

The Association between Oxidative Stress and Miscarriages among Omani Females Attending Sultan Qaboos University Hospital

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Abstract

Objectives: To observe the influence of oxidative stress on spontaneous abortion in Omani women attending Sultan Qaboos University Hospital (SQUH) during the first and second trimesters of pregnancy.

Materials and Methods: This is a case-control study conducted among 103 pregnant women of whom 25 (24.3%) were normal pregnant women, 25 (24.3%) were with history of abortion and 53 (51.45%) with history of spontaneous miscarriages. Reagents from Ransel (Randox Laboratories, Crumlin, UK) were prepared for the assay of glutathione peroxidase (GPx). GPx absorbance within 2 minutes was measured at 340 nm using thermostatically controlled UV-visible Spectrophotometer and the activity was calculated in U/l. The software IBM SPSS Statistics Data Editor Version 19.0.0 was used for data analysis.

Results: One hundred and three women showed decreased GPx absorbance within 2 minutes of recording. However, a decline was more significant in women with spontaneous abortion. Glutathione peroxidase activity was calculated and the results showed 1623.8 U/l in spontaneous abortion subjects compared respectively to 1396.6 U/l and 1545.4 U/l in normal pregnant women and with history of abortions. ANOVA test gave a p-value of 0.171 with a confidence of 82.9%.

Conclusion: This study showed that there is no direct association between oxidative stress and spontaneous abortions due to possible contribution of other factors such as autoimmune diseases or other inflammatory processes that might result in miscarriages. On the other hand, the results may explain how increased oxidants suppress glutathione peroxidase activity; hence more cellular damages.

Keywords: Pregnant; Oxidative stress; Miscarriage; SQUH; Oman

Introduction

Spontaneous abortion and the associated factors are considered to be crucial concerns for the researchers and physicians to study. It is stated as the spontaneous loss of a fetus before the 20th week of pregnancy. A systematic review showed that 30-50% of conceptions end in spontaneous abortion which affect 0.5-3% of women in the reproductive age group [1]. Studies have shown that spontaneous abortions can occur as a consequence of genetic factors, infections, diabetes, hyperprolactinemia, thyroid autoimmunity and oxidative stress [2].

Reactive oxygen species (ROS) is a collective term for oxygen free radicals (i.e., nitric oxide, superoxide) and non-radical oxygen (i.e., hydrogen peroxide, singlet oxygen) compounds which are produced in low concentrations in the body to maintain the normal cellular homeostasis. Previous studies showed that they are essential for embryological development as well as various physiological functions in the female reproductive system such as oocyte maturation, corpus luteum function and leuteolysis [3-5].

Nitric oxide (NO.) is a highly reactive free radical which is essential for numerous physiological mechanisms including digestion, blood

circulation and immune system improvement. Fallopian tube relaxation and contraction is regulated by NO. In addition, it is cardinal for the maintenance of blood flow to the reproductive organs, particularly the uterus by vasodilatation [6,7]. Moreover, mechanisms such as oocyte maturation, ovulation, implantation and formation of blastocyst, are achieved by NO [8].

Antioxidants are biochemical compounds found in diet, exogenous or synthesized by the human body. They are known to have a crucial role in balancing reactive oxygen species for preventing the body from any damage as a consequence of oxidative stress.

Glutathione peroxidase (GPx), a selenium-dependent antioxidant present in low concentrations in the plasma, secreted in response to increase in the levels of oxidants. The level of cellular GPx is regulated via selenium which is known to have a role in the transcription as well as translation of glutathione mRNA and protein respectively [9]. One of the main reactions in which GPx participate in, is reducing hydrogen peroxide to water and alcohol. The other way of reducing is non-enzymatically via direct oxidation of glutathione (GSH) [10,11].

Oxidative stress is defined as a state of imbalance between the production of reactive oxygen species (ROS) and the defence capacity of anti-oxidant systems to readily detoxify them which is known to contribute in causing various types of damages to the body [12].

It has been reported that oxidative stress plays a significant role in pregnancy related crisis including preeclampsia, birth defects, hydatidiform mole, idiopathic recurrent pregnancy loss and spontaneous abortions. Elevated levels of nitric oxide are shown to have an effect on decreasing GPx activity. Earlier study carried out on women with preterm delivery has indicated that increased concentration of NO. in their blood samples immediately after labour tend to suppress GPx activity leading to a variety of systemic damages in neonates' early life [13].

The rationale behind this study was to study the influence of oxidative stress on spontaneous and recurrent miscarriages in Omani females attending SQUH during the first and second trimesters. The main objectives were to observe the activity of GPx and correlate it with oxidant levels in normal pregnancy in comparison to the other categories: history of abortion and spontaneous miscarriages.

Method

Study design

This is a case- control study.

Setting and participants

The study was performed in consensus with Sultan Qaboos University. Ethical approval (MREC #494) was granted by the institutional ethics committee. Informed consent was sought from all participants before initiating this study. The control group was represented by females with normal pregnancy while the comparison subjects were women with history of miscarriage as well as spontaneous miscarriages between the age of 19-40 years. Blood samples were collected in the first and second trimesters, centrifuged at 4°C, plasma was separated, and haemolysates were prepared from packed cells and stored at -80°C until needed.

Reagents

Reagents were prepared from Ransel kit. 10 mL of reagent 1b (R1b) and phosphate buffer were added to R1a which contained glutathione, glutathione reductase and NADPH which can be stored for 48 hours. R2; Cumene hydroperoxide was prepared by mixing 10 µL with 2000 µL of saline. The diluting agent, R3 contents which are viable for four weeks from preparation were reconstituted with 200 ml of saline. A Ransel control which had been assayed once a day was recommended to be used for quality control.

Dilution of samples

Radox Ransel control, reagent blank was prepared by pipetting 20 µl of distilled water and 1000 µl of R1a and 40 µl of Cumene hydroperoxide in cuvette. Samples were diluted via mixing 25 ul of the haemolysate with 750 mL of diluting agent. Then 1000 µl of reagent 1 as well as 40 µl of reagent 2 were added to 20 µl of the diluted sample.

Assay of erythrocyte glutathione peroxidase (GPx)

Measurement of GPx was performed using the reagents prepared from Ransel (Radox Laboratories, Crumlin, UK). This method relies on the conversion of glutathione (GSH) to its oxidized form glutathione disulphide (GSSG) using cumene hydroperoxide (ROOH). Glutathione reductase along with NADPH is essential in reverting

GSSG to the reduced form (GSH). Initial GPx absorbance of samples and reagent blank were read at 340 nm using thermostatically controlled UV-visible Spectrophotometer (Shimadzu UV-1601PC, Shimadzu, Japan). Timer was started simultaneously to record the absorbance after 1 and 2 minutes. Difference in the absorbance (ΔA) was calculated by subtracting the absorbance after 2 minutes from the initial.

Data analysis

The software IBM SPSS statistics Data Editor Version 19.0.0 was used for data analysis. Glutathione peroxidase activity for each sample was calculated using the formula:

$$U/l \text{ of haemolysate} = 8412 \times \Delta A \text{ 340 nm/minute}$$

Results were compared between the three categories involved in the study and also compared to the reference range (4171-10881 U/l) which was measured in the population. Statistical difference between more than two groups was calculated by analysis of variance (ANOVA). Probability (P) values were two-tailed and $P < 0.05$ was considered to be significant.

Results

Glutathione peroxidase absorbance

A total of 103 participants were included in the study. The mean change in initial GPx absorbance as well as after 1 and 2 minutes was recorded using thermostatically controlled UV-visible Spectrophotometer. Table 1 shows that the decline in GPx absorbance is more rapid in females with history of abortions and spontaneous miscarriage in comparison to normal pregnant women.

	Initial absorbance	abs after 1 minute	abs after 2 minutes
Normal pregnancy	1.1406	0.9738	0.8085
History of abortion	1.1331	0.9278	0.7317
Spontaneous abortion	0.892226	0.693628	0.510849

Table 1: Glutathione peroxidase absorbance at 340 nm per minute.

Glutathione peroxidase activity

Glutathione peroxidase activity in normal pregnant women was calculated in units/liter and was shown to be 1396.6 U/l with two extremities in comparison to a higher activity, 1545.4 U/l and 1623.8 U/l of GPx in females with history of abortions and spontaneous abortions respectively. However, there were no significant differences due to the large range of results within one group (Figure 1).

One way ANOVA test

Using IBM SPSS version 19.0.0, one way ANOVA test was done with the test hypothesis that the GPx activity in all three categories of samples are equal. The test gave a p-value of 0.171 (> 0.05), which indicates that the confidence level is equal to 82.9%. Hence, there was no significant difference in the means between the groups.

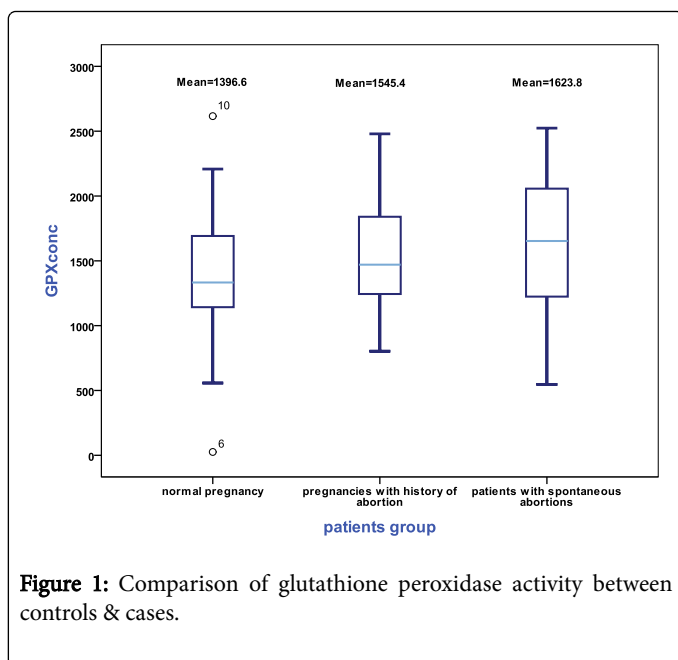


Figure 1: Comparison of glutathione peroxidase activity between controls & cases.

Discussion

The aim of this project is to study the influence of oxidative stress on spontaneous miscarriages in Omani females during the first and second trimesters. Glutathione peroxidase absorbance as well as activity in the blood was used as evidences for its role during normal pregnancy compared to pregnancies with abortions. Normally, GPx activity increases in response to slightly elevated oxidant levels to maintain the balance and prevent cellular damages.

All subjects involved in this study were pregnant; hence they were all experiencing an inflammatory situation which is a predisposing factor for temporary elevated oxidants in the blood. John R Challis et al. Stated that in normal pregnant women, there is a regulated activity of humoral versus cell-mediated immunity which enhances the release of nitric oxide as well as other reactive oxygen species to achieve their role in cellular destruction [13]. In females with altered immunity, there is hyper- excitability of inflammatory cells which results in enhancing NO. release, and hence GPx activity is to maintain the balance required. This explains the reason behind elevated mean GPx absorbance in females with history of abortions and spontaneous abortions more than the normal pregnant subjects.

In addition, it is noticed that spontaneous abortion cases have a rapid decline in GPx absorbance compared to the normal pregnant females. This indicates that those patients have high oxidative status which requires more utilization of GPx for maintenance of homeostasis.

Study observations explain the fact that although oxidative stress causes transient increase in antioxidant levels, the activity starts to diminish gradually with progression of oxidative stress. A study done by Abiaka and Machado showed that pregnant women who had high nitric oxide levels in the blood had much lower GPx activity than the control group with lower NO [14].

According to one way ANOVA test, there was no significant difference in the means of GPx activity among the categories. This might explain that all the subjects involved were pregnant which

indicates that they are all having an inflammatory process with different levels of oxidants released.

In addition, glutathione peroxidase activity depends on the duration of pregnancy. Previous studies have shown that there is a significant decline in GPx activity as pregnancy advanced [15,16] which prove the wide range of GPx activity in the normal pregnant participants; hence, there is high mean GPx activity. Also, other conditions such as heart disease, atherosclerosis and cancer that can predispose to oxidative stress were not considered one of the selection criteria. Thus, elevated GPx levels might not be associated with abortions, or probably exaggerated via the causes mentioned.

Conclusion

This study was successful in demonstrating the relationship between oxidative stress and antioxidant activity. Ransel kits were used to record glutathione peroxidase absorbance in the blood within two minutes to observe its utilization; hence, assuring that regulation and maintenance of balance is occurring in the body. Literatures were reviewed for further evidences that oxidative stress is considered to be a common factor predisposing to spontaneous abortions.

Limitations of the Study

The study has encountered certain difficulties that could have influenced the interpretation of the data. The small sample size could have affected our results; increasing the chance of random errors. The study cannot be generalized because it involved subjects from SQUH only. In addition, unavailability of machines which measure oxidants directly restricted our ability to ascertain from the role of oxidants in inducing abortions accurately.

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