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Original Article

Evaluation of heat shock protein 70, AMH, and key hormonal markers in cellular mechanisms of polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases characterized by hyperandrogenemia and anovulation. The present study aimed to estimate heat shock protein 70 (HSP-70) in PCOS patients. This case-control study involved 90 females aged 15 to 45 years, divided into two groups: 45 controls and 45 PCOS patients. Levels of HSP-70, anti-Müllerian hormone (AMH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and testosterone were measured using enzyme-linked immunosorbent assay (ELISA). The study revealed a significant increase ($p \leq 0.01$) in HSP-70 and AMH levels in PCOS patients compared to controls, with mean ± SD values of 16.49 ± 2.79 and 2386.14 ± 530.09, respectively. Hormonal parameters such as LH, testosterone, and prolactin were elevated, whereas FSH was decreased in PCOS patients. The findings suggest that elevated HSP-70 plays a key role in the pathogenesis of PCOS.

Keywords: Polycystic ovarian syndrome (PCOS); Heat shock protein (HSP-70); AMH.

1. Introduction

Polycystic ovarian syndrome is the prevalent cause of anovulation and hyperandrogenism, which causes endocrine disorders in the reproductive system. A patient with PCOS typically has hyperandrogenism, abdominal adiposity, and delayed or irregular menstrual cycles [1]. Insulin resistance is a critical element in the etiology of PCOS. It was shown to correlate with obesity and dyslipidemia [2]. The fundamental pathogenesis of PCOS is not yet known to scientists [3].

Heat shock proteins represent a vast family of proteins in eukaryotic and bacterial organisms, including hyperthermia, inflammation, infection, and food deficiency promotion of production in (HSPs). HSPs react to nearly all situations that induce physiological stress by folding peptides, unfolding and refolding misfolded proteins, destroying defective proteins, and transporting functional proteins [4,5]. Compared to ovulatory controls of similar age and BMI, women with polycystic ovary syndrome have higher serum HSP-70 levels. These proatherogenic inflammatory markers are linked to ovarian androgenic hormone production, insulin sensitivity, obesity, and fasting lipid levels [6]. Certain investigations have indicated increased HSP70 levels in non-obese PCOS patients [7,8]. AMH is a peptide hormone and known Müllerian inhibitory factor released by granulosa cells in growing primary pre-antral and early antral follicles up to 6 mm in diameter, exhibiting a progressive decline in expression as the follicle matures [9,10]. The diagnosis of PCOS has been generally connected with AMH, and AMH levels are up 2 to 3 times in women with Polycystic ovarian syndrome [11,12]. The relationship between high BMI and women with PCOS is unclear [13-15]. The present study aimed to estimate heat shock protein 70 (HSP) levels in PCOS patients.

2. Materials and Methods

2.1. Study design and participants

This case-control study was conducted to evaluate selected biomarkers associated with polycystic ovary syndrome (PCOS) among women aged 15 to 45 years. A total of 90 participants were enrolled, including 45 clinically diagnosed PCOS patients and 45 healthy controls. Diagnosis was based on clinical symptoms, ultrasound examination, and laboratory criteria according to Rotterdam guidelines. The data were collected and recorded from

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September 2024 to December 2024 at the hospital in the Fertility Unit in Karbala. In addition, information was collected for each group according to the questionnaire.

2.2. Ethical approval and informed consent

The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from all participants prior to inclusion in the study, ensuring compliance with ethical standards for human research IQ.UOK.CAMS.DCL.REC.2

2.3. Inclusion and exclusion criteria

Eligible participants were married women aged between 15 and 45 years. Exclusion criteria included a history of diabetes mellitus, smoking, hormonal contraceptive use, and previous ovarian surgery to minimize confounding factors affecting hormonal levels.

2.4. Sample collection and processing

Venous blood samples (5 mL) were collected from each participant on 2or 3 days of menstrual cycle using standard phlebotomy techniques. Samples were divided into EDTA-coated tubes for whole blood and gel tubes for serum separation. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored under appropriate conditions until analysis.

2.5. Measurement of biochemical and hormonal markers

Levels of heat shock protein 70 (HSP-70), anti-Müllerian hormone (AMH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and testosterone were quantified using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's protocols. Optical density readings were measured at 450 nm.

2.6. Statistical analysis

Data analysis was performed using IBM SPSS software (version 23). Descriptive statistics were computed to summarize means and standard deviations. The Shapiro-Wilk test assessed normality, and Levene's test evaluated variance homogeneity. Group comparisons employed independent t-tests or Mann-Whitney U tests as appropriate. Statistical significance was set at $p < 0.01$.

2.7. Sample size determination

In order to identify clinically significant variations in serum HSP-70 and AMH levels between PCOS patients and healthy controls, the sample size for this case-control study was determined. Applying two independent means with equal group sizes, the comparison formula:

$N= 2 (Z^1 \_ \frac{\alpha}{2} + z^1\_B)^2.a^2\backslash(\mu 1 - \mu 2)^2$

Where n is the sample size per group,

$Z^1 \_ \frac{\alpha}{2}$ is the Z-value corresponding to a 95% confidence level (1.96).

z^1_B is the Z-value corresponding to 80% power (0.84).

"a" is the estimated standard deviation, and $(\mu 1 - \mu 2)$ is the minimum detectable difference in mean values of the biomarker between the two groups.

Based on previous literature assessing HSP-70 and AMH levels in PCOS patients, an estimated pooled standard deviation (α) of 5.0 and a minimum clinically significant difference ($\mu 1$-$\mu 2$) of 3.0 were assumed. Substituting the values into the formula:

$n = 2(1.96 + 0.84)2 .5^2\backslash (3)^2 = 2(7.84) (25) \backslash 9 = 392\backslash 9 \approx 43.56$

As a result, 44 people per group were the estimated sample size. In order to provide sufficient statistical power and account for possible non-responses or exclusions, the final sample size was modified to include 45 PCOS patients and 45 healthy controls, for a total of 90 study participants.

3. Result

3.1. Comparison of serum levels of HSP-70 and AMH between PCOS patients and controls

The results (Table 1) showed a significant increase ($p \leq 0.01$) in serum levels of heat shock protein 70 (HSP-70) and anti-Müllerian hormone (AMH) in PCOS patients compared to healthy controls. Mean values for HSP-70 were 16.49 ± 2.79 ng/mL in patients versus 4.79 ± 0.56 ng/mL in controls, and for AMH, 2386.14 ± 530.09 pg/dL in patients versus 895.01 ± 198.18 pg/dL in controls.

3.2. HSP-70 levels according to demographic characteristics

Significant elevations ($p \leq 0.01$) of HSP-70 in PCOS patients were observed across different demographic criteria, including address (rural vs. urban), age groups (less than 30 and greater than 30 years), type of food (healthy vs. unhealthy), type of delivery (cesarean vs. natural), and BMI categories (normal, overweight, obese), compared to their respective control groups (Table 2).

3.3. HSP-70 levels based on clinical features and fertility indicators

Serum HSP-70 concentrations were significantly higher ($p \leq 0.01$) in PCOS patients regardless of clinical symptoms such as hirsutism and acne, as well as fertility parameters, including type of infertility (primary and secondary) and parity status (nulliparous vs. parous), when compared to controls (Table 3).

3.4. Hormonal profile differences between PCOS patients and controls

Hormonal assays revealed significant increases ($p \leq 0.01$) in luteinizing hormone (LH), prolactin, and testosterone levels in PCOS patients relative to controls, while

Table 1. Comparison of serum levels of HSP70 and AMH in the PCOS patients and the control group.

Parameters	Groups	Mean	Std. Deviation	P-value
HSP(ng\ml)	Control	4.79	0.56	0.0006
	Patient	16.49	2.79	
AMH (pg\dl)	Control	895.01	198.18	0.0001
	Patient	2386.14	530.09	

p-value ($p \leq 0.01$), HSP=Heat Shock proteins, AMH= Anti-mullerian Hormone

Table 2. Estimation of HSP-70 in PCOS patients according to different demographic criteria in PCOS and control groups.

Parameters	Classification	Groups	Control		Patient		P-value
			Mean	Std. Deviation	Mean	Std. Deviation	
HSP-70(ng\ml)	Address	Rural	4.842	0.552	17.176	3.707	0.00002
		Urban	4.724	0.582	16.216	2.328	0.00003
	Age	Less than 30	4.919	0.539	16.409	3.143	0.00001
		Greater than 30	4.696	0.568	16.754	1.199	0.00001
	Nature of food	Healthy	4.738	0.550	16.916	3.211	0.00002
		Unhealthy	4.860	0.579	16.322	2.630	0.00005
	Type of delivery	Ceserian	4.795	0.614	17.443	2.916	0.0002
		Natural	4.795	0.554	16.371	1.274	0.0001
	BMI kg\m²	Normal	4.869	0.723	16.235	3.591	0.00003
		Overweight	4.791	0.434	16.400	2.918	0.00004
		Obese	4.688	0.662	16.235	2.318	0.00006

p-value ($p \leq 0.01$).

Table 3. Comparison of serum levels of HSP-70 according to psychological and fertility indicators in PCOS patients and the control groups.

Parameters		groups	Mean	Std. Deviation	P-value
HSP-70(ng\ml)	Hirsutism	No	15.228	2.695	0.0001
		Yes	16.810	2.753	
		Control	4.795	0.561	
	Acne	No	16.392	2.116	0.0001
		Yes	16.582	3.306	
		Control	4.795	0.561	
	nuliparus\paruslady	nuliparus	16.098	3.208	0.0006
		Parus lady	16.907	2.263	
		Control	4.795	0.561	
	Type of infertility	Primary	16.098	3.208	0.0005
		Secondary	16.907	2.263	
		Control	4.795	0.561	

p-value ($p \leq 0.01$).

Table 4. The levels of different fertility hormones in PCOS patients compared to controls.

Parameters	Level	Mean	Std. Deviation	P-value
T.testosterone(ng\ml)	Control	1.08	0.05	0.0000
	Patient	2.73	0.51	
Prolactin(ng\ml)	Control	20.74	4.33	0.0001
	Patient	64.21	11.66	
FSH(mIU/mL))	Control	5.72	0.70	0.0004
	Patient	2.61	0.61	
LH(mIU/mL)	Control	0.51	0.16	0.0008
	Patient	3.33	0.75	

p-value ($p \leq 0.01$), LH= Lutinizing Hormone, FSH= Follicle-Stimulation Hormone

follicle-stimulating hormone (FSH) levels were significantly decreased in the patient group (Table 4).

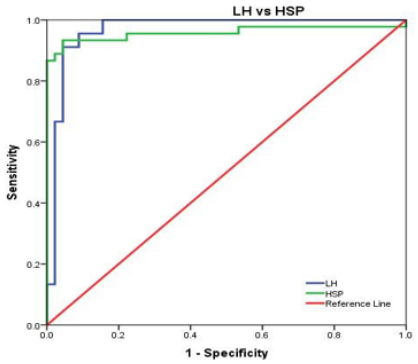
3.5. Diagnostic performance of biomarkers using ROC curve analysis

Receiver operating characteristic (ROC) curve analysis demonstrated high diagnostic accuracy of measured biomarkers, including HSP-70, AMH, testosterone, prolactin, FSH, and LH, with areas under the curve (AUC) ranging from approximately 95.85% to 98.12%, reflecting excellent sensitivity and specificity for differentiating PCOS patients from controls.

As shown in Figure 1, the receiver operating characteristic (ROC) curve analysis illustrates the diagnostic performance of key biomarkers, including heat shock protein 70 (HSP-70), luteinizing hormone (LH), testosterone, anti-Müllerian hormone (AMH), prolactin, and follicle-stimulating hormone (FSH) in patients with polycystic ovary syndrome (PCOS). Panel A displays ROC curves for HSP and LH, Panel B presents curves for testosterone, AMH, prolactin, and the free androgen index, and Panel C shows the curve for FSH. This comprehensive analysis highlights the sensitivity and specificity of these markers in distinguishing PCOS patients from healthy controls (Table 5).

4. Discussion

According to the findings, obesity and PCOS are closely related. Also, the IR seen in PCOS contributes significantly to the development of obesity. However, aberrant visceral fat formation may come from lipid metabolism brought on by an increase in elevated androgen levels in PCOS [15,16]. This agreement with the study found elevated androgen due to elevated weight and increased LH level [17,18]. The researchers found that AMH was elevated due to the pre-antral and tiny antral follicles' enhanced production and release of AMH. Additionally, granulosa cells in PCOS patients' follicles have been shown to produce 75 times as much AMH as healthy cells. Moreover, AMH levels increase proportionately to the antral follicle count (AFC) at a constant 0.2 ng/ml for each follicle [19]. This is in line with the study that found elevated AMH [20,21]. The study also found that the ovarian tissue of PCOS patients had significantly higher levels of HSP70 than that of the healthy group. The most conserved protein, HSP70, is elevated in ovarian tissue and has been linked to a reduction in ovarian follicular cell death. Women



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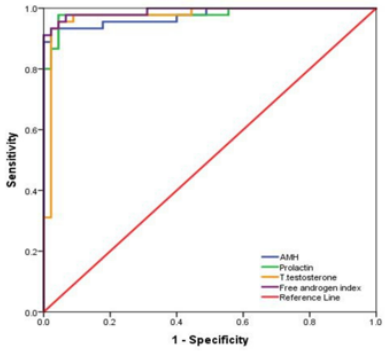

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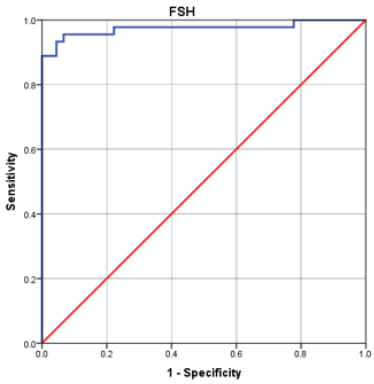

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Fig. 1. Receiver operating characteristic (ROC) analysis for HSP, LH, testosterone, AMH, prolactin, free androgen index, and FSH in PCOS patients. A: Receiver operating characteristic curve for HSP and LH parameters. B: Receiver operating characteristic curve for T. testosterone, AMH, prolactin, and free androgen index parameters. C: Receiver operating characteristic curve for FSH parameter.

Table 5. Area under ROC curve (AUC) analysis for research parameters.

Metrics		T. testosterone	Prolactin	FSH	LH	HSP-70	AMH
Std. Error		0.018	0.013	0.018	0.021	0.025	0.015
Asymptotic Sig.		0.001	0.003	0.009	0.002	0.006	0.008
Asymptotic 95%	Lower Bound	0.938	0.955	0.939	0.925	0.946	0.946
Confidence Interval	Upper Bound	1.000	1.000	1.000	1.000	1.000	1.000
Cutoff Point		1.198	35.143	4.329	2.455	8.151	1465.212
Area Under Curve (AUC)		97.333%	98.123%	97.432%	96.642%	95.852%	97.531%
Sensitivity		95.556%	97.778%	95.452%	91.111%	93.333%	93.432%
Specificity		95.355%	95.456%	93.333%	95.535%	95.556%	97.780%
Accuracy		95.556%	96.667%	94.444%	93.333%	94.444%	95.556%
Positive Predictive Value		95.455%	95.652%	93.478%	95.349%	95.455%	97.674%
Negative Predictive Value		93.478%	97.727%	95.455%	91.489%	93.478%	93.617%

having PCOS may also exhibit apoptosis in their ovarian tissues as a result of this elevation in HSP70, since HSP70 could influence apoptosis by blocking the translocation of the Bcl-2 family protein from the cytosol to the mitochondria [22,23], which also agrees with [24[25]. There is a growing trend in PCOS diagnoses among youth and young adults, which may be related to better diagnostic techniques and more awareness. According to the current study, the age range of 21 to 30 had the highest number of PCOS patients. Studies found that food choices, obesity, and sedentary lifestyles all contribute to the greater frequency of PCOS in younger generations [26].

In the present study, it was found that hirsutism and acne were elevated in PCOS due to increased androgens, which have a clinical effect on women diagnosed with PCOS. Androgen levels increased due to elevated levels of insulin, which stimulate the ovaries and adrenal gland to produce testosterone [27,28]. These findings are in agreement with [29].

It was also revealed that reduced circulation of FSH is linked to a disruption in the hypothalamic-pituitary-ovarian (HPO) axis; higher LH frequency boosts androgen production by theca cells. On the other hand, lower FSH levels hinder the growth of follicles and, as a result, anovulation [30]. These observations are in line with [31]. Total testosterone has shown a significant increase in PCOS patients because high insulin levels and overweight work together to stimulate the production of testosterone in the ovaries and adrenal glands, which feeds a vicious cycle that further increases abdominal obesity [32], which is in agreement with [33].

Previous studies documented comparable results showing that Prolactin's AUC, sensitivity, and specificity were (83.2%, 77%, 88%), respectively [34], testosterone levels were (88.9%, 92.6%, 85.4%) [35], and LH levels were (93.2%; 86.3%: 95%) [36]. On the other hand, FAI sensitivity was 76%, and specificity was 82% [29]. FSH sensitivity was 65.00% and specificity was 87.80% [37]. Moreover, AMH had AUC (0.999) with sensitivity and specificity 99%,100% [36]. HSP70 had sensitivity and specificity of 83.3% and 90.0%, respectively [6].

This study comprehensively evaluated the serum levels of heat shock protein 70 (HSP-70), anti-Müllerian hormone (AMH), and several key reproductive hormones, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and testosterone in women diagnosed with polycystic ovary syndrome (PCOS) compared to healthy controls. The results demonstrated that HSP-70 and AMH were significantly elevated in PCOS patients, supporting their roles as important biomarkers in the cellular and molecular pathogenesis of this syndrome. Elevated levels of HSP-70 suggest an involvement of cellular stress responses and apoptotic regulation in ovarian dysfunction characteristic of PCOS, potentially contributing to follicular arrest and anovulation. Similarly, increased AMH levels reflect enhanced activity of granulosa cells in small pre-antral and antral follicles, consistent with the well-established association between raised AMH and PCOS.

Significant alterations in reproductive hormones were also observed, including marked increases in LH, testosterone, and prolactin, accompanied by a substantial decrease in FSH levels. These hormonal imbalances highlight disruption of the hypothalamic-pituitary-ovarian axis and increased androgen production, reinforcing the complex endocrine dysregulation underlying PCOS. The correlations between elevated HSP-70, AMH, and these hormonal changes underscore the multifactorial mechanisms driving PCOS pathophysiology, including oxidative stress, inflammation, and altered folliculogenesis.

Diagnostic evaluation using ROC curve analysis confirmed that HSP-70, AMH, testosterone, prolactin, LH, and FSH exhibited high sensitivity and specificity in distinguishing PCOS patients from controls, underscoring their clinical utility as predictive biomarkers. This suggests that combined measurement of these markers can improve early diagnosis and disease monitoring, facilitating personalized management strategies.

Moreover, the study's subgroup analyses revealed consistent elevation of HSP-70 across different demographic variables, clinical manifestations, and fertility profiles, indicating its potential as a robust biomarker independent of age, BMI, and reproductive status. In summary, the significant elevation of HSP-70 in conjunction with AMH and key reproductive hormones presents compelling evidence for their integral role in the pathogenesis and clinical presentation of PCOS. These findings not only enhance understanding of the molecular and endocrine alterations in PCOS but also highlight novel avenues for biomarker development, offering potential targets for future therapeutic interventions aimed at mitigating oxidative stress and hormonal dysregulation. Continued research into the mechanistic links between HSP-70 expression and ovarian dysfunction could provide further insights into PCOS etiology and improve outcomes for affected women.

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Authors' contributions

All authors made substantial contributions to data collection, analysis, the preparation of the results and read the final version of the manuscript.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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