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THE RELATIONSHIP OF OXIDATIVE STRESS AND INFLAMMATORY RESPONSE IN FEMALES ASSOCIATED WITH ENDOMETRIOSIS

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Abstract

Endometriosis is considered as a debilitating condition that is characterized by formation of endometrial tissue outside the uterus that consequently adversely impacts female fertility. Number of factors are involved that mediates the gynecological pathology of endometriosis, however the relationship of endometriosis with oxidative stress and inflammatory needs to investigate. In this study, we selected females of age 21-27 years associated with endometriosis and performed a detailed hematological profile. Our data indicated that the hematological profile of patients associated with endometriosis was highly disrupted compared to healthy control females. Number of hematological parameters including RBCs, WBCs, HB, platelets and overall bilirubin level was abnormal in endometritis patients. In addition, we also determined the level of oxidative stress by using the expression profile of different oxidant and antioxidant genes through spectrophotometric examination. We found elevated levels of MDA and reduced expressions of SOD, GSH, and CAT in patients associated with endometriosis relative to healthy patients that suggest the increased oxidative stress level. Furthermore, different vitamins including, Vit. A, Vit. C, Vit. E and Vit. D levels were also reduced in patients of endometriosis. Collectively, our data suggest that hematological profile and oxidative stress of patients associated with endometriosis was highly disruptive and these differential biomarkers may be useful to diagnose the endometriosis patients and also beneficial for developing treatment strategies.

Keywords: Endometriosis, Hematocrit, Hemoglobin, Oxidative stress, Platelets, RBCs, WBCs

INTRODUCTION

Endometriosis is an estrogen dependent gynecological disorder, characterized by the growth of endometrial tissues outside the uterus (1, 2). Growth may be in the form of lesions i.e. peritoneal lesions, cysts on ovary or superficial implants. Endometrium lesions are similar antigenically to eutopic endometrium (3). Presenting symptoms includes pelvic pain, infertility, inflammation and scarring (4-6). Inflammation is due to the endometrial growth on other areas. Cytokines are involved in inflammatory and angiogenesis, this inflammatory response is responsible for symptoms of infertility and pain. Previously, it was shown that approximately 10% of women affected at their reproductive aged (7). However, it was observed that in these affected women 70% of them experience dysmenorrhea during menstruation (8) In Asian women the endometriosis is experienced at the age of 30-40 years. Endometriosis is higher in numbers in Asian women as compared to African and American women (9, 10). However it was also noticed that in some cases endometriosis is asymptomatic. It was observed that endometriosis has a huge impact on physical and mental health of affected individuals that ultimately disrupts the fertility of female (11). Currently, the condition of endometriosis is growing and huge numbers of cases were observed.

Previous reports also indicated its high prevalence in least developed countries due to number of reasons such as the lack of specific test for identification, high cost and less reported cases in 3rd world



countries (12). There are theories which explain its pathophysiology however the exact etiology of endometriosis is still not clear. Theories include Sampson implantation theory, Mayer coelomic metaplasia and induction theory (13). Sampson theory states that for lesions production retrograde menstruation is required and its cells must implant for peritoneal growth of lesions. Coelomic metaplasia postulate that endometriosis origin start from metaplasia of mesothelial cells. While induction theory states that some unknown cells may shed endometrium from mesenchyme to start endometriosis tissue growth. But these theories failed to establish its pathophysiology. Recent work on endometriosis addressed other factors such as increased reactive oxygen species (ROS) and nitric oxide (NO) levels, immune induced inflammation, autoimmunity, low BMI (Body Mass Index) and decreased adipogenesis (14-16).

Oxidative stress is one of the major factors which play an important role in the genesis of endometriosis (17). Oxidative stress is basically imbalance between ROS/RNS and antioxidant levels. ROS damage the cell and heredity material (DNA) (18). In peritoneal cavity ROS cause inflammatory response. In response to stress conditions body developed a system of antioxidants such as catalase, superoxide dismutase (SOD), glutathione and vitamin E and vitamin C, which limit and inactivate ROS levels and they also repair cell damage (19). ROS and inflammatory mediators are produced through different metabolic pathways, which ultimately put deleterious impact on cell proliferation. Inducers of oxidative stress are macrophages and erythrocytes, which transplant into peritoneal cavity via retrograde menstruation and hence peritoneal cavity is involved in ROS production which may lead to endometriosis. How oxidative stress and inflammation impact the endometriosis and these factors have relationship with each other still need to be investigated.

We conducted current study to address these issues in a systematic way. In this study we found increased level of oxidative stress that was evidenced by elevated level of stress marker including: SOD, MDA, GSH, NO, and vitamins A, E, C and D compared to control patients. In addition, our data also indicated increased expressions of different markers related to inflammation. Collectively, our data suggest that oxidative stress and inflammation are highly accompanied in the patients associated with endometriosis and these markers provide differential key points for clinical diagnosis.

MATERIALS AND METHODS

SOURCE OF DATA

Females of age 21-27 were included in this study and have been screened at Jinnah hospital, Lahore. The study was performed by following the guidelines of ethical committee of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. 5.0 ml blood sample was taken from vein of patients. Blood was further processed for the identification of various oxidative markers, antioxidant and various heat shock proteins (HSPs). The samples were collected from the females suffering from endometriosis. Blood samples were centrifuged at 4000 rpm for 10 minutes and serum was separated and collected into EDTA tubes for further analysis.

BIOCHEMICAL ANALYSIS

For biochemical analysis of different biomarkers such as SOD, MDA, CAT, Vit-C, Vit-E and Vit-A have been examined by using spectrophotometric method in control and osteoporotic patients. The levels of SOD were measured by spectrophotometric method of Kakkar *et al.*, (1984) (22). The concentration of MDA was measured by spectrophotometric method of Okawa *et al.*, (1979) (23). CAT was measured by spectrophotometric method of Aebi, (1984) (24). The levels of reduced glutathione and glutathione peroxidase were measured by the methods of Moron *et al.* (1979) (25) and Aydin *et al.*, (2006). The concentration of GSH was estimated by using the method of Moron *et al.*, (1979) (25). Vitamin C and Vitamin E were estimated by the methods of Mohammad, (1991) and Kayden *et al.*, (1973) (26). On the other hand, nitric oxide concentration was typically measured by a well-known method such as colorimetric Griess assay. Vitamin D levels were estimated by using human ELISA kit.

STATISTICAL ANALYSIS

Statistical analysis was performed by SPSS (Statistical Package for the Social Sciences)



measurements 17.0. The after effects of all variables were assessed by utilizing independent sample t-test. Pearson's correlation coefficient was used to determine the correlations between different values of variables of osteoporotic patients.

RESULTS

HEMATOLOGICAL PROFILE OF PATIENTS ASSOCIATED WITH ENDOMETRIOSIS

To evaluate the effect of endometriosis on hematological profile, female of age 21-27 years were selected for this study. Blood sample from healthy control and patients associated with endometriosis were collected for examination of hematological profile by using centrifugation protocol. Our data revealed that hematological profile of patients associated with endometriosis is highly disrupted compared to the healthy control group. We found significantly ($P<0.05$) reduced level of different hematological parameters including, RBCs, HB, platelets, and HCT in patients suffering with endometriosis relative to the healthy individuals (Table I). In addition, elevated level of WBCs and bilirubin was noted in patients compared to the health group (Table. 1). Collectively, our data suggest that endometriosis put adverse impact on hematological profile of female that may be the differential biomarker to diagnose the patients.

Table I. Hematological profile of patients associated with endometriosis

Variables	Control (n= 100)	Patients (n=100)	P-Value
RBCs (million/mm ³)	4.91±1.09	4.4±0.956	0.01256
WBCs (million/mm ³)	8.22±3.056	10.23±2.05	0.0154
Hb g/dl	14.25±4.19	10.25±3.09	0.0196
PLTs 10 ⁹ /L	311.25±24.29	165±15.029	0.01325
Hct %	40.23±9.65	35±14.25	0.01254

RBCs=Red Blood Cells, WBCs=White Blood Cells, Hb=Hemoglobin, PLTs=Platelets, Hct=Hematocrit

RELATIONSHIP BETWEEN ENDOMETRIOSIS AND OXIDATIVE STRESS BIOMARKERS

To determine the relationship of endometriosis with oxidative stress index, sample was collected from patients associated with endometriosis and expressions of different antioxidant genes were examined by using spectrophotometric analysis. We found increased expressions of MDA (an oxidant gene that predict the stress level in cell and tissues) in sample retrieved from patients associated with endometriosis compared to the healthy group as a control (Table II). Besides, our data revealed that the expression level of different antioxidant including, SOD, GSH, and CAT was highly reduced in patients relative to the healthy group (Table II). Furthermore, different vitamins such as vitamin A, vitamin C, vitamin E, and vitamin D were also reduced in endometriosis patients that indicated increased oxidative stress level. Thus, our data suggest that endometriosis may cause increase in level of oxidative stress that consequently lead to poor prognosis.

Table II. Oxidative stress biomarker in patients associated with endometriosis

Variables	Control (n= 100)	Patients (n=100)	P-Value
MDA (nmol/ml)	1.02±0.956	4.25±1.08	0.001452
SOD (U/gHb)	0.236±.059	0.015±0.00156	0.02356
GSH (μmol/L)	9.23±3.29	2.36±0.568	0.03256
CAT (U/gHb)	3.76±0.956	1.33±0.658	0.01452
VIT A (mg/dL)	588.66±24.25	477.09±52.26	0.0145
VIT C (mg/dL)	0.526±.056	0.325±.015	0.02356
VIT E (mg/dL)	0.235±.009	0.223±.0018	0.8452
VIT D (mg/dL)	13.26±2.25	9.8±1.29	0.0623
NO (μmol/L)	20.56±3065	67.58±8.29	0.03256
GSH-Px (μmol/ml)	9.65±1.29	6.8±1.08	0.02356
GSH-Pr (μmol/ml)	2.33±0.59	2.54±0.956	0.03256

MDA=Malonyldialdehyde, SOD=Super Oxide Dismutase, GSH=Reduced Glutathione, CAT=Catalase, NO=Nitric Oxide, GSH-px=Glutathione Peroxidase, GSH-pr=Glutathione

DISCUSSION

Previous studies have shown that multiple factors are involved for developing endometriosis and consequently disrupts the female fertility (5). As we know that endometriosis is highly accompanied with



outgrowth of endometrial tissue outside the uterus that further cause abnormal changes in ovarian structure which ultimately leads to reproductive dysfunction (27, 28). Induction of endometriosis inside female reproductive tract mediates number of stress factors that put negative impacts on pregnancy outcomes. However, identification of these stress factors and their numerical values determination will definitely provide diagnostic approach for patients associated with endometriosis. In this study, we determined the correlation of endometriosis with patients hematological profile and direct relationship with oxidative stress and we found that the hematological profile of patients associated with endometriosis is highly disrupted compared to the control group (29). In addition, endometriosis associated patients have shown highly defective oxidative homeostasis that further impact the reproductive spectrum.

Our findings indicate that hematological profile specifically the RBCs level, HB, and platelets index of endometriosis patients was significantly abnormal relative to the healthy females. Previous studies have also shown that blood profile of endometriosis patients is variable. It was also determined that blood profile has direct relationship with stress factors and our data also observed increased oxidative stress index as antioxidant markers including, SOD, CAT, GSH, and different vitamins working as antioxidant expression are highly reduced in patients associated with endometriosis. In addition, increased expressions of oxidative marker MDA were observed in endometriosis patients. Previously, it was shown that endometriosis patients had elevated level of reactive oxygen species in the form of increased serum MDA level that induced oxidative stress (30). Another study also demonstrated the increased serum MDA level in endometriosis patients relative to the healthy individuals (31). However, different studies have also reported that higher level of MDA was observed in patients associated with endometriosis but no significant difference was observed (32). Our data also revealed that number of antioxidant including SOD, GSH, and CAT has shown reduced expressions in patients relative to the healthy individuals. Increased expression of such type of antioxidant reduced the potential risk factor of miscarriage, increase the embryos developmental competency and also enhance the chances of pregnancy. Collectively, these findings support our notations regarding the involvement of different stress factors in patients associated with endometriosis.

Different types of vitamins are used that provide beneficial effect because these are used as an antioxidant (33). Our results indicated that the level of different vitamins including vitamin A, C, E, and D level was significantly reduced in endometriosis patients (34). Previous reports have shown the involvement of vitamins in reducing oxidative stress that consequently impact the overall female fertility. The lower concentrations of vitamin C in plasma indicated excess production of free radical that ultimately produced ROS (35). Previous studies also have revealed administration of different vitamins can reduce the level of oxidative stress and play a role in oxidative homeostasis. These findings suggest that the vitamins contents are reduced in patients associated with endometriosis relative to healthy females (36).

CONCLUSION

In conclusion, our data suggest that hematological profile of patients associated with endometriosis is highly disturbed especially the level of WBCs and platelets relative to the healthy females. In addition, increased level of oxidative stress was noted as higher expressions of MDS were observed in patient's samples. Furthermore, reduced expressions of antioxidant genes and different types of vitamins acting as an antioxidant was also examined in patient's samples associated with endometriosis. These findings are useful for the diagnostic approach and for developing treatment strategies for endometriosis patients.

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