Medical Science Journal for Advance Research

Vol.4 , No. 4 , January 2024
e-ISSN: 2774-4892

Serum Level Of Heat Shock Protein 70 In Patients With Polycystic Ovarian Syndrome In Iraqi Women

Taif Eimad Ahmed[1*], Asma Ghalib Abid[2], Sahar Radhi Yasir[3], And Dina Akeel salman[4]

[1] *M.B.Ch.B, D.G.O, F.I.C.M.S, Consultant In Obstetrics And Gynaecology Subspecialty In Infertility And IVF Kamal Al-Samarraei Hospital For Infertility Management And IVF, Baghdad, Iraq.*

[2] *M.B.Ch.B., M.Sc., F.I.B.M.S. (Path. Immune) , The Specialized Center For Endocrinology And Diabetes, Baghdad, Iraq.*

[3] *Msc. Medical Microbiology, Kamal Al-Samarraei Hospital For Infertility Management And IVF, Baghdad, Iraq.*

[4] *M.B.Ch.B, D.G.O, F.I.C.M.S, Senior In Obstetrics And Gynaecology. Assistant Professor Collage Of Medicine Al-Mustansiriya University Baghdad, Iraq.*

***E-mail: taifjremad1972@gmail.com**

ABSTRACT

Background: *A common endocrine disease of fertile and postmenopausal woman was <polycystic ovarian syndromes> (PCO). Complex heterogeneous conditions, PCO has many different causes, consequences, and clinical symptoms. Uncertainty still exists regarding the pathogenic alterations of PCO. Recent study has looked into <heat shock proteins> (HSPs) functions as a cause of PCO. Therefore, the aims of the study was determine whether HSP70 serum level differ among PCO women and women as healthy control. And confirmed HSP70 can help improve PCO predictive value.* ***MATERIALS AND METHOD:*** *This was a case control project compose of 90 woman their age (19-42 year), 60 of them PCO diagnosed, while another 30 who are healthy control from May 2022 to November 2022. Testosterones, follicle-stimulating hormones (FSH) and Luteinizing hormones (LH) were tested by specific enzyme linked immunofluorescence assays performed in VIDAS automatic. HSP70 was tested by ELISA method based on the principle of sandwich quantification (sunlong-china).* ***RESULT:*** *Mean HSP70 (ng/ml) was significantly higher in women with PCO in comparison with control group (P<0.05). In addition, compared with healthy groups, hormonal biomarkers such as testosterones, LH, FSH and BMI were found to be significantly elevated in the patients.* ***CONCLUSIONS:*** *Increase level of the HSP70 in women with PCO showed that HSP70 plays key roles in the pathological mechanism of PCOS.*

Keywords: *Polycystic Ovarian Syndromes, Heat Shock Proteins 70*

Article Information
Received: December 12, 2023; Revised: December 31, 2023; Online: January 15, 2024

INTRODUCTION

Polycystic ovary syndrome (PCO) is a disorder characterized by a prevalent polygenic multifactorial disorder affecting a wide population (1). It is a condition present with ovary dysfunction and endocrine problem, Furthermore; it is associated with hyperinsulinemia and metabolic disease. (2). To diagnosis of PCO require two of three criteria are required: (i) oligo-ovulation and / or an ovulation, (ii) hyperandrogen (clinically and / or biochemical), or

https://doi.org/10.46966/msjar.v4i4.161

(iii) polycystic ovary as seen by ultrasound scan and exclusion other causes of high androgen level and irregular menstrual cycles or amenorrhoea. (2)

PCOS can be clinically classified in to four phenotype:

- Excess androgen +ovulatory dysfunctions + polycystic ovary morphology (Phenotype A)
- Excess androgen + ovulatory dysfunctions (Phenotype B)
- Excess androgen + polycystic ovary morphology (Phenotype C)
- Ovulatory dysfunctions + polycystic ovary morphology (Phenotype D) (3)

Normal levels of the circulating androgens are found in 20-40% of PCO patient and often PCO patient associated with hyperandrogenemia have a mild androgen elevation (4, 5).

<Heat shock proteins> HSPs are a group of high conserved and extensively expressed proteins that regulate cellular functions and protein homeostasis. (6) Low levels of HSP are expressed under normal physiological circumstances and dramatically increase as a result cellular stress (7).

They act as indicators of inflammation and tissue damage that are induced in response due to environment, physical and chemicals stress acting likes defensive agent in limitation of damage and facilitation of cellular recovery(8). <Heat shock protein 70> (HSP70) is mostly prominent protein among a set of HSP (9), with potent anti-oxidants, anti-inflammatories, and anti-apoptotic characteristics (7). HSP70 is found in the peripheral circulation of healthy persons, therefore HSP is usually considered to be intracellular proteins with molecular chaperone and cytoprotective functions (10, 11).

The heat shock protein molecular mass was used to give them names. Examples of intensively investigated HSP include <HSP60, HSP70, and HSP90>, which relate to the families of heat shock proteins that are <60, 70, and 90> kilodalton in size, respectively (12). HSP70 has 641 amino acid residues with a molecular weight of 70,052 Da (13).

HSP70 acts in conjunction with a group of co-chaperones and nucleotide interchange factors to make sure that protein are of superior quality, so cell survive is maintained by protection them from environmental stresses (14). As a result, it plays a vital role with proteins homeostasis in association with its transport (15). As mentioned above HSP70 has a direct anti-apoptotic activity, so it can take action as a "friend" of cancer development. Therefore, attempts by researchers enhance methods for cancer therapy by employing the HSP70 inhibitors (16).

The existence of HSP70 on the surface of cancer cells may make them more liable to cytotoxicity of natural killer (NK) cell, which could assist in the development of effective immunotherapy (17). A high serum level of HSP70 has also been linked to ovary damage (18).

Based on these, aims of this study were (i) to determine whether there are differences in serum HSP70 levels among PCO

women and healthy women as control; (ii) to determine whether serum levels of HSP70 could help improve the predictive value for PCO.

METHODS

A case-control study was carried out at Kamal Al – Samarai Hospital, Fertility centre, Bagdad. All samples were collected from May 2022 till November, 2022. The study was executed for 90 female Iraqi individuals their age ranged (19-42) years old within reproductive age and 60 of them diagnosed polycystic ovary syndrome other 30 healthy control group.

Inclusion criteria thirty healthy women in reproductive age have normal menstrual cycles their ages between (20-40) years old were taken as a control group. Each one had a history of regular 26 to 32 day menstrual cycles, absence hirsutism and other manifestations of the hyperandrogenism and thyroid dysfunction that group was checked by gynaecologist and fifty women with PCO "all of them were diagnosed with PCOS according to Rotterdam criteria by gynaecologist" were taken as patient group.

Permission was taken to all subjects to participate in this study furthermore, each person who contributed in this study underwent full history, age, height, weight to calculate their body mass index (BMI), past medical history , past surgical history and Primary or secondary infertility.

No one of these Patients were chronic smokers or Patients with diabetes mellitus, congenital adrenal hyperplasia, thyroid disorders, Cushing's syndrome, hypertension, hyperlipidaemia, take birth control pills or any origin other than polycystic ovarian syndrome and patient that have malignancies were excluded from the study.

Collection of the Blood Samples:

Venous blood samples were collected from each case during 2nd – 5th day of the menstrual cycle (early follicular phase) for those of normal cycle. Five ml of venous blood was drawn using a 5 ml size disposable syringe. The blood samples were left to clot then centrifuged at 3000 rpm for 5 minutes to separate the serum. Serum samples transferred to Eppendorf tubes and stored kept frozen at – 20 °C until use.

Testosterones, follicle Stimulating Hormone (FSH), luteinizing hormone (LH) were measured by a specific enzyme-linked fluorescent immunoassay performed in automated VIDAS. HSP70 was tested by using ELISA method test based on the quantitative sandwich principle by (sunlong-china).

Statistical analyse:

IBM version 26 impact of various factors on study's parameters was assessed using Statistics for the Statistical Package Social Sciences <SPSS>. T-_test was compared to two mean values. A chi-squared test was using for calculation r-degree and coefficients correlation. That was considered significant when p=0.05 was using to compare patient and control (means ±SD) < standard deviation>.

RESULTS:

Table No.1 illustrates the demographics of the study. The purpose of this table is to create comparisons and significant differences. That intended to be significant

when (P<0.005) and presented as Mean ±SD (SD).Independent T-test statistics was applied for parameters that compare patient and control groups. Patients and controls had a mean age of (27.36 ± 5.1) (32.4 ± 6.2) respectively. There was no significant difference in the median age of PCO patients compared to controls. Based on the information provided show mean of the HSP70 levels (14.01 ± 4.0 ng/ml) (9.56 ± 1.7ng/ml) in patients and controls respectively, that significant increase in group of PCO patients (P<0.005) compare with controls group. Table 1 shows that finding results significantly in biomarkers measured in hormone <LH, FSH, Testosterone and BMI> in patients group compare to healthy group, While no significant in Prolactin hormone.

Table: 1 Shows the average HSP70 concentration (ng/ml) increase in PCO patients compared to the control group. Table No. (1): Descriptive statistics of different variables between PCO women and healthy control

Variable	**PCO n.60** **Mean ± SD**	**Control n.30** **Mean ± SD**	**P value**	**R^2**
Age (year)	27.36 ± 5.1	32.4 ± 6.2	0.489	0.15
BMI (kg/m2)	29.86 ± 5.2	26.60 ± 2.2	0.000	0.10
FSH (m IU/ml)	5.82 ± 2.4	7.38 ± 1.1	0.001	0.10
LH (m IU/ml)	6.40 ± 4.2	4.15 ± 0.9	0.000	0.08
Prolactin (ng/ml)	22.36 ± 13.4	13.61 ± 7.4	0.145	0.11
Testosterone	2.29 ± 2.4	0.9000 ± 0.36	0.001	0.09
TSH (m IU/ml)	2.18 ± 1.19	2.30 ± 0.86	0.255	0.003
HSP (ng/ml)	14.01 ± 4.0	9.56 ± 1.7	0.042	0.26

BMI: Body mass index, LH: Luteinizing Hormone

FSH: follicle-stimulating Hormone

SD: standard deviation

R^2 = eta squared (goodness of fit of the model)

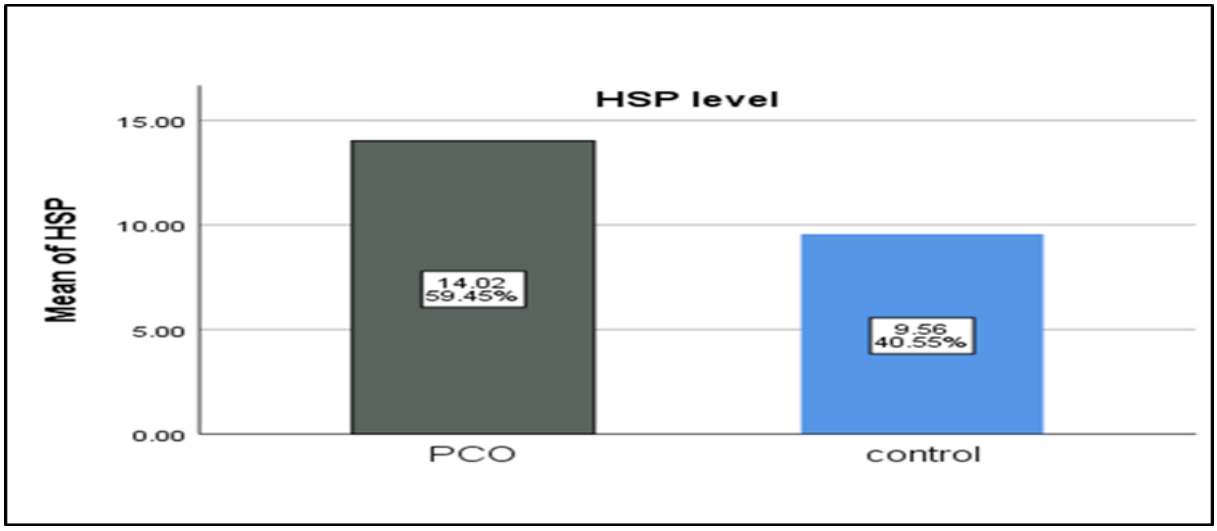

Figure 1: Mean levels of Heat shock protein 70 (ng/ml) in patients group of PCO compared to control group.

ROC curve

ROC curve in Table (2) and figure (2) results indicate that HSP good test to differentiated between PCO and healthy controls with an AUC of 0.873, the optimal cut-off value is 11.3 with accuracy 85.5%

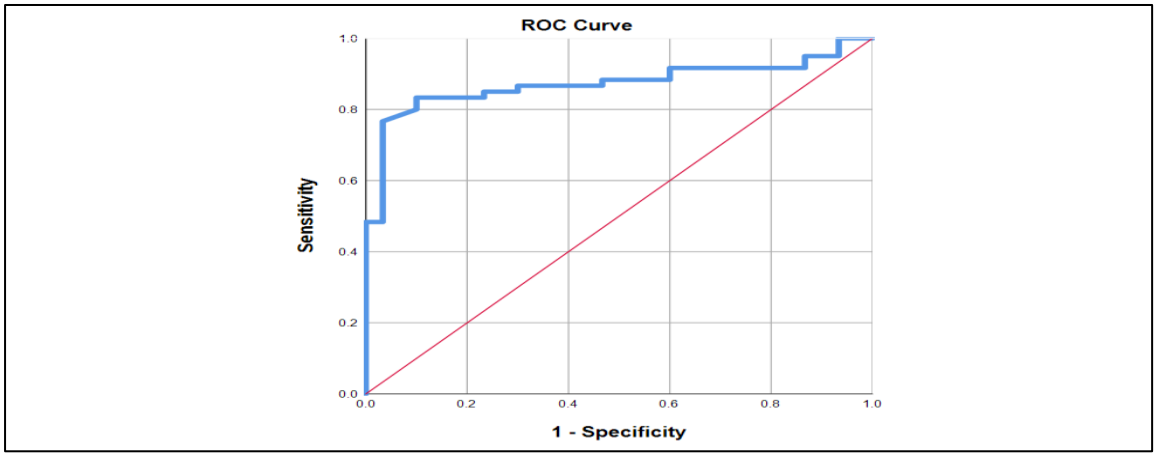

Figure 2: ROC curves for serum HSP to differentiated between PCO and controls.

Table 2: Sensitivity and Specificity, area under curve and Cut-off value for serum HSP in PCO

Marker	Cutoff	AUC	Sensitivity	specificity	PPV	NPP	Accuracy
HSP	11.3	0.873	83.3%	90.0%	94.3%	73.0%	85.5%

In this study finding the percentage of obesity in patients with PCO group was high 32. 22 %while in the control 2.22% as show in figure 3 and types of PCO in patients group of PCO compared to controls group show in figure 4 and statistical difference of variable in table 3.

Table 3: Shows the statistical difference among the study each category of PCO with control groups.

Variable	PCO n.60 Mean ± SD				Control n.30 Mean ± SD	P value	R2
	Type A	Type B	Type C	Type D			
Age	26.88 ±4.8	31.40±7.05	26.00±3.6	29.40±6.1	32.4±6.2	0.001	0.199
BMI	30.54±5.2	28.52±7.2	28.82±2.4	26.84±5.8	26.60±2.2	0.008	0.148
FSH	6.13±1.1	6.90±2.9	3.55±1.5	5.30±3.5	7.38±1.1	0.00	0.214
LH	6.66±4.6	6.33±2.8	4.61±2.1	6.74±4.2	4.15±0.9	0.048	0.106
Prolactin	23.23±15.0	18.73±6.8	21.07±8.1	20.50±10.8	13.6±7.4	0.026	0.122
testosterone	2.65±2.8	1.53±1.0	1.33±0.7	1.27±1.2	0.90±0.3	0.009	0.146
TSH	2.33±1.3	2.29±0.5	1.75±0.6	1.30±0.4	2.30±0.8	0.245	0.061
HSP	14.24±4.5	13.32±2.4	12.88±3.1	14.3±2.5	9.56±1.7	0.000	0.278

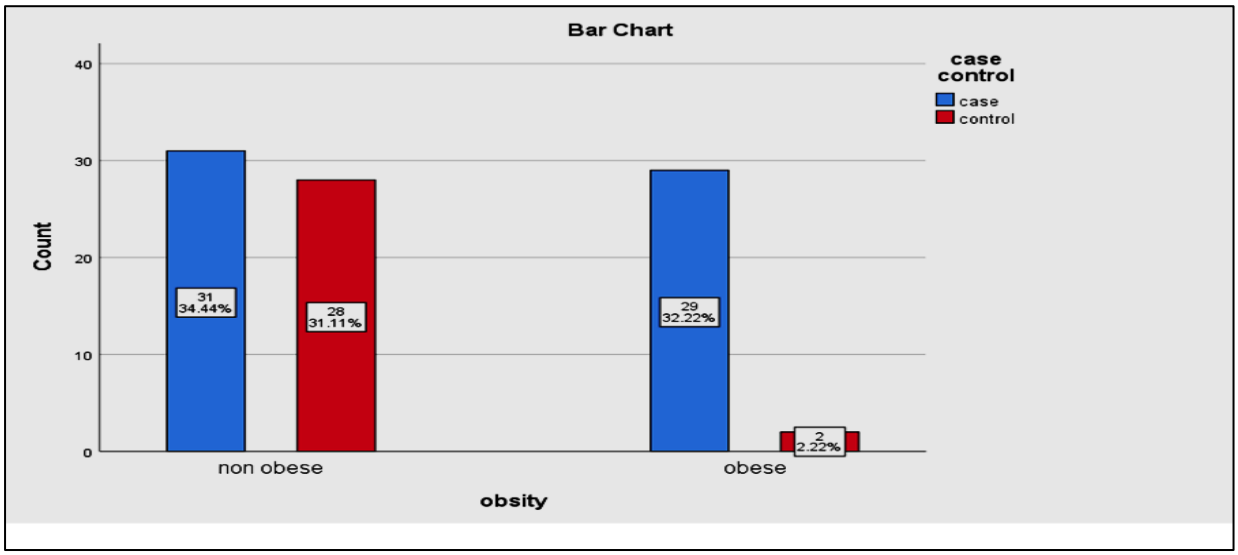

Figure 3: Mean level of obesity with PCO group patients comparing to controls group.

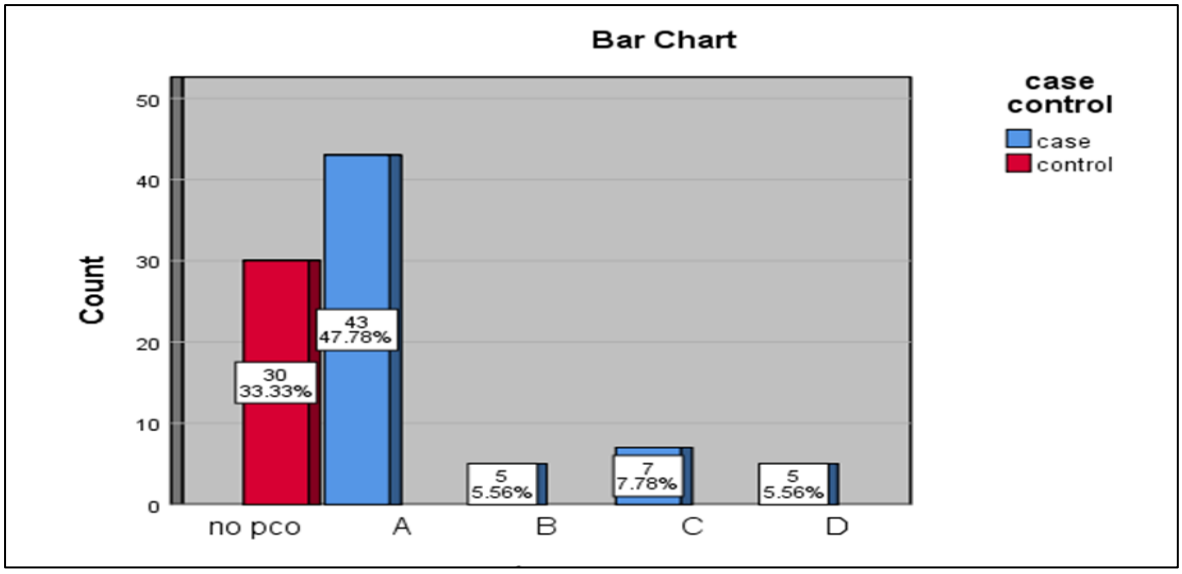

Figure 4: types of PCO in the group of PCO patients in association to controls group.

DISCUSSION

HSP is conserving a significant amount of material and is being expressed widely by family of molecule, also referred as a molecular chaperone which helps to repair non-covalently bound polypeptides (19). HSP plays a significant role for both the proliferation/apoptotic mechanism & in the activity of the steroid hormones, both of which play a significant role in the overall ovary physiology (20).

According to the presented data in table 1 show mean of HSP70 level (14.01 ± 4.0) (9.56 ± 1.7) for patient & control group respectively, Hsp70 was significant higher with the group of PCO patient ($P < 0.05$), and the result concurs to the study increased blood levels of HSP70 in PCO patients (21) HSP70 may contribute with the pathogenesis of Polycystic ovary syndrome PCO (22) Another study, Disagreeing with our results, showed HSP70 significantly increased in tissue of ovary of PCO rats compared to controls, but significantly decrease in serum levels was observed (23).

The regulation of HSP70 expression is mediated by heat shock transcription factor (HSF)-1. (24). Physiological testosterone has been reported to increase the expression of human HSP70 following ischemic pre-conditioning. This may be due to the fact that HSP70 can be phosphorylated by HSF1 and up-regulated by mRNA expression, thus demonstrating that testosterone is necessary for the increased expression of the HSP (25). So in current study testosterone mean level increased significantly ($P<0.05$) in patients PCO group (2.29 ± 2.4) while in control group mean testosterone level (0.9000 ± 0.36)

A high level of luteinizing hormone (LH) leads to the increase LH: follicle stimulating hormone (FSH) ratio commonly found in women with PCO (26). FSH levels become relatively low, causing follicles in the ovary to become resistant to the hormone (27). Our study have significantly increased LH ($P<0.05$) in patients group of PCO (6.40 ± 4.2) while in control group (4.150 ± .9) and significantly decreased FSH ($P<0.05$) in

patients group of PCO (5.82 ± 2.4) while in control group (7.38 ± 1.1). This result agreement with study Malini and Roy George (28).

The result of ROC analysis was agreed with study of Lahieb et al. (21) that Hsp70 was valid test to differentiate between PCO women and healthy controls.

The limitation of this study is the small number of patients and control and short duration of the study. This can be solved by longer duration and larger study size

Phenotype A was the most common phenotype with prevalence of 47.78% followed by phenotype C, B, and D, respectively and has a higher prevalence of obesity, hyperandrogenism. These results are supported by the study conducted (29). This study was the first study to evaluated validity of serum heat shock protein 70 to clinically classified phenotype PCO.

REFERENCES

1. Jacob S, Tang ThH, Balen AH., polycystic ovary syndromes & assisted reproduction In: Gardner DK, Weissman A, Howles CM et al, Text book of assisted reproduction technology. 5th ed... New York. Taylor and Francis.2018.p762
2. Balen A. Polycystic ovary syndromes and secondary amenorrhoea in Edmonds Dk, Lees Ch, Bourne T, Dewhurst's Textbook of obstetrics and genecology . 9th ed. India: Wiley ;2018 p632
3. Teede H, Misso M, Costello M et al .International evidence – based guideline for assessment and management a polycystic ovary 2018.Australia: Monash University 2018 p 13.
4. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006; 91:4237–45.
5. Chang WY, Knochenhauer ES, Bartolucci AA, Azziz R.< Phenotypic spectrum of polycystic ovary syndrome> clinical and biochemical characterization of the three major clinical subgroups. Fertil Steril. 2005; 83:1717–23.
6. Kalmar B, Greensmith L Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev 61: 310-318. doi:10.1016/j.addr.2009.02.003.
7. Lavie L, Dyugovskaya L, Golan-Shany O, Lavie P Heat-shock protein 70: expression in monocytes of patients with sleep apnoea and association with oxidative stress and tumour necrosis factor-alpha. 2010 J Sleep Res 19: 139-147. doi:10.1111/j.1365-2869.2009.00789.x.
8. Hoter, A. and Naim, H.YHeat Shock Proteins and Ovarian

Cancer: Important Roles and Therapeutic Opportunities.Cancers, 2019.11(9), p.1389.

9. Bironaite D, Brunk U, Venalis A Protective induction of Hsp70 in heat-stressed primary myoblasts. Involvement of MAPKs. 2013 J Cell Biochem .

10. Pockley AG, Shepherd J, Corton JM Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies in the serum of normal individuals. Immunol Investig 1998 27: 367-377. doi: 10.3109/08820139809022710.

11. Molvarec A, Tamási L, Losonczy G, Madách K, Prohászka Z et al. Circulating heat shock protein 70 (HSPA1A) in normal and pathological pregnancies. 2010 Cell Stress Chaperones 15: 237-247. doi: 10.1007/s12192-009-0146-5.

12. Buttacavoli, M., Di Cara, G., D'Amico, C., Geraci, F., Pucci-Minafra, I., Feo, S. and Cancemi, P. Prognostic and Functional Significant of Heat Shock Proteins (HSPs) in Breast Cancer Unveiled by MultiOmics Approaches. (2021) Biology, 10(3), p.247.

13. Saibil, H. Chaperone machines for protein folding, unfolding and disaggregation. Nature Reviews Molecular Cell Biology, 2013. 14(10), pp.630–642

14. Hartl, F.U., Bracher, A. and Hayer-Hartl, M. Molecular chaperones in protein folding and proteostasis. 2011. Nature, 475(7356), pp.324–332.

15. Breuninger, S. and Erl, J. Quantitative Analysis of Liposomal Heat Shock Protein 70 (Hsp70) in the Blood of Tumor Patients Using a Novel LipHsp70 ELISA. 2014. Journal of Clinical & Cellular Immunology, 05.(05).

16. Hoter, Rizk and Naim The Multiple Roles and Therapeutic Potential of Molecular Chaperones in Prostate Cancer. (2019). Cancers, 11(8), p.1194.

17. Albakova, Z., Armeev, G.A., Kanevskiy, L.M., Kovalenko, E.I. and Sapozhnikov, A.M. 2020. HSP70 Multi-Functionality in Cancer. Cells, 9(3).

18. Narayansingh, R. M., Senchyna, M., Vijayan, M. M., & Carlson, J. C. Expression of prostaglandin G/H synthase (PGHS) and heat shock protein-70 (HSP-70) in the corpus luteum (CL) of prostaglandin F2α-treated immature superovulated rats. 2004. Canadian Journal of Physio-logy and Pharmacology, 82(6), 363–371.

19. Ellis RJ. Do molecular chaperones have to be proteins? Biochem Biophys Res Commun. 1997;238: 687–92.

20. Velazquez MM, Alfaro NS, Dupuy CR, Salvetti NR, Rey F, Ortega HH. Heat shock protein patterns in the bovine ovary and relation with cystic ovarian disease

[J]. Anim Reprod Sci. 2010; 118(2–4):201–9.

21. Lahieb shakir Mahmood , Shaymaa Z.Nada , Hiba Abdul-Jabbar Al-Wazzan The role of heat shock protein 70 and dysglycemia in Iraqi Women with Polycystic Ovarian Syndrome 2021 Vol.17 No.02 2021

22. Gao, H., Meng, J., Xu, M., Zhang, S., Ghose, B., Liu, J., Yao, P., Yan, H., Wang, D. and Liu, L. Serum Heat Shock Protein 70 Concentration in Relation to Polycystic Ovary Syndrome in a Non-Obese Chinese Population. 2013. PLoS ONE, 8(6), p.e67727. DOI:10.1371/journal.pone.0067727

23. Wu, G., Hu, X., Ding, J. and Yang, J. Abnormal expression of HSP70 may contribute to PCOS pathology. 2019 Journal of Ovarian Research, 12(1 https://doi.org/10.1186/s13048-019-0548-7.

24. Liu J, Tsang S, Wong TM. Testosterone is required for delayed cardioprotection and enhanced heat shock protein 70 expression induced by preconditioning[J]. Endocrinology. 2006; 147(10):4569–77.

25. Kohno H, Takahashi N, Shinohara T, Ooie T, Yufu K, Nakagawa M, Yonemochi H, Hara M, Saikawa T, Yoshimatsu H. Receptor-mediated suppression of cardiac heat-shock protein 72 expression by testosterone in male rat heart [J]. Endocrinology. 2007; 148(7):3148–55.

26. Franks, S.; Hardy, K. What causes anovulation in polycystic ovary syndrome? Curr. Opin. Endocr. Metab. Res. 2020, 12, 59–65.

27. Szeliga, A.; Rudnicka, E.; Maciejewska-Jeske, M.; Kucharski, M.; Kostrzak, A.; Hajbos, M.; Niwczyk, O.; Smolarczyk, R.; Meczekalski, B. Neuroendocrine Determinants of Polycystic Ovary Syndrome. Int. J. Environ. Res. Public Health 2022, 19, 3089.

28. Malini, N.A. and Roy George, K. Evaluation of different ranges of LH: FSH ratios in polycystic ovarian syndrome (PCOS) – Clinical based case control study. 2018; General and Comparative Endocrinology ,260,pp.51–

29. Garima Sachdeva, Shalini Gainder, Vanita Suri, Naresh Sachdeva, Seema Chopra Comparison of the Different PCOS Phenotypes Based on Clinical Metabolic, and Hormonal Profile, and their Response to Clomiphene Indian Journal of Endocrinology and Metabolism ¦ Volume 23 ¦ Issue 3 ¦ May-June 2019 p 326-331.

