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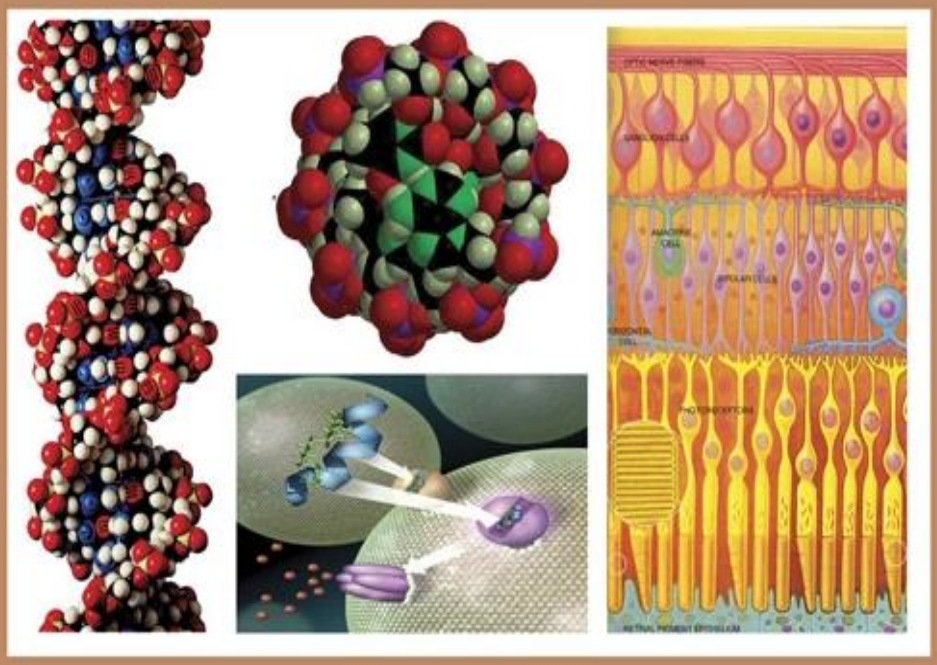

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Evaluation of the Role of Nesfatin-1 and Myonectin as A Diagnostic Marker for Polycystic Ovary Syndrome and Also for Treatment Response with Metformin

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ABSTRACT

This study aimed to evaluate the levels of Nesfatin-1 and Myonectin, to determine the efficacy of metformin therapy in treating patients with newly diagnosed Polycystic Ovary Syndrome-PCOS. Between the 1st September 2022 to 1st November 2022, a total of 90 blood samples were collected from females aged 18 to 35 years old. The samples were divided into three groups, G1 for women newly diagnosed with PCOS before treatment, G2 for the same patients after treatment with metformin, And C is a control group collected from healthy females with no previous history of the disease. The study includes the determination concentration of sex hormones (Follicle stimulating hormones –FSH, Luteinizing hormone-LH and testosterone), in addition to Nesfatin-1, Myonectin, and liver enzymes (Aspartate aminotransferase-AST, alanine aminotransferase-ALT, and alkaline phosphatase-ALP) in sera of samples under investigation.

The results obtained from the present study indicate that the level of FSH significantly increased in G1, and significantly decrease after treatment with metformin. While the LH level didn't show any significant difference between patient groups (G1 and G2) as compared with the control group. The level of testosterone significantly increased after treatment with metformin, with no significant difference between G1 and C. While the level of nesfatin-1, myonectin and ALP significantly elevated in sera of women with PCOS in G1, as compared with C, and then significantly decreased after treatment, With no significant change in the level of AST and ALT between patients and control groups. The results of the ROC curve analysis showed that the area under the curve –AUC values for nesfatin-1 and myonectin in G1 were 0.938 (sensitivity of 96.30%), and 0.935(Sensitivity of 85.19%) respectively. While the AUC for serum nesfatin-1 and myonectin in G2 was 0.910 (Specificity 96%) and 0.793(Specificity 82.76%) respectively. So we can conclude that an increase in the level of nesfatin-1 is most likely to be a diagnostic marker for polycystic ovary syndrome, while decreasing level after treatment with metformin and its return to a normal level may be an indicator of the therapeutic response to the disease.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder that affects 10% of women worldwide (Kumarendran, *et al*. 2018). It is a complex disorder with diverse signs and symptoms, including disruptions in reproductive and endocrine functions that can range from mild to severe. PCOS has a complex and multifactorial etiology. The syndrome is characterized by disrupted menstrual cycles, hirsutism, insulin resistance (IR), hyperandrogenemia, and obesity (Talat, *et al*. 2021; Begum, *et al*. 2017). Diagnosis of PCOS is based on the symptoms and the ultrasonography examination (Usadi and Legro, 2012). The diagnosis of PCOS is determined using the Rotterdam criteria, which requires the presence of at least two out of three criteria: oligo- or anovulation, clinical and/or biochemical indications of hyperandrogenism, and polycystic ovaries while excluding other potential causes such as congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's syndrome (Chang, *et al.* 2094).

The PCOS treatment protocols differ, but initial treatment for irregular menstruation and hyperandrogenism typically involves metformin with contraceptive pills. This protocol is among the recommended treatments for the syndrome (Rocha, *et al*. 2019; Teede, *et al*. 2018). In obese women with PCOS, insulin resistance-IR is a common occurrence, which leads to hyperglycemia, and the resulting hyperinsulinemia increases androgen levels, which in turn enhances the effect of luteinizing hormone (LH) on theca cells (Deshmukh, 2007- Homburg, 2009). Luteinizing hormone stimulates androgen secretion from the ovary, then follicle growth and maturation are repressed (Dunaif, 1997- Rotterdam ESHRE/ASRM, 2004).

Nesfatin-1 is a peptide hormone that functions in glucose metabolism, obesity, energy balance, and potentially gonadal functions. It has been detected in various tissues including the beta cells of the pancreas, adipose tissue, cardiomyocytes, testes, uterus, epididymis, and ovaries (Gonzalez, *et al*. 2009- Gonzalez, *et al*. 2012). The concentration of nesfatin-1 has been found to decrease in the sera of patients with type II diabetes mellitus(T2DM) and IR (Chen, *et al*. 2018) and also decreased in the sera of women with PCOS. This decrease in nesfatin-1 concentration could contribute significantly to the pathogenesis of PCOS (Deniz, *et al*. 2012).

Furthermore, myonectin is a myokine that is involved in lipid and glucose metabolism. If its secretion and function are impaired, it can lead to insulin resistance development, or It is also possible that the expression and secretion of myonectin are affected by IR in skeletal muscle (Gamas, *et al*. 2015). So the present study aimed to evaluate the effect of treatment with metformin on the level of nesfatin-1 and myonectin in sera of patients with PCOS.

MATERIALS AND METHODS

Sample Collection and Study Design: This study was conducted between 1/9/2022 till January 1/11/2022, in which 90 blood samples were obtained from females aged between 18-35 years old. Out of the 90 samples, 30 were collected from women who had recently been diagnosed with PCOS and had not taken any medication (As a first group-G1), while the second group-G2 include the same patients after treatment with metformin, The third group-G3 consisted of 30 blood samples taken from healthy women without a history of PCOS (As a control group-C).

Methods: The study involved the determination of serum concentrations of:

- Sex hormones (including Follicle stimulating hormones –FSH, Luteinizing hormone-LH and testosterone) by using an Enzyme-linked immunosorbent assay-

ELISA kit provided by Monobind company /USA.

- Nasfatin-1 and myonectin by using an ELISA kit provided by Mybiosource company /China.
- Serum liver enzymes (including aspartate aminotransferase-AST, alanine aminotransferase-ALT and Alkaline phosphatease-ALP) by using spectrophotometric methods kits provided by BIOLABO company/Franch.

Statistical Analysis: The statistical analysis of the results obtained in the current study was performed by the SPSS program by using Duncan's Multiple Range test, at a probability ($p \leq 0.05$). Furthermore, the study included an analysis of the Receiver Operating Characteristic (ROC) curve's analysis used to evaluate the area under curve-AUC and cut-off values of serum nesfatin-1 and myonectin .

RESULTS

According to the findings of this study, the level of FSH significantly increased in G1, and significantly decrease after treatment with metformin. While the LH level didn't show any significant difference between patient groups (G1 and G2) as compared with the control group. The level of testosterone significantly increased after treatment with metformin, with no significant difference between G1 and control group, (Table 1).

Table 1: Mean ±standard deviation of sex hormones (FSH, LH and testosterone) levels in sera of patients and control groups.

Parameters	Mean ± SD		
	Control	G1	G2
FSH (mIU/ mL)	6.871±2.204b	8.147±2.004a	6.968±2.377b
LH (IU/ml)	1.8541±7.425a	1.9393±9.048a	2.2292±11.971a
Testosterone (ng/ ml)	0.103±0.047b	0.127±0.055ab	0.139±0.054a

The results also indicate that the level of nesfatin-1 and myonectin significantly elevated in sera of women with PCOS in G1, as compared with the control group, and then significantly decreased after treatment (Table 2).

Table 2: Mean ±standard deviation of nesfatin-1 and myonectin levels in sera of patients and control groups.

Parameters	Mean ± SD		
	Control	G1	G2
Nesfatin-1(pg /ml)	358.851±68.918b	736.405±259.222a	361.650±90.649b
Myonectin (ng/ml)	2.469±0.630c	4.379±0.879a	3.614±0.750b

Also, the study also includes the determination of the activity of liver enzymes in sera of groups under investigation, The results showed that there was no significant difference between patients groups and control in the activity of AST and ALT, While the activity of ALP significantly increased in G1 and G2 as compared with the control group, Table 3.

Table 3: Mean ±standard deviation of liver enzymes (AST,ALT and ALP) in sera of patients and control groups.

Parameters	Mean ± SD		
	Control	G1	G2
AST(IU/L)	11.48±3.767a	11.63±2.822a	11.93±2.563a
ALT(IU/L)	7.37±2.025a	7.17±1.599a	8.00±2.464a
ALP(IU/L)	39.141±10.349c	70.138±18.732a	60.207±17.140b

Figures 1 and 2 illustrates the potential diagnostic significance of serum nesfatin-1 and myonection as demonstrated by the results of ROC curve analysis. The AUC value for nesfatin-1 was 0.938 (sensitivity of 96.30%) with a cut-off is more than 370.533ng/ml for PCOS vs control, While the AUC for myonectin in the group of patients with PCOS was 0.935(The sensitivity 85.19%) and the cut-off value is more than 3.67ng/ml.

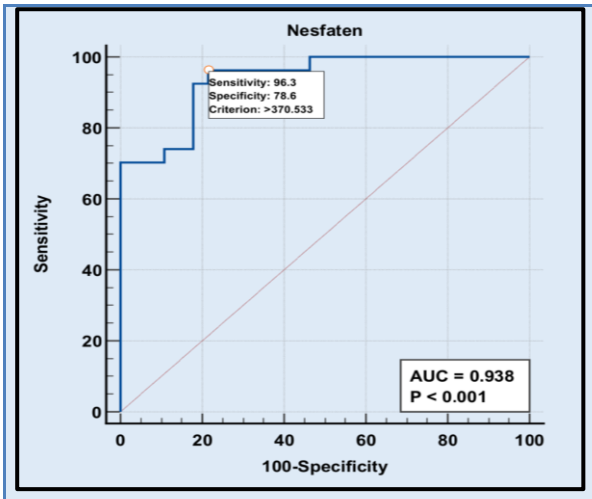

Fig. 1: ROC curve analysis of serum nesfatin-1 in patients with PCOS Vs control.

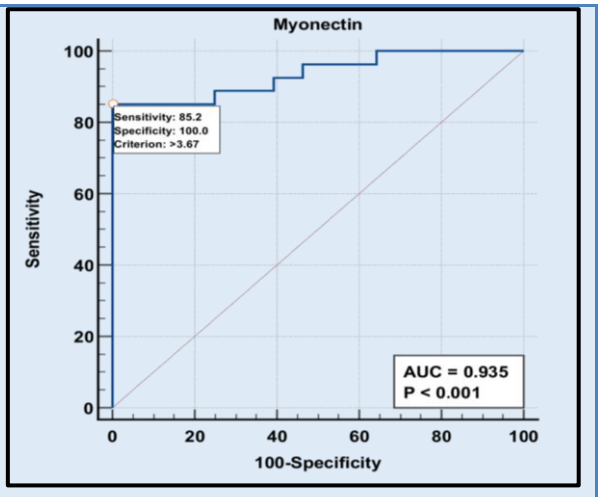

Fig. 2: ROC curve analysis of serum myonectin in patients with PCOS Vs control

On the other hand, the results of ROC curve analysis demonstrated that nesfatin-1 has a high treatment response with metformin as compared with the response of myonection to the treatment. In which the AUC for serum nesfatin-1 was 0.910 (Specificity 96%) with a cut-off of more than 444.861ng/ml. While the AUS for myonection was 0.793(Specificity 82.76%) with a cut-off value of more than 4.2ng/ml, Figures 3 and 4 respectively.

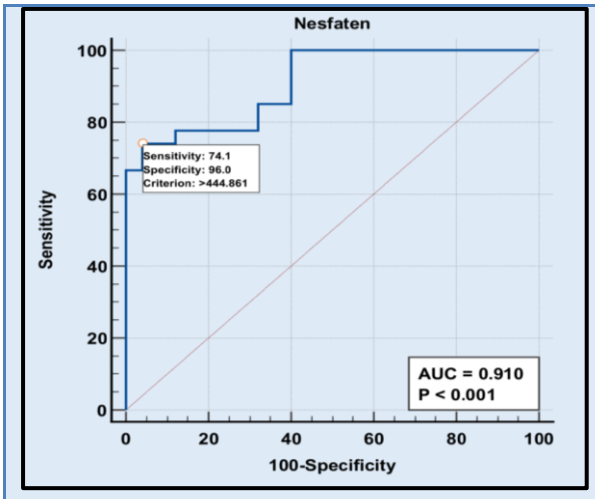

Fig.3: ROC curve analysis of serum nesfatin-1 in G2 Vs G1.

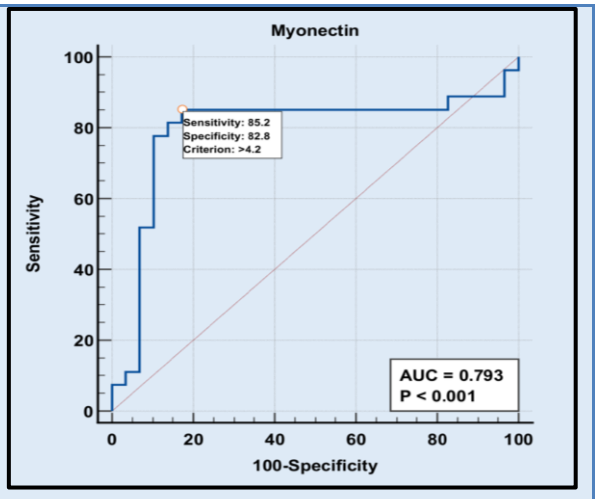

Fig. 4: ROC curve analysis of serum in G2 Vs G1.

DISCUSSION

The results of the current study in group G1 are consistent with the results of the study by (Ademoglu, *et al*. 2014), who indicated that the level of Nesfatin-1 in the sera of women with PCOS significantly increases compared to healthy women. Nesfatin-1 can induce the beta cells of the pancreas to secrete insulin (Zhang, *et al*. 2012), and the peptide has a high correlation with obesity, as well as insulin resistance. These factors are considered risk factors for PCOS; therefore Nesfatin-1 may play a role in the disease mechanism. Additionally, the study by (Faeza, et al. 2022) showed a significant increase in Nesfatin-1 levels in women with the syndrome regardless of age and body mass index, while (Xu, *et al*. 2017) indicated that a decrease in Nesfatin-1 levels contributed to the control of the disease, So the decreasing in the nesfatin-1 level may be considered a potential therapeutic target that requires further studies. This finding agreed with the finding of the ROC curve analysis in which the results of the ROC curve indicate that nesfatin-1 has potential diagnostic significance in identifying patients with PCOS. The high AUC with a sensitivity of 96.30% and a cut-off value of more than 370.533 ng/ml, indicates that the level of nesfatin-1 is most likely to be a diagnostic marker for polycystic ovary syndrome.

The decrease in the level of Nesfatin-1 in G2 after treatment with Metformin and its return to a normal level may be an indicator of the therapeutic response to the disease, and it may be a therapeutic goal targeted by the drug under investigation. And these results also agree with the finding of the ROC curve demonstrating that nesfatin-1 has a high treatment response with metformin.

The increase in the level of Myonectin has been associated with impaired glucose tolerance and the development of type 2 diabetes and insulin resistance. It has been found that individuals with high levels of Myonectin have a risk factor for impaired glucose tolerance and diabetes (Li, *et al.* 2018). Therefore, improved insulin sensitivity may contribute to a decrease in the level of Myonectin. This explains the decrease in its level after treatment with Metformin which improves insulin sensitivity (Herman, *et al*. 2022).

The results of the current study indicate that the activity of liver enzymes didn't show any significant change in the sera of patients with PCOS, while some studies have found an increase in AST and ALT activity in the sera of women with PCOS, there is also evidence that PCOS may not affect AST or ALT activity significantly. The study of (Kim, *et al*. 2018) found that AST levels were not significantly different between women with PCOS and healthy controls. In another study by (Galazis, *et al*. 2014), AST levels were found to be similar between women with and without PCOS.

It is important to note that the effect of PCOS on liver enzymes, may vary depending on the study population, the severity of PCOS, and other factors such as body mass index and IR. Therefore, further research is needed to clarify the relationship between PCOS and liver enzyme activity. While the increase in the activity of ALP may be due to the high levels of androgens (testosterone) in sera of women with PCOS may play a role. Androgens have been shown to stimulate the expression of ALP in osteoblasts, which are cells involved in bone formation. Therefore, it is possible that the androgens present in women with PCOS may stimulate ALP activity in bone cells, resulting in increased circulating levels of the enzyme, or maybe the increase in the activity of ALP due to insulin resistance, which is common in PCOS, may contribute to the increased ALP activity. Insulin is known to play a role in bone metabolism,

and studies have shown that insulin resistance can lead to bone loss. It is thought that the presence of insulin resistance in women with PCOS may result in alterations in bone metabolism, leading to increased activity of ALP (Carmina and Lobo, 2018; Das, *et al*. 2008).

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