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ANTI-OXIDATIVE PROFILE AND THEIR PREDECTIVE POTENTIAL IN PATIENTS WITH RETROGRADE ENDOMETRIOSIS

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ABSTRACT

Endometriosis is characterised by the presence of endometrial stroma and glands outside of the uterus lining and is thought to affect 10-15% women within the reproductive age often leads to infertility and results in reduced quality of life. Among various theories regarding the pathogenesis of endometriosis oxidative stress is one that may be associated with endometriosis etiology. Oxidative stress is detected when an imbalance between oxidants and prooxidants occurs which further leads to inflammatory response in peritoneal cavity and may have an indispensable role in the pathogenesis of endometriosis. Antioxidant system comprises of different components such as super oxide dismutase, catalase and glutathione peroxidase which has the potential to remove harmful free radicals and inhibit cell's structural damage. Non-enzymatic antioxidants such as vitamin C and vitamin E play an important role to influence the complex antioxidant defence system of the body. Imbalance between the production of ROS and prooxidants leads to rise in ROS level and further results in oxidative stress that is implicated as key player in endometriosis pathogenesis. Current study analysed the levels of different oxidative stress biomarkers to quantify the severity and development of endometriosis as well as establish diagnostic biomarker for endometriosis.

Keywords: Reactive oxygen species (ROS), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and vitamin E (vit E)

INTRODUCTION

Endometriosis is a debilitating chronic illness and is one of the most common benign problems experience in gynaecology **[1]**. It is characterised by the presence of endometrial stroma and glands outside of the uterus lining **[2]**. It is estimated that endometriosis affect 10-15% women within the reproductive age often leads to infertility and results in reduced quality of life **[3]**. Our understanding regarding diagnosis and management of endometriosis still remain controversy. There are several theories to identify the pathophysiology of endometriosis however no one could successfully explain endometriosis up to now. Among the various theories regarding the pathophysiology of endometriosis oxidative stress is one that may be associated with endometriosis etiology as well as have generated immense research interest. Oxidative stress is detected when an imbalance between oxidants and prooxidants occurs which further leads to inflammatory response in peritoneal cavity and may have an indispensable role in the pathogenesis of endometriosis **[4]**. Oxidative stress induced by reactive oxygen species (ROS) may stimulate endometrial cell growth and adhesion in the peritoneal cavity to promote endometriosis development. Hence for the management of endometriosis control of oxidative stress can play very vital role **[5]**.

According to retrograde menstruation theory inflammation in peritoneal cavity is induced by refluxed blood components such as dead endometrial tissue, lysed erythrocytes, desquamated menstrual cells and released iron which in turn stimulate the ROS release via activating macrophages as well as also increase ROS production through respiratory burst **[6]**. Increased serum levels IL-6 and TNF-α have been observed in women with endometriosis that are indicator of increased inflammation as well as increased release of IL-6 and TNF-α play an important role in the production of $O_2^{\cdot}$ and H_2O_2 by activating intracellular signalling molecules through NADPH oxidase system **[7, 8]**. SOD enzyme is responsible to for the conversion of superoxide anion to peroxide. The levels of $O_2^{\cdot}$ and H_2O_2 are increased by inflammatory response in peritoneal cavity of women with endometriosis **[9]**. ROS increased levels were also recorded in endometrial granulosa cells of women suffering from endometriosis **[10]**. Pathological rise in ROS concentration cause oxidative stress **[11]**. Previous studies suggest that increased formation of free radicals can cause damage to fertilized eggs

and supress the development of embryo hence during fertilization decrease quality of embryo has been found in women with endometriosis **[12]**. Living organisms have established several mechanisms to protect themselves from reactive oxygen species but in oxidative stress condition there will be the increased production of free radicals or ROS that may have serious consequences. Generation of free radicals can start a chain of reactions that continue until they are removed by some other free radicals most importantly by antioxidants. Antioxidant system comprises of different components such as super oxide dismutase, catalase and glutathione peroxidase which has the potential to remove harm full free radicals and inhibit cell's structural damage. Glutathione peroxidase and superoxide dismutase inhibit the adverse effect of oxidative stress by stimulating their break down as well as by inhibiting the formation of highly toxic hydroxyl radicals. In addition to that superoxide dismutase promote the breakdown of superoxide anion and glutathione peroxidase are responsible for the removal of hydrogen peroxide, peroxide of nucleic acids and proteins and peroxides of lipids to inhibit lipid peroxidation **[13, 14]**. Thus ROS inhibition or inactivation is necessary to maintain less amount of ROS required to retain normal function of cell. To prevent cell damage enzymatic and non-enzymatic antioxidant defence system inactivate or scavenge free radicals. Non-enzymatic antioxidants such as vitamin C and vitamin E play an important role to influence the complex antioxidant defence system of the body **[15]**. Additionally body produce several enzymatic antioxidants including superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase as well as molecule like glutathione that play an important role to scavenge the production of ROS and free radicals in biological system **[16]**. Imbalance between the production of ROS and prooxidants leads to rise in ROS level and further results in oxidative stress that is implicated as key player in endometriosis pathogenesis **[17]**.

Free radicals are thought to play an important role in the pathogenesis of endometriosis because women with endometriosis have intense retrograde mensuration into the peritoneal cavity, hemoglobin induced level of iron is also elevated which is a good source of free radical production in endometriosis **[14]**. In addition to that free radicals favour endometrial cell growth and adhesion in the peritoneal cavity and in the onset of

endometriosis, its associated symptoms, pain and infertility **[17]**. A number of oxidative stress biomarkers have been studied with conflicting findings in endometriosis. Current study was designed to measure the levels of different oxidative stress biomarkers in women with endometriosis and to find their association with the pathogenesis of endometriosis.

MATERIALS AND METHODS

The "Research and Ethics Committee" at Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan approved present study. A total of one hundred consecutive individuals were identified to be enrolled in current study. The subject group of study included fifty (50) women of reproductive age with endometriosis. These women were identified with the confirmed laboratory reports of ultra sound of stage IV endometriosis. Women were excluded if they were alcohol consumer, smokers and taking any type of hormonal therapy. The control group of the study consisted of fifty (50) women of reproductive age without endometriosis who had not any metabolic dysfunction, depression and malnutrition syndrome. Written consent was obtained from the study participants. Five ml (5ml) of venous blood was collected from all individuals of study. Samples were taken immediately to the laboratory for centrifugation. After centrifugation plasma and serum were stored at -70°C for further processing.

Biochemical assay

The concentration of Glutathione (GSH) was determined in the serum of women with endometriosis according to the method of Moron *et al*., 1979 **[18]**. Serum concentration of enzyme catalase was quantified by using the generalized method of Aebi, 1974 **[19]**. Determination of superoxide dismutase activity of study participants was performed by the procedure of Kakkar, (1972) **[20]**. GPx concentration was assessed by spectrophotometric method. Glutathione reductase was analysed by the method of David and Richard (1983) **[21]**. The level of Vitamin C is measured according to the method of Roe and Keuther (1943) **[22]** and the level of Vitamin E was assessed by the reaction of Emmerie-Engel and reported by Rosenberg (1992) **[23]**.

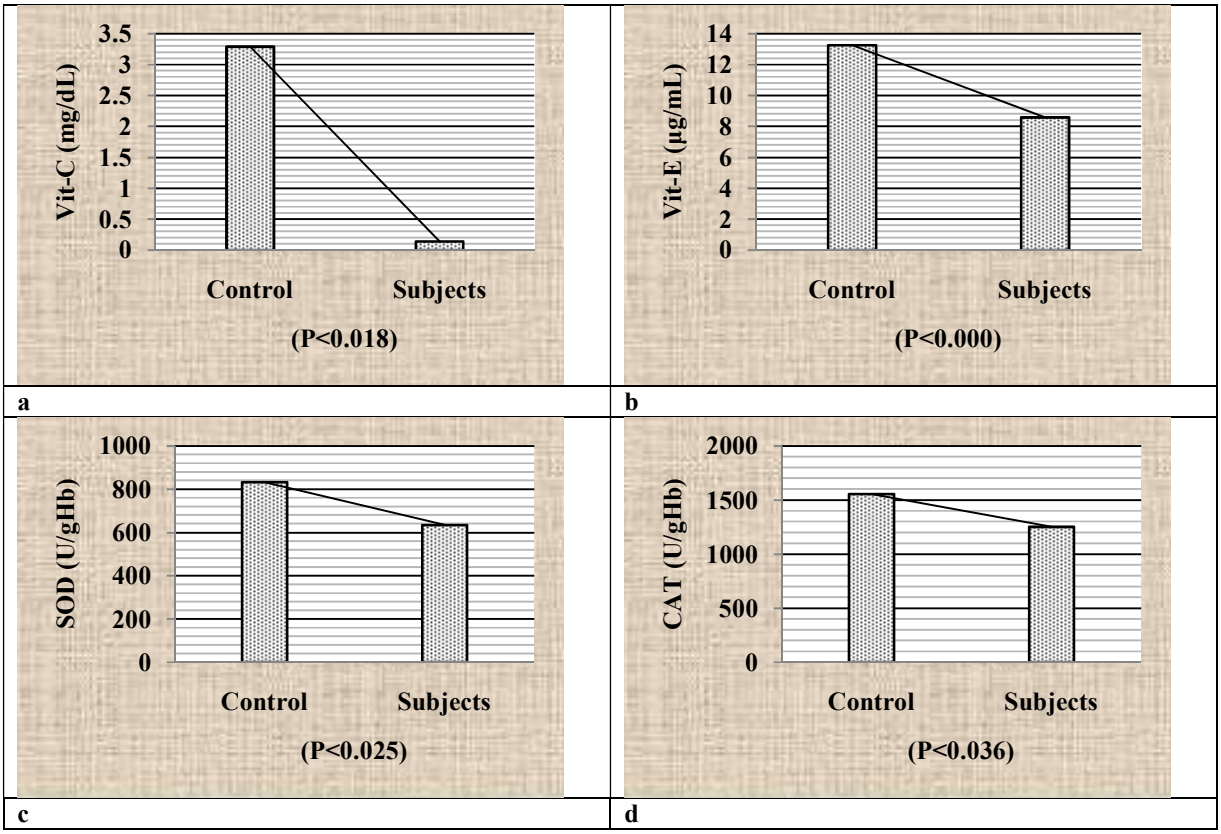
Statistical analysis

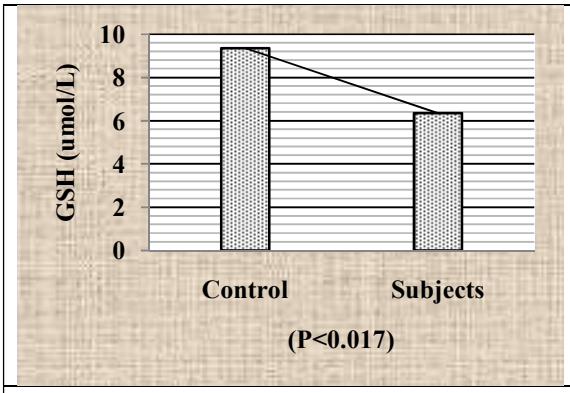
Statistical analysis was carried out by using SPSS version 17.0. The results of all variables were evaluated by independent sample t-test are significantly different ($p<0.05$) from controls. Pearson's correlation coefficient was used to investigate the

association between different variables performed. P-value ($p<0.05$) was estimated by using one way ANOVA.

RESULTS

In present study significant difference of antioxidant profile was observed between subject and controls. The serum levels of Vit-C and Vit-E were found to be 0.14±0.0.08 mg/dL and 8.59±2.19 mg/dL in endometriosis women and 3.29±0.75 mg/dL and 13.26±3.26 mg/dL in control group respectively. The SOD, CAT and GSH activities were significantly lower in women suffering from endometriosis as compared with women without endometriosis (635.26±17.59 Vs. 832.26±16.35 U/gHb; p=0.025), (1253.25±14.26 Vs. 1555.29±17.59 U/gHb; p=0.036) and (6.35±1.59 Vs. 9.356±3.29 umol/L; p=0.017). A slight increase was recorded in the level of GPx in females suffering from endometriosis (2.88±0.047 µmol/ml) as compared with females without endometriosis (1.59±0.026 µmol/ml). In endometriosis females the GRx levels were lower (3.99±1.08 µmol/ml) than those in controls (6.29±1.59µmol/ml).





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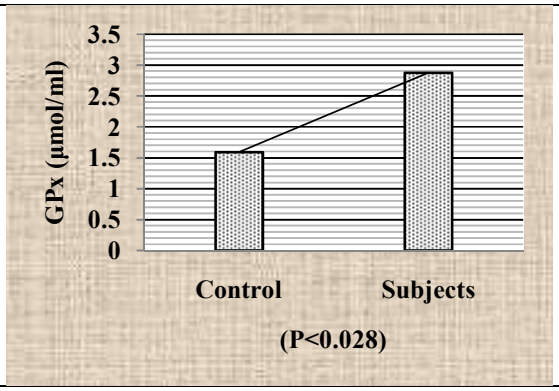

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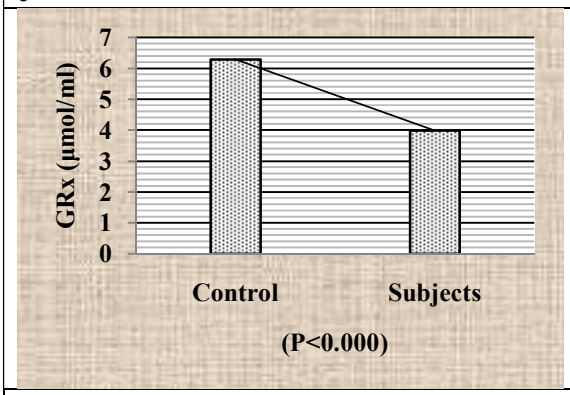

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DISCUSSION

Endometriosis is a chronic inflammatory disorder characterised by alterations in immune system. Several studies demonstrate that inflammation stimulate the production of oxidative stress in various components of peritoneal cavity, endometriotic lesions, peritoneal fluid as well as peritoneum of women suffering from endometriosis **[24, 25]**. Several immunological components such as macrophages, neutrophils and eosinophils trigger the production of ROS and leads to increased oxidative stress in peritoneal cavity. Substantial evidence suggest that due to inefficient immune system various immunological components of peritoneal fluid of women with endometriosis cause an increase in inflammatory mediators which further leads to free radical production **[26]**. In addition to that macrophages and iron overload in endometriosis induce the release of a transcription factor nuclear factor kappa B which also trigger an increase in inflammation associated with endometriosis **[27, 28, 29]**. Studies shows that free radical induced oxidative stress play very vital role in the pathogenesis of endometriosis thereby promoting endometriosis. Various studies have also suggest the significantly reduced

levels of several antioxidants such as SOD, catalase, GSH, GPx, GSR, vit C and vit E in female with endometriosis **[30]**. Oxidative stress and prooxidants are able to moderate stromal cell growth **[31]**. Antioxidant role is controversial in endometriosis mainly due to contradictory sights stated by different authors. Antioxidants system are known to scavenge free radicals and ROS and play an important role in protection of tissue from oxidative damage. A significant reduction in antioxidants potential have been observed in women suffering from endometriosis as compared to women without endometriosis **[14]**. Portz et al., in his study showed that antioxidants protect from endometriosis **[32]**.

With respect to non-enzymatic antioxidants the mean value of vitamin E was decreased significantly in women with endometriosis as vitamin E is considered a structural antioxidant of membrane. Hence as a result of vitamin E deficiency cell's inflammatory response is increased and results in impairment of cellular and humoral immunity **[33]**. Vitamin E is an important lipid soluble antioxidant and can prevent chronic diseases such as endometriosis. Vitamin C is a potent water soluble prooxidants and protect against oxidative stress induced diseases including endometriosis. Vitamin C and vitamin E might be involved in either alone or in combination disease prevention through clearing reactive oxygen species which have been associated with growth and development of endometriosis **[30]**. In current study the concentration of both vitamin C and vitamin E was decreased significantly in women with endometriosis as compared to normal healthy women without endometriosis. Current finding are similar with the findings of previous studies that deficiency of vitamin E and vitamin C may play a key role in disease progression. It has been demonstrated that several oxidative related proteins such as SOD and GPx show differed blood concentration associated with diagnosis of endometriosis. This is because of retrograde menstrual blood flow in to the abdomen and leads to accumulation of ruptured erythrocytes which in turn cause iron overload and results in increased oxidative stress **[34, 35]**. Pejic *et al*., found a decrease in SOD activity in women with endometriosis than in women without endometriosis **[36]**. Decrease of SOD activity might be the result of increase ROS production as shown by various studies. Kong et al., indicated that in response to increased ROS production SOD enzyme may be consumed in oxidative damage prevention since it was observed that increased

production of free radicals and ROS exhaust the capacity of SOD enzyme **[37]**. In current study the activity of SOD and GSH was decreased in women with endometriosis as compared to normal. These results are in accordance with other author's findings. Another intracellular antioxidant enzyme catalase shows a decreased activity in women with endometriosis, suggesting a decreased antioxidant capacity in women suffering from endometriosis. Similar observation was recorded by current study regarding the activity of catalase enzyme. Decrease activity of catalase was shown in women with endometriosis as compared to controls and shown that it is associated with progression of endometriosis. Further studies are required to reveal if alterations of enzymatic and non-enzymatic antioxidant defence system may contribute to the understanding molecular mechanism of endometriosis that may provide further data to develop therapeutic approach to manage endometriosis and improve patient's quality of life.

CONCLUSION

In conclusion current study states that oxidative stress is a phenomenon which is triggered by generation of free radicals which in turn are present in high level in female with endometriosis as well as oxidative stress is a key factor in the progression and development of endometriosis.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

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