WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 299-308. Research Article ISSN 2277– 7105

THE ROLE OF SERUM AND FOLLICULAR FLUID RESISTIN IN ASSESSING THE PREGNANCY OUTCOME IN PATIENT WITH PCOS

Prof. Dr. Nawal Khairy AL-Ani[1*], Dr. Rabab AlwanTaher[1], Dr. Thuraya Husamuldin Abdullah[2]

[1]High Institute for Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University, Baghdad, Iraq.

[2]Al imamin Teaching Hospital, Baghdad, Iraq.

Article Received on
02 Oct 2015,

Revised on 27 Oct 2015,
Accepted on 20 Nov 2015

***Correspondence for Author**
Prof. Dr. Nawal Khairy AL-Ani
High Institute for Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University, Baghdad, Iraq.

ABSTRACT

The hormone resistin, a cysteine-rich protein, is secreted by adipocytes . Serum resistin levels are significantly increased in insulin-resistant mice and genetically or diet-induced obese mice .In addition, neutralization of endogenous resistin with antibodies significantly suppresses hyperglycaemia in diet induced obese mice by increasing insulin sensitivity**.** The objective of this study is to compare level of serum and follicular fluid resistin in infertile women with PCOS undergoing IVF\ICSI and to assess possible correlation between resistin and pregnancy outcome.A total of 90 infertile women(60 women with Poly cystic ovary syndrome and the other 30 women as a control)undergoing controlled ovarian hyperstimulation for intracytoplasmic sperm injection cycle were prospectively recruited for this study in center of fertility and in vitro fertilization at Kamal AL_Samarai Hospital (Baghdad/Iraq)during the period from September 2014 to the end of February 2015. All patients underwent a long standard gonadotrophin releasing hormone agonist protocol(GnRH-a). Serum and follicular fluid resistin hormone levels were measured on the day of oocyte retrieval by using Enzyme linked immuno sorbent assay**.** No significant differences in resistin levels of either serum or follicular fluid between PCOS and control group were found. However, resistin levels in serum were highly significant than that in follicular fluid in both groups.There was a strong positive correlation between BMI and LH(luteinizing hormone)with the resistin in FF and serum. Resistin levels in both serum and follicular did not correlate with estradiol,P4,and testosterone level. No significant correlations

were found between reisistin levels and fertilization rate, clinical pregnancy rate in both PCOS and control groups. We can conclude that Resistin is unlikely to be a major determining factor in the growth and maturation of oocytes during IVF-stimulated cycles in PCOS, so it cannot be used as a test in predicting pregnancy after intracytoplasmic sperm injection cycles.

KEYWORDS: follicular fluid/IVF/ICSI/polycystic ovarian syndrome/resistin.

INTRODUCTION

Polycystic ovarian syndrome:PCOS is aheterogeneous syndrome characterized by oligomenorrhea or amenorrhea,hyperandrogenism,and multiple small subcapsular cystic follicles in the ovary on ultrasonography.[1] It affects nearly 5-10% of women of reproductive age.[2] and nearly 16-80% of the affected women are obese.[3] The syndrome is frequently associated with insulin resistance accompanied by a compensatory hyperinsulinemia and obesity.[4] which have been thought to play an important role in the etiology of PCOS.[5] Abdominal obesity is a feature of overweight PCOS patients.[6] Abdominal obesity is associated with insulin resistance, a high production of free fatty acids and an increased risk of type-2 diabetes and cardiovascular disease.[7] Abdominal obesity is associated with changed secretion of several adipocyte derived peptide hormones, commonly named adipocytokines.The production of adipocytokines has been shown to affect insulin sensitivity and to be an important predictor of the metabolic syndrome.[8]

The hormone resistin, a 12.5 kDa cysteine-rich protein, is secreted by adipocytes Serum resistin levels are significantly increased in insulin-resistant mice and genetically or diet-induced obese mice.In addition, neutralization of endogenous resistin with antibodies significantly suppresses hyperglycaemia in diet induced obese mice by increasing insulin sensitivity.[9] Furthermore, resistin gene expression in obese mice is down-regulated by thiazolidinedione compounds,The relation between PCOS and resistin has been discussed in some studies, which showed that serum resistin levels are not elevated in PCOS women with insulin resistance[10,11], and resistin was therefore not regarded as a major determining factor of PCOS-associated insulin resistance. However, levels of resistin mRNA in adipocytes are increased 2-fold in PCOS patients[10], suggesting that the resistin gene may be a local determining factor in the pathogenesis of PCOS.

Follicular growth and maturation is a complex process regulated by autocrine and paracrine factors[12] and provides the environment in which oocyte maturation occurs. Levels of cytokines such as interleukin (IL)-6,leptin,and tumor necrosis factor(TNF)-a in the follicular fluid affect the success of fertilization and early embryonic development[13], including in women with PCOS.[14] The purpose of this study was to compare levels of serum and follicular fluid resistin in infertile women with PCOS undergoing IVF\ICSI and to assess possible correlation between resistin and pregnancy outcome.

Subjects and methods

This is a prospective case control study which included 90 infertile couples enrolled in assisted reproductive technology (ART) programs to enter their ICSI cycle in Kamal AL-Samarai Hospital, Center of Fertility and IVF(Baghdad/Iraq) during the period from August 2014 to the end of February 2015. All patients were informed about the study and signed a written informed consent.

Subjects

Sixty infertile women who fulfilling at least two of the following three criteria based on the Rotterdam ESHRE/ASRMS sponsored PCOS consensus workshop group:

1) Polycystic ovary on ultrasound.

2) Chronic anovulation or oligovulation.

3) Clinical or biochemical Hyperandrogenism.

All were in good health and had not taken oral contraceptives within the last 3 months. The exclusion criteria also included patients aged more than 43 years or with serum FSH levels >12 mIU/ml. Evidence of endocrine abnormalities such as thyroid dysfunction, diabetes mellitus,cushing's syndrome and congenital adrenal hyperplasia were excluded. Control group included 30 women with regular cycles undergoing ICSI cycle because of(17 with male factor infertility,10 of them with tubal infertility, and 3 with unexplained infertility).They were free of signs and symptoms of PCOS depending on the following criteria.

a. Regular menstrual cycle (26 to 30 days)

b. No history of endocrine disease

c. No use of medication or oral contraceptives

Stimulation protocol and IVF

1. GnRH Agonist–based protocol

All of the patients were treated with a long protocol for ovarian stimulation. For pituitary down-regulation, the patients were treated with a daily administration of subcutaneous injection of GnRH-a, triptorelin (Decapeptyl®; 0.1 mg, Ferring Co, Kiel, Germany) started on the 21st day of the previous menstrual cycle until and including the day of hCG administration. After 2 weeks the pituitary desensitization was completed by reaching the level of E2< 50 pg/ml.

After down regulation with GnRH-a, COH was initiated with recombinant human FSH (rhFSH, Gonal-F®; Merck Serono, Germany) containing 75 IU of FSH activity per ampoule by daily subcutaneous injection. Starting dose of FSH (150-450 IU) from day 2 of next menstrual cycle, and it depends on the women's age and previous response of ovulation induction. The doses of Gonal-F® and follicle growth were monitored by serum E2 level and trans-vaginal ultrasound after the day 6-8 of (Gonal-F®) injection and till the day of Hcg administration.Ovulation induction was induced by the administration of recombinant hCG (rhCG 6500 IU, Ovitrelle®;Merck Serono, Italy) subcutaneously when either one or two lead follicles have reached 18 mm.

2. Oocyte Retrieval

Oocyte retrieval was performed by a gynecologist 34 hours following hCG by an ultrasound-guided transvaginal oocyte aspiration and under general anesthesia or local anesthesia and some form of intravenous sedation. Intracytoplasmic sperm injection was done in male infertility cases. Embryos were transferred 2~3 d after the oocyte retrieval. The luteal phase was supported with transvaginal administration of P (Cyclogest®) 200-400mg twice a day starting from the day of oocyte retrieval until the day of pregnancy test and if the test was positive, P treatment was continued up to 12 gestational weeks.

3. Clinical pregnancy confirmation

A pregnancy test was done 14 day after the embryo transfer. If the test was positive, a transvaginalultrasound study was performed 2-3 weeks later to confirm a clinical pregnancy.

Sample collection

1. Basal Blood Sampling and Hormones Assays

On day 3 of the menstrual cycle before starting COH, blood samples were drawn from each patient for FSH, LH,LH/FSH ratio assays, allowed to clot for 30 minutes and centrifuged at 2500 rpm within 10 minutes to separate the serum. In the same day, basal hormonal status were determined by using miniVIDASsystem. Kit for measurement of FSH and LH were (VIDAS® FSH and VIDAS®LH bioMerieux , France) respectively .

2.Blood and FF Sampling at Day of Oocyte Retrieval

For determination of serum E2, P, testosterone and resistin, blood samples were taken, on the day of oocyte retrieval, immediately before the procedure and allowed to clot for 30 minutes. Sera were obtained after centrifugation at a rate of 1600 rpm for 15 minutes, then the clear sera are stored at -20 °C until assayed. On the other hand, for the determination of E2, P4,testosterone and resistin hormones levels within FF. FF was aspirated by a gynecologist on the day of oocyte retrieval from all follicles under transvaginal ultrasound guidance. The obtained FF was centrifuged at a rate 600 rpm for 5 minutes to separate cellular contents and debris. Lastly the resulting FF supernatant were stored at −70 °C until assayed .Resistin was determined by ELISA (CUSABIO,CSB-E06888h,China), and assays were conducted accordingto the manufacturer's instructions.Steroidal hormones (E2, P and testosterone) were measured in the obtained sera and FF via enzyme-linked immunosorbent assay (ELISA) technique by using diagnostic kits were provided by (MonobindInc.,USA).

Statistical Analysis The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Numeric variables were expressed as(mean ± standard error (SE), while nominal variables were expressed as number and percent. Least significant difference –LSD test was used to significant compare between means and Chi-square test was used to significant between percentage in this study. Estimate of correlation coefficients between some parameters study. Person's correlation coefficient was used to evaluate correlation between numerical variables.

RESULTS

Patient characteristics

The characteristics and hormonal profiles of the PCOS and control groups are shown in Table 1. There were no differences in age, BMI, or duration of infertility between the two

groups. The levels of LH and the LH:FSH ratio were significantly higher in PCOS than in control group (P<0.001). Basal FSH was significantly higher in control group than that in PCOS(P<0.001).

Outcomes of ICSI between PCOS and control group

Table 2 shows the clinical data for the ICSI cycle characteristics and reproductive outcome in the women with PCOS and control group. The total number of oocytes retrieved were significantly higher in PCOS than that in control group (P<0.001). The mean number of mature oocytes [metaphase II (MII)] were more in PCOS than in control groups(P<0.05). There were no significant difference (P>0.05) in both fertilization rate and the number of embryos transferred between PCOS and control. The pregnancy rate in PCOS patients was highly significant (P<0.001) than control.

Resistin and steroids hormone levels in serum and follicular fluid

Table 3 shows the levels of serum on the day of oocytes retrieved and follicular resistin and steroids hormone levels in both PCOS and control groups. There was no significant difference in the levels of serum or follicular resistin between the two groups. However, the serum resistin levels were significantly higher than the follicular fluid resistin levels in both the control and PCOS groups(P<0.001).Serum and follicular fluid levels of both E2 and testosterone were highly significant in PCOS than in control groups, while follicular fluid levels of P4 in PCOS was significantly lower than in control groups(P<0.001).

Table 1 Clinical and baseline hormone concentrations in PCOS and control groups

Characters	PCOS		Control		LSD
	Mean	SE	Mean	SE	
Age(years)	28.33	0.94	28.56	1.24	0.492 NS
BMI kg/m^2)	28.69	1.05	28.33	0.95	0.562 NS
Duration of Infertility	5.72	0.51	5.42	0.40	0.467 NS
Basal LH (mIU/ml)	6.40	0.47	2.81	0.22	0.0001**
Basal FSH (mIU/ml)	4.36	0.26	6.14	0.37	0.0002**
LH/FSH ratio	1.47	0.29	0.457	0.00	0.0033**

Table 4-2. Comparison between patients and control in ICSI Outcome

Parameters	Mean ± SE		P-value
	PCOS	Control	
Oocyte (total)	10.78 ± 0.88	7.33 ± 0.87	0.0155 **
Oocytes (MII)	7.26 ± 0.62	5.27 ± 0.63	0.046 *
FR%	57.76 ± 3.56	55.16 ± 6.08	0.695 NS
ET Number	2.65 ± 0.17	2.23 ± 0.22	0.152 NS
Clinical pregnancy rate (%)	23.33	10.00	0.0001*

Table 3 Serum and follicular fluid (FF) resistin and hormone levels in PCOS and control groups during ICSI-cycles

Parameters	mean± SE		P-value
	PCOS	Control	
FFresistin(ng/ml)	85.31± 6.98	78.99 ± 8.72	0.588 NS
FF E2 (pgm/ml)	4170.37± 100.37	3587.12± 99.04	0.0004 **
FF P4 (ng/ml)	21.73 ± 2.77	30.18 ± 0.13	0.0345 *
FF Testosterone (ng/ml)	9.77 ± 0.81	4.34 ± 0.87	0.0003 **
Serum resistin (ng/ml)	179.85 ± 7.01	178.34 ± 22.24	0.935 NS
SerumE2(pgm/ml)	1463.68 ± 56.18	1008.81 ± 81.04	0.0001 **
Serum P4 (ng/ml)	9.44 ± 1.50	9.06 ± 0.86	0.286 NS
Serum Testosterone (ng/ml)	1.30 ± 0.046	0.83 ± 0.020	0.0001**

Correlation of resistin and steroids hormone and outcomes of ICSI-ET cycle

Strong positive correlation was found between follicular or serum resistin levels and BMI($r=0.58$, $P<0.001$,and $r=0.37$, $P<0.001$ respectively) and LH($r=0.21$,$P<0.05$,$r=0.22$, $P<0.05$respectively),there was strong negative correlation between FF E2 and serum resistin ($r=-0.38$, $P<0.001$).No correlations were found between serum or follicular resistin levels and age,P4, testosterone, basal FSH ,total number of oocyte , fertilization rate, and number of ET.

DISCUSSION

In this study, we found that PCOS women on ICSI cycle showed no significant difference in serum or follicular fluid resistin levels compared to ICSI-treated control subjects, however, resistin levels in serum were higher than that in follicular fluid in both groups. Furthermore, there was no significant difference in serum or follicular fluid resistin levels between the women who succeeded in becoming pregnant and those who did not in both PCOS patients and control group. This is in agreement with results obtained by Seow et al[15], and LU Xiu-e et al.[16] While Shamam et al[17] found that mean serum resistin concentration was increased in women with PCOS compared with the

control group. He also showed that serum resistin level is a strong positive correlation with BMI. This agrees with the fact that adipose tissues usually function as highly specialized endocrine and paracrine organs producing an array of adpokines that include resisten leptin ,TNF-α, and adiponektine.[18] These results agree with the results of this study. Also this study showed strong positive correlation of follicular resistin level and BMI. This is in conflict with what have been mentioned by Seow et al[18] who found a poor correlation between serum and follicular resistin levels and BMI. This is because the number of PCOS patients were very small and statistically feasible. Also LU Xiu-e et al showed that resistin levels in serum and follicular fluid did not correlate with BMI this is because PCOS patients and control group had normal weight (BMI<25 kg/m2).[16] According to the correlation between resistin and the steroid hormones, in the present study, there was significant positive correlation between basal LH and follicular resistin. No correlations were found between serum or follicular resistin levels and serum and follicular fluid(E2, P4, Testosterone) and basal FSH .Also there was no significant correlations found between resistin levels and total number of oocyte, oocytes (MII),FR%, and number of embryos transferred . Both Seow et al[15], and LU Xiu-e et al[16] found in their studies no significant correlation was found between resistin levels and estradiol, LH, or between follicular resistin levels and fertilization rate, implantation rate, clinical pregnancy rate, or early miscarriage rate in PCOS. Their studies showed that resistin does not have correlation with the hormonal and metabolic parameters as well as the outcomes of IVF. These data suggested that resistin is unlikely to be a local determinant factor in steroidogenesis and growth and maturation of oocytes during IVF-ET in cycles PCOS. In the present study, pregnancy rate was highly significant in PCOS patients when compared with control group, this result are agreement with the previous results that found by Esinler et al[19] who found that satisfactory pregnancy rates of PCOS patients were due to sufficient numbers of collected oocytes, fertilized oocytes, and transferred high-quality embryos. In addition in our study the number of PCOS patients(60)more than control (30). In contrast, others found that there was no difference in the pregnancy rate but the clinical abortion rate for PCOS patients was higher than controls. The authors suggested that only cytoplasmic but not nuclear maturity was influenced in PCOS patients.[20]

This study showed that both serum and follicular E2 highly significant in PCOS patients than control group .These results agreed with results obtained by Yi-Ping Zhong et al[21]

who found that compared to the control groups PCOS patients exhibited reduced duration of ovarian stimulation and total Gn dose, as well as increase serum and follicular E2 and number of collected oocyte.This results explained by previous studies have suggested that compared to other infertility patients, PCOS patients exhibited a higher degree of ovary vascularization during the process of ovarian stimulation, which paralleled with vascular endothelial growth factor [VEGF] levels in serum and follicular fluid.

In conclusion, the lack of a significant difference in serum and follicular resistin levels between PCOS and the control groups and between pregnant and non-pregnant group and the fact that resistin levels in the follicular fluid compared to serum were lower in both groups of women during an IVF-stimulated cycle suggest that resistin is unlikely to be a major determining factor in the growth and maturation of oocytes during ICSI-stimulated cycles in women with PCOS.

REFERENCES

1. Franks S, Polycystic Ovary Syndrome .N Eng IJMed., 1995; 333: 853-861.
2. Dunaif A. "Insulin resistance and the Polycystic Ovary Syndrome .mechanism and implications. Insulin action and hyperandrogenism: clinical, histological and biochemical findings" . J Clin Endocrinol Metab., 1997; 21: 1440 -1445.
3. Barber TM, McCarthy MI, Wass JAH *et al*.Obesity and Polycystic Ovary Syndrome.Clin Endocrinol., 2006; 65(2): 137-145
4. Burghen GA , Givens JR and Kit abchi AE. Correlation of hyperandrogensim with hyperansulinemia in Polycystic Ovarian disease. JClinEndocrinolMetab., 1980; 50: 113-116.
5. Chang RJ, Nakamura RM, Judd HL *et al*. Insulin resistance in non obese patients with polycystic ovarian disease. J Clin Endocrinol Metab., 1983; 57: 356 – 359.
6. Douchi T, Ijuin H, Nakamura S*et al*.Body fat distribution in women with polycystic ovary syndrome. Obstetrics & Gynecology., 1995; 86: 16–519.
7. Bjorntorp P. The android woman–a risky condition. Journal of Internal Medicine., 1996; 239: 105–110.
8. Fasshauer M &Paschke R. Regulation of adipocytokines and insulin resistance. Diabetologia., 2003; 46: 1594–1603.
9. Steppan CM, Bailey ST, Bhat S, *et al*. The hormone resistin links obesity to diabetes.

Nature., 2001; 409: 307–312.

10. Seow KM, Juan CC, Wu YL, *et al.* Serum and adipocyte resistin in polycystic ovarian syndrome with insulin resistance. Hum Reprod., 19: 48–53.
11. Panidis D, Koliakos G, Kourtis A,*et al.* Serum resistin levels in women with polycystic ovary syndrome.Fertil Steril., 2004; 81: 361–366.
12. Le´de´e-Bataille N, Lapre´e-Delage, Taupin JL, *et al*.Follicular fluid concentration of leukaemia inhibitory factor is decreased among women with polycystic ovary syndrome during assisted reproduction cycles. Hum Reprod., 2001; 10: 2073–2078.
13. Adashi EY. Growth factors and ovarian function: the IGF-I paradigm. Horm Res., 1994; 42:44–48.
14. Amato G, Conte M, Mazziotti G*etal*.Serum and follicular fluid cytokines in polycystic ovary syndrome during stimulated cycles. Obstet Gynecol., 2003; 101: 1177–1182.
15. Seow Kok-Min,Chi-Chang Juan,Yung-Pei Hsu,et al .Serum and follicular resistin levels in women with polycystic ovarian syndrome during IVF-stimulated cycles. Human Reproduction., 2005; 20(1): 117–121.
16. LU Xiu-e, HUANG He-feng ,LI Mei-gen, et al. Resistin levels of serum and follicular fluid in non-obese patients with polycystic ovary syndrome during IVF cycles. J Zhejiang Univ SCI., 2005; 6B(9): 897-902.
17. Kamila H. Shamam, Hedef D. El – Yassin, Haider Al – Shammaa. Serum resistin levels, and other hormonal and biochemical parameters in patients with polycystic ovary syndrome (PCOS). J Fac Med Baghdad., 2009; 51(20): 200-203.
18. Kershaw EE and Flier JS. Adipose Tissue as an Endocrine Organ. J. of Clinical Endocrinology & Metabolism., 2004; 89(6): 2548–2556.
19. Esinler, U.Bayar, G.Bozdag, and H.Yrali.Outcome of intracytoplasmic sperm injection in patients with polycystic ovary syndrome or isolated polycycysticovaries. Fertility and sterility., 2005; 84(4): 932-937.
20. Baoli Yin ,HaoyingHao, Duo Wie et al .Patients with Polycystic Ovary Syndrome have Successful Embryo arrest. Int J Clin Exp Med., 2015; 8(4): 6247-6251.
21. Yi-Ping Zhong, Ying Ying ,Hai-Tao Wu et al .Comparison of Endocrine Profile and In Vitro Fertilization Outcome in Patients with PCOS, Ovulatory PCO, or normal ovaries. International Journal of Endcrinology., 2011; 2012: 6.