Research Report

Correlation of Total Antioxidant Capacity Measured by Ferric Reducing Ability of Plasma (FRAP) Assay with the Severity of Preeclampsia

Hubungan Kapasitas Antioksidan Total yang diukur dengan Metode Ferric Reducing Ability of Plasma (FRAP) dengan Derajat Beratnya Penyakit Preeklampsia

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Abstract

Objective: To analyze correlation between total antioxidant capacity measured by Ferric Reducing Ability of Plasma (FRAP) assay with severity of preeclampsia.

Method: The cross sectional method was used in this study to compare TAC of four different groups of study, consists of normal pregnancy, mild preeclampsia, severe preeclampsia and eclampsia. The study consisted of 15 women in each group. All of the subjects met the inclusion criteria and were admitted to Dr. Hasan Sadikin Hospital and it's district hospital. The study was conducted from August until September 2011. 3 ml blood samples were taken and were measured by FRAP assay in the laboratory PRODIA Jakarta.

Result: There was no significant difference ($p<0.05$) on subject's characteristics based on number of parity and gestational age among the groups of study. The comparison of TAC measured by FRAP assay based on ANOVA was significant difference ($p<0.01$). The highest mean FRAP assay result appeared in eclampsia which was 1441.1±315.8, while in severe preeclampsia 1118.8±118.3, mild preeclampsia 902.4±102.5 and in normal pregnancy 769.3± 117.1. There was significant ($p<0.05$) positive correlation (ratio 0.880) between TAC measured by FRAP assay with severity of preeclampsia. Based on prevalence ratio with CI 95% subjects with FRAP level ≥ 769.3 had about 2.17 times higher risk to develop mild preeclampsia and subjects with FRAP level ≥ 769.3 had about 2.5 times higher risk to develop severe preeclampsia or eclampsia.

Conclusion: There was a very strong positive correlation between TAC measured by FRAP assay with the severity of preeclampsia.

[Indones J Obstet Gynecol 2011; 35-4: 155-60]

Keywords: FRAP assay, total antioxidant capacity, preeclampsia-eclampsia

Abstrak

***Tujuan**: Untuk menganalisis hubungan antara kapasitas antioksidan total yang diukur dengan metode FRAP dengan derajat beratnya penyakit preeklampsia.*

***Metode**: Penelitian dilakukan secara potong silang dengan membandingkan KAT dengan metode FRAP pada empat kelompok penelitian yaitu kehamilan normal, preeklampsia ringan, preeklampsia berat serta eklampsia. Setiap kelompok berjumlah 15 orang yang memenuhi kriteria inklusi dan datang berobat ke RSUP Dr. Hasan Sadikin Bandung serta rumah sakit jejaringnya, berlangsung dari bulan Agustus - September 2011. Sampel darah dipergunakan sebanyak 3 ml lalu diperiksakan di laboratorium PRODIA Jakarta.*

***Hasil**: Tidak terdapat perbedaan bermakna ($p>0,05$) pada karakteristik subjek penelitian dari segi jumlah paritas dan usia kehamilan. Kadar FRAP pada keempat kelompok penelitian berdasarkan uji ANOVA menunjukkan perbedaan sangat bermakna ($p<0,01$). Kadar FRAP tertinggi ditemukan pada kelompok eklampsia dengan rerata 1441,1±315,8, diikuti preeklampsia berat 1118,8± 118,3, preeklampsia ringan 902,4±102,5 serta kehamilan normal 769,3±117,1. Berdasarkan uji korelasi Rank Spearman terdapat korelasi positif yang bermakna ($p<0,05$) dengan rasio sebesar 0,880 antara kadar FRAP dengan derajat berat penyakit preeklampsia. Berdasarkan uji prevalensi rasio dengan CI 95% didapatkan risiko sebesar 2,17 kali pada kadar FRAP ≥ 769,3 untuk terjadi preeklampsia ringan serta risiko sebesar 2,5 kali untuk terjadi preeklampsia berat atau eklampsia.*

***Kesimpulan**: Didapatkan korelasi positif dengan kekuatan sangat kuat antara kadar FRAP dengan derajat berat penyakit preeklampsia.*

[Maj Obstet Ginekol Indones 2011; 35-4: 155-60]

***Kata kunci**: kadar FRAP, kapasitas antioksidan total, preeklampsia-eklampsia*

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INTRODUCTION

Preeclampsia is described as a pregnancy-specific syndrome that contributes greatly to maternal and fetal morbidity and mortality. Preeclampsia complicates 4 to 8 percent of all pregnancies, and together they form one member of the deadly triad, along with hemorrhage and infection. Eclampsia is seizures that cannot be attributed to other causes in a woman with preeclampsia.[1]

Until 2010 preeclampsia was still a leading cause of maternal mortality, preterm birth, and consequent neonatal morbidity and mortality. In developing countries, where access to safe, emergent delivery is less readily available, preeclampsia claims the lives of more than 60,000 mothers every year.[2]

According to WHO, hypertension in pregnancy contributes 12% of all maternal deaths in the world. Prevalence of hypertension in pregnancy varies all

over the world about 2.6-7.3%.[3] Incidence of preeclampsia is affected by multiple factor such as number of primigravida, socioeconomical status, educational level, etc. The incidence of preeclampsia in Indonesia is about 3-10%.[4]

In Dr. Hasan Sadikin Bandung Hospital in 2002, the incidence of severe preeclampsia was 7.4% and in 2010 was 3.19% (192 cases), while incidence of eclampsia was 1.06% (64 cases).[5]

The high incidence of preeclampsia and its insignificant decline in the recent decades shows that many things are still not know about preeclampsia especially the etiology.

In recent years many efforts have been made in regard to the individuation of pathophysiological factors and possible methods of screening women for preeclampsia before clinical signs and symptoms are apparent. Preeclampsia likely represents the clinical end point of multiple contributory factors and it is unlikely that any single cause will be found. The primary immunologic, genetic, biochemical, inflammatory basis of preeclampsia still remains speculative. Although the etiology of preeclampsia remains unknown it is widely accepted that the disorder are placental origin and endothelial dysfunction.[6-9]

The pathogenesis of endothelial dysfunction remains unclear. One of the hypothesis is associated with oxidative stress, defined as an imbalance between free radical damage and antioxidant protection.[6,8-11]

Oxidative stress, hyperlipidemia and increased iron levels in the maternal compartment in preeclampsia could be responsible for these placental changes by causing oxidative stress in the placenta.[12]

Knowledge of the total responses of the serum antioxidant systems in preeclampsia is limited. Radical-scavenging antioxidants are consumed by the increased free radical activity associated with this condition and the total antioxidant capacity (TAC) has been used to assess free radical activity inderectly. There are conflicting results concerning this topic. Kharb found significantly higher total antioxidant capacity in women with preeclampsia, while Harma found significantly lower values in preeclamptic patients.[11,13]

Because of the difficulty in measuring each antioxidant component of plasma individually and of the interactions that take place among components, method that can measure total antioxidant capacity is used. Many methods that can measure TAC, and there is still no agreed 'golden standard' for this examination.[11,14]

Benzie and his research group in 1996, for the first time described a method to measure the total antioxidant capacity known as the ferric reducing ability of plasma (FRAP). This is a measure of the antioxidant power, based on the reduction of ferrous ions by the effect of the reducing power of plasma constituents, and contributed by low molecular weight antioxidants of a hydrophilic and hydrophobic character. The low molecular weight compounds are Vitamin C, Vitamin E, bilirubin and uric acid. FRAP is said to give more biologically relevant information than individual antioxidant measurements and which may describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma.[14,15]

At low pH, when a ferric- tripyridiltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous (Fe^{2+}) form, an intense blue color with an absorption maximum at 593 nm develops. FRAP is measured total antioxidant capacity which contributes from 60% uric acid, 15% ascorbic acid, 10% thiol protein, 5% of tocopherol and bilirubin.[14]

Knowledge of the total antioxidant capacity in the maternal circulation in pregnancy is limited. We chose to use FRAP because it is cheap, easy and rapid to perform, requires little plasma volume with direct result and its equipment is common in biochemical laboratory. FRAP is regarded as a reliable measurement of over-all ability to resist oxidative damage.[11] Also because FRAP assay used method of reduction of ferric into ferrous ion, this is a distinct advantage because the levels of Fe were found to increase significantly in preeclampsia.[12]

The FRAP value in the maternal circulation could be influenced by the concentration of uric acid. The high contribution of uric acid as an antioxidant that is measured in FRAP examination is thought to be one of the drawbacks of this study because of the presence of increased uric acid in preeclampsia. However Harsem in her research did not found any positive correlation between plasma levels of FRAP and serum concentrations of uric acid in preeclampsia.[16]

Research on TAC with FRAP method in preeclampsia is still rarely done. Throughout our searching, there are two studies that measured TAC with FRAP assay in preeclampsia having results that supported each other. Research conducted by Harsem in Norway in 2006 obtained TAC which was significantly higher in preeclampsia than normal pregnancy[16], while research conducted in Jakarta by Noroyono Wibowo in 2010 obtained values in preeclampsia did not significantly higher than normal pregnancy.[17]

Another study by Zusterzeel in 2001 that measured TAC with FRAP assay in the placental and decidual gained levels in preeclampsia which was significantly lower than in normal pregnancies.[18]

Researchers are interested in adding justification of the above research results, by conducting a different research to analyze the relationship between TAC measured by FRAP assay in plasma and the severity of preeclampsia, especially in the most severe degrees, eclampsia.

METHODS

The method used in this research was cross sectional study to compare TAC measured by FRAP assay of all subjects. Using four different groups consisted of normal pregnancy, mild preeclampsia, severe preeclampsia and eclampsia. The study consisted of 15 women in each group. All of the subjects met the inclusion criteria and were admitted to Dr. Hasan Sadikin Hospital Bandung, Cibabat Hospital, Astana Anyar Maternity Hospital, Majalaya Hospital, and Dr. Slamet Garut Hospital. The inclusion criteria are gestational age > 20 weeks, singleton pregnant, liver and renal function in normal limit, no anemia, no sign of labor, no systemic infection, not having any antioxidant supplement (Vitamin C and E), and no history of chronic hypertension.

3 ml blood samples were taken with vacutainer and EDTA containing vials was used. The total antioxidant capacity by FRAP assay was measured in the laboratory PRODIA Jakarta. The blood samples were kept in ice (2-8°C) for maximum 60 minutes until centrifugation at 2000 G for 10 min at 4°C. The plasma was obtained and stored on vial containing 0,3 ml plasma. FRAP solution contained 300 mmol/liter acetate buffer, pH 3,6; 10 mmol TPTZ (2,4,6 tripyridyl-s-triazine) in 40 mmol/liter HCl; 20 mmol/liter $FeCl_3.6H_20$. Aqueous solution (H_2O) 33 µl was added to 1000 µL FRAP solution at temperature 37°C and used for calibration and control. 33 µl sample of plasma was added to 1000 µl freshly prepared FRAP reagent and incubated for 4 min at 37°C. Absorbance against the blank was read at 593 nm compared with 0 min. The antioxidant capacity was calculated using a calibration curve of known amounts of Fe^{2+}/l. The final results were converted to millimoles of Fe^{2+}/l.[14,16]

All of data were analyzed by SPSS 18.0 for Windows to compare the characteristic of the four subject groups and to compare the TAC measured by FRAP assay in each group. To analyze the correlation of TAC measured by FRAP assay with severity of preeclampsia, Rank Spearman test was used. To calculate the risk of FRAP assay to cause preeclampsia and eclampsia, prevalence ratios with 95% of confidence intervals were used.

RESULT

The study was conducted from August to September 2011 and obtained 60 subjects that met the inclusion criteria, 15 subjects for each group.

Subject's characteristic based on number of parity and gestational age among the groups of study were comparable and not significantly different ($p<0.05$). The mean gestational age from normal pregnancy group was 34.5, mild preeclampsia was 37.3, severe preeclampsia was 38.0 and eclampsia was 35.4. Thus, the four groups of subjects were homogeneous so the proper studies can be compared.

Table 1 showed that the highest mean FRAP level result was found in eclampsia which was 1441.1 ± 315.8, severe preeclampsia was 1118.8 ± 118.3, mild preeclampsia was 902.4 ± 102.5 and in normal pregnancy was 769.3 ± 117.1. Analysis of variance (ANOVA) was found to be significantly different ($p<0.01$) among each group of study.

In post hoc tests based on Games-Howell test it was found significant difference ($p<0.05$) between FRAP levels in normal pregnancy and in mild preeclampsia, whereas among groups of normal pregnancy, severe preeclampsia and eclampsia it was found a very significant difference ($p<0.01$). In these test was also found a very significant difference ($p<0.01$) between groups with mild preeclampsia and severe preeclampsia and between mild preeclampsia and eclampsia, whereas significant differences ($p< 0.05$) was found between severe preeclampsia and eclampsia groups.

Based on Rank Spearman correlation test at the interval confidence of 95%, obtained p-value of 0.021 between TAC measured by FRAP assay and severity of preeclampsia. This indicated that there was a significant correlation between TAC measured by FRAP assay and severity of preeclampsia ($p<0.05$). Correlation value of 0.880 indicated that there was a very strong positive correlation based on the criteria Gamma and Somers'd (Table 2).

Table 2. Correlation between total antioxidant capacity measured by FRAP assay and severity of preeclampsia

Variable correlation	r_S	p
Total antioxidant capacity measured by FRAP assay and severity of preeclampsia	0.880	0.021*

Note: r_S = Rank Spearman correlation coefficient

Table 3 showed that in the normal pregnancy group there were six subjects (40%) with FRAP levels ≥ 769 µmol/liter and 9 subjects (60%) with FRAP levels <769 µmol/liter, while in the mild preeclampsia group there were 13 subjects (86.7%) with FRAP levels ≥769 µmol/liter and 2 subjects (13.3%) with FRAP levels <769 µmol/liter. The analysis showed that prevalence ratio was 2.17 which meant that subjects with FRAP level higher than 769 µmol/liter had risk about 2.17 times to develop mild preeclampsia.

The whole subjects of severe preeclampsia and eclampsia had FRAP levels ≥ 769 µmol/liter. The prevalence ratio was 2.50 which meant that subjects with FRAP level higher than 769 µmol/liter had risk

Tabel 1. Comparison of mean total antioxidant capacity measured by FRAP assay on four groups of study

FRAP level (µmol/liter)	Research groups				Statistic test
	Normal Pregnancy (n=15)	Mild Preeclampsia (n=15)	Severe Preeclampsia (n=15)	Eclampsia (n=15)	
Mean (±SD)	769.3 (117.1)	902.4 (102.5)	1118.9 (118.3)	1441.1 (315.8)	F = 37.412 p < 0.01
Range	554 - 1036	712 - 1048	918 - 1286	1078 - 2160	

Note: F = F test (analysis of variance)

Table 3. Prevalence ratio between mean total antioxidant capacity measured by FRAP assay and severity of preeclampsia

Research groups	Mean FRAP Level of Normal Pregnancy (μmol/liter)		Prevalence Ratio (95% CI)
	≥ 769	< 769	
Normal Pregnancy	6 (40%)	9 (60%)	1.0
Mild Preeclampsia	13 (86.7%)	2 (13.3%)	2.17 (1.13-4.15)
Severe Preeclampsia	15 (100%)	0 (0%)	2.50 (1.35-4.65)
Eclampsia	15 (100%)	0 (0%)	2.50 (1.35-4.65)

Note: x^2: chi square = 24.378; p<0.01
CI = confidence interval

about 2.50 times to develop severe preeclampsia or eclampsia.

DISCUSSION

Characteristics of research subjects

The characteristics of research subjects which were compared in this study consisted of parity and gestational age because it had been speculated that parity and gestational age would be the confounding factor toward the occurrence of preeclampsia. In order to be compared in this study, the characteristics of the subjects among the four groups should be homogeneous.

Davidge, et al stated that the plasma antioxidant activity showed an increase in the second and third trimester of pregnancy so it had been worried that a distant range of gestational age would be a confounding factor of the study's results.[19]

In this study, the characteristics of the study subjects which were compared consisted of the number of parity and gestational age. It was feasible for all four groups to be compared because of the homogeneous characteristics of the data obtained.

Zusterzeel in his study, were trying to find the effect of gestational age on levels of FRAP, testing the correlation between FRAP levels and gestational age but the result was there was no correlation between gestational age and levels of FRAP.[18]

Correlation between total antioxidant capacity measured by FRAP assay and severity of preeclampsia

This study showed that there was a significant increase of total antioxidant levels measured by the FRAP assay among the four research groups: normal pregnancy, mild preeclampsia, severe preeclampsia, and eclampsia. These results were consistent with the results of research conducted by Harsem[16] and Noroyono[17] but contradictory to the results of research conducted by Zusterzeel.[18]

The study conducted by Zusterzeel showed that the total antioxidant levels measured by FRAP assay was significantly lower in severe preeclampsia than in normal pregnancy. There were deferrences in material and specimen used by Zusterzeel in his study. Zusterzeel used placental and decidual tissue of pregnancies with severe preeclampsia compared to normal pregnancy.[18]

Why did it raise some different results that were contrary to this study? The reason of this question could be explained by a study conducted by Serdar which proved that placental and decidual tissue was a source of lipid peroxidation that played an important role in the pathophysiology of preeclampsia. This study determined the lipid peroxidation levels by measuring the peroxidation product of complex unsaturated fatty acid membrane that was malonylaldehyde, which found significantly increased levels of malonylaldehyde in severe preeclampsia, supported by the other literature. Increased level of lipid peroxidation in the placenta and decidua would cause an imbalance between pro-oxidants and antioxidants in preeclampsia and then caused an endothelial damage as the main pathogenesis of preeclampsia.[10,20]

In this study we found that the total antioxidant capacity measured by FRAP assay was significantly higher in mild preeclampsia, severe preeclampsia and eclampsia groups than in normal pregnancy group. This was consistent with the study conducted by Harsem showed that the total antioxidant capacity measured by FRAP method was significantly higher in severe preeclampsia cases than in normal pregnancies.[16] These statement were also consistent with the results obtained by Noroyono in his study which stated that there was an increase of total antioxidant capacity measured by FRAP assay in severe preeclampsia group compared to normal pregnancy.[17]

The existence of increased capacity of total antioxidant in preeclampsia compared to normal pregnancy was also confirmed by Kharb in his study even though he was using the other methods for measuring the total antioxidant capacity.[13] The method that were used was total radical absorption potentials method (TRAP), its working principle is different from FRAP method. FRAP method works based on the reaction of hydrogen atom transfer (HAT) while the FRAP method works based on the reaction of single electron transfer (SET).[21]

Kharb explained that the reason why preeclampsia led an increase of total antioxidant capacity measured by TRAP method was due to high levels of uric acid in serum. The differences in levels of uric acid in both groups were also found in other studies. Uric acid contributed as much as 38-47% to the measurement of total antioxidant capacity by TRAP method, while vitamin C and E respectively contributed as much as 13-17% and 2-8%. Thiol protein (sulfhydryl group) actually had the highest plasma concentrations but it had less effective roles as an antioxidant.[13]

Similarly, the measurement of total antioxidant capacity with FRAP method was also markedly influenced by the levels of uric acid. Uric acid antioxidant contributed as much as 60% to the measurements conducted by the FRAP method, whereas ascorbic acid contributed as much as 15%, thiol protein 10%, tocopherol and bilirubin severally as much as 5%.[14,22]

However, Harsem found no positive correlation between plasma levels of FRAP and serum concentrations of uric acid in the preeclampsia group. The FRAP assay has the restriction that it measures mainly the antioxidant capacity of water-soluble antioxidants (uric acid, Vitamin C, and bilirubin) and to a lesser extent that of hydrophobic components (Vitamin E) and sulfhydryl groups of proteins.[16]

It was found that there was an increase of uric acid levels in preeclampsia and it had been correlated with increased maternal and fetal morbidity, but this increase was more reflective as a result of an increase in severity rather than as a cause of preeclampsia itself. Uric acid had a protective role as antioxidants through a mechanism of metal chelation, reacting with the oxidant (such as hydroxyl radicals and hypochloric acid) to form a more stable product (such as alantoin), and also through the mechanism of peroxynitrite fragmentation.[23]

The role of uric acid levels in affecting the measurement results of total antioxidant capacity and the role of bilirubin as antioxidant remain unclear and require further research.[14]

The study conducted by Gulmezoglu, et al proved that an administration of antioxidants (vitamin C, vitamin E, and allopurinol) in patients with severe preeclampsia showed a marked improvement of the disease.[24] On the other hand, if the administration of antioxidant was carried out before the symptoms of preeclampsia appeared, then this antioxidant supplementation would reduce the incidence of preeclampsia.[17,25]

This might happen because the antioxidant supplementation after the onset of symptoms of preeclampsia was considered too late, since the beginning of the preeclamptic process itself had been occured at the time of trophoblast invasion to desidua.[24]

CONCLUSIONS

There was significant difference of total antioxidant capacity measured by FRAP assay among the groups of study: normal pregnancy, mild preeclampsia, severe preeclampsia, and eclampsia. There is a very strong positive correlation between total antioxidant capacity measured by FRAP assay and severity of preeclampsia with ratio 0.880.

Based on prevalence ratio with CI 95% subjects with FRAP level ≥ 769,3 had about 2.17 times higher risk to develop mild preeclampsia and subjects with FRAP level ≥ 769,3 had about 2.5 times higher risk to develop severe preeclampsia or eclampsia.

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