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ROLE OF EXTRAPOLATIVE FACTORS AND CIRCULATING BIOCHEMICAL INTERPLAY IN FEMALES WITH ENDOMETRIOSIS

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ABSTRACT

Endometriosis is the primary cause of female infertility during reproductive age. It is due to the existence of endometrial glands and stromal tissues outside the wall of uterus. The particular prevalence of the disease is still unclear but approx. 5-10% of women are affected by endometriosis. The aim of this study is to pin point the role of cytokine, matrix metalloproteinases (MMPs) and steroid hormones in progression and aggregation of endometriosis. Hundred patients of endometriosis and hundred healthy age and sex matched individuals were recruited for the current study. The levels of MDA, IsoP-F2α, 8-OHdG, SOD, GSH, CAT, IL-1, IL-2, IL-6, IL-10, IL-11, IL-13, IL-4, TNF-α, MMP-1, MMP-2, MMP-8, MMP-9, MMP-13, cortisol, estradiol and vitamin D were estimated. It shows that levels of MDA were increased significantly in patients (1.99±0.045 nmol/ml) compared to control (0.98±.0015 nmol/ml). The levels ofIsoP-F2α and 8-OHdG were recorded 34.26±4.26 pg/ml and 1.022±0.0015 pg/ml respectively in patients. However, the serum CAT, SOD, GSH and vit D levels in patients were recorded with the significant decline values 3.16±0.015 IU/L, 0.014±0.005 U/L, 6.59±1.6 µmol/L and 14.26±4.66 pmol/L respectively. Serum MMP-8 and MMP-13 were increased in the patients of endometriosis (67.26±8.26 ng/ml and 98.26±4.26 ng/ml respectively). The levels of cytokines IL-4 (5.78±1.28 pg/ml), IL-11 (4.20±1.06 pg/ml)

and IL-13 (6.59±1.13 pg/ml) were elevated in these patients. The serum estradiol and cortisol in these patients recorded 425.58±14.26 pg/ml and 21.25±3.26 µg/dl respectively. Current study clearly depicts that increase in oxidative stress leads to the pathogenicity of endometriosis. Decline in the levels of anti-oxidants is the reason that enhances oxidative stress in such patients. Raised levels of activated macrophages secrete sufficientinflammatory cytokines that are involved in tissue implantation and endometrial cell proliferation. Hence, cytokines and increased oxidative stress activates several MMPs which control the endometriotic lesion throughout the degradation of ECM.

Keywords: Cytokines, Estradiol, Endometriosis, Malondialdehyde, Matrix metalloproteinases, Vitamin D

INTRODUCTION

Endometriosis is one of the most common conditions which have major effect on women during their reproductive years. Commonly, it is identified as the existence of endometrial glands and Stromal tissues outsidethe wall of uterus. Precise prevalence of the disease is still unknown but according to an approximation 5-10% of women in their reproductive years are affected by endometriosis [1]. Women with the disease often experience painful symptoms like chronic pelvic pain, dysmenorrhea, deep dyspareunia, fatigue, infertility issues and ovarian cyst. There are certain theories which explain the development of endometriosis including retrograde menstruation, embryonic rests and metaplasia. One of the most widely accepted theory is known as retrograde menstruation; It is defined as thereverse menstrual flow from the fallopian tubes into the abdomen during menstrual cycle. The growing evidences suggest that the use of combined oral contraceptive pills can decrease the risk of endometriosis. Likewise, exercise and avoiding the consumption of alcohols may also improve the symptoms of endometriosis [2]. The growth and development of endometriotic cells remain associated with inflammation that further leads towards the symptom of excessive pain and female infertility. Whereas,endometric lesions and inflammation is distinguished by excessive production of prostaglandin and other inflammatory cytokines, that triggers pain and fertility issues in female reproductive system. Moreover, estrogen hormone is also involved in the persistence and survival of endometrial lesion that mediate the alteration of inflammatory and immune response. According to different study lines, it has been suggested that pathological angiogenesis is one characteristic of

multiple disorders including endometriosis [3]. Angiogenesis is basically a complex process that initiates due to the deterioration of mature blood vessel by the degradation of extracellular matrix (ECM) and the separation of mural cells.

The patients of endometriosis demonstrate elevated levels of various growth factors such as vascular endothelial growth factor in the peritoneal fluids and ectopic tissue of female reproductive system [4].These growth factors are produced by endothelial cells, neutrophils and stromal cells. Furthermore, patients of endometriosis also represent raised levels of hepatocyte growth factor (HGF), angiogenin, macrophages-migration inhibitory factor, interleukins, tumor necrosis factor and neutrophil activating factor. All of these factors are responsible to enhance angiogenesis that leads to the progression of endometriosis [5]. However, matrix metalloproteinases (MMPs) enzymes are required for the physiological functioning of endometrium wall in uterus. On the other hand, alteration in the activity of matrix metalloproteinases isbelieved to be a significant factor for the progression of endometriosis. Generally, matrix metalloproteinasesare zinc dependent endopeptidase enzymes, implicated in the deprivation of ECM (extracellular matrix) and are also involved in cellular aggression, cellular migration and angiogenesis mechanisms [6]. In addition, MMPs have major role in the cellular activity of epithelial mesenchymal transition. Primarily, matrix metalloproteinases are secreted in the form of latent pro-enzyme and can be stimulated by the proteolytic cleavage of pro-domain. The activities of matrix metalloproteinases can be regulated by tissue inhibitors of metalloproteinases (TIMPs) [7]. The basic role of matrix metalloproteinase (MMPs) in endometriosis is intriguing. According to different studies, it is reported that there are elevated levels of MMP-2,3,7 and 9 in the patients of endometriosis [8]. Following studies were again testified and suggests that the eutopic endometrium of women have increased expressions of MMP-9 as compared to un-affected women [9]. Vitamin D is a fat soluble vitamin which is synthesized by sun exposure, supplements, food intake in the body system. Its deficiency is prevalent in worldwide. It has suggested that vitamin D is implicated in normal cellular growth and regulation. Moreover, it has immuno-regulatory effects during inflammatory processes which enhance the production of anti-inflammatory cytokines as well diminish pro-inflammatory cytokines levels

[10]. Vitamin D has a major role in the suppression of angiogenesis and induction of apoptosis [11]. In some studies, there is decrease level of vitamin D in the patients of endometriosis [12]. Whereas in another studies, it has suggested that higher diary food intake and elevated plasma levels of Vitamin D are linked with reduce risk of endometriosis pathogenicity [13,14].

MATERIALS AND METHODS

SAMPLE COLLECTION

Hundred patients affected by Endometriosis disease were screened at Jinnah Hospital Lahore. Concomitantly, hundred healthy people selected as control individuals. The history and clinical diagnosis of the patients were attained from the hospital medical records. The study was conducted by the ethical committee of Molecular Biology and Biotechnology (IMBB), The University of Lahore. The patients of endometriosis with clinically diagnosis are included in this study. Five ml of venous blood sample was drawn in gel tube from control individuals and patients than separate serum by centrifugation at 4000 rpm within two hours. After the separation of serum, it is than stored at -70ºC until examined. The sample is transferred to the laboratory for further processing. All the reagents and chemicals used during this study were purchased from Sigma chemicals. Hundred female of endometriosis patients with the age range of 20-50 years are included in this study. All the patients with the confirmed clinical reports of stage IV endometriosis were recruited. The patients of endometriosis with the history of taking drugs such as cigarette and alcohol as well as medications including antipsychotic or Parkinson were excluded in this study. Moreover none of the control individuals were included which were on any medications, depression, malnutrition syndrome, history of chronic infections and metabolic dysfunction such as hypertension, diabetes, cancer. MDA (Malondialdehyde) was estimated by the methods of [15, 16]. Glutathione anti-oxidant assay was measured by the method of [17]. The Superoxide dismutase enzyme was analyzed by the method of [18] whereas Catalase (CAT) was measured by the method of [19, 20]. Isoprostanes F2α and 8-OHdG were estimated by commercially available kits. IL-1, IL-2, IL-6, IL-10, IL-11, IL-13, IL-4 and TNF-α were estimated by commercially available ELIZA kits. MMP-1, MMPP-2, MMP-8, MMP-9 and MMP-13 were analyzed by commercially available kits. Cortisol, Estradiol and Vitamin D were also measured by the commercially available kits. Statistical analysis was performed by

SPSS statistics 17.0. The results of all parameters were measured by independent sample t-test. Pearson's correlation coefficient was used to determine the correlations between different variables of endometriosis patients. P-value was calculated by using one way ANOVA.

RESULTS

As shown in the Figure 01, the profile of circulating stress biomarkers of endometriosis patients e.g., Isoprostanes F2α (IsoP-F2α), Malondialdehyde (MDA) and 8-Hydroxyguaniosine (8-OHdG) demonstrate highly significant differences between endometriosis and control patients (Table 1). The mean values of Isoprostanes F2α indicate elevated serum levels in endometriosis subjects (34.26±4.26pg/ml), in comparison to control subjects (0.78±0.007 pg/ml). Generally, lipid peroxidation is evaluated by TBARS and the mean value of MDA in the patients of endometriosis was increased at 1.99±0.045 nmol/ml as compared to control individuals at 0.98±.0015nmol/ml. The serum 8-OHdG value of endometriosis patients was also noted to be raised (1.02±0.001pg/ml) as compared to control individuals (0.07±0.004 pg/ml). Figure 01 represents the profile of circulating anti-oxidants biomarkers in the patients of endometriosis. The lower level of SOD (Superoxide dismutase) in endometriosis patients was recorded (0.014±0.005 U/ml) as compared to control individuals (0.31±0.0017 U/ml). The mean serum level of Catalase (CAT) reduced in endometriosis subjects (3.16±0.015 IU/L) Vs in control individuals (4.26±0.15 IU/L). The Glutathione (GSH) in endometriosis patients was found lower (6.59±1.6 µmol/L), while in healthy individuals (8.06±1.22 µmol/L). The data present in Figure 02 represented the inflammatory biochemical markers of IL-1 (Interleukin-1), IL-2 (Interleukin-2), IL-6 (Interleukin-6), IL-10 (Interleukin-10), IL-11 (Interleukin-11), IL-13 (Interleukin-13), IL-4 (Interleukin-4) and TNF-α (Tumor necrosis factor-α) in the patients of endometriosis compared with control individuals.

The mean serum level of IL-1 increases in endometriosis subjects (7.26±1.02 pg/ml) Vs in control individuals (4.22±0.73 pg/ml). The increase level of IL-2 in endometriosis patients was recorded (6.89±0.42 pg/ml) Vs in control (5.26±0.27 pg/ml). The serum IL-6 in endometriosis patients was found increased (7.26±1.02 pg/ml) while in healthy control (4.66±0.63 pg/ml). The mean value of serum IL-10 was observed remarkably higher (5.16±0.52 pg/ml) in endometriosis patients in contrast to control

individuals (3.99±0.39 pg/ml). Elevated level of IL-11 (Interleukin-11) measured in patients of endometriosis (4.20±0.32 pg/ml) Vs (Versus) healthy control (2.99±0.15 pg/ml). In IL-13, the mean value among endometriosis patients was elevated (6.59±0.54 pg/ml) as compared to healthy controls (5.16±0.41 pg/ml). The level of IL-4 in patient group was increased (5.78±0.38 pg/ml) versus control group (4.99±0.31 pg/ml). Similarly, the mean value of TNF-α (Tumor necrosis factor-α) among endometriosis patients was elevated (33.06±2.55 pg/ml) in comparison to control subjects (21.09±2.06 pg/ml). The data represented in Figure 03 summarized the profile of Matrix metalloproteinases (MMPs) in endometriosis patients and control individuals. The mean value of MMP-1 was observed higher (65.29±8.49 ng/ml) in endometriosis patients in comparison to control subjects (37.06±5.26 ng/ml). In endometriosis patients, MMP-2 (Matrix metalloproteinases) level was found to be elevated (78.26±5.26 ng/ml) as compared to control subjects (41.99±8.77 ng/ml). MMP-8 in endometriosis patients were found to be elevated (67.26±8.26 ng/ml) versus healthy individuals (31.88±7.99 ng/ml). Increased MMP-9 level measured in endometriosis patients (91.56±5.88 ng/ml) in comparison to control subjects (61.25±11.26 ng/ml). Similarly, the level of MMP-13 in patient group was raised (98.26±4.26 ng/ml) versus control group (51.26±4.26 ng/ml). The data presented in Figure 04 shows the clear picture of hormonal profile of Estradiol, Cortisol and Vitamin D in patients of endometriosis as compared to control subjects. The mean value of serum estradiol was reported to be high (425.58±14.26 pg/ml) in endometriosis patients Vs healthy control (226.24±8.26 pg/ml). Likewise, the level of serum cortisol in endometriosis patients was significantly increased (21.25±3.26 µg/dl) as compare to control (13.26±1.25 µg/dl). The serum vitamin D level in endometriosis patients were estimated as 14.26±1.83 pmol/L while in healthy individuals as 22.16±2.26 pmol/L.

DISSCUSION

The aim of this study is to estimate the role of various inflammatory cytokines, matrix metalloproteinases (MMPs), hormonal biomarkers, circulating stress biochemical markers and antioxidant biomarkers in the patients of endometriosis that are susceptible to female infertility. The levels of lipid peroxides have been observed to be highest in the patients with endometriosis that represent the association of reactive oxygen species (ROS) in the pathogenicity of this

disease [21]. MDA is the end product of lipid peroxidation which is synthesized by the decomposition of polyunsaturated fatty acid (PUFA) and arachidonic acid [22]. The present study, MDA levels are elevated in the patients with endometriosis as compared to the healthy individuals similar indications were provided in another set of studies [23]. Increased MDA along with increased ECM degradation which is measured by the levels of MMPs in the patients of endometriosis (MDA Vs. MMP-8,r=0.671***) as shown in the Table 01. Due to oxidative stress various radicals are produced, particularly Hydroxyl radical (OH^-) can interact with lipid, proteins and DNA, causing mutation in genetic makeup [24]. Whenever, OH^- radical reacts with mitochondrial or nuclear DNA, the 8-OHdG (8-hydroxydeoxyguanosine) is synthesized which is the marker of oxidative DNA damage and considered to be pro-mutagenic and carcinogenic. In the present study, there is elevated level of 8-OHdG in endometriosis patients as compared to control subjects. This work is similar to the study of [25].

The Isoprostanes F2α is a complex family of compound which is synthesized by non-enzymatic peroxidation of arachidonic acid (AA) in cell membrane [26]. The elevated levels of Iso-F2α which is a sensitive, reliable and particular marker of lipid peroxidation in women with endometriosis [27].The same trend were also observed in the present study as higher Iso-F2α levels was recorded in endometriosis patients as compare to the age matched healthy subjects.Table 01 shows thatincreased uptake of cytokines results in increased lipid peroxidation(Iso-F2α Vs IL-11, r=0.686**).In endometriosis, the role of antioxidants is still controversial because of contradictory views which were articulated by several authors [28]. Anti-oxidants systems are identified as protective mechanism from the side effects of free radicals. SOD is an important anti-oxidant in all living cells exposed to oxygen. It is an enzyme that catalyzes the dismutation of O_2^- (Superoxide) radical into molecular oxygen and H_2O_2 (Hydrogen peroxide). The superoxide radical is synthesized as by-product of oxygen metabolism and if it is not regulated can cause cellular damage. The study accomplished by Szczepanska*et al*., [21] reviewed that there is significant decrease level of SOD in endometriosis patients as compared to control patients and this work is similar to the current work. Glutathione is an anti-oxidant and a tri-peptide linkage between Glycine, amino group of cysteine and carboxyl group of

glutamate amino acid. It has known to minimize the levels of lipid peroxidation in the cellular membrane and other target cells which are influenced by oxidative insult [29]. In the current study, there is reduced level of GSH in the patients of endometriosis as compared to control individuals. Luciana *et al*., [30] also reported the decreased level of GSH in their studies previously. Similarly the levels of Catalase is also reduced in the patients of endometriosis as compared to control subjects and the research carried out by Hirotaka*et al*., [31] also demonstrated the results which is similar to the present study.

Matrix metalloproteinases are zinc dependentendopeptidase enzymes, which are synthesized in the cells such as wound cells as well as in inflammatory cells and are implicated in both physiological and pathological conditions. They can be activated by inflammatory cytokines and growth factors controlling endometriotic lesion expansion throughout the degradation of extracellular matrix (ECM). Matrix metalloproteinases (MMPs) have a major role in the degradation of matrix throughout the menstruation cycle [33]. MMPs have also linked with malignancy, but their major functions in gynecological malignancies and endometriosis have not been clearly recognized. The research carried out by Di-Nezza*et al*., [33] stated that the expression of MMP2 is increased in endometriotic patients and has major impact in tumor progression which is similar to the current work. Similarly in the recent research work, the level of MMP-8 and MMP-13 is also elevated in the patients of endometriosis as compared to control subjects. According to the Table 01, Increased MMPs results in decreased levels of glutathione (GSH) signifiedin the current studies (MMP-2Vs. GSH, r= -0.681*). In endometriosis, the women in their peritoneal fluid have increased level of activated macrophages, which secrete a wide range of inflammatory cytokines such as TNF-α, Interleukins and VEGF [22, 34]. The cytokines are implicated in the regulation of tissue implantation and endometrial cells proliferation [35]. They are also linked with angiogenic activity in the women with endometriosis. The research carried out by Juha*et al*., [36] reviewed that the levels of interleukin -4, -6 and -10 in the serum of endometriosis patients and concluded that there are elevated level of these cytokines in endometriosis patients as compared to control individuals. This study is similar to the current work in which there are increased level of interleukin -4, -6 and -10

in endometriosis patients as compared to healthy individuals. According to the study of Nasser *et al*., [37], IL-13 is present in ectopic endometrium and expressed in peritoneal fluid of women with endometriosis. The increased serum expression of IL-13 ectopic endometrium indicates that cytokines play a critical role in various inflammatory and immune responses which is significant for endometriosis related infertility. TNF-α is the first cytokines that are produced by endotoxin-activated macrophages which trigger necrosis of tumors as well as differentiation of many target cells. It is pluripotent mediator and angiogenic cytokines which is implicated in the secretion of other cytokines [38]. In this study, there is elevated level of TNF-α in patients of endometriosis as compared to healthy individuals. Another set of study summaries that the levels of TNF-α were higher in the patients [39]. Present study indicated similar results and correlations,increased levels of proinflammatory cytokines remained responsible for increased ECM degradation (MMP-13 Vs. TNF-α, r= 0.633*).

Oxidative stress due to physically or emotionally stimuli may stimulates the neurons which release corticotrophin releasing hormone and resultantly increase serum cortisol concentrations. The growing body of evidence suggested that stress due to elevated levels of serum cortisol may inhibit NK cell activity. These NK cells have the susceptibility to influence cortisol levels and also involved in the suppression of immunity caused by oxidative stress [40]. The research carried out by Lima *et al*., [41] reviewed that there is elevated levels of serum cortisol in the patients with endometriosis as compared to control subjects. This work is similar to the present study, in which the level of cortisol is increase in endometriosis patients as compared to normal individuals. Vitamin D is fat soluble vitamin which is synthesized by sun exposure, food and supplements in the body system. It has a major role to inhibit proliferation, invasion and the synthesis of pro-inflammatory cytokines in patients with endometriosis. It is also involved to reduce the formation of interleukins which trigger the adhesion of endometrial cells in the peritoneal fluid [42]. In the present study there is reduced concentration of Vitamin D in the endometriosis patients as compared to control subjects. In pre-menopausal women, the estrogens can be formed in peripheral tissues and ovaries [43]. In endometriosis patients, there is abnormal level of estrogen

metabolizing enzymes are present that lead to the formation of increase Estradiol (E2) level. The elevated level of E2 is responsible for the proliferation of ectopic endometrium. The increase level of E2 has been observed in the menstrual blood of patients with endometriosis as compared to control subjects [44, 45]. Likewise, situation was observed in the current subjects. In patients of endometriosis levels of estrogen (E2) was increased as compared to healthy subjects which resulted in decreased levels of vitamin D (Estrogen Vs. Vitamin D, r=-0.621*).

CONCLUSION

Several lines of evidences indicate that significant decline in anti-oxidants may lead to enhancement in oxidative stress and consequently production of MDA, isoprostanes F2α, 8-OHdG in the patients of endometriosis. These markers are considered to be pro-mutagenic and carcinogenic. Inflammatory cytokines (TNF-α, Interleukins) are produced by activated macrophages which are implicated in the regulation of tissue implantation and endometrial cell proliferation.The abnormal level of estrogen metabolizing enzymes may lead to the increased formation of E2. That is responsible for the proliferation of ectopic endometrium.Hence, inflammatory mediators and oxidative stress profile must be examined at an early stage of disease to compensate the destructive effects.

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CONFLICT OF INTEREST

Authors declares no conflict of interest

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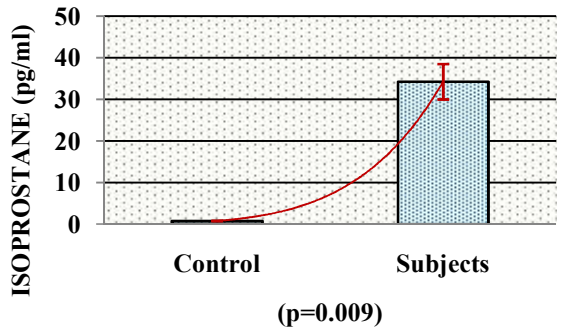

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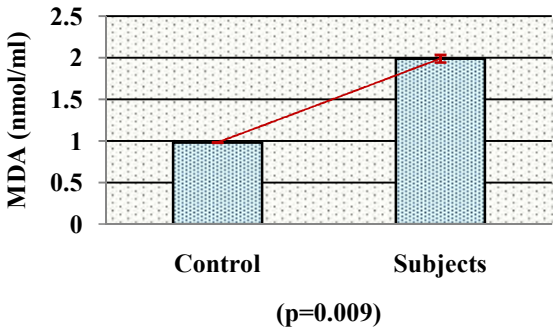

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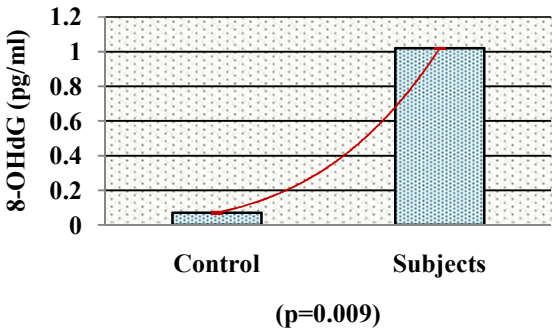

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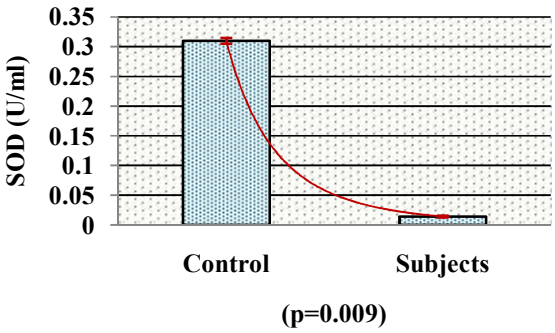

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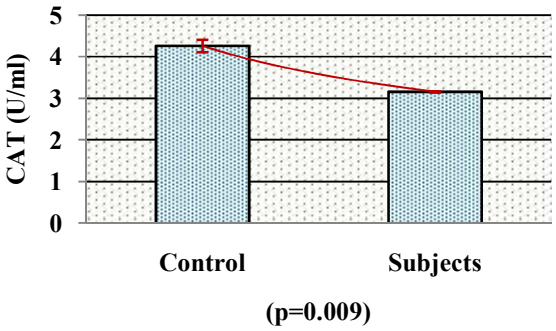

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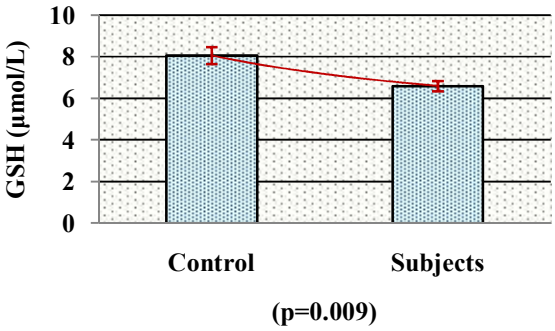
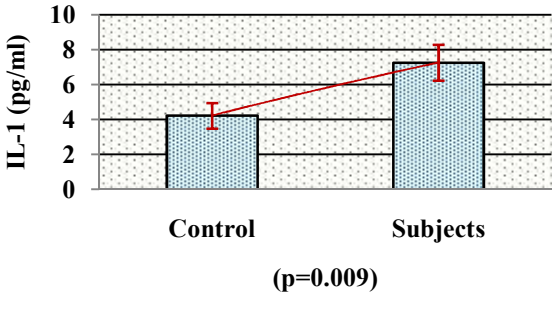


F

Figure 01: Levels of Oxidative Biomarkers and Its Medical Importance Having Role In The Development of Endometriosis



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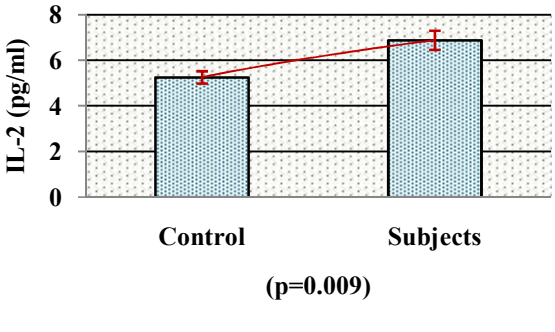

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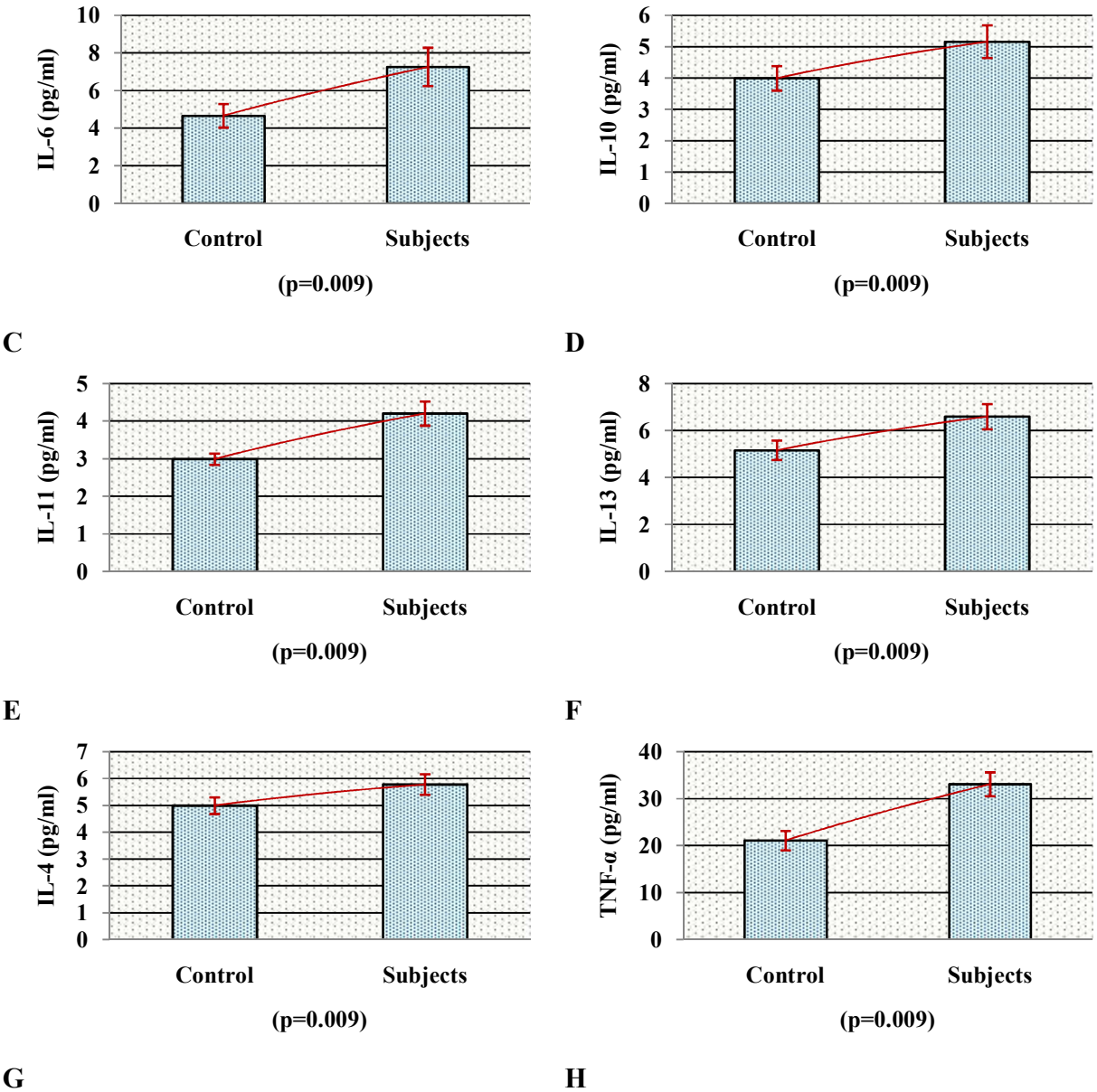


Figure 02: Levels of Inflammatory Biomarkers And Its Medical Importance Having Role In The Development Of Endometriosis

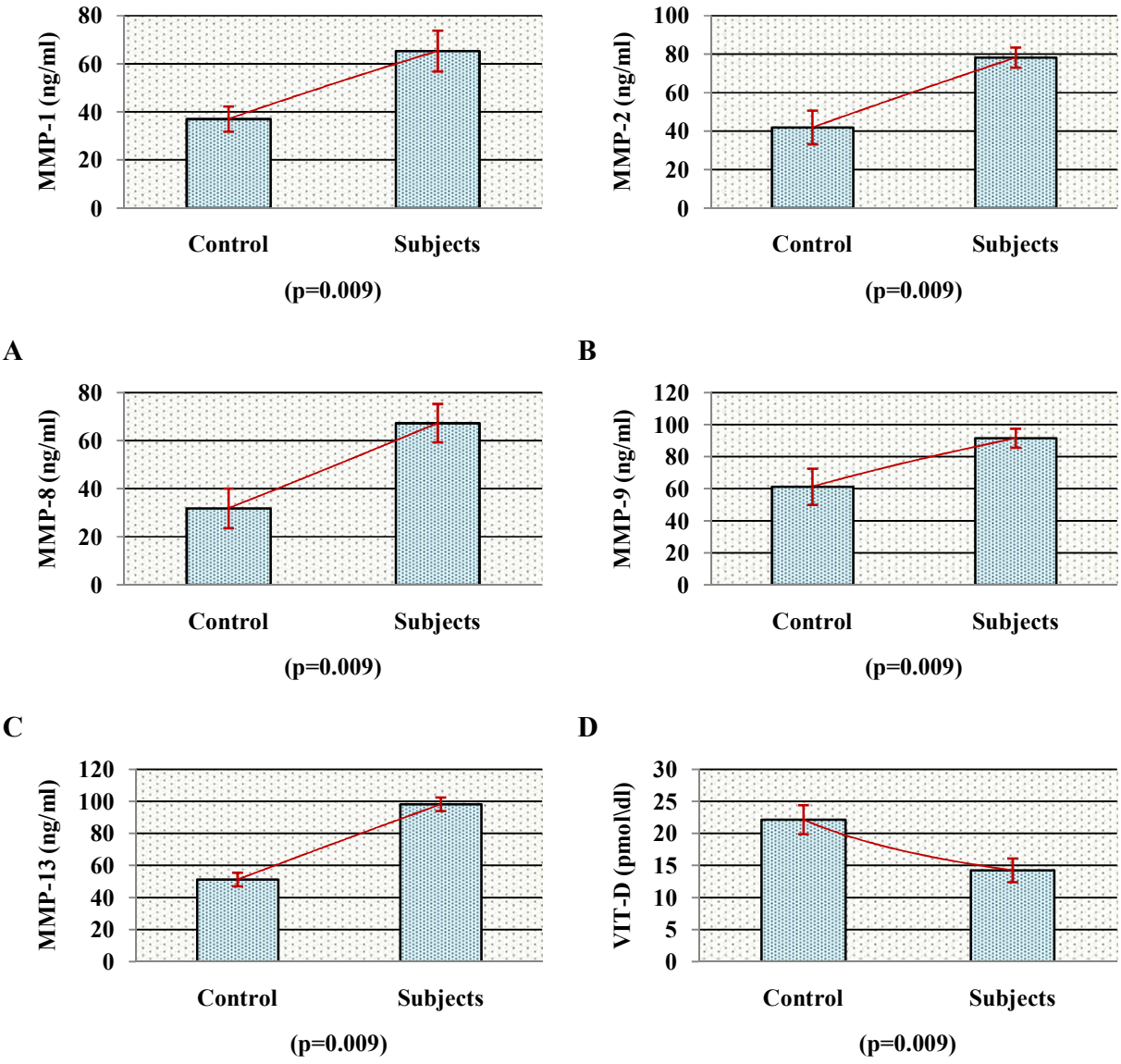
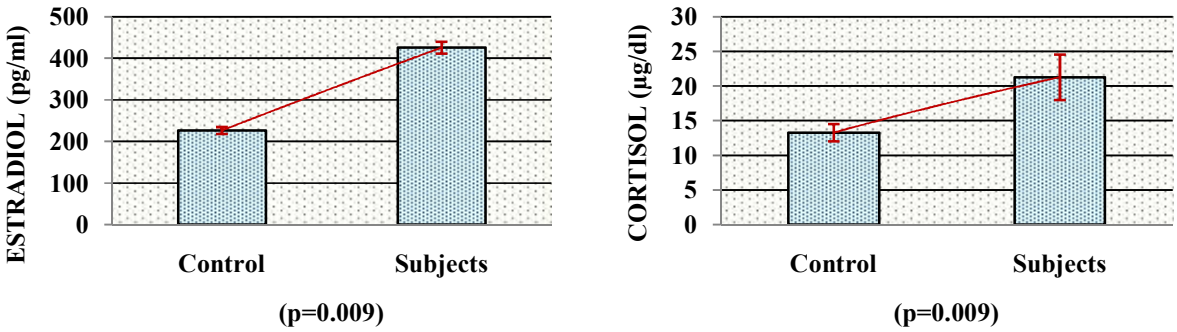


Figure 03: Levels of Matrix MetalloproteinasesOf Medical Importance Having Role In The Development Of Endometriosis



A **B**

Figure 04: Levels of Steroidal Hormone And Its Medical Importance Having Role In The Development Of Endometriosis

Table 1: Pearson s' correlation coefficients matrix of different variables of medical importance having role in the development of endometriosis

VAR.		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
MDA	**A**	1	0.169	0.257*	-0.07	0.175	-0.18	-0.061	0.149	0.075	-0.144	-0.093	0.082	-0.171	-0.110	-0.153	0.354*	0.671***	-0.134	0.242*	0.235	0.454	0.658
IsoP	**B**		1	-0.056	-0.04	-0.01	0.002	-0.036	-0.27	-0.409	-0.270*	0.686**	0.116	0.217	-0.086	-0.115	-0.011	0.249*	0.026	0.020	0.326	0.658	0.568
8-OHdG	**C**			1	-0.27	-0.19	0.265	0.165*	0.593*	-0.235	0.043	-0.099	0.301*	0.125	0.002	-0.173	-0.179	-0.564*	-0.079	-0.211	0.265	0.745	0.356
SOD	**D**				1	-0.07	-0.13	0.297*	0.100	-0.258	-0.035	-0.037	0.073	0.172	0.139	0.090	-0.097	-0.187	0.253*	-0.020	0.125	0.265	0.254
GSH	**E**					1	-0.07	0.092	-0.033	-0.005	-0.206	-0.104	-0.070	-0.166	-0.015	-0.030	-0.681*	0.286*	-0.117	0.232	0.356	0.235	0.235
CAT	**F**						1	0.018	-0.121	-0.079	0.152	0.131	-0.022	-0.052	0.175	0.018	-0.156	-0.254*	-0.159	-0.125	0.452	0.235	0.356
IL-1	**G**							1	0.212*	-0.285	0.454**	-0.188	0.125	-0.041	0.293*	-0.381	-0.519*	-0.686*	0.533*	0.612*	0.262	0.235	0.356
IL-2	**H**								1	0.266*	0.657*	0.174	0.093	0.135	-0.654	0.097	-0.170*	-0.325*	0.892*	0.234*	0.356	0.265	0.356
IL-6	**I**									1	-0.512*	0.529***	0.523*	0.670*	0.341*	0.141	-0.018	-0.498*	-0.170	-0.031*	0.356	0.235	0.235
IL-10	**J**										1	-0.65**	-0.228	-0.246*	-0.710	-0.171	-0.174*	0.432*	0.255*	0.101	0.156	0.235	0.325
IL-11	**K**											1	-0.241	-0.226	-0.129	0.093	-0.010	-0.127	-0.068	0.045	0.654	0.658	0.353
IL-13	**L**												1	0.591**	0.633*	-0.115	-0.111	0.073	0.027	-0.161	0.125	0.235	0.325
IL-4	**M**													1	0.219	0.041	0.021	-0.153	-0.076	-0.054	0.654	0.235	0.235
TNF-α	**N**														1	-0.065	0.056	-0.542*	-0.321	0.633*	0.235	0.245	0.356
MPP-1	**O**															1	0.020	-0.044	0.255*	0.302*	0.425	0.25	0.235
MPP-2	**P**																1	0.276*	-0.319	-0.392*	0.125	0.658	0.658
MPP-8	**Q**																	1	-0.101	-0.108	0.356	0.458	0.458
MPP-9	**R**																		1	0.110	0.235	0.252	0.568
MPP-13	**S**																			1	0.265	0.234	0.231
ESTRADIOL	**T**																				1	0.235	-0.621*
CORTISOL	**U**																					1	0.235
Vit-D	**V**																						1