The Correlation of Subfatin and Vitamin D3 in Obese and Normal Weight PCOS Patients

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Abstract:

Background: Polycystic ovarian syndrome (PCOS) is a complex endocrine problem affecting women with an estimated global prevalence of 9%, due to inherited and environmental causes. PCOS mainly impacts women aged 15-35 years, and women who are at their earliest or final period of pregnancy. Subfatin is a newly identified hormone that controls metabolism and is thought to be a biomarker for PCOS and metabolic syndrome. Vitamin D3 has an important function in the development of PCOS.

Objective: To explore the effect of obesity on Subfatin and D3 levels, and the impact of the pathophysiology of PCOS on Subfatin and D3 levels.

Methods: The total number of participants was 120: 80 females with PCOS, divided to 40 obese and 40 normal-weight, and 40 healthy females (controls). The current investigation was carried out from July to December 2024 at Kamal Al-Samarrai hospital in Baghdad, Iraq. The enzyme-linked immunosorbent assay (ELISA) was used to determine the subfatin serum levels. Body mass index, follicle-stimulating hormone (FSH), testosterone, estrogen, D3, and luteinizing hormone (LH) levels were all assessed. The data were examined with the origin lab software version 25. The study groups were compared using Analysis of variance (ANOVA).

Results: Serum levels of subfatin, waist circumference, LH, estrogen, and testosterone were significantly higher ($P < 0.001$) in PCOS women compared to the control group. FSH besides the D3 levels was lower in PCOS cases than in healthy females.

Conclusion: High levels of subfatin and low vitamin D3 levels might be implicated in the fundamental mechanisms of PCOS, irrespective of body weight.

Keywords: Hyperandrogenism; Obesity; Polycystic Ovary Syndrome; Subfatin; Vitamin D3.

Introduction

Polycystic ovarian syndrome (PCOS) is a multifactorial condition affecting women in their reproductive years. It appears in females with hyperandrogenism, oligomenorrhea, amenorrhea, acne, hirsutism, insulin resistance, obesity, and infertility (1). PCOS is known to cause more than 40% of female infertility. Additionally, females having PCOS are more likely to experience endometrial cancer. Other metabolic illnesses seen in PCOS individuals include diabetes, hepatic steatosis, dyslipidemia, and cardiac issues (2). Women with PCOS usually have insulin resistance, which means, their body is unable to efficiently use glucose, resulting in greater glucose levels and higher levels of insulin secretion. Diabetes can develop over time if glucose levels in the blood are consistently high (3). Adipokines are cytokines released by adipose tissue that may influence a variety of biological processes. Insulin action in the body's peripheral tissues (especially the liver and skeletal muscles) is impeded

by the presence of this molecule, which causes insulin resistance. Subfatin, a recently discovered hormone that regulates metabolism, is being studied as a potential biomarker for metabolic disorders. In rat models, thermal stimulation significantly increases subfatin expression in the brown adipose tissue, and subsequent workout also enhances its expression in the skeletal muscles (4). Initial studies identified subfatin as an adipokine with pleiotropic effects on glucose homeostasis, including enhanced insulin sensitivity, promotion of fat tissue browning, and increased energy expenditure. A previous study has revealed that subfatin may protect against a variety of cardio-metabolic and inflammatory immunological diseases (5). The aim of this study is to explore the effect of obesity on Subfatin and D3 levels, and the impact of the pathophysiology of PCOS on Subfatin and D3 levels.

Methods:

The total number of participants in the current study is 120: 80 were females with PCOS, distributed to 40

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obese and 40 normal-weight, and the remaining 40 were healthy females without PCOS (controls). The age range for each group was 18- 45 years. The study was carried out from July to December 2024 in the Kamal Al-Samarrai Hospital in Baghdad, Iraq. The diagnostic criteria were based on Rotterdam guidelines for PCOS depending on hyperandrogenemia, oligoamenorrhea, and ovarian morphology in ultrasonography.

The project was approved by the local ethical committee at the University of Baghdad/ College of Science for Women. The authors signed an ethical consideration's approval, approval of participants from Kamal Al-Samarrai Hospital was obtained in accordance with ethical standards. The subfatin concentrations in the serum were measured employing the enzyme-linked immunosorbent assay (ELISA) technique. The luteinizing hormone, testosterone, and estrogen levels were all analyzed. The BMI was measured in addition to waist circumference. Patients suffering from diabetes, androgen-secreting tumors, pregnant women, and those who had administered drugs such as metformin or dopamine were excluded from the study. The data were examined with the Origin Lab software, version 25. The study groups were compared using ANOVA.

Blood Sampling: Five millilitres of venous blood was drawn in the morning from each woman during the follicular phase (the second or third day of her menstrual cycle). The blood samples were collected in a gel tube, and left to clot at room temperature, and then centrifuged at 2000 rounds per minute, for ten minutes. The serum samples were transferred to Eppendorf tubes and frozen at -20°C for further analysis, while the hemolyzed sample was rejected.

Determination of Hormone Levels: The hormones (LH, FSH, Estrogen and testosterone) were quantified in human serum using the electrochemiluminescence immunoassay (ECLIA) technique, using Cobas e 411 immunoassay analyzers (Roche and Hitachi Companies). The test was based on the sandwich principle.

Biochemical Assay

Determination of Subfatin and D3 Levels: The subfatin kit used the Sandwich enzyme immunoassay technique (ELISA), developed by Mybiosource/USA. The D3 amounts were evaluated using the ECLIA kits from Roche, Germany.

Results

In the current study, the Mean ± S.D of BMI for obese PCOS women was significantly higher ($P < 0.001$) than that of normal weight PCOS women and the control group. There was no significant difference in BMI level between the control group and normal weight PCOS women. The waist circumference of obese PCOS women was higher than that of normal weight PCOS women and the control group. The waist circumference for normal weight PCOS women is significantly higher than that of the control group (Table 1).

Table1: Mean ± S.D for BMI and waist circumference in the study groups

Groups	Mean ± S.D.	
	BMI (kg/m^2)	waist circumference (cm)
Control group	22.5 ± 1.92	73.8± 7.02
Normal weight PCOS	23.8± 1.42 [a]	79.6± 5.00 [a]
Obese PCOS	34.8±3.65 [b]	98.1±11.23 [b]
P – Value	< 0.001	< 0.01

(a) represents the comparison of both patient groups with control.
(b) represents the comparison among patient groups.

Subfatin of obese PCOS women showed significantly higher levels than for normal weight PCOS women and the control group. The level of subfatin of normal weight PCOS women was significantly higher than that of the control group. There is a significant difference in the mean level of subfatin between the three groups ($P < 0.001$). Vitamin D3 in the control group showed a significantly higher level than that of the normal weight and obese PCOS women, and the mean level of Vitamin D3 in the obese PCOS was significantly higher than that of normal weight PCOS ($P < 0.001$), as shown in Table 2.

Table 2: Mean ± S.D for Subfatin and Vitamin D3 levels in the study groups

Groups	Mean ± S.D		*P* – Value for Vitamin D3 and subfatin
	Subfatin (ng/ml)	Vitamin D3 (ng/ml)	
Control group	47.0 ± 6.45	41.0 ± 2.07	
Normal weight PCOS	52.2± 8.26 [a]	30.2± 8.01 [a]	< 0.001 Sig
Obese PCOS	58.0±3.68 [b]	33.3±3.18 [b]	

(a) represents the comparison of both patient groups with control
(b) represents the comparison between both patient groups

The descriptive plots for FSH, LH, Estrogen, and testosterone in figure (1,2,3& 4) show that the highest concentration for FSH was in the control group, while the lowest concentration was in obese PCOS women, with is a significant difference in the level between the three groups ($P < 0.001$). The highest concentrations for LH, estrogen and testosterone were in obese PCOS women, while the lowest concentrations were in the control group, respectively, there is a significant difference between the three groups ($P < 0.001$).
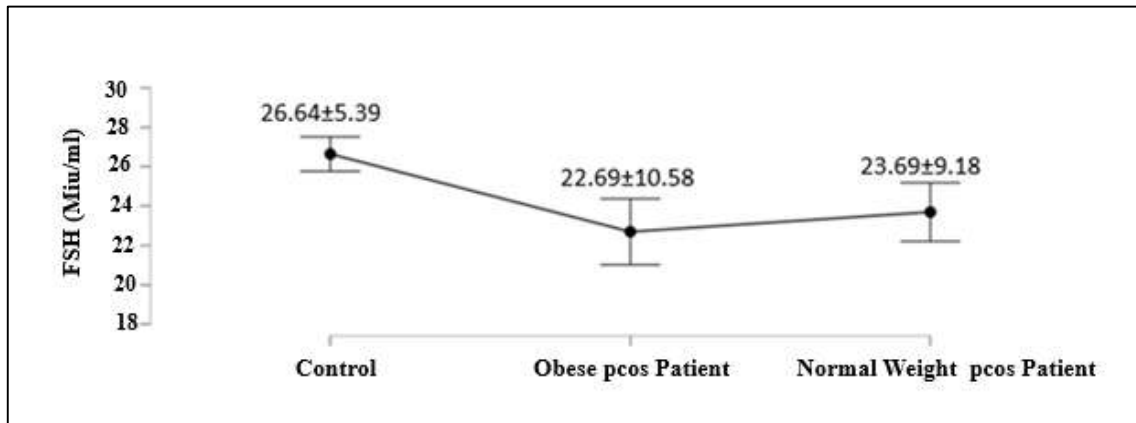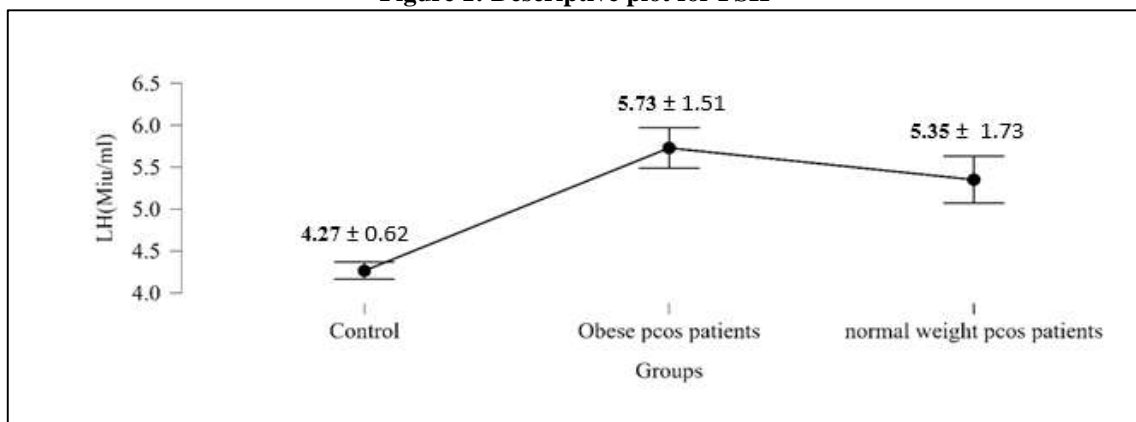

Figure 1: Descriptive plot for FSH
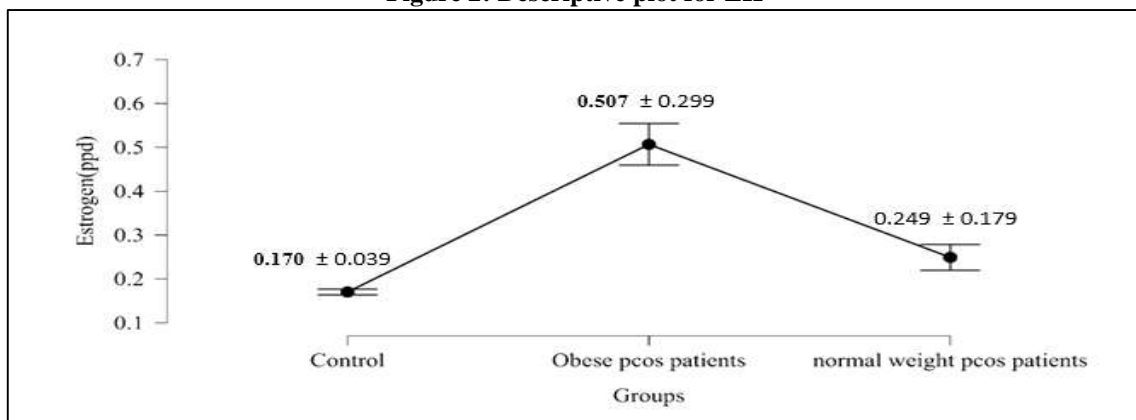

Figure 2: Descriptive plot for LH

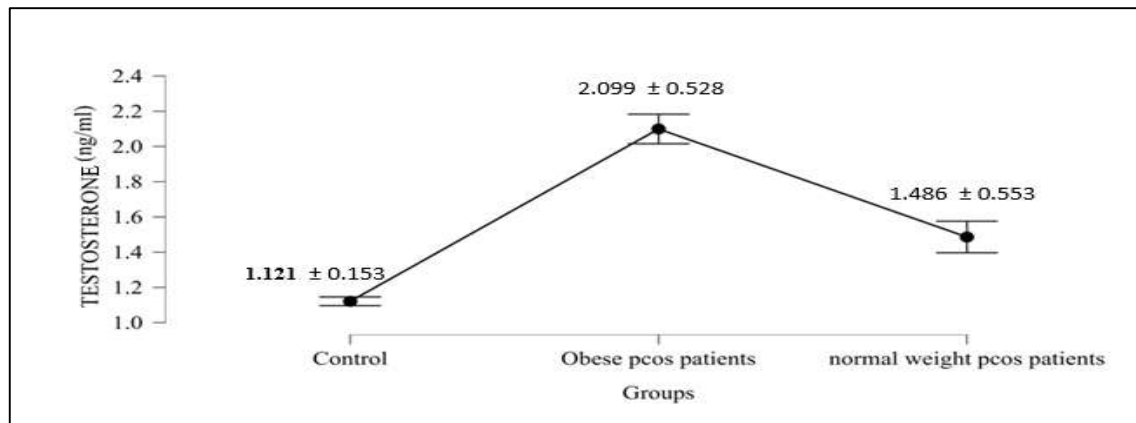**Figure 3: Descriptive plot for Estrogen**

Figure 4: Descriptive plot for Testosterone

Discussion

Obesity, particularly abdominal obesity, is a clinical predictor of high insulin levels, more severe lipid alterations, and increased production of inflammatory substances (6). There are specific established effects of visceral fat on metabolic dysfunction through adipokine and fatty acid release (7). Increasing weight, diseases of the heart, type 2 diabetes, malignancy, and emotional disorders are some of the medical diseases that are linked to D3 deficiency (8). Obesity remains a significant factor in vitamin D3 deficiency because vitamin D3 is fat-soluble. As obesity leads to hypertrophy or hyperplasia of adipocytes, it becomes an essential site for D3, resulting in small levels of D3 entering the blood, which leads to a decrease in D3 levels (9,10). Vitamin D deficiency is more common among people with PCOS and obesity since the accumulation of vitamin D in adipose tissue reduces its availability (11). The current study indicates that obese PCOS women have a greater BMI than normal weight females with PCOS and the control group. This is consistent with an earlier study, which found that PCOS women seem to have a higher BMI than controls (12).

The results of the current study that subfatin levels in obese PCOS patients are higher than those in normal weight PCOS patients and controls contradict those of Fouani et al who reported that subfatin was lower in the sera of PCOS patients than in the sera of the controls (13). It was also reported that high circulating levels of subfatin stimulated energy expenditure and the gene expression associated with anti-inflammatory cytokines and improved glucose tolerance in obese/diabetic mice (14).

The finding of the current study that PCOS cases had significantly greater LH levels than controls is consistent with earlier studies (15,16). Other studies found that LH concentrations in patients' sera were considerably higher in PCOS females (17,18). In comparison, women with PCOS had substantially reduced FSH levels (19). Increased LH secretion is hypothesized to have multiple implications. LH triggers ovarian thecal cells for testosterone production. Thus, androgen creation in the ovarian thecal cells is enhanced, which can lead to hyperandrogenism. Elevated serum LH concentrations are related to hold a lower chance of conception and a higher risk of miscarriage [20]. Furthermore, serum follicle-stimulating hormone (FSH) levels were lower in obese PCOS patients, but not statistically significant when compared to PCOS individuals of normal weight. This observation is consistent with the earlier study (21).

The findings of our study that testosterone levels were highest in obese PCOS women, and lowest in the control group, were consistent with the studies of Abdalla *et al.* (22), and Ibraheem *et al.* (23). The finding of the current study that vitamin D3 level in the normal weight and obese PCOS females was lower than that in the control group, is in agreement with the finding of Gokosmanoglu *et al.,* who found that D3 deficiency may have a role in the pathophysiology of PCOS (8).

Limitations

1. Recruitment from a single center which could restrict the applicability of the results to other groups.
2. Absence of longitudinal follow-up: The variations in biochemical markers over time were not evaluated.
3. Unaccounted confounders: Certain lifestyle elements, including diet, physical activity, and sun exposure (which influences vitamin D levels), were not entirely managed.

Conclusion

High levels of subfatin and low vitamin D3 levels might be implicated in the fundamental mechanisms of PCOS, irrespective of body weight.

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 for re-publication attached to the manuscript. Authors sign on ethical considerations' Approval-Ethical Clearance: The project was approved by the local ethical committee in (Place where the research was conducted or samples collected and treated) according to the code number (83743) on (20.08.2024).

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Data availability: Upon reasonable request, the corresponding author will make the data sets generated and/or analyzed during the current work available.

Authors' contributions
Study conception & design: (Walaa N. Adulwahid & Reem M. Obaid). Literature search: (Walaa N. Adulwahid & Reem M. Obaid). Data acquisition: (Walaa N. Adulwahid & Reem M. Obaid). Data analysis & Interpretation: (Reem M. Obaid). Manuscript preparation: (Walaa N. Adulwahid & Reem M. Obaid). Manuscript editing & review: (Walaa N. Adulwahid & Reem M. Obaid)

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العلاقة بين السبفاتين وفيتامين D3 لدى مريضات متلازمة تكيس المبايض المصابات بالسمنة وذوات الوزن الطبيعي
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الخلاصة

الخلفية: متلازمة تكيس المبايض (PCOS) هي مشكلة غدد صماء معقدة تصيب العديد من النساء، لأسباب وراثية وبيئية. تصيب متلازمة تكيس المبايض بشكل رئيسي النساء اللواتي تتراوح أعمارهن بين 15-35 عاما، والنساء في المراحل المبكرة أو الأخيرة من الحمل. السبفاتين هو هرمون تم اكتشافه حديثا يتحكم في عملية التمثيل الغذائي، ويعتقد أنه مؤشر حيوي لمتلازمة تكيس المبايض ومتلازمة التمثيل الغذائي. لفيتامين D3 دور مهم في تطور متلازمة تكيس المبايض .

الهدف: إستكشاف تأثير السمنة على مستويات السبفاتين وفيتامين D3 وتأثير الفسيولوجيا المرضية لمتلازمة تكيس المبايض على مستويات الدهون تحت الحمراء وفيتامين D3.

المنهجية: بلغ العدد الإجمالي للمشاركين 120 مشاركا؛ منهم 80 أنثى مصابة بمتلازمة تكيس المبايض (موزعة على 40 من ذوات الوزن الزائد و40 من ذوات الوزن الطبيعي) والـ 40 المتبقية من الإناث الأصحاء. أجريت الدراسة الحالية في الفترة من تموز إلى كانون الأول 2024 في مستشفى كمال السامرائي ببغداد، العراق. واستخدمت تقنية مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) لتحديد مستويات السبفاتين. وقيمت مستويات كل من مؤشر كتلة الجسم، والهرمون المنبه للجريب(FSH) ، والتستوستيرون، والإستروجين، وفيتامين د3، والهرمون الملوتن (LH). وفحصت البيانات باستخدام برنامج Origin Lab الإصدار 25. وقورنت مجموعات الدراسة باستخدام تحليل التباين.(ANOVA)

النتائج: كانت مستويات السبفاتين، ومحيط الخصر، والهرمون الملوتن، والإستروجين، والتستوستيرون أعلى بشكل ملحوظ (P < 0.001) لدى كل من النساء ذوات الوزن الزائد والوزن الطبيعي المصابات بمتلازمة تكيس المبايض مقارنة بالمجموعة الضابطة. ولم يكن مستوى مؤشر كتلة الجسم في المجموعة الضابطة أقل بشكل ملحوظ من مستوى النساء ذوات الوزن الطبيعي المصابات بمتلازمة تكيس المبايض، ولكنه أقل بشكل ملحوظ من مستوى النساء المصابات بمتلازمة تكيس المبايض المصابات بالسمنة. كانت مستويات هرمونFSH ، بالإضافة إلى مستويات فيتامين D3 في المصل، أقل لدى حالات متلازمة تكيس المبايض مقارنة بالنساء الأصحاء.

الإستنتاج: قد يكون لارتفاع مستويات السبفاتين وانخفاض مستويات فيتامين د3 دور في الآليات الأساسية لمتلازمة تكيس المبايض، بغض النظر عن وزن الجسم.

الكلمات المفتاحية: فرط الأندروجين، العقم، السمنة، متلازمة تكيس المبايض، السبفاتين.