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The Effect of Interleukin-2 –330 T/G Polymorphism in People with Schizophrenia Among The Batak and Javanese Ethnic Groups

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ABSTRACT

Background: Schizophrenia is a complex disorder involving multiple genes with mild to moderate effects and non-genetic risk factors such as environmental and psychological influences that alter brain chemistry. Significant reduction in interleukin-2 production by peripheral lymphocytes is an immunological finding replicated in schizophrenia across various countries. Investigations on the interleukin-2 –330 T/G polymorphism in people with schizophrenia (PWS) are still minimal, and the study location involves only a few countries with different results. Therefore, this study aims to examine the effect of interleukin-2 –330 T/G polymorphisms in people with schizophrenia among the Batak and Javanese ethnic groups in Indonesia, particularly in North Sumatra, **Method:** This study used purposive non-probability sampling to recruit people with schizophrenia with 120 Batak and 120 Javanese subjects who were hospitalized at Prof. M. Ildrem Mental Hospital, Medan, Indonesia. The interleukin-2 -330 T/G polymorphism was examined by the PCR method. **Result:** The results showed that the genotype frequency of the Batak people with schizophrenia is as follows: GG 11.7%, TG 53.3% and TT 35%. Furthermore, the group of Javanese people with schizophrenia is as follows: GG 23.3%, TG 44.2% and TT 32.5%. The OR for the genotypic comparison of GG was found to be 2.154 with 95% CI 0.992-4.678, p=0.053, while that of the TG genotype was 0.892 with 95% CI 0.505-1.574 and p=0.693. The T allele was higher than the G allele in Batak and Javanese ethnic groups, as demonstrated by p=0.713, OR=0.919 with 95% CI 0.641-1.318. **Conclusion:** There is no statistically significant difference between the occurrence frequency of alleles. In addition, there is no significant relationship between the GG and the TG genotype of the interleukin-2 – 330T/G polymorphism in people with schizophrenia among the Batak and the Javanese ethnic groups.

Keywords: genotype, alleles, schizophrenia, ethnicity, Indonesia.

1. INTRODUCTION

Schizophrenia affects 20 million people worldwide and is characterized by distortions in thinking, perception, emotion, language, low self-esteem, and behavior. The experienced feeling includes hallucinations which involve hearing voices, seeing false things, and delusions. This disease is a variable psychopathological clinical syndrome, but very disturbing, involving cognition, emotion, perception, and other aspects of behavior. It usually begins before the age of 25 years, sometimes persists for life, and can occur in all social classes. The exact cause of schizophrenia is still unknown, and several previous studies mentioned various phenotypes of the disease arising from different factors, including genetic susceptibility and environmental influences. Evidence also supports the idea that genetic factors play an important role in schizophrenia causes (1-3).

Interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) mediate immune and inflammatory responses as well as activate cytokines which play a vital role in the central nervous system. They can also be released from activated glial cells. In schizophrenics, elevated levels of the soluble interleukin-2 receptor (sIL-2R) have been observed along with significantly higher production of interleukin-2 and interferon-gamma (IF-γ). Interleukin-2 is a potent T cell growth factor which has been assumed for many years to amplify lymphocyte responses in vivo and also influences cell survival, differentiation, and formation of immune memory cells. Addition-

ally, it acts as a negative regulator of immune activation and has a profound effect on immune function by contributing to the formation as well as the dissemination of antigen-specific immune responses. Animal and human studies showed that low doses of interleukin-2 promote the expansion of regulatory T cells in-vivo and suppress autoimmune disease. In schizophrenia, aggregate measures of pro-inflammatory cytokine levels namely interleukin-2, IFN-γ, and transformed TNF- are negatively associated with prefrontal cortical thickness, especially in individuals at risk of developing psychotic disorders. Various studies have also reported how specific cytokines relate to brain structure and symptoms in people with schizophrenia, including the pro-inflammatory cytokines IL-1β, interleukin-2, IL-6, IL-12, IFN-γ, and TNF-, and the anti-inflammatory cytokines IL-4 and IL-10 (4-8).

Schwarz et al. (9) in Germany stated that the interleukin-2 –330 T/G polymorphism is functionally associated with increased production of interleukin-2 with the GG genotype, while the TT and GT genotypes are associated with decreased in-vitro production of cytokines. The TT genotype was significantly associated with schizophrenia, but the GT gene type was less common; hence, it could be concluded that some of the in vitro production of the interleukin-2 polymorphism was reduced. Meanwhile, Watanabe et al. (10) in Japan did not find a significant relationship between interleukin-2 polymorphisms and schizophrenia in the Japanese population using a single marker and haplotype analysis. Peripheral blood lymphocytes from individuals with the GG genotype produced significantly more interleukin-2 than others with both TT and TG genotypes. The study also concluded that there was no relationship between the TT and GG genotypes with schizophrenia. The genotypic frequency of the interleukin-2 -330T/G polymorphism was not significantly different in schizophrenia.

Furthermore, Samojedny et al. (4) in Poland found statistically significant differences in the distribution of genotype and allele frequencies for the interleukin-2 polymorphism in people with schizophrenia. The presence of the TT genotype and T allele correlates with an increased risk of paranoid schizophrenia. This study also reported that the GG genotype was associated with high levels of interleukin-2, while the TT and GT genotypes were associated with decreased cytokine production. Another study by Frydecka et al, (11) in Poland stated that the interleukin-2 -330 T/G polymorphism was located in the binding domain for transcription factors in the gene promoter. The analysis of peripheral blood lymphocytes stimulated by anti-CD3/CD28 antibodies revealed that the interleukin-2 -330T/G GG genotype contributed to the increased production of interleukin-2, while the GT and TT genotypes are associated with decreased production.

A pro-inflammatory study related to ethnicity has also been carried out in Indonesia, especially in North Sumatera by Amin et al in 2019 (12). Furthermore, another study by Damanik et al., in 2020 investigated the relationship between the INF-+874 A/T gene polymorphism and schizophrenia in Batak ethnic group, and Saragih et al, in 2021 also examined the difference between the frequency of the G and the C allele variant –G174C IL-6 in the schizophrenia group of the Batak ethnic (13, 14). In general, investigations on cytokines gene polymorphisms in schizophrenics are limited. Based on the literature review performed, no studies in Indonesia, especially in North Sumatera, have examined the comparison between interleukin-2 –330 T/G polymorphisms in people with schizophrenia among the Batak and Javanese ethnic groups.

2. MATERIAL AND METHODS

A one-time unpaired categorical comparative analytical study was conducted by comparing groups of people with schizophrenia from the Batak and Javanese ethnic groups using the cross-sectional method. The sample included people with schizophrenia in the Inpatient Unit of the Mental Hospital of North Sumatera Province between March-September 2022. The method used non-probability sampling with a purposive type.

The sample size was calculated using the comparative-categorical-unpaired-table 2x2. This was carried out by setting the effect size (P1-P2) (15).

Sample size formula is:

$$n1 = n2 = \left(\frac{2a\sqrt{2PQ} + 2\beta\sqrt{\mathrm{P1Q2} + \mathrm{P2Q2}}}{\mathrm{P1} - \mathrm{P2}}\right)$$

n1 = number of samples in group 1 (Batak ethnicity)

n2 = number of samples group 2 (Javanese ethnicity)

Alpha (α) = type one error, set at 5%, 95% confidence level

Zα = the standard alpha value of 5% is 1.96

Beta (β) = type two error, set at 20%

Zβ = the standard value of beta 20% at 0.842

P_1 = the proportion of the T allele in the people with schizophrenia of the Batak ethnic based on the literature is 0.557

$Q_1 = 1\text{-}P_1 = 1\text{-}0{,}557 = 0{,}443$

$P_1\text{-}P_2$ = t h e minimum proportion difference between the people with schizophrenia of the Batak and Javanese ethnic groups considered significant, was set at 0.2

P_2 = the proportion of the T allele in the people with schizophrenia among the Javanese ethnic group, namely the proportion of the T allele in the people with schizophrenia in the Batak ethnic group minus the minimum difference that is considered significant = 0.557-0.2 = 0.357

$Q_2 = 1\text{-}P_2 = 1\text{-}0{,}357 = 0{,}643$

$P = (P_1+P_2)/2 = (0{,}557+0{,}357)/2 = 0{,}914/2 = 0{,}457$

$Q = 1\text{-}P = 1\text{-}0{,}457 = 0{,}543$

Hence:

$$n1 = n2 = \left(\frac{1{,}96\sqrt{2K0{,}457K0{,}543}+0{,}842\sqrt{0{,}557x0{,}443+0{,}357x0{,}643}}{0{,}2}\right)^2$$

$$n1 = n2 = \left(\frac{1{,}96\sqrt{0{,}496}+0{,}842\sqrt{0{,}247+0{,}229}}{0{,}2}\right)^2$$

$$n1 = n2 = \left(\frac{1{,}96\sqrt{0{,}496}+0{,}842\sqrt{0{,}476}}{0{,}2}\right)^2$$

$$n1 = n2 = \left(\frac{1{,}96(0{,}704)+0{,}842(0{,}69)}{0{,}2}\right)^2$$

$$n1 = n2 = \left(\frac{1{,}38+0{,}581}{0{,}2}\right)^2$$

$$n1 = n2 = \left(\frac{1{,}961}{0{,}2}\right)^2$$

$$n1 = n2 = (9{,}805)^2$$

$$n1=n2=96.138$$

$$n1=n2=97$$

Based on the literature, the proportion of the T allele in people with schizophrenia is 55.7%. This study hypothesized that the proportion of the T allele in the Javanese is different from the Batak ethnic group, with a difference of at least 20%. Using a type I error set at 5% and a type 2 error set at 20%, this study will require 97 schizophrenics for each Batak and Javanese ethnic group (15, 16). Finally, the samples recruited were 120 people with schizophrenia from each Batak and Javanese ethnicities.

The subjects were schizophrenics who fulfilled the inclusion and exclusion criteria. The inclusion criteria included people with schizophrenia from Batak and Javanese ethnicities diagnosed based on International Classification of Disease and Related Health Problems 10th edition (ICD-10) criteria, having a Positive and Negative Syndrome Scale (PANSS) score of 80-120, aged 18-45 years, having the same ethnicity in two generations of first-degree family and above, cooperative as well as willing to be interviewed. Meanwhile, the exclusion criteria were having a history of other psychiatric disorders, a history of neurological diseases, endocrine disorders, autoimmune diseases, and a history of taking alcohol as well as other addictive substances except for nicotine and caffeine. Data collection was carried out by screening those who met the inclusion and exclusion criteria. Furthermore, the subjects were asked to read a statement about the study and sign a letter of approval after an explanation of the objectives. The subjects were assessed by an interview where the PANSS value between 80-120 indicated the readiness to participate (17, 18). Blood was then taken from the subject, followed by laboratory examinations, statistical analysis, and preparation of results. In some cases where the family card was not found, information was obtained directly from the patient and confirmed through the family via telephone. The telephone number data of each patient's guardian is included in the medical record. Furthermore, interviews were conducted with patients using the MINI ICD-10. Venous blood was taken by the laboratory staff of the Prof. M. Ildrem Mental Hospital, with the following steps: a) The tools and materials were prepared b) An identity label was provided on the study subject's blood tube, c) A tourniquet was used at 3-4 inches from the puncture site, the subject was then asked to clench the palm of the hand until the vein was clearly visible, d) The puncture site on the median cubital vein at the elbow crease was cleaned with 70% alcohol by rotating it from the inside out, and allowed to dry, e) The median cubital vein was punctured at an angle of 45 degrees with the needle facing up, f) Blood was allowed to flow into the needle, the subject was asked to open the fist and blood was withdrawn up to 5 ml, g) The tourniquet was removed, and the needle was pulled while still pressing the puncture hole with an alcohol swab, h) The puncture site was covered with plaster, i) The blood tube was placed in a cooler, and then taken to the Faculty of Medicine Universitas Sumatera Utara integrated laboratory for further processing.

In the PCR and allele-specific PCR examinations, primers obtained from the previous study were blasted to confirm the primer and enzyme sequences, as well as reverse primers that can identify the site. The examination was carried out at the integrated laboratory. Blood samples were taken in the amount of 5 ml from the anterior cubital vein. The blood was placed into a vacutainer containing ethylenediamine tetraacetic acid (EDTA) and stored at 4-8° C until DNA isolation was performed through the salting out method. A total of 2 ml peripheral blood samples containing EDTA was put into a falcon tube, then mixed with 6 ml of red blood cell (RBC) lysis solution (blood ratio: RBC = 1:3), containing 199mM EDTA, 100 mM $KHCO_3$, and 1.45 mM NH_4Cl. The tubes were homogenized by inverting and incubating at room temperature 27°C for 10 minutes. Furthermore, the tube was inverted again before centrifugation at a speed of 1500 rotation per minute (rpm) for 10 minutes at room temperature; consequently, the supernatant and pellet (sediment) were white, and then the supernatant was discarded. The above procedure was repeated 3 times to obtain a pellet free of red blood cells.

The pellet formed was added with cell lysis solution (CLS) containing 10 mM Tris-HCl, 0.25 mM EDTA, and 20% SDS. Furthermore, 1.334 ml of CLS was added and carried up-down with a transfer pipette slowly until the mixture became homogeneous. The mixture was incubated in a water bath at 37°C for 30-60 minutes, and then protein precipitation (PP) (5 M ammonium acetate) up to 867 µl was added and vortexed sufficiently. The mixture was centrifuged again at 3000 rpm for 15 minutes at 4°C to obtain a supernatant and brown pellet on the tube wall. The supernatant was transferred to a new tube containing 767 µl of cold isopropanol solution, and the tube was inverted 25-30 times until DNA strands were seen floating in the solution. Next, the DNA in isopropanol was incubated overnight at 20°C room temperature, then centrifugation was carried out at 3000 rpm for 5 minutes and the supernatant was discarded. About 867 µl of cold 70% ethanol was added for washing and inverted, then centrifuged again at 3000 rpm for 5 minutes at 4°C to obtain the supernatant and DNA pellet. The DNA pellets were dried for 2 hours at room temperature or 1 hour at 37°C. Drying was carried out by

placing the tube in an inclined position on a dry tissue base. The DNA pellet was dissolved by adding 300 µl of TE solution containing 10mM Tris-HCl, 0.25 EDTA, and then incubated for 2 hours at 37°C in a water bath. Furthermore, the DNA was transferred to a 1.5 ml Eppendorf tube and stored at -20°C for the next stage. The single base polymorphism at position -330 in the interleukin-2 promoter region was read by PCR sequence-specific primer (PCR-SSP) oligonucleotide primer (forward): 5'CTGACATGTAAGAAGCAATCTAT3', and (reverse): 5'CTCAGAAAATTTTCTTTGTCC3' for the G allele examination. Meanwhile, the T allele examination was read with PCR sequence-specific primer (PCR-SSP) oligonucleotide primer (forward): 5'TTCACATGTTCAGTGTAGTTTTAT3', and (reverse) 5'TGTTACATTAGCCCACACTTA3'. PCR was performed with 50 ng of DNA in a total volume of 10 µl containing 1 µl of the reaction mixture, 0.4 µl of $MgCl_2$, 0.25 µl of each primer, and 0.1 µl of each experimental mixture according to the instructions for 45 cycles including 0 seconds at 95 °C, ramp speed at 20°C/s, and then increased to 10 seconds at 58°C, ramp speed 20°C/s, as well as for acceleration 10 seconds at 72°C, ramp speed 20°C/s . After amplification was obtained by maintaining the reaction at 40°C for 30 seconds and then slowly raising the temperature to 95°C at a rate of 0.1°C/s, T and G alleles were obtained at 54°C and 51°C respectively.[9]

The data collected were analyzed using a statistical package for the service solution software program. Data analysis began with a variable analysis; the results were presented in the form of a frequency distribution table. This analysis includes the number, percentage, and confidence interval of the percentage for data with categorical types. The variable analyzed was interleukin-2 -330 T/G polymorphism in people with schizophrenia among the Batak and Javanese ethnic groups. The subsequent analysis was a comparative test to examine the differences in the distribution of the allele for the interleukin-2 -330 T/G polymorphism. It was found that both variables were normally distributed, and the conditions for chi-square were fulfilled; hence, the test was conducted according to the Hardy-Weinberg Equilibrium. The Kolmogorov-Smirnov test was used to examine data normality for a sample size of more than 50 for each group. Additionally, the odds ratio (OR) value was calculated. An OR value of more than one is a risk factor, and an OR of less than one indicates that the independent variable is a protective factor.

3. RESULTS

A total of 240 blood samples were collected and analyzed by PCR in the integrated laboratory of the Faculty of Medicine, Universitas Sumatera Utara. Consequently, 240 bands of agarose gel electrophoresis were read and analyzed. The reading was carried out according to a study by Samojedny (6).

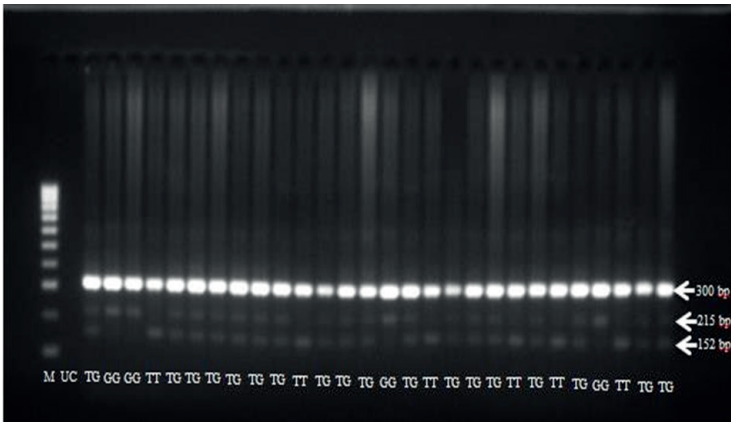

Figure 1. Agarose gel electrophoresis interleukin -330 T/G. Row 1= marker, 25 base pair ladder, Row 2=PCR product, Row 3,4,5= GG homozygote, Row 6 TG heterozygote, Row 7= TT homozygote.

	Batak ethnic	Javanese ethnic	p-value
	(n=120)	(n=120)	
Gender			
Male	93 (77.5)	82 (68.3)	
Female	27 (22.5)	38 (31.7)	0.146*
Age (years)	36 (24-44)	34 (26-50)	0.044**
Onset	25 (21-34)	25 (17-35)	0.188**
Duration of illness	10 (1-20)	9 (4-18)	0.574**
PANSS	97.5 (81-120)	101 (81-110)	0.16**

* Chi-square with continuity correction
** Mann-Whitney U

Table 1. Demographic characteristics

The demographic characteristics analyzed were gender, age, onset, duration of illness, and total PANSS score at the time of examination. The variables of age in both groups, onset, duration of illness, and total PANSS score were numerical scale variables. The normality test used the Kolmogorov-Smirnov test because the number of samples for each group was more than 50. After the normality test for the Batak ethnic, it was found that the variables of age, onset, duration of illness, and total PANSS score were not normally distributed; therefore, the data were presented in the median form (minimum-maximum). For the Javanese, it was found that the age variable was not normally distributed; hence, the data was presented in the median (minimum-maximum). Regarding the onset, duration of illness, and total PANSS score, the data were not normally distributed, then an attempt was made to normalize the data with the Log10 transformation before the comparison test was carried out but this attempt failed. Meanwhile, the Mann-Whitney U test was used for the comparison between the two groups.

Based on the results, the categorical variable in the people with schizophrenia in both groups was gender. The data were presented in a frequency distribution. Furthermore, to examine the equality between the two groups, a comparative test was conducted. Gender is a categorical variable as well as phenotype; the relationship between the two was tested using the unpaired cat-

egorical comparisons in a 2x2 table, where the chi-square test requirements were fulfilled.

Table 1 shows the demographic characteristics of the people with schizophrenia from the Batak and Javanese ethnic groups. In both groups, the majority of the samples were male. In the Batak ethnic group, the male sample was 93 or 77.5%, while in the Javanese, the males amounted to 82 or 68.3%. There was a non-significant difference between the gender groups with a p-value = 0.146. Furthermore, the median age of the Batak ethnic group was 36 years, with the minimum and maximum scores being 24 and 44 years, respectively. In the Javanese ethnic group, the median age was 34 years, with a minimum and maximum score of 26 and 50 years. This shows that there was a significant difference in terms of age between groups with a p-value = 0.044. In the Batak ethnic group, the median onset was 25 years, with a minimum and maximum score of 21 and 34 years. For the Javanese ethnic group, the median age of onset was 25 years, with the minimum and maximum scores being 17 and 35 years. There was no significant difference in terms of disease onset between the two groups as demonstrated by p=0.188. In the Batak ethnic group, the median length of illness was 10 years, with a minimum and maximum value being 1 and 20 years respectively. Meanwhile, the median length of illness was 9 years for the Javanese ethnic group with a minimum and maximum score of 4 and 18 years. There was no significant difference in terms of length of illness between the two groups p=0.574. The median total score for PANSS in the Batak ethnic group was 97.5, with the minimum and maximum scores being 81 and 120. In contrast, the median total score for PANSS in the Javanese ethnic group was 101 with the minimum and maximum scores being 81 and 110. Therefore, there was a non-significant difference in the total PANSS score between the two groups as demonstrated by p=0.16.

The interleukin-2 gene -330 T/G consists of two alleles, namely G and T. Allele variables are categorical (nominal), and the data were presented in a frequency distribution. The relationship between the allele of the interleukin gene -330 T/G with schizophrenia was expressed by the odds ratio (OR) obtained from the risk estimate of the chi-square test.

The frequency of the G allele in the Batak ethnic group was 104 times or 43.3%, while the T allele was 136 times or 56.7%. In the Javanese ethnic group, the frequency of the G allele occurrence was 109 times or 45.4%, while that of T was 131 times or 54.6%. Furthermore, the p-value obtained was 0.713, and the OR was 0.919 with a 95% confidence interval between 0.641 to 1.318. This shows that there is a non-significant difference between alleles and schizophrenia in the Batak and Javanese ethnic groups.

Allele	People with schizophrenia of the Batak ethnic (n=120)	People with schizophrenia of the Javanese ethnic (n=120)	*p*-value	OR (95% CI)
G	104 (43.3)	109 (45.4)		0.919 (0.641-1.318)
T	136 (56.7)	131(54.6)	0.713	

Table 2. Comparison between the allele of the interleukin-2 -330 T/G gene and schizophrenia in the Batak and Javanese ethnics obtained by the chi-square test

Genotype	People with schizophrenia of the Batak ethnic (n=120)	People with schizophrenia of the Javanese ethnic (n=120)	*p*-value	OR (95% CI)
GG	14 (11.7)	28 (23.3)	0.053	2.154 (0.992-4.678)
TG	64 (53.3)	53 (44.2)	0.693	0.892 (0.505-1.574)
TT	42 (35)	39 (32.5)	Comparison	

Table 3. Comparison of genotypes of interleukin-2 –330 T/G polymorphisms in Batak and Javanese people with schizophrenia obtained by logistic regression procedures.

		p-value
	People with schizophrenia in the Batak ethnic	People with schizophrenia of the Javanese ethnic
Chi-square	0,16	0,231

If P < 0.05–not consistent with HWE.
Not accurate if <5 individuals in any genotype group.

Table 4. HWE values in people with schizophrenia among the Batak and Javanese ethnics with interleukin-2 -330 T/G polymorphism

The genotype of interleukin -330 T/G is a combination of G and T alleles, consisting of GG, TG, and TT genotypes. Besides, genotype and phenotype are categorical variables. The data were presented in a frequency distribution and then continued with the chi-square test. However, because there were 3 genotype variables, the risk estimate from the chi-square test cannot be obtained. The comparison between the genotypes and the incidence of schizophrenia was then expressed in terms of ORs obtained from logistic regression, using the TT genotype as a comparison.

Based on the results, the genotype frequency of GG, TG, and TT in the Batak ethnic group was 14 (11.7%), 64 (53.3%) and 42 (35%), while for the Javanese ethnic group, it was 28 (23.3%), 53 (44.2%), and 39 (32.5%). Furthermore, the logistic regression analysis results showed that the p-value for the GG genotype was 0.053 and the OR=2.154 with a 95% CI between 0.992-4.678. The p-value for the TG genotype was 0.693 and the OR=0.892 with 95% CI between 0.505-1.574. This shows that there are differences in the GG genotype among people with schizophrenia in the Batak and Javanese groups but there is no difference in the TG genotype.

According to the Hardy-Weinberg Equilibrium (HWE) formula, when the frequencies of the alleles A and a (from the biallelic locus) are p and q, then (p+q) = 1. This means $(p+q)^2 = 1$, it is true that $(p+q)^2 = p^2 +$

$2pq + q^2 = 1$. This p^2 formula corresponds to the genotype frequencies of AA homozygous, q^2 to aa, and 2pq to Aa. 'AA, Aa, aa' are the three possible genotypes for the biallelic locus; like most SNPs, the total frequency will be 1. In this study, a test was carried out according to the steps above, using online software with the following results (19, 20).

Based on the analysis results obtained using the HWE formula assisted by online software, the p-value for the Batak and Javanese ethnic groups was 0.16 and 0.231, respectively (19, 20).

4. DISCUSSION

Based on the demographic characteristics, most of the samples were males. However, there was no significant difference in gender between the two groups, a significant difference was found in age. Charlson et al, on the Global Epidemiology and Burden of Schizophrenia, reported that no gender difference was observed in the prevalence globally and about 70.8% of cases were found in the age group of 25-54 years with the highest prevalence in the 40s and only a few in the older age group (18). Furthermore, the median onset of people with schizophrenia in the Batak ethnic group was 25 years, with a minimum and maximum value of 21 and 34 years. For the Javanese ethnic group, it was found that the median onset was 25 years, with a minimum and maximum value of 17 and 35 years. The median length of illness in the Batak ethnic group was 10 years with a minimum and maximum value of 1 and 20 years, while in the Javanese ethnic group, it was 9 years with a minimum and maximum value of 4 and 18 years, respectively. Schizophrenia can occur at any age and most schizophrenic disorders are characterized by a prodromal stage that lasts for several years and leads to social consequences. The onset of schizophrenia shows a sharp increase that reaches its peak at the age of 15 to 25 years in males, while in females, it ranges from 15 to 30 years (first peak) and 44-49 years (second peak) (20).

The median total PANSS score in the Batak ethnic group was 97.5 with a minimum and maximum score of 81 and 120, while in the Javanese group, it was 101 with a minimum and maximum score of 81 and 110. Kozma et al. examined 1028 schizophrenia patients in 2010 in the United States by examining the factors that led to the hospitalization and followed for 52 weeks. The total score of PANSS, as well as the Personal and Social Performance Scale (PSPs), were the rating scales analyzed. The PANSS score is divided into 3 parts: low (<75), medium (75-94), and high (>95). Moreover, the hazard ratio for hospitalized patients with a total score of high PANSS was 5.45 (p<0.001 95% CI 2.59-11.46) and 2.31 (p=0.01 95% CI 1.11-3.20) in patients with a medium total PANSS score, compared with a low score (21).

These results showed that the frequency of the G and T alleles in people with schizophrenia in the Batak ethnic group was 43.3% and 56.7%. Meanwhile, in the Javanese ethnic group, it was 45.4% and 54.6%, respectively, then the chi-square analysis obtained p value = 0.713, which means that there is a non-significant relationship between the alleles and schizophrenia. The OR value obtained was 0.919 with a 95% CI between 0.641-1.318. This means that people with the G allele have the possibility (odds) of experiencing schizophrenia by 0.713 times compared to others who have the T allele. When the OR value is below 1, then the G allele is a protective factor for the occurrence of schizophrenia.

This study was conducted on people with schizophrenia from different ethnic groups. Meanwhile, in previous studies, many people with schizophrenia had healthy controls in the same ethnicity. The results obtained are related to the distribution of allele frequencies and are not consistent with a study conducted by Samojedny et al, in Poland. The study included 115 schizophrenia subjects with a mean age of 43.3 ± 12.6 years and 135 healthy controls with a mean age of 41.3 ± 9.0 years. It was found that the proportion of the G and T alleles in the schizophrenia group was 44.3% and 55.7%, while in the control group, it was 55.6% and 44.4% respectively. Furthermore, the value of p<0.05 means that there is a significant difference between the frequency of alleles and the schizophrenia incidence (4). However, the results obtained in this study are in agreement with Watanabe et al, who examined 536 people having schizophrenia in Japan, with a mean age of 40.1 ± 14.2 years and 510 healthy controls with a mean age of 37.4 ± 10.2 years (10). A p-value> 0.05 was found, which means that there is no significant difference between the frequency of alleles and the incidence of schizophrenia.

In this study, the genotype of the interleukin-2 -330 T/G polymorphism was a combination of G and T alleles, consisting of the GG, TG, and TT genotypes. In the Batak ethnic group, the genotype frequency of GG, TG, and TT was 14 (11.7%), 64 (53.3%) and 42 (35%), while for the Javanese ethnic group, it was 28 (23.3%), 53 (44.2%), and 39 (32.5%) respectively. Furthermore, the logistic regression analysis results showed that the p-value for the GG genotype was 0.053 and the OR=2.154 with a 95% CI between 0.992-4.678. In this case, it can be interpreted that there are differences in the GG genotype among people with schizophrenia in the Batak and Javanese ethnic groups. The p-value for the TG genotype was 0.693, and the OR = 0.892 with a 95% CI between 0.505-1.574. This implies that there is no difference in the TG genotype between the two ethnic groups.

The results are not in accordance with a study conducted by Frydecka et al, on the Polish population. This study involved 151 schizophrenics with a mean age of 38.0 ± 11.9 years and 279 controls with a mean age of 38.7 ± 8.8 years. The proportion of GG, TG, and TT genotypes in the schizophrenics' group was 10.7%, 46.3%, and 43%, while in the control group, it was 9.9%, 43.1%, and 47%, with a p-value = 0.72. This shows that there is a significant association of polymorphisms in people with schizophrenia among the Polish population (11). However, Watanabe et al, in Japan, linked the same gene in the Japanese population. The study involved 536 schizophrenics with a mean age of 40.1 ± 14.2 years and 510 healthy controls with a mean age of 37.4 ± 10.2 years, as

well as a p-value = 0.701. This shows that there is no significant difference between the genotype polymorphism and the schizophrenia incidence in the Japanese population (10). In the genetic estimates of most populations, the Hardy-Weinberg Equilibrium (HWE) is usually assumed. This means that the genotype probability is solely determined by the allele frequency without any distortion of the transmission ratio, as well as the selection of genotypes. When this assumption is not fulfilled, the estimate will not be accurate, and the statistical methods using allele frequencies might be invalid (19).

The chi-square values with the HWE formula found in the interleukin-2 -330 T/G polymorphism in the Batak and Javanese ethnic groups were 0.16 and 0.23. Considering that the p-value> 0.05 and no individual from each subject is less than 5, therefore, the result satisfies the Hardy-Weinberg equilibrium. This means that the allele frequency remains constant from one generation to another (19).

There are several limitations to this study, namely a) the subjects used were in one center, b) the small number of subjects, c) the inability to control all confounding factors affecting cytokines, and d) differences in the number of male and female subjects. Nevertheless, this is the first study in Indonesia and the Province of North Sumatera to examine schizophrenics from the Batak and Javanese ethnic groups, particularly in relation to the genotype and allele of the interleukin-2 –330T/G polymorphism. Aside from comparing the alleles and genotypes in the interleukin-2 -330 T/G polymorphism, an odds ratio analysis was also carried out to determine whether the distribution of alleles and genotypes is a risk or protective factor. The results can be used as a reference for future studies with more population and ethnicity due to the inconsistency associated with previous reports. Future studies are recommended to take samples and controls from several centers. Additionally, future investigation is also expected to control all confounding factors that affect cytokines.

5. CONCLUSION

Based on the results, there was no significant difference between the occurrence frequency of alleles in people with schizophrenia in the Batak and Javanese ethnic groups. Individuals with the T allele had the same odds of developing schizophrenia as those with the G allele. The IL-2 –330T/G polymorphism allele is not a risk factor for schizophrenia. The genotype frequency for GG, TG, and TT in the Batak ethnic group was 14, 64, and 42, while in the Javanese group, it was 28, 53, and 39. The p-value for the GG genotype was 0.053 with OR = 2.154 (95% CI 0.992-4.678), while the p-value for the TG genotype was 0.693, with OR = 0.892 (95% CI 0.505-1.574). This means that there is no significant relationship between the GG and the TG genotypes of the interleukin-2 –330T/G polymorphism.

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