ORIGINAL ARTICLE

# DIFFERENTIAL EXPRESSION OF PROPHETIC FACTORS AND THEIR IMPULSIVE INTERPLAY TO DEVELOP ENDOMETRIOSIS IN YOUNG FEMALES

**Arif Malik[1], Iram Qamar[3], Huma Ali[1], Rabia Rasool[1], Sulayman Waqar[1], Gulshan Parveen[1], Mahmood Husain Qazi[2]**

[1]Department of Biochemistry, Institute of Molecular Biology and Biotechnology, University of Lahore - Pakistan

[2]Department of Physiology, Centre for Research in Molecular Medicine, University of Lahore - Pakistan

## ABSTRACT

**Objective:** The present study was conducted to assess the differential expression of 8-hydroxydeoxyguanosine, Malondialdehyde, estrogen and progesterone levels in young females suffering from endometriosis.

**Material and Methods:** Serum, saliva and urine samples of fifty endometriosis patients and thirty controls were analyzed for MDA spectrophotometrically and 8-hydroxy-2'-deoxyguanosine (8-OHdG), and estradiol (E2), and progesterone levels by using ELISA kits.

**Results:** The serum concentrations of 8-OHdG were observed to be increased in endometriosis patients(0.3291±0.0.17887ng/ml) than the concentrations in controls(0.0498±0.02477ng/ml, p=0.000). 8-OHdG levels in saliva of endometriotic women were higher (0.391±0.001961ng/ml) as compared to control group(0.0003±0.000015ng/ml). Likewise, urinary concentrations of 8-OHdG were increased in endometriosis (0.2014±0.012456ng/ml) in comparison with controls (0.313±0.00263ng/ml, p=0.000).The levels of MDA in serum (3.4940±0.96nmol/ml), saliva (0.2715±0.13762nmol/ml) and urine (1.8897±0.91083nmol/ml) were higher in females with endometriosis when compared with controls. The estradiol level in serum of patients were elevated (249.3289±14.71pg/ml) as compared to healthy subjects (70.9388±6.95512pg/ml). In saliva (54.8499±9.9585pg/ml) and urine (15.0499±6.74889pg/ml) were higher and significant than levels observed in saliva (19.9150±7.59245pg/ml) and urine (3.0547±0.81pg/ml) of controls. The concentration of progesterone was found to be higher in controls than in women suffering from endometriosis in all type of sample.

**Conclusion:** Increased oxidative stress and DNA damage have intrusive effects in host body and contribute in the progression of endometriosis in females. Moreover, progesterone and estradiol are found to play their significant roles in the disease.

**Key Words:** 8-hydoxydeoxyguanosine (8-OHdG), Endometriosis, Malondialdehyde, Oxidative stress.

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## INTRODUCTION

Endometriosis is a chronic painful gynecologic disease which is characterized by the presence of endometrial glandular epithelium and stroma outside the uterus. It is benign and mostly found in the ovaries, pelvic peritoneum and the recto-vaginal septum but can also be found in the pleura, pericardium and brain rarely. This estrogen-dependent condition can cause severe morbidity which includes pelvic pain, multiple surgeries and sterility.[1] Patients may experience symptoms such as dysmenorrhea and dyspareunia. It is a puzzling disease which is relatively common among women of reproductive age group with prevalence assumed to be approximately 10%.[2] Regardless of countless studies on endometriosis, its etiology still remains obscure. It is suggested that the disease has multifactorial characteristics and the pathogenesis involves a combination of several hormonal, genetic, environmental, anatomical and immunological factors.[3] Endocrine disruptors that include environmental toxins may modulate, stimulate or block the steroidal receptors and play their role in

**Arif Malik** (Corresponding Author)
Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore - Pakistan
Cell: +92-321-8448196
Email: arifuaf@yahoo.com

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pathogenesis of endometriosis.[4] Initiation and progression of endometriosis are influenced by production of estrogen and dysregulation of progesterone secretion.[5] Vigano, states that women having menorrhagia, short menstrual cycles and longer duration of menstruation as well as women who are nulliparous have higher incidence of endometriosis.[2] As the data suggests that endometriosis is linked with local inflammatory changes, the focus of many studies has been on inflammatory markers and oxidative stress in endometriosis so that less invasive methods of diagnosing the disease can be established.[6] Oxidative stress (OS) is thought to be a critical factor in pathogenesis of endometriosis. Presence of iron, macrophages, and contaminants in environment for example polychlorinated biphenyls, all of which generatereactive oxygen species (ROS) in the peritoneal fluid can cause imbalance in ROS production and antioxidants scavenging them. This results in increase in tissue proliferation and formation of adhesions.[7] Only a moderate dose of ROS lead to the initiation and progression of endometriosis while higher doses do not cause this effect as higher doses are cytotoxic and lead to apoptosis of cells so oxidative stress has a biphasic dose-response.[8] Oxidative stress may increase the production of vascular endothelial growth factors (VEGF) and thus can promote angiogenesis in endometrial implants outside the uterus. Oxidative stress stimulates the expression of a glycoprotein called glycodelin. Glycodelin may enhance VEGF expression in ectopic endometrial implants thus acting as an autocrine factor.[9]

Increased cytokines and various other immune mediators are reported due to increase in macrophage activity. Jackson et al showed that there is a weak trend of increased amounts of thiobarbituric acid reactive substances (TBARS) in women having endometriosis after correcting for the confounding factors like gravidity, age, BMI, and levels of vitamin E and lipids in serum.[13] TBARS are considered as an overall measure of oxidative stress.[7] It was also found that there is an increased production of Nitric oxide (NO) and increased lipid peroxidation in endometrium of affected women. Nitric oxide (NO) is a free radical that is considered to be pro-inflammatory and increases OS in peritoneal fluid, thus causing decreased fertility.[10] 8-hydroxy 1-deoxyguanosine levels were found to be significantly higher in women with endometriosis in contrast to women diagnosed of having male factor, tubal, or idiopathic infertility. Lipid peroxide and 8-hydroxy 1-deoxyguanosine levels were 6-fold higher in endometriosis of ovaries in contrast to normal endometrial tissue.[11]

Due to oxidative stress, various free radicals are produced specially hydroxyl radicals (OH-) that act together with lipids, proteins and DNA. The interrelation between DNA and free radicals alter the genetic sequence. When ever hydroxyl radicals (OH-) interact with nuclear and mitochondrial DNA, the 8-hydroxy-2'-deoxyguanosine (8-OHdG) is formed which is the biomarker of oxidative stress.[12]

## MATERIAL AND METHODS

This study was reviewed and approved by Research and Ethics Committee at University of Lahore. Fifty patients of mean age 20-40 years who attended the Out Patient Department of Obstetrics and Gynae with endometriosis, Lahore (Jinnah Hospital, Lahore) during the period September 2014 to November 2014 was enrolled in the study and fifty healthy patients were selected. History of Periodontal therapy (PDL) or antibiotic therapy in past three months, history of systemic disease that may affect periodontal status e.g. Diabetes, Hypertension, Cardiovascular, Gastrointestinal disorders and liver disease, Pregnancy or Lactation, Smokers, and any other active oral pathology were excluded from the study because we only want to observe the levels of oxidative parameters in the patients suffering from endometriosis. Informed consent was taken from all the patients under study. The experimental protocol was approved by the Research Ethical Committee of The Institute of molecular biology and biotechnology, The University of Lahore. Five (5) ml of venous blood samples, Urine and saliva sample were also taken. The sample bottles were centrifuged within one hour of collection, and stored at -70°C until assayed. Following parameter was assayed in all three samples types: Malondialdehyde (MDA) was determined by spectrophotometrically, 8-OHdG, progesterone and estrogen were estimated with help of ELISA kits (Abcam-Biochemicals). Provided 96 wells platewas assayed with help of ELISA reader. Plates were prepared with their respective protocols. Appropriate amount of samples, standard and controls were added to wells through the pipette. Reading was taken at mentioned wavelengths followed by the steps of washing and incubation. Data processing and statistics was done using SPSS version 16. The data was expressed as Mean $\pm$ SD. Independent (t) test were used for comparison of endometriosis and controls. Pearson correlation coefficient (r) was also calculated. The changes were considered significant at p-values $\leq 0.05$. ROC curve was measured in different mediums of patients i.e., Serum, Saliva and Urine to check the sensitivity and specificity.

## RESULTS

According to the Figure 1, the concentrations of the biomarker of DNA damage occurring due to oxidative stress 8-hydroxy-2'-deoxyguanosine (8-OHdG)

showed a relatively similar trend. The serum concentrations of 8-OHdG were observed to be increased in patients with endometriosis (0.3291±0.017887ng/ml) than the concentrations in control subjects (0.0498±0.02477 ng/ml), the difference being significant statistically (p=0.000). The 8-OHdG levels in saliva of women having endometriosis were higher (0.391±0.001961 ng/ml) as compared to control group (0.0003±0.000015 ng/ml) with a statistically significant difference (p=0.000). Similarly, urinary concentrations of 8-OHdG were increased in females with endometriosis (0.2014±0.012456 ng/ml) in comparison with controls (0.313±0.00263ng/ml) and differed significantly (p=0.000) with each other. The data obtained from the Figure 2 reveals that the levels of malondialdehyde (MDA) in serum were higher in females with endometriosis (3.4940±0.96 nmol/ml) when compared with controls (1.4070±0.34 nmol/ml) and the difference was found out to be statistically significant (p=0.001). The MDA concentration in saliva was also increased in patient group (0.2715±0.13762 nmol/ml) in comparison with control group (0.0027±0.00125 nmol/ml) with statistically significant difference (p=0.000). The urinary levels of MDA were increased in endometriosis patients (1.8897±0.91083 nmol/ml) than those found in controls (0.0546±0.06382 nmol/ml) with significant statistical difference (p=0.000).As shown in figure 03, the concentrations of estrogen which is the key hormone in endometriosis pathogenesis were also measured. The levels of estrogen in serum of patients were elevated (249.3289±14.71 pg/ml) as compared to subjects without endometriosis (70.9388±6.95512 pg/ml). Estrogen levels in patients saliva were also higher (54.8499±9.9585 pg/ml) than levels observed in saliva of controls (19.9150±7.5924 pg/ml) with a statistically significant difference (p=0.000). Showing a similar trend there were increased urinary concentrations of estrogen in women with endometriosis (15.0499±6.74889 pg/ml) in comparison with control group (3.0547±0.81 pg/ml) the difference found to be statistically significant (p=0.000).The levels of progesterone showed different trend than estrogen. As illustrated in figure 04, the concentration of progesterone in serum was found to be higher in controls (104.594±11.15 nmol/L) than in women suffering from endometriosis (58.2344±8.62203 nmol/L) the difference being significant statistically (p=0.006). Progesterone concentrations in saliva of patients were also decreased (3.8984±1.23 nmol/L) compared to control subjects (25.8357±5.0815 nmol/L) with a significant difference statistically (p=0.011). Similarly, urinary concentrations were observed to be lower in patient group (16.8003±3.75179 nmol/L) in comparison to the concentrations found in control group (28.7377±8.94 nmol/L) with the level of significance (0.000).

According to the Figure 5, Receiver operating curve (ROC) was measured in different mediums of patients i.e., Serum, Saliva and Urine to check the sensitivity and specificity. Levels of Estrogen were assayed in mediums and it remained most sensitive in the Urine, then in Serum and at last in Saliva. Area under curve (AUC) remained (0.999, 0.970 and 0.945) respectively. The level of progesterone remained most sensitive and specific in serum, then urine and at last in saliva. The AUC in serum remained most (0.841) while in saliva (0.674) and at last in urine (0.580). Levels of MDA and 8-OHdG remain significantly sensitive and specific in serum recorded (0.967 and 0.966) respectively.

## DISCUSSION

Endometriosis is a common, chronic and debilitating gynecological disorder of women of the reproductive age group. It is a multi-factorial condition that involves a complex interaction of various factors which include hormonal imbalance, immunological disruption, gene mutations, chromosomal aberrations, epigenetic variations as well as environmental factors causing oxidative stress and chronic inflammation. The results obtained in this study suggest that oxidative stress may play a key role in endometriosis. Results from earlier studies also found significantly increased oxidative stress in endometriotic tissue, blood and peritoneal fluid of patients with endometriosis.[10,13,14]

8-OHdG, a by-product of DNA damage by ROS, may result in G:C to A:T transversion mutation if it is present during DNA replication. DNA repair that excises 8-OHdG adducts leads to the excretion of 8-OHdG in tissues, blood and urine from where it can be measured as an indicator of oxidative damage to DNA. Studies show that urinary 8-OHdG is a reliable marker of oxidative DNA injury.[15] In the present study, the levels of 8-OHdG in serum, saliva and urine were observed to be significantly increased in women having endometriosis in comparison to the control group strongly suggesting the presence of oxidative DNA damage in endometriosis patients. These results are in accordance with the results obtained in studies performed by Polak, who observed significantly higher 8-OHdG levels in peritoneal fluid of endometriosis patients,[16] Da-Broi, who observed increased 8-OHdG concentrations in serum and follicular fluid of women having endometriosis related infertility,[17] Carvalho, who found higher 8-OHdG levels in tissue and peritoneal fluid of patients with different stages of endometriosis.[18] These results propose that endometriosis is associated with higher oxidative injury as well as increased frequency of mutations in DNA. Furthermore, no statistically significant correlation was found between salivary 8-OHdG and salivary MDA, es-

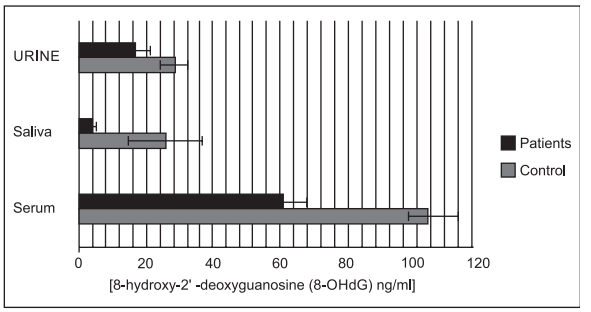



Figure 1: Level of (8-ohdg) in young females with endometriosis

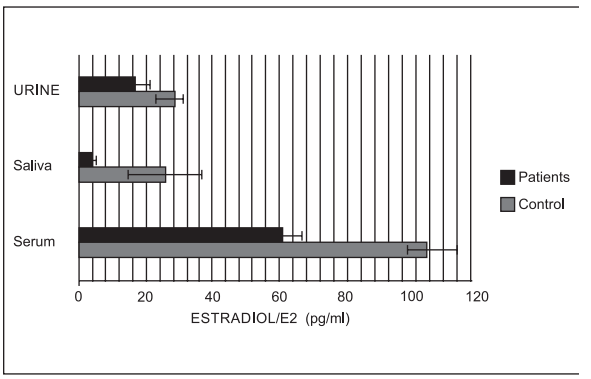



Figure 2: Level of (mda) in young females with endometriosis

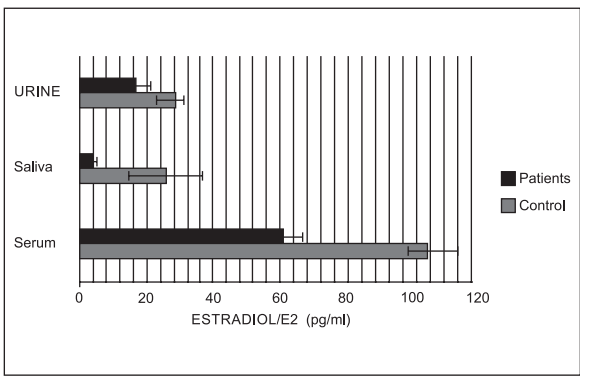



Figure 3: Level of (estradiol) in young females with endometriosis

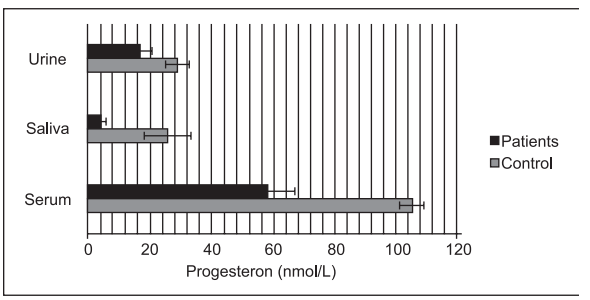
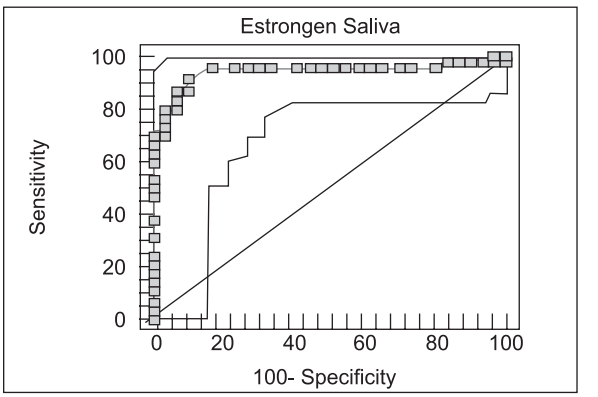
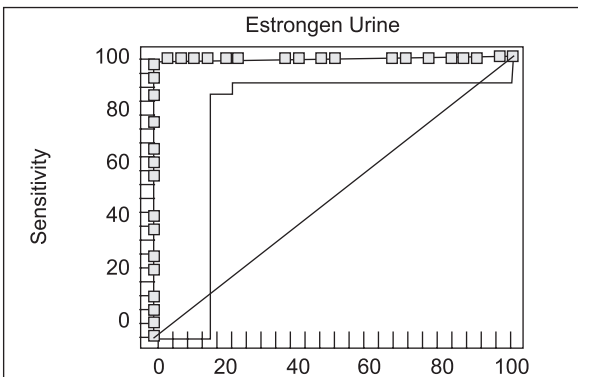


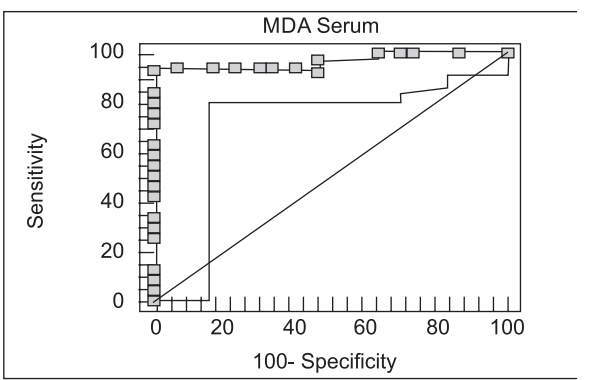

Figure 4: level of (progesterone) in young females with endometriosis



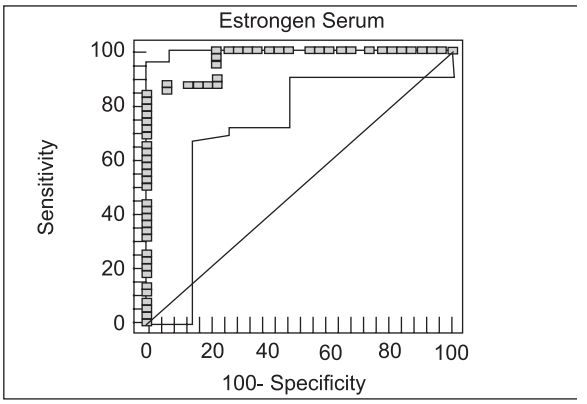

Area under the ROC curve (AUC)=0.970
Standard Errora=0.0149
95% Confidence intervalb=0.905 to 0.995
z statistic=31.638
Significance level P (Area=0.5)=<0.0001



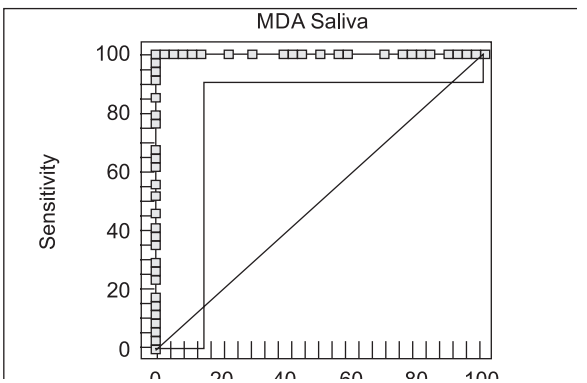

Area under the ROC curve (AUC)=0.999
Standard Errora=0.000943
95% Confidence intervalb=0.954 to 1.000
z statistic=529.623
Significance level P (Area=0.5)=<0.0001



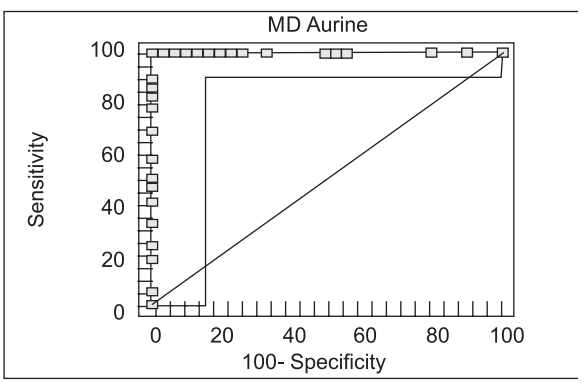

Area under the ROC curve (AUC)=0.967
Standard Error[a]=0.0195
95% Confidence interval[b]=0.901 to 0.994
z statistic=23.975
Significance level P (Area=0.5)=<0.0001
[a]DeLong et al., 1988, [b]Binomial exact



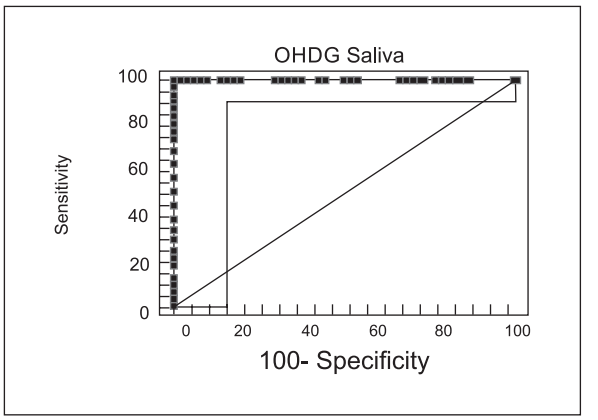

Area under the ROC curve (AUC)=1.000
Standard Errora=0.000
95% Confidence intervalb=0.955 to 1.000
Significance level P (Area=0.5)=<0.0001



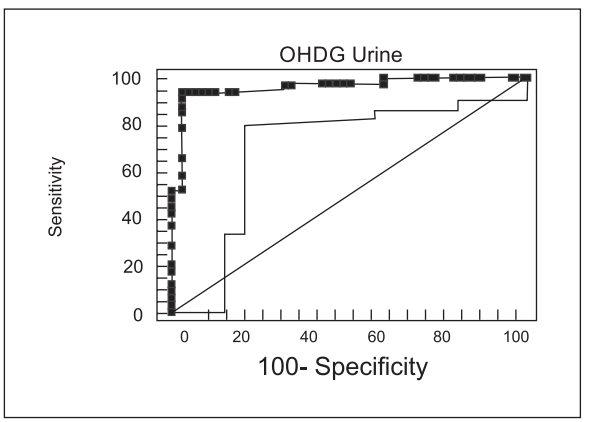

Area under the ROC curve (AUC)=1.000
Standard Errora=0.000
95% Confidence intervalb=0.955 to 1.000
Significance level P (Area=0.5)=<0.0001



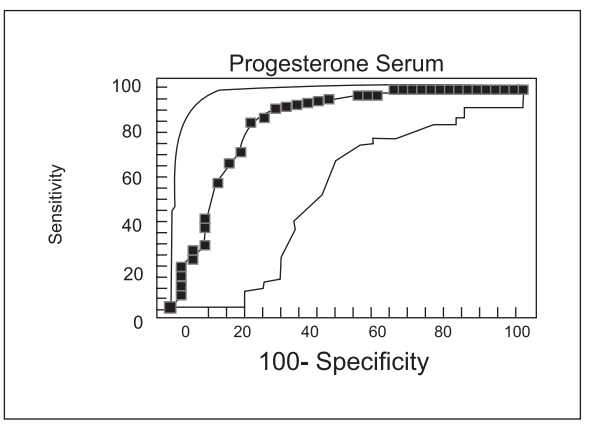

Area under the ROC curve (AUC)=1.000
Standard Errora=0.000
95% Confidence intervalb=0.955 to 1.000
Significance level P (Area=0.5)=<0.0001



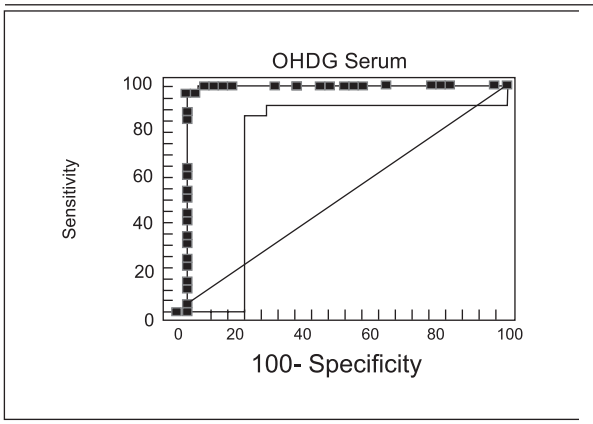

Area under the ROC curve (AUC) =1.000
Standard Errora=0.000
95% Confidence intervalb=0.955 to 1.000=
Significance level P (Area=0.5)=<0.0001



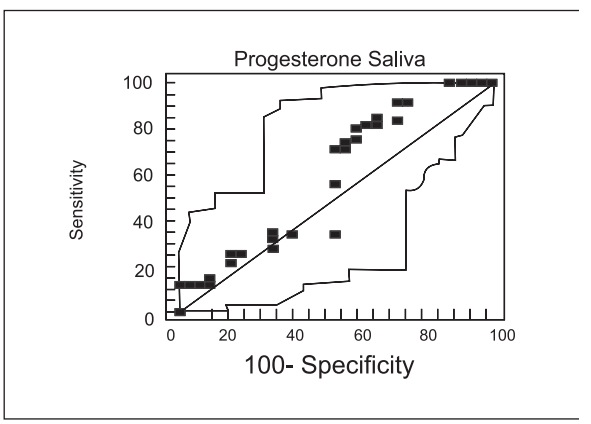

Area under the ROC curve (AUC)=0.962
Standard Errora=0.0212
95% Confidence intervalb=0.894 to 0.992
z statistic=21.822
Significance level P (Area=0.5)=<0.0001



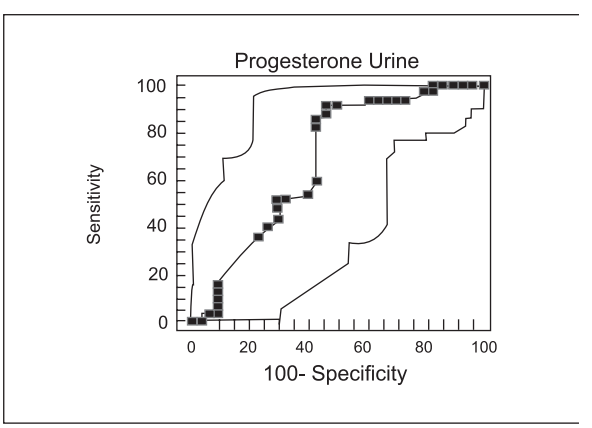

Area under the ROC curve (AUC)=0.841
Standard Errora=0.0513
95% Confidence intervalb=0.742 to 0.913
z statistic=6.644
Significance level P (Area=0.5)=<0.0001




Area under the ROC curve (AUC)=0.966
Standard Errora=0.0333
95% Confidence intervalb=0.899 to 0.994
z statistic=13.984
Significance level P (Area=0.5)=<0.0001




Area under the ROC curve (AUC)=0.580
Standard Errora=0.0710
95% Confidence intervalb=0.464 to 0.689
z statistic=1.122
Significance level P (Area=0.5)=0.2619




Area under the ROC curve (AUC)=0.674
Standard Errora=0.0691
95% Confidence intervalb=0.560 to 0.775
z statistic=2.524
Significance level P (Area=0.5)=0.0116

trogen and progesterone (8-OHdGVs MDA, r= -0.261, 8-OHdGVs estrogen, r = -0.050, 8-OHdGVs progesterone, r = -0.140). Also no statistically significant correlation of serum 8-OHdG and serum MDA, estrogen and progesterone was seen in this study (8-OHdGVs MDA, r = 0.146, 8-OHdGVs estrogen, r = 0.067, 8-OHdGVs progesterone, r = -0.040).

MDA is a relatively stable physiologic keto-aldehyde formed as a byproduct in peroxidation of unsaturated lipids. MDA can be analyzed in several biological samples including tissues, serum, plasma and urine.[19] In the present study the levels of MDA were measured in serum, saliva and urine of endometriosis patients and compared with MDA levels in control group. The levels were found to be significantly higher in diseased group. This depicts the presence of oxidative stress in these fluids in women suffering from endometriosis. These results are consistent with the findings of study performed by Mier-Cabrera, in which MDA levels were significantly increased in the peritoneal fluid in women having endometriosis compared with normal women.[20] Shanti et al found higher levels of oxidatively modified lipid-protein complexes along with higher concentrations of serum autoantibodies to malondialdehyde-modified low-density lipoprotein which represents damage produced by peroxidized lipids in endometriosis patients.[21] Statistically insignificant correlation was observed among MDA in saliva and salivary 8-OHdG, estrogen and progesterone (MDA Vs8-OHdG, r = -0.261, MDA Vs estrogen, r = -0.004, MDA Vs progesterone, r = -0.019). Also no statistically significant correlation was found between serum MDA and serum 8-OHdG, estrogen and progesterone (MDA Vs8-OHdG, r = 0.146, MDA Vs estrogen, r = -0.048, MDA Vs progesterone, r = -0.031). In the same way urinary MDA and urinary 8-OHdG, estrogen and progesterone did not show any significant statistical correlation (MDA Vs8-OHdG, r = -0.094, MDA Vs estrogen, r = -0.104, MDA Vs progesterone, r = -0.194).

Estrogen is the most important hormone playing a key role in pathogenesis of endometriosis. Its biologically active form estradiol (E2) is involved in the growth as well as the inflammatory reactions going on in the ectopic tissue.[22] There is local estrogen production from androgens due to the aromatase expression in endometriotic implants. Aromatase helps in the maintenance of ectopic lesion on even low estrogen levels.[4] In females with endometriosis the molecular changes induced by progesterone are masked in the eutopic tissue indicating the resistance to the action of progesterone in diseased women. There may be decrease in progesterone receptor (PRs) levels along with deficient PR isoform (PR-B). Progesterone causes the induction of secretion of several paracrine factors by

stromal cells in normal endometrial tissue. The function of these factors is the induction of 17β-hydroxysteroid dehydrogenase type-2 (17β-HSD-2) that is involved in the formation of estrone (E1) by the metabolism of the biologically active form E2. The in-vivo deficiency of PR-B as well as the significantly reduced progesterone receptor A (PR-A) levels in ectopic endometrial tissue could be responsible for the failure of production of paracrine factors induced by progesterone by the stromal cells in endometrial tissue. Consequently the deficient metabolism of the active form E2 results in significantly increased concentrations of this mitogen in endometriosis patients.[22] In the present study the levels of estrogen in the saliva, serum and urine of women with endometriosis were analyzed to be significantly higher in comparison to control subjects. However this study could not establish any statistically significant correlation between salivary estrogen and salivary 8-OHdG, MDA and progesterone (estrogen Vs8-OHdG, r = -0.050, estrogen Vs MDA, r = -0.004, estrogen Vs progesterone, r = 0.098). Likewise the correlation between serum estrogen and serum 8-OHdG, MDA and progesterone came out to be insignificant statistically (estrogen Vs8-OHdG, r = 0.067, estrogen Vs MDA, r = -0.048, estrogen Vs progesterone, r = -0.022). Also the urinary estrogen and urinary 8-OHdG, MDA and progesterone were statistically insignificantly correlated.

The endometrial tissue proliferation induced by estrogen is antagonized by progesterone. There is increased volume of cells lining the uterine wall due to the action of progesterone leading to endometrial thickening and blood vessel invasion. In endometriosis, gene polymorphisms may lead to decreased progesterone receptor stability resulting in the loss of capacity of receptor to inhibit ER activation. This causes inadequate receptor control leading to increased exposure of endometrium to estrogen. Extracellular matrix metalloproteinases (MMPs) may also be regulated by progesterone. In this study the levels of serum, salivary and urinary levels of progesterone were examined to be significantly decreased in diseased women as compared with the levels seen in control group which is concurrent with Li Y et al.[23] Nevertheless the correlation between progesterone in saliva and 8-OHdG, MDA and estrogen in saliva was observed to be insignificant statistically (progesterone Vs8-OHdG, r = -0.140, progesterone Vs MDA, r = -0.019, progesterone Vs estrogen, r = 0.098). The serum progesterone and serum 8-OHdG, MDA and estrogen were also correlated insignificantly statistically (progesterone Vs8-OHdG, r = -0.031, progesterone Vs MDA, r = -0.040, progesterone Vs estrogen, r = -0.022).Likewise the correlation between urinary progesterone and urinary 8-OHdG, MDA and estrogen was also statistically not significant (progesterone Vs8-OHdG, r = 0.062, progesterone Vs MDA, r = -0.194, progesterone Vs estrogen, r = -0.149).

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## REFERENCES

1. Giudice LC, Kao LC. Endometriosis. The Lancet 2004; 364:1789-99.
2. Vigano P, Parazzini F, Somigliana E, Vercellini P. Endometriosis, Epidemiology and aetiological factors. Best Practice and Research in Clinical Obstetrics and Gynaecology 2004;18:177-200.
3. VinatierD, CossonM, Dufour P.Is endometriosis an endometrial disease?Eur J ObstetGynecolReprod Biol. 2000:113-25.
4. Barbosa CP, Souza AM, Bianco B, Christofolini D. The effect of hormones on endometriosis development. Minerva Ginecol 2011;63:375-86.
5. Templeton DM, Liu Y. Genetic regulation of cell function in response to iron overload or chelation. Biochimica et BiophysicaActa (BBA)-General Subjects 2003;20:113-24.
6. Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR, et al. Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. Hum Reprod 2000;17: 426-31.
7. Donnez J, Van Langendonckt A, Casanas-Roux F. Current thinking on the pathogenesis of endometriosis. GynecolObstet Invest 2002;54:52-58.
8. Foyouzi N, Berkkanoglu M, Arici A, Kwintkiewicz J, Izquierdo D, Duleba AJ. Effects of oxidants and antioxidants on proliferation of endometrial stromal cells. Fertile Steril 2004;82:1019-22.
9. Park JK, Song M, Dominguez CE, Walter MF, Santanam N, Parthasarathy, et al. Glycodelin mediates the increase in vascular endothelial growth factor in response to oxidative stress in the endometrium. Am JObstetGynecol 2006;195:1772-77.
10. Szczepanska M, Kozlik J, Skrzypczak J, Mikolajczyk M. Oxidative stress may be a piece in the endometriosis puzzle. FertilSteril 2003;79:1288-93.
11. Saito H, Seino T, Kaneko T, Nakahara K, Toya M, Kurachi H. Endometriosis and oocyte quality. GynecolObstet Invest 2002;53:46-51.
12. Yamaguchi K, Mandai M, Toyokuni S, Hamanishi J, Higuchi T, Takakura K, et al. Contents of endometriotic cysts, especially the high concentration of free iron, rae a possible cause carcinogenesis in the cysts through the iron-induced persistent oxidative stress. Clin Cancer Research 2008;14:32-40.

13. Jackson L, Schisterman E, Day-Rao R, Browne R, Armstrong D. Oxidative stress and endometriosis. Hum Reprod 2005;20:2014-20.

14. Matsuzaki S, Schubert B. Oxidative stress status in normal ovarian cortex surrounding ovarian endometriosis. FertilSteril 2010;93:2431-32.

15. Szymanska-Chabowska A, Beck A, Poręba R, Andrzejak R, Antonowicz-Juchniewicz J. Evaluation of DNA Damage in People Occupationally Exposed to Arsenic and Some Heavy Metals. Polish J of Environ Stud 2009;18:1131-39.

16. Polak G, Wertel I, Barczynski B, Kwasniewski W, Bednarek W, Kotarski J. Increased levels of oxidative stress markers in the peritoneal fluid of women with endometriosis. EurJObstGyn Rep Bio 2013;168: 187-90.

17. Da BroiMG,de Albuquerque FO, de Andrade AZ, Cardoso RL, Jordao Junior AA, Navarro PA. Increased concentration of 8-hydroxy-2'-deoxyguanosine in follicular fluid of infertile women with endometriosis. Cell Tissue Res 2016;366:231-42.

18. Carvalho LF, Abrao MS, Biscotti C, Sharma R, Nutter B, Falcone T.Oxidative cell injury as a predictor of endometriosis progression.ReprodSci 2013;20: 688-98.

19. Grotto D, Maria LS, Valentini J, Paniz C, Schmitt G, Garcia SC, et al. Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. Quimica Nova 2009;32:169-74.

20. Mier-Cabrera J, Jimenez-Zamudio L, Garcia-Latorre E, Cruz-Orozco O, C Hernandez, Guerrero C. Quantitative and qualitative peritoneal immune profiles T-cell apoptosis and oxidative stress-associated characteristics in women with minimal and mild endometriosis. BJOG Int J ObstetGynaecol 2011;118:6-16.

21. Shanti A, Santanam N, Morales AJ, Parthasarathy S, Murphy AA . Autoantibodies to markers of oxidative stress are elevated in women with endometriosis. FertilSteril 1999;71:1115-18.

22. Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, et al. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. Molecular and cellular endocrinology 2006;27:94-103.

23. Li Y, Adur MK, Kannan A, Davila J, Zhao Y, Nowak RA, et al. Progesterone Alleviates Endometriosis via Inhibition of Uterine Cell Proliferation, Inflammation and Angiogenesis in an Immunocompetent Mouse Model. PLoS One 2016;11:e0165347.

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Following authors have made substantial contributions to the manuscript as under:

**Malik A:** Conceptualized the theme of data.

**Iram Q:** Written the text.

**Ali H:** Written / reviewd the data.

**Rasool R:** Edited the data.

**Waquar S:** Analytically reviewed and edited the text.

**Parveen G:** Reviewed the discussion.

**Qazi MH:** Done final revision.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.