Metabolic effects of Anti-Müllerian hormone (AMH) and Myonectin in Polycystic ovary syndrome Iraqi women

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Abstract
Background and aim: poly cystic ovarian syndrome is a common reproductive dysfunction associated with metabolic dysregulation. This study aims to investigate the metabolic effects of Anti-Müllerian Hormone (AMH) and Myonectin in Iraqi women with PCOS.
Patients and methods: A case-control study was conducted in Baghdad, Iraq. The overall sample size (n = 90) that met the inclusion criteria was divided into two groups as follows: 60 patients who were diagnosed with PCOS and 30 healthy matched women. Blood sample taken for biochemical and hormonal analysis. Lipid profile and fasting blood levels measured by enzymatic spectrophotometric method. HbA1c by by an enzymatic, colorimetric method by Roche (Germany). Enzyme-linked immunosorbent assay ELISA is used for measuring AMH and Myonectin. Enzyme-linked fluorescence immunoassay (ELFA) used to measure LH and FSH.
Results: Anti-Müllerian Hormone (AMH) were notably higher in patients, with a highly significant P value of 0.0001, while myonectin levels were substantially lower in patients compared to controls, with a P value of 0.0001. Body Mass Index (BMI), PCOS patients had significantly higher values than controls (P = 0.002). Correlation analysis revealed that AMH levels had a strong negative correlation with Myonectin levels ($r = -0.697$, $p < 0.0001$). AMH has strong positive correlation with LH ($r = 0.618$, $p < 0.0001$), and negatively and significantly correlated with FSH. AMH shown to be positively correlated with glycemic markers (FBS and HbA1c) and triglycerides (TG). Myonectin shown to be negatively associated with FBS, HbA1c, TG, total cholesterol and LDL, and positively linked to HDL.
Conclusion: PCOS represents a potential link between reproductive dysfunction and metabolic dysregulation. AMH and Myonectin could be used as biomarkers for diagnosis of PCOS and shown to have an influence on metabolic changes observed in PCOS.
Keywords: Polycystic ovary syndrome; Body Mass Index; Anti-Müllerian hormone; Myonectin

Introduction:

Polycystic Ovary Syndrome (PCOS) represents the commonest endocrine pathology in female at reproductive age period. The global prevalence of the disease is increasing and nowadays affecting approximately 6–20% according to Rotterdam's criteria which is dependable for diagnosis of PCOS (1). The common features observed in PCOS patients are hyperandrogenemia, anovulation and polycystic ovarian morphology (2). Farther than the reproductive implications of PCOS, variable metabolic changes been recognized in those patients including: insulin resistance, increase body weight, abnormal lipid profile and increased risk of type 2 diabetes mellitus (3-5). In Iraq, we have deficient knowledge about the prevalence of PCOS and the metabolic consequences associated with it, this acquired our attention for further research in Iraqi population.

Anti-Müllerian Hormone (AMH), an ovarian glycoprotein secreted by granulosa cells, and regarded as a biomarker to have a role in pathogenesis and progression of PCOS. As a degree of follicular disfunction marker, AMH shown to be elevated in women with PCOS and its level directly associated with worsening overran reserve (6). Recently, studies suggested that AMH might have a role in metabolic changes observed in PCOS, through its contribution to insulin resistance and disturbances of lipid metabolism (7). Though the exact mechanisms that causing these changes are still not well understood specifically in in Iraqi women as an ethnic group.

Myonectin, a skeletal muscle derived peptide, emerging as marker that played a role in regulation of glucose and lipid metabolism (8). Regarding PCOS, where the metabolic disturbances is obvious, Myonectin might act as a noticeable link between metabolic changes and muscular activity. Few studies investigated the role of myonectin in PCOS patients, and its relation to hormonal changes observed in those patients remain not fully explored.

This study aims to investigate the metabolic effects of AMH and myonectin in Iraqi women with PCOS. By examining the interplay between these two biomarkers, we hope to shed light on the underlying mechanisms driving metabolic dysfunction in PCOS and provide insights into potential therapeutic targets. Given the unique genetic and environmental factors influencing Iraqi

women, this research may also contribute to a deeper understanding of PCOS in diverse populations.

Patients and methods:

A case control study was approved by ethical committee of the College of medicine at Mustansiriyah University department of Chemistry &biochemistry in Baghdad, Iraq according to the national and international standard (Declaration of Helsinki). The study included (60) PCOS patients diagnosed with PCOS according to Rotterdam's criteria and (30) age matched control subject. All participant included in the study were recruitment from the Gynecology department of Al-Yarmouk teaching hospital/ Baghdad, Iraq from (November 2023 to March 2024). Verbal consents were obtained from all participants. Fasting 5 mL blood obtained, direct measure of HbA1c by an enzymatic, colorimetric method by Roche (Germany). The serum was separated into two portions; the first portion was used for measurements (LH, FSH) by VIDAS is an automated enzyme-linked fluorescence immunoassay (ELFA) test (Biomerieux / USA). The second portion was frozen at -20 °C until it was used for measuring AMH (ELISA kits supplied by USCN (CEA228Hu) Germany Company, and Myonectin ELISA kits for erythroferrone supplied by USCN (SEU540Hu) Germany. Fasting blood sugar and lipid profile measured using spectrophotometrically kits supplied by Linear / Spain.

Statistical analysis:

Data analysis was done by GraphPad Prism 8.0.2 (CA) software and Microsoft excel. The data presented as mean ± SD. The data tested for normality, and the difference between the two independent means was tested using the Student t test when normally distributed and Mann Whitney's test for non-normally distributed data. correlation analysis performed using Pearson's correlation and its t test were used for quantitative variables. Whenever the probability value is ≤0.05, it is considered significant.

Results

This study involved 60 patients with PCOS and 30 control subjects. The basic characteristics of the two groups are shown in Table 1. Hereby compares key health and biological characteristics between patients (n = 60) and control participants (n = 30), several significant differences were observed. There was no age difference between PCOS patient compared to controls thanks for good age matching. When examining Body Mass Index (BMI), patients had significantly higher values than controls (P = 0.002).

Table 1: Basic characteristics of the study population.

†Student t-test or Mann Whitney's test

*Significant at 0.05 level

Levels of Anti-Müllerian Hormone (AMH) were notably higher in patients, with a highly significant P value of 0.0001, indicating a substantial difference in this reproductive hormone levels as shown in figure 1. In contrast, in figure 2

Characteristic	PCOS (n = 60)	Control (n=30)	P value†
Age	**28.5±5.7**	**27.3±4.6**	**0.374**
BMI (Kg/m^2)	**28.90±5.553**	**25.261±3.622**	**0.002***
AMH (pg/ml)	**3.524±0.780**	**1.283±0.667**	**0.0001****
Myonectin (ng/ml)	**0.461±0.427**	**4.090±1.518**	**0.0001****
LH (m IU/ml)	**9.060±2.060**	**5.317±1.419**	**0.0001***
FSH (m IU/ml)	**4.770±1.012**	**5.635±1.223**	**0.001***
FBS (mg/dl)	**98.809±7.879**	**93.771±3.726**	**0.002***
HbA1C %	**5.614±0.606**	**5.084±0.486**	**0.0001***
Total Cholesterol (mg/dl)	**224.86±37.709**	**202.157±26.509**	**0.005***
Triglycerides (mg/dl)	**119.05±34.597**	**87.064±18.424**	**0.0001***
LDL (mg/dl)	**88.197±18.532**	**73.907±12.048**	**0.0001***
HDL (mg/dl)	**46.283±6.103**	**60.936±5.796**	**0.0001***

Myonectin levels were substantially lower in patients compared to controls, with a P value of 0.0001, highlighting a pronounced difference in this muscle-related protein.

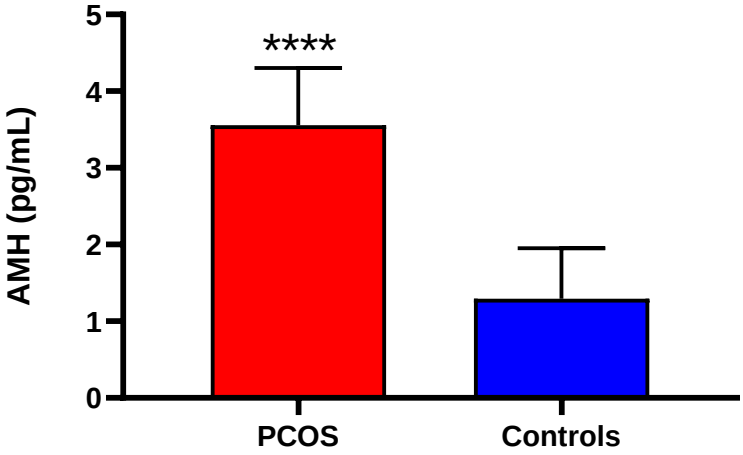

Figure 1: A comparison of AMH between PCOS patients and controls, red bar for PCOS patients, blue bar for control subjects, student t test used comparing differences between 2 means, ** significant <0.0001**.

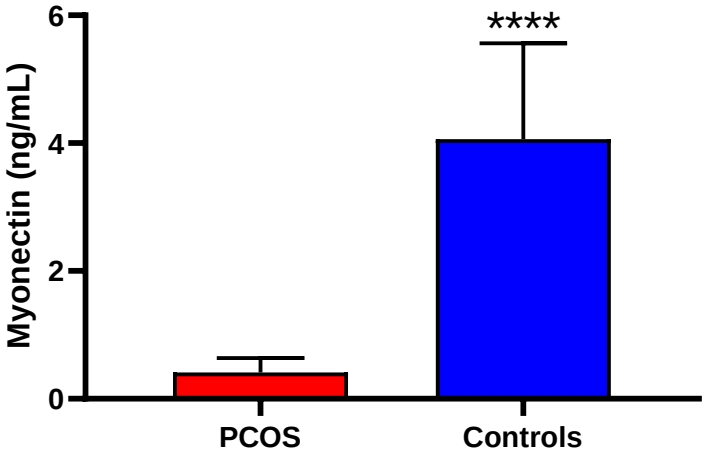

Figure 2: A comparison of Myonectin between PCOS patients and controls, red bar for PCOS patients, blue bar for control subjects, student t test used comparing differences between 2 means, ** significant <0.0001**.

To investigate the relationship between AMH, Myonectin and both hormonal and metabolic parameters represented by BMI and fasting blood sugar and glycated hemoglobin (HbA1c) and lipid profile in polycystic ovarian syndrome, correlation analysis was done and shown in table (2). It revealed that **AMH** levels had a

strong negative correlation with **Myonectin** levels (r = -0.697, p < 0.0001). **AMH** has strong positive correlation with **LH** (r = 0.618, p < 0.0001). Regarding BMI, a negative correlation observed with **Myonectin,** while a positive correlation obtained with **AMH** (r = 0.4667, p < 0.023). AMH shown to be positively correlated with FBS (r =0.414, p < 0.0001) and HbA1c (r =0.462, p < 0.0001), regarding correlation analysis with lipid profile AMH shown only correlated positively with TG (r =0.394, p < 0.0001), while total cholesterol, LDL and HDL no significant correlation observed. Myonectin show strong negative correlation with FBS, HbA1c, TG, cholesterol and LDL, while strong positive correlation observed with HDL cholesterol (r =0.708*, p < 0.0001).

Table 2: Correlation of AMH and Myonectin to ovarian hormones and metabolic parameters of PCOS patients.

		AMH	Myonectin
Myonectin	R	-0.697*	1.000
	P value	0.0001	
BMI	R	0.4667*	-0.3627*
	P value	0.023	0.043
LH	R	0.618*	-0.627*
	P value	0.0001	0.0001
FSH	R	-0.299	0.256
	P value	0.005	0.066
FBS	R	0.414	-0.299
	P value	0.0001	0.005
HbA1c	R	0.462	-0.353
	P value	0.0001	0.001
Cholesterol	R	0.178	-0.279
	P value	0.09	0.008
TG	R	0.394	-0.421
	P value	0.0001	0.0001
LDL	R	0.127	-0.329
	P value	0.082	0.002
HDL	R	-0.246	0.708*
	P value	0.0651	0.0001

Pearson's correlation considered as followings; weak to Moderate Correlation (r = 0.3 to 0.5), moderate to Strong Correlation (r = 0.5 to 0.7), Strong Correlation (r = > 0.7) and significance level adopted < 0.05.

Discussion

The main results obtained from the current study showed that AMH levels were significantly higher in PCOS patients than in controls, these differences proved statistical significance (P values <0.05). Simultaneously, Myonectin levels were significantly lower (P values <0.05) in PCOS patients than in controls.

Previous studies emphasizes the significance of elevated AMH as a stable biomarker to detect PCOS in as much as this elevation of AMH is connected with the rise in amount of small antral follicles that are most often connected with the PCOS phenotype(9, 10) The findings of the present study were in accordance with the aforementioned findings as AMH was significantly elevated in PCOS relative to control. In addition, it has been reported that Myonectin may affect metabolism as an extra-ovarian hormone. It is sensitive advantage to Myonectin levels were significantly lower in PCOS patients compared to controls, have an average for patients (0.461 ng/ml ±0.427) controls (4.090 ng/ml ±1.518) These results are consistent with the findings by Demir and Guler [11].

The levels of Luteinizing Hormone (LH) were significantly higher in the PCOS group compared to the control group with a p-value of 0.0001. This result confirms what is already known about LH levels in PCOS patients and the dysregulation of the hypothalamic-pituitary-ovarian axis (HPO axis), where LH, was always reported to be in elevated concentrations. Similarly, Follicle-Stimulating Hormone (FSH) level among PCOS group was statistically lower than control group .The raised LH/FSH ratio is a hallmark of PCOS and is suggestive of disturbed folliculogenesis and anovulation (12). The correlation analysis regarding ovarian hormones revealed that LH is positively correlated with AMH and negatively correlated with Myonectin. The changes observed in FSH correlation analysis is not the same. AMH shown to be negatively correlated with FSH, but no observed significant association of FSH with Myonectin in PCOS patients. These observations could reveal the fact that LH changes regarded the leading hormone responsible for PCOS morphology and metabolic changes associated with the condition. Conversely, FSH is less likely affected by hormonal changes.

Regarding glycemia markers, Fasting blood sugar (FBS) and glycated hemoglobin (HbA1C) levels were higher than those of the control group (93.771 ± 3.726 mg/dl and 5.084 ± 0.486%, 9. respectively) with p-values of 0.002 and 0.0001, in any case. These findings suggest impaired glucose tolerance and an increased risk of insulin resistance in patients with PCOS, consistent with existing literatures (13, 14). Furthermore, a prominent finding of this study reveals that positive correlation between AMH levels and both FBS and HbA1c which suggested that an elevated AMH is associated with dysregulated glycemia, highlighting a potential link of reproductive health and glycemia. These finding is consistent with previous studies linked a high AMH and insulin resistance (15, 16). Moreover, Myonectin shown to be negatively associated with glycemic markers in PCOS patients. A low level in Myonectin level observed in PCOS patients is accompanied with higher FBS and HbA1c compared to controls, which allied with the proposed function of Myonectin as a metabolic regulator. In agreement with these results, previous study observed that Myonectin improve insulin sensitivity and enhanced peripheral glucose uptake (17).

Lipid profile analysis represents a good marker for monitoring metabolic status and an index for cardiovascular risk associated with PCOS. Results in this study revealed that, in PCOS patient all lipid parameters shown to be significantly differ from what observed in control patients. The observed dyslipidemia in PCOS group could link the reproductive health and cardiovascular risk factors (18). Obvious important observations regarding correlation analysis of lipid profile in PCOS group is that positive correlation between AMH and triglycerides (TG) ($r = 0.394$, $p < 0.0001$), with no significant links to total cholesterol, LDL, or HDL, suggesting a specific relationship between AMH and TG. The high TG level and the positive correlation of TG with AMH could not merely a sign of metabolic disturbances but also high TG level could have an impact in pathogenesis and progression of PCOS. These findings also observed by other study (19). The justification of absence of correlation of AMH with cholesterol metabolism could attributed to other factors

like diet, other hormones and genetic factors rather that the direct effects of AMH.

The results show a **positive correlation between AMH and triglycerides (TG)** ($r = 0.394$, $p < 0.0001$), with no significant links to total cholesterol, LDL, or HDL, suggesting a specific relationship between AMH and TG. Elevated TG levels, often tied to metabolic dysregulation and conditions like PCOS, may reflect an underlying metabolic disturbance influencing AMH production or vice versa. This correlation highlights a potential interplay between reproductive hormones and lipid metabolism.

However, the results of the current study stressed on hormonal and metabolic changes observed in PCOS, but the role of AMH and myonectin shown to be prominent. Both ovarian, (AMH), and extraovarian peptide myonectin could consider as a key player in metabolic changes recognized in PCOS and might contribute to the metabolic disturbances in those patients.

Conclusions:

The patients with Polycystic ovarian syndrome were significantly differs from control women subjects in their metabolic and hormonal parameters. AMH levels were significantly higher and Myonectin lower in PCOS compared to control. The disturbed hormonal and metabolic dysregulation stressed on the need for more and deep research in the pathogenesis and management in PCOS, with potential therapeutic use of peptides like myonectin to reverse those disturbances.

Recommendations:

Further research is required to shed light of the mechanisms underlying the metabolic changes that observed in PCOS and their relation to hormonal disturbances observed in this syndrome. Further researches also recommended on strategies for raising myonectin by exercise, weight loss and drugs which could served as regulator of metabolic disequilibrium.

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Conflicts of interest

There are no conflicts of interest.

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