

RESEARCH ARTICLE

Evaluation of Serum TNF- α in Insulin Resistant Non PCOS Patients Undergoing ICSI: A Randomized Clinical Trial

Manar Alhoda Z. Al-Faqheri, Mohammad O. Selman,
Manal T. Al-Obaidi

High Institute of Infertility Diagnosis and Assisted Reproductive
Technologies, Al- Nahrain University, Baghdad- IRAQ.

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Abstract

Background: With the rapid evolution of assisted reproductive technologies, more and more studies are concerned about the possible influential factors on the success rates of these procedures, with the efforts are now mainly directed toward defining these factors in a trial to increase these rates. Of these factors, inflammation is considered among the most remarkable and is in the focus of the updated studies. However, most of these studies are evaluating the role of inflammation in patients with polycystic ovarian syndrome(PCOS) , while the impact of this inflammation in insulin resistant non PCOS patients is underestimated and not well evaluated .

Objectives: This study is a trial to elucidate the importance of one of the inflammatory mediators , Tumor Necrosis Factor-alpha (TNF – α) , in insulin resistant patients undergoing IVF (ICSI) cycles .

Patients and Methods: A group of 27 non-PCOS infertile patients were selected for this clinical randomized prospective study from patients attending the high institute for infertility diagnosis and assisted reproductive technologies / Al-Nahrain University and subjected to intra-cytoplasmic sperm injection (ICSI) procedure. BMI was measured for all patients and they were classified according to WHO classification into 3 groups : Acceptable , overweight and obese . Fasting plasma level of glucose and insulin was investigated and insulin resistance index (HOMA) was calculated. Serum TNF- α has been measured using ELISA and the relation with insulin resistance was investigated. Finally, the pregnancy rate, as a primary reproductive endpoint was compared according to TNF - α and insulin resistance levels between different study subjects.

Results: Of the 27 infertile patients , 17(63 %) were non obese and 10 (37%) were obese , 15(55.5%) were having HOMA index < 1.6 and 12 (44.5 %) were having HOMA index $\geq$ 1.6 . BMI was found to be positively correlated with higher HOMA index (P = 0.01 and R= 0.4 in spearman correlation). Investigating the relation between TNF- α with BMI revealed that TNF- α levels are increasing with the increment in BMI, However , the difference in the mean TNF- α between the three BMI groups failed to achieve statistical significance (P=0.1). In contrast to that, when stratifying for HOMA index , TNF- α was significantly correlated with it (spearman correlation R= 0.4 , P=0.01) , and patients with HOMA index $\geq$ 1.6 were found to have higher levels of TNF- α (P = 0.03) and to be more obese (P=0.02) than those with HOMA index <1.6.

The effect of insulin resistance was also implicated on pregnancy rates , as those with high insulin resistance were shown to have significantly lower pregnancy rates when compared to those with low indices of resistance ($P = 0.03$) . However, the impact TNF- α on pregnancy rates was apparently less significant ($P= 0.5$).

Conclusion: Rate of asymptomatic bacterial vaginosis was 32.6 % in pregnant women compared to 73.3% in non- pregnant group, being highly significant higher in those women who failed to get pregnant.

Keywords: IVF, Bacterial vaginosis, oocyte & ICSI

Introduction:

Tumor necrosis factor α (TNF- α), also known as cachectin, is a powerful pro-inflammatory cytokine that can play an important role in the immune system during many events such as inflammation, cell proliferation, differentiation and apoptosis(1) .It was discovered in 1975 as a cytokine which showed cytotoxic activity after immune system stimulation causing tumor necrosis.

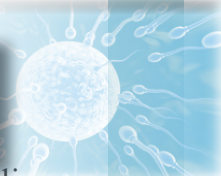
Emerging data suggested that the coding gene of TNF- α is expressed in adipose with higher levels being detected in the models of rodent obesity. These findings suggested that elevated levels of fat-derived TNF- α is a proposed factor for inducing insulin resistance linked with obesity(2). Moreover, another evidence of this effect has been derived from trials investigated the effect of normalization of TNF- α in obese animal models, which obviously detected at least partial normalization in insulin sensitivity(3)(4). Likewise, obese mice that are lacking TNF- α receptors are thought to be protected against insulin resistance(5).

The explanation of this effect may go in multiple directions, the best known is the direct augmenting effect of TNF- α on serine phosphorylation of IRS-1 (insulin receptor substrate-1) in tissues that are sensitive to insulin (6). This will lead subsequently to the reduction in the expression of insulin-sensitive glucose transport protein known as GLUT-4, and a decrease in glucose transport (7).In addition , the action and the synthesis of Peroxisome proliferator-activated receptor gamma PPAR γ (key regulators of glucose and lipid metabolism(8) is antagonized by TNF- α , while PPAR γ agonists (Thiazoli-

dinediones) are known to inhibit the effect of TNF- α on fat cells , as well as it's synthesis , giving a further clue about the relation between TNF- α and insulin resistance .

The reproductive effect of TNF- α mediated insulin resistance in PCOS has been discussed in many previous researches and tumor necrosis factor- α was found to be influential on many reproductive steps including follicular formation and maturation(9)(10) , steroidogenesis induced by gonadotrophins by the stimulation of theca-luteinized cell proliferation (11) synthesis of androgen (12) and may inhibit the differentiation of cultured granulosa cells(13) . Furthermore, elevated follicular fluid tumor necrosis factor- α concentration were reported to be related to a lower quality of oocytes, resulting in lower pregnancy rates (14).

In contrary, data about the impact of TNF- α mediated insulin resistance related to obesity on the reproductive parameters and pregnancy rates in non-PCOS patients is scarce . This study was carried out in a trial to elucidate the important role of TNF- α and the consequent insulin resistance in non PCOS patients and its impact on pregnancy rates .



Patients, Materials and Methods:

27 Iraqi infertile non polycystic ovarian syndrome patients were selected from those attended the high institute for infertility diagnosis and assisted reproductive technologies / Al-Nahrian University - Baghdad and subjected to intra-cytoplasmic sperm injection (ICSI) procedure. The study was approved by the local medical ethical committee of college of medicine, Al-Nahrain University, and written consent was obtained from patients to participate in the study.

Patients were divided according to their BMI into: Acceptable BMI (<25 Kg/m²), Overweight (25-29.9 Kg/m²) and Obese (30+ Kg/m²), and according to HOMA index into : < 1.6 and ≥ 1.6 .

Body mass index (BMI):

The BMI was measured by dividing the weight in kilograms by the height in squared meters (kg/m²). The females with BMI of ≤ 18.5 considered as underweight, 18.5-24.9 was normal, 25-29.9 as overweight and ≥ 30 as obese (15).

Fasting glucose test:

Fasting glucose test is the measurement of glucose level in venous blood in fasting state. The centrifuge tubes of the serum which were collected and stored at day of oocyte retrieval were thawed and analysis done using automated spectrophotometer.

Insulin level measurement:

Insulin levels were analyzed through an enzyme linked immunosorbent assay (ELISA) technique. The kit were purchased from KONO and labelled as human insulin ELISA kit. Assay range: 0.5mu/L-16mu/L.

Homeostatic model assessment (HOMA):

The HOMA represents the dynamic relationship between glucose and insulin predicting fasting glucose and insulin concentrations for a wide range of possible combinations of β -cell function and insulin resistance. HOMA is calculated

ed by multiplying fasting plasma insulin (FPI) by fasting plasma glucose (FPG), then dividing by the constant 22.5, i.e. $HOMA-IR = (FPI \times FPG) / 22.5$ (16). In this study , we used a HOMA level of 1.6 as an indicator for insulin resistance (17).

TNF- α assay in the serum:

The TNF- α levels were analyzed through an enzyme linked immunosorbent assay (ELISA) technique .The kit were purchased from KONO and labelled as human TNF- α ELISA kit. Assay range: 20ng/L-400ng/L. Since there is no clear cut off value for serum TNF- α , a level of 121 ng\L (the median value of the sample size) was used in this study to compare between study subjects .

Results:

In table 1,figure 1 body mass index is classified into 3 categories

Table 1. Classification of Body mass index

parameters	BMI category			Total
	Acceptable	overweight	Obese	
N (%)	5 (18.5%)	12(44.5%)	10 (37%)	27 (100%)
Range	19.6 - 24.5	26-29.1	30.4-43.1	19.6 - 43.1
Mean	22.6	27.4	34.8	29.1
SD	13.2	0.87	4	5.5

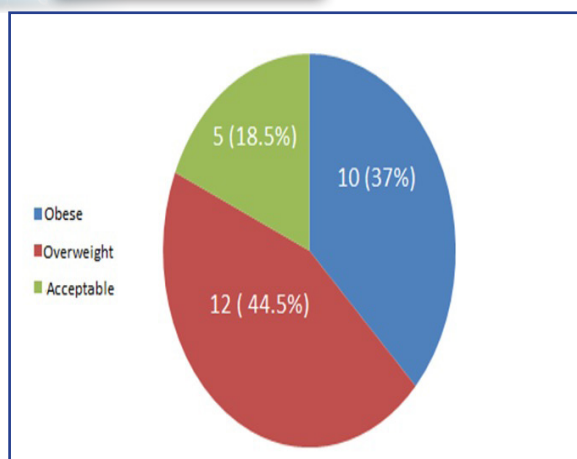


Figure (1): Classification of Body mass index

In figure 1 body mass index is classified into acceptable (5 cases) with a mean of 22.6, overweight (12 cases) with a mean of 27.4 and obese (27 cases) with a mean of 34.8

In table 2, figure 2 classification of patient according to HOMA insulin resistance.

Table 2. Classification of HOMA insulin resistance

parameters	HOMA IR		Total
	<1.6	≥ 1.6	
N (%)	15(55.5%)	12(44.5%)	27
Range	0.5 -1.4	1.6 -3.5	0.5-3.5
Median	1	2.2	1.4

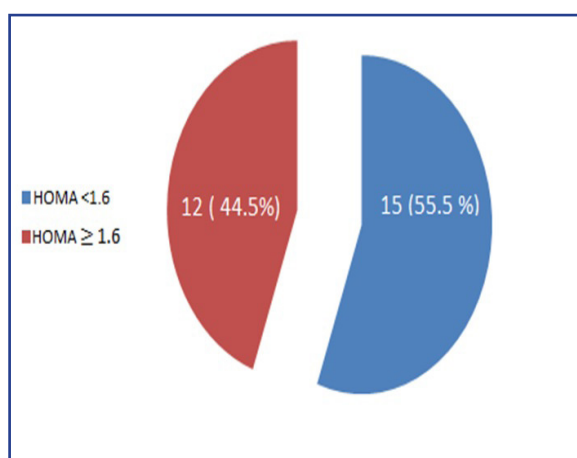


Figure (2): Classification of HOMA insulin resistance

In figure 2 patients were classified into two groups according to their HOMA insulin resistance (group of HOMA<1.6 15 cases with a median of 1 and group of HOMA ≥1.6 12 cases with a median of 2.2)

In figure (3): A positive correlation was found between BMI and HOMA index. The value of R is 0.46687 and the two-tailed value of P is 0.01. By normal standards, the association between the two variables would be considered statistically significant

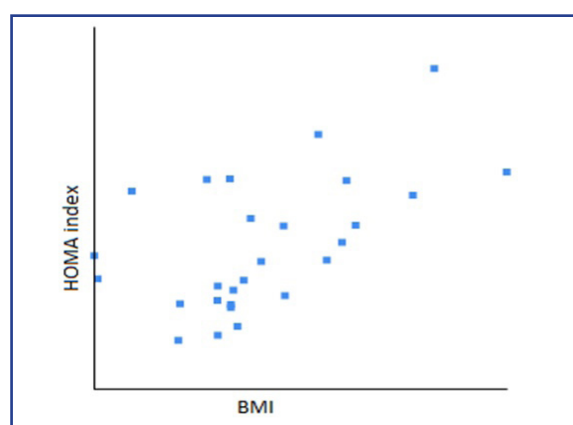
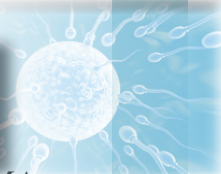


Figure (3): correlation between BMI and HOMA index

In table 3 although there is an increment of serum TNF-α with higher BMI, this increment failed to achieve statistical significance

Table3. Relation between BMI and TNF-α

BMI	S. TNF -a		P value
	Median	Range	
Acceptable	81.5	53.5	0.1
Overweight	131.75	186.5	
Obese	133	182.5	
Total	121	187.5	
Acceptable * Overweight = 0.1			
Acceptable * Obese = 0.06			
Overweight * Obese = 0.6			
Non obese * obese = 0.3			



The value of R is 0.31302 and the two-tailed value of P is 0.11188. By normal standards, the association between the two variables would not be considered statistically significant

In table 4 median of TNF- α and median of BMI were significantly higher with HOMA index ≥ 1.6

Table 4. TNF- α and BMI stratified by HOMA index

HOMA - Index	S. TNF -a		P Value
	Median	Range	
<1.6	90.5	187.5	0.03
≥1.6	160	177	
	BMI (Mean)		
<1.6	26.5		0.02
≥1.6	32.3		

In figure (4) , A statistical significant correlation was found between serum TNF- α and HOMA index. The value of R is 0.47382 and the two-tailed value of P is 0.01254. By normal standards, the association between the two variables would be considered statistically significant.

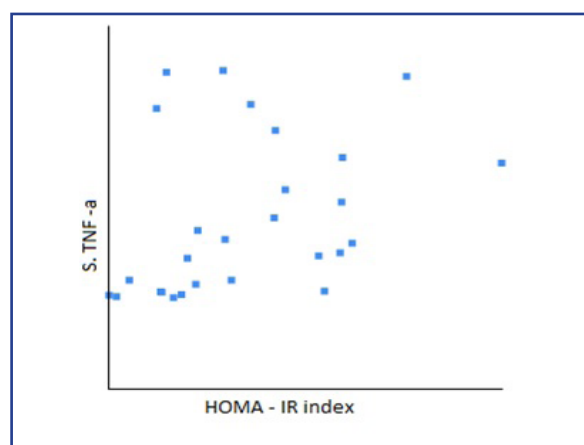


Figure (4): correlation between serum TNF- α and HOMA index

Table (5): Number of cases having HOMA INDEX ≥ 1.6 stratified by TNF- α median

TNF- α	No. (%)	HOMA index		P value
		No of cases <1.6	No of cases ≥ 1.6	
<121	13 (48.5%)	10	3	0.03
≥ 121	14 (51.5%)	9	5	
Total	27 (100%)	19	8	

In table (5) when TNF- α increased above 121 the number of cases with HOMA index ≥ 1.6 is also increased.

Table(6): Predicting pregnancy outcome

HOMA - Index	No. (%)	pregnancy outcome		P value
		positive	negative	
<1.6	15 (55.5%)	11	4	0.03
≥ 1.6	12 (44.5%)	4	8	
Total	27 (100%)	15	12	

In table (6) significant lower pregnancy rate achieved when HOMA index ≥ 1.6

Table(7): Predicting pregnancy rate stratified by TNF- α

TNF- α	No. (%)	pregnancy outcome		P value
		positive	negative	
<121	13 (48.5%)	8 (61.5%)	5 (38.5%)	0.5
≥ 121	14 (51.5%)	7 (50%)	7 (50%)	
Total	27 (100%)	15	12	

In table (7) pregnancy rate was lower with TNF- α levels ≥ 121 however it failed to achieve a statistical significance.

Discussion:

The relationship between inflammation, insulin resistance and obesity was meticulously examined in previous trials for PCOS patients so as to demonstrate the proper connection between them and to implicate the conclusions of these trials on the daily practice and management of PCOS patients with infertility. On the other hand, the effect size of these three variables on non-PCOS patients and the importance of considering it in the evaluation of those patients was under-investigated. Despite being important features in PCOS patients, obesity and insulin resistance are present in other non PCOS patients as well, a fact that may answer the question of being concerned about these parameters and its effect while the patient is not a case of PCOS.

Obese persons, whether they are known cases of polycystic ovarian syndrome or not, have higher levels of insulin resistance in comparison to normal weight women. This obesity induced insulin resistance will induce hyperinsulinemia that usually present in both: fasting and postprandial states (18). This severe obesity related hyperinsulinemia results in a greater anabolic activity, which may lead to fast deposition of adipose tissue, and more obesity as a result (19). Consequently, once obesity is imposed, it will promote further weight gain. This positive correlation between obesity and insulin resistance was also demonstrated in this study, where patients with insulin resistance index ≥ 1.6 showed a significantly higher body mass index when compared with those having an index < 1.6 (Figure 3 & table 4).

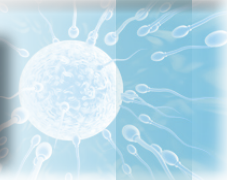
Additionally, many proteins, known as adipokines, are produced from the adipose tissue, including the pro-inflammatory cytokines like tumor necrosis factor (TNF- α) and interleukin 6 (IL-6) (20) through leptin induced inflammation (20) and hypoxia induced adipocyte death in the fatty tissue (21). Based on that, it has been suggested that the fatty tissue in obese patients is the source of the inflammatory mediators, with higher lev-

els of these mediators being detected in obese patients. This link between TNF- α and BMI was also demonstrated in this study, however, the difference in serum level of TNF- α between the three groups of BMI failed to achieve statistical significance (Table 3).

On the other hand, TNF- α was proposed as a progenitor and mediator of insulin resistance (22). As mentioned, this may be explained by the effect of TNF- α on serine phosphorylation of insulin receptor substrate-1 (IRS-1)) and the reduction in the expression of insulin-sensitive glucose transport protein known as GLUT-4. The impact of TNF- α on insulin resistance was shown in this study by the higher number of cases with insulin resistance ≥ 1.6 in patients having serum TNF- $\alpha \geq 121$ (table 5) as well as the positive correlation between the two variables (Figure 4) and the significant difference in serum TNF- α between the two HOMA index categories (Table 4).

The relation between inflammation and metabolic parameters was previously demonstrated by the most comprehensive and updated meta-analysis assessing TNF- α level in PCOS women done by Gao *et al* in 2016 (23). This meta-analysis concluded that high serum TNF- α concentration in PCOS patients was related mainly to insulin resistance but not to the BMI. Therefore, a high TNF- α level may be involved in promoting insulin resistance. Validation of this result in non-PCOS insulin resistant patients by this study revealed a comparable outcome, where higher levels of serum TNF- α was found to be mainly linked to insulin resistance regardless the BMI status.

The practical aspect of this relation between inflammation, insulin resistance and BMI is demonstrated in multiple different forms, ranging from testing these factors as important features in Diabetes to its effect on reproduction. In this study, however, the effect on the pregnancy rate as a primary reproductive endpoint in patients undergoing intracytoplasmic sperm injection was investigated.



Comparing to PCOS , women with insulin resistance usually need higher doses of gonadotrophin during stimulation, and higher levels of resistance to insulin is also linked to a higher risk of multiple follicular development and higher rates of cancellation(24) (25).Likewise , Hyperinsulinaemia is also associated with lower fertilization rates after IVF, and less implantation rates (26) . on the other side , the direct effect of serum TNF- α on IVF outcome is more vague .It has been suggested that TNF- α may influence hormone synthesis, placental architecture , the development of embryo and follicle , steroid synthesis, uterine cyclicality, as well as placental differentiation (27). Additionally , TNF- α may stimulate the apoptosis of human primary villous trophoblast cells, inhibit the mouse fetal development and the human trophoblast cells proliferation (28).Furthermore, In this study , Findings showed that pregnancy rates are directly and inversely correlated with insulin resistance (Table 6) while there was no direct effect of serum TNF- α on pregnancy (Table 7) . In other words,it is not the high levels of serum TNF- α that may negatively affect the pregnancy outcome, but it is may be the consequences of its elevation (including insulin resistance) that has the direct link to pregnancy outcome .

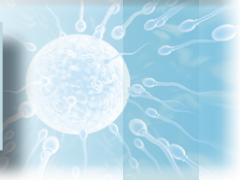
Conclusion:

Insulin resistance is an important underestimated feature in non-PCOS patients that is directly correlated with high levels of serum TNF- α , which may in turn represent a potential promoter of insulin resistance, leading to more complications during assisted conception. The ability of insulin sensitizing drugs to antagonize and reduce this negative effect should be in area of future researches, representing another trial to prepare infertile patients well before going to assisted conception.

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