Interleukin–33, Oxidative Stress in Prediabetic Polycystic Ovary Syndrome Patients with Insulin Resistance

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Summary:

Background: Polycystic ovary syndrome (PCOS) is one of the common female endocrine disorders of uncertain etiology, which causes menstrual disorders as well as infertility. Interleukin–33(IL-33) is considered as a strong risk marker of inflammation and may have possible role in pathogenesis of PCOS.

Objectives: The present study is designed to investigate the possible role of IL-33 in pathogenesis of PCOS and its relation with glycated hemoglobin (HbA1C),insulin resistance(IR) and oxidative stress in prediabetic PCOS patients.

Subjects and Methods: The study involved 30 healthy women as control group and sixty six infertile Iraqi women with PCOS which were divided into two groups according to glycated heamoglobin(HbA1c) value and homeostasis model assessment for insulin resistance (HOMA-IR). The first group (G1) consist of 30 women with HbA1c $\leq$ 6.5% and HOMA –IR<3.8 as PCOS without diabetes and without insulin resistance. The second group(G2) consist of 36 patients with HbA1c $\geq$ 6.5 % and HOMA-IR $\geq$ 3.8 as PCOS with pre diabetes type 2 and insulin resistance. Ten milliliters of blood were collected from all subjects by vein puncture during 2^{nd} – 4^{th} day of the menstrual cycle. Two ml of blood were collected in EDTA tube for HbA1c analysis. The serum which obtained from the remained blood were used for determination of (insulin, fasting blood glucose(FBG), Malondialdehyde(MDA) , Total antioxidant capacity(TAC) , uric acid, glutathione(GSH), albumin, and IL-33) .

Results: Results revealed a significant elevation in HbA1c, insulin, HOMA-IR, MDA, TAC, uric acid and IL-33 in the patients group when compared with healthy women. On the other hand, a significant decreased in GSH and albumin were found in patients group when compared with control group. Also there are significant differences in the results between patients in group 1 and group 2. A significant positive correlation between IL-33 and HbA1c levels was noticed in G1 and G2 .A significant positive correlation between IL-33 and HOMA-IR was noticed in group 1 while a negative significant correlation was found in group 2. No correlation founded between IL-33 levels and TAC concentration in group 1, but a positive correlation noticed with group 2. A significant negative correlation was observed between IL-33 and MDA/TAS ratio in G1, while a significant positive correlation in G2 was found.

Conclusion: high levels of IL-33 in patient groups may be considered as a novel cytokine involved in the pathogenesis of PCOS. Also the elevation in oxidative stress in PCOS patients could be a reason for disturbed follicular development and ultimately infertility.

Key words: PCOS,IL-33,Insulin Resistance, Oxidative stress,TAC.

Introduction:

Polycystic ovary syndrome is one of the most common female endocrine disorders of uncertain etiology.The disorder causes multiple abnormal cysts in enlarged ovaries so they do not produce the normal number of eggs and do not ovulate normally (1).

Insulin resistance (IR) is a pathological state in which insulin action is impaired in target tissues including liver, skeletal muscle and adipose tissue (2).As many as 70% of PCOS women are IR and 10% have diabetes mellitus(3). Lucky *et al* (4) have been postulated that reactive oxygen species (ROS) have potent role in physiological processes such as folliculogenesis, oocyte maturation, endometrial cycle, luteolysis, implantation, and embryogenesis.Antioxidant that prevent or limit the damaging effects of oxygen radicals have been reported to have important roles in the female reproductive system and in the pathogenesis of female infertility. Changes in antioxidant concentrations in serum and peritoneal fluid have been studied in idiopathic infertility, tubal infertility, and endometriosis patients (5).The sum of endogenous plus exogenous antioxidants represents the total

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antioxidant capacity of extracellular fluids. Changes of these antioxidants reflect their consumption during acute oxidative stress states (6).

Under certain conditions increase in oxidant and decrease in antioxidant can not be prevented and the oxidants / antioxidant balance shifts towards the oxidative stress status

which is considered to be one of the main cause of molecular damage to cellular and tissue structure and is known to increase in patients with PCOS(7).Insulin resistance encourages oxidative stress because hyperglycemia and higher levels of free fatty acids lead to ROS production (8).

Interleukin-33(IL-33) the new member of the IL-1 family of cytokines, is expressed by many cell types following pro-inflammatory stimulation and is thought to be released on cell lyses (9).Interleukin-33 function as a classical cytokine by binding to its specific plasma membrane receptor and by recruiting the IL-1 receptor accessory protein (IL-1RAP) into a trimetric complex inducing signaling pathways similar to those of IL-1 family (10). IL-33, in its own turn, stands out as a cytokine with dual function, through the activation of the toll –interleukin -1 receptor super family (ST2L) membrane receptor complex it acts as a traditionally conceived cytokine. On the other hand, IL-33 performs the role of a nuclear transcription factor. Originally, IL-33 was recognized as a nuclear factor of high endothelial venues IL-33/ ST2 pathway can play a protective role in glucose homeostasis and metabolic–induced tissue damage in addition to being protective against atherosclerosis by inducing a T-Helper cell (Th1) to Th2 switch of immune responses .Thus, the effects of IL-33 are either pro- or anti- inflammatory depending on the disease and the model (11).

Therefore, IL-33, free radical generation and status of antioxidant defense mechanism appears to be closely associated in their common outcome of PCOS with prediabetic and IR. Taking these observation into consideration ,the present study was aimed to explore the relationship of IL-33 with ,HbA1C,IR and oxidative stress in women with prediabetic PCOS patients.

Subjects and Methods:

The study included a control group of 30 healthy females with regular menstrual cycles and with ultrasonographically normal ovaries and sixty six infertile Iraqi women with PCOS, age range of (18-35) year who were recruited in consult a team clinic of the high institute for infertile diagnosis and assisted reproductive technologies, AL-NAHREN University in Baghdad for the period from March 2012 to July 2012. All the patients in the study were clinically diagnosed as patients with PCOS when at least two of the following three features were present, oligo or anovulation, and or biochemical signs of hyperandrogenism and polycystic ovaries examined by ultrasound. Patients were divided into two groups according to HbA1c value (12) and HOMA-IR(13) :

Group 1(G1): consists of 30 women with HbA1c $\leq$ 6.5% and HOMA –IR<3.8 as PCOS without diabetes and insulin resistance.

Group 2(G2): consists of 36 women with HbA1c $\geq$ 6.5 % and HOMA-IR $\geq$ 3.8 as PCOS with diagnosed diabetes and insulin resistance.

Ten milliliters of blood were collected from all subjects by vein puncture during 2^{nd} – 4^{th} day of the menstrual cycle. Two ml of blood were collected in EDTA tube for HbA1c analysis. The serum were obtained from remind blood after centrifuge stored and frozen until analysis other parameters(insulin, fasting blood glucose (FSG),Malondialdehyde (MDA), Total antioxidant capacity (TAC), uric acid, glutathione, albumin, and IL-33) .

Insulin resistance was assayed by calculating the homeostasis model assessment for insulin resistance (HOMA-IR) HOMA-IR was estimated using the following formula (13):fasting glucose x fasting insulin] / 22.5.FBG, HbA1C,insulin,uric acid ,albumin were determined by using standard procedures of the biochemistry laboratory of hospital.

MDA was determined in serum as a thiobarbituric reactive species according to the method of Buege and Aust (14). TAC was measured according to the method of Miller(15) by using Randox kit(cat No. Nx 2331) .

Reduced glutathione was determined based on the reaction of aliphatic thiol compounds with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) at pH8.The absorbance of the yellow chromate was measured at 412nm and is directly proportional to GSH concentration(16).

Serum IL-33 levels were measured by using specific enzyme-linked immunosorbent assay (ELISA) kit (Ray Bio Human IL-33 for *in vitro* quantitative measurement of human IL-33 in serum) ,according to the manufactures protocol.

Data are presented as mean ± SD. The differences between two groups were analyzed by Student's t-test by using SPSS. Pearson's correlation coefficient was used to examine the relation of IL-33 with IR and TAC in patients groups. P-value of < 0.05,<0.001 considered to be significant.

Results:

The descriptive characteristic of the PCOS patients and healthy control are presented in table (1). The results of HbA1c showed a highly significant increase in G1 (6.91± 0.88 %) and G2 (8.33 ± 0.5 %) compared to healthy control (4.57± 1.43%). A highly significant elevation (P<0.001) observed between the two patients groups. Also a highly significant increase in insulin and HOMA–IR in patients groups G1 and G2 was found when compared with control group. Also in G2 compared to G1 was observed.

Table 1: Descriptive characteristics and some biochemical parameters in healthy control and patients groups.

Parameters	Control	Group 1		Group 2		P**-value
	Mean ±SD	Mean ±SD	P-value	Mean ±SD	P*-value	
Age(Yrs)	27.80±3.92	27.53±6.12	NS	26.07±5.96	NS	NS
WHR	0.79±0.03	0.82±0.07	NS	1.13±0.42	<0.001	<0.001
HbA1c %	4.57±1.43	6.91±0.88	<0.001	8.33± 0.5	<0.001	<0.001
FSG (mg/dl)	91.27±13.09	94.37±8.9	NS	95.43±19.67	NS	NS
Insulin (µIU/ml)	5.09±1.9	10.18±3.62	<0.001	20.98±2.49	<0.001	<0.001
HOMA-IR	1.16±0.51	2.36±0.84	<0.001	4.95 ± 1.17	<0.001	<0.001

P-value: between control &G1, P*-value : between control & G2,P**-value: between G1& G2,NS :non significant

Table (2) showed the MDA levels of G1 (1.28±0.58 µmol/L) G2 (1.24±0.53 µmol/L) and control group (0.36 ±0.17 µmol/L) there was a highly significant increase when compared PCOS patients with control groups ,while a non significant difference between G1 and G2 was found.

The results of TAC showed a highly significant increase in G1 (3.59±0.67 mmol/L) and G2 (5.64±0.73 mmol/L) compared to healthy control group (0.75±0.32 mmol/L) .No significant difference was found between the two PCOS groups .

The values of MDA/TAS ratio present in table 2 showed a significant difference between G1 (0.37±0.18) and G2 (0.38±0.2) compared to healthy women (0.63±0.56). While, a non significant difference was found between the two patients groups

Results of GSH in table (2) demonstrated that a significant decrease in G1 (2.36 ± 0.54 mmol/L) and G2 (2.57±0.27 mmol/L) when compared to control group (8.14±1.56 mmol/L).

The concentration of uric acid significantly increase in G1 (14.77±3.03mg/dl) and G2 (16.92±3.63 mg/dl) compared to control group (4.69±0.60 mg/dl) , but no significant difference observed between the two PCOS patients groups.

There was a highly significant decrease in albumin levels when compared G1 (53.96±1.79 g/l) and G2 (40.91±1.51 g/l) with control group (63.41±2.95g/l) .Also, a highly significant increase was found in G1when compared with G2.

Data in table (2) showed a significant increase in IL-33 concentration in G1 (47.16±27.65 ρg/ml) and G2 (126.08±28.80 ρg/ml) when compared to the control group (25.29±13.1ρg/ml) .Also, a highly significant increase was noticed in group 2 compared to group 1 .

Table 2 : Serum levels of some oxidant &antioxidant and IL-33 in healthy control and patients groups.

Parameters	Control	Group 1		Group 2		P**-value
	Mean ±SD	Mean ±SD	P-value	Mean ±SD	P*-value	
MDA (µmol/L)	0.36±0.17	1.28±0.58	<0.001	1.24±0.53	<0.001	NS
TAC (mmol/L)	0.75±0.32	3.59±0.67	<0.001	5.64±0.73	<0.001	<0.05
MDA/TAS	0.63±0.56	0.37±0.18	< 0.05	0.38±0.2	< 0.05	NS
GSH (mmol/L)	8.14±1.56	2.36±0.54	<0.001	2.57±0.27	<0.001	NS
Uric acid(mg/dl)	4.69±0.60	14.77±3.03	<0.001	16.92±3.63	<0.001	NS
Albumin (g/L)	63.41±2.95	53.96±1.79	<0.001	40.91±1.51	<0.001	<0 .001
IL-33 (ρg /ml)	25.29±13.1	47.16±27.65	<0.001	126.08±28.80	<0.001	<0 .001

A significant positive correlation between IL-33 and HbA1c levels was observed in G1 (r= 0.139, p< 0.001) and G2 (r= 0.24, p<0.001) .

Also a significant positive correlation between IL-33 and HOMA-IR was noted in group 1 (r =0.38 ,p<0.001) while a negative significant correlation was found in group 2 (r= - 0.53 , p<0.001) .Moreover no correlation founded between IL-33 levels and TAC concentration in G1 , but a positive correlation noticed with G2 (r= 0.241 ,p<0.001).A significant negative correlation was observed between IL-33 and MDA/TAS ratio in G1, while a significant positive correlation in G2 was found.

Discussion:

Insulin resistance and hyperinsulinemia are supposed to play an important role in the pathogenesis of PCOS. In PCOS the compensatory hyperinsulinemia in response to insulin receptors defect in the muscles directly stimulates testosterone production by ovarian thecal cells, promoting the hyperandrogenic state.In addition hyperinsulinemia inhibits the hepatic production of sex hormone binding globulin, further increasing circulating free testosterone levels. Insulin impedes ovulation, either by directly affecting follicular development, or by indirectly increasing intra ovarian androgen levels or altering gonadotropin secretion (5,17). The present data show that IR increased in G2 significantly compared to G1.and that could be due to a frequent abnormality in both obese and non obese women with PCOS which could be related to metabolic disorders in PCOS regarding glucose metabolism as result of insulin resistance as presented by increased HOMA-IR values among the PCOS patients. Women with IR have a tendency to progress from high insulin with normal glucose levels to abnormally high glucose levels, that is pre-diabetes or type 2 diabetes because, beta cell function tends to deteriorate . When beta cells can no longer produce the excessive amounts of insulin needed in IR to control glucose levels, insulin levels fall allowing abnormally high glucose levels to develop resulting initially in pre- diabetes and ultimately type 2 diabetes and these results are in agreement with other reported data (18). Oxidative stress in this study shows a significant increase in G2 and G3 compare to G1 which are in agreement with other study. Oxidative stress may influence not only on cardiovascular system but also female reproductive system. Although endothelial dysfunction which may precede manifest CVD even in young women with PCOS. In PCOS oxidative stress in response to stimulation between plasma testosterone or androstendione and ROS generation. Also, the ovarian steroidogenic enzymes responsible for androgen production are stimulated by oxidative stress and inhibited by antioxidants(19). In the present study the lipid peroxidation product (MDA) employed as biomarker for lipid peroxidation. The increasing in MDA could be due to increased generation of ROS due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. Similar reports of elevated MDA levels have been reported in patients with PCOS While, MDA levels were elevated in PCOS patients group with IR and without IR .This observation suggests that the presence of IR in PCOS patients has no effect on MDA levels (20).

The increase in IL-33 concentration could be due to that IL-33 have a potent protective role in various experimental chronic heart failure (CHF) studies through inhibition of the nuclear factor kappa B (NF-$_k$B) cascade .Authors hypothesized that IL-33 levels may be increased in patient with advanced CHF and may play a part in the regulation of oxidative response in these patients .It has been reported that IL-33 reduced oxidative stress (21,22). It seemed ,that hyperandrogenism was correlated with serum level of IL-33 and it is important factor of increased level of IL-33(23). Granne *et al*. suggested that IL-33 may play a significant role in pregnancies complicated by pre-eclampsia(24).Furthermore, recent study demonstrated that serum IL-33 is abnormally elevated in women with endometriosis (DIE) and suggested IL-33 as a novel cytokine involved in the pathogenesis of DIE (25). A conclusion could be drown from this study for the first time that PCOS patients complicated with IR showed increased IL-33 levels so, IL-33 may be considered as a novel cytokine involved in the pathogenesis of PCOS.

Authors Contribution:

First and second authors are supervisors, third authors is a Ph.D candidate

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