Original Article

The association between hormonal variation, antioxidant status and oxidative stress in Iraqi women with endometriosis

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Abstract:

Background: In the last years, Endometriosis affects up to 10 percent of reproductive aged Iraqi women. Little is known about the etiology of endometriosis in Iraqi women.

Objectives: The aim of this study is to investigate the hormonal changes, antioxidant status, Coenzyme Q_{10} and oxidative stress in women patients with endometriosis.

Patients and methods: 30 endometriotic women mean aged (31.16±5.61 year) who were undergoing laparoscopy to participate in this study, and (n=30) healthy women volunteers mean aged (31.96±5.42 year) as control group.

Results: The results in this study revealed a highly significant increased ($P<0.01$) in estradiol (E_2), uric acid (UA) and oxidative stress (MDA). The results of the Coenzyme Q_{10} (CoQ_{10}) and vitamins (A, E, β-carotene, C) showed a highly significant decreased ($P<0.01$) in the sera levels of patients with endometriosis as compared to control. This study showed, there was a significant negative correlation between the levels of Luteinizing Hormone (LH), Follicle Stimulating Hormones (FSH), Testosterone (Test.), E_2 and vitamins, whereas a significant positive correlation was observed in UA level with concomitant increase in MDA levels.

Conclusions: The results of this study concluded the increase of oxidative stress and the decrease of antioxidant vitamins and CoQ_{10}. Thus increasing the growth and adhesion of endometrial cells in the peritoneal cavity, which will lead to increase the risk factors of endometriosis.

Keywords: Endometriosis, antioxidants status, oxidative stress, hormone changes and CoQ_{10}.

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Introduction:

Worldwide, approximately 176 million women between the ages of 15 and 49 are affected by endometriosis (1). Endometriosis is one of the most frequent benign chronic gynecological disorders, and it influences female health negatively by causing abdominal pelvic pain and infertility. It has been estimated that endometriosis affects approximately 10% of women of reproductive age and up to 50% of infertile women (2). FSH is a major promoter for orchestrating follicular development and differentiation in the granulosa cells of preovulatory follicles. LH plays a key role in initiation of the ovulatory process of preovulatory follicles by activating multiple cellular signaling pathways (3). Estrogen has been shown to play a critical role in the growth of endometriotic tissues (4). The role of antioxidants in endometriosis is controversial, mainly because of contradictory views expressed by various authors. Antioxidant systems are known to protect tissues from the adverse effects of free radicals. A lower total antioxidant potential has been found in the peritoneal fluid of infertile women with endometriosis compared with women with idiopathic infertility (5). Reactive oxygen species (ROS) can affect a variety of physiological functions in the reproductive tract, and excessive levels can result in precipitous pathologies affecting female reproduction. ROS production may be amplified in the setting of endometriosis due to menstrual reflux, which subjects the peritoneal cavity to pro-inflammatory hemoglobin and heme molecules released from transplanted erythrocyte debris. Peritoneal fluid containing ROS - generating iron, macrophages, and environmental contaminants such as polychlorinated biphenyls may disrupt the oxidant/ antioxidant balance, resulting in increased proliferation of tissue and adhesions (6). CoQ_{10} (2 ,3-dimethoxy-5-methyl-6-decaprenyl benzoquinone), also known as ubiquinone, is a lipid soluble component of all eukaryotic cells, It is an endogenously synthesized provitamin serves as a lipid soluble electron carrier in the mitochondrial electron transport system, as well as an important intracellular antioxidant (7). The objectives of this study therefore

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was to determine the levels of (LH, FSH, Test. and E_2) in endometriosis patients which will help to assess the contribution of endocrine abnormalities and antioxidant status (A, E, β-carotene, C) vitamins to endometriosis in the study population. And estimated the CoQ_{10} as an index of antioxidant capacity and attempted to find the correlation between all these parameters with lipid peroxidation, given by oxidative stress (MDA) level's determination.

Patients and Methods:

The present investigation was carried out at departments of Gynecology and Obstetrics related to the following hospitals: Baghdad Teaching Hospital-Medical city and Kamal Al-Samarray Hospital from January to July 2015, in Baghdad, Iraq. The data collected from 30 women patients as referred by a laparoscopy for endometriosis investigation mean aged (31.16±5.61) year, a total of 30 healthy females volunteers mean aged (31.96±5.42) year served as control. To eliminate the factors which might affect this study, we excluded 30 women with smoking, and suffering from chronic or acute diseases, such as hypertension, diabetes mellitus, diseases of the liver, kidney and all patients with hormonal therapy treatment.

Anthropometric Measurements: Body mass index (BMI) was calculated as weight (kg) divided by the height^2 (m^2). Patients were taken as obese if their body mass index was 29.9 (8). Waist:hip ratio (WHR) was calculated by dividing waist by hip measurements (9).

Analysis of Samples:

Specimen collection: Fasting blood samples (10 mL) were collected and placed into containing tubes during 2-5 days of the menstrual cycle. After centrifugation at 1500×g for 10 min. the sera were removed and retained for assay of the level of vitamin C and all the parameters, respectively. Serum samples were stored at -80C° until analysis.

Laboratory assessments: Serum concentration of LH, FSH, Test. and E_2 levels were measured by mini-VIDIS assay using kit supplied by Bio Merieux Sa-France. Uric acid was measured with an enzymatic colorimetric assay using a kit supplied by Cromatest-Spanish. Coenzyme Q10 was measured with a competitive inhibition enzyme immunoassay technique using a kit supplied by HCUSABIO-China. The vitamins (A, E & β-carotene) were determined by a reverse-phase HPLC technique consisting of a Shimadzu-Japan binary solvent metering pump, a Rheodyne7125 injector with a 150µL sample loop, column (25cm×4.6mm inner diameter), 5µm partical size, injection volume 50 µL and 10AV UV–Visible detector operating at λ_{max} 280 nm and 450 nm (10-11). Vitamin C determination is based on the oxidation of ascorbic acid in serum by Cu^{2+} to form dehydroascorbic acid that react with the acidic 2,4-dinitrophenyl hydrazine to form a red bis-hydrazone which is measured as (A 520 nm) (12). Malondialdehyde formed from the breakdown of poly unsaturated fatty acids serves as a convenient index of peroxidation reaction. The thiobarbituric acid method of (13), was used to measure MDA.

Statistical analysis: All data were expressed as mean ± standard deviation (mean ± SD). Statistical analysis was performed using LSD, considering $p<0.05$ as the lowest limit of significance. Statistical analysis was performed using a software program (SPSS 13 for Windows, USA). One-way analysis of variance (ANOVA) was used to compare means with least significant difference (LSD).

Results:

Table1 showed the demographic and hormonal characteristics in endometriotic patients and control group. There was non significant difference in the age of endometriosis patients when compared to control group. While there was a highly significant increases ($p<0.01$) in BMI, W/H ratio and E_2. Non significant differences were detected in LH, FSH, LH/FSH ratio and Test. levels. Table2 revealed all vitamins (A, E, β-carotene, C and CoQ_{10}) displayed a highly significant decreased ($p<0.01$), while a highly significant increased ($p<0.01$) was observed in the levels of serum uric acid and MDA in women with endometriosis compared to control group. Table3 showed the results of correlation between oxidative stress index (represented by MDA level) and concentration of hormones, antioxidant vitamins and uric acid in endometriosis patients. There was a significant negative correlation between the levels of LH, FSH, Test., E_2, vitamins A, E, β-carotene and C, whereas a significant positive correlation was observed between uric acid and CoQ_{10} with concomitant increase in MDA levels.

Table1. Demographic and hormonal characteristics in endometriosis patients and control group.

Parameters	Control (N=30) Mean ± SD	patients (N=30) Mean ± SD	P-Value
Age (years)	31.96±5.42	31.16±5.61	NS
BMI (Kg/m^2)	25.30±2.66	28.94±3.77	0.001**
Waist/Hip ratio	0.79±0.08	0.90±0.11	0.001**
LH (mIU/mL)	4.96±2.40	5.80±2.79	NS
FSH (mIU/mL)	6.60±1.84	7.68±3.61	NS
LH/FSH	0.75±0.19	0.75±0.15	NS
Test. (ng/mL)	0.27±0.11	0.33±0.16	NS
E_2(pg/mL)	51.18±14.44	72.34±13.4	0.001**

NS: Non significant, **the mean difference is a highly significant at the $p< 0.01$ level.

Table2. Serum levels of antioxidant status and MDA in endometriosis patients and control group.

Parameters	Control (N=30) Mean ± SD	patients (N=30) Mean ± SD	P-Value
Vit. A (mg/dL)	0.04±0.01	0.02±0.00	0.001**
Vit. E (mg/dL)	0.98±0.29	0.73±0.17	0.001**
β-caroten (mg/dL)	0.17±0.05	0.10±0.03	0.001**
Vit. C (mg/dL)	1.68±0.20	1.07±0.19	0.001**
CoQ_{10} (ng/mL)	42.31±3.64	30.12±9.67	0.001**
UA (µmol/L)	232.43±30.70	367.27±27.59	0.001**
MDA (µmol/L)	0.65±0.12	1.74±0.39	0.001**

**the mean difference is a highly significant at the p< 0.01 level.

Table3. Correlation coefficients and the significant levels of different serum chemical components in patients with endometriosis.

Component vs. MDA	Slope	Intercept	R^2	r	P-Value
LH (mIU/mL)	-0.085	2.163	0.193	-0.440*	0.028
FSH (mIU/mL)	-0.059	2.118	0.161	-0.401*	0.038
LH/FSH	-0.708	2.192	0.016	-0.127	NS
E_2 (pg/mL)	-0.010	2.635	0.166	-0.408*	0.031
mL)/Test. (ng	-1.483	2.475	0.178	-0.423*	0.020
Vit. A (mg/dL)	-39.85	2.354	0.192	-0.439*	0.028
Vit. E (mg/dL)	-1.163	2.620	0.168	-0.411*	0.033
β-caroten (mg/dL)	-9.338	2.497	0.218	-0.468*	0.024
Vit. C (mg/dL)	-1.289	2.992	0.314	-0.561**	0.003
CoQ10 (ng/mL)	0.042	0.551	0.324	0.569**	0.007
Uric acid (µmol/L)	0.005	-0.471	0.118	0.438*	0.032

NS: non significant, *Correlation is significant at the p<0.05 level, **correlation is a highly significant at the p< 0.01 level.

Discussion:

Obesity plays an important role in endometrial proliferation and the most important study that examined the relationship between BMI and endometrial thickness and suggested the presence of a significant relationship between BMI and endometrial thickness is a prospective cross-sectional study conducted by (15). Hediger and coworkers found a greater waist to hip ratio is associated with risk of endometriosis (16). Our results were in agreement with a study done by Hsin et al., which reported in there study, there was no significant changes in FSH and LH levels (14). Endometriosis is a hormonally dependent disease and as a result is chiefly found in reproductive aged women (17). Estrogen plays many roles throughout a woman's body and in her cycle, yet one of the most important ones is to stimulate the thickening of the endometrium. This is an important, normal process that happens in all women. If estrogen levels are high, however, the endometrium grows very thick, and much larger than normal. This provides a 1) more tissue out of which ectopic endometria can be made and 2) more tissue from which prostaglandins and other inflammatory markers can be released. Increased exposure to estrogens is a common link among several known risk factors for endometriosis (18). All of the different forms of estrogen are synthesized from androgens, specifically testosterone and androstenedione, by the enzyme aromatase. The significantly increased in E_2 levels in this study was agreement with Bulun study which found an increased level of E_2 in endometriosis patients that caused by increased expression of aromatase (CYP19A1, which catalysis conversion of androstenedione to estrone (E_1)), 17β-hydroxysteroid dehydrogenase type 1 ((HSD17B1), which catalyzes conversion of E_1 to E_2) (19). Estrogens have been shown to have in vitro antioxidant effects on membrane phospholipid peroxidation (20). The antioxidant activity of estrogens is associated with the phenol structure of E_2 and its metabolites (21). Sex hormones are influenced by diet in many ways. A prime example is estrogen and conditions related to estrogen/progesterone imbalance, including hormonal cancers, endometriosis and infertility. Hormonal balance can be improved through nutritional and lifestyle interventions. Antioxidants may enhance specific pathways of estrogen metabolism and detoxification (22). Antioxidants properties of nutritional supplementation are associated with a significant reduction in inflammatory endometriosis-related markers, and with a suppressive effect on the endometrial-cell survival in vitro (23). ROS may affect the growth of endometrial tissue. The presence of endometriosis increased oxidative stress and depletion of antioxidants may contribute to excessive growth of endometrial cells. Oxidative stress is thought to promote angiogenesis and the growth and proliferation of endometriotic implants. Our results were in good agreement with study has shown decreased levels of oxidative stress markers in people who consume antioxidant rich diets or take antioxidant supplements (24). In certain populations, women with endometriosis were observed to have a lower intake of vitamins A, C and E than fertile women without the disease (25). In other study vitamin E is limit the proliferation of endometriotic cells (23). Uric acid is the major antioxidant in human plasma, it is correlates and

predicts in conditions associated with oxidative stress like obesity. The rise of uric acid represents an attempted protective response by the host, so the uric acid may function either as antioxidant (primarily in plasma) or pro-oxidant primarily within the cell (26). Therefore, we suggest that uric acid may have a contributory role in the pathogenesis of endometriosis. Indeed, uric acid is the most abundant aqueous antioxidant, accounting for up to 60% of plasma antioxidative capacity. The antioxidative effect of uric acid is evidenced by its ability to directly scavenge free radicals or to form stable complexes with transition-metal ions, such as iron, thereby preventing ascorbate oxidation and lipid peroxidation (27). This study was carried out to investigate the role of CoQ_{10} and oxidative stress in endometriosis patients. CoQ_{10} is believed to exert its effects via three main mechanisms. First, it participates in oxidative phosphorylation as a coenzyme for three critical mitochondrial enzyme systems, complexes I, II, and III. To a lesser degree, free CoQ_{10} in the cytosol may also contribute to electron transfer outside of the mitochondria as well. By increasing ATP production, it is thought to improve energy function in tissues with high oxidative demands. Second CoQ_{10} has significant antioxidant activity. It exists in the cell in both oxidized and reduced forms and is one of the few substances for which there are enzymes whose sole function is to restore their reduced state. CoQ_{10} also serves to restore oxidized α-tocopherol and thus is important for the function of this important antioxidant as well. Finally, due to its lipid solubility, it is present in the cell membrane phospholipid layer and may influence membrane stability as well (28). LH was ascertained to be correlated negatively with MDA level, suggesting antioxidative, protective role in the reproductive system of these patients. The protective role of LH may be produced through the action of estradiol (31). FSH are produced from the pituitary gland which stimulates ovary to develop and mature follicle. Many studies done earlier have shown that the increased level of reactive oxygen species play a critical role in the folliculaogenesis as well as corpus lutea function such as (32). This result is in agreement with current study an inverse negative relationship between FSH and MDA level in endometriotic patients. Our findings suggest that ovarian E_2 production may play an important antioxidant role, an inverse correlation between E_2 and lipid peroxide production with aging have been reported (33). A positive association between oxidative stress and endometriosis (29). Traberta et al., found increased β-carotene consumption was associated with increased endometriosis risk (30).

Conclusion:

from all theafore mentioned observations it can be concluded that increased generation of reactive oxygen species and concomitant impairment of the antioxidant system occurs in patients with endometriosis. These findings indicate that sex hormones have a strong impact on oxidative stress and endometriosis. This suggests that oxidative stress might play a role in the development and progression of endometriosis as well as results of the present study showed the clear decline in the level of coenzymeQ_{10} , may contribute to the risk of women with endometriosis.

Authors Contributions:

Tamara S. Naji: Collecting samples, analysis of data and writing the manuscript.

Salwa H. N. Al- Rubae'i: Designer research, analysis of data and performed statistical analysis.

Kisma M. Turki: provided essential materials/ reagents and revision of the writing.

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