

Endothelin an Inflammatory Marker and its Association with PCOS

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ABSTRACT

Background: Endothelin is an inflammatory marker secreted from endothelium and disturbed in inflammatory condition.

Aim: To evaluate the effects of endothelin in polycystic ovarian syndrome (PCOs).

Methods: This study was comprised of two groups of women, group A, 48 women with polycystic ovarian syndrome using Rotterdam criteria and group B was composed of 48 women of reproductive age having no PCOS. Detailed information about age, body mass index (BMI), duration of menstrual cycle, marital status and clinical features like obesity, infertility, acne and hairs on face were noted. A total 5ml venous blood was collected by using aseptic techniques and then centrifuged immediately; serum was separated and stored at -20°C after proper labeling. Samples were analyzed for estimation of serum endothelin by using enzyme linked immunosorbent assay kit (ELISA). Statistical analysis was done by using statistical package for social sciences (SPSS) 21. The p value <0.05 was considered to be statistically significant.

Result: After performing ELISA test of endothelin, it was revealed that mean±SD of endothelin for PCOS women was 5.2±1.05 and mean±SD of healthy women was 3.65±0.51 and median (IQR) of endothelin in PCOS women was 4.9 (1.28) and median (IQR) of healthy women was 3.65 (0.85) and p value of endothelin, generated by Mann-Whitney U test was < 0.001.

Conclusion: It is concluded that the level of endothelin is increased in PCOS as compared to healthy women in which the level of endothelin is decreased or within normal ranges.

Keywords: Endothelin, Inflammation, Polycystic ovarian syndrome, Enzyme linked immunoassay absorbant,

INTRODUCTION

Endothelin was first introduced by scientist through the extraction of bovine endothelial cells by culture media.¹ It has three isoforms with different regions of expression and binding to different receptors.² Endothelin is a potent circulating vasoconstricting peptide having 21 amino acids in healthy individuals which maintains cell-growth, cardiovascular homeostasis, endocrine function, inflammation and reproductive function.³ Endothelin maintains the balance between vasoconstriction and vasodilatation. Endothelin is secreted from endothelium by the action of endothelium converting enzyme which is located on endothelial cell membrane. Endothelin is synthesized from pro-endothelin (which has 39 amino acids) by the action of proteolytic enzymes.

Endothelin has three isoforms that is ET-1, ET-2 and ET-3 having regions for expression and binding of receptors. Endothelial cells produce more endothelin in response of oxidative stress, hypoxia, presence of oxidized LDL and pro-inflammatory cytokines.² Endothelin is a major vasoconstrictor which is produced and secreted by endothelial cells act on smooth muscle cells by activating the G-protein coupled receptor and is involved in the regulation of various metabolic processes. Endothelin has regulatory effect on adiponectin expression and secretion through IP3 and calcium pathway. A recent study¹⁵ suggested that endothelin decreases adiponectin secretion by acting through endothelin receptor kinase pathway

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Dysregulation of endothelin can lead to increased chances of metabolic disorder and type 2 diabetes Mellitus.

Studies⁴ suggested that endothelin acts as an agonist of calcium channels and it controls the action of inositol phosphate and induces utilization of calcium from cell. Activation of calcium channel may cause hyperpolarization of membrane and sustained depolarization occurs. This action shows that vasoconstricting effect of endothelin is inhibited by calcium channel blocker. Binding of platelets to the endothelial cell receptors cause release of endothelin which causes endothelin dysfunction⁴. Normally endothelin remains in balance but when over expression occur can contribute to high blood pressure, atherosclerosis and other metabolic disorders.

Polycystic ovarian syndrome was originally described in 1935 by Stein and Leventhal as a syndrome consisting of decrease flow of menstruation, acne, growth of facial hair and anovulation in association with enlarged polycystic ovaries because small cysts develop in ovary that's why it is called Polycystic Ovarian Syndrome⁵. It is most common disorder in females of 20-40 years of age affecting 5% to 10% of pre-menopausal women. Genetic factors, lifestyle and cousin marriages are the important risk factors in the development of PCOS. When the cells of pancreas are resistant to the action of insulin blood sugar level is raised. Inflammatory effect can lead to increased level of androgen and decreased level of estrogen which can interfere with signals from brain and cause anovulation⁶⁷.

The underlying mechanism is not yet clear but it can occur due to failure of ovaries to ovulate due to presence of ovarian cysts, hyperandrogenism and increase in body mass index⁸. One of the important biochemical changes

which occurs in Polycystic Ovarian Syndrome is volume of ovary is increased upto 10cm³ with multiple small follicles.⁸ Normally endothelin is not present in blood but whenever endothelial dysfunction occurs due to inflammation and any oxidative stress then endothelin appears in plasma. Normal level of endothelin ranges up to 5 pmol/l.²

A study related to it suggests that the level of endothelin is disturbed in patients of PCOS. Biochemically, in PCOS insulin resistance occurs which can lead to increased levels of endothelin causing endothelial dysfunction at an early stage.⁹ This endothelial dysfunction can make patients of PCOS prone to cardiovascular disease as compared to normal healthy women of the same age¹⁰. Endothelial disease becomes more evident after fourth decade of life. Due to endothelial injury causing abnormal vascular reactivity¹¹, increased levels of endothelin in PCOS can lead to cardiovascular disease in upto 7% of the cases and more chances of development of atherosclerosis¹². Hormonal imbalance is also associated with increased endothelin level and endothelial dysfunction. Women with PCOS also appear to be more likely to have carotid artery disease early in life and the carotid atherosclerosis index in such patients is associated with age, BMI, diastolic blood pressure and LDL cholesterol level. Carotid change is a surrogate marker for CAD and stroke¹³. Endothelin has two type of distinct receptors that are endothelin A (ETA) and endothelin B (ETB) and they are located on 4 and 13 chromosomes respectively.

Endothelin has more affinity for ETA. Eosinophilic airways inflammation can stimulate the endothelin and levels are increased which precipitates the release of inflammatory cytokines².

MATERIALS AND METHODS

The research was authorized by the Ethical Committee of the University of Health Sciences Lahore. It was conducted in the Biochemistry Department, Post Graduate Medical Institute Lahore in October 2018. Samples were collected by clinically and ultrasonographical diagnosed cases of PCOS patients. About 5ml of venous blood samples were collected in serum vials and shifted to the laboratory on ice where they were allowed to clot at room temperature. The centrifugation of samples was done at 5000 rpm for 5 min. The serum obtained in eppendorf tubes was stored at -20°C until used. Samples were analyzed by using ELISA kit. Glory science kit was used to estimate the levels of endothelin in controls and patients. A total of ninety six sample size was finalized after considering the inclusion and exclusion criteria. Group A comprised of females with PCOS. Group B were healthy females without PCOs. The data were entered and analyzed using SPSS (Statistical Package for Social Sciences) version 21. The mean±standard deviation, median and inter-quartile range

(IQR) was the quantitative variables. Normal data distributions were verified by Shapiro-Wilk's statistics. Independent t-test was used to compare average values amongst groups of the same variables. Data was considered statistically significant with p value ≤0.05. Whereas p value >0.05 was considered as non-significant statistically. Mann-Whitney U test was used to compare the normal distributed variables. BMI and endothelin were normally distributed while age was non-normally distributed. Chi square test was applied to estimate the pattern of menstrual cycle in both group A and B.

RESULTS

The Mean ± S.D and Median/IQR of age in PCOS females was 29.5±4.9 years while it was 28.4±6.1 years in normal controls. The difference of age between two groups was not statistically significant (p 0.302; Table 1). Mean±SD BMI of PCOS group was 30.5±1.5 and in control group 24.4±2.5 (Table 2).

Out of forty eight, seventeen PCOS females had regular menstrual cycle and thirty one had irregular menstrual cycles. In control group forty six females had regular menstrual cycles and two had irregular menstrual cycle. Chi square test was applied to see the statistical significance of menstrual cycle pattern which was 38.8 and p value was <0.05 which is statistically significant (Fig. 1). The Mean±SD of serum endothelin levels in PCOS group was 5.2±1.05 pmol/l whereas in control group it was 3.65±0.51 pmol/l. There is significant increased level of endothelin in PCOS women (p<0.001; Table 3 & Figure 2). This figure shows that level of endothelin was increased in PCOS women as compared to healthy women.

Table 1: Comparison of age between PCOS women and healthy women

Age	Patient (n=48)	Control (n=48)
Mean ± SD	29.5208±4.868	28.4375±6.1225
Median/IQR	29.00/7.50	27.000/11.00

P> value .302*

Table 2: Comparison of BMI between PCOS women and healthy women

BMI	Patient (n=48)	Control (n=48)
Mean±SD	30.500±1.5298	24.4042±2.488
Median/IQR	30.00/1.75	24.500/4.90

Statistically significant**

P value .000*

Table 3: Serum endothelin in PCOS women and healthy women

Endothelin	Patient (n=48)	Controls (n=48)
Mean±SD	5.2±1.05	3.65±0.51
Median (IQR)	4.9 (1.28)	3.65 (0.85)

*Statistically significant; **Mann-Whitney U test

P value <0.001*

Figure 1: Pattern of menstrual cycle in patients and control

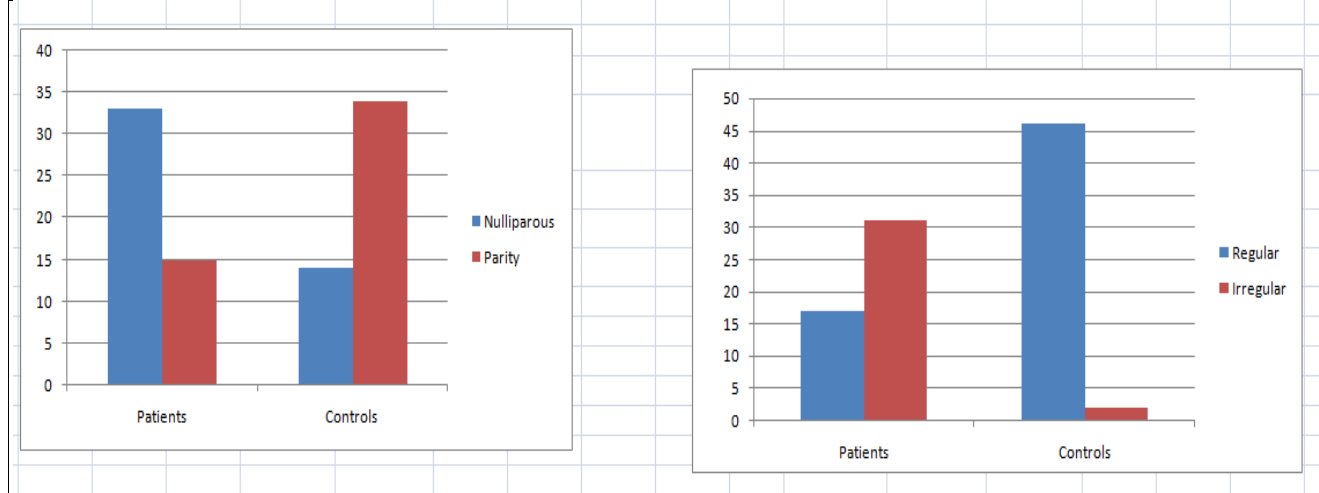
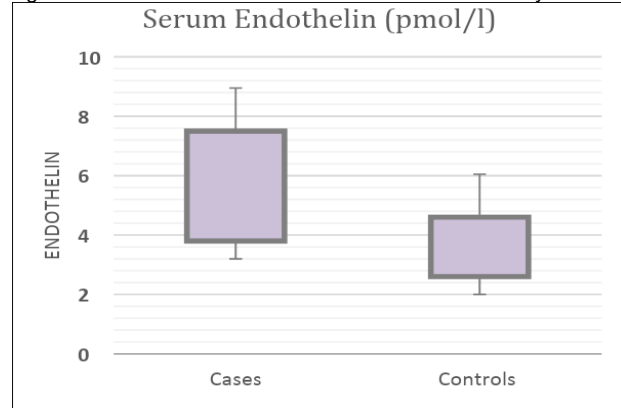


Figure 2: Serum endothelin in PCOS women and healthy women



DISCUSSION

In this study different variables like endothelin, age and BMI were evaluated to see their importance in inflammatory condition like PCOS. Endothelin is a potent circulating vasoconstricting peptide which maintains cell growth, CVS homeostasis, endocrine function, inflammation and reproductive function in healthy people. In our study we observed that slightly higher than normal levels of endothelin in PCOS but in some patient value of endothelin within normal range was noted which is consistent with parent study which shows significant increase in endothelin levels in women with PCOS as compared to healthy women showing p (0.020).

PCOS is common hormonal disorder among women of reproductive age group and most common cause of sterility and early stage abortions. Some evidences of autosomal transmission related to strong familial clustering is noted. Series of genes renders the ovaries susceptible to increase insulin resistance and increase androgen secretion while blocking follicular maturation.

In PCOS levels of endothelin are increased which was associated with hyperinsulinemia, increased insulin resistance and decreased glucose utilization leading to endothelial injury and increase in risk of developing CVD

and metabolic disorders¹⁴. Endothelin was considered as an important biochemical marker of endothelial dysfunction. In a study it was noted that increased level of endothelin can lead to more intimal thickness of vessel and chances of developing atherosclerosis is increased¹⁵. Endothelial injury is early sign in development of vascular pathological changes as endothelin levels are changed in PCOS.

In another study endothelin was an important marker in the initiation of lung injury by activation of neutrophils releasing cytokines from monocyte. Advanced glycation product is another factor which is increased in PCOS due to which insulin resistance is increased, it can lead to endothelial damage. Endothelin beta receptors mediate vasodilation but it is deficient in polycystic ovarian syndrome and vascular changes are vasoconstriction primarily. Insulin sensitizer like metformin can decrease insulin resistance in inflammatory conditions like PCOS.

According to present study, age of PCOS females was 29.52±4.868 years while that of control group was 28.437±6.122 years it is supported literature. The (mean±SD) of age in females with PCOS was (25.2±1) and (25.1±0.7) in control group, this is well supported by a study.¹⁹ The mean age in patients with PCOS (24.3±4.6) and (24.1±3.6) in healthy women.

The little changes of age in different studies may be due to ethnic differences. It was concluded from these studies that PCOS is more common among women of 18 to 40 years of age. The prevalence of polycystic ovarian syndrome in the general population appears to be decreased with age, and it was observed that only 7.8% in women older than 35 years suffered from PCOS, as compared to 21.6% women <35 years of age.¹⁶

The results of the current study show higher BMI in PCOS group when compared with control group and p-value has been found to be 0.021 which is consistent with some earlier studies showing that PCOS is more common in women with greater BMI than those with lower BMI.¹⁷ Chi square test was applied to assess the menstrual cycle pattern in both groups which is statistically significant. Obesity does not have an important effect on menstrual pattern in PCOS and basically menstrual pattern

sometimes is regular in obese and non-obese women.¹⁸ There are common symptoms like hirsutism, acne, loss of hair, increase body fat and irregularity in menstruation which can be altogether absent in patients with PCOS. Sometimes PCOS occur at younger age without any menstrual disturbances and less BMI.

Synthesis of endothelin is increased by the oxidative stress and mechanical stress which induces vasoconstriction, fibrosis and vascular injury and these effects are regulated by ETA and ETB receptors. Receptors blockers have significant effects in lowering the level of endothelin and maintain the blood pressure.

CONCLUSION

Alteration in serum levels of endothelin might lead to inflammatory response at cellular level leading to hemostatic change and endothelial dysfunction. In this study we have concluded that level of endothelin was increased in PCOS women as compared to women with no health issue and this parameter helpful in evaluation of complication associated with Polycystic Ovarian Syndrome.

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