis cases in China. World J Gastroenterol 2002; 8:158-61.
10. Gong W, Lv N, Wangetal B. Risk of ulcerative colitis-associated colorectal cancer in China: a multi-center retrospective study. Dig Dis Sci 2012; 57:503-7.

11. Regueiro MD. Diagnosis and treatment of ulcerative proctitis. J Clinic Gastroenterol 2004; 38:733-40.
12. Shirazi KM, Somi MH, Bafandeh Y, Saremi F, Mylanchy N, Rezaeifar P, Abedi Manesh N, Mirinezhad SK. Epidemiological and clinical characteristics of inflammatory bowel disease in patients from northwestern iran. Middle East J Dig Dis 2013; 5: 86-92.
13. Balai H, Asadzadeh A, Aghdaei H, Farnood A, Habibi M, Mafi AA, Firouzi F, Sharifian A, Shahrokh S, Lahmi F, Zojaji H, Naderi N, Zali MR. Time trend analysis and demographic features of inflammatory bowel disease in Tehran. Gastroenterol Hepatol Bed Bench 2015; 8: 253-61.
14. Björck S, Dahiström A, Ahlman H. Treatment of distal colitis with local anaesthetic agents. Pharmacol Toxicol 2002; 90:173-80.
15. Ozdil S, Akyüz F, Pinarbasi B, Demir K, Karaca C, Boztas G, Kaymakoglu S, Mungan Z, Besisik F, Cakaloglu Y, Okten A. Ulcerative colitis: analyses of 116 cases (do extraintestinal manifestations effect the time to catch remission?). Hepatogastroenterology 2004; 51:768-70.
16. Kühbacher T, Scheiber S, Fölsch UR. Ulcerative colitis: conservative management and long term effects. Langenbecks Arch Surg 2004; 5:350-53.
17. Su C, Lichtenstein GR. Diagnosis of Crohn's disease: A wolf in sheep's clothing? Am J Gastroenterol 2000; 95:3345-50.
18. Ozin Y, Kılıç MZY, Nadir I, Cakal B, Disibeyaz S, Arhan M, Dagli U, Tunc B, Ulker A, Sahin B. Clinical features of ulcerative colitis and Crohn' s disease in Turkey. J Gastrointestin Liver Dis 2009; 18:157-62.
19. Al-Shamali MA, Kalaoui M, Patty I, Hasan F, Khajah A, Al-Nakib B. Ulcerative colitis in Kuwait: a review of 90 cases. Digestion 2003; 67:218-224.
20. Karlén P, Löfberg R, Broström O, Leijonmarck CE, Hellers G, Persson PG. Increased risk of cancer in ulcerative colitis: A population-based cohort study. Am J gastroenterol 1999; 94:1047-52.
21. Ekbom A, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer: a population –based study. N Engl J Med 1990; 336:357-9.
22. Tahara T, Inoue N, Hisamatsu T, Kashiwagi K, Kanai T, Watanabe M, Ishii H, Hibi T. Clinical significance of microsatellite instability in the inflamed mucosa for the prediction of colonic neoplasms in patients with ulcerative colitis. J Gastroenterol Hepatol 2005; 20:710-5.
23. Karaman A, Hamurcu Z, Donmez H, Bitken N, Karaman H, Baskol M, Gursoy S, Guven K, Takaishi H, Yucesoy M. Investigation of genome instability in exfoliated colonic epithelial cells and in mitogen-stimulated lymphocytes of patients with ulcerative colitis. Digestion. 2012; 85:228-35.

ORIGINAL ARTICLE

The association between the serum levels of matrix metalloproteinase 9 and colorectal cancer

Ismar Rašić[1], Azra Rašić[2], Goran Akšamija[1], Svjetlana Radović[3], Nedžad Šehović[4]

[1]Clinic for General and Abdominal Surgery, [2]Clinic for Oncology; Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [3]Department of Pathology, School of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [4]Regional Hospital Haugesund, Gastro Surgical Department, Haugesund, Norway

ABSTRACT

Aim To determine the serum levels of matrix metalloproteinase 9 (MMP-9) concentration and their association with the stage and histopathologic sizes of colorectal cancer (CRC).

Methods One hundred and two patients with clinically diagnosed and histologically confirmed colorectal cancer ready for surgical treatment were included in the study. In each patient, preoperative peripheral venous blood samples were taken for determination of the concentration of MMP-9 using ELISA immunoassay test. Resected tumour specimens were studied pathologically according to the criteria of the TNM classification. All patients were divided into groups according to the TNM classification. The control group presented 30 subjects of the appropriate age and gender with no family history of cancer, clinical signs of malignancy or inflammatory bowel disease.

Results The serum levels of MMP-9 were progressively increased in patients with CRC reaching the highest value in the fourth stage of CRC. It was also confirmed that the serum concentrations of MMP-9 were significantly higher in patients with pericolonic lymph nodes involvement compared to the patients with no involvement of lymph nodes, 456.4 (445.9-464.7) ng/mL vs. 438.4 (418.4-447.8) ng/mL ($p<0.001$). Significantly higher serum levels of MMP-9 were found in the patients with metastatic CRC, 458.5 (452.0-468.1) ng/mL compared with the CRC patients without metastasis, 445.8 (436.9-456.5) ng/mL ($p<0.001$).

Conclusion It was confirmed that serum concentration of MMP-9 presented the significant independent risk factors for the progression of CRC.

Key words: colorectal cancer, proteolytic enzyme, serum, risk, progression

Corresponding author:
Ismar Rašić
Clinic for General and Abdominal Surgery, Clinical Centre of the University of Sarajevo, Bolnička 25, 71 000 Sarajevo, Bosnia and Herzegovina
Phone: +387 33 297 179;
Fax: +387 33 221 518;
E-mail: rasicismar@gmail
ORCIDE ID: http://www. orcid.org/0000-0003-3675-4173

Original submission:
04 April 2017;
Revised submission:
20 April 2017;
Accepted:
28 April 2017.
doi: 10.17392/908-17

Med Glas (Zenica) 2017; 14(2): 229-235

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in human pathology, which was estimated as the third cause of mortality due to cancer disease in the United States in 2016 and the second most common cause of death from cancer in the European Union (EU) according the statistics for 2015 (1,2). More than a half of colorectal cancers are still diagnosed only when the disease involves regional or distant organs (1). This cancer is a heterogeneous disease that occurs only by complex and partially known sequence of molecular events. It is believed that a significant role in the pathogenesis of this disease has disrupted the relationship of apoptosis and proliferation of tumour cells, increased oxidative stress and impaired antioxidant protection (3-5). In addition, proteolytic enzymes play a sophisticated role in cancer development and progression due to their abilities to degrade various substrates (6). Matrix metalloproteinases (MMP) are multigenic family of structurally similar proteolytic enzymes. These are zinc-dependent endopeptidases, which have the capacity to degrade almost any extracellular matrix component (7). It is believed that tumour cells perform overexpress of protease enzyme activity causing the action of enzymes in the adjacent stromal cells for the purpose of degradation of the basement membrane and invasion of the surrounding tissue (8).

Higher activity of MMP and their over-expression has been confirmed in several malignant conditions, such as colon cancer (9), lung cancer (10), pancreas cancer (11), ovarian cancer (12), prostate cancer (13), breast cancer (14) and brain cancer (15), and its correlation with tumour aggressiveness and its malignant potential. Some studies have presented conflicting results regarding the expression of the most commonly related MMP-2 (gelatinase A), MMP-7 (matrilysin) and MMP-9 (gelatinase B) with the prognosis in patients with colorectal cancer (16,17). Recent publications about this cancer indicate that serum levels of matrix metalloproteinases 9 (MMP-9) are elevated in the patients with colorectal cancer (18,19) and can act even in non-proteolytic way (20). Immunohistochemical staining confirmed that the MMP-9 is very positive in the colorectal cancer cells and confirmed that tumour associated macrophages are significant sources of MMPs in the process of carcinogenesis (21). However, the question of the association of serum levels of MMP-9 with the different histopathologic size and progression of colorectal cancer still remains open (22).

The aim of the study was to analyse the serum concentration of MMP-9 in the patients in various depth of wall invasion, lymph nodes involvement and stage of colorectal cancer, estimated on the basis of preoperative, intraoperative findings and histological findings made after surgical resection of tumour altered organ, and to determine predictive significance of MMP-9 in the disease progression.

PATIENTS AND METHODS

Patients and study design

The research was designed as a cross-sectional study conducted from January 2014 to December 2015 at the Clinic for General and Abdominal Surgery, Clinical Centre of the University of Sarajevo (CCUS). A total of 102 patients of both genders with clinically diagnosed and histologically confirmed colorectal cancer who met the inclusion criteria were included in the study. Inclusion criteria were clinically diagnosed and histologically confirmed colorectal cancer and a need for surgical treatment of CRC. Exclusion criteria were evidence of neoplasm on an organ that is not linked to colorectal cancer, patients treated by preoperative radiotherapy or chemotherapy, those with inflammatory bowel disease or a known history of familial adenomatous polyposis and refusing surgical treatment.

A control group included 30 healthy volunteers of the appropriate age and gender without family history of cancer or clinical signs of malignant or inflammatory bowel disease, who went for preventive examinations at the Gastroenterology Counselling Centre. Informed consents were obtained from all participants. The study was approved by the Ethics Committee of the Clinical Centre and the Ethics Committee of the School of Medicine, University of Sarajevo.

Methods

Preoperative confirmation of the diagnosis of colorectal cancer was prepared on the basis of clinical examination, colonoscopy with biopsy following the histological evidence of tumour

lesions and additional radiological findings (chest X-ray, ultrasound and computed tomography of the abdomen and pelvis, and if necessary, other radiological imaging). Complete preoperative diagnostics was carried out in the institutions of the Clinical Centre of the University in Sarajevo.

A sample of 5 mL of peripheral venous blood was collected from each CRC patient for MMP-9 determination. Blood sample was taken before the surgical treatment of colorectal cancer in BD Vacutainer test tube with no additive, while the control blood was taken on the day of physical examination. Each test tube with blood sample was labelled with patient's number and promptly transported to the laboratory, where it was immediately centrifuged at 1.006 x g for 10 minutes at room temperature followed by separation of serum. All serum samples were stored at -80°C until analysis of the serum concentration of MMP-9. Serum concentration of MMP-9 was quantified on duplicate aliquots of each sample using the technique of enzyme-linked immunosorbent assay (ELISA) at the Department of Clinical Immunology of CCUS according to the manufacturer's instructions (R&D Systems, Inc; RD-DMP900). Reading of the results was carried out spectrophotometrically at 450 nm on a plate reader BioTek ELX50, with the correction wavelength at 540 nm or 570 nm. The measurement concentration of MMP-9 was expressed in nanograms per millilitre (ng/mL).

Surgical treatment of colorectal cancer (right hemicolectomy, left hemicolectomy or resection of the rectum, synchronous operation colon with metastasectomies) was performed as part of the therapeutic process under general anaesthesia. Oncology surgery principle "en bloc" resection of colon cancer with associated lymph-vascular arcade was complied with during the operative treatment. After surgical resection and macroscopic examination of surgically obtained samples of colon cancer, samples of the tumour mass and lymphatic nodes were taken for microscopic analysis.

Resected tumour specimens were studied histologically. Histological examination included determination of histological type of malignancy according to the categorization of the World Health Organization, the depth of tumour invasion in the intestinal wall (pT) and the number of pericolic lymph nodes infiltrated with cancerous tissue (pN) (23). The TNM classification of the American Joint Committee on Cancer of 2010 (24) was used for staging of colorectal cancer, in which "T" marks the degree of invasion of the intestinal wall, "N" level of involvement of lymph nodes and "M" means metastases and spread staging. Staging of the colorectal cancer was marked with numbers I to IV. All patients were divided into groups according to the TNM classification (24).

Statistical analysis

All data were expressed as the mean ± standard deviation (SD) or as median and interquartile range. The normality of data distribution was determined by Kolmogorov-Smirnov test or Shapiro-Wilk test. Comparison of mean values between two groups was performed using Student's t-test for variables with normal distribution and using Mann Whitney U test for variables without normal distribution. Kruskall-Wallis test was used for statistical evaluation of more than 3 groups. A model of regression analysis was applied to examine the impact of MMP-9 on stage, depth of CR-wall invasion (pT) and carcinoma infiltration lymph nodes. The level of significance was set at $p<0.05$.

RESULTS

Clinical and histopathologic colorectal cancer was more commonly confirmed in older people, mean age was 66.2 (range 47-78) years of which 61 (60%) were males. Adenocarcinoma was found in 100% of cases, predominantly grade 2, in 72 (70.6%) patients. The most common location of colorectal cancer was in the area of rectum, 34 (33.3%), and the rarest in the area of cecum, three (2.9%). According to the depth of tumour invasion of the intestine, histopathological pT3 was most common, in 65 (63.3%), while pT2 was the least common, in nine (8.9%) patients. Distant metastasis was found in 29 (28.4%) patients, mostly liver metastasis, in 26 (25.4%) patients. The serum concentration of MMP-9 was significantly higher in patients with CRC compared to the control group, 446.8 (433.2-454.3) vs. 323.6 (236.2-374.1) ng/mL ($p<0.001$) (Table 1).

Table 1. Demographic and clinical characteristics of the control group and colorectal cancer group patients

Demographic and clinical parameter	Control (n=30)	CRC (n=102)	p
Age (years) (median; min, max)	64.1 (47-78)	66.2 (51-78)	0.06
Males (No, %)	14 (46.7%)	61 (60%)	0.20
MMP-9 (ng/mL) (median and interquartile range)	323.6 (236.2-374.1)	446.8 (433.2-454.3)	0.001
Localization of CR cancer (No, %)			
Cecum		3 (2.9%)	-
Ascendens		15 (14.7%)	-
Transversum		3 (2.9%)	-
Descendens		6 (5.9%)	-
Sigmoid		22 (21.5%)	-
Rectosigmoid		19 (18.6%)	-
Rectum		34 (33.3%)	-

MMP-9, matrix metalloproteinase 9; CRC, colorectal cancer

Statistically significant differences in serum MMP-9 concentration in different stages of colorectal cancer and pericolonic lymph nodes infiltrated with cancerous tissue was found ($p < 0.01$) (Table 2). The serum level of MMP-9 was statistically significantly higher in the third stage of CRC as compared to the second stage of CRC, 454.8 (447.0-461.6) and 437.9 (403.7-444.8) ng/mL, respectively ($p<0.001$). Registered dynamics of the change of this parameter according to the depth of the CR-wall invasion (pT) indicated that the concentration of MMP-9 in serum is significantly higher in pT4 group as compared to pT3 group of the CRC patients, 459.5 (453.1-464.6) and 447.0 (436.8-457.0) ng/mL, respectively ($p<0.001$).

Table 2. Serum concentration of matrix metalloproteinase 9 (MMP-9) according to the stage of colorectal cancer (CRC), depth of CR-wall invasion (pT), lymph node involvement and metastatic disease

Characteristics	MMP-9 (ng/mL) (median and interquartile range)	p
Stage of CRC		
I stage	436.1 (409.9-442.8)	
II stage	437.9 (403.7-444.8)	
III stage	454.8 (447.0-461.6)*	<0.01
IV stage	458.5 (452.0-468.1)*	
	stage II vs stage I	0.145
	stage III vs stage II	0.000
	stage IV vs stage II	0.000
	stage IV vs stage III	0.102
Depth of CR-wall invasion (pT)		
pT2	450.4 (438.8-454.8)	
pT3	447.0 (436.8-457.0)	<0.01
pT4	459.5 (453.1-464.6)*	
	pT3 vs. pT2	0.396
	pT4 vs. pT2	0.081
	pT4 vs. pT3	0.000
Lymph node involvement		
N0	438.4 (418.4-447.8)	
N1	456.4 (445.9-464.7)*	<0.01
N2	456.6 (449.8-464.5)*	
	N1 vs. N0	0.000
	N2 vs N1	0.543
	N2 vs N0	0.000
Metastatic disease		
M0	445.8 (436.9-456.5)	
M1	458.5 (452.0-468.1)	<0.001

*p<0.001, the difference between stages III – II and stages IV - II, between pT4 and pT3, between N1 and N0, and between N2 and N0.

Statistically significant differences were confirmed in the average level of MMP-9 in sera in cohort group of patients according to the existing infiltration of the lymph nodes ($p<0.01$). Mean value of the MMP-9 serum concentration was higher in the group N1 (the abstraction of 1-3 regional lymph node with cancer tissue) compared to N0 group of patients with no lymph node presentation in cancerous tissue, as well as in group N2 (metastases to 4 or more regional lymph nodes) compared to N0 group without lymph nodes involvement. It was also confirmed that the serum level of MMP-9 was significantly higher in patients with metastatic CRC than in those without metastasis, 458.5 (452.0-468.1) vs. 445.8 (436.9-456.5) ng/mL ($p<0.001$).

The model of regression analysis showed that MMP-9 serum concentrations were an independent predictor for the stage of colorectal cancer ($p<0.001$) as well as of the carcinoma infiltration lymph nodes ($p<0.05$) (Table 3).

Table 3. Matrix metalloproteinase 9 (MMP-9) as an independent predictor of colorectal cancer (CRC) progression

	B	SE	Beta	t	p	CI 95.0% (lower – higher)	
Constant	-1.711	0.692		-2.472	0.015	-3.083	-0.339
MMP-9	0.007	0.001	0.351	6.390	0.000	0.005	0.009
The dependent variable: the stage of CRC							
Constant	-2.143	1.149		-1.865	0.066	-4.426	0.141
MMP-9	0.006	0.002	0.246	2.632	0.010	0.002	0.011
Dependent variable: carcinoma infiltration of the lymph nodes							

SE, standard error; CI, confidence interval; B, regression coefficient

DISCUSSION

Matrix metalloproteinases are involved in the remodelling of extracellular matrix in a variety of normal and pathological processes, such as morphogenesis, angiogenesis, tissue repair. In some solid tumours this enzyme enhances tumour invasion and metastasis ability (25). Several studies have shown that matrix metalloproteinase can play an important role as indicators of colorectal cancer and its progression (26,27). The question of the significance of these enzymes, particularly MMP-9

and their serum levels correlation with progression of colorectal cancer remains open (22).

The results of our study indicate that the concentration of MMP-9 in the serum of patients with colorectal cancer was significantly higher than in the control group. Significant increase in serum levels of MMP-9 was observed with disease progression. Although the highest value was found in patients in the fourth stage of colorectal cancer, we found that serum MMP-9 levels were significantly increased in stage III patients compared to stage II patients, as well as in stage IV patients compared to stage II patients. Biasi et al. reported a statistically significant increase in the serum enzymatic activity and protein levels of MMP-9 only for the second and the third stage of colorectal cancer, comparable to the tendency we found (28). They also confirmed by immunohistochemistry that MMP-9 was clearly produced in large amounts in tumours at stage two and third.

Metastasis of cancer is a complex, multifactorial and multistage process, involving stromal invasion, penetration into the bloodstream from the primary site, the secondary site extravasation and growth of new tumours (29). These processes require degradation of extracellular matrix by proteolytic enzymes, among which the most important are the matrix metalloproteinases (6). In our research, it was found that the concentration of matrix metalloproteinase 9 in patients with colorectal cancer was significantly higher in patients with metastatic form of the disease, suggesting that MMP-9 is included in the cancer invasion and formation of metastases. A study of Hurst and colleagues also indicates elevated serum levels of MMP-9 in patients with colorectal cancer (18). Wilson et al. demonstrated that higher serum MMP-9 concentrations were significantly associated with the presence of colorectal neoplasia, with the suggestion that MMP-9 probably has the highest predictive value when used as part of a panel of biomarkers (19).

Conversely, some authors did not show clear association of MMP-9 with significant colorectal pathology despite robust sampling protocols in evaluation of accuracy of a serum matrix metalloproteinase 9 test in indicating colorectal cancer or its precursor conditions in a symptomatic population (30). Recent study of Jonsson et al. demonstrated significant differences regarding concentrations of some matrix metalloproteinases including MMP-9, using plasma vs serum (31). In this study all MMPs expressed higher concentration in serum compared with plasma. The authors of this study considered that measurements in serum could reflect the release of proteases by involved blood cells during the clotting process and that the use of anticoagulant in the collected blood prevent this artefact, and therefore they recommended the use of plasma samples for future studies of these proteases.

Herszényi et al. showed that the matrix metalloproteinases and their tissue inhibitors (TIMPs) are particularly important in the process of tumour invasion, progression and metastasis of colorectal cancer (32). The results of our study also suggest that MMP-9 plays an important role during the metastasis of colorectal cancer. Research by Ting and associates suggests that genetic variations in MMP-2 and MMP-9 may be potential predictors of survival without the existence of distant metastases after curative surgery (33). Recent studies have also shown that the level of gene expression and enzyme activity of MMP-2 and MMP-9 correlates with the initiation, progression, angiogenesis, metastasis and recurrence of colorectal cancer (34-36). In a study Said et al. (37) the level of expression of MMP-1, -2, -7, -9 and -13 is correlated with poor outcome, and MMP-12 may be protective. Such findings suggest that the matrix metalloproteinase can be an attractive therapeutic target.

The results of linear regression analysis in our study indicate that serum MMP-9 concentration is statistically significant independent positive predictor of stages of colorectal cancer and carcinoma infiltration of pericolonic lymph nodes.

In conclusion, a significant increase in serum concentrations of MMP-9 in relation to the stage of CRC, depth of CR-wall invasion, lymph nodes involvement and present of metastatic disease indicates the involvement of MMP-9 in carcinogenesis and progression of human colorectal cancer. The search for non-invasive markers and useful parameters progression of neoplastic diseases of the colon is of great importance. Our findings suggest that serum MMP-9 could establishes itself as a "liquid biopsy" parameter, but there is a need for additional clinical studies with a large group of patients with CRC and meta-analysis to determine the "cut-off" values for different stages of colorectal cancer.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATIONS

Competing interests: none to declare.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66:7-30.
2. Malvezzi M, Bertuccio P, Rosso T, Rota M, Levi F, La Vecchia C, Negri E. European cancer mortality predictions for the year 2015: does lung cancer have the highest death rate in EU women? Ann Oncol 2015; 26:779-86.
3. Fearonn ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61:759-67.
4. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interac 2006; 160:1-40.
5. Gopčević KR, Rovčanin BR, Tatić SB, Krivokapić ZV, Gajić MM, Dragutinović VV. Activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in different stages of colorectal carcinoma. Dig Dis Sci 2013; 58:2646-52.
6. Herszényi L, Barabás L, Hritz I, István G, Tulassay Z. Impact of proteolytic enzymes in colorectal cancer development and progression. World J Gastroenterol 2014; 20:13246–57.
7. Iyer RP, Patterson NL, Fields GB, Lindsey ML. The history of matrix metalloproteinases: milestones, myths, and misperceptions. Am J Physiol Heart Circ Physiol 2012; 303:H919-30.
8. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clinl Oncol 2009; 27:5287-97.
9. Hong SW, Kang YK, Lee B, Lee WY, Jang YG, Paik IW, Lee H. Matrix metalloproteinase-2 and -7 expression in colorectal cancer. J Korean Soc Coloproctol 2011; 27:133-9.
10. El-Badrawy MK, Yousef AM, Shaalan D, Elsamanoudy AZ. Matrix metalloproteinase-9 expression in lung cancer patients and its relation to serum MMP-9 activity, pathologic type, and prognosis. J Bronchology Interv Pulmonol 2014; 21:327-34.
11. Mroczko B, Lukaszewicz-Zajac M, Wereszczynska-Siemiatkowska U, Groblewska M, Gryko M, Kedra B, Jurkowska G, Szmitkowski M. Clinical significance of the measurements of serum matrix metalloproteinase-9 and its inhibitor (tissue inhibitor of metalloproteinase-1) in patients with pancreatic cancer: metalloproteinase-9 as an independent prognostic factor. Pancreas 2009; 38: 613-8.
12. Zhang W, Hu X-x, Yang X-z, Wang Q, Cheng H, Wang S-m, Yan-ling Hu, Yang Z-j, Li L. Combined detection of serum matrix metalloproteinase 9, acetyl heparinase and cathepsin L in diagnosis of ovarian cancer. Chin J Cancer Res 2012; 24: 67–71.
13. Zhang L, Shi J, Feng J, Klocker H, Lee C, Zhang J. Type IV collagenase (matrix metalloproteinase-2 and -9) in prostate cancer. Prostate Cancer Prostatic Dis 2004; 7:327-32.
14. Provatopoulou X, Gounaris A, Kalogera E, Zagouri F, Flessas I, Goussetis E, Nonni A, Papassotiriou I, Zografos G. Circulating levels of matrix metalloproteinase-9 (MMP-9), neutrophil gelatinase-associated lipocalin (NGAL) and their complex MMP-9/NGAL in breast cancer disease. BMC Cancer 2009; 9:390.
15. Hormigo A, Gu B, Karimi S, Riedel E, Panageas KS, Edgar MA, Tanwar MK, Rao JS, Fleisher M, DeAngelis LM, Holland EC. YKL-40 and matrix metalloproteinase-9 as potential serum biomarkers for patients with high-grade gliomas. Clin Cancer Res 2017; 23:1422-31.
16. Herszényi L, Hritz I, Lakatos G, Varga MZ, Tulassay Z. The behavior of matrix metalloproteinases and their inhibitors in colorectal cancer. Int J Mol Sci 2012; 13:13240-63.
17. Yang B, Su K, Gao J, Rao Z. Expression and prognostic value of matrix metalloproteinase-7 in colorectal cancer. Asian Pac J Cancer Prev 2012; 13:1049-52.
18. Hurst NG, Stocken DD, Wilson S, Keh C, Wakelam MJ, Ismail T. Elevated serum matrix metalloproteinase 9 (MMP-9) concentration predicts the presence of colorectal neoplasia in symptomatic patients. Br J Cancer 2007; 97:971-7.
19. Wilson S, Damery S, Stocken DD, Dowswell G, Holder R, Ward ST, Redman V, Wakelam MJ, James J, Hobbs FD, Ismail T. Serum matrix metalloproteinase 9 and colorectal neoplasia: a community-based evaluation of a potential diagnostic test. Br J Cancer 2012; 106:1431-8.
20. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 2010; 141:52-67.
21. Chu D, Zhao Z, Zhou Y, Li Y, Li J, Zheng J, Zhao Q, Wang W. Matrix metalloproteinase-9 is associated with relapse and prognosis of patients with colorectal cancer. Ann Surg Oncol 2012; 19:318–325.
22. Emara M, Cheung P-Y, Grabowski K, Sawicki G, Wozniak M. Serum levels of matrix metalloproteinase-2 and -9 and conventional tumor markers (CEA and CA 19-9) in patients with colorectal and gastric cancers. Clin Chem Lab Med 2009; 47:993–1000.
23. Hamilton SR, Aaltonen LA. (Eds). Pathology and Genetics of Tumours of the Digestive System. In: Kleihues P, Sobin LH. (series eds.) World Health Organization Classification of Tumours. Lyon: IARCPress, 2000:104.
24. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. AJCC cancer staging manual (7th ed). New York: Springer-Verlage, 2010.
25. Sun J. Matrix metalloproteinases and tissue inhibitor of metalloproteinases are essential for the inflammatory response in cancer cells. J Signal Transduct 2010; 2010:985132.

26. Bendardaf R, Lamlum H, Pyrhönen S. Prognostic and predictive molecular markers in colorectal carcinoma. Anticancer Res 2004; 24:2519-30.
27. Vihinen P, Kähäri V. Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets. Int J Cancer 2002; 99:157-66.
28. Biasi F, Guina T, Maina M, Nano M, Falcone A, Aroasio E, Saracco GM, Papotti M, Leonarduzzi G, Poli G. Progressive increase of matrix metalloprotease-9 and interleukin-8 serum levels during carcinogenic process in human colorectal tract. PLoS ONE 2012; 7: e41839.
29. Talmadge JE, Fidler IJ. AACR centennial series: the biology of cancer metastasis: historical perspective. Cancer Res 2010; 70:5649-69.
30. Damery S, Nichols L, Holder R, Ward ST, Warmington S, Wilson S, Wakelam MJ, James J, Ismail T. Assessing the value of matrix metalloproteinase 9 (MMP9) in improving the appropriateness of referrals for colorectal cancer. Br J Cancer 2013; 108:1149-56.
31. Jonsson A, Hjalmarsson C, Falk P, Ivarsson ML. Levels of matrix metalloproteinases differ in plasma and serum - aspects regarding analysis of biological markers in cancer. Br J Cancer 2016; 115:703-6.
32. Herszényi L, Hritz I, Lakatos G, Varga MZ, Tulassay Z. The behavior of matrix metalloproteinases and their inhibitors in colorectal cancer. Int J Mol Sci 2012; 13:13240-63.
33. Ting WC, Chen LM, Pao JB, Yang YP, You BJ, Chang TY, Lan YH, Lee HZ, Bao BY. Genetic polymorphisms of matrix metalloproteinases and clinical outcomes in colorectal cancer patients. Int J Med Sci 2013; 10:1022-7.
34. Asano T, Tada M, Cheng S, Takemato N, Kuramae T, Abe M, Takahashi O, Miyamato M, Hamada J, Moriuchi T, Kondo S. Prognostic values of matrix metalloproteinase family expression in human colorectal carcinoma. J Surg Res 2008; 146:32-42.
35. van der Jagt MFP, Wobbes T, Strobbe LJA, Sweep FCGJ, Span PN. Metalloproteinases and their regulators in colorectal cancer. J Surg Oncol 2010; 101:259-69.
36. Zucker S, Vacirca J. Role of matrix metalloproteinases (MMPs) in colorectal cancer. Cancer Metastasis Rev 2004; 23:101-17.
37. Said AH, Raufman J-P, Xie G. The role of matrix metalloproteinases in colorectal cancer. Cancers 2014; 6:366-75.

Udruženost između serumskih nivoa matriks metaloproteinaze 9 i kolorektalnog karcinoma

Ismar Rašić[1], Azra Rašić[2], Goran Akšamija[1], Svjetlana Radović[3], Nedžad Šehović[4]

[1]Klinika za opću i abdominalnu hirurgiju, [2]Klinika za onkologiju; Klinički centar Univerziteta u Sarajevu, Sarajevo, Bosna i Hercegovina, [3]Katedra za patologiju, Medicinski fakultet, Univerzitet u Sarajevu, Sarajevo, Bosna i Hercegovina, [4]Regional Hospital Haugesund, Gastrosurgical Department, Haugesund, Norwey

SAŽETAK

Cilj Utvrditi nivoe koncentracije matriks metaloproteinaze 9 (MMP-9) u serumu i njihove povezanosti sa stadijem i patohistološkom veličinom kolorektalnog karcinoma (KRK).

Metode Stotinu i dva pacijenta sa klinički i patohistološki potvrđenom dijagnozom kolorektalnog karcinoma, koji su bili za hirurško liječenje, uključeni su u studiju. U svakog pacijenta preoperativno su uzeti uzorci periferne venske krvi za određivanje koncentracije MMP-9 pomoću ELISA-testa. Resecirani uzorci tumora proučavani su patološki prema kriterijima TNM klasifikacije, te su i pacijenti podijeljeni u grupe prema TNM klasifikaciji. Kontrolnu grupu predstavljalo je 30 ispitanika odgovarajuće dobi i spola, bez porodične historije karcinoma, kliničkih znakova maligniteta ili upalne bolesti crijeva.

Rezultati Serumski nivoi MMP-9 progresivno su se povećavali u pacijenata sa KRK-om dostigavši najveću vrijednost u četvrtom stadiju bolesti. Također je potvrđeno da su koncentracije MMP-9 u serumu bile značajno više u bolesnika sa zahvaćenim u odnosu na bolesnike bez zahvaćenih perikoličnih limfnih čvorova 456,4 (445,9-464,7) ng/mL nasuprot 438,4 (418,4-447,8) ng/mL ($p<0,001$). Statistički značajno veći serumski nivoi MMP-9 nađeni su u pacijenata sa metastatskim kolorektalnim karcinomom, 458,5 (452,0-468,1) ng/mL, u odnosu na pacijente bez metastatske bolesti, 445,8 (436,9-456,5) ng/mL ($p<0,001$).

Zaključak Potvrđeno je da je serumska koncentracija MMP-9 predstavljala značajan neovisan faktor rizika za progresiju KRK-a.

Ključne riječi: karcinom debelog crijeva, proteolitički enzim, serum, rizik, progresija

ORIGINAL ARTICLE

Early results of the conservative treatment of distal radius fractures-immobilization of the wrist in dorsal versus palmar flexion

Maki Grle[1], Miro Miljko[2], Ivana Grle[3], Faruk Hodžić[4], Tarik Kapidžić[4]

[1]Department for Orthopaedics, [2]Department for Radiology, [3]Department for Physiotherapy; University Clinical Hospital Mostar, [4]Department for Traumatology and Orthopaedics, Cantonal Hospital Zenica; Bosnia and Herzegovina

ABSTRACT

Aim To evaluate immobilization with dorsal forearm plaster splint with the wrist in dorsal flexion vs palmar flexion in patients with distal radius fracture.

Methods In the prospective study (2012-2014) 122 patients (of which 22 patients lost) with fractures of the distal radius type A2, A3 and C according to the AO classification were investigated. At the end there were 50 patients in each of the two groups: the dorsiflexion (DF) group had a total of 37 women and 13 men, mean age was 63.48 ± 14.70, and in the palmar flexion (PF) group there were respectively 38/12, and the mean age was 64.20 ± 12.99. In both groups measurements of radiological, clinical and functional parameters were conducted. Patient related wrist evaluation survey (PRWE) and SF12 questionnaire were used for evaluation of pain and function of the wrist and physical and mental condition, respectively.

Results The study showed excellent results in both groups but there was significant improvement in the range of motion (ROM) on every measurement in the DF group: dorsal flexion 47.70±15.29; ulnar deviation 24.10±7.80; radial deviation 11.50±5.65 vs PF 22.80±19.04; 16.00± 9.31; 4.80± 494 ($p<0.001$). Also, radiological parameters showed significant improvement until the end of the follow-up. Functional parameters showed significant improvement of physical component of SF-12 in the DF group ($p<0.014$).

Conclusion Immobilization with forearm plaster splint on the dorsal side and with the wrist in dorsiflexion gives better early clinical, radiological and functional results in patients with fractures of type A2, A3, C1-3 in patients of all age groups, compared to immobilization with the wrist in palmar flexion.

Keywords: wrist, distal radius fracture, conservative treatment, splints

Corresponding author:
Maki Grle
Department for Orthopaedics,
University Clinical Hospital Mostar
Bijeli Brijeg bb, 88000Mostar,
Bosnia and Herzegovina
Phone: +387 36 336 297;
Fax; +387 36 336 297;
E-mail: grlemaki@gmail.com
ORCID ID: http://www.orcid.org/0000-0003-3816-4521

Original submission:
28 October 2016;
Revised submission:
08 November 2016;
Accepted:
11 April 2017.
doi: 10.17392/888-17

Med Glas (Zenica) 2017; 14(2): 236-243

INTRODUCTION

According to the Arbeitsgemeinschaft für Osteosynthesefragen (AO) classification, which is the preferred classification system, distal radius fractures are divided into extra-articular (A), partial articular (B) and complete articular (C) (1-4). There is a consensus about the best way of treatment only for the B type fracture, e.g. open reduction and internal fixation (5,6). Fractures without displacement can be treated conservatively and only displaced fractures without stability parameters (shortening of the radius <3mm, articular step<2 mm, and dorsal inclination <10°) should be treated surgically (4). However, the same authors cannot suggest the best type of treatment for the fractures with displacement which are stabile after reposition, means within stability parameters (7-9). On the other hand, any type of surgical treatment of stabile fractures with displacement does not give better results in terms of improvement of function versus conservative treatment (10-14). Today there are different conservative approaches in traumatology in terms of the type of immobilization,duration of immobilization, immobilization level, repositioning techniques and rehabilitation (15,16). For most authors, the treatment of distal radius fracture consists of immobilization with forearm dorsal plaster splint with the wrist in a position of palma reflexion and ulnar adduction (14,17). Ligamentotaxis is based on the preservation of the positions of fracture fragments using strained surrounding soft tissues (18). The first ligamentotaxis techniques consisted of the placement of the wrist in position of maximum palmare flexion which led to a great number of complications in terms of neuropathy of median nerve or extensor pollicis tend on rupture. Therefore, it was replaced by a modification of the same position as suggested by Charlney and Bölerwith the 20° of palmar flexion and 20°of ulnar adduction, which reduced the incidence of these complications (19,20). On the other hand, better functional, clinical and radiological results in the intra and extra articular fractures of the distal radius were demonstrated in patients with the same type of immobilization, but with the wrist in the position of dorsal flexion and ulnar adduction (21-23).

The aim of this study was to compare two types of ligamentotaxis or immobilisation of wrist in dorsal and palmar flexion. Based upon our experience and above mentioned positive results, immobilisation of the wrist in dorsiflexion/adduction should give better results than the immobilisation in palmar flexion/ulnar adduction in patients with distal radius fracture.

PATIENTS AND METHODS

Patients and study design

In this prospective cohort study122 patients admitted to the Trauma Centre of the University Clinical Hospital Mostar, Bosnia and Herzegovina, from spring of 2012 to spring 2014 with distal radius fracture.

Inclusion criteria were patients with distal radius fracture and the age above 25 years. Exclusion criteria were previous fracture in the same place, associated diseases such as diabetes mellitus and rheumatoid arthritis, open distal radius fractures, ulna fracture (except fracture of the styloid process), patients with unstable distal radius fractures (fractures that are unstable immediately after the reposition or on the control after 7-days and Smith's fracture). Patients were selected in the groups, oral clarification was made by an examiner obtaining informed patients' consents. Institutional review board and the Ethical Committee of the University Clinical Hospital Mostar approved the investigation. The patients were divided in two groups: with immobilisation of the wrist in dorsal flexion and ulnar deviation (DF group), and in group with immobilisation of the wrist in palmar flexion and ulnar deviation (PF group).

At the beginning there were 62 patients in the DF group and 60 in the PF group. During the follow-up period 22 patients were lost (15 patients did not come to the control and seven did not satisfy stability parameters). In the PF group seven patients were lost because they did not come to the control and three patients were opted to another type of treatment due to instability.

At the end of the investigation 50 patients remained in each group; mean age was 63.48±14.70 in DF group and 64.20±12.99 in PF group; 25 were males and 75 females.

Methods

After hematoma block with 4mL of 2% lidocaine injected in the fracture site, fractures were manipulated with traction by two assistants with

forearm in pronation. Upon the reduction of fragments, the immobilisation with plaster splint on the dorsal side was done. In the DF group with constant counter traction of two assistants, the surgeon was giving a pressure on the distal radius fragment while the assistant was bringing the wrist in 20° of dorsal flexion and minimal deviation. In the PF group everything was done in the same way, except for the wrist that was positioned in 20°of palmar flexion and minimal ulnar deviation which was completely done by the surgeon, while the upper arm counter traction was done by the assistant. After wrist anteroposterior (AP) and lateral radiographs (L) was done, the measurements of radiological parameters of volar inclination and the radial height and the radial inclination were taken, and in the case of intra articular fracture ‘’step off’’ was measured. Radial height was measured on AP: two lines perpendicular to the radial shaft were drawn; one was drawn along the articular surface and the second one along the styloid tip (a normal measure 9.9mm - 17.3mm). Radial inclination was measured on AP: the angle of the distal radial surface with respect to a line perpendicular to the shaft (a normal slope should be 15° - 25°). Volar inclination was measured on L: the angle of the distal radial surface with respect to a line perpendicular shaft (10°- 25° was considered normal). If the fracture was stable (shortening of the length of radius bone<3mm, dorsal inclination 10° and intra articular step<2mm), the patient would have the next control in 7days; in case that the control radiograph demonstrated the stability, the next control was in four weeks after the immobilisation, and in case of instability at the first appointment the patient left the study and was referred to a different form of the treatment.

After 4 weeks from the beginning of the study the immobilization was removed, a new radiograph control was conducted during which again the radiological measurement of radial height (RH) in millimetres (mm) and radial inclination (RI) and palmar inclination (PI) in degrees (°) were measured, and in addition, clinical measurements of the range of motion (ROM) with goniometer (expressed in degrees) (dorsal and palmar flexion DF/PF, ulnar/radial deviation UD/RD), grip strength (GS) (mmHg, with pressure gauge) were made.

Patient rated wrist evaluation surveys (PRWE) were conducted examining pain and function of the wrist. The PRWE questionnaire consisting of 15 questions related to pain and disability in daily activities of the wrist. The PRWE allows the patient to assess their pain and disability from 0-10 with two sets of questions with regard to pain- 5 items (0 = no pain, 10 = strongest ever) and the function- 10 items (6 specific and 4 common activities; 0 = feasible without difficulty, 10 = impossible). The total number of points for both groups of questions was 100 (0 = no difficulty), where items for pain and function carry the same number of points (24).

The SF 12 questionnaire (short form) that examined the general physical and mental condition of the patient were also conducted. The SF 12 is a questionnaire that measures the quality of health through subjectively described physical and mental condition. It consists of 12 questions taken from the larger questionnaire SF-36. At the end of the questioning, the questionnaire obtained separate sums for both domains (physical and mental component of SF 12 survey SFPCS/SFMCS) through the sum of all 12 questions (25).

The next examination was made after two months when all radiographic, clinical and functional measurements were made. The patients were observed for all related recognized complications during the follow-up.

Statistical analysis

Data were processed using descriptive and inferential statistical methods. Continuous variables were presented as arithmetic mean and standard deviation. Distribution of the sample population was tested using Kolmogorov-Smirnov test. It was assessed the normality of the distribution for all measures and for each group. Student t-test used for testing of differences for continuous variables was used. The p-value of <0.05 was taken as statistically significant for all measurements.

RESULTS

Two groups of patients were equalac cording to all observed parameters, i.e. they were comparable.

On the first and second measurement, patients in DF group had a significantly greater range of motion. The parameter of range of motion was significantly higher on each control in the DF group (DF 40.70°, UD 24.10°, RD 11.50°; p<0.001) vs PF group (Table 1).

Table1. Comparison of clinical parameters between dorsiflexion (DF) and palmar flexion (PF) groups

Variable	Group				t	p
	DF		PF			
	$\bar{X}$	SD	$\bar{X}$	SD		
Age	63.48	14.70	64.20	12.99	0.260	0.796
After immobilisation removal						
Palmar flexion(°)	47.80	16.39	42.50	21.07	1.404	0.164
Dorsal flexion(°)	40.70	15.29	22.80	19.04	5.184	<0.001
Ulnar deviation (°)	24.10	7.80	16.00	9.31	4.714	<0.001
Radial deviation (°)	11.50	5.65	4.80	4.94	6.312	<0.001
Strength (mmHg)	49.50	19.20	43.40	15.99	1.726	0.087
Two months after immobilisation removal						
Palmar flexion (°)	63.60	13.52	64.90	14.41	0.465	0.643
Dorsal flexion(°)	60.70	14.95	53.90	20.78	1.878	0.064
Ulnar deviation (°)	29.00	4.95	24.40	6.52	3.974	<0.001
Radial deviation (°)	17.80	5.55	14.80	7.28	2.317	0.023
Strength (mmHg)	76.80	23.40	70.10	16.80	1.645	0.104

Radio logical parameters demonstrated a significant difference between the two groups in terms of better restoration of anatomy in the DF group (RH 11.67 mm; p=0.003; RI 5.34°; p<0.001), except for palmar inclination that was significantly better resort on the first measurement in PF group (PI 11.84°; p=0.001). Both types of immobilization give radiological results that have a positive effect on the improvement of the patient's condition, but with statistically better improvement in the DF group (p=0.001) (Table 2).

Table 2. Comparison of radiological parameters between dorsiflexion (DF) and palmar flexion (PF) groups

Variable	Group				t	p
	DF		PF			
	$\bar{X}$	SD	$\bar{X}$	SD		
Age	63.48	14.70	64.20	12.99	0.260	0.796
After reposition						
Radial height (mm)	11.67	1.67	10.36	2.53	3.056	0.003
Radial inclination (°)	24.32	3.14	20.00	4.98	5.191	<0.001
Palmar inclination (°)	5.34	6.28	11.84	11.16	3.589	0.001
After immobilisation removal						
Radial height (mm)	10.41	1.73	9.34	1.81	3.017	0.003
Radial inclination (°)	20.64	4.43	18.18	4.63	2.713	0.008
Palmar inclination(°)	3.30	7.01	3.50	6.26	0.150	0.881
Two months after immobilisation removal						
Radial height (mm)	10.18	1.83	9.12	1.89	2.851	0.005
Radial inclination (°)	20.02	4.76	17.34	4.52	2.889	0.005
Dorsal inclination (°)	2.70	7.35	3.18	5.91	0.360	0.720

The PRWE survey showed no significant differences between the two groups. The results of the SF12 survey showed a significant difference on the second control a in physical component between the two groups with a better out come in the DF group (SFPCS43.10, p=0.014). Functional results showed a particularly significant decrease in pain and increase in functions proven through results of the SF 12 survey (Table 3).

Table 3. Comparison of functional parameters between dorsiflexion (DF) and palmar flexion (PF) groups

Variable	Group				t	p
	DF		PF			
	$\bar{X}$	SD	$\bar{X}$	SD		
After the immobilisation removal						
PRWE	73.17	17.56	73.12	23.74	0.012	0.990
SFMCS	61.58	10.30	63.84	9.67	1.131	0.261
SFPCS	32.14	5.64	30.08	5.80	1.800	0.075
Two months after the immobilisation removal						
PRWE	27.13	22.53	25.87	20.05	0.295	0.769
SFMCS	60.32	10.04	61.90	8.76	0.839	0.404
SFPCS	43.10	8.35	39.26	7.00	2.492	0.014

PRWE, Patient related wrist evaluation; SFPCS, Short Form Physical Component Survey; SFMCS, Short Form Mental Component Survey;

There was a significant difference between males and females in the power grip strength in terms of a stronger grip strength in males (62.40mm Hg; p=0.001). Palmar flexion increased in both sexes, but again significantly in males (PF 70.40°, p=0.010). The functional results of the PRWE survey and the SF12 questionnaire scores demonstrated a significant improvement in males (PRWE 17.46, p=0.013; SFPCS33.28; p=0.030) (Table 4).

Table 4. Comparison of clinical, radiological and functional parameters by gender

Variable	Gender				t	p
	M		W			
	$\bar{X}$	SD	$\bar{X}$	SD		
Age	53.00	14.37	67.45	11.62	5.067	<0.001
After immobilisation removal						
Palmar flexion(°)	50.00	19.69	43.53	18.58	1.485	0.141
Dorsal flexion(°)	39.00	22.64	29.33	17.69	1.946	0.060
Ulnar deviation(°)	22.40	8.79	19.27	9.61	1.441	0.153
Radial deviation (°)	9.40	7.68	7.73	5.71	1.154	0.251
Strength (mmHg)	62.40	18.83	41.13	14.01	5.189	<0.001
PRWE	74.56	23.37	72.67	19.98	0.392	0.696
SFPCS	33.28	5.84	30.39	5.62	2.207	0.030
SFMCS	64.00	6.34	62.28	10.96	0.960	0.340
Two months after immobilisation removal						
Palmar flexion(°)	70.40	14.78	62.20	13.08	2.627	0.010
Dorsal flexion(°)	63.40	18.01	55.27	18.10	1.948	0.054
Ulnar deviation(°)	28.00	5.59	26.27	6.37	1.213	0.228
Radial deviation (°)	18.00	7.22	15.73	6.35	1.493	0.139
Strength (mmHg)	93.80	20.07	66.67	15.73	6.953	<0.001
PRWE	17.46	18.83	29.55	21.22	2.530	0.013
SFPCS	44.68	8.64	40.01	7.34	2.632	0.010
SFMCS	62.56	5.24	60.63	10.42	1.211	0.229
After reposition						
Radial height (mm)	11.08	2.06	10.99	2.30	0.167	0.867
Radial inclination (°)	22.28	4.81	22.12	4.66	0.147	0.883
Palmar inclination (°)	7.72	9.49	8.88	9.66	0.522	0.603
After immobilisation removal						
Radial height (mm)	10.12	1.76	9.79	1.87	0.765	0.446
Radial inclination (°)	20.12	4.09	19.17	4.86	0.875	0.384
Palmar inclination (°)	4.16	6.43	3.15	6.70	0.662	0.510
Two months after immobilisation removal						
Radial height (mm)	9.96	1.79	9.55	1.97	0.929	0.355
Radial inclination (°)	19.48	4.33	18.41	4.95	0.960	0.339
Palmar inclination (°)	4.72	5.13	2.35	7.00	1.559	0.122

PRWE, Patient related wrist evaluation; SFPCS, Short Form Physical Component Survey; SFMCS, Short Form Mental Component Survey;

Data comparison revealed that there was a significantly better grip strength at the second measurement and better results of functional score in patients without complications (GS 74.95 mm Hg, p=0.003; PRWE 24.30; p<0.001) (patients with complication had Morbus Sudeck and were in DF group). The patients with complications had significantly reduced grip strength, and worse functional results. More common complications were found in the group with dorsal immobilisation (Table 5).

Table 5. Comparison of clinical, radiological and functional parameters by presence of complication

Variable	Complication				t	p
	NO		YES			
	$\bar{X}$	SD	$\bar{X}$	SD		
Age	63.54	14.01	68.50	9.85	0.852	0.397
After immobilisation removal						
Palmar flexion (°)	45.64	18.63	37.50	24.44	1.019	0.311
Dorsal flexion (°)	31.70	19.24	32.50	23.61	0.097	0.923
Ulnar deviation (°)	20.11	9.30	19.17	12.81	0.235	0.815
Radial deviation (°)	8.09	6.27	9.17	6.65	0.408	0.684
Strength (mmHg)	46.76	17.62	41.67	22.29	0.675	0.501
PRWE	73.41	21.07	69.00	16.26	0.502	0.617
SFPCS	31.03	5.91	32.33	3.56	0.532	0.596
SFMCS	63.09	9.70	56.83	13.64	1.493	0.139
Two months after immobilisation removal						
Palmar flexion(°)	65.37	12.81	46.67	19.66	2.299	0.067
Dorsal flexion(°)	58.09	18.01	45.00	20.74	1.712	0.090
Ulnar deviation(°)	26.91	6.10	23.33	7.53	1.377	0.172
Radial deviation(°)	16.33	6.69	15.83	5.85	0.177	0.860
Strength (mmHg)	74.95	19.86	50.00	17.89	2.998	0.003
PRWE	24.30	19.39	60.50	20.60	4.417	<0.001
SFPCS	41.62	7.88	34.33	4.84	2.232	0.028
SFMCS	61.63	8.69	53.00	16.17	1.295	0.250
After reposition						
Radial height(mm)	11.10	2.27	9.67	.82	1.537	0.128
Radial inclination (°)	22.19	4.72	21.67	4.27	0.265	0.791
Palmar inclination (°)	9.06	9.58	1.17	6.34	1.986	0.050
After immobilisation removal						
Radial height (mm)	9.92	1.87	9.17	1.17	0.970	0.334
Radial inclination (°)	19.48	4.69	18.33	4.80	0.580	0.564
Palmar inclination (°)	3.66	6.48	0.67	8.07	1.565	0.121
Two months after immobilisation removal						
Radial height (mm)	9.71	1.94	8.67	1.37	1.295	0.198
Radial inclination (°)	18.74	4.79	17.67	5.43	0.531	0.597
Palmar inclination (°)	3.17	6.52	0.67	8.07	1.378	0.171

PRWE, Patient related wrist evaluation; SFPCS, Short Form Physical Component Survey; SFMCS, Short Form Mental Component Survey

DISCUSSION

Fracture of the distal radius breaks the continuity of the shaft, and the flexor and extensor muscles indirectly cause dislocation of the fragments (20). It is therefore very important to cancel this force. Palmar flexion is required at the point of fracture to tighten intact periosteum from the dorsal side of the fracture and to give more stability to the fracture (20). The dorsal carpal ligaments are attached only to the triquetrum, while most of the palmar flexion occurs in the mid carpal joint, and in palmar flexion immobilisation, those ligaments are not tense and they are unable to stabilize the fracture displacement (20). Therefore, in the dorsiflexion position of the wrist radiotriquetral and radiocapitate ligaments are tense. They are attached to the distal row of carpal bones causing in that way a double positive effect on fracture reposition preservation through the stabilization of those two rows and relaxation of extensor apparatus. This kind of "S" shape immobilisation performed by this technique stabilizes the fracture fragments in both types of fractures, especially in intra articular fractures (26). There is no consensus on the best treatment of fractures of the distal part of the radius, even though the fracture has been recognized and therapeutic approaches investigated in detail for the past 200 years (27,28). The good clinical practice in most centres throughout the world is made up of conservative treatment of these fractures with the wrist in a palmar flexion of 20° and mild ulnar deviation and the results of this treatment are satisfactory especially in older people (29). However, in people under 65 years of age, results, particularly short-term, are not very satisfactory (30). Therefore, Guptain the 90's worked on different types of conservative treatment with the immobilization under elbow cast with the wrist in the dorsal flexion of 20° and mild ulnar deviation, and showed better radiological results with better flexibility of wrist and faster recovery of the hand grip strength (20). In this study, which is different from Gupta´s study, under elbow plaster splint immobilization was used instead of complete cast in both groups. Data in this study showed that immobilization in dorsiflexion really improves the mobility of the wrist in earliest period, which is certainly important for a faster recovery and a quicker return to work activities. Radiological measurement has demonstrated a significant difference in all of the observed parameters in the first control. Reduction of the radial height significantly affects the results of the initial range of motion of the wrist and this study observed a significantly better preservation of this parameter in the DF group and that had an impact on better functional result of the treatment (31,33). In the second control, palmar inclination

showed no significant difference between the two groups in this study. The reason of this equalization is again a better stabilization of reposition of this parameter in the DF group, which in the first control was significantly better restored in the PF group, probably because of better knowledge about the technique of the reposition by the orthopaedist. Palmar inclination is normally around 12° and when it is over 10° of dorsal and over 15° of palmar, it has a negative influence on the incidence of pain, reduction of grip strength and mobility of the wrist (33). It is extremely important to preserve this parameter for better functional outcome in patients with fractures of the distal radius, as it has been observed in the DF group in our study (32,35).

The survey data confirm the fact that better radiological and also clinical results give a better functional outcome (35).The comparison between the genders in this study showed significant difference between males and females in the improvement of the power grip strength, range of motion (ROM) and function. These data only partially correlate to the global studies that have shown that regardless of gender, better grip strength and mobility give better functional results, whereas this study shows that these results are evident only in males (35). A significantly better improvement of grip strength and better results of function in all parameters were observed in patients without complications in our study. There was no significant difference in terms of incidence of complications between two groups and the data confirm that patients with poor clinical parameters have more complications, and these patients have shown worse functional results, but there is no parameter that could be used for early detection of possible complication.

In this study stronger exclusion criteria were applied, similarly to other studies, in order to get more statistically relevant results. Both types of immobilization give radiological results that have a positive effect on improving the condition of the patient, but with statistically better improvement in the DF group, thus confirming the hypothesis of the study.

In conclusion, immobilization with forearm plaster splint on the dorsal side and with the wrist in dorsiflexion gives better early clinical, radiological and functional results in patients with fractures of type A2, A3, C1, C2, C3 according to AO classification in patients of all age groups, compared to the treatment with immobilization of the wrist in palmar flexion and ulnar deviation. The results of this study have shown that conservative treatment can be an excellent tool for the treatment of the major parts of distal radius fractures and that the immobilization in dorsal flexion of the wrist gives better preservation of reduction of the fracture of distal radius compared to other immobilization techniques.

FUNDING

No specific funding was received for this study

TRANSPARENCY DECLARATION

Competing interests: None to declare.

REFERENCES

1. Lofthus CM, Frihagen F, Meyer HE, Nordsletten L, Melhuus K. Epidemiology of distal forearm fractures in Oslo. Osteoporos Int 2008; 19:781–6.
2. Hove LM, Fjeldsgaard K, Reitan R, Skjeie R, Sorensen FK.Fractures of the distal radius in a Norwegian city. Scand J Plast Reconstr Surg Hand Surg 1995; 29:263–7.
3. Mulders MA, Rikli D, Goslings JC, Schep NW. Classification and treatment of distal radius fractures: a survey among orthopaedic trauma surgeons and residents. Eur J Trauma Emerg Surg 2016 [Epub ahead of print]
4. Kreder HJ, Hanel DP, McKee M, Jupiter J, McGillivary G, Swiontkowski MF. Consistency of AO fracture classification for the distal radius. J Bone Joint Surg Br 1996; 78:726-31.5.
5. Ring D. Intra-articular fractures of the distal radius. J Hand Surg Am 2002; 2:61-77.
6. Brug E, Joosten U, Püllen M. Fractures of the distal forearm. Which therapy is indicated when? Orthopade 2000; 29:318-26.
7. Handoll HHG, Huntley JS, Madhok RD. External fixation versus conservative treatment for distal radial fractures in adults. Cochrane Database Syst Rev 2007; (3):CD006194.
8. Brogren E, Petranek M, AtroshiI. Incidence and characteristics of distal radius fractures in a southern Swedish region. BMC Musculoskelet Disord 2007; 8:48.
9. American Academy of Orthopaedic Surgeons. The Treatment of Distal Radius Fracture - Guideline and Evidence Report. Rosemont (IL): American Academy of Orthopaedic Surgeons, 2009. http://www.aaos.org/research/guidelines/drfguideline.pdf (17 January 2012).

10. Blatter G, Papp P, Magerl FA. Comparison of 2 methods of plastic cast fixation in treatment of loco classico radius fracture. Unfallchirurg 1994; 97:534–40.
11. Grabicki M, Grzegorzewski A. Evaluation of distal radius fracture treatment non-operative vs. operative with percutaneous pinning. Chir Narzadow Ruchu Ortop Pol 2010; 75:205-10.
12. Handoll HH, Madhok R. Surgical interventions for treating distal radial fractures in adults. Cochrane Database Syst Rev 2003; (3):CD003209.
13. Kreder HJ, Agel J, McKee MD. A randomized, controlled trial of distal radius fractures with metaphyseal displacement but without joint incongruity: closed reduction and casting versus closed reduction, spanning external fixation, and optional percutaneous K-wires. J Orthop Trauma 2006; 20:115-21.
14. Jordan RW, Naeem R, Jadoon S, Parsons H, Shyamalan G. Cast immobilisation versus wire fixation in the management of middle-aged and elderly patients with distal radius fractures. J Hand Surg Asian Pac 2016; 21:18-23.
15. Arora R, Lutz M, Deml C, Krappinger D, Haug L, Gabl MA. Prospective randomized trial comparing nonoperative treatment with volar locking plate fixation for displaced and unstable distal radial fractures in patients sixty-five years of age and older. J Bone Joint Surg Am 2011; 93:2146-53.
16. Al Khudairy A, Hirpara KM, Kelly IP, Quinlan JF. Conservative treatment of the distal radius fracture using thermo plastic splint: pilot study results. Eur J Orthop Surg Traumatol 2013;23:647-50.
17. Grafstein E, Stenstrom R, Christenson J, Innes G, McCormack R, Jackson C, Stothers K, Goetz TA. Prospective randomized controlled trial comparing circumferential casting and splinting in displaced Colles fractures. CJEM 2010; 12:192-200.
18. Melone CP. Open treatment for displaced fractures of the distal radius. Clin Orthop 1986; 202:103-11.
19. Lakshmanan P, Sayana MK, Purushothaman B, Sher JL. Ligamentotaxis for Barton's and paediatric distal radial fractures. J Orthop Surg 2009; 17:28-30.
20. Broos PLO, Fourneau IAM, Stoffelen DVC. Fractures of the distal radius current concepts for treatment. Acta Orthopædica Belgica 2001; 67:212-13.
21. Gupta A. The treatment of Colles' fracture. Immobilisation with the wrist dorsiflexed. J Bone Joint Surg Br 1991; 73:312–5.
22. Viegas SF, Tencer AF, Cantrell J, Chang M, Clegg P, Hicks C, O'Meara C, Williamson JB. Load transfer characteristics of the wrist: Part II. Perilunate instability. J Hand Surg Am1987; 12:978-85.
23. Rikli DA, Honigmann P, Babst R, Cristalli A, Morlock M, Mittlmeier T. Intra articular pressure measurement in the radioulnocarpal joint using a novel sensor: in vitro and in vivo results. J Hand Surg Am 2007; 32:67-75.
24. Rajan S, Jain S, Ray A, Bhargava P. Radiological and functional outcome in extra-articular fractures of lower end radius treated conservatively with respect to its position of immobilization. Indian J Orthop 2008; 42:201–7.
25. Macdermid JC, Tottenham V. Responsiveness of the disability of the arm, shoulder, and hand (DASH) and patient-rated wrist/hand evaluation (PRWHE) in evaluating change after hand therapy. J Hand Ther 2004;17:18-23
26. Ware J, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 1996; 34:220-33.
27. Mandziak DG, Watts AC, Bain GI. Ligament contribution to patterns of articular fractures of the distal radius. J Hand Surg Am 2011; 36:1621–5.
28. Nazar MA, Mansingh R, Bassi RS, Waseem M. Is there a consensus in the management of distal radial fractures? Open Orthop J 2009; 3:96–9.
29. Alluri RK, Hill JR, Ghiassi A. Distal radius fractures: approaches, indications, and techniques. J Hand Surg Am 2016; 41:845-54.
30. Grabicki M, Grzegorzewski A. Evaluation of distal radius fracture treatment non operative vs. operative with percutaneous pinning. Chir Narzadow Ruchu Ortop Pol 2010; 75:205-10.
31. Nellans KW, Kowalski BS, Chung KC. The epidemiology of distal radius fractures. Hand Clin 2012; 28:113–25.
32. Warwick D, Field J, Prothero D, Gibson A, Bannister GC. Function ten years after Colles' fracture. Clin Orthop 1993; 295:270–4.
33. Tsukazaki T, Iwasaki K. Ulnar wrist pain after Colles' fracture. 109 fractures followed for 4 years. Acta Orthop Scand 1993; 64:462–4.
34. Leung F, Ozkan M, Chow SP. Conservative treatment of intra-articular fractures of the distal radius factors affecting function an outcome. HandSurg 2000; 5:145-53.
35. Fujii K, Henmi T, Kanematsu Y, Mishiro T, SakaiT, Terai T. Fractures of the distal end of radius in elderly patients: a comparative study of anatomical and functional results. J Orthop Surg (Hong Kong) 2002; 10:9–15.

Rani rezultati konzervativnog liječenja pacijenata s prijelomom distalnog okrajka palčane kosti – usporedba imobilizacije ručnog zgloba u dorzalnoj ili palmarnoj fleksiji

Maki Grle[1], Miro Miljko[2], Ivana Grle[3], Faruk Hodžić[4], Tarik Kapidžić[4]

[1]Odjeljenje za radiologiju,[2] Odjeljenje za radiologiju,[3] Odjeljenje za fizikalnu medicinu i rehabilitaciju; Sveučilišna klinička bolnica Mostar,[4] Odjeljenje za traumatologiju i ortopediju, Kantonalna bolnica Zenica; Bosna i Hercegovina

SAŽETAK

Cilj Procijeniti prednosti imobilizacije podlaktičnom gips-longetom s ručnim zglobom u dorzalnoj fleksiji naspram položaja ručnog zgloba u palmarnoj fleksiji kod pacijenta s prijelomom distalnog dijela palčane kosti.

Metode U ovoj prospektivnoj kohortnoj studiji (2012-2014) bila su ispitivana 122 pacijenta (od kojih su 22 izgubljeni za vrijeme praćenja) s prijelomom distalnog okrajka palčane kosti tip A2, A3 i C prema AO klasifikaciji. Na kraju su dobivene dvije skupine od po 50 pacijenta: u dorzifleksijskoj (DF) grupi bilo je ukupno 37 žena i 13 muškaraca srednje životne dobi od 63.48 ± 14.70 godina, a u palmarnofleksijskoj (PF) grupi bilo je 38 žena i 12 muškaraca srednje životne dobi od 64.20 ± 12.99 godina. Mjereni su radiološki, klinički i funkcionalni parametri u obje grupe. PRWE-anketa i SF-12 upitnik bili su korišteni za procjenu razine bola i funkcije zgloba, te općeg tjelesnog i psihičkog stanja pacijenta.

Rezultati Pokazali su značajno poboljšanje kliničkih parametara opsega pokreta na svakom mjerenju u DF grupi: dorzalna fleksija 47.70±15.29; ulnarna devijacija 24.10±7.80; radijalna devijacija 11.50±5.65, naspram PF grupe: 22.80±19.04, 16.00± 9.31, odnosno 4.80± 4,94 ($p<0,001$), te radioloških i funkcionalnih parametara kroz anketu SF-12 u DF grupi ($p<0,014$).

Zaključak Imobilizacija ručnog zgloba u dorzifleksiji daje bolje rane kliničke, radiološke i funkcionalne rezultate naspram imobilizacije u plamarnoj fleksiji kod pacijenata s prijelomom distalnog radijusa tip A2, A3, C prema AO klasifikaciji.

Ključne riječi: ručni zglob, prijelom distalnog radijusa, konzervativno liječenje, imobilizacije.

ORIGINAL ARTICLE

Warfarin should not be used for thromboprohylaxis in elective major orthopaedic surgery: a Croatian perspective

Robert Kolundžić[1,2], Marijana Šimić Jovičić[3], Maja Đinkić[4], Tadija Petrović[1], Tomislav Crnković[2,5], Vladimir Trkulja[6]

[1]Department of Trauma Surgery, University Hospital Centre "Sestre Milosrdnice", Zagreb, [2]School of Medicine, Josip Juraj Strossmayer University, Osijek, [3]Department of Orthopaedics, Children's Hospital, Zagreb; Croatia, [4]Department of Orthopaedic Surgery, Karaborg Hospital, Lidkoping, Sweden, [5]Department of Orthopaedic and Trauma Surgery, County General Hospital, Požega, [6]Department of Pharmacology, School of Medicine, University of Zagreb; Zagreb, Croatia

ABSTRACT

Aim To identify modes of venous thromboembolism (VTE) prophylaxis in patients undergoing elective major orthopaedic surgery (total hip or knee arthroplasty, THA/TKA) at a single university-associated hospital in Croatia.

Methods A retrospective analysis of consecutive patients subjected to THA or TKA over a two-year period (2014-2015) with a focus on anticoagulation during the first 15 post-surgical days (period of highest VTE risk).

Results Of 603 identified patients three (0.5%) were not anticoagulated (haemophilia) and others received perioperative doses of low molecular weight heparins (LMWH). Overall, 228 (37.8%) patients received prophylaxis not involving warfarin, and 372 continued with short-term LMWH with switching to warfarin. They contributed a total of 1218 international normalized ratio (INR) values (median=3, range=1-8). These were consistently below the target INR range across the observed period. Between post-surgical days 6 and 15 (after the initial titration), 438 values were taken in patients treated with LMWH+warfarin and 92.7% were below, and only 6.8% within the target range; 580 values were taken in patients already switched to warfarin, 74% were below and only 25% within the range.

Conclusion The prevailing mode of VTE prophylaxis was in a clear contrast to (then) actual professional guidelines, with inadequate monitoring and poor anticoagulation. There is no reason to expect a substantially different situation at other institutions across the country. The prevailing practice of VTE prophylaxis in major orthopaedic surgery in Croatia should be promptly abandoned and up-dated in agreement with the current state of the art.

Key words: hip, knee, arthroplasty, venous thromboembolism

Corresponding author:
Vladimir Trkulja
Department of Pharmacology, Zagreb
University School of Medicine
Šalata 11, 10000 Zagreb, Croatia
Phone: +385 1 45 66 830;
Fax: +385 1 49 200 49;
E-mail: vtrkulja@mef.hr
Robert Kolundžić: ORCID ID: http://www.orcid.org/0000-0001-7008-6979

Original submission:
21 March 2017;
Revised submission:
29 May 2017;
Accepted:
2017.
doi: 10.17392/902-17

Med Glas (Zenica) 2017; 14(2): 244-249

INTRODUCTION

Elective total hip (THA) and knee arthroplasty (TKA) are highly successful procedures that convey great improvements in functional ability and quality of life in patients suffering a variety of pathological hip/knee conditions. Both surgeries have a successful and long-standing history in Croatia (1). However, both procedures are associated with an increased risk of post-surgical venous thromboembolic incidents (VTE) – deep venous thrombosis or pulmonary embolism. In the 1970s, the estimated risk of such events was in the range of 20-25% but introduction of thromboprophylaxis, improvement of surgical techniques and post-surgical management (e.g., early ambulation) have greatly reduced their occurrence (2). As estimated by the American College of Chest Physicians (ACCP), the critical period for the occurrence of VTE lasts for 30-35 post-surgical days and a contemporary risk of symptomatic VTE associated with elective THA/TKA without prophylaxis is estimated at 2.9% over the first 15 post-surgical days and up to 4.6% during the 30-35 days (2). While there are different mechanical and pharmacological means of thromboprophylaxis, a thorough systematic review conducted by ACCP demonstrated that pharmacological means should be preferred except when explicitly contraindicated (2). Based on a comprehensive evaluation of clinical trial data of individual pharmacological means vs. no treatment, mechanical methods or vs. each other, which assessed the risk of VTE and the risk of relevant bleedings (i.e., major or non-major clinically relevant), ACCP also suggested that low-dose acetylsalicylic acid (alone or combined with mechanical means) and unfractionated heparin should not be used in this setting, emphasized the role of low molecular weight heparins (LMWH) particularly in comparison to vitamin K antagonists and depicted the emerging role of the new orally active anticoagulants (NOACs) (2).

There are no formal surveys on thrombophrophylaxis in this setting in Croatia, but an *ad hoc* survey among the members of the Croatian Orthopaedic Society during the 2016 annual assembly confirmed the common knowledge - the prevailing mode of thrombophrophylaxis related to THA/TKA consists of a short-term peri-operative (up to several days after the surgery) use of LMWH (first dose before the surgery) with concomitant introduction of vitamin K antagonists (almost exclusively warfarin) and subsequent continuation of treatment with warfarin, and is driven by the policy of the Croatian Health Insurance fund which reimburses only the "in-hospital" use of LMWH. However, ACCP has demonstrated (2) that even under the conditions of controlled clinical trials with extremely high rate of appropriate warfarin use (time in therapeutic range, TTR), i.e. with international normalized ratio (INR) values between 2.0 and 3.0 (targeted value 2.5%), >60%), this strategy was inferior to the exclusive use of LMWH as it resulted in several excess cases of VTE and 40 excess cases of major bleeding/1000 treated.

An observational study in the USA (2009-2011) included 596 THA/TKA patients treated with such an approach, who received warfarin over 10-35 days and were monitored through specialized anticoagulation clinics at two university hospitals (3). The average TTR was 36% and median time to the first INR within the target range was 12-13 days. A similar study (4) embraced 3313 THA/TKA patients (2005-2009). The median number of determined INR values over the 28-35 post-surgical days per patient was 5 (excluded were the initial 5 days of "early titration"). The average TTR was 28%, only 33% of the determined INR values were within the range, average proportion of time with INR <2.0 was 64%, and patients who did not experience a single INR value within the range had 5.3 times higher risk of symptomatic VTE (incidence of VTE in the cohort was 3.3%). Overall, data suggest that even with stringent monitoring and careful titration, warfarin does not seem to achieve the desired level of anticoagulant activity during the period of the highest VTE risk.

Warfarin-based anticoagulation is specific in that it successfulness greatly depends on close monitoring. There are no specialized anticoagulation clinics in Croatia and no structured monitoring systems (e.g., registries) of long-term anticoagulation e.g. in stroke prevention or VTE treatment/prophylaxis. A recent study demonstrated a poor practice of warfarin-based anticoagulation in Croatian patients with atrial fibrillation scheduled for direct current cardioversion (5). The present analysis aimed to evaluate anticoagulation adequacy in elective major orthopaedic surgery in a specialized Croatian hospital.

PATIENTS AND METHODS

Study design

This retrospective analysis refers to a 2-year period (January 1 2014 – December 31 2015) at the Department of Trauma Surgery, University Hospital Centre "Sestre Milosrdnice", Zagreb, Croatia in order to identify modes of venous thromboembolism (VTE) prophylaxis in patients undergoing elective major orthopaedic surgery (total hip or knee arthroplasty, THA/TKA).

The study was approved by the Ethics Committee of the University Hospital Centre "Sestre Milosrdnice", Zagreb, Croatia.

Eligible for inclusion were consecutive patients who underwent primary elective THA/TKA.

Methods

Two researches independently searched the hospital electronic database system for the specific procedure codes matched with the unique patient identification numbers. Results were mutually rechecked and verified through hard-copy archives. Demographic, comorbidity, co-medication data and data on peri-operative anticoagulation treatment, as well as laboratory data on coagulation monitoring tests were extracted for a period of 15 post-surgical days (Table 1). We restricted the observational period to the first 15 post-surgical days as this is the period of the highest risk for VTE.

RESULTS

A total of 603 patients were enrolled, mostly subjected to THA, 434 (72%) (Table 1).

Table 1. Characteristics of patients who underwent elective major orthopaedic surgery (total hip or knee arthroplasty)

Characteristic	No (%) of patients
Total	603
Total hip / knee arthroplasty	434 (72) / 169
Age (range, years)	69 (20-97)
Males	273 (45.3)
Previous use of antiplatelet	76 (12.6)
Previous use of anticoagulants	33 (5.4)
History of pulmonary embolism	1 (0.2)
History of deep venous thrombosis	20 (3.3)
History of myocardial infarction or angina	23 (3.8)
History of transitory ischemic attack or stroke	10 (1.7)
Atrial fibrillation or flutter	25 (4.1)
Chronic heart failure	71 (11.8)
Venous insufficiency	50 (8.3)
Diabetes mellitus	71 (11.8)
Thrombocytopenia	6 (1.0)
Haemophilia	3 (0.5)

Apart from three patients with haemophilia, all underwent anticoagulation and received a pre-surgical and a post-surgical low molecular weight heparin (LMWH) dose (Figure 1). For 228 (37.8%) patients anticoagulation did not include warfarin, however for 120 (19.9%) patients only short-term LMWH treatment could be verified without any information about further prophylaxis. Overall 103 (17.1%) patients received LMWH prophylaxis during the observed period, and sporadic patients were treated with new orally active anticoagulants (NOACs) or LMWH, and acetylsalicylic acid. The majority of the patients, 372 (61.5%) underwent prophylaxis with LMWH switching to warfarin and contributed a total of 1218 INR values (median=3, range 1-8 per patient). Almost 1/3 one third of the patients, 113 (30.4%) contributed only 1 or 2 INR values over the observed 15 days (Figure 1).

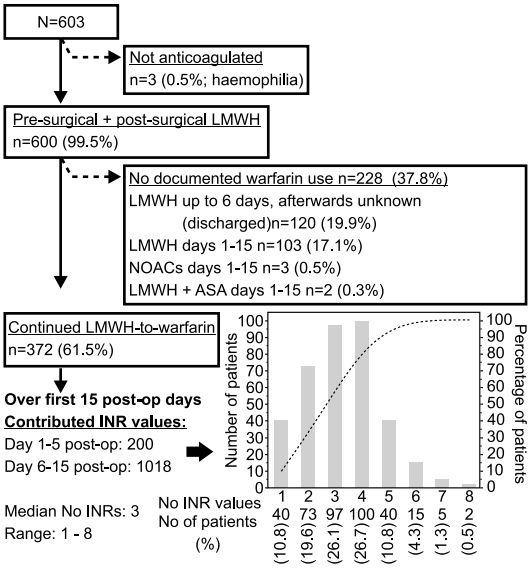

Figure 1. Patient eligibility diagram. Histogram depicts distribution of patients (absolute counts and cumulative relative distribution) across the number of contributed international normalized ratio (INR) values

ASA, acetylsalicylic acid; LMWH, low molecular weight heparin; NOACs, new orally active anticoagulants

Regardless of the treatment under which INR values were provided, over time they were consistently below the target INR range. Considering the period between days 6-15 (after initial titration would be expected to be accomplished), 92.7% out of 439 values taken in patients under LMWH + warfarin treatment were below the target as well as 74% out of 580 values taken in

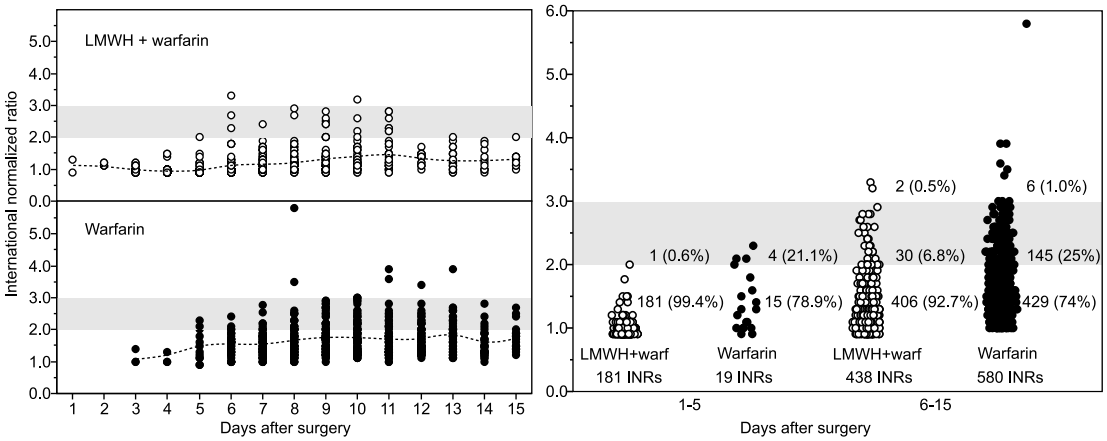

Figure 2. International normalized ratio (INR) values in the subset of patients anticoagulated by the "LMWH-to-warfarin" treatment. Shaded areas indicate the target INR range. Individual values (circles) across the observed 15 days by treatment (left) and individual values at days 1-5 and 6-15 by treatment (right) with the absolute number (percentage) of values below, within and above the range
LMWH, low molecular weight heparin; Warf, warfarin

patients already switched to warfarin, with only 25% within the target range (Figure 2).

DISCUSSION

Although conducted at a single centre and thus not representative for the entire country, the present results confirmed three expectations. First, the initial use of LMWH with switching to warfarin was the most predominant practice. Second, in the setting of major orthopaedic surgery there was no "centralized structured" monitoring of successfulness of anticoagulation: at the institution at which the surgery was indicated and performed, and at which the anticoagulation was initiated, only sparse data on a laboratory marker (i.e., INR in patients treated with warfarin) of its successfulness were recorded (30.4% patients contributed only 1 or 2 INR values over the observed period) and there were practically no records on the overall outcome (i.e., records of VTE/bleedings during the first month post-surgery). Third, although TTR could not be determined due to paucity of the international normalized ratio (INR) values, data on percentage of values within the target INR range, particularly those taken between days 6 and 15 (after the "initial titration" period) strongly indicate the failure of the procedure to achieve a known strongly predictive (for clinical outcomes) laboratory goal during the period of the highest VTE risk. While patients treated with both LMWH and warfarin could be considered "protected" (on the account of LMWH), the fact that 92.7% of their INR values were below the target clearly demonstrated that they were not fit to continue only warfarin treatment. On the other hand, the fact that 74% of the values taken in patients treated with warfarin were only below the target clearly documented excessive "under-protection" from the VTE risk.

In 2012 the American College of Chest Physicians (ACCP) clearly recommended the preference of pharmacological over mechanical (various forms) thromboprophylaxis in major orthopaedic surgery (except where the former were explicitly contraindicated) and strongly advised (based on randomized controlled trial data) against the use of vitamin K antagonists (e.g., warfarin) due to inferior balance between the VTE and bleeding risks as compared to LMWH (2). Observational data with stringent anticoagulation monitoring (3,4) demonstrated that warfarin inherently did not seem to be appropriate for a setting in which a rapid onset of a relative short anticoagulation is needed (30 days overall, with the highest risk over first 15 post-surgical days): median time to target INR has been estimated to equal the period of the highest risk (12-13 days) (3), time in therapeutic range over the first 30 post-surgical days has been repeatedly reported to be around 30% (3,4) with around 30% of INR values within the range (4). The present data are in line with these observations and actually indicate that the situation is even worse when there is no structured monitoring of the warfarin effect. In one large cohort (3313 THA/TKA patients) treated with warfarin between post-sur-

gical days 5-30 (4), the rate of symptomatic VTE was 3.3% - almost as if no anticoagulation was installed: the best estimate of the contemporary VTE risk without thromboprophylaxis is 4.6% (cumulatively over 30-35 days) and appropriate prophylaxis is expected to reduce it by 65-70%, relatively (2). Clearly, it is time to completely abandon the practice of warfarin use in this setting. In 2012, ACCP recommended the use of LMWH (during the entire period of the increased risk) (2). In the meantime, NOACs have emerged as a new valid option: three have gained regulatory approval in this setting (dabigatran, rivaroxaban, apixaban) and have demonstrated a comparable VTE/bleeding risks balance as compared to LMWH (6,7) with a convenience of simpler oral dosing. Nowadays, therefore, the most adequate options for VTE prophylaxis in major orthopaedic surgery should be identified among LMWH and NOACs and should take into account not only population estimates of their efficacy and safety, but also individual pharmacological characteristics (e.g., pharmacokinetics, dependence on renal function) and co-morbidity (e.g. existing long-term anticoagulation due to non-valvular atrial fibrillation or long-term VTE prophylaxis for other reasons) in order to apply the optimum choice for each individual patient.

In conclusion, the presented insight into thromboprophylaxis in major orthopaedic surgery at a single university-affiliated centre in Croatia disclosed that the prevailing mode of VTE prophylaxis was in clear contrast to (then) actual professional guidelines, with inadequate monitoring and poor anticoagulation. There is no reason to expect a substantially different situation in other institutions across the country. The prevailing practice of VTE prophylaxis in major orthopaedic surgery in Croatia should be promptly abandoned and up-dated in agreement with the current state of the art.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Conflicts of interest: none to declare

REFERENCES

1. Kolundžić R, Orlić D. Forty years of total hip replacement in Croatia at the department of orthopedic surgery Zagreb – orthopedic surgery of the 20th century. Lijec Vjesn 2011; 133:343-51.
2. Falck-Ytter Y, Francis CW, Johanson NA, Curley C, Dahl OE, Schulman S, Ortel TL, Pauker SG, Colwell CW Jr; American College of Chest Physicians. Prevention of VTE in orthopedic surgery patients. Antithrombotic therapy and prevention of thrombosis 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2012; 141(suppl):e278S-e325S.
3. Barnes GD, Kaatz S, Golgotiu V, Gu X, Leidal A, Kobeissy A, Haymart B, Kline-Rogers E, Kozlowski J, Almany S, Leyden T, Froehlich JB. Use of warfarin for venous thromboembolism prophylaxis following knee and hip arthroplasty: results of the Michigan Anticoagulation Quality Improvement Initiative (MAQI2). J Thromb Thrombolysis 2013; 35:10-4.
4. Nordstrom BL, Kachroo S, Fraeman KH. Warfarin prophylaxis in patients after total knee or hip arthroplasty – international normalized ratio patterns and venous thromboembolism. Curr Med Res Opinion 2011; 27:1973-85.
5. Dubravčić M, Cukon P, Car S, Puljević D, Trkulja V. Croatia needs a registry of patients undergoing direct current cardioversion for persistent atrial fibrillation/flutter. Croat Med J 2016; 31:403-5.
6. Trkulja V. Safety of apixaban for venous thromboembolism prophylaxis: the evidence to date. Drug Health Patient Saf 2016; 19:25-38.
7. Trkulja V, Kolundžić R. Rivaroxaban vs. dabigatran for thromboprophylaxis after joint-replacement surgery: exploratory indirect comparison based on meta-analysis of pivotal clinical trials. Croat Med J 2010; 51:113-23.

U tromboprofilaksi u bolesnika podvrgnutih elektivnim velikim ortopedskim zahvatima ne bi trebalo korisiti varfarin: iskustvo iz Hrvatske

Robert Kolundžić[1,2], Marijana Šimić Jovičić[3], Maja Đinkić[4], Tadija Petrović[1], Tomislav Crnković[2,5], Vladimir Trkulja[6]

[1]Klinika za traumatologiju, Klinički bolnički centar "Sestre milosrdnice", Zagreb, [2]Medicinski fakultet, Sveučilište Josip Juraj Strossmayer, Osijek, [3]Odjel za ortopediju, Dječja bolnica Zagreb, Zagreb; Hrvatska, [4]Klinika za ortopediju, Bolnica Karaborg, Lidkoping, Švedska, [5]Odjel za ortopediju i traumatologiju, Županijska opća bolnica, Požega, [6]Zavod za farmakologiju, Medicinski fakultet Sveučilišta u Zagrebu, Zagreb; Hrvatska

SAŽETAK

Cilj Identificirati modalitete prevencije venskog tromboembolizma (VTE) u bolesnika podvrgnutih elektivnim velikim ortopedskim zahvatima (totalna artroplastika kuka ili koljena) u jednom sveučilišnom medicinskom centru u Hrvatskoj.

Metode Retrospektivna analiza podataka o uzastopnim bolesnicima, podvrgnutim totalnoj artroplastici kuka ili koljena, tijekom dvije godine (2014-2015), s fokusom na antikoagulacijsko liječenje tijekom prvih 15 postoperativnih dana (razdoblje najvišeg rizika za VTE).

Rezultati Od ukupno 603 bolesnika, troje (0.5%) nisu bili antikoagulirani (hemofilija), dok su ostali primili preoperativne doze niskomolekularnog heparina (LMWH). U ukupno 228 (37.8%) bolesnika antikoagulacijski tretman nije uključivao varfarin, dok su 372 bolesnika tretirana kratkotrajnom primjenom LMWH-a s prijelazom na varfarin. Tijekom promatranog razdoblja ukupno je izmjereno 1.218 vrijednosti internacionalnog normaliziranog omjera (INR) (medijan=3, raspon= 1-8, po bolesniku). Vrijednosti su konzistentno bile ispod terapijskog INR-raspona. U razdoblju između šestog i petnaestog postoperativnog dana (nakon početne titracije), ukupno je izmjereno 438 INR-vrijednosti u bolesnika koji su primali i LMWH i varfarin, te je 92.7% njih bilo ispod terapijskog raspona, a samo 6.8% vrijednosti bilo je u terapijskom rasponu. Istodobno, izmjereno je ukupno 580 INR-vrijednosti u bolesnika koji su već bili prebačeni na varfarin – 74% vrijednosti bilo je ispod, a samo 25% vrijednosti bilo je u terapijskom rasponu.

Zaključak Opaženi dominantni oblik VTE profilakse u izravnoj je suprotnosti s profesionalnim smjernicama, s neadekvatnim nadzorom i nedostatnom antikoagulacijom. Nema razloga očekivati da je stanje bitno drugačije u ostalim institucijama u zemlji. Taj predominantni oblik VTE profilakse u velikim ortopedskim zahvatima u Hrvatskoj treba što prije napustiti i uvesti postupke u skladu s trenutno važećim standardima dobre kliničke prakse.

Ključne riječi kuk, koljeno, artroplastika, venski tromboembolizam

ORIGINAL ARTICLE

Croatian rotatory oblique three-dimensional osteotomy (CROTO) – a modified Wilson's osteotomy for adult hallux valgus intended to prevent dorsal displacement of the distal fragment and to reduce shortening of the first metatarsal bone

Robert Kolundžić[1,5], Mladen Mađarević[2], Vladimir Trkulja[3], Tomislav Crnković[4,5], Igor Šmigovec[2], Daniel Matek[2]

[1]Department of Trauma surgery, University Hospital Centre "Sestre Milosrdnice", [2]Department of Orthopaedic Surgery, University Hospital Centre, [3]School of Medicine, Zagreb University; Zagreb, [4]Department of Orthopaedic and Trauma Surgery, County General Hospital, Požega, [5]School of Medicine, Josip Juraj Strossmayer University Osijek, Osijek; Croatia

ABSTRACT

Aim To evaluate biomechanical and clinical outcomes of a newly developed modification of the Wilson's osteotomy for hallux valgus: a three-dimensional subcaptial correction of the metatarsal head position with a simultaneous lateral and plantar shift with derotation intended to reduce displacement of the distal fragment and shortening of the first metatarsal bone.

Methods Thirty four feet (28 female patients) underwent the new procedure and were evaluated before and 12 to 84 months (median=25.5) after the surgery.

Results Plantar shift of the distal fragment was achieved in all feet. Shortening of the first metatarsal was moderate: ≤6 mm in 32/34 feet, 7 and 10 mm in the remaining two. Median difference in metatarsal index post- vs. pretreatment was -4.0. The hallux valgus angle, intermetatarsal and distal metatarsal articular angles were reduced in all feet. The American Orthopaedic Foot and Ankle Society score improved in all feet (median increase= 51.5).

Conclusion The method allows for a lateral and plantar shift with derotation of the distal fragment and a mild/moderate shortening of the first metatarsal bone.

Key words: hallux valgus, surgery, metatarsal

Corresponding author:
Robert Kolundžić
Department of trauma surgery, University Hospital Centre "Sestre Milosrdnice"
Draškovićeva 19 10000 Zagreb, Croatia
Phone: +385 98 382 519;
+3851 4697000;
Fax: +385 1 4610 365;
E-mail: robert.kolundzic@zg.t-com.hr
ORCID: http://www. orcid.org/0000-0001-7008-6979

Original submission:
21 March 2017;
Revised submission:
31 March 2017;
Accepted:
27 June 2017.
doi: 10.17392/903-17

Med Glas (Zenica) 2017; 14(2): 250-256

INTRODUCTION

Hallux valgus is a common clinical problem (1-4). More than 130 different surgical methods have been described for correction of hallux valgus since the first description of surgery of bunions in 1871, and osteotomies of the first metatarsal bone have been described in various ways and sites (5-10). However, there has been no compelling evidence of advantages of any of these methods over any other particular type of surgery (11).

Metatarsalgia is one of the late complications of osteotomy procedures caused by a shift of the forefoot load distribution towards the lesser metatarsals, which is attributed to shortening of the first metatarsal and dorsal displacement of the distal fragment (5,6). Osteotomy of the first metatarsal bone as described by Wilson is an established procedure for the treatment of the adult hallux valgus. It is fast and easy to perform, and we have been using it for a number of years. A drawback of Wilson's osteotomy is shortening of the first metatarsal bone (3). Here we describe and present the first results of a modified technique – a three-dimensional subcapital correction of the metatarsal head position with a simultaneous lateral and plantar shift with derotation that we developed with the intention to prevent dorsal displacement of the distal fragment and to reduce shortening of the first metatarsal bone and its consequences, while keeping the procedure simple.

PATIENTS AND METHODS

Patients

The study included 34 feet in 28 consecutive female patients presented at the Department of Orthopaedic Surgery, University Hospital Centre Zagreb, in the period between January 2004 and June 2007 who gave a written consent to be treated by the new method after being informed about the details of the procedure. Ethics Committee at the University Hospital Centre Zagreb approved the use of the method.

Surgical treatment was indicated when the following criteria were cumulatively met: hallux valgus angle >20 degrees, intermetatarsal angle >10 degrees, negative metatarsal index and pain due to pressure over the medial side of the first metatarsophalangeal joint.

Methods

Operative procedure and postoperative care. All surgeries were performed by the same surgeon under spinal anaesthesia with a pneumatic high-thigh tourniquet. For the procedure, a short (3 to 5 cm) medial longitudinal skin incision was made over the head of the first metatarsal bone. The capsule was incised medially in a U fashion, with the capsule flap based distally. A bunion was removed using chisel. Oblique osteotomy of the metatarsal head was performed through the metaphysis using a thin 2.5 mm bade on air powered oscillator (Synthes, Switzerland). The osteotomy was made from the medial distal part of the bone towards the lateral proximal part, as seen in the horizontal plane, at an angle of 25 degrees on the line perpendicular to the metatarsal shaft (Figure 1). At the same time, in the sagittal plane, the osteotomy was made from the distal dorsal towards the proximal plantar part of the bone at an angle of 15 degrees (Figure 1A). Therefore, it allowed for a correction of the metatarsal head position with a simultaneous plantar shift (Figure 1B) and the distal fragment could be rotated if necessary (Figure 1C).

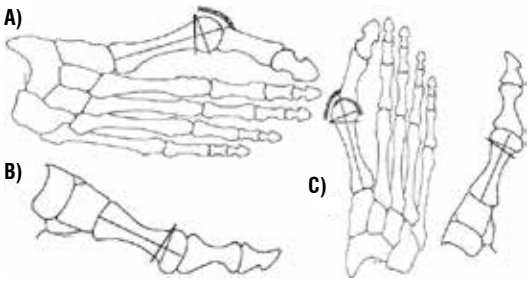

Figure 1. First metatarsal bone osteotomy scheme in A) horizontal and B) sagittal plane and C) the way of derotation. Dotted lines represent diaphyseal lines, full lines represent osteotomy (Kolundžić R., 2004)

The amount of shift was set as the width of a plantar cortex. Osteotomy angles were set arbitrarily. The fragments were fixed using one Kirschner wire inserted from distal to proximal in a paraosteal position in relation to the hallux and the head of the first metatarsal and then into the dorsomedial part of the medullary canal of the first metatarsal bone, driven as far as the base of the first metatarsal for better stabilization (Figure 2). The head position and the osteotomy site checked and capsulorrhaphy was performed followed by a skin suture. Elastic bandage was applied. Three days postoperatively, the bandage

was removed and replaced by plaster boots with heels adjusted for walking that were used for the next five weeks. After the plaster boot removal, Kirschner wire was removed and range-of-movement (ROM) exercises of metatarsophalangeal joints commenced. Gradually, full weight bearing was allowed after eight weeks.

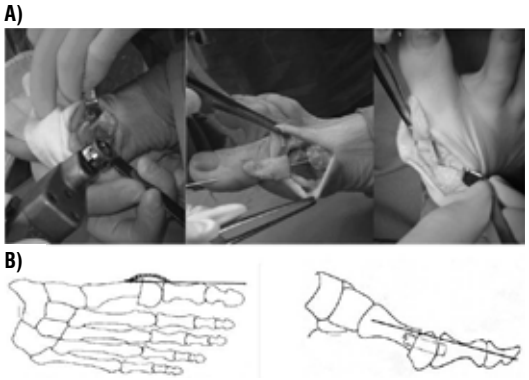

Figure 2. A) Intra-operative photographs: oscillator used to perform the osteotomy (left), Kirschner wire in place, lateral view (middle), Kirschner wire in place, anteroposterior view (right); B) Schematic representation of postoperative correction with Kirschner wire in place in horizontal and sagittal plane (Kolundžić R., 2004)

Patient evaluation. All patients and feet were evaluated radiologically and clinically before and minimum 12 months after the treatment by the same observer. The technique used for securing standardized X-rays was anteroposterior, lateral and sesamoid skyline views.

Anteroposterior (AP) weight bearing views were obtained with the patient standing on a shallow inverted wooden tray placed on the floor, just big enough to contain and protect the grid cassette, with feet 3 cm apart. With a source-to-film distance of 100 cm, the ray is directed 15 degrees backward, towards the hindfoot and is centered midway between the navicular bones. Non-weight bearing lateral oblique views were taken with the patient sitting on the standard couch with the foot rolled 15 degrees inward on a grid cassette placed on the floor. The beam is directed vertically at the centre of the cuboid from a source positioned 100 cm away. For the radiological assessment, pre- and postoperative radiographs of the forefoot were scanned and analysed using custom-designed computer software (Delphi, Visual Pascal – Borland Software Corporation, Austin, Texas, USA). The following parameters were determined: length of the first metatarsal, hallux valgus angle, first intermetatarsal angle, metatarsal index, distal metatarsal articular angle, position of the sesamoid bone and the amount of the sagittal shift (in millimeters) (only postoperatively). The axes of the first metatarsal, second metatarsal and the proximal phalanx were drawn by bisecting the diameters of their bases and joining the central points (12). Length of the first metatarsal on AP radiographs was determined by measuring the first metatarsal axis from the base to the tip of the metatarsal head. Hallux valgus angle (HVA) and first intermetatarsal angle (IMTA) were measured as described by Millner (13) and Schneider et al. (14,15). Metatarsal index (MTI) is a measurement of relative metatarsal protrusion on AP radiographs. It was determined by measurements in arcs cantered on the transverse tarsal line as described by Hardy and Clapham (16). Distal metatarsal articular angle (DMA) was measured as described by Gudas and Marcinko (17). The position of the sesamoid bone was classified into 4 levels according to Smith et al. (18). The amount of the sagittal shift was measured on lateral radiographs as a distance between 2 parallel lines, one drawn on the dorsal cortex of the proximal and the other drawn on the dorsal cortex of the distal fragment of first metatarsal bone. For clinical evaluation, the status of the forefoot was assessed using the American Orthopaedic Foot and Ankle Society (AOFAS) classification, i.e. the Hallux Metatarsophalangel-Interphalangeal Scale system (Table 1) (19).

Table 1. Preoperative and postoperative values (median, range) of radiological findings and of the American Orthopaedic Foot and Ankle Society (AOFAS) scores and differences between postoperative and preoperative ("post-pre") values with 95% confidence intervals (CI) (N= 34)

Parameter	Preoperatively	Postoperatively	Difference (95% CI)*	p
Length of 1st metatarsal bone (mm)	64 (55 to 70)	60 (52 to 66)	-4.0 (-4.5 to -3.0)	<0.001
Hallux valgus angle (degrees)	35 (20 to 50)	15.5 (0 to 35)	-18.5 (-22.5 to -15)	<0.001
Intermetatarsal angle (degrees)	15 (10 to 22)	5 (2 to 15)	-9.0 (-10 to -7.5)	<0.001
Metatarsal index	-2 (-6 to 4)	-4 (-10 to -1)	-4.0 (-4.5 to -3.0)	<0.001
Distal metatarsal articular angle (degrees)	15 (1 to 30)	9 (1 to 25)	-6.0 (-8.5 to -3.0)	<0.001
AOFAS score	44 (14 to 78)	95 (64 to 95)	51.5 (45.5 to 59.0)	<0.001

*Median difference with two-sided p-value from the Wilcoxon signed ranks test

Statistical analysis

Individual data and summary statistics on patients' demographics and pre- and postoperative radiological and clinical findings are presented. Differences between the postoperative and preoperative ("post – pre" difference) radiological parameters and clinical status scores are shown as median differences with the approximate 95% (exact 97.6%) confidence intervals (CI) calculated using the binomial method.

RESULTS

Thirty-four feet in 28 patients were operated-on (median age at surgery 44 years, range 19 to 58) and were evaluated before surgery and after a median follow-up of 25.5 months (range 12 to 84) at median age of 46 years (range 20 to 60). The sagittal shift of the first metatarsal was achieved in all feet and varied from 1-9 mm (median 3 mm). The first metatarsal was shorter after than before the operation in all feet with a median difference of -4 mm (Table 1), but the shortening never exceeded 10 mm. Distribution of feet regarding the shortening was: -1 mm, n=5; -2 mm, n=6; -3 mm, n=4; -5 mm, n=7; -6 mm, n=6; -7 mm, n=1 and -10 mm, n=1. The foot with a 10 mm (excessive) shortening of the first metatarsal (patient No. 14, foot No. 16) presented with an overall "fair" clinical status based on the AOFAS score at follow-up. It was painful with limitation of activities and motion restriction in the first metatarsophalangeal joint (Figure 3A). At follow-up, the metatarsal index was lower than before the treatment in 31/34 feet and the median "post-pre" difference was -4.0 (Table 1). The hallux valgus angle and the intermetatarsal angle were reduced by the surgery in all feet, with median "post-pre" differences being -18.5 and -9 degrees, respectively (Table 1). Typical pre-operative and post-operative X-ray findings are depicted in Figure 4. The AOFAS score illustrating clinical status was higher at follow-up than before treatment in all feet, with a median "post-pre" difference of 51.5 points (Table 1). Clinical status level improved in 33/34 feet, most frequently from "poor" to "excellent" (Table 2). The clinical status was graded "fair" only for the mentioned foot with a 10 mm shortening of the first metatarsal. In one foot for which the clinical status level remained "poor", the AOFAS score improved from 49 to 64 points. The "poor" result was due to metatar-

Table 2. Pre- to post-surgical "transition" of the overall clinical status and sesamoid bone position by foot (N= 34)

AOFAS clinical status					Position of the sesamoid bone				
Pre-surgery	**n**		**Post-surgery**	**n**	**Pre-surgery**	**n**		**Post-surgery**	**n**
Poor	33	→	Excellent	25	Severely rotated	15	→	No rotation	4
		→	Good	6			→	Slightly rotated	10
		→	Fair	1			→	Moderately rotated	1
		→	Poor	1	Moderately rotated	9	→	No rotation	9
Fair	1	→	Excellent	1	Slightly rotated	10	→	Slightly rotated	8
							→	No rotation	2
Total	**34**			**34**		**34**			**34**

AOFAS, American Orthopaedic Foot and Ankle Society

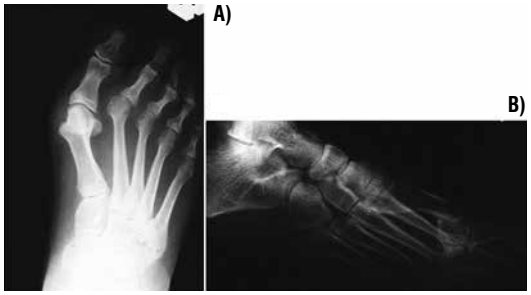

Figure 3. Postoperative complication: A) excessive shortening of the first metatarsal bone (Patient No. 14, foot No. 16), B) metatarsal head dislocation (Patient No. 22, foot No. 26) (Kolundžić R., 2008)

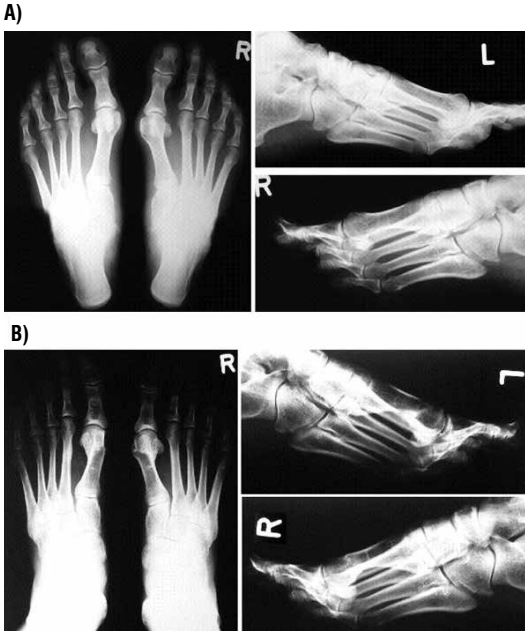

Figure 4. A) X-ray of both feet under load before corrective osteotomy of the first metatarsal bone (Patient No. 11, feet 11 and 12); B) X-ray of both feet under load 5 years postoperatively (Patient No. 11, feet 11 and 12) (both feet left, right left feet right above, right feet right below) (Kolundžić R., 2011)

sal head dislocation followed by a malunion (patient No. 22, foot No. 26) (Figure 3B).

There were no infections and no nonunions at the osteotomy site. We observed delayed union in one patient. Superficial skin irritation by the Kirschner wire occurred in one foot. A variable level of derotation of the sesamoid bone was achieved in all feet (Table 2).

DISCUSSION

A number of techniques (with a variety of modifications) are available for surgical treatment of the adult hallux valgus, but it appears that there is no equivocal evidence of advantages of any of these methods over any other particular type of surgery (11). Metatarsalgia and calluses are apparently the major late complications of surgical procedures and are due to a shift of the forefoot load distribution towards the lesser metatarsals, which is attributed to shortening of the first metatarsal and dorsal displacement of the distal fragment (5-11, 20). Merkel has suggested that the first metatarsal shortening greater than 10 mm is likely to result in a higher degree of patients' dissatisfaction and an increased frequency of metatarsalgia (20). Wilson's osteotomy is an established procedure for treatment of the adult hallux valgus. It is fast and easy to perform and we have been using it routinely for a number of years. It does, however, result in a shortening of the first metatarsal (13, 21-25). We have developed a simple modified technique that includes a three-dimensional subcapital correction of the metatarsal head position with a simultaneous plantar shift intended to prevent dorsal displacement of the distal fragment and to compensate for the metatarsal shortening. Magnan and Pezze described a similar percutaneous technique (26). Their method allows for a good lateral displacement of the first metatarsal head but does not allow for a plantar shift of the distal fragment. Xarchas et al. have described a similar method of the lateral and plantar shifting, but without the derotation of the head of the metatarsal bone (8). They also use a K-wire for fixation, but so that the wire is placed through the head of the metatarsal bone into the diaphysis. The advantage of our technique is that we place the K-wire in parallel with the proximal phalanx and over the surface after the bunionectomy. Furthermore, with the derotation of the head of the metatarsal bone we achieve additional balancing of the muscules and of the ligamentous-sesamoide complex. The present data suggest that our open osteotomy indeed allows for a plantar shift and the amount of the first metatarsal shortening achieved in the present series could be considered moderate – it was ≤6 mm in 32/34 feet (7 mm and 10 mm in the remaining two), with a median shortening of 4 mm. Mađarevic et al. reported a mean shortening of 5.5 mm in 28 feet treated by the standard Wilson's procedure (24). A similar mean shortening (5.1 mm, range 1 to 11.5 mm) with this method (but with a reduced osteotomy angle) was reported for a series of 46 feet by Yildrim et al. (25). Klosok et al. (23) reported an average 10 mm (range 6 to 20 mm) shortening in a series of 37 feet, whereas Klareskov et al. (22) reported a mean shortening of 8 mm in a 77-feet series and Pouliart et al. (27) reported a 8.5 mm mean shortening in a 32-feet series. Grace at al. (21) summarized three older studies on Wilson's osteotomy (published prior to 1981) and calculated an overall mean shortening of 7.8 mm for a total of 118 feet.

Taking into account the mentioned published data that refer to a total of 338 feet, an n-weighted mean shortening of 7.6 mm could be calculated. An approximate comparison to the median shortening of 4 mm reported in the present series (the mean value was 3.97 mm) suggests that the described method apparently achieved its main purpose of reducing the first metatarsal shortening. The method also appears to perform well in respect to standard clinical (AOFAS score and clinical status) and other radiological (relevant angles) criteria. A single "poor" result according to AOFAS score in one patient was due to metatarsal head dislocation followed by a malunion, whilst a single "fair" result according to AOFAS score in another patient was due to excessive (10 mm) shortening of the first metatarsal bone. However, whether or not this method indeed confers radiological (primarily the first metatarsal shortening) and clinical benefits (reduced incidence and/or severity of metatarsalgia) over other established methods is yet to be evaluated in prospective randomized trials.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Conflicts of interest: None to declare

REFERENCES

1. Coughlin M. Hallux valgus. J Bone Joint Surg Am 1998; 78:932-66.
2. Donley BG, Tisdel CL, Sferra JJ, Hall JO. Diagnosing and treating hallux valgus: a conservative approach for a common problem. Cleve Clin J Med 1997; 64:469-74.
3. Kilmartin TE, Wallace WA. The etiology of hallux valgus: a critical review of the literature. Foot 1993; 3:157-67.
4. Vanore JV, Christensen JC, Kravitz SR, Schuberth JM, Thomas JL, Wei LS, Zlotoff HJ, Mendicino RW, Couture SD. Clinical practice guideline. First metatarsophalangeal joint disorders. Joint disorders panel of the American college of foot and angle surgeons. Diagnosis and treatment of first metatarsophalangeal joint disorders. Section 1: Hallux valgus. J Foot Ankle Surg 2003; 42:112-23.
5. Helal B. Surgery for adolescent hallux valgus. Clin Orthop 1981; 157:50-63.
6. Helal B, Gupta SK, Gojaseni P. Surgery for adolescent hallux valgus. Acta Orthop Scand 1974; 45:271-95.
7. Guler O, Yilmaz B, Mutlu S, Cerci MH, Heybeli N. Distal oblique metatarsal osteotomy for hallux valgus deformity: a clinical analysis. J Foot Ankle Surg 2017; 56:497-504.
8. Xarchas KC, Mavrolias D, Kyriakopoulos G. Modified Wilson's Osteotomy for hallux valgus deformity. A new approach. Open Orthop J 2014; 8:361-7.
9. Ben-Ad R. Fixation updates for hallux valgus correction. Clin Podiatr Med Surg 2014; 31:265-79.
10. Chandler LM. First metatarsal head osteotomies for the correction of hallux abducto valgus. Clin Podiatr Med Surg 2014; 31:221-31.
11. Ferrari J, Higgins JP, Prior TD. Interventions for treating hallux valgus (abductovalgus) and bunions. Cochrane Databse Syst Rev 2004; 1:CD000964.
12. Venning P, Hardy RH.Sources of error in the production and measurement of standard radiographs of the foot. Br J Radiol 1951; 24:18-26.
13. Miller JW. Distal first metatarsal displacement osteotomy: its place in the schema of bunion surgery. J Bone Joint Surg Am 1974; 56-A:923-31.
14. Schneider W, Csepan R, Knahr K. Reproduciblity of the radiographic metatarsophalangeal angle in hallux surgery. J Bone Joint Surg Am 2003; 85-A:949-59.
15. Schneider W, Knahr K. Metatarsophalangeal and intermetatarsal angle: different values and interpretation of postoperative results dependent on the technique of measurement. Foot Ankle Int 1998; 19:532-36.
16. Hardy RH, Clapham JCR. Observations on hallux valgus, J Bone Joint Surg Br 1951; 33:376-91.
17. Gudas CJ, Marcinko DE. The complex deformity known as hallux abductor valgus. In: Marcinko DE, ed. Comprehensive textbook of hallux valgus reconstruction. St Louis: Mosby 1992, 1-17.
18. Smith RW, Reynolds JC, Stewart MJ. Hallux valgus assessment: report of Research Committee of American Orthopaedic Foot and Ankle Society. Foot Ankle 1984; 5:92-103.
19. Kitaoka HB, Alexander IJ, Adelaar RS, Nunley JA, Myerson MS, Sanders M. Clinical rating system for the ankle-hindfoot, midfoot, hallux and lesser toes. Foot Ankle Int 1994; 15:349-53.
20. Merkel KD, Katoh Y, Johnson EWJr, Chao EY. Mitchell osteotomy for hallux valgus: Long term follow-up and gait analysis. Foot Ankle 1983; 3:189-96.
21. Grace D, Hughes J, Klenerman L.A comparison of Wilson and Hohmann osteotomies in the treatment of hallux valgus. J Bone J Surg Br 1988; 70-B: 236-41.
22. Klareskov B, Dalsgaard S, Gebuhr P. Wilson shaft osteotomy for hallux valgus. Acta Orthop Scand 1988; 59:307-9.
23. Klosok JK, Pring DJ, Jessop JH, Maffulli N. Chevron or Wilson metatarsal osteotomy for hallux valgus. A prospective randomized trial. J Bone J Surg Br 1993; 75-B:825-29.
24. Mađarevic M, Kolundžić R, Matek D, Šmigovec I, Crnkovic T, Trkulja V. Mitchell and Wilson metatarsal osteotomies for the treatment of hallux valgus: comparison of outcomes two decades after the surgery. Foot Ankle Int 2006; 27:877-82.
25. Yildrim Y, Saygi B, Aydin N, Cabukoglu C, Bautista S. Components of the Wilson osteotomy that are effective on hallux valgus repair. J Foot Ankle Surg 2007; 46:21-6.
26. Magnan B, Pezze L,Rossi N,Bartolozzi P. Percutaneous distal metatarsal osteotomy for correction of hallux valgus. J Bone J Surg Am 2005; 87:1191-99.
27. Pouliart N, Haentjens P, Opdecam P. Clinical and radiographic evaluation of Wilson osteotomy for hallux valgus. Foot Ankle 1996; 17:388-94.

Hrvatska modifikacija Wilsonove osteotomije za liječenje haluks valgusa u odraslih: rotatorna kosa trodimenzionalna osteotomija u svrhu prevencije dorazlnog pomaka distalnog ulomka i skraćenja prve metatarzalne kosti

Robert Kolundžić[1,5], Mladen Mađarević[2], Vladimir Trkulja[3], Tomislav Crnković[4,5], Igor Šmigovec[2], Daniel Matek[2]

[1]Klinika za traumatologiju, Klinički bolnički centar "Sestre milosrdnice, Zagreb, [2]Klinika za orotopediju, Klinički bolnički centar "Zagreb", Zagreb, [3]Medicinski fakultet Sveučilišta u Zagrebu, Zagreb, [4]Odjel za ortopediju i traumatologiju, Županijska opća bolnica Požega, Požega, [5]Medicinski fakultet Sveučilišta Josip Juraj Strossmayer, Osijek; Hrvatska

SAŽETAK

Cilj Procijeniti biomehaničke i kliničke rezultate nove modifikacije Wilsonove osteotomije za liječenja haluks valgusa: trodimenzionalne subkapitalne korekcije položaja glave prve metatarzalne kosti s istodobnim lateralnim i plantarnim pomakom s derotacijom u svrhu prevencije pomaka distalnog ulomka i skrećenja prve metatarzalne kosti.

Metode Novom su metodom operirana 34 stopala u 28 žena, a evaluirana su prije operacije i 12 do 84 mjeseca nakon operacije (medijan=25.5 tjedana).

Rezultati Plantarni pomak distalnog fragmenta ostvaren je u svim stopalima pri čemu je skraćenje prve metatarzalne kosti bilo umjereno: ≤6 mm u 32/34 stopala i 7 i 10 mm u preostala dva. Medijan-razlika metatarzalnog indeksa, nakon zahvata u odnosu na stanje prije zahvata, iznosio je -4.0. Kut haluks valgusa, intermetatarzalni kut i kut distalnog metatarzalnog zgloba smanjeni su u svim stopalima. Skor Američkog društva za stopalo i skočni zglob povećan je u svim stupalima (medijan povećanja=51.5).

Zaključak Metoda omogućuje plantarni i lateralni pomak distalnog ulomka, uz derotaciju i blago do umjereno skraćenje prve metatarzalne kosti.

Ključne riječi haluks valgus, operacija, metatarzalna kost

ORIGINAL ARTICLE

Serum homocysteine levels in patients with probable vascular dementia

Salem Alajbegović[1], Orhan Lepara[2], Almira Hadžović-Džuvo[2], Alma Mutevelić-Turković[2], Lejla Alajbegović[3], Asija Zaćiragić[2], Nesina Avdagić[2], Amina Valjevac[2], Nermina Babić[2], Amela Dervišević[2]

[1]Department of Endocrinology and Diabetes, Cantonal Hospital, Zenica, [2]Department of Human Physiology, School of Medicine, University of Sarajevo, Sarajevo, [3]Representative office of Berlin-Chemie AG in Sarajevo; Bosnia and Herzegovina

ABSTRACT

Aim To investigate total homocysteine (tHcy) serum concentration in patients with probable vascular dementia (VD) and in age-matched controls, as well as to determine an association between tHcy serum concentration and cognitive impairment in patients with probable VD.

Methods Serum concentration of tHcy was determined by the Fluorescence Polarization Immunoassay on the AxSYM System. Cognitive impairment was tested by the Mini Mental Status Examination (MMSE) score. Body mass index (BMI) was calculated for each subject included in the study.

Results Age, systolic, diastolic blood pressure and BMI did not differ significantly between the two groups. Mean serum tHcy concentration in the control group of subjects was 13.35 μmol/L, while in patients with probable VD it was significantly higher, 19.45 μmol/L (p=0.002). A negative but insignificant association between serum tHcy concentration and cognitive impairment in patients with probable VD was found.

Conclusion Increased tHcy concentration in patients with probable VD suggests the possible independent role of Hcy in the pathogenesis of VD.

Key words: neurotoxicity, cognitive impairment, Mini-Mental State Examination

Corresponding author:
Orhan Lepara
Department of Human Physiology, School of Medicine, University of Sarajevo
Čekaluša 90, 71 000 Sarajevo, Bosnia and Herzegovina
Phone: +387 33 226 478; fax +387 33 203 670;
E-mail. orhan.lepara@gmail.com
Salem Alajbegović ORCID ID: http://www.orcid.org/0000-0003-3191-6356

Original submission:
24 May 2017;
Revised submission:
22 June 2017;
Accepted: 04 July 2017.
doi: 10.17392/916-17

Med Glas (Zenica) 2017; 14(2): 257-261

INTRODUCTION

Vascular dementia represents a clinical syndrome that includes a wide spectrum of cognitive dysfunctions resulting from brain tissue death due to ischemia caused by vascular disease (1). Homocysteine (Hcy) is a thiol-containing amino acid involved in the cycle of methionine as the demethylation product of methionine and in the transsulfuration pathway (1,2). Hyperhomocysteinemia, or increased serum concentration of total homocystein (tHcy) is a risk factor for cardiovascular disease and seems to be an independent risk factor for dementia (1,2).

Hyperhomocysteinemia is associated with relative deficiencies of folate, vitamins B6 and B12, as well as with older age, male sex, estrogen deficit, renal insufficiency and also with use od different substances like caffeine, dopamine or anticonvulsant drugs (2).

Elevated homocysteine levels can increase oxidative stress and cause endothelial cell dysfunction, smooth muscle cell proliferation and impaired fibrinolysis (2,3). While this could be a contributory factor, data from several incidence studies show that levels in normal elderly people prognosticate the occurrence of stroke and dementia (2-4). Moreover, it is possible that deficiency of vitamin B12 or folic acid could result in metabolic derangements other than hyperhomocysteinemia (2-4). Thus, hyperhomocysteinemia should, at the present time, be regarded only as an indicator of susceptibility to disease, and therapies that could reduce Hcy levels are not guaranteed to reduce the risk and incidence of either strokes or dementia (5).

Homocysteine promotes changes associated with atherosclerosis but the pathological mechanisms triggered by Hcy accumulation are poorly understood (6). The effect of atherosclerosis on dementia has also been attributed to its relation to cerebral infarction or to systemic or local factors that underlie both atherosclerosis and cognition (7).

Several studies have shown significantly higher serum Hcy concnentration in patients with VD compared to control subjects (8). Studies have shown a correlation between low folic acid and high Hcy levels with cognitive functions in vascular dementia and AD, which is consistent with the idea of secondary elevation of total Hcy in demented people. (9).

The aim of this study was to investigate serum tHcy concentration in patients with probable VD with age-matched controls. We also aimed to determine whether there is an association between serum tHcy concentrations and cognitive impairment tested by Mini Mental Status Examination (MMSE) score at patients with probable VD.

PATIENTS AND METHODS

Patients and study design

The study was designed as a cross-sectional study which included 20 patients (17 females and 3 males) with probable VD aged 65 years old and more, institutionalized at the specialized unit at the Health-Care Hospice for persons with disabilities in Sarajevo, Bosnia and Herzegovina. Furthermore, in this study 19 community-dwelling, age-matched apparently healthy controls without dementia were included.

For both groups of subjects, exclusion criteria were positive medical history of chronic inflammatory diseases (asthma and rheumatoid arthritis), thyroid disease, hepatic and renal insufficiency or cancer.

An approval for the study was obtained by the Ethics Committee of Clinical Centre, University of Sarajevo. All procedures on human subjects were performed according to the Declaration of Helsinki, 1975. Written informed consent was obtained from all participating subjects and caregivers.

Methods

Probable vascular dementia was clinically diagnosed by standardized clinical examination conducted by a specialist neurologist and psychiatrist by the NINDS-AIREN criteria (10).

Global cognitive function was tested with the MMSE test which has been used for rapid screening of those with cognitive and/or intellectual deficit (11). The test evaluates orientation, short term memory, serial subtraction, constructional capacities and use of language. The total score was 30, where the score of 24 was considered abnormal, and the score less than 17 was considered as dementia. All patients with probable VD had a score ≤ 12 while subjects in the control group had a score from 26 to 30.

The Hachinski ischemic score (HIS) differentiates patients with VD from those with AD (12). The original scale consists of 13 items where each scale item represents a specific clinical event which has a certain numeric value. A score

less or equal 4 means Alzheimer's dementia, 4-7 indicates mixed dementia, and a total score of ≥7 refers to vascular dementia. Patients in this study with probable VD had a score ≥7.

Non-fasting blood samples were drawn from the antecubital vein into siliconized tubes. After venipuncture, blood samples were put on ice. The median time between venipuncture and centrifugation was 50 min (interquartile range: 30-70 min). Serum samples were stored at or below -20 ºC.

Serum tHcy concentration was measured by using a fluorescence polarization immunoassay on the AxSYM System at the Institute for Chemistry and Biochemistry, Clinical Centre of the University of Sarajevo (13). The reference interval for tHcy concentration with the use of this method was from 3.36 to 20.44 μmol/L.

Body Mass Index (BMI) for all participants was calculated as weight (kg) divided by the square of height in meters (m^2). Height was measured with stadiometer and body weight was measured with the use of Toledo self-zeroing weight scale.

Trained staff measured blood pressure using a mercury sphygmomanometer on the right arm after a 5 minute rest.

Statistical analysis

Data were presented as mean ± SEM. Data distribution was determined using the Shapiro-Wilk test. Data were statistically analyzed by using Student's t test. Additionally, Pearson correlations were used as measures of association for the continuous variables. Statistical significance was set at $p<0.05$.

RESULTS

No differences emerged in age, systolic and diastolic blood pressure between the groups. No difference in BMI was found between the two groups. Subjects with probable VD had statistically significantly lower MMSE scores compared with the control group ($p<0,001$) (Table 1).

Mean serum tHcy concentration in the control group was 13.35 μmol/L, while in patients with probable VD the mean serum tHcy concentration was significantly higher, 19.45 μmol/L ($p=0,002$) (Figure 1).

A negative but insignificant correlation was noted between tHcy concentration and MMSE score in patients with probable VD ($r=-0,05$).

Table 1. Baseline characteristics of patients with probable vascular dementia and the control group*

Variables	Patients with probable vascular dementia	Controls
Age (years)	76,9±1,4	77,8±1,1
SBP (mmHg)	132,0±6,6	130,5±4,4
DBP (mmHg)	80,0±3,4	81,3±1,9
BMI (kg/m²)	23.9±0,5	25,6±0,8
MMSE score	8,0±1,3†	27,6±0,3

*Data are presented as mean ± S.E.M.; †$p<0,001$; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; MMSE, Mini Mental State Examination;

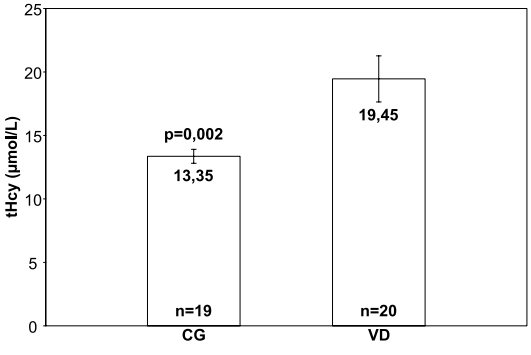

Figure 1. Mean serum total homocysteine concentration (tHcy) in the control group (CG) and in patients with probable vascular dementia (VD)
Data are presented as mean ± S.E.M.

DISCUSSION

Previous cross-sectional studies have found elevated Hcy levels in patients with VD (8,14,15). It is believed that there are several mechanisms between increased Hcy levels and the occurrence of VD. One of them explains that homocysteine has a direct neurotoxic effect which play a significant role in the etiology of VD and is also associated with cerebrovascular disease (14). More recently, observational studies have begun to link high concentrations of homocysteine to Alzheimer`s disease and other dementias (15).

The main finding of this study is a significantly higher serum tHcy concentration in patients with probable VD compared with the control group. Our observations are consistent with those of Malaguarnera et al. who also found elevated serum concentration levels of Hcy in patients with VD compared with the control group of subjects (8). Follin et al. showed a significant increase in plasma Hcy levels in patients with AD and VD compared with controls. These results showed that the VD group had the highest levels and also elevated Hcy levels showed a great involvement in dementia (16). Several studies also reported

that persons with elevated serum homocysteine concentrations have a significantly greater risk of developing dementia than the persons without elevated homocysteine (17,18), but the results have not been entirely consistent (19). It is unclear whether low concentrations of vitamin B-12 or folate acid were responsible for the greater risk of dementia or elevated serum homocysteine concentrations in those studies. Haan et al. reported significant associations of dementia or cognitive impairment with elevated plasma homocysteine concentrations in a 4.5-year follow-up of a cohort study, conducted after the introduction of mandatory folic acid fortification in the United States of 1779 Mexican Americans aged 60 years (20).

While there is a strong correlation between hyperhomocysteinemia on one side and cerebrovascular disease and dementia on the other, a correlation should never be taken to indicate causation (2,3,21). Homocysteine itself is thought to be toxic to blood vessels and can be shown in vitro to cause excitotoxic damage like high levels of glutamate (2,3,21). Oxidative stress can be one of the mechanism involved in endothelial dysfunction. Therefore, oxidative stress could affect endothelial cells in large vessels (e.g. the carotid arteries) or smaller-caliber arterioles (such as penetrating blood vessels in the brain) resulting in local thrombosis and cerebral ischemia (2,3,21).

Kloppenborg et al. (22) found that patients with symptomatic atherosclerotic disease and higher homocysteine levels are associated with higher presence of lacunar infarcts and slightly worse cognitive function. The same statement about high level of plasma homocysteine as an independent risk factor for cerebral large-artery atherosclerosis was also reported by Wang at al. (23). Alternatively, large vessel intracranial atherosclerosis could be a marker for dysfunction of small cerebral vessels and their endothelium that might be the proximate cause of cognitive deterioration, either through disruption of the communication between neurons and blood vessels (the neurovascular unit) that underlies activity induced vasodilatation, or through disruption of the blood-brain barrier (7).

A Swedish study involving patients with early (EOAD) and late (LOAD) onset of AD showed that there was no difference in tHcy or its determinants between the EOAD group and age- and sex-matched controls. In contrast, patients with VD or mixed AD/VD showed increased tHcy. Total Hcy was also elevated in patients with LOAD and a history of cerebrovascular disease (CVD) compared with both AD patients without history of CVD and with controls. These findings suggest that elevated tHcy contributes to dementia mainly through vascular mechanisms (24).

When interpreting our results a number of limitations should be considered. A limitation of the study was a small sample size. As we analyzed cross sectional data our study did not demonstrate a causal relationship. Increased Hcy concentration in patients with probable VD observed in our study suggest a possible role for Hcy in the pathogenesis of this disease. These findings imply that the serum Hcy level may be a potential biomarker in the diagnosis of vascular dementia. Further studies are needed to establish that elevated homocysteine is a risk or a consequence of VD as well as evaluate the potential biomarkers role of homocysteine in this disease.

FUNDING

No specific funding was received for this study.

TRANPARENCY DECLARATIONS

Competing interests: none to declare.

REFERENCES

1. Kalaria RN. Neuropathological diagnosis of vascular cognitive impairment and vascular dementia with implications for Alzheimer's disease. Acta Neuropathol 2016; 131:659–85.
2. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J 2015; 14:6.
3. Schalinske KL, Smazal AL. Homocysteine imbalance: a pathological metabolic marker. Adv Nutr 2012; 3:755-62.
4. Ashjazadeh N, Fathi M, Shariat A. Evaluation of homocysteine level as a risk factor among patients with ischemic stroke and its subtypes. Iran J Med Sci 2013; 38:233-9.
5. Spence JD, Howard VJ, Chambless LE, Malinow MR, Pettigrew LC, Stampler M, Toole JF. Vitamin Intervention for Stroke Prevention (VISP) trial: Rationale and design. Neuroepidemiology 2001; 20:16–25.
6. Oksana Tehlivets. Homocysteine as a risk ractor for atherosclerosis: Is its conversion to S-Adenosyl-L-homocysteine the key to deregulated lipid metabolism. J Lipids 2011; 2011:702853.
7. Dolan H, Crain B, Troncoso J, Resnick SM, Zonderman AB, and OBrien RJ. Atherosclerosis, dementia, and Alzheimer disease in the Baltimore longitudinal study of aging cohort. Ann Neurol 2010; 68:231–40.

8. Wang B, Zhong Y, Yan H, Cui L. Meta-analysis of plasma homocysteine content and cognitive function in elderly patients with Alzheimer's disease and vascular dementia. Int J Clin Exp Med 2014; 5118-23.
9. Cai C, Xiao R, Van Halm-Lutterodt N, Zhen J, Huang X, Xu Y, Chen S, Yuan L. Association of MTHFR, SLC19A1 genetic polymorphism, serum folate, vitamin B12 and Hcy status with cognitive functions in Chinese adults. Nutrients 2016; 8:10.
10. Román GC, Tatemichi KT, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, Amaducci L, Orgogozo JM, Brun A, Hofman Al. Vascular dementia: diagnostic criteria for research studies: Report of the NINDS-AIREN International Workshop. Neurology 1993; 43:250-60.
11. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12:189-98.
12. Loncarević N, Mehmedika Sulić E, Alajbegović A, Kucukalić A. The neurologist role in diagnostics and therapy of the Alzheimer's disease. Med Arh 2005; 59:6-9.
13. Pernet P, Lasnier E, Vaubourdolle M. Evaluation of the AxSYM homocysteine assay and comparison with the IMx homocysteine assay. Clin Chem 2000; 46:1440-1.
14. Khedr EM, Hamed SA, El-Shereef HK, Shawky OA, Mohamed KA, Awad EM, Ahmed MA, Shehata GA, Eltahtawy MA. Cognitive impairment after cerebrovascular stroke: Relationship to vascular risk factors. Neuropsychiatr Dis Treat 2009; 5:103–16.
15. Lepara O, Alajbegovic A, Zaciragic A, Nakas-Icindic E, Valjevac A, Lepara D, Hadzovic-Dzuvo A, Fajkic A, Kulo A, Sofic E. Elevated serum homocysteine level is not associated with serum C-reactive protein in patients with probable Alzheimer's disease. J Neural Transm 2009; 116:1651-6.
16. Folin M, Baiguera S, Gallucci M, Conconi MT, Di Liddo R, Zanardo A, Parnigotto PP. A cross-sectional study of homocysteine-, NO-levels, and CT-findings in Alzheimer dementia, vascular dementia and controls. Biogerontology 2005; 6:255-60.
17. Seshadri S. Homocysteine and the risk of dementia. Clin Chem 2012; 58:1059-60.
18. Moretti R, Caruso P, Dal Ben M, Conti C, Gazzin S, Tiribelli C. Vitamin D, homocysteine, and folate in subcortical vascular dementia and Alzheimer dementia. Front Aging Neurosci 2017; 9:169.
19. Luchsinger JA, Tang MX, Shea S, Miller J, Green R, Mayeux R. Plasma homocysteine levels and risk of Alzheimer disease. Neurology 2004; 62:1972–6.
20. Haan MN1, Miller JW, Aiello AE, Whitmer RA, Jagust WJ, Mungas DM, Allen LH, Green R. Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging. Am J Clin Nutr 2007; 85:511–7.
21. Lehotský J, Tothová B, Kovalská M, Dobrota D, Beňová A, Kalenská D, Kaplán P. Role of Homocysteine in the Ischemic Stroke and Development of Ischemic Tolerance. Front Neurosci 2016; 10:538.
22. Kloppenborg RP, Nederkoorn PJ, van der Graaf Y, Geerlings MI. Homocysteine and cerebral small vessel disease in patients with symptomatic atherosclerotic disease. The SMART-MR study. Atherosclerosis 2011; 216:461-6.
23. Wang YJ, He Y, Jiang WJ, Du B, Jin M. Correlation between plasma level of homocysteine and cerebral large-artery atherosclerosis. Zhonghua Nei Ke Za Zhi 2006; 45:744-7.
24. Nilson K, Gustafson L, Huktberg B. Relation between plasma homocysteine and Alzheimer's Disease. Dement Geriatr Cogn Disord 2002; 14:7-12.

Nivo homocisteina u serumu pacijenata s mogućom vaskularnom demencijom

Salem Alajbegović[1], Orhan Lepara[2*], Almira Hadžović-Džuvo Almira[2], Alma Mutevelić-Turković[2], Lejla Alajbegović[3], Asija Zaćiragić[2], Nesina Avdagić[2], Amina Valjevac[2], Nermina Babić[2], Amela Dervišević[2]

[1]Odjeljenje za endokrinologiju i dijabetes, Kantonalna bolnica Zenica, [2]Katedra za fiziologiju čovjeka, Medicinski fakultet, Univerzitet u Sarajevu, Sarajevo, [3]Berlin-Chemie AG, Predstavništvo u Sarajevu; Bosna i Herzegovina

SAŽETAK

Cilj Ispitati koncentraciju ukupnog homocisteina (tHcy) u serumu pacijenata s mogućom vaskularnom demencijom (VD) i kod ispitanika kontrolne grupe, te utvditi povezanost između nivoa tHcy i kognitivnog oštećenja kod pacijenata s mogućom VD-om.

Metode Serumska koncentracija tHcy određivana je metodom fluorescentno-polarizacijskog imunoeseja na AxSYM sistemu. Kognitivno oštećenje je testirano skorom minimentalnog statusa (MMSE). Indeks tjelesne mase (ITM) izračunat je za svakog ispitanika uključenog u studiju.

Rezultati Starosna dob, sistolni i dijastolni krvni pritisak i ITM nisu se značajno razlikovale između dvije ispitivane grupe. Prosječna koncentracija tHcy u kontrolnoj grupi iznosila je 13.35 μmol/L, dok je u grupi pacijenata s mogućim VD-om bila signifikantno viša i iznosila je 19.45 μmol/L (p=0.002). Utvrđena je negativna, ali ne signifikantna povezanost između serumske koncentracije tHcy i kognitivnog oštećenja kod pacijenata s mogućim VD-om.

Zaključak Povećan nivo tHcy kod pacijenata s mogućim VD-om sugeriše na moguću nezavisnu ulogu Hcy u patogenezi VD-a.

Ključne riječi: neurotoksičnost, kognitivno oštećenje, minimentalni status

ORIGINAL ARTICLE

Mental diseases and criminal offences committed by persons placed at the Forensic Department of the Penitentiary in Zenica, Federation of Bosnia and Herzegovina

Edin Bjelošević[1], Adisa Krehmić[2], Halima Hadžikapetanović[1], Sanel Čoralić[3], Sonja Bjelošević[1]

[1]Mental Healthcare Centre, Healthcare Centre Zenica, [2] School of Medicine, University of Zenica, [3]Forensic Department, Zenica Penitentiary; Zenica, Bosnia and Herzegovina

ABSTRACT

Aim To investigate an impact of various biological, psychological and social factors on perpetration of criminal offences by persons with mental disorders and to examine legal requirements for placement of persons with mental disorders, who committed criminal offences.

Methods This retrospective, descriptive study based on the analysis of data collected from records of the Zenica Penitentiary, Forensic Department (age, qualifications, employment status, marital status, mental disorders, information related to earlier treatments, type of committed criminal offense, duration of the security measure of mandatory psychiatric treatment and custody) included 154 examinees.

Results The study included 154 male examinees. An average age of the examinees was 34 years. An average duration spent at the Forensic Department was 3 years, murder was committed by 68 (44.15%) examinees, and 34 (22.07%) examinees committed attempted murder. Eighty-five (55. 19 %) examinees suffered from schizophrenia, 30 (19.48%) had delusional disorder, and 19 (12.33%) had mental retardation.

Conclusion The highest number of committed crimes was in correlation with schizophrenia, (the highest number of examinees suffered from schizophrenia). It is necessary to work on the establishment of a forensic hospital in the territory of the Federation of Bosnia and Herzegovina in order to create good conditions for rehabilitation of patients with mental disorders who committed criminal offences.

Key words: schizophrenia, accountability, murder

Corresponding author:
Edin Bjelošević
Mental Healthcare Centre, Healthcare Centre Zenica
Fra Ivana Jukića 2, 72000 Zenica,
Bosnia and Herzegovina
Phone: +387 32 444 411;
Fax: +387 32 242 113;
E-mail: edinvesna@gmail.com
ORCID ID: http://www. orcid.org/ 0000-0002-2359-3641

Original submission:
15 March 2017;
Revised submission:
06 April 2017;
Accepted:
17 April 2017.
doi: 10.17392/905-17

Med Glas (Zenica) 2017; 14(2): 262-268

INTRODUCTION

The World Health Organization describes mental health as a state of wellbeing in which every individual realizes his or her own potential, can cope with the normal stress of life, can work productively and fruitfully, and is able to contribute to his or her community. From this positive standpoint mental health is a basis for human wellbeing and efficient functioning of individuals and a community (1). Experts from the field of mental health have been trying to define normal and abnormal behaviour. Although there is often a very thin line between normal and abnormal behaviour, over time some consensus has been reached on what is abnormal behaviour and experience, and it is contained in modern diagnostic guidelines, Diagnostic and Statistical Manual of Mental Disorders (DSM 5) (2) and International Classification of Diseases and Causes of Death (ICD -10) in the form of diagnostic criteria of mental disorders (2,3). According to ICD-10 mental disorders are placed in the group F from F00 to F99 (3).

There is a question when a person is mentally incapacitated or with reduced mental capacity.

''A mentally incapable person is the one who, at the time of perpetrating the criminal office, was incapable of comprehending the significance of his acts or controlling his conduct due to a permanent or temporary mental disease, temporary mental disorder or retardation (mental incapacity)'' (4). If the capacity of a perpetrator to comprehend the significance of his act and his ability to control his conduct were considerably diminished due to any of the mental conditions referred to in paragraph 1 of Criminal Code (CC) of the Federation of Bosnia and Herzegovina (FB&H), he may be punished less severely (considerably diminished mental capacity) (4). If a perpetrator committed an unlawful act in the state of mental incapacity there are conditions stipulated by the law to place him by coercion in a medical institution (5).

In FB&H there is no adequate medical institution for placement, treatment, resocialization and rehabilitation of persons who committed a criminal offence in the state of mental incapacity due to permanent or temporary mental disorder or mental retardation. Such persons are placed at the Forensic Department of the Penitentiary in Zenica in order to serve their security measures of compulsory psychiatric treatment and custody. From the professional, psychiatric, ethical and legal aspect, their placement in the Forensic Department of the Penitentiary Facility in Zenica rather than a medical institution is of great concern.

There are high levels of stigma and discrimination reported by persons with mental disorders (6). Stigmatization prevents natural resocialization of persons with mental retardation, which affects the course of a therapeutic process (7). Forensic psychiatry is applied psychiatry for legal purposes (8). In the field of criminal law forensic mental health is a wider area, which involves the assessment and treatment of persons with mental disorders, whose behaviour resulted or could have resulted in the perpetration of criminal offences (8). A research conducted in the USA has discovered that 95% of the public believe that a legal intervention could prevent expected damage if it were anticipated that a person with mental disorder could be violent towards other persons (9). Having been discharged from hospital, 18.7% of patients with mental disorders committed at least one violent act within the first 20 weeks after discharge (10). The Law on Mental Health clearly stipulates that custody in a hospital needs to be therapeutic, or "in the interest of protection of health, security or protection of others" and that civil rights of individuals must not be violated (11). The research has shown that there is 5% of persons suffering from schizophrenia among those convicted of murder (12).

The aim of the study was to examine an impact of different biological, psychological and social factors to the perpetration of criminal offences by persons with mental disorders and examine legal conditions for placement of persons with mental disorders who committed a criminal offence. The purpose of the study was to underline the legality of placement of persons with mental disorders in the institution where they are placed.

EXAMINEES AND METHODS

Design and study sample

In this retrospective and descriptive study, data gathered from protocols of the Forensic Department of the Penitentiary in Zenica, FB&H, were analysed. The study included a period from the establishment of the Forensic Depar-

tment in 1996 until 2015. The study included a total number of 154 examinees placed at the Forensic Department of the Zenica Penitentiary to serve their security measures of compulsory psychiatric treatment and custody, but also those who served their imprisonment sentences. All examinees were males as the Penitentiary in Zenica admits only male perpetrators of criminal offences. According to the current data of the Forensic Department, out of the total number of examinees who served their security measures of compulsory psychiatric treatment, there were 18 persons with mental or psychiatric disorders, who are defined by Article 3 paragraph 1 and paragraph 2 of the Law on Protection of Persons with Mental Disorders in the FB&H as "mentally ill person with a mental disorder, a person with insufficient mental development, alcohol or drug addict or persons with other mental disorders" and ''a person with serious mental disorders is a person with such mental disorders that prevent him from comprehending significance of his acts or is not able to control his will or his abilities are reduced to the extent that he/she needs psychiatric assistance", respectively (13).

Data collected for the purpose of the analysis included the examinees' age, place of residence, property or financial status, qualifications, professional experience, marital status and family environment, socio-pathological aspects such as alcoholism and other forms of toxic mania, diagnoses of diseases or mental disorders, data on past medical treatments, information on individual characteristics and personality, type of committed crime, duration of the measure of compulsory psychiatric treatment and custody.

An approval for the study was obtained from Zenica Penitentiary.

Statistical analysis

Methods of descriptive statistics, ANOVA test, χ^2 test were used for the analysis of the data: age, education level, marital status, employment status**,** type of criminal offence broken down by age, type of criminal offence broken down by qualifications of a perpetrator, duration of the security measure, number of murders and attempted murders.

The $p<0.05$ was considered as statistically significant.

RESULTS

The study included 154 examinees placed at the Forensic Department of the Penitentiary in Zenica. All the examinees were males. The examinees were mostly in the age groups of 30-39 and 20-29 years of age, 53 (32%) and 49 (30%), respectively. Majority of the examinees had secondary school degree, 67 (43.50%), and primary school, 49 (31.81%), followed by the examinees without primary education, 19 (12.33%). As far as the employment is concerned, most examinees were unemployed, 87 (56.49%), followed by the retirees, 38 (24.67%), and 16 (10.38%) examinees were employed. There were 61 (39.61%) single persons, 35 (22.72%) divorced and 34 (22.07%) examinees were married (Table 1).

Table 1. Demographic characteristics of examinees

Characteristic	No (%) of examinees
Age (years)	
Under 19	4 (2.59)
20-29	49 (31.81)
30-39	53 (34.41)
40-49	34 (22.07)
50-59	16 (10.38)
60-69	9 (5.84)
Total	154 (100)
Education	
No qualifications	19 (12.33)
Primary school	49 (31.81)
Secondary school	67 (43.50)
College	0
University	3 (1.94)
Student	1 (0.64)
Unknown	12 (7.79)
Total	154 (100)
Employment status	
Employed	16 (10.38)
Unemployed	87 (56.49)
Pensioner	38 (24.67)
Student	1 (0.64)
Unknown	12 (7.79)
Total	154 (100)
Marital status	
Married	34 (22.07)
Single	61 (39.61)
Divorced	35 (22.72)
Widower because of wife's murder	13 (8.44)
Unknown	11 (7.14)
Total	154 (100)

Of 154 examinees 85 (55. 19 %) were diagnosed with schizophrenia, 30 (19.48%) had the diagnosis of delusional disorder, 31 (20.12%) had the diagnosis of specific personality disorder, 19 (12.33%) were diagnosed with mental retardation, 10 (6.49%) with persistent delusional disorder, four (2.59%) examinees had the diagnosis of acute brief psychotic disorder, four had a manic episode, three (1.94%) had the diagnosis of PTSD and one (0.64%) examinee had the di-

agnosis of a depressive episode and one had dissociative disorder (Ganser syndrome) (specific developmental speech disorder).

Ninety-four (62%) examinees had one diagnosis, 43 (29%) examinees had two diagnoses and 13 (9%) had more than two diagnoses.

Of the total number of examinees, 54 (33%) abused alcohol, 13 (8%) examinees consumed drugs and 97 (59%) did not abuse either alcohol or drugs.

Of the total of 109 criminal offences against life and body, 68 (62%) examinees committed murders, 34 (31%) attempted murder, and seven (7%) examinees inflicted serious bodily injuries to their victims. Of 68 murders in total, 37 (54.41%) were committed against family members and 31 (45.58%) against other persons. Attempted murder was noted in 34 cases, of which 13 (38%) were against family members, while 21 (61.76%) were against other persons (Table 2).

Table 2 . Distribution of 102 murders and attempted murders according to persons against whom such crimes were committed

Murders and attempted murders against	No (%) of murders	No (%) of attempted murders
Mother	8 (11.76)	2 (5.88)
Father	6 (8.83)	0
Wife	14 (20.58)	6 (17.66)
Other family members	9 (13.23)	5 (14.7)
Other persons	31 (45.58)	21 (61.76)
Total	**68 (100)**	**34 (100)**

The most frequent criminal offences were committed by the examinees aged 20-29 years, 65 (28.13%), those aged 30-39, 65 (28.13%) and examinees in the age 40-49, 58 (25.10%). Most frequent criminal offences in these age groups were against life and body (total of 107) in 21 (19.62%), 41 (38.31%) and 26 (24.29), cases, respectively, (p=0.052) against property (total of 50) in 20 (40%), 11 (22%) and 10 (20%), respectively, and against public health order and legal transactions (total of 36) in nine (25%), 10 (27.77%) and 10 (27.77%), respectively (Table 3).

The highest number of the examinees, 30 (19.48%), were pronounced the measure of mandatory psychiatric treatment and custody in the period between six months and one year, as well as one to two years (Table 4).

Table 4. Duration of the security measure of mandatory psychiatric treatment and custody

Duration of the security measure of mandatory psychiatric treatment and custody	N (%)
Up to 5 months	12 (7.79)
6 – 12 months	30 (19.48)
1 - 1 year and 12 months	30 (19.48)
2 years – 2 years and 12 months	21 (13.63)
3 years – 3 years and 12 months	16 (10.38)
4 years – 4 years and 12 months	15 (9.74)
5 years – 5 years and 12 months	7 (4.54)
6 years – 6 years and 12 months	5 (3.24)
7 years – 7 years and 12 months	1 (0.64)
8 years – 8 years and 12 months	3 (1.94)
11 years – 11 years and 12 months	5 (3.24)
13 years – 13 years and 12 months	2 (1.29)
14 years – 14 years and 12 months	1 (0.64)
15 years – 15 years and 12 months	1 (0.64)
17 years – 17 years and 12 months	3 (1.94)
18 years – 18 years and 12 months	2 (1.29)
Total	**154 (100)**

The examinees with the secondary school degree most frequently committed criminal offences, 99 (43.42%) (p<0.001), followed by the examinees with primary school qualifications 65 (28.50%) (Table 5).

An analysis of pronounced security measure of mandatory psychiatric treatment and custody because of the perpetration of one or more cri-

Table 3. Distribution of examinees according to the type of the criminal offence broken down by age

Criminal offence against	No (%) of examinees in age group (years)							
	<19	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**Total**	**p**
life and body	1 (0.93)	21 (19.62)	41 (38.31)	26 (24.29)	9 (8.41)	9 (8.41)	107 (46.32)	0.052
freedom and rights*		3 (33.33)		1 (11.11)	3 (33.33)	2 (22.22)	9 (3.98)	
against marriage, family and youth†		3 (100)					3 (1.29)	
people's health‡		1 (50)		1 (50)			2 (0.86)	
sexual freedom and morality§		2 (50)	2 (50)				4 (1.24)	
property¶	5 (10)	20 (40)	11 (22)	10 (20)	4 (8)		50 (21.64)	
environment, agriculture and natural resources#				1 (100)			1 (0.43)	
public safety of persons and property**		6 (31.57)	1 (5.26)	9 (47.36)	2 (10.52)	1 (5.26)	19 (8.22)	
public order and legal transactions††	2 (5.55)	9 (25)	10 (27.77)	10 (27.77)	2 (5.55)	3 (8.33)	36 (15.58)	
TOTAL	**8 (3.46)**	**65 (28.13)**	**65 (28.13)**	**58 (25.10)**	**20 (8.65)**	**15 (6.49)**	**231 (100)**	

*six (66.66%) were related to endangering security and three (33.33%) to infringing inviolability of dwelling; †all were related to abduction of a child; ‡both were related to unauthorized production and sale of narcotic drugs; §all were related to rape; ¶11 (21%) were thefts, 22 (43%) were aggravated thefts, 3 (6%) were robberies, 7 (14%) were aggravated robbery, 1 (2%) was embezzlement and 7 (14%) were criminal offences of malicious mischief; #forest theft; **16 (84.21%) were offences of provoking general danger and 3 (15.78%) were serious criminal offences against public safety and property; ††10 (28%) were attacks against staff while carrying out security work, 8 (23%) against family members, 9 (26%) against other persons, 1 (3%) was removal or damage of an official seal, and 7 (20%) were related to illicit possession of weapons or explosive substances

Table 5. Examinees according to the type of criminal offences broken down by qualifications

Criminal offences against	No (%) of examinees by the qualifications							
	No qualifications	Primary school	Secondary school	University	Student	Unknown	Total	p
life and body	12 (11.11)	25 (23.14)	52 (48.14)	5 (4.62)		14 (12.96)	108 (47.36)	<0.001
freedom and rights		1 (14.28)	5 (71.42)			1 (14.28)	7 (3.07)	
marriage, family and youth			2 (100)				2 (0.87)	
people's health		2 (100)					2 (0.87)	
sexul freedom and morality	1 (25)	1 (25)	2 (50)				4 (1.75)	
property	7 (14)	20 (40)	13 (26)		3 (6)	7 (14)	50 (21.92)	
environment, agriculture and natural resources		1 (100)					1 (0.43)	
public safety of persons and property	3 (15.78)	6 (31.57)	8 (42.10)			2 (10.52)	19 (8.33)	
public order and legal transactions	4 (11.42)	9 (25.71)	17 (48.57)		1 (2.85)	4 (11.42)	35 (15.35)	
TOTAL	**27 (11.18)**	**65 (28.50)**	**99 (43.42)**	**5 (2.19)**	**4 (1.75)**	**28 (12.28)**	**228 (100)**	

minal offences showed that 105 (68.18%) examinees committed one criminal offense and 49 (12.82%) committed more criminal offences (data not shown).

Before the crime perpetration 104 (67.54%) examinees had been treated previously, and 50 (32.46%) had not been treated (data not shown).

Forty-five (29%) examinees had been previously convicted and 109 (71%) had not been convicted before (data not shown).

Reasons for the termination of the security measure of mandatory psychiatric treatment and custody were as follows: in three (1.94 %) examinees, the security measure of mandatory psychiatric treatment and custody ceased because of suicide, the natural death occurred in four (2.59 %) examinees, 23 (14.93%) were transferred to prison, 108 (70.12%) were released, and in 16 (10.38%) examinees the security measure was retained (data not shown).

Of the total of 154 examinees, 130 (84.41%) were found mentally incapacitated and the security measure of mandatory psychiatric treatment and custody was pronounced to them, while 23 (15%) examinees were found to have diminished mental capacity and in addition to the security measure of mandatory psychiatric treatment and custody, an imprisonment sentence was pronounced, in one (0.64%) examinee the security measure of mandatory psychiatric treatment and custody was pronounced with a suspended imprisonment sentence (data not shown).

DISCUSSION

The study included 154 examinees placed at the Forensic Department of the Penitentiary in Zenica, Federation Bosnia and Herzegovina. Of the total number of examinees 55.19% had the diagnosis of schizophrenia. In addition to schizophrenia, the highest number of examinees, 19.48%, had the diagnosis of delusional disorder, 20.12% were diagnosed with specific personality disorder, and 12.33% with mental retardation; Pond et al. study showed 32.4% psychosis and 19.7% personality disorder (14).

Persons with schizophrenic disorders are four times more likely to commit violence than persons without schizophrenic disorder (15), which is confirmed by our study. The studies of criminality in patients in psychiatric hospitals and mental disorders among imprisoned offenders suggest a link between major mental disorders (schizophrenia and big emotional disorders) and crime. It was found that men with most serious mental disorders or a handicap had higher likelihood of committing criminal offences than men without disorders and were four times more likely to commit violence. Criminal behaviour occurred in more than half of those cases at the age of 18 years (16). In our research, we received information that the majority of people with mental disorders committed a crime in the age of 20-29 years, 28.13%.

The results of this study have shown that persons with more serious mental disorders and persons with intellectual difficulties have a higher risk of violent behaviour and perpetration of criminal offences confirming results of previous studies (16). Research conducted in relation to readmission to custody or prison has shown that 30.8% of persons returned to prison indicating that early detection of deteriorated condition leads to improvement in mental health and decrease in the number of returns to prison (17). It is in line with the research conducted by Evans et al. showing that 68% examinees had been previously treated and that 29% had an earlier measure of treatment

(17). Suicidal behaviour is a significant problem in American prisons, e.g. suicidal ideas precede suicidal behaviour in 16% of the prison population (18). Our study has found that 1.94% examinees committed suicide.

There is a huge problem in FB&H related to the lack of an adequate medical institution for custody, treatment, resocialization and rehabilitation of persons who committed criminal offences in the state of mental incapacity due to temporary mental disease or disorder. In Bosnia and Herzegovina the psychiatric hospital with a forensic department has been constructed in Sokolac town. Patients from the Federation of Bosnia and Herzegovina who committed criminal offences do not have access to it. In Germany patients are placed in forensic mental hospitals (19). Early signs of psychotic symptoms, physical aggression in childhood needs to be taken seriously by parents, teachers and mental health professionals. Such symptoms may later lead to aggressive behaviour (20). Early preventive strategies aimed at decreasing physical aggression among young children are likely to be more effective in decreasing violent behaviour in people with psychosis (21,22).

Individuals who meet diagnostic criteria for schizophrenic disorders had a higher risk of being violent in the age of 26, which was confirmed by a study conducted by **Walsh et al. (22). A study of** Cechova-Vayleux et al. found that the average age of perpetrators was 37.8 years, they all had a psychiatric anamnesis, previous criminal records and history of violence against others. It corresponds to our research, indicating that the highest number of criminal offences was committed at the age 20 to 29 and **30 to 39 years (23).** Out of the total number of murders in the presented study 54.41% were committed against family members. Similar results were obtained by Hanlon et al., 33% (24). The highest number of our examinees had secondary (43**.**42%**)** and **primary school education** (28.50%), which was similar to the results of a study by Ghoreishi et al. (25).

In conclusion, the process of resocialization is affected by many factors ranging from the deinstitutionalization process to stigmatization of persons with mental disorders in the society. It is necessary to work intensively on the opening of a forensic hospital in the territory of the Federation of Bosnia and Herzegovina in order to improve conditions for custody, treatment, resocialization and rehabilitation of persons who committed criminal offences in the state of mental incapacity due to permanent or temporary mental disease or disrder. In addition, legal conditions for placement of mental patients in medical institutions are to be created, because such an institution does not exist in the Federation of Bosnia and Herzegovina and therefore, new perpetrators of criminal offences caused by mental disorders could not be placed in an adequate institution. Early detection of mental disorders and adequate support and work with such persons are also important in order to reduce their aggressiveness and perpetration of criminal offences.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

REFERENCES

1. Anonymous. The World Health Report 2001. Mental Health: New understanding, New Hope. Geneva: World Health Organization, 2001. http://www.Who.int/whr/2001/en/ (07 February 2017)
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. (5th ed.). Arlington, VA: American Psychiatric Publishing, 2013.
3. Hrvatski zavod za javno zdravstvo. Međunarodna klasifikacija bolesti i srodnih zdravstvenih problema – deseta revizija, svezak 1. Zagreb: Medicinska naklada, 1994.
4. Parlament Federacije Bosne i Hercegovine. Kazneni zakon Federacije Bosne i Hercegovine (KZFBiH). Sarajevo: Službene novine Federacije BiH“, broj 36/03, 37/03, 2003.
5. Parlament Federacije Bosne i Hercegovine. Zakon o krivičnom postupku Federacije Bosne i Hercegovine (ZKPFBiH). Sarajevo: Službene. novine Federacije BiH broj 35/03, 37/03, 56/03, 2003.
6. Mezey G, Youngman H, Kretzschmar I, White S. Stigma and discrimination in mentally disordered offender patients – a comparison with a non-forensic population. J Forens Psychiatry Psychol 2016; 27:1-13
7. Clement S, Schauman O, Graham T, Maggioni F, Evans-Lacko S, Bezborodovs N, Morgan C, Rüsch N, Brown JS, Thornicroft G. What is the impact of mental health-related stigma on help-seeking? A systematic review of quantitative and qualitative studies. Psychol Med 2015; 45:11-27.

8. Mullen PE. Forensic mental health. Br J Psychiatry 2000; 176:307-11.
9. Monahan J, Steadman H, Robbins CP, Silver E, Appelbaum P, Grisso T, Mulvey PE, Roth HL. Developing a clinically useful actuarial tool for assessing violence risk. The Br J Psychiatry 2000; 176:312-19.
10. Monahan J, Steadman H, Robbins P, Appelbaum P, Banks S, Grisso T, Heilbrun K, Mulvey E, Roth L, Silver E. An actuarial model of violence risk assessment for persons with mental disorders. Psychiatr Serv 2005; 56:810-15.
11. Gareth R, Reed J. Patients or prisoners? Time to reconsider the voting rights of mentally disordered offenders. BJPsych Bull 2016; 40:169-72.
12. Friedman R A. Violence and mental illness — how strong is the link? N Engl J Med 2006; 355:2064-6.
13. Parlament Federacije Bosne i Hercegovine. Zakona o zaštiti osoba s duševnim smetnjama. Službene novine Federacije BiH, br: 37/01. Sarajevo, 2001.
14. Pondé MP, Freire AC, Mendonça MS. The prevalence of mental disorders in prisoners in the city of Salvador, Bahia, Brazil. J Forensic Sci 2011; 56: 679-82
15. Arseneault L, Cannon M, Murray R, Poulton R, Caspi A, Moffitt TE. Childhood origins of violent behaviour in adults with schizophreniform disorder. Br J Psychiatry 2003; 183:520-25.
16. Hodgins S, Sheilagh H. Mental disorder, intellectual deficiency, and crime. Arch Gen Psychiatry 1992; 49:476-83.
17. Evans C, Forrester A, Jarrett M, Huddy V, Campbell C, Byrne M, Jamieson-Craig TK, Valmaggia LR. Early detection and early intervention in prison: improving outcomes and reducing prison returns. J Forens Psychiatry Psychol 2017; 28:91-107.
18. Schaefer KE, Esposito-Smythers C, Tangney J. Suicidal ideation in a United States jail: demographic and psychiatric correlates. J Forens Psychiatry Psychol 2016; 27:698-704.
19. Schalast N, Redies M, Collins M, Stacey J, Howells K. EssenCES, a short questionnaire for assessing the social climate of forensic psychiatric wards. Crim Behav Ment Health 2008; 18: 49-58.
20. Poulton R, Caspi A, Moffitt TE, Cannon M, Murray R, Harrington H. Children's self-reported psychotic symptoms and adult schizophreniform disorder: a 15-year longitudinal study. Arch Gen Psychiatry 2000; 57:1053 -8.
21. Dodge KA. Investing in the prevention of youth violence. International Society for the Study of Behavioural Development Newsletter 2002; 2: 8-10.
22. Walsh E, Gilvarry C, Samele C, Harvey K, Manley C, Tyrer P, Creed F, Murray R, Fahy T.
23. Reducing violence in severe mental illness: randomised controlled trial of intensive case management compared with standard care. BMJ 2001; 323:1093-6..
24. Cechova-Vayleux E, Leveillee S, Lhuillier JP, Garre JB, Senon JL, Richard-Devantoy S. Female intimate partner homicide: clinical and criminological issues. Encephale 2013; 39: 416-25.
25. Hanlon ER, Brook M, Demery JA, Cunningham MD. Domestic homicide: neuropsychological profiles of murderers who kill family members and intimate partners. J Forensic Sci 2016; 61:163-70.
26. Ghoreishi A, Kabootvand S, Zangani E, Bazargan-Hejazi S, Ahmadi A, Khazaie H. Prevalence and attributes of criminality in patients with schizophrenia. J Inj Violence Res 2015; 7:7–12.

Duševna oboljenja i krivična djela osoba smještenih na Forenzičkom odjelu Kazneno-popravnog zavoda Zenica, Federacija Bosne i Hercegovine

Edin Bjelošević[1], Adisa Krehmić[2], Halima Hadžikapetanović[1], Sanel Čoralić[3], Sonja Bjelošević[1]

[1]Centar za mentalno zdravlje, JU Dom zdravlja Zenica, [2] Medicinski fakultet Univerziteta u Zenici, [3]Forenzički odjel Kazneno-popravnog zavoda Zenica; Zenica, Bosna i Hercegovina

SAŽETAK

Cilj Ispitati utjecaj različitih bioloških, psiholoških i socijalnih faktora na izvršenje krivičnih djela od strane lica s mentalnim poremećajem, te ispitati zakonske uvjete u kojima su smještene osobe s mentalnim poremećajima, a koje su počinile krivično djelo.

Metode U ovom retrospektivnom deskriptivnom istraživanju analizirani su podaci prikupljeni iz protokola Forenzičkog odjela Kazneno-popravnog zavoda Zenica (dob, školska sprema, radni status, bračno stanje, mentalni poremećaji, podaci koji se odnose na ranije liječenje, vrsta počinjenog krivičnog djela, trajanje mjere bezbjednosti obaveznog psihijatrijskog liječenja i čuvanja) za 154 ispitanika.

Rezultati Istraživanje je obuhvatilo 154 ispitanika (muškaraca) prosječne starosne dobi 34 godine. Prosječan broj godina provedenih na Forenzičkom odjelu je 3 godine; ubistvo je počinilo 68 (44,15%) ispitanika, a 34 (22,07%) pokušaj ubistva. Od shizofrenije je bolovalo 85 (55,19%) ispitanika, 30 (19,48%) je imalo sumanuti poremećaj, a 19 (12,33%) mentalnu retardaciju.

Zaključak Najveći broj počinjenih djela bio je u korelaciji sa shizofrenijom (najveći broj ispitanika bolovao je od shizofrenije). Potrebno je raditi na podizanju forenzičke bolnice na području Federacije Bosne i Hercegovine, kako bi se osigurali dobri uvjeti za rehabilitaciju psihičkih bolesnika koji su počinili krivično djelo.

Ključne riječi: shizofrenija, uračunljivost, ubistvo

AUTHOR'S CORRECTION

Molecular epidemiology and antimicrobial susceptibility of AmpC- and/or extended-spectrum (ESBL) ß-lactamase-producing *Proteus* spp. clinical isolates in Zenica-Doboj Canton, Bosnia and Herzegovina

Selma Uzunović[1], Amir Ibrahimagić[1], Branka Bedenić[2,3]

[1]Department for Laboratory Diagnostics, Institute for Public Health and Food Safety, [2]School of Medicine, University of Zagreb, [3]Clinical Department of Clinical and Molecular Microbiology, University Hospital Center Zagreb; Zagreb, Croatia

Volume 13, no. 2, p. 103–112, 2016. Page 103: The byline should appear as shown above.

TENPRIL®

ramipril

JEDINI ACE INHIBITOR ODOBREN ZA PRIMJENU U SEDAM INDIKACIJA

Zlatni standard za sve ACE inhibitore

Pakovanje:
Tableta 2,5 mg x 30 (Br. rješenja: 04-07.3-1-7789/15)
Tableta 5 mg x 30 (Br. rješenja: 04-07.3-1-7790/15)
Tableta 10 mg x 30 (Br. rješenja: 04-07.3-1-7791/15)

Lijek se izdaje na ljekarski recept.
Bosnalijek d.d., Jukićeva 53, Sarajevo, BiH

BH.TEN.2016.01.

INDIKACIJE:
Liječenje hipertenzije, kardiovaskularna prevencija: smanjenje kardiovaskularnog morbiditeta i mortaliteta u pacijenata: s manifestnom aterotrombotičkom kardiovaskularnom bolesti (anamneza koronarne bolesti srca ili moždanog udara, ili periferne vaskularne bolesti); s dijabetesom i s najmanje jednim kardiovaskularnim faktorom rizika, **liječenje bubrežne bolesti**: incipijentna glomerularna dijabetička nefropatija koja je definirana s prisustvom mikroalbuminurije; manifestna glomerularna dijabetička nefropatija koja je definirana s makroproteinurijom, u pacijenata s najmanje jednim kardiovaskularnim faktorom rizika; manifestna glomerularna nedijabetička nefropatija koja je definirana s makroproteinurijom ≥ 3 g/dan, **liječenje simptomatskog zatajenja srca, sekundarna prevencija nakon akutnog infarkta miokarda**

KONTRAINDIKACIJE:
Preosjetljivost na aktivnu supstancu, na bilo koju od pomoćnih supstanci, angioedem u anamnezi (nasljedni, idiopatski ili zbog prethodnog angioedema uz ACE inhibitore ili uz antagoniste receptora angiotenzina II, značajna bilateralna stenoza bubrežne arterije ili stenoza bubrežne arterije u jedinom funkcionalnom bubregu, drugi i treći trimestar trudnoće, hipotenzija.

NEŽELJENI EFEKTI:
Suhi kašalj, angioedem, hiperkalijemija, bubrežno ili jetreno oštećenje, pankreatitis, teške kožne reakcije i neutropenija/agranulocitoza.

POSEBNA UPOZORENJA:
Pacijenti s teškom hipertenzijom, pacijenti s dekompenziranim kongestivnim zatajenjem srca, pacijenti s hemodinamički relevantnom zaprekom protoka krvi kroz lijevu komoru, prilikom punjenja ili pražnjenja (npr. stenoza aortalnog ili mitralnog zaliska), pacijenti s unilateralnom stenozom bubrežne arterije i s drugim funkcionalnim bubregom itd.

DOZIRANJE:
Hipertenzija: Početna doza je 2,5 mg na dan. Doza se može povećavati do maksimalne dnevne doze od 10 mg. Obično se doza primjenjuje jedanput na dan.
Kardiovaskularna prevencija: Početna doza je 2,5 mg TENPRILA jedanput na dan. Dozu bi trebalo povećati do ciljne doze održavanja od 10 mg TENPRILA jedanput na dan.
Liječenje bubrežne bolesti: Početna doza je 1,25 mg TENPRILA jedanput na dan. Preporučuje se dozu udvostručiti nakon dvije sedmice liječenja na dozu od 2,5 mg jedanput na dan, a potom, nakon daljnjih dvije sedmice, na dozu od 5 mg jedanput na dan.
Simptomatsko zatajenje srca: Preporučena početna doza je 1,25 mg na dan. Maksimalna dnevna doza je 10 mg. Preferira se lijek primjenjivati režimom dva puta na dan.
Sekundarna prevencija nakon akutnog infarkta miokarda: početna doza je 2,5 mg dva puta na dan. Doza održavanja je 5 mg dva puta na dan.

Za sve detaljnije informacije o lijeku koristiti posljednji odobreni Sažetak glavnih karakteristika lijeka i Uputstvo o lijeku.

BOSNALIJEK