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The role of Annexin A1 in Secondary Infertility

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Abstract:

Objective: The aim of current study was to assess role of Annexin A1 (AnxA1) levels in women have Secondary Infertillity.

Materials and methods: The study conducted in tikrit City, from July to December 2022. Blood samples were obtained from 60 Secondary Infertility Patients, grouped in to two categories: - PCOS and obesity, and 30 from healthy subjects (control) whose age ranged between 24 to 40 years. Biochemical measurements of sex hormones levels by Minividas , while AnxA1 measured by kit is based on sandwich enzyme-linked immune-sorbent assay technology (ELISA) .

Results: There are a highly significant decrease ($p<0.0001$) in the serum levels of AnxA1 in obesity group when compared with control group, no significant difference ($P>0.05$) in PCOS group when compared control group, and a highly significant decrease ($p<0.0001$) in the serum levels of AnxA1 in obesity group when compared with PCOS group. The optimal criterion for ANXA1 in groups is estimated from ROC curves, and according to results, the test is positive if the test ≤4188.667/ criterion values for obese/control groups, and is positive if the test ≤5122/ criterion values for PCOS/ control groups. Results indicated that the ANXA1is closely related obese secondary infertility more than PCOS secondary infertility. The results revealed that LH and T levels were elevated a highly significant ($p<0.001$), and FSH levels were decreased a highly significant ($p<0.001$) in patients group when compared with control group.

Conclusion: ANXA1 level was found to be not change in PCOS, and it sensitive to obesity.

Keywords: ANXA1, PCOS, obesity, Infertility, Androgens.

دور Annexin A1 في العقم الثانوي

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مستخلص

ان الهدف من الدراسة الحالية هو تقييم دور مستويات (AnxA1) Annexin A1 لدى النساء المصابات بالعقم الثانوي. أجريت الدراسة في مدينة تكريت خلال الفترة من يوليو إلى ديسمبر 2022. تم الحصول على عينات دم من 60 مريضة بالعقم الثانوي، تم تقسيمهم إلى فئتين: – متلازمة تكيس المبايض والسمنة، و30 من الأشخاص الأصحاء (السيطرة) الذين تراوحت أعمارهم بين من 24 إلى 40 عامًا. تعتمد القياسات البيوكيميائية لمستويات الهرمونات الجنسية بواسطة Minividas، بينما يتم قياس AnxA1 بواسطة مجموعة تعتمد على تقنية مقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA). لقد تبين ان هناك انخفاض معنوي كبير ($P<0.0001$) في مستويات مصل AnxA1 في مجموعة السمنة بالمقارنة مع مجموعة السيطرة، ولا يوجد فرق كبير ($P>0.05$) في مجموعة متلازمة تكيس المبايض عند مقارنتها بمجموعة السيطرة، وانخفاض كبير للغاية ($P <0.0001$) في مستويات مصل AnxA1 في مجموعة السمنة بالمقارنة مع مجموعة متلازمة تكيس المبايض. يتم تقدير المعيار الأمثل لـ ANXA1 في المجموعات من منحنيات ROC، ووفقًا للنتائج، يكون الاختبار إيجابيًا إذا كانت قيم الاختبار ≤4188.667/ المعيار لمجموعات السمنة/ السيطرة، ويكون إيجابيًا إذا كانت الاختبار ≤5122/ قيم المعيار لـ PCOS/ مجموعات التحكم. أشارت النتائج إلى أن ANXA1 يرتبط بشكل وثيق بالعقم الثانوي الناتج عن السمنة المفرطة أكثر من العقم الثانوي لمتلازمة تكيس المبايض. أوضحت النتائج أن مستويات LH و T ارتفعت بدرجة معنوية عالية ($p<0.001$)، كما انخفضت مستويات FSH بشكل معنوية عالية ($p<0.001$) في مجموعة المرضى بالمقارنة مع مجموعة السيطرة. خلال هذه الدراسة وجد أن مستوى ANXA1 لا يتغير في متلازمة تكيس المبايض، كما أنه حساس للسمنة.

الكلمات المفتاحية: ANXA1، متلازمة تكيس المبايض، السمنة، العقم، الأندروجينات.

1. Introduction

The failure to conceive within a year despite regular unprotected sexual activity is considered infertility, which is a significant public health concern globally [1]. While secondary infertility refers to couples who are unable to conceive after a year of unprotected sexual activity after a previous pregnancy, primary infertility is the inability to conceive after a year of unprotected sexual activity without any past conception. [2].

Obesity and overweight are both characterized by an unhealthy or excessive fat buildup that can be harmful to health. Body mass index (BMI) is a recommended method by the World Health Organization (WHO) for identifying adult overweight and obesity. Weight in kilos divided by height in meters squared is what determines BMI (kg/m2). Adults are considered overweight or obese if their BMI is ≥ 30 [3]. Excess adipose tissue (especially visceral fat) in women increases the risk of subfertility for several reasons. The association between obesity and subfertility is ovulation vs anovulation. Low sex hormone binding proteins, excessive amounts of androgens, and a constellation of insulin-related disorders all contribute to hormonal dysregulation. Together, this result in dysfunctional hypothalamic gonadotropin hormone production, which lowers progesterone levels and the number of follicles [4,5].

Luteinizing hormone (LH) and hormones follicle stimulating (FSH) released from the pituitary, and they are a complex hetero-dimeric molecules glycoprotein which consist of α-subunit common and β-subunit specific [6,7]. With the feedback control of gonadotropin releasing hormone (GnRH), LH and FSH fluctuate periodically and serve various functions at various phases of follicle formation and ovarian steroids, which influences the overall reproductive outcomes in female reproductive physiology, including oocyte quality, ovulation, and ovulation timing. While, Hypothalamic-pituitary axis dysregulation is a disease that is frequently linked to polycystic ovarian syndrome (PCOS) [8,9]. Androgens which include: testosterone, dihydrotestosterone, and materials that can be bioconverted to testosterone (T). Circulating T is produced (25%) by the ovaries, (25%) by the adrenal cortex, and (50%) from conversion of

adrenal androgens in peripheral tissue. In addition, target tissues' intracellular testosterone synthesis contributes significantly to the effect of female androgens. [10].

Annexin A1 (AnxA1) which discovered in the 1980s, is a Annexins superfamily member, which are a group of polypeptides that have high homology and are differentiate by her ability of binding phospholipids in manner that a calcium-dependent. AnxA1 is an anti-inflammatory polypeptide that acts on innate immune cell response and is one of the glucocorticoids' downstream mediators. In addition to being highly expressed in leukocytes: neutrophils, macrophages and monocytes, AnxA1 is also expressed in several organs and tissues, including: heart, spleen ,liver, , colon, brain ,pancreas, adipose and prostatic tissue, and arteries [11].

AnxA1 is composed from 346 amino acids (aa) and have a molecular weight Approximately 37 kDa. Structurally, it is composed of a C-terminal (from Pro44 to Gly344) portion and an N-terminal (from Ala2 to Asp43) region that is specific for AnxA1 protein [12,13].

2. Materials and methods

The study conducted in tikrit City, from July to December 2022. Blood samples were taken from 60 Secondary Infertility Patients, grouped in to two categories: - PCOS and obesity, and 30 from healthy subjects (control) whose age ranged between 24 to 40 years. Biochemical measurements of sex hormones levels by minividas while AnxA1 measured by kit is based on sandwich enzyme-linked immunesorbent assay technology (ELISA) from Sun Long Biotech - China. Statistical Analysis: Data were analyzed by XLSTATE statistical package software. A statistic used are Student's t-test and curves or receiver operating characteristics (ROC), to compare means.

3. Results & Discussion

A. AnxA1

The mean (±SD) of AnxA1 concentration in serum of control group and Secondary Infertility Patients groups are illustrated in table (1) and figure (1). There is a highly significant decrease ($p<0.0001$) in the serum levels of AnxA1 in obesity group when compared with control group, no significant difference ($P>0.05$) in PCOS

group, and a highly significant decrease ($p<0.0001$) in AnxA1 serum levels in obesity group when compared to PCOS group.

Table (1): The Mean ± SD of ANXA1(ng/ml) levels for Patients and Control Groups

Groups	Control	Secondary Infertility Patients	
		PCOS	Obesity
ANXA1(ng/ml)	4638.6±472.5	4609.2±450.1	4081.9±531.4
P value			
Control/PCOS	Control/ Obesity	PCOS / Obesity	
P>0.05	P = 0.0001	P = 0.0001	

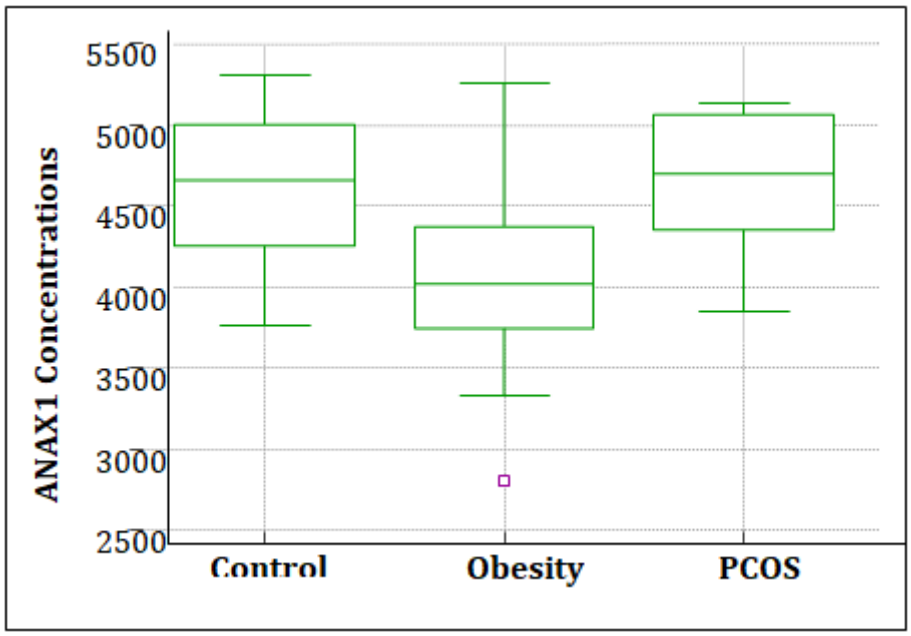

Figure (1): The Mean ± SD of ANXA1(ng/ml) levels for Patients and Control Groups

Curves of ROC were used to investigation about the relationship between parameter and disease, and to determine the approximate criterion values for ANXA1. Sensitivity, area under the curve (AUC) and specificity were measured to diagnostic accuracy. Table (2) and figure (2) show the diagnosis validity of ANXA1 levels in groups.

Table (2) : Predictive values of serum ANXA1 in all groups

Groups	Sensitivity	Specificity	Criterion	AUC
Control/ Obese	62.16%	76%	≤4188.667	0.784
Control/ PCOS	100.00%	17.39%	≤5122	0.515

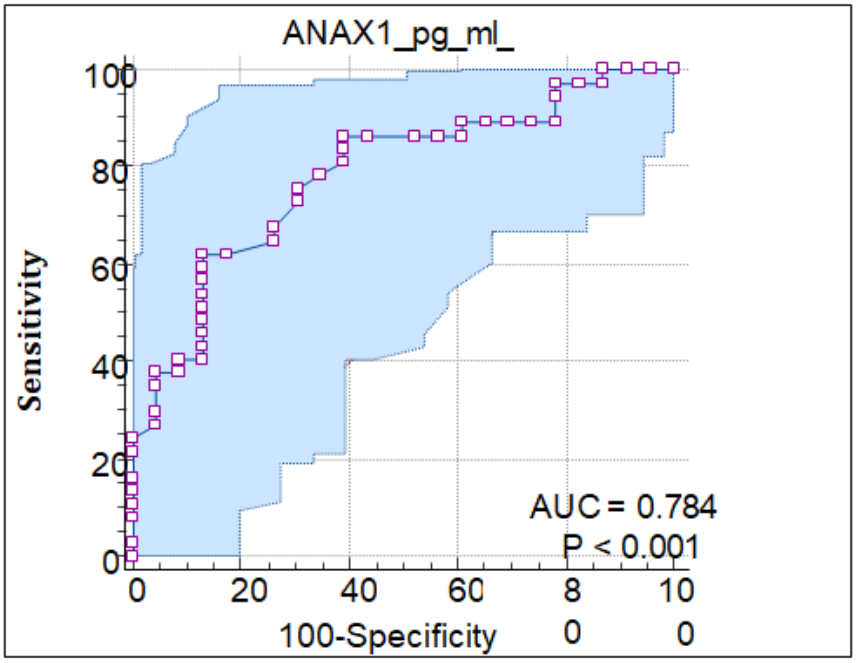

Figure (2): The ROC curve of ANAX1 in obesity/Control

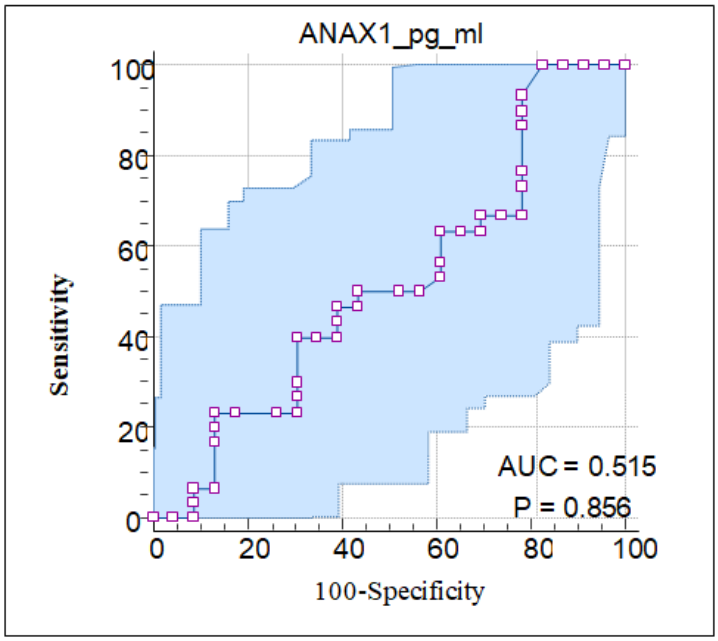

Figure (3): The ROC curve of ANAX1 in PCOS / control

The optimal criterion for ANXA1 in groups is estimated from ROC curves, and according to results, the test is positive if the test ≤4188.667/ criterion values for obese/control groups, and is positive if the test ≤5122/ criterion values for PCOS/control groups. Results indicated that the ANXA1is closely related obese secondary infertility more than PCOS secondary infertility

Annexin A1 (AnxA1) is a powerful pro-resolving mediator that affecting in the regulation of body weight and metabolic health conditions. Relevant for glucose metabolism pathway and fatty acid uptake by adipose tissue, several studies suggested that: AnxA2 contribute to coordinate and regulate glucose transporter type 4 (GLUT4) translocation and to support the fatty acid transporter. Also, AnxA6 has been linked to the control of lipolysis in adipocyte and to adiponectin release [14].

Beneficial effects of AnxA1 were also described in muscle and beta-cells in pancreas, which both a highly relevant for homeostasis of glucose [15]. Also, saturated fatty acid-palmitate, which is a highly elevated in the serum of obese patients, induced happened insulin resistance and over suppressed AnxA1 expression in L6 cells in myotubes [16]. AnxA1, which is generated by mesenchymal stromal cells, would, on the other hand, enhance the glucose-induced insulin release from human islets while displaying protective actions on pancreatic beta-cells. [17].

Recent researches indicates that the AnxA1 axis is crucial for understanding obesity, associated inflammation, and other problems such insulin resistance, type 2 diabetes, and atherosclerosis [18-21]. The ANXA1 anti-inflammatory actions may be compromised or change by obesity which may alteration the ANXA1's autocrine and paracrine effects action on adipose tissue to reduce inflammation. However, the data would suggest: this effect may be can reversed as a response to a highly weight loss, therefore, helping to reduce systemic inflammation and the likelihood of acquiring co-morbidities related to obesity. However, plasma ANXA1 levels were also decreased significantly in patients with suffer from lipodystrophy and inversely correlated with the BMI, in a similar manner that is observed with obese in individuals, suggesting that the degree of obesity or adiposity may not be the main cause of attenuated or reduced plasma ANXA1 levels, Despite the negative correlation

between these variables [22.23].

B. FSH, LH and T

The mean (±SD) of FSH, LH and T Levels in serum of control group (normal individuals), and serum of total secondary infertility patients (PCOS and obesity) are illustrated in table (3). The results revealed that LH and T levels were elevated a highly significant ($p<0.001$), and FSH levels were decreased a highly significant ($p<0.001$) in patients group when compared with control group.

Table (3) : Mean ±SD of FSH, LH and T Levels

Parameters / Groups	Mean ± SD		
	LH	**FSH**	**T**
Control	9.251 ± 1.670	20.159 ± 2.435	0.726 ± 0.090
Total Patients	30.36 ± 3.470	7.638 ± 0.744	21.584 ± 2.532
P Value	**<0.001**	**<0.001**	**<0.001**

Results were consistent with earlier research showing that PCOS was linked to higher levels of LH, estrogen, and T [24,25]. Additionally, the elevated levels of testosterone may be a significant factor in the continued rise in abdominal adiposity [26]. The biochemical defining feature of PCOS is hyperandrogenemia. In 75–90% of PCOS patients with oligomenorrhea, high levels of androgens are seen, and their concentrations frequently rise with the severity of the phenotypic [27].

Gonadotropin-releasing hormone (GnRH) pulsatility, which specifically increases LH production, is more frequent in PCOS patients. Theca cell hyperplasia and an increase in testosterone synthesis by LH stimulation of many steroidogenic enzymes in the ovarian theca cells [28,29]. Being obese increases the hormonal and clinical symptoms of PCOS, and PCOS patients have a higher chance of becoming obese [30]. Young women of reproductive age have the highest prevalence of PCOS, and research has linked it to long-term difficulties in oncology, metabolism, and reproduction. The discovery of a considerably abnormal LH/ FSH ratio suggests that PCOS

should be identified and treated in adolescence [31].

4. Conclusion:

ANXA1 level was found to be not change in PCOS, and it sensitive to obesity. clinical studies described in the current manuscript would indicate that lower plasma concentrations of ANXA1 protein are associated with higher pro-inflammatory status and poorer health outcomes.

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