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RESEARCH ARTICLES

Correlation of Ferritine and Brain Derived Neurotrophic Factor (BDNF) Levels in Preeclampsia

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Abstract

***Objective**: This study aims to determine the correlation between ferritin and BDNF serum levels in preeclampsia.*

***Methods**: This was an observational analitical study with cross-sectional design involving 66 pregnant women with preeclampsia which were taken from Dr. M. Djamil Central General Hospital Padang and examined for ferritin and BDNF serum levels using the ELISA method from January-October 2020. The data normality test was performed using the Kolmogorov-Smirnov test, followed by the Pearson correlation test and simple linear regression test.*

***Results**: Ferritin serum level was normal (181.76 ± 212.08 ng/ml). The mean of BDNF levels were found to be lower than normal pregnancies (0.505 ± 0.314 ng/ml) (normal range of BDNF levels was 8-46 ng/ml). There was an insignificant positive correlation between ferritin levels and serum BDNF levels, with correlation coefficient (R) 0.147 (weak correlation) (p = 0.240).*

***Conclusion**: Ferritin serum level have positive correlation with BDNF serum level in preeclampsia.*

***Keywords*: Preeclampsia, ferritin, iron deficiency anemia, Brain Derived Neurotrophic Factor**

INTRODUCTION

Brain Derived Neurotrophic Factor (BDNF) is a neurotrophic factor that has a structure similar to growth factors. BDNF is expressed massively in the central and peripheral nervous systems.[1] BDNF is also expressed in large amounts in blastocysts. BDNF plays an important role in regulating synaptogenesis and synaptic plasticity and is involved in learning and memory mechanisms in the central nervous system.[1,2] BDNF can protect nerve cells from injury induced by hypoglycemia, ischemia, hypoxia and other neurotoxicities.[1,2] BDNF also plays a role in various cells for growth of axons and dendrites. In addition, BDNF plays a role in antiapoptotic, anti-inflammatory, antiepileptic and neuroprotective against glutamate and sodium oxide (NO) toxicity.[2] BDNF is also involved in the regulation of hemostasis, metabolic function, eating behavior and body weight.[1] In addition to the above functions, BDNF and Natural Killer- 4 (NK4) stimulates angiogenesis in the heart, skeletal muscle, and skin by binding to the tropomyosin receptor kinase N (TrkB) receptor on endothelial cells. The neurotrophin BDNF has been shown to have an important role in the differentiation, proliferation, survival of cytotrophoblasts and the development and maturation of the

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placenta by increasing the growth and survival of trophoblast cells during the peri and post-implantation period based on in vitro and in vivo studies. BDNF also regulates angiogenesis and protects endothelial progenitor cells. Placental BDNF also plays a role in fetoplacental angiogenesis[3]

Based on the previous studies described above, the incidence of iron deficiency anemia, which is characterized by a decreasing in serum ferritin levels, results in a decreasing in maternal BDNF levels.[4,5] The decrease in BDNF levels starting even before the conception period increases the risk of preeclampsia during pregnancy through the mechanism of disrupting the development and implantation of blastocysts, decreasing MMP-9 signaling, disrupting trophoblast invasion so that it ends in an imbalanced state of angiogenesis which results in tissue hypoxia. Placental hypoxia will cause endothelial dysfunction and an increasing in free radicals.[6,7] Endothelial dysfunction and oxidative stress due to increased levels of free radicals cause manifestations of preeclampsia.[8] Based on the above background, the authors want to conduct a study on the correlation between serum ferritin levels and serum BDNF levels in pregnant women with preeclampsia.

METHOD

This research is an analytic observational study with a cross sectional study approach. The study sample was all pregnant women with gestational age more than 20 weeks diagnosed with preeclampsia who fulfilled the inclusion and exclusion criteria. Inclusion criterias were mothers with gestational age > 20 weeks with a diagnosis of preeclampsia and were willing to be research respondents and had signed an informed consent form. Exclusion criteria were mothers with a history of hypertension outside of pregnancy (anamnesis), mothers with a history of malignancy (based on history), leukocytes more than 16,900/mm3[9], and mothers with diabetes mellitus (with current blood sugar values <200 mg/dL).

All samples have taken anamnesis, physical examination, ultrasound examination and the patient was managed by the therapeutic procedure of Dr. M. Djamil Central General Hospital Padang. Furthermore, blood sample was taken for ferritin and BDNF levels according to the procedure of Dr. M. Djamil Central General Hospital Padang. The blood samples were centrifuged in Clinical Pathology Laboratory of Dr. M. Djamil Central General Hospital Padang, and then the serum of the samples were delivered to Biomedical Laboratory Faculty of Medicine, Andalas University for ferritin and BDNF levels examination using ELISA method.

Data processing using univariate analysis to determine the frequency distribution of each research variable, namely ferritin levels, BDNF levels, maternal age, gestational age, parity, systolic blood pressure and diastolic blood pressure, MAP, and BMI before pregnancy. The next step is bivariate analysis to see the relationship between the independent variable, namely ferritin and the dependent variable, namely BDNF. Normality test was performed

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using the Kolmogorov Smirnov test (n < 100). If the data is normally distributed, the Pearson Correlation test is used, while if it is not normal, Spearman's Rank Correlation test is used.

RESULT

Characteristics of research subjects based on maternal age, gestational age, BMI, parity, BDNF, Hb, systolic blood pressure, diastolic blood pressure, MAP, leukocytes, ferritin levels, and BDNF levels can be seen in table 1 below.

Table 1. Characteristics of Research Subject and Variable

Characteristics of Respondents Variable	Mean ± SD	F	%
Mother's age (years old)			
<20 years old		8	12,1
25-35 years old		36	54,5
>35 years old		22	33,3
Mean of mother's age (years old)	32,02 ± 6,46		
Gestational age (weeks)			
<34 weeks		30	45,5
>34 weeks		36	54,5
Mean of gestational age (weeks)	34,71 ± 4,08		
BMI (kg/m^2)			
Normoweight		20	30,3
Overweight		18	27,3
Obesitas		28	42,4
Mean of BMI (kg/m^2)	27,84 ± 6,16		
Parity			
Primipara		19	28,8
Multipara		47	71,2
Systolic blood pressure (mmHg)	169,86 ± 19,10		
Diastolic blood pressure (mmHg)	103,15 ± 12,98		
Mean Arterial Pressure (mmHg)	125,76 ± 13,65		
Hb			
Hb > 11 g/dL		38	57,6
Hb < 11 g/dL		28	42,4
Mean of Hb g/dL	11,37 ± 2,04		
Leukocytes /mm^3	12.243,65 ± 3.779,81		
Random bloo sugar (mg/dL)	102,42 ± 31,25		
Feritin (ng/ml)	181,76 ± 212,08		
BDNF (ng/ml)	0,505 ± 0,314		

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Based on Table 1, it is known that the average age of pregnant women is 32.02 ± 6.46 years old, with the frequency of pregnant women aged 25-35 years (including in healthy reproductive age) 36 people (54.5%), pregnant women who have a high risk with the age of less than 20 years as many as 8 people (12.1%) and pregnant women with the age of more than 35 years as many as 22 people (33.3%). The mean gestational age with preeclampsia in this study was 34.71±4.08 weeks. There were 36 (54.5%) pregnant women with late onset preeclampsia (gestational age >34 weeks), and 30 (45.5%) pregnant women with early onset (gestational age <34 weeks) preeclampsia. Pregnant women who experienced the most preeclampsia in this study had an average BMI of 27.84 ± 6.16 with a BMI (Body Mass Index) classified as obese (28 people; 42.4%), pregnant women with overweight as many as 18 people (27 people). ,3%) and 20 pregnant women with normoweight (30.3%). There were 19 primiparous pregnant women (28.85%), while the multiparous pregnant women who experienced preeclampsia in this study were 47 people (71.2%).

Based on the table above, the mean systolic blood pressure of pregnant women with preeclampsia is 169.86 ± 19.10 mmHg, the average diastolic blood pressure is 103.15 ± 12.98 mmHg with the mean MAP (Mean Arterial Pressure) 125.76 ± 13.65 mmHg. The average Hb level of pregnant women in this study was 11.37 ± 2.04 g/dL. Almost half of pregnant women who had preeclampsia in this study had Hb <11 g/dL, 28 (42.2%) and 38 (57.6%) pregnant women had Hb>11 g/dL. The mean leukocyte level of pregnant women with preeclampsia in this study was 12,243.65 ± 3,779.81/mm3 with a normal interpretation (reference normal leukocyte values in pregnant women was 5,900-16,900/mm3).[9] The average blood sugar during pregnancy in this study was 102.42 ± 31.25 mg/dL, this result is classified as normal blood sugar with a normal reference value of 60-200 mg/dL.[9] The average ferritin level was found to be normal in this study, with a mean value of 181.76 ± 212, 08 ng/ml, with a normal ferritin reference value of 11–306.8 ng/ml.[10] The mean BDNF level of pregnant women with preeclampsia in this study was found to be lower than normal pregnancy, namely 0.505 ± 0.314 ng/ml (with a normal range of BDNF levels). 8-46 ng/ml).[11]

Correlation of Feritin and BDNF Levels in Preeclampsia

To determine the correlation between ferritin levels and BDNF levels in preeclampsia which was analyzed using the Pearson test, can be seen in table 2 below:

Table 2. Correlation Between Ferritin Levels and BDNF Serum Levels in Preeclampsia

Variable	Mean ± SD (ng/ml)	R	p-*value*
Feritin (ng/ml)	181,76 ± 212,08	0,147	0,240
BDNF (pg/ml)	0,505 ± 0,314		

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Based on table 2, it is known that there is a positive correlation between ferritin levels and BDNF levels, with a correlation coefficient of 0.147 (weak correlation) and there is no significant relationship between ferritin levels and BDNF levels.

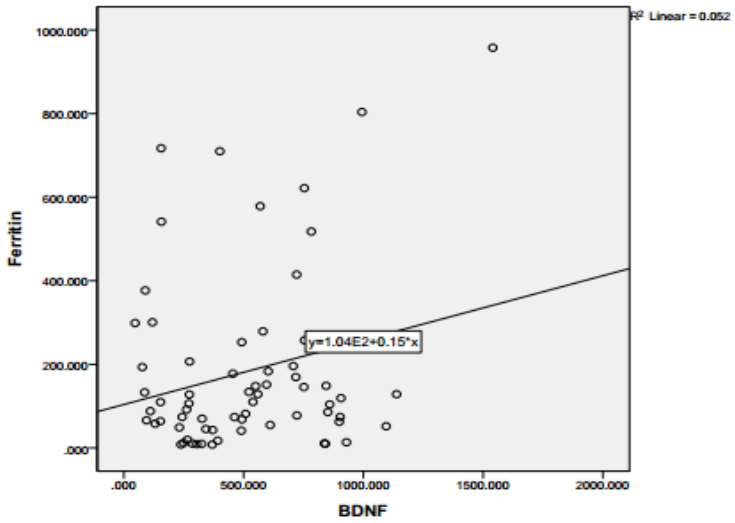

Figure 1. Scatter Plot Graph of Ferritin Levels adn BDNF Levels

Based on the scatterplot graph above, it can be concluded that the correlation between ferritin levels and BDNF levels is positively correlated, which means that if ferritin levels decrease, BDNF levels will also decrease. The value of r square obtained is 0.1, which means that ferritin levels affect BDNF levels by 10%.

Table 3. Simple Linear Regression Table of Ferritin Levels and BDNF Serum Levels

Variable	R	R Square	F	p-value	A	B
Ferritin BDNF	0,229	0,052	3,531	0,065	444,177	0,339

Table 3 explains the value of the correlation or relationship (R) which is 0.229, and the coefficient of determination (R Square) is 0.052, which implies that the effect of the independent variable (ferritin) on the dependent variable (BDNF) is 5.2%. It is known that the calculated F value = 3.531 with a significance level of 0.065, then the regression model cannot be used to predict the BDNF variable or in other words there is no effect of the ferritin (X) variable on the BDNF variable (Y). The consistent value of the ferritin variable is 444.17 with the X regression coefficient of 0.33; states that for every 1% addition of the BDNF value, the ferritin value increases by 0.33. The regression coefficient is positive, so it can be said that the direction of the influence of the variable X on Y is positive. The tcount value is 1.879 < ttable

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1.997, so it can be concluded that the ferritin (X) variable has no effect on the BDNF variable (Y).

DISCUSSION

In this study, the mean age of pregnant women was 32.02 ± 6.46 years with 22 (out of 66) pregnant women >35 years old, and 8 pregnant women <25 years old. Age over 35 years is a risk factor for preeclampsia.[12] This may be due to aging of uterine blood vessels and increased arterial stiffness leading to gradual loss of cardiovascular vascular compliance leading to endothelial dysfunction. Advanced age (>35 years) is an independent risk factor for poor maternal and perinatal outcomes in preeclamptic pregnant women.[13]

In this study, the mean gestational age was 34.71±4.08 weeks, where there were 36 (54.5%) pregnant women with late onset preeclampsia (gestational age >34 weeks), and 30 (45.5%) pregnant women. with early onset (gestational age <34 weeks) preeclampsia. The ratio of late and early onset preeclampsia in this study was 1:1,2. Early-onset preeclampsia is preeclampsia that develops before 34 weeks of gestation, and generally has an abnormal placental etiology associated with hypoxia due to failure of spiral artery modification and endothelial dysfunction. Maternal and fetal complications in early-onset preeclampsia are more severe than those of late-onset preeclampsia.[14] Late-onset preeclampsia is preeclampsia that occurs at more than 34 weeks' gestation, and is generally caused by decompensated conditions of oxidative stress in the placenta resulting from endothelial dysfunction. The impact of oxidative stress decompensation is what causes vasoconstriction and decreased blood flow to many organs.

Pregnant women who experienced the most preeclampsia in this study had a BMI (Body Mass Index) which was classified as obese (as many as 28 people; 42.4%). Pregnant women with obese BMI have a 5.8 times higher risk factor for severe preeclampsia compared to pregnant women with normoweight BMI.[15] These results are in accordance with previous literature which states that obesity is a risk factor for severe preeclampsia. In obese mothers, severe preeclampsia can occur through the mechanism of hyperleptemia, metabolic syndrome, inflammatory reactions and increased oxidative stress that causes endothelial damage and dysfunction.[16]

In this study, there were 19 primiparous pregnant women (28.85%), while the multiparous pregnant women who experienced preeclampsia in this study were 47 (71.2%). This is associated with the first exposure to chorionic villi (of fetal origin) and associated maternal immunologic incompetence is more likely to occur during the first pregnancy and may increase the risk of developing preeclampsia.[17]

Almost half of pregnant women who had preeclampsia in this study had Hb <11 g/dL, 28 (42.2%) and 38 (57.6%) pregnant women had Hb>11 g/dL. This condition results in

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impaired blood supply to the fetus, accompanied by severe anemia in the mother, which contributes to a poor outcome.

The mean systolic blood pressure of pregnant women with preeclampsia in this study was 169.86 ± 19.10 mmHg, the average diastolic blood pressure was 103.15 ± 12.98 mmHg with the mean MAP (Mean Arterial Pressure) 125.76 ± 13.65 mmHg. Combination tests based on maternal characteristics and blood pressure at 30-33 weeks of gestation can effectively identify women at high risk for subsequent development of preeclampsia.[18]

The mean leukocyte level of pregnant women with preeclampsia in this study was 12,243.65 ± 3.779.81/mm3 with a normal interpretation (referral of normal leukocyte values in pregnant women was 5.900-16,900/mm3). It is important to know the average leukocyte value in each sample in this study to exclude leukocyte values that exceed the normal reference value of leukocytes in pregnant women. A high leukocyte value indicates the occurrence of infection or an inflammatory process which is biased towards this study.

The average ferritin level was found to be normal in this study, namely 181.76 ± 212.08 ng/ml, with a normal ferritin reference value of 11–306.8 ng/ml. Ferritin decreases in pregnant women with poor nutritional intake, especially iron, causing iron deficiency conditions and ultimately affecting hemoglobin values during the first trimester. One of the stages of iron deficiency anemia is decreased levels of iron reserves/iron stores in the form of ferritin. There are many other factors or conditions that affect maternal serum ferritin levels including infection, inflammation (acute and chronic), oxidative stress (which activates the ferritin pathway), siderosis, hemochromatosis, thalassemia, repeated transfusions, liver disease such as liver cell damage or alcoholic liver disease. (as part of the acute-phase response or as a result of ferritin release from damaged hepatocytes), rheumatoid arthritis and malignancies (such as acute leukemia, Hodgkin's carcinoma, carcinoma of the lung, colon, and liver).[19]

The condition when pregnant women experience iron deficiency anemia in the first trimester is what causes changes in nerve morphology, reduces levels of BDNF which plays an important role in the trophoblast invasion process so that there is a disturbance in the trophoblast invasion mechanism and ultimately causes preeclampsia. This study was conducted in a cross-sectional manner, where the value of ferritin levels examined in pregnant women with preeclampsia diagnosed on arrival was in the second and third trimesters of gestation, thus skipping the golden period in the examination of ferritin and hemoglobin levels in the blood of pregnant women, especially pregnant women who were detected. had anemia during laboratory tests. At 25 weeks of gestation, there was a positive correlation between superoxide concentrations and ferritin levels in pregnant women with preeclampsia.[20]

The mechanism of increasing ferritin levels in some of the studies above can be explained by the theory of oxidative stress. The adverse effects of the placenta result in the

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spiral arteries being smaller than normal in size during the last half of pregnancies with preeclampsia. This event will cause obstructive lesions of the spiral arteries called acute atherosclerosis, causing placental ischemia. When the tissue becomes ischemic, reactive oxygen species such as superoxide and hydrogen peroxide are produced.[21-23] The presentation of the placenta in preeclamptic patients shows a histologic appearance with severe vascular damage in the area of attachment of decidua cells in the infarct area, this corresponds to cell damage and detachment. Fe. Catabolic amounts of transition metal ions, especially Fe, appear in the ischemic state of the placenta through destruction of red blood cells from thrombotic, necrotic, and hemorrhagic areas, these substances can generate highly reactive hydroxyl radicals through Fenton chemistry. These radicals can initiate the process of lipid peroxidation, which, if not controlled, can cause endothelial cell damage. [20-24]

The mean BDNF level of pregnant women with preeclampsia in this study was found to be lower than normal pregnancy, namely 0.505 ± 0.314 ng/ml (with a normal range of BDNF levels 8-46 ng/ml).[11] BDNF levels are influenced by many factors, namely genetics, hormones, body weight, gender, age, nutrition, lifestyle factors, oxidative stress, and inflammation. Nutritional factors, especially iron, play an important role in the production and signaling mechanism of BDNF in the brain. Iron deficiency affects the hippocampus as evidenced by decreased energy metabolism, impaired morphology and nerve transmission, and increased susceptibility to infarction.[4]

The low level of BDNF is also influenced by the increased incidence of oxidative stress due to pathological hypoxia and an imbalance of pro-angiogenic factors and anti-angiogenic factors causing endothelial disorders, dysfunction and damage, resulting in clinical manifestations of preeclampsia. Oxidative stress has been shown to regulate the expression of neurotropins which are known to influence the process of angiogenesis. Increased oxidative stress can lead to downregulation of neurotrophins. Oxidative stress is higher in pregnant women with preeclampsia.[5] Decreased BDNF will lead to downregulation of MMP-9 which plays a role in trophoblast invasion and trophoblast and placental growth and development thereby increasing cell apoptosis.[25] BDNF is also known to play an important role in the regulation of angiogenesis and has been reported protects endothelial progenitor cells by increasing the expression of superoxide dismutase (SOD).[25] This imbalance of proangiogenic factors and anti-angiogenic factors will lead to clinical manifestations of preeclampsia. Thus, it can be hypothesized that activation of the fetomaternal BDNF/TrkB system under oxidative stress can cause preeclampsia.[5,25]

Based on the results of the study, it is known that the correlation between ferritin levels and BDNF levels in preeclampsia is positively correlated (R = 0.1), which means that the lower the ferritin level, the lower the BDNF level, with a weak correlation value. The results of the analysis showed that there was no significant difference between ferritin levels and BDNF levels in preeclampsia (p>0.05). The cause of the weak correlation found in this study is the

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time of taking ferritin samples. This study was conducted in a cross-sectional manner, where the value of ferritin levels examined in pregnant women with preeclampsia diagnosed on arrival was in the second and third trimesters of gestation, thus skipping the golden period in the examination of ferritin and hemoglobin levels in the blood of pregnant women, especially pregnant women who were detected. had anemia during laboratory tests. In addition, other factors that affect ferritin levels such as infection, inflammation (acute and chronic), oxidative stress (which activates the ferritin pathway), siderosis, hemochromatosis, thalassemia, repeated transfusions, liver disease, such as liver cell damage or alcoholic liver disease. as part of the acute phase response or as a result of ferritin release from damaged hepatocytes), rheumatoid arthritis and malignancies (eg acute leukemia, Hodgkin's carcinoma, carcinoma of the lung, colon, and liver)[19]; and other factors that affect BDNF levels such as genetics, hormones, body weight, nutrition, oxidative stress, and inflammation[4] which were not investigated in this study due to limitations.

There are no studies that have assessed the correlation between ferritin levels and BDNF levels in preeclampsia. Research by Yusrawati et al. in 2018 regarding differences in BDNF levels in neonates born to normal mothers and mothers with low ferritin levels, it was found that the plasma BDNF concentration in neonates born to normal mothers was 3.81±1.37 ng/mL and plasma BDNF concentrations in neonates born from mothers with low ferritin levels was 2.78±1.19 ng/mL (p=0.015), and it was concluded that neonates born to mothers with low serum ferritin levels had low BDNF levels. One of the suspicions of a correlation between ferritin levels and BDNF levels is supported by the studies described above.

It is necessary to conduct further research on the correlation of serum ferritin and BDNF levels in pregnant women with anemia who have a final diagnosis of preeclampsia, compared with normal pregnant women. Pregnant women were then checked for their iron profile and BDNF during preconception, first, second and third trimesters. It would be better if the study was conducted with a prospective cohort design with a larger population and sample size to better see the relationship between ferritin and BDNF in preeclampsia.

CONCLUSION

From the results of the study, it can be concluded that there is a positive correlation between ferritin levels and BDNF levels in preeclampsia.

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