

## Study The Role of Paraoxonase, Ceruloplasmin and Estrogen in Sera of Endometriotic Patients in Baghdad.

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**Abstract:** Endometriosis is a chronic inflammatory autoimmune disease, is among one of the most challenging of the 21st century that affects women in reproductive age. Seventy five consecutive married women endometriotic patients with age range (25-40) year were enrolled in this study, divided into three groups, the first included (25) newly diagnosed endometriotic patients (without any treatment), the second consisted of twenty five endometriotic patients who were treated with zoladex for 3 to 5 months, the third involved twenty five patients with recurrent endometriosis (post treatment of zoladex) for one to two years ago. Patients groups were compared with two matched age and sex control groups, control group included twenty five healthy women while pathological control group involved twenty five women suffering from infertility caused by gynecological disorders not linked with endometriosis. The present study highlights the role of Paraoxonase, Ceruloplasmin and estrogen in the pathogenesis of endometriosis. The present study have reported that PON-1 activity was low in sera of patients while it was increased after treatment. Furthermore, this is the second study that proves a positive relation between CP and endometriosis and the first dealing with zoladex role on decreasing CP level in endometriosis. Lastly, estrogen was higher in endometriosis compared with control and pathological control groups, while it was decreased by zoladex action.

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### I. Introduction

Endometriosis is a common benign chronic – inflammatory gynecologic disorder (1,2), defined as the presence and proliferation of functional endometrial glands (endometrial - like tissue) outside the normal location (uterine cavity) (3), mainly causes pain and infertility (4). Endometriosis gets its name from the word endometrium, which is the tissue lining the uterus (womb) (5). The humoral immune response may explain endometriosis in general terms, in accordance with the characteristics of an autoimmune disease (6). It has been suggested that increased oxidative stress appears to be common contributory factor in the pathogenesis of endometriosis, as well two recent studies have indicated the important role of oxidative stress besides inflammation in the pathogenesis of endometriosis (7).

Broadly, the pathophysiological mechanism of endometriosis is unknown, but recent studies suggest a link with oxidative stress. Paraoxonase1 (PON-1) is an antioxidant serum enzyme which circulates associated with HDL (High Density Lipoprotein), and prevent oxidative modification of low-density lipoproteins (LDL) (8). It hydrolyzes various arylesters, lactones and organophosphate compounds. Its name derives from paraoxon, a metabolite of an ordinary pesticide called parathion, which is hydrolyzed by PON-1 with modest catalytic efficiency. The PON-1 has an appreciable arylesterase activity, being phenylacetate the typical substrate, enzyme commission number related to PON-1 is 3.1.1.2 (8). Serum paraoxonase-1 (PON-1) is an antioxidant enzyme which exerts its direct and indirect effects through HDL and LDL (9). Ceruloplasmin (CP) is an abundant protein of the  $\alpha_2$ -globulin fraction of human blood serum that contains seven copper atoms per molecule and accounts for 95% of the total circulating Cu in healthy adults. The polypeptide chain has a total of 1,046 amino acid residues (MW 120,085) and has attachment sites for four glucosamine oligosaccharides; together these account for the total molecular mass of human ceruloplasmin (132 kDa) (10). The most important role of CP is Cu transport. CP is a positive acute-phase protein, which means that its level in plasma is elevated in disorders accompanied by inflammation, infection, trauma, injury...etc (11, 12). Estrogen is a steroid hormone, synthesized in ovaries and placenta, transported by the blood from its site of synthesis to its target organs. Because of their hydrophobicity, they must be complexed with a plasma protein (13).

### II. Materials and methods

#### Patients selection and sampling:

In the present study, (75) consecutive married infertile women of reproductive age (25-40) years were enrolled, who attended Department of Gynecology & Obstetrics related to the following hospitals: Baghdad Teaching Hospital, Al-Yarmook Teaching Hospital and Kamal Al-Samarray Hospital / Baghdad. They have undergone either laparoscopy or laparotomy, moreover diagnosis of patients groups was confirmed by histopathology after surgical exploration. First group (G1) included (25) newly diagnosed endometriotic

patient, they were not given any treatment related to Gynecology or anti-inflammatory medications, while the second group (G2) consist of (25) endometriotic patient who were treated with zoladex for 3 to 5 months, they received Goserlin acetate (Zoladex) depot injection (3.6 mg) subcutaneously in the lower abdomen every 28 days after the first day of diagnosis. Finally, third group (G3) involves (25) patients of recurrent endometriosis, they were post treatment of Goserlin acetate (Zoladex) for 1 to 2 years ago, but symptoms of the disease are returned again and diagnosis revealed recurrence of endometriosis. The three groups of patients were compared with two control groups, also with age in range (25-40) years. The first (healthy control or C) include (25) healthy women and the second (Pathological control or PC) involved (25) women who were undergone laparoscopic diagnosis for infertility in Baghdad Teaching Hospital, and laparoscopy revealed that infertility is caused by pathological conditions unrelated to endometriosis just like tubes blockage and retroverted uterus. Five milliliters (5 mL) of venous blood were collected from all subjects enrolled in this study (from 23 April 2013 to the end of October 2013), placed into plain tubes until coagulation was performed. Serum was separated from blood cells by centrifugation at 4000 r.p.m for 3 min, subsequently serum was divided into small portions and kept frozen (-20°C) until analysis.

#### **Laboratory tests:**

Paraoxonase (PON) was determined by quantitative sandwich enzyme linked immunosorbent assay (ELISA) technique. Antibody specific for PON has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-36 $\gamma$  present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-36 $\gamma$  is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-36 $\gamma$  bound in the initial step. The color development is stopped and the intensity of the color is measured. Ceruloplasmin (CP) was determined by immunosorbent assay. The procedure consisted of an immunoprecipitation in a gel between an antigen and its homologous antibody. Reacted. Estrogen was determined by a Single ELISA method. This include antibody, enzyme – antigen conjugate and native antigen.

#### **Statistical Analysis:**

The results expressed as mean  $\pm$  SEM. Student's t-test was applied to compare the significance of the difference between all the studied groups. P-value ( $p < 0.05$ ), ( $p < 0.001$ ) and ( $p > 0.05$ ) considered statistically significant, highly significant and non-significant respectively. The correlation coefficient (r) test is used for describing the association between the different studied parameters.....

### **III. Results**

Paraoxonase-1 activity in sera of patients and control groups was shown in table (1). Results have revealed that PON-1 activity was highly significant decreased ( $p < 0.001$ ) in sera of G1 (780.3 $\pm$ 22.0) mLU/mL compared with groups C (1037.8 $\pm$ 59.0) mL U /mL and PC (996.6 $\pm$ 68.9) mLU/mL, while a significant increase ( $p < 0.05$ ) was observed in G2 (890 $\pm$ 41.7) mLU / mL compared with G1 (780.3 $\pm$ 22.0) mLU/mL. Conversely, a significant decrease ( $p < 0.05$ ) was noticed in sera of G2 (890.6 $\pm$ 41.7) mL U/mL compared with group C (1037.8 $\pm$ 59.0) mL U /mL. Also there was a non-significant difference ( $p \geq 0.05$ ) in G3 (779.2 $\pm$ 17.6) mL U / mL compared with G1(780.3 $\pm$ 22.0) mL U / mL. Results also have revealed that CP levels were significantly increased ( $p < 0.05$ ) in sera of G1 (4.9 $\pm$ 1.9) g/L compared with groups C (25.1 $\pm$ 1.9) g/L and PC (25.2 $\pm$ 0.4) g/L, while a significant decrease ( $p < 0.05$ ) was noticed in G2 (25.9 $\pm$ 2.6) g/L compared with G1(49.6 $\pm$ 1.9) g/L. Also there were non-significant differences ( $p < 0.05$ ) between G2 (25.9 $\pm$ 2.6) g/L and group C (25.1 $\pm$ 1.9) g/L and between G3 (35.6 $\pm$ 4.1) g/L and G1(49.6 $\pm$ 1.9). Estrogen levels in sera of studied groups were also shown in table (1). Our results have reported that E2 levels were highly significant increased ( $p < 0.001$ ) in sera of G1 (86.5 $\pm$ 11.7) pg/mL compared with groups C (38.6 $\pm$ 3.0) pg/mL and PC (44.8 $\pm$ 3.3) pg/mL, while a significant decrease ( $p < 0.05$ ) was noticed in G2 (46.3 $\pm$ 2.8) pg/mL compared with G1(86.5 $\pm$ 11.7) pg/mL. Also, there were a non-significant difference ( $p \geq 0.05$ ) in G2 (46.3  $\pm$ 2.8) pg/mL compared with group C (38.6 $\pm$ 2.0) pg/mL and in G3 (65.2 $\pm$ 4.6) pg/mL compared with G1 (86.5 $\pm$ 11.7) pg/mL.

**Table 1:** PON activity , CP and Estrogen levels in sera of the five groups enrolled in this study.

Parameter	Mean ±SEM C	Mean ±SEM PC	Mean ±SEM G1	Mean ±SEM G2	Mean ±SEM G3	C vs G1 T.Test	PC vs G1 T.Test	G1 vs G2 T.Test	CvsG2 T.Test	G1vs G3 T.Test
PON-1	1037.8 ±59.0 mL U/ mL	996.6 ±68.9 mL U/ mL	780.3 ±22.0 mL U/ mL	890.6 ±41.7 mL U/ mL	779.2 ±17.6 mL U/ mL	H.S	H.S	S	S	N.S
CP	25.1 ±1.9 (g/L)	25.2 ±0.4 (g/L)	49.6 ±1.9 (g/L)	25.9 ±2.6 (g/L)	35.6 ±4.1 (g/L)	S	S	S	N.S	N.S
E2	38.6 ±3.0 (pg/mL)	44.8 ±3.6 (pg/mL)	86.5 ±11.7 (pg/mL)	46.3 ±2.8 (pg/mL)	65.2 ±4.6 (pg/mL)	H.S	H.S	S	N.S	N.S

#### IV. Discussion

Paraoxonase is a calcium-dependent esterase with 43KDa molecular weight and 354 amino acids residue which is found exclusively associated with HDL in serum. PON protects LDL from oxidative stress by destroying biologically active phospholipids (14 , 15 , 16) A recent study has observed lower serum PON-1 activity and HDL levels in the endometriosis group compared with the control group. Serum PON-1 is an antioxidant enzyme associated with HDL which prevents development of oxidative changes of LDL. Besides blocking oxidation of HDL , PON-1 substantiates the anti-atherogenic role of HDL in the reverse cholesterol transport. It also facilitates faster clearance of LDL. Therefore, with PON-1 deficiency , the impact of oxidative stress increases , while levels of HDL decrease (9). This study is in agreement with the high significant increase of PON-1 in G1 compared with group C.

On the other hand , the high significant increase of PON-1 in G1 compared with PC group indicates the severity of oxidative stress in endometriotic patients compared with other infertile women. However , infertility may be caused by hormonal , immunological abnormalities besides the effect of oxidative stress on female infertility (17). Indeed , Oxidative stress occurs when there is an imbalance in the interplay between reactive oxygen species (ROS) and the antioxidant defense systems whereby the oxidants super cedes the antioxidants. One of the most important oxidative processes is oxidation of lipids and lipoproteins. Oxidized low-density lipoprotein (Ox-LDL) induces foam cell formation from macrophages that plays a key role in early atherogenesis. Under oxidative stress , not only LDL , but other serum lipids are exposed to oxidation. As it is well established PON-1 activity is decreased in dyslipidemia accompanied by higher ox-LDL levels (17). Moreover , a recent study has demonstrated that PON-1 acts as protecting HDL and LDL of lipid peroxidation , degrading cholesterol esters oxidized lipids and phospholipids present in the lipoproteins, erythrocytes and macrophages. PON-1 may otherwise be inactivated by oxidized lipids. Furthermore , PON-1 inhibits cholesterol biosynthesis by macrophages by stimulating cholesterol efflux (8). Some authors have also suggested the possibility of endometriosis is a disease caused by or associated with oxidative stress . In the presence of pelvic endometriosis, there was activation of macrophages in the peritoneal cavity, which could promote increased production of reactive oxygen and nitrogen species and, consequently oxidative stress, resulting in lipid peroxidation, its degradation products and the products formed by its interaction with low-density lipoproteins and other proteins. Oxidative stress also damages mesothelial cells and can induce the appearance of adhesion sites for endometrial cells, favoring the development and progression of endometriosis (8). In fact , The excess production of ROS is accompanied by a decreased level of antioxidants that usually eliminates these molecules. Resulting accumulation of reactive oxygen species ROS may contribute to the propagation and maintenance of endometriosis and associated symptoms (18).

The significant increase of PON-1 in G2 compared with G1 indicates that zoladex has a crucial role in alteration of PON-1 activity but the significant decrease of PON-1 in G2 compared with group C reveals that PON-1 activity was not returned to the normal value in control subjects, although it was altered. In this regard , PON-1 is associated with HDL in serum , thus any change in HDL levels in serum can alter PON-1 activity. Ultimately , the non-significant difference of PON-1 activity in G3 compared with G1 reports that endometriosis is a recurrent disease (19). It has been reported that 47% of the endometrial lesions appear again after removal of endometriotic tissue in patients (20).

A recent study has reported relationships between inflammatory diseases and raised plasma concentrations of inflammatory markers such as CP , thus CP might be a mediator in a specific inflammatory pathway that causally links inflammatory diseases (21) , according to this study ceruloplasmin has been reported to possess both oxidative and anti-oxidative functions. It was shown that ceruloplasmin catalyses the oxidation of Fe<sup>2+</sup> to Fe<sup>3+</sup> , as well as the oxidation of Cu<sup>1+</sup> to Cu<sup>2+</sup>. The reaction reduces O<sub>2</sub> to H<sub>2</sub>O without releasing superoxide or hydrogen peroxide. This so-called ferroxidase activity is essential for iron homeostasis and

is thought to be responsible for the ability of ceruloplasmin to block free radicals-induced proteolysis and DNA damage. The ferroxidase activity is increased, for example, during inflammation and infection. By contrast, ceruloplasmin has been reported to possess oxidative effects and may induce LDL oxidation. As an acute phase reactant, its concentrations are commonly raised in patients with chronic diseases (22).

However, the significant decrease of CP in sera of G2 compared with G1 and the non-significant difference between G2 and group C indicate the reactive role of zoladex in alternating CP levels. The levels of CP may be expected to decrease simultaneously with oxidative stress after treatment of endometriosis aimed at preventing oxidative damage. CP also may be useful for monitoring endometriosis during pharmacology therapy (9). Indeed, elevation of CP levels in G1 revealed that CP has prooxidative activity in patients related to G1. Interestingly, zoladex has increased HDL-c levels and PON-1 activity for the same patients, this means that zoladex was able to induce parameters which have antioxidant properties. Therefore, it is expected that zoladex inhibits parameters have oxidative properties such as CP. Lastly, the non-significant differences between G1 and G3 indicate that endometriosis is a recurrent disease (19), because oxidative damage was come back.

Endometriosis is a complex and challenging disease that involves aberrant adhesion, growth, and progression of endometrial tissues outside of the uterine cavity, and there is evidence to suggest that estrogen plays a key role in development, progression and growth of endometriosis (20). Estrogen promotes the survival and persistence of endometrial lesions, as may altered immune and inflammatory processes. Numerous in vivo clinical studies have described the ectopic expression and regulation of estrogen receptor (ER) in the different types of endometriosis compared to normal or eutopic endometrium. Endometriosis is a proliferative, estrogen-dependent disorder (lesions are estrogen-stimulated) (20).

Other research demonstrated that estrogen is the driven force of endometrial proliferation and ectopic lesions may have an increased responsiveness to estrogen, thus enhancing the development of endometriosis (23). Consequently, infertility disorders not necessarily linked with estrogen defect and this is in agreement with the high significant increase of estrogen in sera of G1 compared with groups C and PC. Conversely, the significant decrease of Estrogen in sera of G2 compared with G1 and the non-significant difference between G2 and group C indicate the importance of zoladex in depressing estrogen hormone. Gonadotropin-releasing hormone (GnRH) agonist treatment (including zoladex and other drugs) has been studied more extensively than other medical treatment regimes. They are modified forms of GnRH that bind to receptors in the pituitary but have a longer half-life than native GnRH and thereby in down-regulation of the pituitary-ovarian axis and hypoenestrogenism (24). Indeed, GnRH agonists are medicines that work by causing a temporary menopause. The treatment causes the ovarian to stop production estrogen, which causes the endometriosis implants to shrink (25). Lastly, the non-significant difference between G3 and G1 indicate that endometriosis is a recurrent disease. This suggests that hormonal treatment does not lead to a complete suppression of endometriotic foci and that recurring lesions appear to grow from the residual loci. It has been reported that 47% of the endometrial lesions appear again after removal of endometriotic tissue in patient (26).

## V. Conclusions

Paraoxonase-1 activities were decreased in sera of patients but we show highly significant increase under treatment. Moreover, This is the second study that proves a positive relation between CP and endometriosis and the first dealing with zoladex action in decreasing CP levels in endometriotic patients, revealing that it could be considered a good biochemical marker for endometriosis. Lastly, Estrogen was increased in patients indicating its proven positive relationship with endometriosis, while it was decreased under treatment.

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