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Effect of Interlukin-36 gamma and Tumor Necrosis Factor alpha on Patients with Polycystic Ovary Syndrome on Governorate Messan in Iraqifemale

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Abstract
Background: Polycystic ovary syndrome PCOS is a heterogeneous disorder. PCOS affects 6–10% of women during their reproductive life.Its complete phenotype is manifested by ovulatory dysfunction, hyperandrogenism and polycystic ovaries.
Objective: The aim of this study is to evaluate a new recent member of the IL-1 super family of cytokinesinterleukin-36(IL-36) levels in serum that has a crucial roleindiagnosis diseasesand in order to evaluate its utility as a clinical biochemical parameter inPolycystic ovary syndrome.
Methods: The present study was conducted on 100 subjects which divided in to 4 groups. First group includes 25healthy individuals as control group . Second group includes 25 female with PCOS aspatient group newly diagnosis .Third group includes 25 patients with PCOS after 12 months from diagnosis. Fourth group also 25 patients after 24 months and more. All subjects attendingfrom teaching AL-Sadder hospital and AL-Zahrawi hospital in Governorate of Messan in Iraq. Parametersmeasured in the sera of patient and healthy groups were interleukin-36 (IL-36), tumor necrosis factor alpha and ceruloplasmin concentration.
Results: A recent member of super family cytokines Interterleukin-36(IL-36) was determined in serum of polycystic ovary syndrome. Higher significant elevation was found when compared with healthy control.
Conclusion: From this study a conclusion was drawn, that evaluation of concentration of a new superfamilycytokines (IL-36) could be considered as clinical biochemical parameter in polycystic ovary syndrome in Iraqifemale patients in Governorate of Missan. Also this study may demonstrated a relation between increased IL-36levels and increased TNF-α and ceruloplasmin levels.
Keywords: polycystic ovary syndrom, interleukin-36 (IL-36), cytokines, TNF-α,Ceruloplasmin

Introduction

Polycystic ovary syndrome PCOS is a heterogeneous disorder. PCOS affects 6–10% of women during their reproductive life (Goodarzi et al., 2011). Its complete phenotype is manifested by ovulatory dysfunction, hyperandrogenism and polycystic ovaries (Dinka et al. 2013). Patients with PCOS are in the high risk group for coronary heart disease because of their abnormal lipid profile, insulin resistance and obesity (Wild et al. 2010). All these findings show that there is an increase in prevalence of cardiovascular disease (CVD) and DM in PCOS with time (Ali et al. 2011).Women with PCOS are prone to inflammation. They also develop heart disease, diabetes, high blood pressure, asthma, thyroid disorders and other disease caused by chronic inflammation at an alarming rate(Rourk, Kay and Lyle.2006). Interleukin (IL-36) cytokines (previously designated as novel IL-1family member cytokines, IL-1F5-IL-1F10) constitute a novel cluster of cytokines structurally and functionally similar to members of the IL-1cytokines cluster (Ravisankaret al.2012).In addition, seven novel IL-1 family members have been identified on the basis of their sequence homology; three-dimensional protein structure, gene location and receptor binding profile .These proteins are now termed IL-36 Ra, IL-36α, IL-36β, IL-36 γ (Dinarelloet al.2010). IL-36α, IL-36β, IL-36 γ bind a heterodimeric receptor consisting of the IL-36 receptor (IL-36 R) subunit and the IL-1 receptor accessory protein (IL-36 AcP) (Twoneet al.2004).Interleukin-36 cytokines and IL-36R are abundantly expressed by keratinocytes and other epithelial cell types (Solenneet al.2012).Interestingly, it has recently been shown that γδ T-cells can express IL-36β under specific conditions (Yang et al., 2010). IL-36α, IL-36β, and IL-36γ can induce IL-17 and TNF expression in keratinocytes, which can be synergized by the cytokine IL-22 (Carrier et al., 2011).IL-36 γ is highly expressed in epithelial cells of the intestine, lungs, and skin; however IL-36 has previously been characterized in human

female reproductive tract (FRT). IL-36R and its ligands are expressed in skin and internal epithelial tissues exposed to pathogens, such as trachea, lung and esophagus, but also in the brain, gut and kidney (Kumar et al.2000, Towne and Sim.2012, Ichii etal.2010).Several studies suggest that IL-36 exerts pro-inflammatory effects contributing to the pathogenesis of psoriasis and lung inflammation (Towne. 2012, Chustz et al. 2011, Tortola et al, 2012). In addition, we recently described that IL-36 stimulates cytokine production by dendritic cells (DC) more efficiently than other IL-1 family members(Vigne et al.2011). In addition, IL-36 acts in synergy with IL-12 to induce the polarization of nave CD4+ T cells into T helper (Th)1cells (Vigne et al. 2012). Consistently, IL-36 enhances Th1 responses in vivo (Vigne et al. 2012, Vinge et al. 2011).These observations led to the hypothesis that IL-36, being expressed in epithelia and in immune cell, might acts as an early danger signal to active cells of the innate and adaptive immune system.Depending on context, this activation might enhance host responses against pathogens, amplify pathological inflammation, as illustrated by the occurrence of generalized pustular psoriasis in patients with mutated IL-36Ra (Onoufraids et al. 2011, Merrakchi et al. 2011).

Tumor necroses factor alpha (TNF-α) is an adipokine involved in systemic inflammation and is a member of group cytokines that stimulate the acute phase reaction. It is produce chiefly by activated macrophages (M1), although it can be produced by many other cell types such as CD+ lymphocytes, natural killer NK cells and neurons (Swardfager et al.2010). The primary role of TNF-α is in the regulation of immune cells being an endogenous pyrogen, is able to induce fever, apoptotic cell death, cachexia inflammation and to inhibit tumor genesis and viral replication and respond to sepsis vial IL-1 and IL-6 producing cells (Swardfager et al.2010).PCOS is a proinflamatory state as evidenced by elevated plasma concentrations ofhighsensitive C-reactive protein(hsCRP). Inobesity-related diabetic syndrome, TNF-α is overexpressed in adipose tissue andinduces insulin resistance through acute and chronic effects on insulin sensitive tissues.Chronic exposure to TNF-α decreases the expression of glucose transporter 4 (GLUT4),the insulin-sensitive two glucose transport protein. Because decreased GLUT4 expression hasbeen identified in PCOS, it is possible that TNF-αcontributes to this post receptor defect. The sourceof excess circulating TNF-αin PCOS is likely to be adipose tissue in theobese. In lean women with PCOS increased visceral adiposity has been proposed as a sourceof excess TNF-α(Gonzáles et al. 2005).

Ceruloplasmin is one of the acute phase proteins and the majority of the antioxidant ceruloplasmin activity in serum depends on the level of Cu+2 containing protein, this single glycoprotein containing six or seven copper atoms (Walfe.2009).Ceruloplasmin contains six cupredoxin domains and has a molecular weight of 120 kDa (Ganna, and Ross.2013).Ceruloplasmin is the major copper- carrying protein in the blood and in addition plays a role in iron metabolism. It was described in 1948 (Vinayaket al.2010).Ceruloplasmin known to have antioxidant property, its level is increased in plasma in infections and malignant conditions and inflammatory disorder, especially in Hodgkins disease, its level decreased in Wilson's disease and used in the diagnosis of the disease (Shivananda. 2007).

Material and method

This study was carried out inMissan Governorate, Iraq. The subjects consisted of one hundred (100) female patientsdiagnosed of polycystic ovary syndrome that attended the teaching AL-Saddar hospital and AL-Zahrawi hospital in Governorate of Missan, Iraq..They were diagnosed by physician at the hospital using Ultra sound .The patients were divided into four groups depending on duration of PCOS diseases, (G2) from 3monthes to 1years ,(G3)from 1years to 2 years, (G4) from 2years to 4 years and more . In addition, to group (G1) of 25 healthy were enrolled in the study as a control group.

1-Sample Collection and Preparation:

About 10 ml of venous blood were collected from the patients in the different centers using the standard vein puncture technique and its after obtaining a consent from the patient and the center management. This was discharged into a plain tube without additives and allowed to clot. The serum from the sample was separated after centrifugation at 3,000 rpm and stored frozen at -20 0C.Untilanalyses of the samples.

2-Assay method

2.1-Determination of serum IL-36 gamma levels (pg/ml)

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL1F9 has been pre-coated onto a micro plate.Standards and samples are pipetted into the wells and any IL1F9 present is bound by the immobilized antibody.

2.2-Determination of serum TNFα levels (pg/ml)

The DEMEDITEC TNF-α human ELISA is a sandwichα assay for determination of TNF-α in serum.

Determination of serum cerluplasmin level (g/L)

The procedure consists in animmuno precipitation in a agarose between an antigen and its homologous antibody.It is performed by incorporating one of the two immune reactants (usually antibody) uniformly throughout a layer of agarose gel, and then introducing the other reactants (usually antigen) into well punched in the gel. Antigen diffuses radially out of the well into the surrounding gel- antibody mixture, and a visible ring of

precipitation forms where the antigen and antibody reacted (Berne . 1974).

Statistical analysis

Results were expressed as Mean± SEM. Student-test was used to show the difference between groups variation was considered significant when P-values are ≤ 0.05. The correlation coefficient (r) test is used to describe the association between the different studied parameters.

Result

Table (1) shows that levels of IL-36, TNF-α and CP concentration in sera of G1, G2, G3 and G4 for control andpatientsrespectively. For IL-36 was a highly significant increase inG1vsG2 and non-significant between G1vsG3 and significant in G1vsG4 when compared between control and patient group. also a non-significant increase for TNF-αin G1vsG3 and G1vsG4,but increase significant G1vsG2 when compared between control and patient group. While non-significant increase in CP levels for all groups G1vsG2, G1vsG3, G1vsG4 compared with control and patientsgroups. Table (2) shows positive correlation for IL-36 ,TNF-α, CP and duration of disease, the positive correlation inG4 for IL-36 (r =0.319), TNF-α for G3and G4 (r = 0.0002, r = 0.099), as shown in figure (1,2,3) respectively, while there was negative (-ve) correlation G2 and G3 for IL-36 (r =- 0.267 , r =- 0.309), TNF-α G2 (r = -0.202), CP in G2,G3 and G4(r = -0.398,r = - 0.215 and r = -0.156)and duration.

Discussion:-

Modulation of immune responses by the action of differentcytokines is essential for optimal protection against invadingmicroorganisms. Over the past decade, cytokines of the IL-1 familyhave been recognized for their crucial roles in innate and adaptiveimmune responses. These cytokines target distinct CD4 T-cellsubsets. IL-1 has a key role in the differentiation of Th17 cells,while IL-18 and IL-33 are known to enhance Th1 and Th2polarization, respectively(Sims, Towne and Blumberg.2010). In our previous study, we demonstratedthat the newly named IL-36 cytokines exerted stimulatory effectson DCs and CD4 T cells promoting Th1 responses in *vitro* and in*vivo*.This direct effect of IL-36 on immune cells was alsodemonstrated in human monocyte-derived DCs. (Mutambaet al.2012). The results of the present study showed the serum of IL-36 level was high-significantly in patients with polycystic ovary syndrome patients than in healthy control. No data in the literature was found concerning the level of lL-36 in such patients.Seventy five women with PCOS and 25 controls were included and it was found that IL-36 levels was elevated inG2(689.74±137.94) and (561.46±112.29) in G3 and level of 1L-36 in G4(439.69 ±87.93) was low.The process of an inflammatory response the invading pathogens or damaging insults is of critical importance to the homeostasis of the human body. Puder et al. demonstrated that the increase in low grade chronic inflammation and insulin resistance in women with PCOS is associated with central fat excess. Independently of each other, both total body fat as well as central fat excess has a major impact on serum levels of inflammatory mediators, on the white blood cell WBC, and on estimates of insulin resistance(Puder et al.2005).In this study, serum activity TNF-α it was found that levels in G2 and G4 waselevated(8.39 ±1.67, 7.26± 1.45) compared with level in G3 was low(5.72±1.14).Also in this study, serum TNF-αactivity was non-significantly increased in patients with PCOS than healthy controls. This result was contrast to that found ina study done by Pudder et.al whomeasured inflammatory markers like TNF-α, C-reactiveprotein and white blood cells who found that to be non-significantly in obese women with PCOScompared to control group (Puder et al.2005).Also this study was contrast with study of Nadia andHaydar (Nadia and Haydar.2010).Another proinflammatory cytokine is IL-18, which wasreported to be increased in PCOS.IL-18 induces theproduction of TNF α which promotes the synthesis ofIL-6, which is also considered a strong risk marker forcardiovascular disease. Collectively, the above findingsindicate that low-grade chronic inflammation could be anovel mechanism contributing to increased risk of coronaryheart disease in PCOS (Thozhukat and Stephen.2010).So that,in this study we showthe levels of CP for groups found in G2was elevated (24.86±4.972) compared with level in G3and G4 was lower (13.16 ±2.63 ,14.07 ±2.21), and the activity of CP was non-significant increase in G2,G3 and G4than control healthy.Ceruloplasmin is an abundant glycoprotein in human plasma and is mainly produced by the liver; (Ganna, and Ross.2013).The present study is in agreement with other suggested data that believe Ceruloplasmin is high in a variety of neoplastic and inflammatory states since it behaves as an acute phase reactant, although levels raise more slowly than do those of other acute phase reactants, Increases are described in autoimmune disease (Vinayaket al.2010).

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Table (1):- Level of IL-36, TNF-α and CP in sera of the four group studies

parameter	Mean ±SEMG1	Mean ±SEMG2	Mean ±SEM G3	Mean ±SEMG4	G1vsG2	G1vsG3	G1vsG4
IL-36Pg/ml	330.25± **6**6.05	510.**7**4±102.14	370.46±72.09	439.69±87.93	HS	NS	S
TNF-α	6.61±1.32	**8.39**±1.67	5.72±1.14	7.26± 1.45	S	NS	NS
CP	12.39±2.47	24.86 ±4.97	13.16 ±2.63	14.07 ±2.21	HS	NS	S

P values< 0.05 considered significant (S)
P values< 0.001 considered high significant (HS)
P values>0.05 considered non- significant (NS)

Table (2): Correlation analysis between biochemical parameters among three studied groups

		G2	G3	G4
IL-36Pg/ml& months	**r**	**NS**	**NS**	**NS**
	P	**P>0.05**	**P>0.05**	**P>0.05**
TNF-α &months	**r**	**NS**	**S**	**NS**
	P	**P>0.05**	**P<0.05**	**P>0.05**
CP&months	**r**	**NS**	**NS**	**NS**
	P	**P>0.05**	**P>0.05**	**P>0.05**

P values< 0.05 considered significant (S)
P values< 0.001 considered high significant (HS)
P values>0.05 considered non- significant (NS)
R values mean correlation coefficient

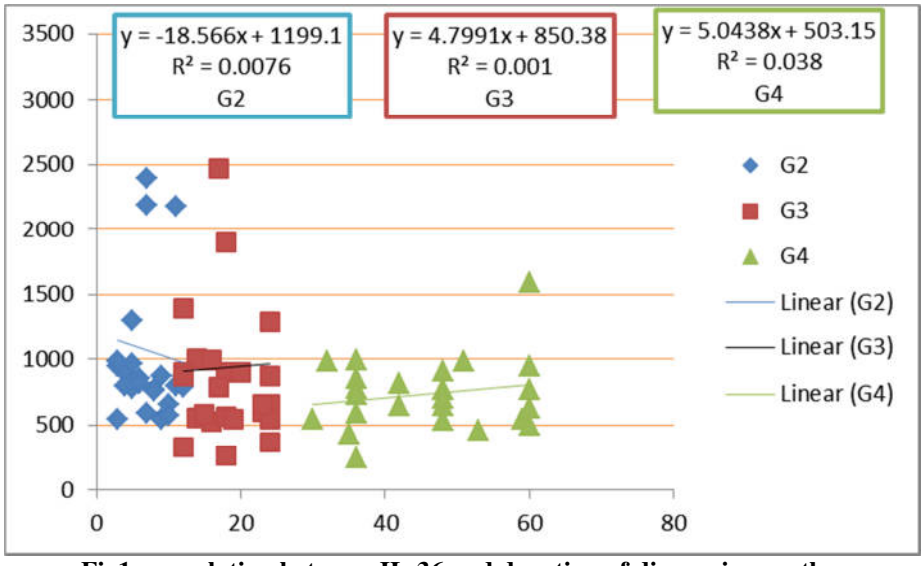

Fig1: correlation between IL-36 and duration of disease in months.

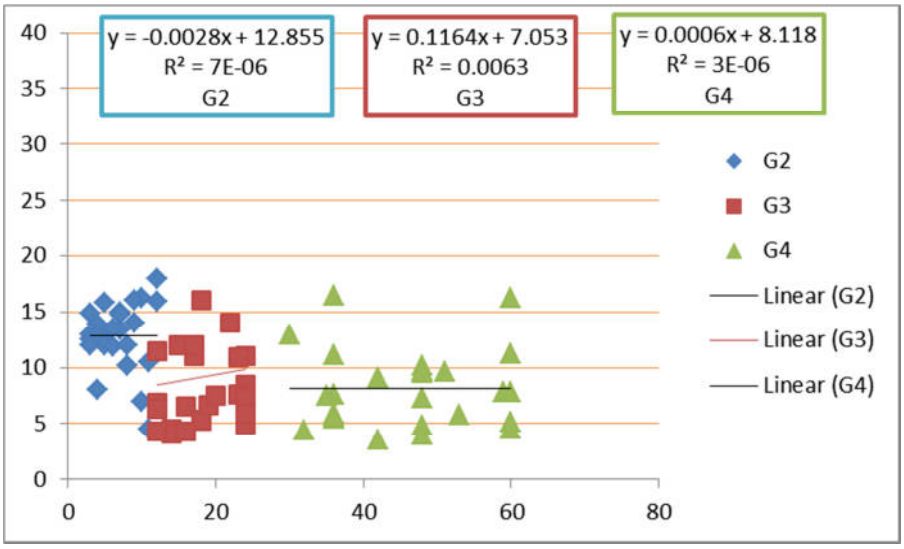

Fig2: correlation between TNF- α and duration of disease in months.

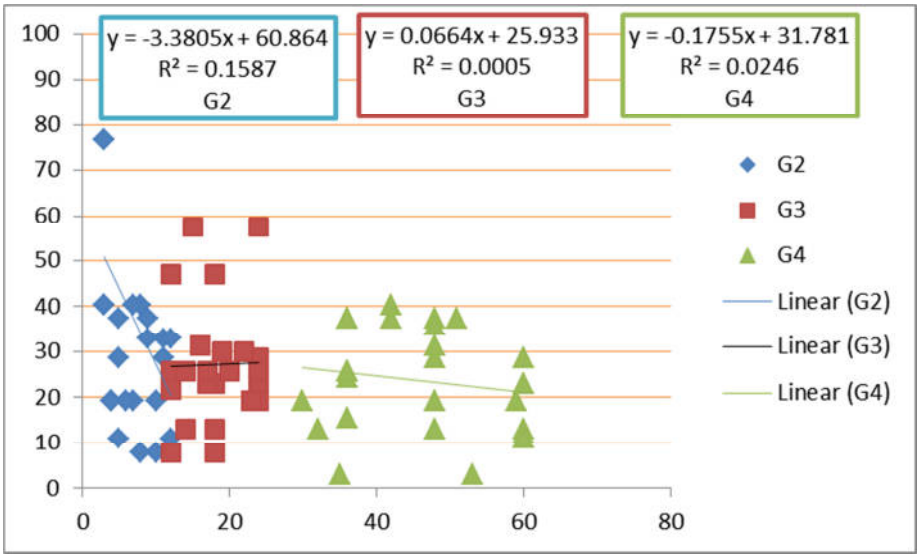

Fig3: correlation between CP and duration of disease in months.