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Comparison of Immunoreactivity Serum Neuregulin 1 in Bataks Ethnic with Schizophrenia Paranoid and Bataks Ethnic Healthy Control

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Abstract

Protein measurements in blood are often used to investigate the pathological contribution of individual molecules. Neuregulin1 (NRG1) proteins influences the development of white matter connectivity and is implicated in genetic susceptibility in schizophrenia (NRG1 proteins in rat, Frenzel, NRG1 1 genetic variation Fei Wang). Neuregulin 1 affects the regulation of central nervous system myelination by inducing the migration and differentiation of oligodendrocytes in the CNS. The objective of the present study is to make comparison of immunoreactivity serum Neuregulin 1 in Bataks ethnic with schizophrenia and Bataks ethnic healthy control. Serum samples were collected from 42 Bataks ethnic with schizophrenia (21 men, 21 women) and 30 control subjects (15 men, 15 women). Neuregulin 1 was measured by ELISA using antibody against NRG 1 beta 1. The differences between Bataks ethnic with schizophrenia and healthy control were assessed using Mann Whitney test (significant value $p < 0,05$). Mean immunoreactivity of serum Neuregulin 1 in schizophrenic patients 14,51 pg/ml (SD ± 6,81) and mean immunoreactivity of serum neuregulin 1 in control subjects 13,12 pg/ml (SD ± 2,49). Immunoreactivity of serum neuregulin 1 in Batak ethnics with schizophrenia was significantly higher than in Bataks ethnic healthy control (p=0,036).

Keywords: *serum neuregulin- schizophrenia- Bataks ethnic*

1. Introduction

Schizophrenia is a complex genetic disorder that carries lifetime morbid risk of 1% across different populations with different cultures (Stefansson et al., 2002; Williams et al., 2003; Li, Collier dan He, 2006; Mc Grath et al., 2009; Nieratschker, Nothen dan Rietscel, 2010). Schizophrenia paranoid is one of the most common type of schizophrenia which is characterized by preoccupation with one or more delusion or frequent auditory hallucination (Sadock dan Sadock, 2007; Goldberg, David dan Gold, 2011; Yeganeh et al., 2011). Neuregulin1 (NRG1) proteins are implicated in the differentiation and myelination of Schwann cells and oligodendrocytes, the migration of CNS neuronal precursors along radial glia, synaptogenesis, plasticity and regulation of neurotransmitter receptors (Kircher et al., 2009). NRG1 seems to play a major role in neurodevelopment, both during fetal gestation and postnatal reorganization and myelination processes, which continue until early adulthood. Evidence shows that NRG1 signalling is altered in schizophrenia (Stahl, 2008; Hall et al., 2006; Dammann et al., 2008; Kircher et al., 2009; Buonanno, 2010; Haraldsson et al., 2010).

2. Subjects and Methods

This study was approved by the Research Ethics Committee of Medical Faculty University of Sumatera Utara. Forty–two Bataks ethnic patients with schizophrenia paranoid from Pempropsu mental hospital and thirty Bataks ethnic as healthy control were recruited as participants. All subjects were Batak ethnic with schizophrenia paranoid, cooperative, age between 15 and 55 years old. Exclusion criteria for all subjects were having severe medical illness: especially heart disease, having other psychiatric disorder and pregnant. Written informed consent was obtained from all participants after giving a full explanation of the study protocol. Semi-structured interviews using MINI-ICD X were carried out for all participants. Diagnoses of schizophrenia paranoid were made based on ICD X criteria. Control subjects were recruited primarily from the staff of participating hospitals and associated laboratories. We matched the ages and genders of the control healthy volunteers to those of the patients examined. Serum samples were collected from 42 Bataks ethnic with schizophrenia paranoid and 30 healthy control subjects.

2.1. Blood sampling

Blood was collected in tubes between 9 and 12 a.m. Within 1 hour of collection, blood was coagulated at 37^0C for 60 min. Serum was separated by centrifugation at 4^0C for 15 min and stored at -80^0C until use for analysis.

2.2. NRG enzyme immunoassay

We have established an enzyme-linked immunosorbent assay (ELISA), using Ab100614 human NRG1 beta 1 (Neuregulin 1 beta 1) ELISA (Enzyme-Linked Immunosorbent Assay). Ab100614 Human NRG1 beta 1 (Neuregulin 1 beta 1) ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human NRG1 beta 1 in serum, plasma (collect plasma using EDTA or citrate as an anticoagulant. Heparinized plasma are not recommended), cell culture supernatants and urine. This assay employs an antibody specific for human NRG1 beta 1 coated on a 96-well plate. Standards and samples are pipetted into the wells and NRG1 beta 1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human NRG1 beta 1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of NRG1 beta 1 bound.

2.3. Statistical Analysis

Univariat analysis was performed to describe each variable and was described by frequency table. The putative confounding factors included age and gender were analyzed between the Batak ethnics with schizophrenia and without schizophrenia by using X^2 test.

Bivariat analysis was performed to analyze whether there are differences between immunoreactivity NRG1 serum in Batak ethnics with schizophrenia and healthy control. The immunoreactivity NRG1 serum data was analyzed by using t-independent test if the data was normally distributed, or Mann-Whitney test if it was not normally distributed. Statistical analysis was performed using SPSS software (version 15.0). The probability level of $p<0,05$ and confidence interval 95% was considered to be statistically significant.

3. Result

Serum of Bataks ethnic with schizophrenia paranoid (n = 42) and Bataks ethnic healthy control (n = 30) were obtained. We matched age and gender of healthy control to those of the patients examined. Minimizing the influence of differences in sampling conditions, we did consistent procedures which involved the time of blood collecting and coagulation procedure.

NRG1 immunoreactivity serum of Bataks ethnic schizophrenia paranoid patients (mean 14,51 ± 6,81 pg/ml) was significantly higher than those in Bataks ethnic healthy control (mean 13,12 $\pm$ 2,49 pg/ml, p = 0,036). There was no statistically difference between NRG1 immunoreactivity serum in Bataks ethnic schizophrenia paranoid men (mean 15,29 $\pm$ 9,52 pg/ml) than in Bataks ethnic healthy control men (mean 14,02 $\pm$ 3,07 pg/ml, p = 0,574). NRG1 immunoreactivity serum in Bataks ethnic schizophrenia paranoid women (mean 13,72 $\pm$ 1,72 pg/ml) was significantly higher than in Bataks ethnic healthy control women (mean 12,22 $\pm$ 1,30, p=0,012).

To estimate the correlations among levels of immunoreactivity NRG 1, age, onset, duration of illness and dose of antipsychotic medication, we performed Spearman correlation analysis. There were no correlation among age (R=0,09, p=0,46), age at onset (R=0,15, p=0,34), duration of illness (R=0,14, p=0,36), dose of antipsychotic medication (R=-0,16, p=0,31) and levels of immunoreactivity neuregulin 1.

Tabel 1. Characteristic of Study Participants

Variabel	Batak Ethnic		*P*
	With schizophrenia paranoid n= 42	Healthy control n= 30	
Age (years)	37,76 ± 7,88	36,13 ± 7,09	0,699
Duration of illness	7,55 ± 4,12		
Age at onset	30,21 ± 3,09		
Dose of antipsychotics medication*)	1052,38 ± 651,92		
Antipsychotic medication			
- Haloperidol (3-15mg/day) and Chlorpromazine(300-1000mg/hari)	19		
- Risperidone (2-6mg/day)	23		
Endogen factor			
(+)	7 (16,67%)		
(-)	35 (83,33%)		
Sex			0,277
Men	21 (50 %)	15 (50%)	
Women	21 (50%)	15 (50%)	
Psychosocial stresor			0,533
(+)	16 (38,10%)	12 (40%)	
(-)	26 (61,90%)	18 (60%)	

Tabel 2. Comparison of NRG1 Immunoreactivity Serum of Bataks Ethnic Schizophrenia Paranoid Patients and Bataks Ethnic Healthy Control

	Bataks ethnic schizophrenia paranoid patients n=42	Bataks ethnic healthy control n=30	*P*
NRG1 immunoreactivity serum	14,51±6,81	13,12±2,49	**0,036**
Men	15,29 ± 9,52	14,02 ± 3,07	0,574
Women	13,72 ± 1,72	12,22 ± 1,30	**0,012**

4. Discussion

We established ELISA for NRG1 and measured NRG1 immunoreactivity in serum to evaluate the pathological influences of schizophrenia on NRG1 protein. We found that NRG1 immunoreactivity Bataks ethnic schizophrenia paranoid patients were significantly higher than those in healthy control. NRG1 expression is induced by adult ischemic and traumatic brain injury, as well as by neonatal hypoxia. Thus, it is possible that the more abnormal expression of NRG1 is induced in human embryos or neonates carrying these SNPs by these environmental insults, the more severely this factor might impair brain development to increase the risk of schizophrenia. The latter report is consistent with our present findings on NRG1expression in serum. Shibuya et al (2010) found that immunoreactivity NRG1 healthy control serum were significantly higher than those in patients. There were several studies examining the expression of NRG1 mRNA in postmortem brains of patients with schizophrenia, most have reported an increase in mRNA levels. The hippocampus and prefrontal cortex of schizophrenia patients contain higher levels of type-I mRNA than samples from control subjects (Hashimoto et al., 2004; Law et al., 2006; Petryshen et al., 2005) report an increase in mRNA encoding type-III NRG1 precursor (SMDF), while Zhang et al. (2008) detect decreases in type-I and type-II mRNAs encoding NRG1 precursors (HRG-b3 and GGF2) in patient lymphocytes.

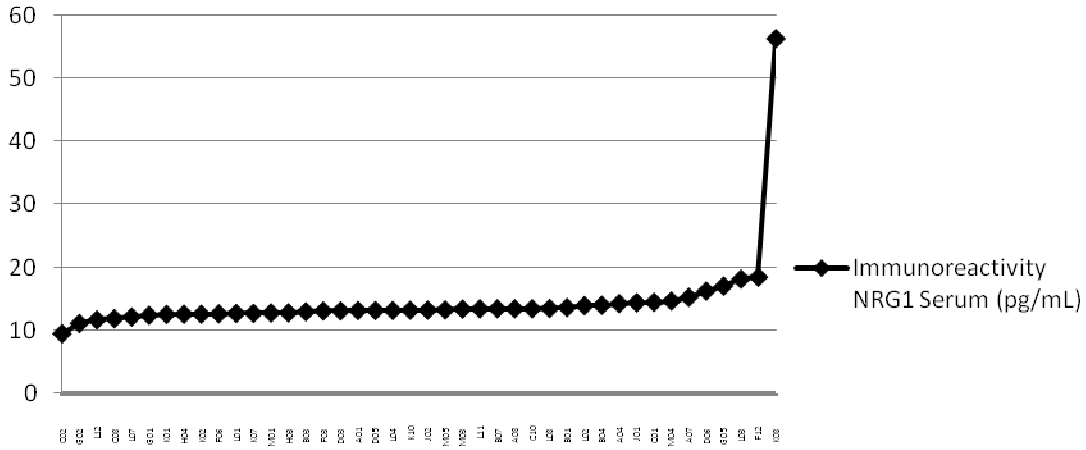

Figure 1. Immunoreactivity NRG1 Serum Curve. The concentration variation from 9,474 pg/mL to 56,279 pg/mL

We found there were no significantly correlations among age, age at onset, duration of illness, dose of antipsychotic medication and level of immunoreactivity NRG1 serum. There was slight correlation between age and level of immunoreactivity NRG1 serum. It might because of matching that have done. There were slight correlation among age at onset, duration of illness and level of immunoreactivity NRG1 serum. Hahn suggested that chronic haloperidol treatment significantly reduces NRG1-mediated ErbB4 activation in mice (Hahn et al., 2006). It is possible that the protein expression levels of NRG1 and ErbB receptors are also modified by chronic antipsychotic drug treatment, and these alterations might contribute to the pharmacological action of antipsychotic drugs.

5. Strength and Limitation

It was the first study to investigate immunoreactivity NRG1 serum in Bataks Ethnic and in Indonesia. We also had controlled confounding factors such as age, gender and psychosocial stressor which in previous study these factors had association in immunoreactivity NRG1.

In this study, duration of medication and severity of illness were not evaluated. Theoretically, the course of schizophrenia is influenced by those factors. Furthermore, it could modulate the pharmacotherapy, at the end it was possible to influence expression of the protein.

We hope that the ELISA system for NRG1 peptide will help to explore its biological role in schizophrenia pathogenesis or screening tools for schizophrenia.

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