Research Article

Lower Glutathione Peroxidase Serum Level Compared to Normal Pregnancy

Kasus dengan Ancaman Keguguran Menunjukkan Kadar Glutation Peroksidase yang lebih Rendah dibandingkan pada Kehamilan Normal

Kadek Pramarta, I.G.P. Surya

Department of Obstetrics and Gynecology
Faculty of Medicine University of Udayana/
Sanglah Hospital
Denpasar

Abstract

Objective: To determine the difference of glutathione peroxidase (GPx) in threatened miscarriages and normal pregnancy.

Method: This is an analytic cross sectional study with 42 samples divided into two groups. Group 1 consists of 21 cases of threatened miscarriages with < 20 gestational age and group 2 is divided into 21 normal pregnancies of < 20 weeks gestational age. We took 3 cc of blood samples from the cubiti veins and mixed it with EDTA. Its GPx quantities were than examined at the Pathology Lab at Sanglah General Hospital. Data was then analyzed using the Shapiro Wilk Test and the independent t-test with $p < 0.05$.

Result: From this research, we obtained the mean GPx levels on the threatened miscarriages was 49.92 ± 14.17 U/g Hb lower than the mean of normal pregnancy levels, which was 88.94 ± 30.11 U/g Hb.

Conclusion: The quantities of GPx between threatened miscarriages and normal pregnancies are statistically different, in which the quantity of GPx in threatened miscarriages is lower compared to normal pregnancy.

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Keywords: GPx, normal pregnancy, threatened miscarriages

Abstrak

***Tujuan**: Untuk mengetahui perbedaan kadar Serum GPx pada abortus iminens dan kehamilan normal.*

***Metode**: Potong lintang. Jumlah sampel adalah sebesar 42 sampel, di mana 21 kasus abortus iminens dengan usia kehamilan < 20 minggu dan 21 kasus dengan kehamilan normal < 20 minggu. Pengambilan darah pada vena cubiti sebanyak 3 cc kemudian dimasukkan ke dalam tabung EDTA, lalu diperiksa kadar GPx pada Laboratorium Patologi Klinik RSUP Sanglah Denpasar. Dari data yang terkumpul dilakukan pengujian normalitas data dengan Shapiro-Wilk Test, kemudian dilakukan analisis data dengan t-independent sample test dengan tingkat kemaknaan p < 0,05.*

***Hasil**: Dari penelitian ini didapatkan kadar rerata GPx pada abortus iminens 49,92 ± 14,17 U/g Hb lebih rendah dari kehamilan normal dengan kadar rerata 88,94 ± 30,11 U/g Hb.*

***Kesimpulan**: Perbedaan kadar GPx antara abortus iminens dan kehamilan normal berbeda bermakna secara statistik, di mana kadar GPx pada abortus iminens lebih rendah dibandingkan kehamilan normal.*

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***Kata kunci**: abortus iminens, GPx, hamil normal*

Correspondence: Kadek Pramarta, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Udayana, Denpasar, Telephone: 081999912515, Email: ikp_love@yahoo.com

INTRODUCTION

Abortion is the most common complication found in pregnancy. It is estimated that spontaneous abortion (miscarriage) happens in 7% of women since the time of conception. However, the patients are not fully aware of it since it happens at the same time with the next menstrual period. In the cases where the patients are aware of, 15-20% of them end up with spontaneous abortion or ectopic pregnancy.[1] The probability of someone to experience recurrent spontaneous abortion is increased alongside the frequency of someone having abortion. Even after experiencing it three to four times, the possibility of subsequent abortion is as much as 45% and 54.3%, respectively.[2] More than 80% of abortion takes place at the first trimester,[3] that is until the 14th week of gestational age.

Abortion is the termination of pregnancy, either spontaneously or induced, before the fetus is considered as viable. Generally, abortion is defined as the termination of pregnancy before the 20th week of gestational age or when the fetal weight is less than 500 gr. Theoretically, the incident of abortion can be caused by embryonic factors, such as chro-

mosomal abnormality, or maternal factor such as renal disease, diabetes mellitus, acute infection, trauma, abnormality of reproductive system, and placental factor. Beside that, abortion can also be caused by imbalance between oxidant and antioxidant in uteroplacental tissue, which holds an important role in many diseases, including abortion.

Glutathione peroxidase is an enzymogen peroxide which functions in catalyzing hydrogen peroxide (H_2O_2) and hydroxyl peroxide, hence its role in preventing lipid peroxidation on the cell membrane and its works as free radical binding agent[4]. By the presence of GPx, reduced glutathione (GSH) reacts with H_2O_2 or organic hydroperoxide (RO OH), forming glutathione disulfide (GSSG) and H_2O. Glutathione peroxidase can be found in mitochondria, cytosol, and extracellular space. In human, 8 types of this substance have been found. However, the function of each enzyme hasn't yet to be fully explained. Glutathione peroxidase serum plays an important role in the pregnancy. In the early pregnancy, GPx functions on the endometrium to ensure the successful implantation by protecting blastocyst form free radical. On the desidua, GPx serum protects the cell from damage due to activation of TNF-α. Tumor Necrosis Factor alpha induces cell damage through superoxide. Thus, high level of GPx serum during normal pregnancy will protect the conception from TNF-α. On the placenta, GPx serum works as its protector from lipid peroxidase. Lipid peroxidase can cause cell damage through enzymatic reaction, altering unsaturated fatty acid into lipid peroxide, which in turn disturbs the stability of cell membrane, hence inducing cell damage. In normal pregnancy, the level of lipid peroxidase will reduce, whereas GPx serum will increase in line with the advancing gestational age.

Though oxidative stress is only giving small portion to the etiology of imminent abortion, but the impact of it, that is Glutathione peroxidase (GPx) serum level can be measured, and by knowing the difference of its level in normal pregnancy, we could assume its role in miscarriage. Thus, this substance can be administered from outside, so it can reduce the incident of imminent abortion.

From previous studies, some researcher found a reduction of GPx serum level in spontaneous abortion. Meanwhile, the level of GPx erythrocyte in spontaneous abortion is still controversial, some researchers did find an increment, but in another study, it was found that the level was not different compared to normal pregnancy.

METHOD

This is an analytical cross sectional study. The sample of this study is all pregnant mother who came to delivery room of emergency department and obstetric and gynecology polyclinic of Sanglah General Hospital Denpasar with the diagnosis of imminent abortion and normal early pregnancy with the gestational age of < 20 weeks who fulfilled the inclusion criteria. The inclusion criteria were pregnant mother with the gestational age of < 20 weeks with the diagnosis of imminent abortion who came to emergency department and obstetric and gynecology polyclinic of Sanglah General Hospital Denpasar, and agreed to be enrolled in this study. The exclusion criteria were mola hidatidosa, early pregnancy with uterine abnormality, and early pregnancy with uterine myoma. The sample of blood was taken from cubital vein as much as 3cc then it was contained in EDTA tube. The level of GPx was then investigated in the laboratory of Clinical Pathology, Sanglah General Hospital Denpasar. The data analysis of this study was using Statistical Package For The Social Sciences (SPSS) for windows 16.0. The data were then analyzed descriptively and the result would be presented in a table. To test the normality of the data, Shapiro-Wilk test was used. The comparability of the characteristics in imminent abortion and normal early pregnancy was tested using t-independent test, and for the variable of age and gestational age, Mann-Whitney test was used. The difference of the average level of GPx was tested using t-independent test.

RESULT

During the period of this study, 42 blood samples were collected, which consist of 21 samples taken from imminent abortion group and the same amount from normal early pregnancy group.

Table 1. Age average, Parity, Gestational age of the group of imminent abortion and normal pregnancy

Characteristic	Imminent abortion	Normal pregnancy	p
Maternal age (years)	26.19 ± 4.54	28.81 ± 6.94	0.156
Parity	1.19 ± 1.07	1.47 ± 1.28	0.523
Gestational age (weeks)	9.80 ± 3.57	9.28 ± 2.83	0.601

Table 1 showed that the difference of maternal age between the group of imminent abortion and

the group of normal early pregnancy was not statistically significant (p>0.05). The difference in parity was not statistically significant (p>0.05). Thus, from the data above, the influence of confounding variable was found to be reducible in the imminent abortion and normal early pregnancy group.

To identify the difference of average level of GPx, t-independent test was used in this study. The analysis results is presented in Table 2.

Table 2. The Difference of Average Serum Level of GPx of the Imminent Abortion and Normal Pregnancy Group

Group	Serum level of GPx		p
	Average	SD	
Imminent abortion	49.92	14.17	0.001
Normal Pregnancy	88.94	30.11	

Table 2 showed that the average level of GPx in the group of imminent abortion was as much as 49.92 with SD of 14.47. Meanwhile, it was 88.94 with SD of 30.11 in the group of normal pregnancy. The difference of the result found in the two groups was statistically significant (p<0.05). Thus, the average level of GPx in imminent abortion was found to be lower compared to the level in normal early pregnancy.

DISCUSSION

Vaginal bleeding before 20 weeks of gestational age is the most common complication, around 15-20% in young pregnant woman. This complication is commonly known as abortus. Chromosomes factor plays a role in 75% of abortion cases. But the test itself spends much cost.[5] The role of reactive oxygen in pathogenesis of abortus has been studying (1-5%) in the form of O_2- (superoxide anion), OH- (hydroxil), and H_2O_2 (hydrogen peroxide) which will attack sincitiotrophoblast. Because of the unstable state of the free radical production as the aerobic cells metabolism, antioxidant is needed as body defense for either in enzymatic or non enzymatic form. Enzymatic defenses such as Gluthation peroxidase, catalase, and SOD. And the non-enzymatic form are vitamin C, vitamin E, and vitamin B.[6]

Characteristic of Sample

The mean age of patients with imminent abortion and normal pregnancy is 26.19 and 28.81 years old (p>0.05) which is statistically insignificant. Previous study[6] showed that the mean age of patient with imminent abortus is 15-20% and the incidence is increasing as the mother's ages becoming older, reaching as high as 35% in women aged 38 years old. The parity average in this research is 1.19 ± 1.07 for the imminent abortus group and 1.47 ± 1.28 in normal pregnancy. It isn't significantly different (p>0.05). The mean gestational age is 9.80 ± 3.57 weeks for imminent abortus group and 9.28 ± 2.83 for normal pregnancy. Statistically, it isn't significantly different (p>0.05). In Okanet al (2006) research, the mean gestational age is 5.7 ± 2.0 weeks. In less than 10 weeks of pregnancy, there is no blood flow to the fetus. If this happened earlier, it will destroy sincitiotrophoblast by the effect of high pressure oxygen, causing misscarriage.[7] Jauniaux, et al stated that the earlier blood flow will increase free radical production such as O_2^- which destroy syncitiotrophoblast and increase apoptosis, causing damage and detachment of placenta from uterine wall make the bleeding in young pregnancy.

GPx Level in Imminent Abortus

Through this research, it was found that the average level of GPx in immnens abortus group is around 49.92 ± 14.47, lower than in normal pregnancy, which is 88.94 ± 30.11. Statistically, the difference is significant, with p<0.05. In imminent abortus, pathological condition occured when there is a failure in artery spiralis remodelling which causes placental ischemic that will produce free radical. Fetus grows and develops in low state of oxygen especially in implantation period, because an increasing O_2 can induce free radical forms which is toxic for the fetus especially syncitiotrophoblast. Normally, somatic cells in aerob state produce free radical as much as 1-5%. Anion superoxide as one of important free radical is neutralized by GPx enzyme. The GPx enzyme level in imminent abortus is also affected by endogen and exogen factors. In two third of abortus cases, there is anatomic proof of placentation defect which characterize by thinner or fragmented trophoblast covering sheet, less endometrium invasion by tropho-blast and unfinished occlusion of end spiralis artery. This is correlated with no physiologic changes in most of big spiralis arteries and cause prematus onset of maternal circulation through the whole placental.

Gluthation peroxidase is seleno-enzyme which is first found in mammals.[8] The level is high in kidney,

liver, and blood, moderate in the lens and erytrocyte and low in alveoli and blood plasm.[9] This enzyme needs gluthatione as substrate donor for binding with H_2O_2 or organic hydrogenperoxide (ROOH) in forming gluthatione disulphide (GS-SG), water and hydroxi phase of that organic matter (ROH). Nevertheless, now it is found that another substrate such as thioredoxin, glutaredoxin and other protein with CXXC motif can be also used by GPx for binding with hydrogen peroxide.[8] Now it is known there are 8 kinds of GPx in human, from GPx 1 to GPx 8. Most of these are selenoprotein (GPx1, GPx2, GPx3, GPx4, and GPx6), whereas in GPx5, GPx7 and GPx8, the active site of selenocysteine waste is replaced by cysteine. The function of these GPx is not fully known.[8] The mechanism of how glutathione peroxidase specifically affect the occurence of abortus is not fully understood. But research in mice, showed the inactivation of GPx4 expression gene causes death.[8,10] Some research about gluthation peroxidase level in aborted and normal pregnancy find decreasing level of Gluthation peroxidase in the erythrocyte and plasm in spontaneous abortus.[11,12] Ozkaya found that erythrocyte GPx level in abortus with bleeding is indifferent compare to the level in the normal pregnancy. In habitualis abortus, Simsek found that GPx plasm level was in significantly different with the normal pregnancy. In normal pregnancy, Jauniaux found that placental tissue glutathione peroxidase level in first trimester has positive correlation with age of pregnancy[9], found the decreasing of erythrocyte GPx level in the pregnancy age of 15-20 weeks compare with 6-8 weeks, then increase significantly in 26-30 weeks and the peak is at aterm.

CONCLUSION

From this research we could conclude that the average level of GPx in abortus imminent group is 49,92 ± 14,17 U/g Hb. The average level of GPx in normal pregnancy is 88,94 ± 30,11 U/g Hb.

SUGGESTION

Further research is still need to be done by using the results of this research in the effort of preventing the occuring of imminent abortus. Based on this research, it is necessary to give exogen antioxidant to the high-risk pregnant women to prevent the occurence of imminent abortus.

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