ORIGINAL ARTICLE

Immunological and molecular detection of Tumor necrosis factor-α (TNF-α) in Iraqi Women with Polycystic Ovarian Syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among adult women in the developed world and is characterized by anovulation, androgen excess (primarily ovarian, but also adrenal in origin) and the appearance of polycystic ovaries on ultrasound. Diagnostic criteria are expert-based and debated as they do not incorporate known metabolic abnormalities related to aberrant insulin action, such as glucose intolerance, diabetes, and dyslipidemia that affect many women with the syndrome. Symptoms that are most troublesome to patients include hirsutism, obesity, infertility and menstrual disorders. The level of TNF-α increase significantly in PCOS than control groups. The SNP polymorphisms in the *TNF-α* gene have been associated with altered TNF-*α* secretion and are linked with PCOS and pregnancy problems. TNF-*α* genetic polymorphisms might be a risk factor for PCOS.

Keyword: polycystic ovarian syndrome, tumor necrosis factor- alpha, single nucleotide polymorphism

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an ovarian disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries. It may be the most common female endocrinopathy in the developed world. However, it most likely represents a heterogeneous disorder and one whose pathophysiology and etiology are debated. PCOS affects young women with oligo-ovulation (which can lead to oligomenorrhea), infertility, acne and hirsutism. It also has notable metabolic sequelae, including an elevated risk of diabetes and cardiovascular risk factors, and long term treatment should also consider these factors. These multiple stigmata have led to a multi-pronged treatment approach, with most therapies targeting individual symptoms. The search for the single unifying theory to this disorder will hopefully yield the single best treatment, but this quest remains one of the Holy Grails of reproductive endocrinology. This chapter will discuss the diagnosis, clinical evaluation, pathophysiology, and treatment of women with PCOS. Tumor necrosis factor-α (TNF-α) a pro-inflammatory cytokine is secreted by ovarian macrophages, granulose-luteal cells, and immune cells [(1)]. In addition to interference in immune and inflammation responses, differentiation, proliferation, and cell death, TNF-α has a role in PCOS patients with obesity, insulin resistance, hyperandrogenism, and PCOS patients with hyperandrogenism[2]. In contrast, the production of TNF-α in granulosa cells in PCOS patient's decreases aromatase gene expression. The process occurs via the inhibition of adenylate cyclase and the cyclic adenosine monophosphate (cAMP) signaling pathway, resulting in the reduction of 17-β-estradiol production from the ovary; consequently, elevated ovarian androgen is one of the most common characteristic of PCOS patients[3].

TNF-α induces serine phosphorylation in insulin receptor substrate-1 (IRS-1), resulting in the inhibition of tyrosine kinase activity in the insulin receptor and leading to insulin resistance and hyperinsulinemia. The process is also the cause of a low production of sex hormone–binding globulins in the liver, which increases the free androgen serum level[4].

The direct relationship between the serum levels of TNF-α and androgen in PCOS patients has been identified in some studies. TNF-α is encoded by a gene located on chromosome 6p21.3, and it has a promoter of 1100 bp in length. Nucleotide substitution in this region can affect transcription factors' binding affinity, and subsequently, the level of gene expression. Therefore, different concentrations of serum TNF-α can be produced, leading to many sorts of disorders[5].

Studies in Chinese, Korean, and South Indian populations have revealed a relationship between polymorphisms in the promoter region of the TNF-α gene and PCOS, hyperandrogenism, type 2 diabetes, and obesity [(6)]. In a case-control study of a Korean population, G allele carriers of single-nucleotide polymorphism (SNP) rs361525 in the TNF-α gene showed an association with overweight/obesity susceptibility[7]. It has been demonstrated that a G238A TNF-α SNP in the promoter region could be associated with diabetes, and the 238A/ 308G haplotype has been shown to elevate the TNF-α serum level in an Indian population[8].

Overall, based on different studies, it has been shown that TNF-α SNPs can elevate the serum levels of TNF-α, and this could be associated with PCOS[9,10]. The adipose tissue plays a central role in the relationship between cytokines and insulin resistance.

The expression of TNF-*α* and TNFR2 in adipose tissue is increased in obesity. TNF-*α* expression correlates with indexes of insulin resistance and decreases with weight loss in parallel with the improvement in insulin sensitivity[11,12].

MATERIALS AND METHODS

In this study, 250 Iraqi women aged between 15-50 years were studied. The patients were divided into two groups: study group (n=125, PCOS women) and age-matched controls group (n=125 normal women). The blood sample was obtained on the 2nd day of menstruation cycle. TNF-α

concentrations were determined in both groups, and after this determine the TNF-α SNPs (TNF-*α*-1031G/A) by using real time PCR.

RESULTS

The level of TNF- α showed significant differences in PCOS patients in comparison with healthy control groups. The level was 2.24E2± 135.93 (Pg /ml), 1.41E2± 141.16 (Pg /ml) for PCOS patients and healthy control respectively. The level ranging between 18 and 693 pg/ml, table (1).

Detection of the tumor necrosis factor- alpha (TNFα-1031 G/A), Single nucleotide polymorphism (SNP) analysis was performed for TNF-α genotype (GG/GA/AA) in the results as shown below in table (2), and figure (1). The GG genotype was detected in 10/125 (8%) of PCOS, and the GA/AA genotype was detected in 115/125 (92%) of PCOS, in comparison to healthy controls, GG genotype was detected in 0/125 (0%) of healthy controls, and the GA/AA genotype was detected in 125/125 (100%) of healthy controls. There was highly significant difference (P<0.001) in frequencies of TNF-1031 genotypes between PCOS patients and healthy controls. The G allele of the TNF-1031 SNP was highly frequent in PCOS cases, in comparison to with healthy controls (odds ratio= 2.65, *P* value <0.0001). In addition, the current results showed highly significant association of G allele of TNF-1031 gene (A/G) with PCOS (P<0.0001), table (3).

Table 1: Tumor necrosis factor α (TNF- α) level in PCOS patients and healthy control groups

Serum TNF- α (Pg /ml)	N	Mean	Std. Deviation	P-value
Patients	125	2.24E2	135.93	<0.0001
Controls	125	1.41E2	141.16	

**E2: ×100*

Table 2: Genotype polymorphism of TNF-α for the PCOS patients and healthy control

P<0.0001			TNF1031			Total
			AA	GA	GG	
Patient-control	Patients	Count	51	64	10	125
		% within Patient_control	40.8%	51.2%	8.0%	100.0%
		% within TNF1031	37.5%	61.5%	100.0%	50.0%
	Controls	Count	85	40	0	125
		% within Patient_control	68.0%	32.0%	.0%	100.0%
		% within TNF1031	62.5%	38.5%	.0%	50.0%
Total		Count	136	104	10	250
		% within Patient_control	54.4%	41.6%	4.0%	100.0%
		% within TNF1031	100.0%	100.0%	100.0%	100.0%

Figure 1: Genotype polymorphism of TNF-1031 for the PCOS patients and healthy control group

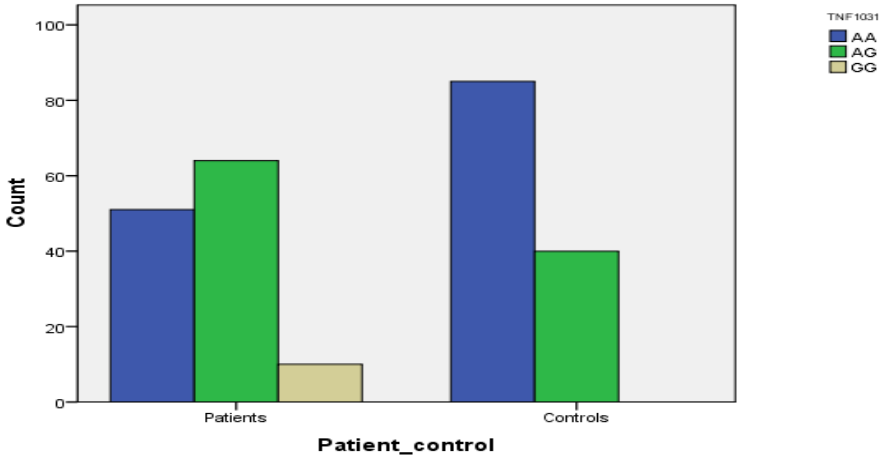

Table 3: Allele polymorphism of TNF-1031 for the PCOS patients and healthy control

P<0.0001 Odds ratio=2.65, P<0.0001			TNF1031		Total
			G allele	A allele	
Patient-control	Patients	Count	84	166	250
		% within Patient_control	33.6%	66.4%	100.0%
		% within TNF-1031	67.7%	44%	50.0%
	Controls	Count	40	210	250
		% within Patient_control	16%	84%	100.0%
		% within TNF-1031	32.2%	56%	50.0%
Total		Count	124	376	500
		% within Patient_control	24.8%	75.2%	100.0%
		% within TNF-1031	100.0%	100.0%	100.0%

DISCUSSION

Serum TNF-α is elevated in overweight/obese adolescents with PCOS. Chronic inflammation in adolescents with PCOS render them at a potential increased risk for the development of atherosclerosis, type 2 diabetes, cancer, infertility, and other comorbidities. Every effort should be made to identify adolescents with PCOS early and initiate aggressive therapy to prevent future complications[14].

Tumor necrosis factor-alpha (TNF-α) is a major proinflammatory cytokine and expressed mainly in monocytes, macrophages and adipose tissue. Serum levels of TNF-α were elevated in both obesity and T2DM. TNF-α played a role in the pathogenesis of insulin resistance, it inhibited tyrosine phosphorylation of insulin receptor and insulin receptor substrate-1 in muscle and fat cells. Elevated levels of TNF-α were reported to be associated with an increased risk of future myocardial infarction [(14, 15)]. Therefore, TNF-α may be a key mediator which is linked to T2DM and cardiovascular diseases in women with PCOS. Therefore, TNF-α may be a useful biomarker for the diagnosis of PCOS and the treatment of T2DM and cardiovascular diseases in women with PCOS[16].

The present study suggests that the PCOS condition induces an inflammatory state exacerbated when obesity is present, where a higher TNF-α signaling is observed, all of which could affect glucose uptake in the tissue and may cause fertility failures in these women; this result is actually in harmony with another study conducted in 2016[17].

The TNF-alpha gene resides within the class III region of the major histocompatibility complex and is located on the short arm of chromosome 6 (6p21.3). A single nucleotide polymorphism (SNP) located at position −308 in the promoter region of the *TNF-alpha* gene gives rise to a G-A exchange, which has been associated with elevated serum TNF-alpha concentrations in certain clinical states [(18)]. The distribution of genotypes of TNF-α1031G/A between patients and health controls revealed very interesting results. There were very significant differences in TNF-α1031G/A between patients and controls with the majority of patients with GA genotypes being 51.2%, while most of the healthy controls carrying the AG genotype were 32.0% and most importantly, only small proportion of PCOS patients have GG genotype were found in 8.0% and GG genotype not detectible in health controls, while AA genotype detectible in 40.8% of PCOS patients and 68% of healthy control groups. These results clearly indicate that the G allele is a risk factor for polycystic ovarian syndrome.

Certain polymorphisms in the *TNF-α* gene have been associated with altered TNF-*α* secretion and are linked with PCOS and pregnancy complications. TNF-*α* genetic polymorphisms might be a risk factor for PCOS [(18)]. Here, the results showed a significant difference in the allele frequencies of *TNF-α*-1031G/A in patients with PCOS. A strong association was reported previously in Korean, South Indian, and Chinese Han populations [(19)]. Other studies reported non-significant association of TNF-α-*1031G/A* genotype frequency in PCOS patients[20,21].

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