Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN: 2521-3520 (online)
Volume 28, Issue 3, 2017
DOI: http://doi.org/10.23851/mjs.v28i3.551

Research Article

Study the Effect of Interleukin36 Gamma and AMH in Iraqi Women with PCOS

Wafa R. Alfatlawi
Applied Science Department, Applied Chemistry Branch, University of Technology, IRAQ
Correspondent Author Email: wafaaragee@yahoo.com

ArticleInfo

Received
13 Nov. 2016

Accepted
12 Jun. 2017

Abstract

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders and affect approximately (5-10) % of women of reproductive age. Anti-Müllerian hormone (AMH) is a homodimeric glycoprotein, a member of the transforming growth factor-β superfamily, it is secreted exclusively from women by granulose cells of ovarian follicles and it is considered as the precise marker of follicle pool size. AMH has been shown to be a good surrogate marker for polycystic ovary syndrome (PCOS). Interleukins are considered as strong risk markers of inflammation. Interleukin-36 gamma (IL36) also known as interleukin-1 family member 9 (IL1F9) is a protein that in humans is encoded by the IL36G gene. Serum samples were collected on day 2 of the menstrual cycle. Serum IL36ɣ, FSH and LH concentration were measured by using ELISA. This study aimed to evaluate the association between IL36ɣ and AMH and study the relationship between obesity and AMH of women in the age of reproductive (25-35) yrs. This study included 28 infertile women with PCOS their husbands were apparently normal (hormones and seminal fluid analysis), their aged (25-35) years, and 20 healthy women aged (25-33) years as control. All control women & patients were from outpatients unit of Alkadumia teaching hospital at Baghdad and all the parameters were measured in Sigma Laboratory. Serum IL-36 ɣ elevated in PCOS patients mainly those with high AMH levels. This hormone increased in PCOS patients compared with control. There was a non-significant difference between patients and control to LH and FSH levels.

Keywords: AMH, PCOS, IL-36, FSH.

الخلاصة

يعتبر متلازمة تكيس المبايض من امراض الغدد الصماء الشائعة والتي تصيب النساء وتؤثر على مايقارب (5-10)% من النساء في سن الانجاب .ان هرمون الانتي موليرين (AMH) دايمر متجانس من البروتين السكري وهو احد عوامل النمو من النوع *B* بيتا ويفرز في النساء من الخلايا الحبيبية من جريبات المبيض . ان مستوى هذا الهرمون يعتبر عامل دقيق لحجم الجريبات في المبيض وهو ايضا عامل يدل على وجود متلازمة تكيس المبايض PCOS .
ان الانترلوكينات هي عوامل خطورة تسبب الالتهاب واحد اعضائهاهو الانترلوكين36 كاما والذي يعرف ايضا بالانترلوكين 1 ويرمز له (IL1F9) اي الانترلوكين1 احد اعضاء العائلة 9 وهو عبارة عن بروتين يوجد في الانسان ويشفر من قبل الجين IL36G .لقد تم جمع العينات في اليوم الثاني من ايام الدورة الشهرية لقياس هرمونات LHو FSH واستخدمت تقنية الايلايزا ELIZA . ان الهدف من هذه الدراسة هو تقييم العلاقة بين IL36ɣ و AMH ودراسة العلاقة بين السمنة وAMH لدى النساء في عمر الانجاب (25-35) سنة. تضمنت هذه الدراسة 28 من النساء العقيمات والمصابات بمتلازمة تكيس المبايض واللاتي كان ازواجهن طبيعيين (تبين ذلك من خلال تحليل الهرمونات والسائل المنوي) وكانت اعمار المريضات (25-35) سنة وتم الحصول على العينات للنساء السليمات والمريضات من المرضى الخارجيين في مستشفى الكاظمية التعليمي في بغداد وكان عدد النساء السليمات 20 امرأة استخدمت كمجموعة ضابطة وتراوحت اعمارهن (25-35)سنة وتم قياس جميع العوامل في مختبر سكما التخصصي . لقد تبين من خلال النتائج ان هناك زيادة في تركيز IL36ɣ لدى النساء المصابات بمتلازمة تكيس المبايض وخاصة اللاتي لديهن زيادة في تركيز AMH مقارنة مع المجموعة الضابطة ولاتوجد علاقة معنوية بين المرضى والمجموعة الضابطة عند قياس مستوى الهرمونات LH و FSH .

Introduction

Polycystic ovary syndrome defined as endocrine disorder in women in the age of reproduction and the diagnosis of this disorder

remain one of the most challenging issues in the medicine of endocrinology, gynecology, and reproduction. There are many criteria of PCOS have been proposed: 1-Clinical (hirsuitism, acne, and irregular menstrual cycles) 2-Biological (increased LH/FSH ratio, increase serum testosterone or androstenedione) 3-Ultrasound U/S. The Rotterdam European Society for Human Reproduction /American Society of Reproductive Medicine (ESHRE/ASRM) hypothesized that PCOS consider a true syndrome when the tow of the following available: 1-an ovulation 2-hyperandrogenism 3-PCOS on Ultrasound. at the present time the increase of ovarian volume (10ml) and the number of follicles in each ovary measuring 2-8mm very specific and classify as PCOS [1] [2].

Infertility is inability of a couple to get pregnancy after one year of their marriage. There are many causes of infertility like: age, 4% of couples in the first stage of their life was infertile and this ratio will increase to 20%at their 30 years. Sometimes the problems with their female and also 10% of couples have unexplained infertility. Most causes of female infertility are due to underlying medical problems, these causes include: ovarian factors such PCOS, hypothalamic-pituitary factors, and tubal (ectopic) peritoneal factors, uterine fibroid and cervical factors. Also age, smoking, sexually transmitted infections, and being overweight or underweight can all affect [3] [4].

Anti-Müllerian hormone (AMH) defined as glycoprotein and its structure near to inhibin and activin, and it is consider as transforming growth factor (TGF) superfamily [5]. It is also considered as a local growth factor and a cellular differentiation factor [6]. The concentration of AMH decrease with age in women in the first phase of follicles of the menstrual cycle and this change of the concentration of AMH starts before any change of the hormones of FSH, E2, and inhibin B [7]. Obesity in women is associated with many conditions like pregnancy loss, anovulation and late pregnancy complications (pre-eclampsia, gestational diabetes). In PCOS obesity is correlated to failure and or delayed response to many treatments including gonadotropins, clomiphene citrate and laparoscopic ovarian diathermy. Many studies reported that after losing 6% of initial body weight, the obese women with PCOS improved spontaneous ovulation rates and pregnancy. Therefore, the losing of weight prior to conception improves live birth rate in women with obesity with or without PCOS. Treatment of obesity may include important lifestyle therapy, exercise and diet, bariatric surgery and pharmacological treatment [8]. This study aimed to evaluate association between IL36ɣ and AMH and study the relationship between obesity and AMH of women in the age of reproductive (25-35) yrs.

Materials and Methodology

This study included 28 infertile women with PCOS, their husbands were apparently normal (hormones and seminal fluid analysis), their age (25-35) years, and 20 healthy women their age range (25-33) years as control. All control women and patients were from outpatients unit of Alkadumia Teaching Hospital in Baghdad and all the parameters were measured in Specialist Sigma Laboratory. A total of 28 infertile women at reproductive age were enrolled in this study, a complete history was recorded on specially prepared data sheet including; age, weight, height, cycle history, hirsuitism. PCOS women were already on treatment with metformin at 1500-1700mg\day for at least 2 months. Women with PCOS were based on Rotterdam Consensus Group Criteria for Definition of PCOS. The control group had regular cycles, no endocrine abnormalities, and normal ultrasonic ovarian morphology. In this study diabetic and pregnant women were excluded. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). When there were two independent groups, they were compared by Student's t-test. The degree of association between continuous variables was calculated by Spearman's correlation coefficient (r). Values were expressed as mean ± SD A P-value < 0.05 was considered to be statistically significant, P-value <0.01 was considered to be statistically highly significant.

Volume 28, Issue 3, 2017 DOI: http://doi.org/10.23851/mjs.v28i3.551

Results and Discussion

Table 1 showed Demographic characteristics of studied groups. There were significant difference ($P < 0.05$) in BMI in infertile women when compared with healthy control group (37.65 ±5.47), (22.2 ±2.7) respectively.

Table 1: Demographic Characteristics of PCOS Women and Control.

	PCOS patients	Controls	P-value
	Mean ±SD	Mean ±SD	
No.	28	20	
Age (years)	29.1 ±2.66	29.2 ±2.70	NS
BMI (kg/m^2)	37.65 ±5.47	22.2 ±2.7	<0.05

The levels of IL36Ɣ showed significant increase ($P<0.05$) between PCOS patients and control (437.85±48.94),(427±46.31) Pg/ml respectively ,also AMH showed a significant statistical increase between PCOS patients (3.74± 0.61) ng/ml and control group $P<0.05$(1.74±0.39) ng/ml. There were non-significant difference between patients and control in LH and FSH levels as shown in Table 2. The correlation between IL36Ɣ with different biochemical parameters were shown in Table 3 and there was non-significant correlation between IL36Ɣ with AMH, LH and FSH.

Table 2: The Biochemical Parameters of PCOS Patients and Control.

	PCOS patients	Controls	P-value
	Mean ±SD	Mean ±SD	
IL36Ɣ Pg/ml	437.85±48.94	427±46.31	>0.05
AMH ng/ml	3.74±0.61	1.74±0.39	<0.05
LH	5.83±0.605	4.85±0.41	NS
FSH	5.65±0.84	4.23±0.49	NS

Humburg et al study the clinical significant of AMH and have confirmed AMH increasing levels that is mean two to three times more in PCOS than the women with normal ovary [10]. While the study of Cook CL et al reported that significantly increase levels of AMH in an-ovulatory patients when it is compared with control [11]. Women with PCOS have high levels of concentration of AMH in spite of they are lean or obese when compared with control and this has been reported in many studies that deal with samples from a community that include lean and overweight women. The concentration of AMH increased in PCOS women than non PCOS women and this in any case of insulin resistance and adiposity status [12]. Also another study reports the similar results that non obese adolescents with PCOS had very high levels of AMH 1.5 times than control [13].

Table 3: Correlation coefficient (r) and P-value between IL36Ɣ with different biochemical parameters studied.

	IL36Ɣ	
variable	r*	p-value
AMH	0.037	NS
LH	0.082	NS
FSH	0.066	NS

*r=Regression

The secretion of AMH by granulose cells from small antral and prenatal follicles in the ovary and this hormone decreased the aromatase induction by FSH in antral in the follicles and make inhibition in recrument of primordial follicles [14].The increase of production of AMH induces the decrease in the sensitivity of follicles to FSH at levels of receptors, which is very necessary for growing of this follicles, it leads to an increase of the numbers of antral follicles on the detriment of their size and the number of small antral follicles (size2-5mm) increases, therefore the selection of the dominant follicle. Like this situation classified clinically by anovulation cycles, manifesting themselves as oligo or amenorrhea [15]. The study of Sighinolfi et al reported that the overweight and also obese fertile women when compared with normal weight women have lower concentration of FSH, LH, inhibin B, and estradiol in the first phase of follicles growth and with direct inhibition of body mass on gonadotropin and estradiol production, independent of age [16], while other studies

showed a negative results of the effect of obesity on the biochemical parameters of ovarian reserve [17].

Pigny and his coworkers suggested that the women with PCOS have high levels of AMH in comparison with control group (mean serum AMH level was markedly increased in the PCOS group (47.1 +/- 22.9 vs. 20.8 +/- 11.6 pmol/liter in controls; $P < 0.0001$) and this results agree with our results [18]. The biological and functional roles of IL36ɣ in the healthy subject and disease were rarity on its data .The super family of interleukin-1 of cytokines comprises a group of essential mediators of inflammation more than the other cytokine family. The ligands and receptors of interleukin-1 were related with both acute and chronic inflammation and the pivotal action of interleukin 36 cytokines stay wily in immune responses [19] [20]. Our results show that serum levels of IL36ɣ were increased in PCOS women when compared with healthy subject ($p_{<0.05}$).

Since interleukin 36 consider proinflamatory marker some studies report that they used drug to decrease the levels of this interleukin especially in cardiovascular disease and this will shred a new light on the treatment of PCOS also this need to further studies based on large subjects [21].

Studies on the impact of obesity and weight loss on AMH levels are few; some results show that obese in reproductive age (34-48) years had lower levels of AMH when compared to healthy subject [22]. The study of Foster A.M. et al reported that there are three hypothesis: 1- The effect of obesity on the catabolism of AMH 2- The relation between obesity and ovarian dysfunction 3-The ovarian potential could reduce by obesity, also none of these hypothesis supported or rejected. It is necessary to elucidate the effect of obesity on the function of ovary [23].

In our study there was no significant association between AMH and Il36 ɣ $P<0.05$ (Table 3). Mostly cytokines have autocrine modes from its action and there are many studies suggested the role of follicles cytokines in PCOS women and the levels of this cytokines and function in the ovary. Study of Freeman E. *et al* [24] of cytokines showed that the evaluation of this cytokines have influence from systemic point of view in order to get pregnancy and to avoid consequences. Interleukin 36 was evaluating in PCOS women presenting infertility factors and this can explain the effect of ovarian response [25] [26] [27]. Increased levels of interleukin 36 not intrinsic characteristic in women with PCOS, but it are a useful monitoring clinical parameter in order to treat PCOS [28].

Conclusions

The conclusion of this study was: high levels of IL36ɣ in PCOS women with its associated with increased the levels of AMH and that possibly due to increase the number of small antral follicles .IL36ɣ could be considered as clinical biochemical parameter in PCOS in Iraqi female patients.

Acknowledgment

I would like to thank the staff of Sigma laboratory to their help in this study, especially Dr. Fatin Saad Maki and Dr. Ban Almamoori to their assistance.

References

[1] Cristescu C. and Neagu M., "Anti-Műllerian hormone a prognostic marker for metformin therapy efficiency in the treatment of women with infertility and polycystic ovary syndrome." Vol.15, 4, 2012 .

[2] HimabinduY., Sriharibabu KM "Determination of Anti-Müllerian hormone level in Polycystic Ovary Syndrome, Infertile, and Healthy Iraqi Women.," Vol. 24,3, 2013.

[3] Shevell T, Malone FD and Vidaver J. , ", Assisted reproductive technology and pregnancy outcome.," Vol. 106,5, 2005 .

[4] Makar RS and Toth T., "The evaluation of infertility." vol. 117, 2002 .

[5] Himabindu M Sriharibabu, KK Gopinathan, "Anti-mullerian hormone and antral follicle count as predictors of

ovarian response in assisted reproduction," Vol. 6,1, 2013 .

[6] Gelbaya TA, Majumder K, and Laing I, "The use of anti-Mu¨ llerian hormone and antral follicle count to predict the potential of oocytes and embryos," Vol. 150, 2010 .

[7] DeVet A, Laven JS, JS,and DeJong FH, , " Antimullerian hormone serum levels:aputative marker of ovarian aging.," Vol. 77, 2002 .

[8] Motta AB" The role of obesity in the development of polycystic ovary syndrome," Vol. 18, 17, 2012 .

[9] Jenny A VisseFrank H de Jong, Joop S E Laven1 and Axel P N Themmen, "Anti-Müllerian hormone: a new marker for ovarian function," Vol. 131, 2006 .

[10] Homburg R.and Crawford G., "The role of AMH in anovulation associated with PCOS: a hypothesis," Vol. 29, 2014 .

[11] Cook CL, Siow Y, Brenner AG and Fallat ME, "Relationship between serum mulerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women," Vol. 77,1, 2002 .

[12] Cassar S, Teede H., "Polycystic Ovary Syndrome and Anti-Müllerian Hormone: Role of insulin resistance, androgens, obesity and gonadotropins," Vol. 81,6, 2014 .

[13] Sopher AB. Laura D., "Anti-Mullerian hormone may be a useful adjunct in the diagnosis of polycystic ovary syndrome in nonobese adolescents," Vol. 27, 2014 .

[14] Carlsson IB, Scott JE, Visser JA, Ritvos O, Themnen AP, Hovatta O., "Anti-Mullerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro.,"Vol. 21, 2006 .

[15] La Marca A. and Volpe A., " Anti-Mullerian hormone in female reproduction: is measurement of circulating AMH a useful tool?," Vol. 64, 2006 .

[16] Sighinolfi G, Radi D, Argento C , " Anti-Mullerian hormone as a predictive marker in assisted reproductive technology (ART).," Vol. 16, 2009 .

[17] Pergola G., Maldera S., and Tartagni M, "Inhibitory effect of obesity on gonadotropin, estradiol, and inhibin B levels in fertile women.," Vol. 14,11, 2006 .

[18] PignyP. ,Merlen E., and Y., " Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest.," Vol. 88, 2003 .

[19] Piltonen T, Morin P., Koivunen R, and Perheentupa A." Serum anti-Mullerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with polycystic ovary syndrome," Vol. 20, 2005 .

[20] Gresnigt S. and, Rösler B., "The IL-36 receptor pathway regulates Aspergillus fumigatus-induced Th1 and Th17 responses.," Vol. 43,2, 2013 .

[21] Vigne S., and Palme G. " IL-36R ligands are potent regulators of dendritic and T cells.," vol. 118, 2011 .

[22] Dinarello C., and Arend W., " IL-1 Family Nomenclature.," Vol. 11, 2010 .

[23] Foster AM, Baliwag J, and Chen CS, "IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin," Vol. 192, 12, 2014 .

[24] Freeman E, Gracia C, Sammel MD, Lin H, Lim LC. and Strauss JF, " Association of anti-Müllerian hormone levels with obesity in late reproductive age women.," Vol. 87, 2007 .

[25] Seifer D and MacLaughlin D,, " Müllerian inhibiting substance is an ovarian growth factor of emerging significance.," Vol. 88, 2007 .

[26] Simón C1, Mercader A, and Frances A., " Hormonal regulation of serum and endometrial IL-1 alpha,IL-1 beta and IL-1ra: IL-1 endometrial microenvironment of thehuman embryo at the apposition phase under physiological

andsupraphysiological steroid level conditions.," Vol. 31, 1996 .

[27] Karagouni EE, Chryssikopoulos A, Mantzavinos T, Kanakas N and Dotsika EN., "Interleukin-1beta and interleukin-1alpha may affect the implantation rateof patients undergoing in vitro fertilization-embryo transfer.," Vol. 70, 1998 .

[28] Zheng P. ,Yifan S. ,Xiaolan L., Hongyu Z., Chunming L. and Shengming D., PLOS ONE J, ". Interleukin-6 Levels in Women with Polycystic Ovary Syndrome," Vol. 11, 2, 2016 .