

Original Research

The Reliability of Serum Neuregulin-4 as a Marker of Polycystic Ovarian Syndrome with Respect to Adiposity Parameters

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is a chronic endocrinopathy of unexplained etiology linked to obesity. Neuregulin 4 (NG-4) is an adipokine synthesized primarily by brown adipose tissue; that keeps glucose and lipids in hemostasis. Earlier research tested serum NG-4 correlation with metabolic parameters in PCOS; herein, we aimed to examine serum NG-4 validity as a marker for PCOS with respect to obesity parameters and the influence of obesity on NG-4 concentrations. **Methods:** A cross-sectional study recruited 120 women into two groups; PCOS cases (60/120) and healthy controls (60/120). For every participant, three sets of data were recorded; anthropometric data (age, height, and weight for calculation of body mass index (BMI), waist/hip ratio, and systolic and diastolic blood pressure) hormonal levels, including serum (luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio, prolactin, anti-Müllerian hormone (AMH), testosterone, and insulin) and biochemical biomarkers (fasting blood sugar, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), high and low-density lipoproteins (HDL and LDL), and NG-4). **Results:** Serum NG-4 levels were significantly higher among PCOS vs. healthy controls. The univariate analysis confirmed a significant correlation of NG-4 to BMI, hormonal, and metabolic parameters. None of the obesity parameters were correlated with serum NG-4; only PCOS had an effect on serum NG-4 with $p < 0.001$ in multivariate analysis. At a cutoff value of 32 (ng/mL), NG-4 showed the highest sensitivity and specificity in discriminating PCOS cases with an area under the curve (AUC) of 0.97, $p < 0.001$. **Conclusions:** Serum NG-4's strong relation to hormonal and biochemical parameters that define PCOS independent of BMI and waist-to-hip ratio makes it a reliable biomarker in diagnosing and following up PCOS cases.

Keywords: polycystic ovarian syndrome; diagnosis; serum neuregulin-4; obesity; body mass index; waist to hip ratio; insulin resistance

1. Introduction

The global incidence of obesity has reached pandemic levels. Women are more prone to being obese as they acquire more adipose tissue with each pregnancy. Obese women suffer from adverse reproductive outcomes; they are less likely to conceive spontaneously and more likely to miscarry, in addition to adverse metabolic diseases like hypertension, diabetes, and ischemic heart disease. Obesity is indisputably associated with increased insulin resistance (IR), diabetes mellitus (DM), and polycystic ovary syndrome (PCOS) [1,2].

Obesity prevalence has increased along with obesity-related co-morbidities such as PCOS, a prevalent endocrinopathy affecting around 13% of childbearing women [3]. PCOS is distinguished by hyperandrogenism and reproductive and metabolic abnormalities and is linked to insulin resistance, which is independent of but exacerbated by obesity. Affected cases suffer from central obesity, which correlates to IR, increased androgen, and chronic anovulatory cycles [4].

Metabolic syndrome (MeS) is a disease of modern society, with multiple components like central obesity, increased fasting blood sugar, hypertension, and an abnormal

lipid profile. These interact to escalate the life risk of DM and vascular diseases. Around half of PCOS women develop MeS alongside its associated risks of premature atherosclerosis, vascular diseases, and endometrial cancer [5]. A growing body of evidence has proven an intimate link between obesity and polycystic ovary syndrome. Obese women have higher odds of having PCOS; likewise, obesity in those affected will increase the severity of the syndrome [6,7]. Adipose tissue's (AT) role as an independent endocrine system is well established. AT secretes numerous adipokines, adipocyte-secreted cytokines whose concentration is related to body fat mass and degree of obesity; many influence IR and overall vascular risk [8].

Adiponectin levels were found to be inversely linked to gestational age in normal-weight females; however, this negative correlation is not observed in obese and overweight pregnant women [9,10].

Neuregulin 4 (NG-4) is a protein molecule that belongs to the neuregulin family, an adipokine synthesized in many tissues, primarily brown adipose tissue. NG-4 activates the Epidermal Growth Factor receptor to bind with ErbB3 and ErbB4 receptors (ErbB stands for Human Epidermal Growth Factor Receptor), triggering cell-to-cell sig-



naling by phosphorylation [11]. Serum NG-4 keeps the sugar and lipids hemostasis in obese individuals via reducing hepatic lipogenesis, increasing fatty acid β -oxidation, and energy consumption. Studies have linked low NG-4 expression with obesity, IR, diabetes, and non-alcoholic fatty liver diseases [12].

Many scientists have pursued biomarkers that may affect MeS to unveil their role in obesity-related metabolic diseases [13]. Correlation of NG-4 with metabolic parameters in PCOS was researched earlier [14], yet the data regarding NG-4 performance with respect to adiposity parameters (including body mass index (BMI) and waist-to-hip ratio) are scarce and sometimes conflicting. NG-4 is primarily secreted by adipose tissue, and since PCOS is a heterogeneous syndrome where obesity affects most affected, the current study aimed to examine obesity's impact on NG-4 concentrations and validate NG-4's role in PCOS cases as a screening biomarker. Introducing new biomarkers can improve our understanding and management.

2. Materials and Methods

2.1 Study Design and Setting

A cross-sectional observational study was conducted in the Gynecology department of The University teaching hospital/in Baghdad, Iraq, between October 2021 and April 2022. The scientific and ethical committees at the College of Medicine/Mustansiriyah University approved its protocol (ethical approval no. 164, date: 12/July/2021). Participants were invited to enroll after the study aim and methodology were explained; they gave informed consent before enrollment; the Declaration of Helsinki was followed.

2.2 Study Population

During the study period, 120 women who satisfied our criteria were recruited into two groups; PCOS cases (60/120) and healthy controls (60/120).

The study recruited women in the age range of (18–35) years. There was no limit on BMI as we were interested in the levels of NG-4 in a wide weight range. PCOS cases were defined based on the revised Rotterdam 2003 consensus, where two out of three pre-requisites confirm the diagnosis [15]:

- Polycystic ovaries on ultrasound scan.
- Menstrual irregularity; oligomenorrhea and /or amenorrhea.
- Evidence of hyperandrogenism, whether clinical or lab.

Healthy controls were age and BMI-matched to PCOS cases. They had regular menstrual cycles (ranging from 28–35 days), showing no signs of hyperandrogenemia, and those mentioned above were the study inclusion criteria.

Exclusion criteria:

- Past medical history of diabetes mellitus, hypertension, thyroid disease, and blood dyscrasias.
- Past surgical history of bariatric surgery.

- Drug intake, including insulin sensitizers, lipid-lowering agents, oral contraception pills, anti-androgenic medications, and the current use of fertility drugs. Those who were on aspirin, steroids, and smoker were all excluded.

- A participant with missing or incomplete data. All were described in the study flow chart; see Fig. 1.

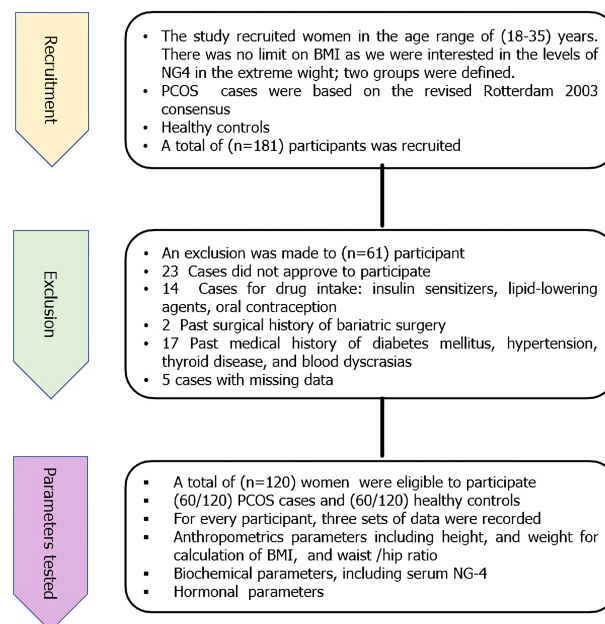


Fig. 1. The study flowchart.

2.3 For Every Woman Enrolled, Three Sets of Data were Collected

Anthropometric data (age, height, and weight for calculation of BMI, waist-to-hip ratio, and systolic and diastolic blood pressure) hormonal levels including serum (luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio, prolactin, anti-Mullerian hormone (AMH), testosterone, and insulin) and biochemical biomarkers (Fasting blood sugar, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), high and low-density lipoproteins (HDL and LDL), and serum NG-4).

Essential demographic criteria were recorded, including age and blood pressure, which was monitored with a mercurial sphygmomanometer as they were sitting in the cubital fossa and was repeated to confirm the diagnosis if it was found to be abnormal. Anthropometric readings were done twice in a barefoot standing pose. The BMI was estimated based on the formulae: weight (kg)/squared height (m^2). Women were considered obese with a BMI of ≥ 30 kg/m^2 ; the normal BMI was set in the range of 18.5–24.9 kg/m^2 . The waist diameter was measured in centimeters midway between the patient's lowest rib and the iliac crest at the end of a gentle expiration, from where we create the waist-to-hip ratio.

Insulin resistance (IR) was calculated by homeostasis model assessment of insulin resistance (HOMA-IR) where: (Fasting glucose (mg/dL) \times Fasting insulin (IU/mL)/405). HOMA-IR normal level was set at 1.0 (rang 0.5–1.4); any reading >1.9 was considered insulin resistance [16].

Hormonal and biochemical Assessment: Blood samples were collected at 10 AM following 12 hours of night fasting; on Day 2 of the menstrual cycle (whether natural or induced). The blood was centrifuged for ten minutes at 3000 revolutions; clear serum was separated and stored at -20°C until analysis. Neuregulin 4 (NG-4) serum levels were estimated using a sandwich enzyme-linked immune-sorbent assay technology ELISA reader from (E-EL-H0890, Human Company, Berlin, German).

2.4 The Study Sample Size was Estimated According to the Equation

Sample size = $r + 1 (P^*)(1 - P^*)(Z\beta + Z\alpha/2)^2 / r(P1 - P2)^2$ [17]

Where r = control to case ratio, which = to 1;

P^* is average No. Exposed = No. of exposed cases + No. of exposed control/2;

$P1 - P2$ = expected differences in No. Depending on earlier research ($P1$ and $P2$ are the cases and controls);

Number respectively. $Z\beta$ is the standard normal variant for 80% power of the study (0.84);

$Z\alpha/2$ is the standard normal variant at a significant level of 0.05 p -value (1.96);

Sample size = $r + 1 (P^*)(1 - P^*)(Z\beta + Z\alpha/2)^2 / r (P1 - P2)^2$;

Sample size = 56;

So, the required sample size is 56 for cases with sufficient controls = 112 (we recruited 120 women).

2.5 Statistics

All statistical analysis was performed using the Statistical Package for the Social Sciences software, version 22.0 (SPSS Inc., Chicago, IL, USA). The normality of the data was tested using Shapiro-Wilk Test. The continuous parameters were expressed as mean \pm standard deviation (SD). The anthropometric data, biochemical, and hormonal parameters of females with and without PCOS were analyzed using a student t -test. The difference in serum NG-4 across the study subgroups was graphically expressed by box and whiskers plots.

Pearson's univariate correlation analysis assessed the relationship between serum NG-4 and other study parameters. Multivariate linear regressions were done between serum NG-4 versus HOMA-IR, testosterone, AMH, HDL, and LDL to examine the relationship between serum NG-4 in PCOS against PCOS parameters that showed statistical differences in univariant correlation.

Analysis of Co-variance; ANCOVA was performed to elucidate the effect of age and obesity parameters (BMI and waist-to-hip ratio) and PCOS on serum NG-4 levels. Fi-

Table 1. The primary demographic criteria of the study participants (n = 120) data were expressed as means \pm standard deviations; the statistical significance was set at $p < 0.05$.

Parameters	Healthy controls, n = 60	PCOS cases, n = 60	p -value
Age (years)	28.34 \pm 4.71	27.33 \pm 5.79	0.29
BMI (kg/m ²)	25.69 \pm 4.24	28.10 \pm 5.39	0.007*
Waist/hip ratio	0.95 \pm 0.13	0.99 \pm 0.11	0.07
SBP (mm/Hg)	129.11 \pm 25.85	131.47 \pm 26.86	0.35
DBP (mm/Hg)	75.60 \pm 8.40	79.8 \pm 13.34	0.47

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. *Refers to statistically significant value: $p < 0.05$.

nally, the receiver operator curve (ROC) curve was constructed to estimate the serum NG-4 cutoff value linked with the highest sensitivity and specificity among PCOS women. A p -value of <0.05 was considered significant for all.

3. Results

The analysis involved 120 eligible women divided into healthy controls (60/120) and PCOS cases (60/120) based on Rotterdam criteria. In Table 1, the primary demographic criteria of the study participants were described; the age, systolic, and diastolic blood pressure showed no statistical differences across the two sub-groups; conversely, BMI was significantly higher among PCOS cases; $p = 0.007$. As for the waist-to-hip ratio, it showed a trend of increase in PCOS cases. Table 2 described the hormonal and biochemical biomarkers among the two groups. Significantly higher hormonal and biochemical parameters in PCOS cases were reported, including serum (fasting blood sugar, HOMA-IR, triglyceride (TG), and LDL) and serum (LH/FSH ratio, prolactin, AMH, testosterone, insulin) as $p < 0.05$. The HDL was significantly high among controls. Serum NG-4 was meaningfully higher in PCOS women compared to controls; 39.52 ± 7.5 vs. 22.48 ± 5.71 ng/mL; $p < 0.0001$, shown in Fig. 2.

Table 3 highlights Pearson's correlation of serum NG-4 versus the study parameters. Only BMI had a significant correlation ($r = 0.21$, $p = 0.019$) among anthropometric criteria, while the waist-to-hip ratio was not ($p = 0.54$). Hormonal parameters show a significant correlation to serum NG-4, including serum (LH/FSH ration, AMH, testosterone, and insulin) as p (0.02, <0.0001 , 0.02, 0.0004), respectively. Interestingly, the strongest correlation was with AMH, $r = 0.55$, $p < 0.0001$. While serum prolactin was insignificant in correlation analysis, HOMA-IR, TG, LDL, and HDL were strongly and significantly correlated with NG-4; $r = (0.56, 0.47, 0.51, \text{ and } -0.54)$, respectively. Multivariate linear regression was constructed by taking serum NG-4 as an independent factor versus PCOS parameters that showed statistical differences in Pearson's correlation. Analysis signifies a strong association to (AMH, HOMA-

Table 2. A comparison of the hormonal and biochemical biomarkers in healthy controls (n = 60/120) vs. PCOS cases (n = 60/120); data were expressed as means \pm standard deviations, significance was set at $p < 0.05$.

Parameters	Healthy controls, n = 60	PCOS cases, n = 60	p-value
LH/FSH Ratio	1.32 \pm 0.64	2.08 \pm 1.47	0.0003*
Prolactin (ng/mL)	14.81 \pm 5.79	20.98 \pm 4.81	0.002*
AMH (ng/mL)	4.88 \pm 1.31	8.24 \pm 2.47	<0.0001*
Serum testosterone (ng/mL)	0.62 \pm 0.25	1.18 \pm 0.73	0.004*
Serum insulin (mIU/mL)	5.47 \pm 0.22	8.87 \pm 1.38	0.0002*
Fasting blood sugar (mg/L)	91.0 \pm 11.30	114.92 \pm 11.56	<0.0001*
HOMA-IR	1.59 \pm 0.44	3.97 \pm 1.83	<0.0001*
TG (mg/dL)	87.02 \pm 19.26	119.7 \pm 38.39	<0.0001*
Serum HDL (mmol/L)	68.67 \pm 11.99	46.67 \pm 10.85	<0.0001*
Serum LDL (mmol/L)	94.47 \pm 13.36	130.15 \pm 31.78	<0.0001*
Serum NG-4 (ng/mL)	22.48 \pm 5.71	39.52 \pm 7.5	<0.0001*

FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, anti-Mullerian hormone; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NG-4, neuregulin-4. *Refers to statistically significant value: $p < 0.05$.

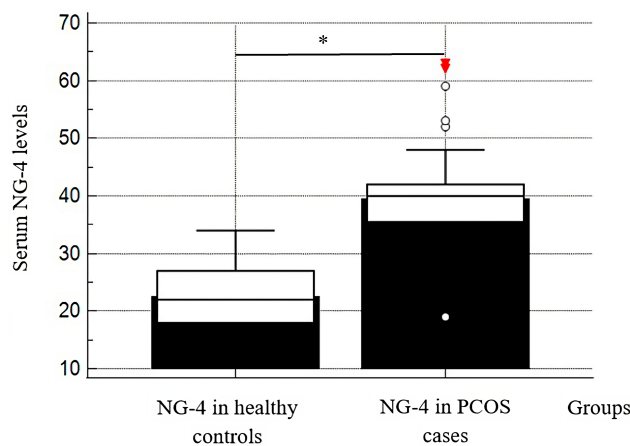


Fig. 2. Box and whisker chart showing serum NG-4 levels in healthy controls vs. PCOS cases; serum NG-4 was meaningfully higher in PCOS vs. controls; 39.52 ± 7.5 vs. 22.48 ± 5.71 ng/mL; *Refers to statistically significant value: $p < 0.0001$.

IR, testosterone, and HDL), $\beta = (1.65, 1.73, 1.44, \text{ and } -0.14)$ shown in Table 4. Only serum LDL was insignificant, $p = 0.057$.

Analysis of Co-variance; ANCOVA was performed to verify the effect of study demographic criteria (age, waist-to-hip ratio, and BMI) and PCOS on serum NG-4 described in Table 5. Only PCOS was influential on serum NG-4 with $p < 0.001$; neither obesity parameters nor age was of value. The BMI failed to show statistical value even in sub-group analysis by one-way ANOVA shown in Table 6. Finally, the ROC calculated serum NG-4 cutoff value (>32 ng/dL) with respective 90% and 96.7% sensitivity and specificity in distinguishing PCOS cases. The area under the curve (AUC) was 0.97, $p < 0.001$, highlighted in Fig. 3.

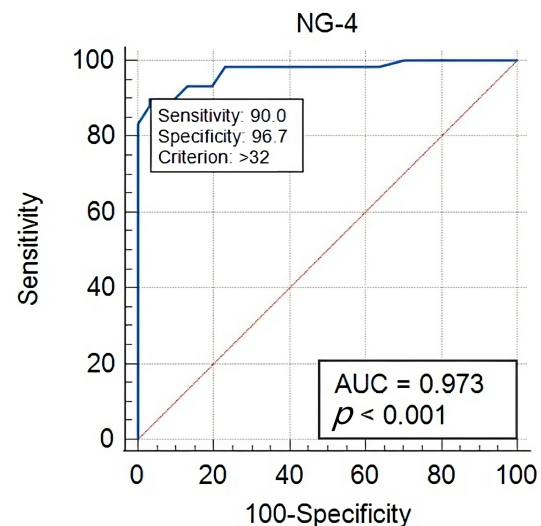


Fig. 3. ROC curve for serum NG-4 cutoff value that discriminates PCOS cases. At a cutoff value (>32 ng/dL), serum NG-4 had 90% and 96.7% sensitivity and specificity in distinguishing PCOS cases. The AUC was 0.97, $p < 0.001$.

4. Discussion

Serum NG-4 levels were significantly higher among PCOS cases. Only BMI was significantly linked to NG-4 among anthropometric criteria. As for the hormonal parameters, HOMA-IR, TG, LDL, and HDL were significantly correlated to NG-4. The multivariate analysis confirmed NG-4 was closely associated with AMH, HOMA-IR, testosterone, and HDL. However, none of the obesity parameters nor the age were correlated to serum NG-4; in fact, only PCOS was influential on serum NG-4 with $p < 0.001$. Although univariate analysis showed a significant link between BMI and NG-4, the multivariate analysis denies that.

Table 3. Pearson's correlations of serum NG-4 versus all study parameters, including anthropometric, hormonal, and biochemical parameters. The correlation (r) coefficient is interpreted as a positive correlation if (r = 1) and an inverse correlation if (r = -1). The strength of correlation (r) is interpreted as weak if r 0.2 to 0.4, moderate if r 0.4 to 0.6.

NG-4 vs. study Parameters (n = 120)	Correlations (r)	p-value
BMI	0.21	0.019*
Waist/hip ratio	0.06	0.54
LH/FSH Ratio	0.22	0.02*
Prolactin (ng/mL)	0.17	0.07
AMH (ng/mL)	0.55	<0.0001*
Testosterone (ng/mL)	0.21	0.02*
Serum insulin (mIU/mL)	0.31	0.0004*
HOMA-IR	0.56	<0.0001*
TG (mg/dL)	0.47	<0.0001*
LDL (mmol/L)	0.51	<0.001*
HDL (mmol/L)	-0.54	<0.001*

BMI, Body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, anti-Mullerian hormone; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NG-4, neuregulin-4. *Refers to statistically significant value: $p < 0.05$.

NG-4 is an adipokine of the brown adipose tissue (BAT), a type of fat that boosts metabolism and energy expenditure by burning calories. BAT has anti-glycemic, anti-lipidemic, and anti-obesity effects. BAT levels were reduced in PCOS women, implying an underlying mechanism for weight gain and metabolic alterations in affected women. Research showed an inverse relationship between BAT activity and age, adiposity, and BMI. Young and lean women have higher BAT activity [11,12].

There is much controversy in the literature regarding the NG-4 role; earlier research discussed the protective role of NG-4 against developing metabolic diseases related to MeS. Lower serum levels of NG-4 were found in cardiovascular disease cases, coronary heart disease, type 2 DM, and obese individuals. That implies that NG-4 was the link between adiposity parameters and MeS [18–20]. Based on animal studies, an inverse correlation between NG-4 and the MeS is mediated via the positive stimulatory effect of NG-4 on pancreatic beta cells, maintaining sugar hemostasis and increasing insulin sensitivity [19,21].

However, after 2017, the picture was different; in line with our analysis, Temur *et al.* [22] discussed the higher levels of NG-4 among PCOS women in a case-control study; their study linked NG-4 with IR and elevated inflammation biomarkers in affected cases in univariate and multivariate analysis. Neither BMI nor waist-to-hip ratio were linked to NG-4 levels on univariate analysis.

Another study by Kurek *et al.* [23] confirmed that NG-4 was higher in obese vs. non-obese PCOS cases; moreover,

Table 4. Multivariate linear regression was constructed where serum NG-4 was taken as an independent factor versus PCOS parameters that showed statistical differences in Pearson's correlation. To explore the prediction risk of PCOS by serum NG-4.

Parameters (n = 120)	β -coefficient	p-value
AMH	1.65	<0.0001*
Testosterone	1.73	0.007*
HOMA-IR	1.44	0.004*
HDL	-0.14	0.011*
LDL	0.72	0.057

AMH, anti-Mullerian hormone; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein. *Refers to statistically significant value: $p < 0.05$.

Table 5. Analysis of Co-variance; ANCOVA for serum NG-4 versus study demographic criteria; the test aims to verify the effect of study demographic criteria and PCOS on serum

NG-4.		
Parameters (n = 60)	F-ratio	p-value
PCOS	179.32	<0.001*
Age	0.023	0.88
Waist/hip ratio	1.48	0.23
BMI	0.16	0.80

*Refers to statistically significant value; $p < 0.05$.

higher levels were found for obese vs. non-obese healthy controls. Their study examined NG-4 levels in different ranges of obesity; and confirmed a positive correlation with BMI, obesity, diabetogenic, and atherogenic profiles. They confirmed that obesity was the most crucial factor affecting NG-4 secretions, as BMI was independently correlated to NG-4 [23], contradicting our results where BMI failed to correlate to NG-4 on multivariate, and only PCOS had a significant correlation.

Cao *et al.* [24] investigated NG-4 among two groups of obese adolescents with and without PCOS. Their result agreed with ours; NG-4 was significantly higher among PCOS girls. After one year of lifestyle modification, a significant reduction of NG-4 was found. Weight reduction was recommended to resolve many health problems linked to PCOS, which suggests a prognostic avenue for NG-4 in PCOS management [24].

The AT mass in the body secretes NG-4; reducing the latter will consequently reduce the total level of secreted NG-4, which manifests as improved metabolic and hormonal parameters. Positive association of NG-4 and MeS parameters (higher blood sugar, disturbed lipid profile, and hyperandrogenemia) may be interpreted as adjusting the body to the chronic inflammatory status among PCOS cases [25,26]. PCOS and obesity share common metabolic parameters, which further signifies the importance of lifestyle

Table 6. One-way ANOVA for serum NG-4 versus BMI highlighting the insignificant effect of BMI on serum NG-4 concentration in PCOS cases (n = 60).

Parameters	Mean \pm SD	F-ratio	p-value
BMI ≤ 25 (n = 21)	38.60 \pm 8.38	0.176	0.84
25 < BMI ≤ 30 (n = 24)	39.96 \pm 7.65		
BMI > 30 (n = 15)	39.94 \pm 6.94		

modification regarded as a cornerstone in PCOS management [24,26].

Interestingly, NG-4 correlation to adiposity markers was absent upon conducting co-variance analysis (ANCOVA). None of the demographic criteria were influential on serum NG-4; neither BMI, waist-to-hip ratio, nor age was significant. The link between NG-4 levels and BMI in disease pathophysiology was addressed by other authors who found no such link. In obese patients, high NG-4 levels were correlated with low BMI and systolic blood pressure compared to cases with low NG-4 levels [27].

In patients with acute non-fatty liver disease, NG-4 levels were unaffected by BMI based on Dai *et al.* [28] study. Similarly, in patients with cardiovascular disease, NG-4 concentration was unaffected by BMI upon assessing the atherogenic risk [20]. Likewise, NG-4 in Preeclamptic women showed no correlation with BMI [29].

Another controversial point regarding NG-4 is the consequences on the human cardiovascular system (CVS). Some proposed a negative impact of NG-4 on CVS health by promoting atherosclerotic changes, while others discussed; a positive link between high BAT and reduced CVS risks in a study that followed patients for five years for biomarkers of subclinical atherosclerosis. The study recommended BAT as a valuable modulator of cardiovascular risk. Hopefully, future studies might provide further information about NG-4's impact on the cardiovascular system [30,31].

The current study showed that PCOS scored the strongest correlation to NG-4; $p < 0.001$; even when subgroup analysis was done according to BMI classification, the NG-4 had an insignificant correlation with obesity parameters. That point was not discussed before in PCOS and formed our study highlight. This result can be interpreted in more than one way:

First, PCOS is a heterogeneous syndrome; many theories were suggested for its pathogenesis, like genetic factors, ethnicity, inflammation, and the imbalance of oxidative and antioxidative stress.

Women with a genetic predisposition will develop PCOS once certain risks are triggered. Should the women develop obesity or be exposed to inflammatory insults or oxidative stress, that will trigger multiple pathways that participate in PCOS development. That is why not all PCOS cases are obese; lean PCOS cases (30% of all cases) possess many challenges in management since they do not have AT

to lose [32].

Second, NG-4 is a brown AT adipokine, and PCOS women suffer more from increased visceral fat (white AT manifested as abdominal obesity) and reduced brown AT. Higher visceral facts contribute to much more severe PCOS symptoms and worse long-life risks [18,21,32]. In light of the data mentioned above, NG-4 does not underlie the true activity of brown AT, and NG-4 levels are unaffected by obesity parameters, including BMI and waist-to-hip ratio.

Serum NG-4 is recommended diagnostic marker of PCOS, showing strong relation to all hormonal and biochemical parameters that define PCOS. NG4 can provide valuable insights into the mechanism underlying this elusive syndrome. Tracking biomarkers over time can guide clinicians to evaluate the treatment plans and allow targeted interventions. Neuregulin-4 cutoff value scored the highest sensitivity and specificity in discriminating PCOS cases with an AUC of 0.97, $p < 0.001$.

The independence of NG-4 with obesity and BMI makes it a reliable biomarker in diagnosing and following up PCOS for prognostic purposes. The current results may have future implications, PCOS syndrome is a heterogeneous syndrome with a genetic predisposition [2], and our result can be incorporated into screening those destined to develop this syndrome and offer preventive strategies, like dietary and lifestyle changes. Accordingly, providing more personalized management. There are potential confounders for NG-4 estimation, including IR, age, and ethnic group [11], which should be kept in mind when assessing its levels in practice. Effects of NG-4 on hyperandrogenism, infertility, and menstrual cycle irregularity are scarce and urge for more research. Overall, there are challenges associated with NG-4 implementation in practice; still, there is encouraging potential to revolutionize PCOS management.

4.1 Study Limitation

This study was a cross-sectional design, and the long implication of NG-4 cannot be evaluated [33]. Our study did not consider the participant's ethnicity or the different phenotypes of PCOS [34]. We were interested in elucidating obesity's effect on PCOS rather than PCOS presentation. Finally, being a single center may limit our results impact and hinders globalization.

4.2 Study Strengths

The current study has resolved a critical dispute in earlier research; the effect of obesity on NG-4 in PCOS cases. We have shown the causal link with BMI and explored the lack of any effect of obesity on NG-4 in PCOS women. NG-4 is a reliable diagnostic marker with high sensitivity and specificity on the ROC curve; it outstands many adipokines tested in earlier works [7,24,35]. The current study is well-powered, had strict inclusion, and its participants were carefully followed since it was conducted in a single center.

Many adipokines were tested and were found to have altered levels among PCOS cases, which signifies that adipokines contribute to PCOS pathology. Having that said, and due to inconsistent results, most of these adipokine's roles in PCOS are not vivid. Our current knowledge of the NG-4 role in PCOS is far from complete. Further longitudinal studies are recommended to explore more implications of this adipokine in metabolic syndrome.

5. Conclusions

Serum NG-4 levels were significantly higher among PCOS cases; it was significantly correlated with hormonal and biochemical biomarkers in PCOS cases. Serum NG-4 discriminates PCOS cases with high sensitivity and specificity independence of obesity parameters. Its reliability opens the door for diagnostic and therapeutic applications in practice.

Abbreviations

PCOS, Polycystic ovarian syndrome; NG-4, Neuregulin 4; BMI, body mass index; LH/FSH, luteinizing hormone/follicle-stimulating hormone; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL and LDL, high and low-density lipoproteins; IR, insulin resistance; DM, Diabetes Mellitus; MeS, Metabolic syndrome; AT, Adipose tissue's; ANCOVA, Analysis of Co-variance; ROC, receiver operator curve.

Availability of Data and Materials

The data supporting this study is available on reasonable request from the corresponding author.

Author Contributions

Conceptualization—WN and ZAH; Methodology—WN and MNAH; Software—WN; Investigations and data curation—MNAH; Writing, reviewing and editing—WN and ZAH; Drafting and supervision—WN. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board. The scientific and ethical committees at the College of Medicine/Mustansiriyah University approved its protocol (ethical approval no. 164, date: 12/July/2021). Informed Consent Statement: informed was obtained from all subjects involved in the study.

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Conflict of Interest

The authors declare no conflict of interest.

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