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Association of Neuregulin-4 Levels and Body Mass Index with Hyperandrogenism in Polycystic Ovary Syndrome Patients

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Abstract

Background: Polycystic Ovary Syndrome (PCOS) is a common endocrine and metabolic disease that affects approximately 15% of women of childbearing age worldwide. It is one of the causes of infertility and is characterized by hirsutism, acne, persistent or interrupted anovulation, and hyperandrogenemia. Neuregulin-4 (NRG-4) is an adipokine hormone from the protein neuregulin family. Its level is greater in PCOS than in control women, and Neuregulin-4 is linked to body fat percentage and liver fat, as well as insulin resistance (IR).

Objectives: To estimate the serum NRG-4 levels in women diagnosed with (PCOS), to compare it with non-PCOS healthy control and to explore the effect of hyperandrogenism on the obtained result.

Methods: A case-control study was conducted in the Gynecology and the Infertility Clinics in Kut - Zahraa Hospital, Medical City, from September to December 2022. The study included 120 women aged (18-42) years, 60 of whom were diagnosed with polycystic ovaries, and the other 60 were the control group.

Results: Women with PCOS had a significantly higher NRG-4 LH, LH/FSH ratio, testosterone, free testosterone and Dehydroepiandrosterone sulfate (DHEA-S) than the control groups. In this study, NRG-4 was significantly higher in obese and overweight PCOS women than in normal-weight women.

Conclusion: The mean serum NGR-4 concentration is significantly increased in women with PCOS and could be a part of metabolic syndrome. NGR-4 levels were associated with obesity, hyperandrogenism (testosterone, free testosterone and DHAES) and may have a role in the development of PCOS.

Keywords: BMI; Free testosterone; Neuregulin-4; Polycystic Ovary Syndrome

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Introduction:

PCOS is characterized by hyperandrogenism, reproductive and metabolic problems, and is connected to IR, which is independent of but increased by obesity [1]. PCOS is the most common endocrine condition among females of reproductive age [2].

Independent of obesity, IR is more common in women with this illness and contributes to the metabolic and reproductive abnormalities identified in this syndrome [3]. PCOS has an impact on women's health and quality of life [4]. The enzyme 5-reductase transforms testosterone in females into dihydrotestosterone (DHT) [5].

Hyperandrogenism Ovulation is impacted by an increase in androgen production when PCOS is present because the ovaries may create excessive levels of androgens[6]. In other words, the ova do not grow normally and do not come out of the follicles where they are developing. Acne and hirsutism can also result from hyperandrogenism [7]. As a result of androgen exposure, gonadotropin-releasing hormone (GnRH) pulse velocity might increase, altering the LH: FSH ratio and leading to ovarian stoppage and dysplasia [8]. Neuregulin-4 is a new adipokine released from brown adipose tissue and expressed in many organs [9]. The nervous system has several descriptions of neuregulins. They control a variety of processes involved in the growth and operation of neurons, including myelination, neurotransmission, and synaptic plasticity [10]. Furthermore, a secreted adipokine has recently been shown to have an important role in the development of metabolic illnesses in obese humans and animals, including type 2 diabetes mellites (T2DM) and non-alcoholic fatty liver disease (NAFLD) [11] .

Subjects and Methods:

This case-control study was conducted under the Department of Biochemistry, College of Medicine, University of Baghdad. Patient and methods in the Gynecology Clinic and the Infertility clinic at Baghdad Teaching Hospital Medical City and Al-Zahra Teaching Hospital in Wasit, from 1st September to 30th December 2022. The study included 120 women aged 18 to 42 years who were allocated to one of two groups: Group

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1 included 60 women diagnosed to have PCOS by a consultant gynaecologist according to the Rotterdam criteria (Rotterdam, 2004), while group 2 included 60 healthy women as controls.

Exclusion criteria: Included pregnant lactating women, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, other adrenal gland diseases, thyroid disorders and diabetes mellitus.

Fasting blood samples of 5 ml were collected from cases and controls during the 2^{nd} – 5^{th} day of the menstrual period. Serum investigations included: Neuregulin-4 and free testosterone measured using (ELISA); Serum testosterone, FSH, LH and DHEA-S measured using Cobas E411.

Statistical analysis: The significance of the difference of different percentages (qualitative data) were tested using the Pearson Chi-square test (χ^2-test) with the application of Yate's correction or Fisher Exact test whenever applicable and ANOVA test were used to analysis mean numerical data across many variables. Pearson's correlation determined the study variables' link.

Results:

The mean ± SD age in the PCOS group was 27.2 ± 6.39 years versus 27.8 ± 6.33 years for the control group. Figure 1 shows a similar distribution for both study groups by age groups with the highest proportion being for those < 25 years of age, 23 (38.3%) for PCOS cases and 25 (41.7%) controls.

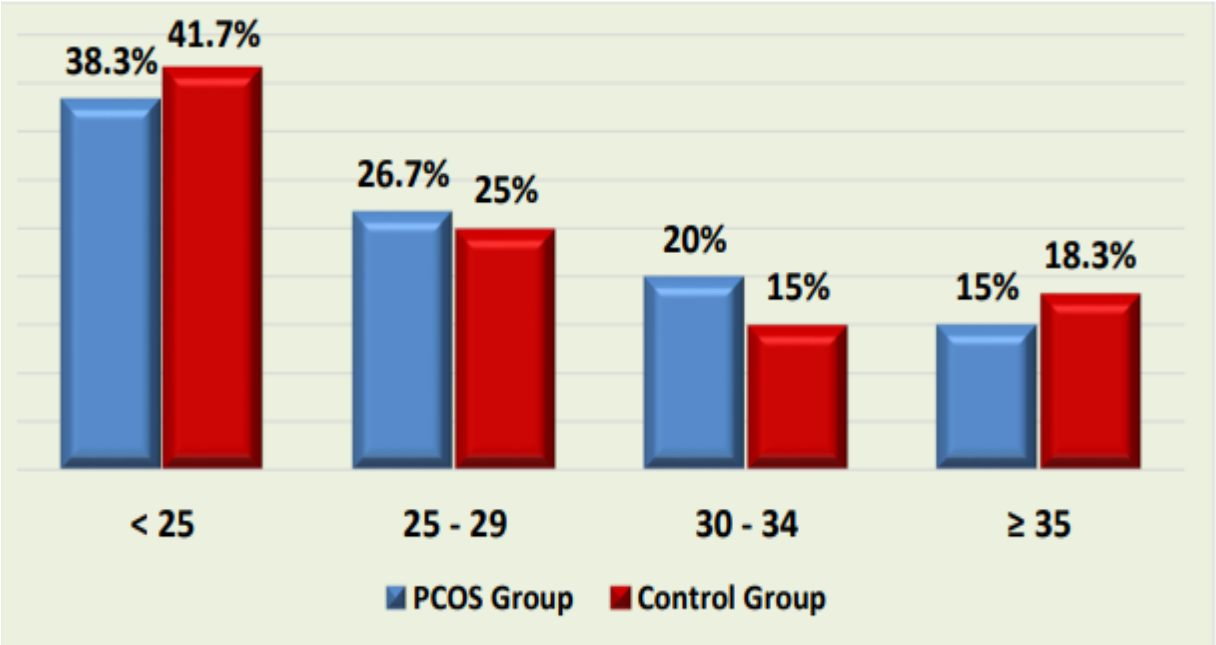

Figure 1: Distribution of the study groups by age groups

The mean body mass index (BMI) of the PCOS group was 28.2 ± 6.06 kg/m^2 and of the control group was 28.6 ± 6.24. Figure 2 shows the distribution of the study groups according to BMI levels with the highest proportion of PCOS women and controls being overweight, 22 (36.7%) and 21 (35%) respectively, followed by the obese.

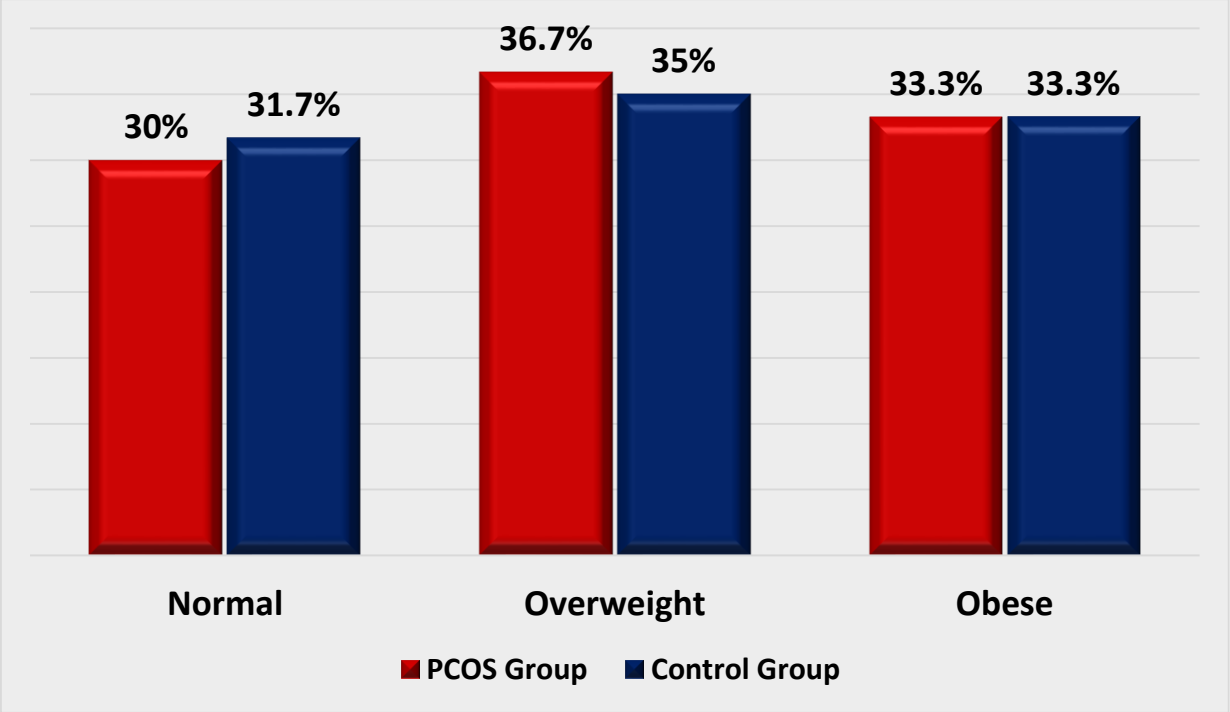

Figure (2): Distribution of the study groups according to BMI levels

In this study, no associations were found between the age groups and BMI levels of PCOS cases and their controls ($P \geq 0.05$), table (1).

Table (1): Distribution of the two study groups by age groups and BMI levels

Patients Characteristics	Study Groups		P- Value
	PCOS Group No. (%)	Control Group No. (%)	
Age (Years)			
< 25	23 (38.3)	25 (41.7)	0.863
25 - 29	16 (26.7)	15 (25.0)	
30 - 34	12 (20.0)	9 (15.0)	
≥ 35	9 (15.0)	11 (18.3)	
BMI			
Normal	18 (30.0)	19 (31.7)	0.975
Overweight	22 (36.7)	21 (35.0)	
Obese	20 (33.3)	20 (33.3)	
Total	60	60	

The comparison of hormonal parameters between the two study groups revealed that the mean ± SD levels of LH, LH/FSH ratio, testosterone, free testosterone and DHEAS were significantly higher in the PCOS group compared with control groups. The mean FSH level was not significantly different between the two study groups. The mean ± SD levels of NRG-4 were significantly higher for the PCOS group compared to the control group, Table 2.

Table 2: Comparison of the mean values of hormonal parameters between the two study groups

Hormonal Parameters	Study groups		*P* - Value
	PCOS Group Mean ± SD	Control Group Mean ± SD	
LH (mIU/ml)	13.9 ± 8.48	4.8 ± 1.58	0.001
LH/FSH Ratio	2.6 ± 1.63	0.8 ± 0.26	0.001
Testosterone (ng/ml)	0.5 ± 0.23	0.2 ± 0.13	0.002
F. Testosterone (pg/ml)	3.0 ± 0.93	1.4 ± 0.60	0.001
DHEAS (ng/ml)	254.0 ± 81.89	175.2 ± 73.01	0.001
FSH (mIU/ml)	6.0 ± 2.49	6.1 ± 1.60	0.658
NRG-4 (ng/ml)	38.2 ± 8.64	24.4 ± 7.82	0.001

LH: luteinizing hormone; FSH: follicular stimulating hormone; Free testosterone,
DHEAS: Dehydroepiandrosterone sulfate, NRG-4: Neuregulin-4

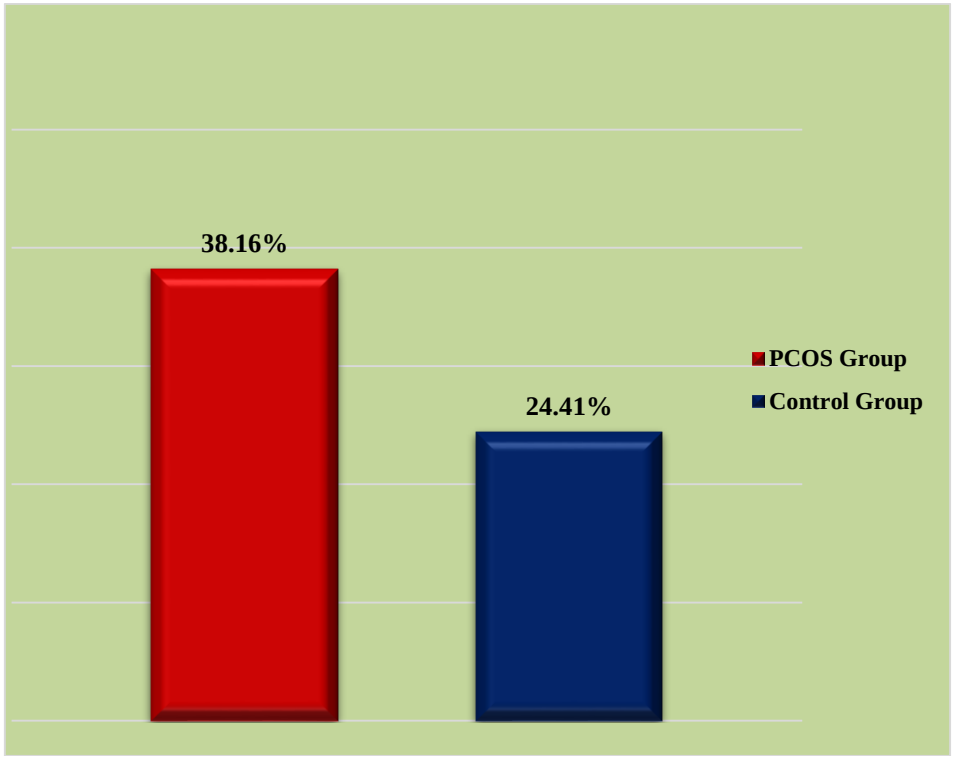

Figure (3): Distribution of study groups according to percentages of NRG-4

Table 3 shows the mean values of the NRG-4 for the three BMI categories. The ANOVA test showed that the difference between these means was statistically significant ($p \leq 0.05$).

Table (3): Comparison of mean ±SD level of NRG-4 according to BMI levels of the PCOS group.

BMI (kg/m^2)	NRG-4 Mean ± SD	P - Value
Normal	35.9 ± 7.54	0.010
Overweight	35.8 ± 6.99	
Obese	42.8 ± 9.63	

Post hoc tests (LSD) were run to confirm the differences in the mean ± SD level of NRG-4 according to the BMI of the PCOS group and revealed that obese patients had a significantly higher NRG-4 level than patients with normal weight and overweight patients (Table 4).

Table (4): Post hoc tests (LSD) for the differences in mean ±SD levels of NRG-4 by BMI of the PCOS group

	BMI			P - Value
	Normal Mean ± SD	Overweight Mean ± SD	Obese Mean ± SD	
NRG-4 Level (ng/ml)	35.9 ± 7.54	35.8 ± 6.99	-	0.958
	35.9 ± 7.54	-	42.8 ± 9.63	0.011
	-	35.8 ± 6.99	42.8 ± 9.63	0.007

The Pearson's correlation analysis, revealed a positive correlation between NRG-4 levels and BMI, LH/FSH

ratio testosterone, free testosterone, and DHAE-S (Table 5).

Table (5): Correlation of NRG-4 levels with biochemical and hormonal parameters

Parameters	NRG-4 (ng/ml)	
	r	P - Value
BMI (kg/m^2)	0.295	0.001
LH (mIU/ml)	0.363	0.001
LH/FSH Ratio	0.393	0.001
Testosterone (ng/ml)	0.353	0.001
F. Testosterone (pg/ml)	0.536	0.001
DHEAS (ng/ml)	0.233	0.010

Discussion:

The results of the current study regarding the non-significant differences between the age and BMI of the two study groups agree with other published work (12). Higher serum LH concentrations in PCOS cases compared to the control group agrees with the results of Temur, where increased LH production is a frequent sign of PCOS. Furthermore, poor ova formation and failure to ovulate are caused by a combination of high LH levels, which lead to high testosterone levels and low FSH levels. Inadequate amounts of progesterone generated by the ovary as a result of insufficient ovulation may cause a woman's menstrual cycle to cease completely [13]. The speed and intensity of the LH spike growing in women having PCOS, leading to higher 24-h production, and the frequency of hypothalamus gonadotropin-releasing hormone (GnRH) pulse are assumed to be the reason for this elevation in LH production [14]. LH/FSH Ratio was higher in the PCOS group compared to the control group. Which agrees the findings of Ray et al., and Kumar and Agarwal who reported that this is consistent with the most common clinical symptom of PCOS in women with an abnormally high LH/FSH ratio in 70% of PCOS women [14].

Testosterone, free testosterone and DHEA-S were all significantly higher in the PCOS group compared to the controls in the current study, similar to the findings of Temur *et al*. Increased fasting insulin levels induce the ovaries to release testosterone, causing testosterone levels to increase. Insulin inhibits hepatic sex hormone binding globulin SHBG synthesis, increasing free testosterone and exerting a stimulatory effect on ovarian androgen production, promoting hyperandrogenism [15]. As a result of high IR, the risk of T2DM in PCOS patients significantly outnumbers diabetes estimates in the general population [16].

The significantly higher serum NRG-4 levels in the PCOS group than in the control group agree with the findings of Temur *et al*. Obesity, as well as glucose and lipid metabolic problems, can all be blamed for the elevation in NRG-4 in PCOS patients [15]. This is demonstrated as an increased NRG-4 level in PCOS females with elevated BMI. The result of the current study showed a significantly higher NRG-4 in obese and overweight PCOS women than in normal weight PCOS women as well as a significantly higher level in obese PCOS than in overweight ones**.**

Several investigations also showed similar findings of elevated NRG-4 in obese PCOS subjects compared to non-obese patients. High insulin and ovarian androgen production are primarily caused by obesity. Moreover, increased adipose tissue can result in hyperinsulinemia and IR [17].

A significant positive correlation was found between the NRG-4 levels and LH and LH/FSH ratio, which is supported by the findings The disease occurs due to manipulations that occur in hypothalamic-pituitary-gonadal (HPG) axis. The primary hormones that fall under this axis include GnRH, LH, and FSH and estrogen in females. That is, there is a defect in the regulation of the pulsatile secretion of GnRH, which leads to FSH concentration decreases as LH increases, resulting in higher androgens and lower estrogen (18).

A significant positive correlation was also found between the NRG-4 and the level of testosterone, free testosterone and DHAE-S in the PCOS women group. Other studies revealed that NRG-4 indirectly regulates the hormone environment via binding to Erb-b2 receptor tyrosine kinase 4 (ErbB4) receptors. It is documented that testosterone or androgen-like progestins can cause IR in women (19).

Conclusions

The mean serum NGR-4 concentration is significantly increased in women with PCOS and could be a part of metabolic syndrome. NGR-4 levels were associated with obesity, hyperandrogenism (testosterone, free testosterone and DHAES) and may have a role in the development of PCOS.

Authors' declaration:

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the figures and images, which do not belong to the current study, have been given permission to re-publicate the attached manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in (Iraqi Ministry of Health, Medical City Department, Baghdad Teaching Hospital, and Kut-AL-Zahraa Hospital) according to the code number (495) on (ISU.495.2.11.22).

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Author contributions:

Study conception & design: (Manal K. Rasheed). Literature search: (Mustafa M. Ghalib). Data

acquisition: (Mustafa M. Ghalib & Afraa M. Al-Naddawi). Data analysis & interpretation: (Mustafa M. Ghalib & Manal K. Rasheed). Manuscript preparation: (Mustafa M. Ghalib). Manuscript editing & review: (Manal K. Rasheed& Afraa M. Al-Naddawi).

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ارتباط مستويات النيوروكلين 4 ومؤشر كتلة الجسم مع فرط الأندروجين لدى مريضات متلازمة تكيس المبايض

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الخلفية: متلازمة المبيض المتعدد الكيسات هي أحد أمراض الغدد الصماء والأيض السائدة التي تصيب ما يقرب من 15٪ من النساء في سن الإنجاب على مستوى العالم. وهو أحد أكثر أسباب العقم شيوعًا، ويتميز بالشعرانية وحب الشباب والإباضة المستمرة أو المتقطعة وفرط الأندروجين في الدم. النيوروكلين 4 هو هرمون من عائلة بروتين النيوروكلين ويعمل بمثابة أديبوكين ويكون مستواه أعلى لدى النساء المصابات بمتلازمة تكيس المبايض ويرتبط نيوروكولين 4 بنسبة الدهون في الجسم وكذلك دهون الكبد ويزيد بمقاومة الأنسولين.
الأهداف: الهدف من هذه الدراسة هو تقدير مستويات النيوروكلين-4 في المصل لدى النساء المصابات بمتلازمة تكيس المبايض والمقارنة مع نساء ليس لديهن تكيس مبايض ومعرفة مدى تأثير فرط الأندروجين على النتيجة التي تم الحصول عليها.
المرضى وطرق العمل/ المواد وطرق العمل:أجريت دراسة حالة وضبط في عيادة أمراض النساء ومركز العقم في مستشفى بغداد التعليمي في مدينة الطب من سبتمبر إلى ديسمبر 2022. وشملت الدراسة 120 امرأة تتراوح أعمارهن بين (18-42) سنة، 60 منهن تم تشخيصهن بمتلازمة تكيس المبايض، وكان 60 منهن مجموعة ضابطة.
النتائج: اظهرت النتائج ان النساء المصابات بمتلازمة تكيس المبايض لديهن مستويات عالية لكل من NRG-4 و LH و FSH / LH والتستوستيرون والتستوستيرون الحر و DHEA-S عند المقارنة بالمجموعة الضابطة. وكانت مستويات النيوروكلين 4 أعلى بشكل ملحوظ في النساء البدينات وذوات الوزن الزائد المصابات بمتلازمة تكيس المبايض مقارنة بالنساء ذوات الوزن الطبيعي.
الاستنتاجات: توضح الدراسة الحالية أن متوسط تركيز النيوروكلين 4 في الدم يزداد بشكل ملحوظ لدى النساء المصابات بمتلازمة تكيس المبايض ويمكن أن يكون جزءًا من متلازمة التمثيل الغذائي. كذلك وجد ارتباط بين النيوروكلين 4 والسمنة وفرط الأندروجين (التستوستيرون والتستوستيرون الحر وDHAES) في النساء التي لديهن متلازمة تكيس مبايض وهذا قد يكون له دور في تطوير متلازمة تكيس المبايض.
مفتاح الكلمات: :النيوروكلين-4؛ مؤشر كتلة الجسم؛ متلازمة المبيض المتعدد الكيسات؛ التستوستيرون الحر.