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ROLE OF HYDROGEN PEROXIDE PRODUCTS AND THEIR IMMINENT ROLE IN PATIENTS WITH DEGENERATING ENDOMETRIOSIS

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ABSTRACT

Endometriosis is an inflammatory condition characterised by existence of endometrial tissue in extra uterine locations, being aggressive and invasive can cause a significant decrease in quality of life. Various scientific evidence suggests that peritoneal fluid of women suffering from endometriosis has elevated concentration of proinflammatory cytokines, reactive oxygen species as well as rich in low density lipoprotein which are able to upregulate the formation of oxidised lipid components in an inflammatory milieu along with increased number of active macrophages. Consequently formation of inflammatory response together with the existence of local oxidative environment possibly play an active role in the pathogenesis of endometriosis. It has been reported that lipidperoxidation end products are increased in serum of women with endometriosis. Current study was aimed to identify the levels of lipidperoxidation biomarkers in women diagnosed with advance stage of endometriosis in order to determine the status of lipidperoxidation as well as lipidperoxidation end product induced DNA damage in women with endometriosis.

Keywords: 8-hydroxy-2'-Deoxyguanosine (8-OHdG), Malondialdehyde (MDA), 4-Hydroxynonenal (4-HNE), Isoprostanes, reactive oxygen species (ROS)

INTRODUCTION

Endometriosis is an inflammatory condition characterised by existence of endometrial tissue in extra uterine locations and remains one of the main cause of pelvic pain and infertility in women of reproductive age, being aggressive and invasive can cause a significant decrease in quality of life **[1]**. An estimated prevalence of endometriosis is 10-15%. Pathogenesis of endometriosis is poorly ununderstood, it has been postulated that the etiology of endometriosis is associated with inflammation as increased concentration of different inflammatory mediators such as activated microphages, different cytokines as well as B cells and T cells in the pelvic cavity. It is still unclear to what extent peritoneal environment stimulate the development and progression of endometriosis **[2]**. Various scientific evidence suggests that peritoneal fluid of women suffering from endometriosis has elevated concentration of interleukin-1, tumor necrosis factor alpha, reactive oxygen species as well as rich in low density lipoprotein which are able to upregulate the formation of oxidised lipid components in an inflammatory milieu along with increased number of active macrophages. Consequently formation of inflammatory response together with the existence of local oxidative environment possibly play an active role in the pathogenesis of endometriosis **[3]**. Reactive oxygen species and free radicals aggravate endometriosis progression through inducing various chemoattractant and growth promoting activity of endometrial cells **[4]**. Mechanism for the formation of oxidative stress process comprises three different stages, in first stage rise in ROS production occurs, in second phase there will be the mobilization of antioxidant and in last phase oxidative damage to major biomolecules occurs **[5, 6]**. There are several markers for the evaluation of distinct stages of oxidative stress. Hydroperoxides can be used as a marker of first stage of oxidative stress and for second phase of oxidative phase can be estimated by measuring the levels of various antioxidants which are important to protect cells against oxidative stress as well as inhibit the initiation of lipidperoxidation. Third stage of oxidative stress can be assessed by evaluating the levels of different lipidperoxidation biomarkers including MDA, 4-Hydroxynonenal (4-HNE) and isoprostane, advanced oxidation protein products (AOPP) for protein oxidation and 8-OHdG (8-hydroxy-2'-Deoxyguanosine) as a marker of DNA damage **[7]**. Due to the existence of double bond in their molecular

structure lipids are effected by oxidative damage mostly hence they are important indicators of free radicals and lipidperoxidation. The presence of these markers in endometriosis confirms the hypothesis that lipidperoxidation play an important role in the initiation and progression of endometriosis **[8]**. Lipidperoxidation primarily occurs by ROS induced peroxidation of unsaturated fatty acid, though membrane cholesterol and saturated fatty acids can also undergo peroxidation **[9]**. The reaction products of peroxidised lipid radicals are highly stable and leads to the formation of peroxyl radicals through interaction with oxygen. This peroxyl radical stimulate a chain reaction as it takes hydrogen from other fatty acids and results in the formation of new radical and a lipid peroxide and continue in a sequence process named as progression **[10]**.

Lipid metabolism and its association with inflammatory mediators may play a critical role in the formation of oxidative stress. The levels of lipid have been analysed in endometriosis patients which demonstrate that women with endometriosis resulting in increased levels of low density lipoprotein, triglyceride and total cholesterol whereas the level of high density lipoprotein has been decreased in women with endometriosis **[11]**. Murphy and his colleagues proposed that pelvic endometriosis triggers the activation of macrophages, these activated macrophages might trigger oxidative stress in peritoneal cavity which in turn leads to formation of peroxides of lipids and their degradation products as well as form various other products by interacting with low density proteins and some other proteins **[12]**. Peroxidised lipids characterised by lipid-peroxidation when undergoes decomposition may results in the formation of malondialdehyde, may considered as foreign substance and stimulating an antigen response following antibodies production. This could leads to the oxidative damage to endometrial cells peritoneal cells and red blood cells which further upregulate the activation and recruitment of mononuclear phagocytes disseminating oxidative damage to pelvic cavity **[13]**. One of the end product of lipidperoxidation is MDA, because of its stability it can be used as accumulative marker to measure lipidperoxidation **[14]**. A number of studies have demonstrated that oxidative stress induced increased production of ROS and free radicles are able to cause oxidative damage of biomolecules including proteins, lipids and nucleic acids **[15]**. Evidence of reactive oxygen species and free radicals has been perceived in the peritoneal

cavity and endometrial tissue of women with endometriosis. Peritoneal fluid of endometriosis women comprises elevated concentration of lipidperoxidation products containing malondialdehyde (MDA), (4-HNE), isoprostane and 8-hydroxy-2-deoxyguanosine (8-OHdG) **[2, 16]**. The objective of current study was to evaluate the concentration of reliable lipidperoxidation markers including malondialdehyde (MDA), (4-HNE), isoprostane and 8-hydroxy-2-deoxyguanosine (8-OHdG) in women with endometriosis.

MATERIALS AND METHODS

The material used in current study comprised one hundred blood samples in total were taken after obtaining informed consent at Jinnah Hospital Lahore, Pakistan. Current study comprised two group. Fifty female patients of endometriosis who were clinically diagnosed stage IV endometriosis were selected for participation in subject group. Additionally fifty healthy women of reproductive age who were non-smoker, having no history of cancer, diabetes, hypertension and depression were assigned as control in current study. Women who were on antioxidant, immunosuppressive or anti-inflammatory and hormonal treatment were excluded. The protocols followed in current study was ethically approved by the "Research and Ethics Committee" at Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan. Five ml blood samples were obtained from 100 participants. Samples were centrifuged at 4000 rpm for 15 minutes, serum was aliquoted and stored at 70°C until measurements.

Biochemical analysis

Malondialdehyde (MDA) was estimated calorimetrically according to the method of Ohkawa *et al*., 1979 by the measurement of Thiobarbituric acid reactive substances (TBARS) **[17]**. The OxiSelect™ HNE Adduct Competitive ELISA Kit was used to determine 4-HNE levels. 8-Isoprostane EIA standard of isoprostanes was added in every test tube for the determination of Isoprostane. About 50 µl sample and 8-isoprotane AChE Tracer was added in each well. Afterwards 8-isoprotane EIA antiserum was added in the wells. Thin plastic film was used to cover the plates, allowed to stand at 4^{o}C for 18 hours. Wells were then emptied and rinsed with wash buffer for five times. Then about 200 µl of Ellman's reagent and 5 µl of tracer were added into it. Finally wavelength was observed at 405-420nm **[18]**. 8-hydroxy-2deoxyguanosine (8-OHdG) was determined with the help of commercially

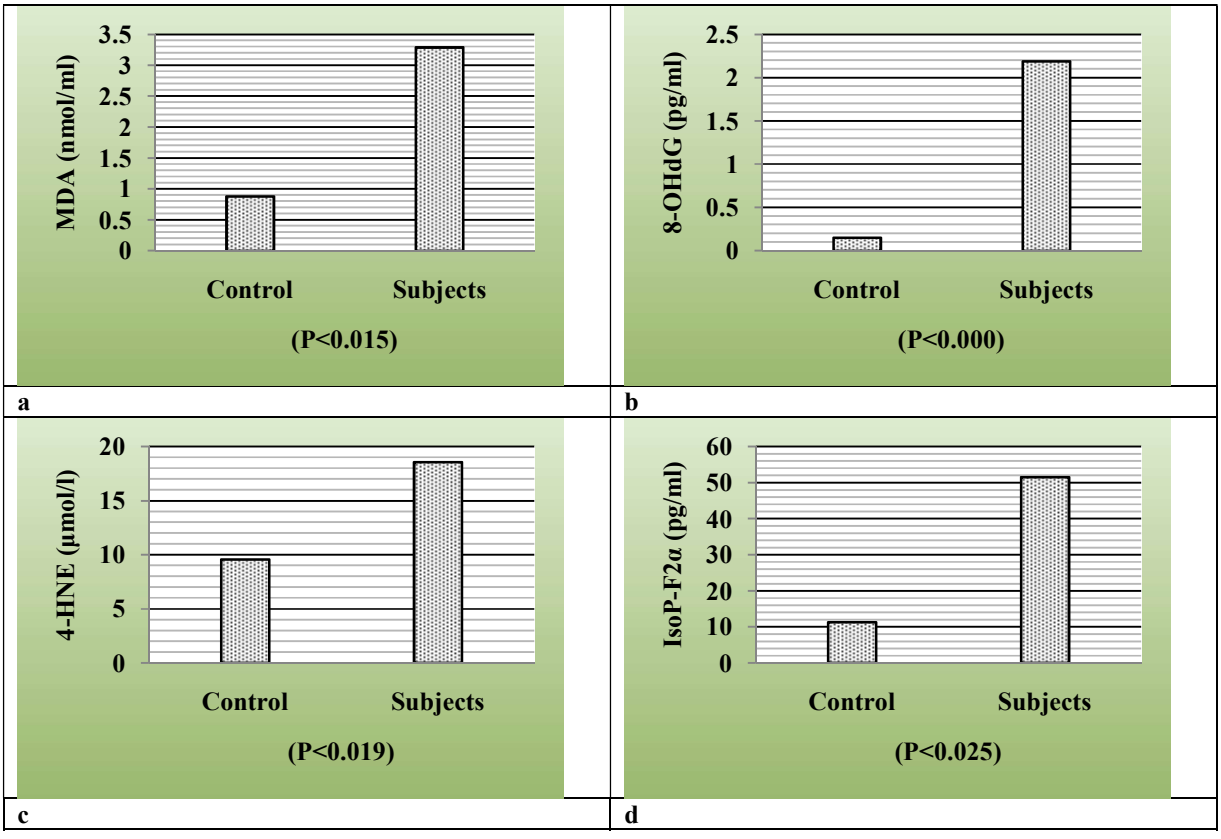
available ELISA kit by Glory Science Co. Ltd. USA.

Statistical analysis

To perform all statistical analysis SPSS version 7.0 was used. Test applied to analyse all variables was Independent T-test. A p value ($p < 0.05$) was considered statistically different.

RESULTS

In current study the levels of malondialdehyde (MDA), (4-HNE), isoprostane and 8-hydroxy-2-deoxyguanosine (8-OHdG) were measured. The MDA level was found to be significantly higher in women with endometriosis p=0.015. The levels of 4-HNE and isoprostane were differed significantly between subject and controls (18.59±1.26 Vs. 9.59±2.17 µmol/l) and (51.59±8.29 Vs. 11.26±2.99 pg/ml) with p=0.019 and p=0.025 respectively. Higher DNA damage is associated with the severity of endometriosis hence a marker of DNA damage 8-OHdG was also evaluated in current study. A significantly p=0.000 elevated level of 8-OHdG was observed in women suffering from endometriosis (2.19±0.064 pg/ml) when compared to controls (0.15±0.0019 pg/ml).



DISCUSSION

Oxidative stress and altered antioxidant defence system has been observed as a potential factor contributing to the pathophysiology of endometriosis. Current study indicates that women suffering from endometriosis have impaired antioxidant defence system and increased concentration of lipidperoxidation products as an oxidative stress biomarker. Recent reports suggest that although oxidative stress play an active role in normal functioning of endometrium cells but it also contribute to the etiology of endometriosis **[19]**. Increased production of free radicals and reactive oxygen species has the potential to cause damage to cell and impairs cell function as well as numerous studies implicates oxidative stress as a consequence, certainly related to inflammation **[20, 21]**. Therefore in the present study we analysed the concentration of malondialdehyde (MDA), (4-HNE), isoprostane and 8-hydroxy-2-deoxyguanosine (8-OHdG) in endometriosis patients that may contribute to initiate a chain of events associated with the etiology of endometriosis. Objective of current study was to examine whether all these variables studied including MDA, 4-HNE, isoprostane and 8-OHdG produced during oxidative stress and inflammation could serve as reliable biomarkers in discriminating the women with endometriosis from those without endometriosis. In support of our objectives the findings of current study showed that all variables studied shown significantly increase levels in women with endometriosis as compared to normal women. Findings of current study are consistent with previous studies suggesting that oxidative stress and lipid peroxidation play an important role in the pathogenesis of endometriosis.

Imbalance between oxidants and antioxidants results in oxidative stress **[22]**. Free radicals, ROS and RNS are highly reactive and unstable molecules that can cause damage to cell, impair cell function and disease by binding to cell structures including lipids, nucleic acid, proteins and carbohydrates. To avert cell damage cells have antioxidant defence system that scavenge and neutralize excessive free radicals **[23]**. In endometriosis oxidative stress might be induced by disturbance of balance between free radicals production and antioxidant levels. Evidence from previous studies suggest that oxidative stress is a prospective factor that may contribute in development and progression of endometriosis **[24]**. Recently a study by Amreen and his colleagues reported that

increased level of oxidative stress is associated with the progression of endometriosis **[25]**. ROS are second messenger of cell proliferation and are associated with the formation of endometriosis hence oxidative stress markers are increased in endometriosis. Santulli and his colleagues showed that higher level of oxidative stress and increased production of free radicals are associated with deep infiltration in endometriosis **[26]**. Furthermore women with endometriosis have increased concentration of lipidperoxidation end products such as MDA, 4-HNA and isoprostanes indicating the involvement of ROS induced lipidperoxidation in the development of endometriosis, also demonstrating the increased concentration of MDA **[27, 28]**. These findings support the results of current study where significant increase in the level of MDA, isoprostane and 4-HNE was recorded in endometriosis women. Additionally increased DNA damage and reduction in DNA repair was observed in advance stages of endometriosis indicates that instability of cell associated with higher oxidation may play a major role in endometriosis progression suggesting that these reliable markers are indicator of disease severity **[29, 30]**. One of the most important indicator of DNA damage resulting from oxidative stress is 8-OHdG, a DNA guanine base oxidation product as well as a well validated biomarker studied in other publications. There has been increasing evidence that oxidative stress can cause DNA damage in endometriosis tissue **[31]**. All these findings suggest that endometriosis induces increased oxidative stress and more frequent DNA damage which occurs in peritoneal cavity of women with endometriosis **[32-40]**. In current study an increase in 8-OHdG level was observed in women with endometriosis. As 8-OHdG is a reliable biomarker to measure free radicle induced DNA damage indicating that women with endometriosis have higher amount of DNA damage **[41-44]**.

CONCLUSION

In conclusion the possible role of MDA, isoprostane and 4-HNE (lipidperoxidation end products) and 8-OHdG (DNA damage biomarker) in the pathogenesis of endometriosis provide insights in the medical treatment of endometriosis.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

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