ABSTRACT

Aim Schizophrenia is a mental disorder and one of the suspected causes is cytokines. One of them is tumour necrosis factor-alpha (TNF-α). Cytokines have the potential to affect cognitive function. The study aimed to find a correlation of TNF-α level with the Mini-Mental State Examination (MMSE) score in patients with schizophrenia (PwS), and comparing the level of TNF-α levels between PwS and healthy controls.

Methods We conducted a cross-sectional analytic study and the study designs were correlation and comparative analysis, i.e. using a Mann-Whitney U test. A total number of 100 subjects were collected, and they were divided into two groups of PwS and control group, respectively.

Results The results found that most of the PwS subjects were 39 men (78.0%), while the control group were 28 men (56.0%). The differences in TNF-α levels between PwS and control groups were found to be significant p <0.001, there was no significant correlation between TNF-α level and the score of MMSE of the PwS with p = 0.938, with a very weak correlation that was r = -0.011, and a negative correlation direction.

Conclusion There was a significant difference between TNF-α level of PwS and control group, i.e. PwS group had lower TNF-α level compared to the control group. The TNF-α level of PwS group had a very weak effect on the cause of cognitive dysfunction in PwS group, yet the higher level of it could reduce MMSE score in PwS group.

Key words: cytokines, cognitive dysfunction, psychotic disorders
INTRODUCTION

Schizophrenia is a mental disorder that usually appears in late adolescence or early adulthood, which affects about 21 million people worldwide (1). Thus far, the aetiology of schizophrenia is unknown, but several hypotheses related to this have been stated: schizophrenia is a neurodevelopmental disease; it is a neurodegenerative disease; and it is a progressive neurodevelopmental disease (2). Another important point is the implication of cytokines in the aetiology or pathology of schizophrenia (3). One of the cytokines that play an essential role for schizophrenia is tumour necrosis factor-alpha (TNF-α). TNF-α level also increased significantly in patients with schizophrenia (PwS) compared to healthy controls (4-7). Studies with the same results were reported by O’Brien, Scully, and Dinan (8), i.e. there were significant differences in TNF-α level in the schizophrenia group of 13.49 pg/ml ± 0.42 and 6.79 pg/ml ± 0.42 in the control group. This result is supported by Czerski et al. (9) who found a significant difference in the frequency of TNF-α between PwS and control group, 87.9% and 82.9%; this frequency increased to 90.7% of those who had a history of schizophrenia in the first and second-degree relatives. Similar results have also been reported by Rosario García-Miss et al. (10) who found that there was a significant difference between PwS and control group regarding the TNF-α values of 10.63 ± 4.65 pg/mL and 6.74±4.00 pg/mL, TNF-α levels in PwS were higher than in the group.

Inflammation is thought to have a role in the pathophysiological process of schizophrenia, and currently, various studies are being conducted to find out the anti-inflammatory effect in PwS (11). A literature review from Baune et al. (12) concluded that TNF-α has a neuroprotective or neurodegenerative function. Therefore, it remains debatable whether TNF-α can maintain or decrease cognitive function. A study conducted by Xiu et al. (13) found that TNF-α could mediate cognitive severity towards PwS. Thus far, different results have been found in previous studies. Therefore, more research is needed to support the statement of cytokines, such as conducting a study of the TNF-α, which has a relation with schizophrenia.

Malayan-Mongoloid people are one of the races living in Medan, Indonesia. It consists of two sub-races, i.e. Proto Malayan and Deutro Malayan. Our study focused on the Proto Malayan type only.

The aim of this study was to investigate the effect of TNF-α on the cognitive function of patients of the Malayan-Mongoloid race with schizophrenia in North Sumatera, and it was the first study in North Sumatera which assessed this.

PATIENTS AND METHODS

Patients and study design

This cross-sectional analytical study was carried out at the Outpatient and Inpatient Installation of the North Sumatera Psychiatric Hospital (R. S. J. Provsu) Prof. M. Ildrem during the period of 3 months in 2019. This psychiatric hospital is a referral psychiatric hospital in North Sumatra Province, and had an inpatient capacity of 400 beds. Patients who came to this psychiatric hospital were almost entirely Malayan-Mongoloid Race. The target population was PwS that were the patients of the Outpatient and Inpatient Installation of North Sumatera Psychiatric Hospital. The patients included the PwS group, and the healthy control group that fulfilled the inclusion and exclusion criteria. Inclusion criteria of PwS were: schizophrenic patients diagnosed based on the 10th edition of the International Classification of Disease and Related Health Problems criteria (14), aged 15-40 years, Malayan-Mongoloid Race, cooperative and willing to be interviewed. Exclusion criteria were: having a history of previous psychiatric disorders, having a general medical condition that affected brain structure, and obesity (defined by body mass index - BMI of ≥ 30). The inclusion criteria for the control group were: age 15-40 years, Malayan-Mongoloid Race, cooperative and willing to be interviewed, and no family history of having a mental disorder. Exclusion criteria were: having a history of previous psychiatric disorders, having a general medical condition that affected brain structure, and obesity. We took the control group from the people who lived near the hospital.

The sample size calculation used the following formula:

\[ n_1 = n_2 = \frac{2 \left( z_{\alpha/2} + z_{\beta} \right)^2 s}{n_2 - \bar{x}_2} \]

The sample size was based on the sample size table with alpha 5% two-sided beta hypothesis of 10%, in which the assumption of the standard
deviation ratio was 1, it was found that n1 = n2 = 21 subjects. In this study, 50 subjects were taken for each group.

Next, the subjects and their relatives were asked to read the letter of statement of the research and sign the consent form after an explanation about the participation in the study. The study was approved by the Health Research Ethical Committee of Faculty of Medicine, Universitas Sumatera Utara.

Methods

Blood plasma sampling was carried out as followed: blood was drawn with a sterile syringe (aseptic) from a vein in the area where the upper arm meets the forearm (median cubital vein) for 6 mL. The blood was then kept in a vacutainer containing ethylenediaminetetraacetic acid (EDTA) and stored at 4-8 °C until the serum and plasma were separated.

The enzyme-linked immunosorbent assays (ELISA) examination was then performed using the human Quantikine TNF-alpha kit (R&D systems, Minneapolis, MA, USA) and read the results using the Thermo-Fisher machine. The cognitive function of the PwS was measured using Mini-Mental State Examination (MMSE) rating scale (15) that had been validated in the Indonesian language by Geriatric Psychiatry Section of Indonesia Psychiatry Association (16); the total score was divided into normal (24-30), probable cognitive disorder (17-23), and definite cognitive disorder (0-16). The rating scale itself only took less than 10 minutes to complete, and was relatively easy to use.

Statistical analysis

Statistical analysis began by normalizing the data using Saphiro-Wilk normality test. We found that the result was abnormal data, and continued the analysis using Mann-Whitney U test to compare the difference between the TNF-α level of PwS and control, and the Spearman correlation test to find the relationship of TNF-α level with MMSE score in the PwS group.

RESULTS

Among 100 patients analysed (50 patients in each PwS and control group) the males predominated over female patients in both groups, 38 (76.0%) in PwS and 28 (56.05) in control group, with higher average age of 35.42±2.78 years in PwS group. Higher BMI, of 23.79±2.98, in the control group was found. The average MMSE score was 21.00±4.56 in PwS group (Table 1).

The TNF-α level for PwS was 3.24 (0.65-43.80) pg/dL, while for the control group it was 16.25 (4.80-56.10) pg/dL (p<0.001) (Table 2). The boxplot comparison of these two groups was shown in Figure 1. We found that the PwS group had the lowest TNF-α level i.e. 0.65 pg/dL, much less varied in the TNF-α level and lower median compared to the control group. The highest of the TNF-α level was found in the control group, i.e. 56.10 pg/dL.

There was no significant correlation between TNF-α level and MMSE score (p=0.938); the correlation coefficient was very weak, and the direction was negative (r= -0.011). The higher TNF-α level resulted in lower MMSE score (Figure 2).
DISCUSSION

The results in this study found predominance of male PwS patients, and it was the same as reported by Lv et al. (17), Garcia-Miss et al. (10), Kunz et al. (18), Tian et al. (19), Naudin et al. (4), and Kubistova et al. (20). Different results found that there were more females with schizophrenia than males as reported by Kowalski et al. (6), and Hope et al. (21). It was explained by the fact that males with schizophrenia were more frequently hospitalised compared to females, because males are less adherent to the treatment, and often commit suicide (22), or because females with schizophrenia were more responsive to the treatment and less than 50% experienced inpatient care (23).

In this study the mean of TNF-α level in the PwS group was lower compared to the control group. Similar results were reported by Chiang et al. (24) who found lower TNF-α in males. Other studies found the same results such as the study by Tian et al. (19), and Zhu et al. (25). We assumed that the similarities that we found were due to all of these studies conducted in Mongoloid race. Many studies showed different results, e. g. the TNF-α level in the PwS group was higher compared to the control group (4,26-30), and most of them were done in Caucasian race, except one study that was done in Indonesia.

Some conditions that potentially caused lower TNF-α level included antipsychotic drugs consumed by PwS (14,31), the chronicity of the schizophrenia (17), and variations in vitamin D levels in the body (32). Cytokines were thought to be involved in the regulation of several neurotransmitters, such as dopamine, serotonin, norepinephrine, and glutamate (33). A low level of inflammatory cytokines in the brain could still affect complex brain functions such as memory, mood, anxiety, cognition, and nerve activity (34,35). Cytokines, in this case, TNF-α had an essential role in regulating complexity including immunity and inflammation. Thus, the low TNF-α level in the PwS group indicated that there had been a defect during the induction of inflammatory pathways or active inhibition of these cytokines (17).

Our results show that there was no significant correlation between TNF-α level and MMSE score, and it had a very weak correlation coefficient. In schizophrenia, cognitive dysfunction happened before the appearance of positive and negative symptoms (12). The existence of TNF-α had an important role related to immunity and inflammation in the brain (36), and changes in the level of TNF-α start when the PwS is still in the mothers’ womb (37). One mechanism that allows TNF-α to influence schizophrenia is through neuregulin-1. The neuregulin-1 gene along with the erbB4 receptor acted for the occurrence of plasticity, myelination, and the formation of long-term potentiation, thus it had an important role in the cognitive function (12). The results of our study were supported by a study conducted by Hennessy et al. (38), which found that high TNF-α could induce a decrease of working memory. In addition, inhibition of TNF-α could also improve memory loss and spatial learning (39). The existence of TNF-α was also important in learning activities and memory because the presence of TNF-α could interfere with both processes. As stated before, inhibition of TNF-α could restore cognitive function (40). Some of the mechanisms offered related to the above statement are TNF-α having a contribution in astrogliosis, apoptosis, neurogenesis and permeability in the endothelial cell layer; thus, it can influence the cognitive function (41). This process started with prenatal inflammation resulting in abnormalities of cytokine levels including TNF-α resulting in neurodevelopment disorders. Specifically, concerning cognition, these effects included its relationship with the quality of life and overall function of PwS, which later influenced the outcome of the disease (42). This is supported by a literature review written by Misiak et al. (43), they also confirm that TNF-α has contributed to cognitive impairment in PwS.
In conclusion, no relationship between TNF-α and cognitive function of PwS was found. We thought that the age of the patients affected our results. Based on our findings of the coefficient correlation, the level of TNF-α may have a very weak effect on the cognitive function of PwS. It contradicts with previous studies that suggested that cytokines had an important role in influencing the cognitive function.

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TRANSPARENCY DECLARATIONS

Competing interests: None to declare.

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