

Research Article

The Level of 8-OHdG Serum was Higher in Women with Blighted Ovum***Tingkat 8-OHdG Serum pada Blighted Ovum Lebih Tinggi dari Kehamilan Normal***

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Abstract

Objective: To determine the difference of 8-hydroxy-2-deoxyguanocytine (8-OHdG) serum level in women experiencing blighted ovum and women with normal pregnancy.

Method: This was a cross sectional study with 82 samples, divided into two groups. Thirty two cases of blighted ovum and 51 cases of normal pregnancies, with 7-12 weeks gestational age. The examination of 8-OHdG serum level, was performed with the venous blood taken from the cubiti veins, and its 8-OHdG serum quantities were than examined at the Pathology Lab. at Sanglah General Hospital. Data was analyzed with the Shapiro Wilk Test and the t independent test with alpha 0.05.

Result: The average 8-OHdG serum level in women with blighted ovum and normal pregnancies were 0.177 (SD 0.06) ng/ml and 0.111 (SD 0.01)ng/ml, and significantly different ($p < 0.05$). The cut off value of 8-OHdG serum level was 0.138 ng/ml with sensitivity of 96.1% and specificity of 80.6%.

Conclusion: The level of 8-OHdG serum was higher in women with blighted ovum than in women with normal pregnancies.

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Keywords: 8-OHdG serum level, blighted ovum, and normal pregnancy.

Abstrak

Tujuan: Untuk mengetahui perbedaan 8-hidroksi-2-deoxyguanocytine (8-OHdG) serum blighted ovum dan kehamilan normal.

Metode: Penelitian ini merupakan penelitian potong lintang dengan 82 sampel dibagi menjadi dua kelompok. 31 kasus blighted ovum dan 51 kasus kehamilan normal, dengan 7-12 minggu usia kehamilan. Pemeriksaan 8-OHdG kadar serum, diambil dari vena cubiti, dan jumlah serum 8-OHdG yang sedang diperiksa di Laboratorium Patologi di Rumah Sakit Umum Sanglah. Data dianalisis dengan Shapiro Wilk Test dan uji independent dengan alpha 0,05.

Hasil: Rata-rata 8-OHdG serum untuk blighted ovum dan kehamilan normal adalah 0,177 (SD 0,06) ng/ml dan 0,111 (SD 0,01) ng/ml, dan berbeda nyata ($p < 0,05$). Cut off nilai tingkat serum 8-OHdG adalah 0,138ng/ml dengan sensitivitas 96,1% dan spesifisitas 80,6%.

Kesimpulan: Tingkat 8-OHdG serum pada blighted ovum lebih tinggi dari kehamilan normal.

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Kata kunci: 8-OHdG kadar serum, blighted ovum, dan kehamilan normal.

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INTRODUCTION

Abortion is a commonly found obstetric complication in early pregnancy which sometimes is caused by the lack of embryo in the gestational sac (blighted ovum).¹ The prevalence of the failure of conception progress in 10-13 weeks of gestational age is about 2.8%, and blighted ovum happens in 37.5% of pregnancy loss.² According to the Obstetric Polyclinic of Sanglah Hospital's data, the incidence rate of blighted ovum was 5% of all cases in 2009.

The certain cause of blighted ovum is still unknown. In blighted ovum pregnancy, the conception's result develop into blastocyst, however, the

inner mass cell and embryonic pole are never formed.³ Some experts assumed that free radical has a role in inducing complication in early pregnancy by triggering chromosomes changes that can eventually lead to abnormality of the chromosomes. Free radical like Reactive Oxygen Species (ROS) is a very unstable and reactive substance that can cause cell damage. Some scientific evidence described that the significantly maternal-fetal blood stream is formed at the end of the first three months. Thus, the embryo development happens in an environment with relatively lower in oxygen level.⁴ In a physiological pregnancy, embryo is protected from ROS by the development balance and prevention of the harmful oxydative stress.

The oxydative stress causes anomaly and retardation in embryo development correlated with DNA cell membrane damage and apoptosis. Apoptosis results in fragmented embryo, caused limitation in the implantation capability, which finally ended in the low rate of successful fertilization.⁵

There are 3 kind of ROS, superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl (OH^\cdot).⁵⁻⁷ The hydroxyl radical has a capability to cause damage in DNA chain, yet its level can not be measured directly because of its excessive reactivity. The serum level of 8 Hydroxy-2-Deoxyguanocyne (8-OHdG) is a DNA damage marker which could be examined using ELISA method.⁸ So far, studies have not showed the average levels of 8-OHdG in pregnant women with blighted ovum. This study was aimed to determine the difference level of 8-OHdG serum level in women with blighted ovum and normal pregnancies.

METHOD

This study was an analytical cross sectional study with consecutive sampling method. This study was done from June 2012 until December 2012 in Polyclinic and Emergency Room of Obstetric and Gynecologic Department of Sanglah General Hospital, Denpasar, Bali, Indonesia. The population of the samples were women with 7-12 weeks of gestational pregnancy both normal or with blighted ovum. Samples had to fulfill the inclusion and exclusion criteria and agreed to join in the research. There were 31 women with blighted ovum pregnancy and 51 women with normal pregnancy participated in this study. The samples then was managed as the therapy guidelines of Obstetric and Gynecologic Department of Sanglah General Hospital with the following steps: anamnesis (name, maternal age, parity, last menstrual period), physical examination (consciousness, body weight and height, blood pressure, heart and respiration rate, and gynecologic examination), supporting tests

(pregnancy test and ultrasonography by supervisor), and 8-OHdG serum level examination.

For 8-OHdG serum level examination, blood samples were taken from cubital vein using plain tube. The next step, the tube was labelled with the patient's identity and identification number, moved to a tube polipropilen labelled, and centrifuged 2700 rpm for 10 minutes. The serum then was thinned 1:20 with sample diluents and at least 150 μ l of thinned samples were ready to be examined in duplicate using Assay Design's DNA Damage ELISA method. After being processed, the 8-OHdG value would be appear automatically on the screen of the machine. The process was done in Clinical Pathology Laboratory of Sanglah General Hospital, Denpasar.

All statistical analysis was done using 16th version of SPSS for windows and data normality test using Shapiro-Wilk Test. The characteristic comparison of women with normal pregnancy and blighted ovum was examined using t-independent test. The difference of the average level of 8 Hidroxy-2-Deoxyguanocyne was examined with t-independent test.

RESULT

In this study, there were 82 women who fulfill the inclusion and exclusion criteria, consisted of 31 women with blighted ovum and 51 women with normal pregnancy. According to the analytic results, there were no differences between samples in the group with maternal age, parity, and gestational age variables ($p > 0.05$). The average of 8-OHdG serum level in blighted ovum was 0.177ng/ml (SD 0.06) whilst in normal pregnancy was 0.111ng/ml (SD 0.01). Importance analysis using t-independent test showed that 8-OHdG serum levels in the two group was significantly different ($p < 0.05$).

Table 1. The Average of Maternal Age, Gestational Age, and Parity in Blighted Ovum Group and Normal Pregnancy Group.

Characteristic	Blighted ovum n=31	Normal pregnancy n=51	p
Maternal Age (years old)	30.42 (SD 5.36)	30.06 (SD 6.36)	0.793
Parity	1.58 (SD 0.80)	1.31 (SD 1.09)	0.240
Gestational Age (weeks)	8.45 (SD 1.06)	8.63 (SD 1.99)	0.651

Table 2. The Difference of The Average 8-OHdG Serum Level in Blighted Ovum Group and Normal Pregnancy Group.

Group	8-OHdG Serum Level (ng/ml)		p
	Average	SD	
Blighted ovum	0.177	0.06	0.001
Normal Pregnancy	0.111	0.02	

DISCUSSION

From analytical study results, there were no significant differences between maternal age, parity and gestational age characteristic, showing that those variables were homogen, and have insignificant influences.

This study found the average of 8-OHdG serum level in blighted ovum was 0.177 (SD 0.06) ng/ml, higher than in normal pregnancy group which was 0.111 (SD 0.02) ng/ml. There was a statistically significant difference in the two groups ($p < 0.05$). The cut off point used for the 8-OHdG serum level according to the ROC curve was 0.138 ng/ml with sensitivity value of 96.1% and specificity value of 80.6%. From those results, the average of 8-OHdG serum level in early pregnancy was higher than in normal pregnancy. It is caused by the imbalance between the antioxydant and oxydant level, resulting in the inability of the antioxydant to neutralize the harmful oxydant. Thus, the cell damage happens and terminate the early pregnancy. Most of blighted ovum is caused by chromosome anomaly.

In a physiological pregnancy, embryo is protected of ROS by means of the development balance and prevention of the harmful oxydative stress. The oxydative stress causes anomaly and retardation in embryo development correlated with DNA cell membrane damage and apoptosis. Apoptosis results in fragmented embryo, caused limitation in the implantation capability, which finally ended in the low rate of successful fertilization.³

Oxydative stress can trigger some of pathological effects in reproductive functions. Reactive oxygen species can be found in the peritoneal fluid of a patient with endometriosis, idiopathic infertility, and those who went Fallopian tube ligation. However, ROS level in patients with endometriosis and control group was not significantly different, so that with those who suffering idiopathic infertility and in control group. Thus, ROS may have an important role in patients with idiopathic infertility.

Some studies described that low concentration ROS found in follicle fluid, can be used as a potential marker to predict the success rate in in vitro fertilization (IVF) patients. There was a higher tendency of blastocyst development in a low ROS concentration in hydrosalping fluid. Thus, low level of ROS can be a marker of normal secretoric of the Fallopian tube. Concisely, the physiologically low level of ROS has an important role in normal reproductive function, whilst high level will cause pathologic condition that will affect the human fertility. The treatment strategies have to be aimed to decrease the ROS level and maintain its low level, to keep the normal cell function.⁴

Embryo can survive and grow in a low oxygenation environment, especially in the implantation stage. Physiologically, in an anaerob environment, cells of the human body produce 1-5% of free radicals. In two third of the abortion cases, there are anatomical evidence of placentation defect with a thinner or fragmented trophoblastic layer characteristic, decreased trophoblastic endometrium invasion and blocked of imperfect spiral arteries end, correlated with the unchanged spiral arteries and eventually cause premature onset of maternal circulation on the whole placenta.⁴

Because of it physiological and pathological functions, human body create a mechanism to maintain its concentration within some levels. Female reproductive system has a plenty enzymatic antioxydant to prevent and maintain the balance so that ROS will not be able to cause cellular molecule damage.⁸

CONCLUSION

There was a significant differences between 8 Hydroxy-2-Deoxyguanocyne (8-OHdG) serum level in women with blighted ovum and normal pregnancy ($p < 0,05$). The cut off point of 8-OHdG serum level used in this study was 0.138 ng/ml with sensitivity value of 96.1% and specificity value of 80.6%. Ad-

vanced studies based on this study results were still needed to find the screening method for early detection of the possibility of a pregnancy to develop blighted ovum and consideration of antioxidant supplementation.

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