Original Article

The Relationship of Calcyphosina with Lipid Profile in Polycystic Ovary Syndrome Women

Shahad Ahmed[1*], Khalid F. Al-Rawi[2], Rafal Mustafa Murshid[3]

[1*]Department of Chemistry, College of Education for Pure Sciences, University of Anbar, Ramadi, Iraq. E-mail: shahad201920202020@gmail.com

[2]Department of Chemistry, College of Sciences University of Anbar, Ramadi, Iraq.

[3]Head of Department of Obstetrics and Gynecology, College of medicine, University of Anbar, Ramadi, Iraq.

Abstract

Objectives: The current study was designed to monitor the relationship between Lipid Profile and calcyphosin a level by studying the correlation of calcyphosin a concentration in blood serum with T. Cho and TG in women with PCOS in Anbar Governorate.

Methods: This study included 100 Iraqi women (age: 18-45 years) who were divided into two groups, polycystic ovary syndrome women group (60 women) and healthy women group (40 women). Body mass index was measured for all participating women. CAPS levels were evaluated using ELISA technology, while levels of (Total Cholesterol) were assessed using a spectrophotometer.

Results: The mean CAPS was significantly higher in the group of women with PCOS than in the group of healthy women. This study also showed that there are no statistically significant changes in the level of In comparison to a control group of healthy women, PCOS women had higher levels of total cholesterol, triglycerides, good cholesterol (HDL), and low-density lipoprotein (VLDL). However, the study's findings and statistical analysis revealed that PCOS patients also had significantly higher levels of VLDL.

Conclusion: Serum CAPS levels can be used as a novel biomarker in the detection of PCOS and may be an effective biomarker in the diagnostic test of PCOS.

Keywords: CAPS As Well As Polycystic Ovary Syndrome.

DOI: 10.47750/pnr.2022.13.S03.146

INTRODUCTION

One of the broadest endocrine disorders affecting women of childbearing age, polycystic ovarian syndrome (PCOS) affects 5–10% of females. Chronic anovulation, polycystic ovary, and chemical or clinical hyperandrogenism are all related with PCOS. Infertility, hyperandrogenism, obesity, abnormal glucose metabolism, insulin resistance (IR), hirsutism, and acne vulgaris are some of the clinical characteristics of PCOS [1]. According to NIH and Rotterdam criteria, PCOS prevalence in Iran was found to be 6/8% and 19/5%, respectively, in 2014.[2]. The selective estrogen-receptor modulator clomiphene citrate (CC) used to be the first-line treatment for oligoovulatory and anovulatory women with PCOS. However, treatment with the aromatase-inhibitor letrozole results in higher live-birth and ovulation rates [3][4][5].

Concerning women with PCOS, there is potentially an interaction between bone derived markers and downstream metabolic and hormonal imbalances [6]. A high prevalence of vitamin D deficiency and even an inverse association with insulin sensitivity markers have been shown for PCOS patients [6], [7]. Women with PCOS and vitamin D insufficiency have been shown to benefit from vitamin D treatment in terms of increased insulin sensitivity and decreased androgen levels; however, similar advantages do not appear to apply to women without COS who are vitamin D deficient.[8]. The CAPS gene in humans encodes the protein calcyphosin (CAPS) [9]. A Ca-binding protein that is encoded by this gene may help control ion transport. R2D5 antigen is the name given to a comparable protein that was first identified in the dog thyroid as a possible crucial regulatory protein. This gene's alternative splicing results in two transcript variants [10].EF-hand protein CAPS is involved in a variety of physiological activities, including the Ca2+-phosphatidylinositol and cyclic AMP signaling pathways [11].Ca2+-loaded CAPS' crystal structure was established up to 2.65oA. An excellent indication of ovarian reserve is anti-Mullerian hormone (AMH), a member of the beta-transforming growth factor family called a bipolar non-associated glycoprotein.[12].

The hormone concentrations can be used to evaluate the ovarian reserve and responsiveness to induction [13].Cholesterol is a waxy, fatty molecule that is present in

the cell walls of every organ in the body, including the neurological system, heart, and liver[14]. Women with polycystic ovarian syndrome tend to have higher rates of insulin resistance (PCOS).[15] and intolerance to glucose[16],Low levels of HDL cholesterol in the blood and high levels of triglycerides are two conditions that are usually associated with an abnormal lipoprotein pattern.[17].

An increased mobilization of free fatty acids is hypothesized to contribute to insulin resistance and obesity (FFA)[18].A rise in plasma FFA is typically associated with insulin resistance and glucose intolerance.[19], They additionally serve as a substrate for hepatic VLDL synthesis, increasing blood triglyceride levels. Plasma lipids may continue to decline as a result of a gradual degradation of glucose tolerance brought on by P-cell fatigue and reduced insulin action.[20].This study sought to determine the effects of anthropometric factors, sex hormones, insulin sensitivity, glucose tolerance, and anthropometric factors on the plasma FFA and serum lipid profile in PCOS-affected women and control patients over a wide range of body mass index (BMI).

Materials and Method

This study was conducted in Al-Ramadi Teaching Hospital for Women and Children, in Anbar Governorate - Ramadi during the period from October 2021 to April 2022.This study included 100 samples split into two groups: The first group was 60 samples of women that is patients that suffering polycystic ovary syndrome (PCOS) that mean hormonal disorder in group and 40 samples of women that is control group don't have hormonal disorder. Their ages range from (15-45 years). These groups do not have a chronic or infectious disease. A questionnaire was used for the participants in the search that showed information on (age, weight, height, Smoker, Other disease, Drugs...).

collected from the participating women during (2-4 days of the menstrual cycle), 5 ml of whole blood was taken and left to clot for half an hour at room temperature, then it was separated by centrifugation for 15 minutes at 5000 xg. The serum was isolated in Eppendorf tubes and placed. In the refrigerator at -20 ° C pending biochemical assessment. Each participant in the study had their anthropometrics measured. CAPS levels were measured using an ELISA kit (BT LAB Inc, China) with a Microplate Reader System and (Total Cholesterol) levels were measured by Spectrophotometer (Mindray Semi-Auto Chemistry Analyzer).

Statistical Analysis

Utilizing GraphPad Prism version 7, data analysis was done. Simple metrics of mean. The data were presented using standard deviation (SD), standard error of the mean (SEM), and values. The Students' t-test was used to determine the significance of the difference between two independent means, and the analysis of variance was used to determine the significance of the difference between more than two independent means (ANOVA). Statistical significance was considered when the P value was 0.05 or less.

For the purpose of determining the significance of the correlation between two quantitative variables, the Pearson correlation was calculated. Values below 0.3 show no connection, between 0.3 and 0.5 suggest a weak correlation, between 0.5 and 0.7 indicate a moderate correlation, and above 0.7 indicate a strong correlation. The correlation coefficient value (r) can be positive (direct correlation) or negative (inverse correlation). The coefficient of determination (r2) was also computed in addition to correlation. When r is 0.58, for instance, the r2 is 0.34, which suggests that 34% of the variance in the values of y can be explained by understanding the values of x, or the opposite is true when r is 0.58.

Receiver operator curve (ROC) analysis was also applied. The area under the ROC curve offers perception into how well a tested parameter may differentiate between three groups (one of which is a control group). The ROC analysis is useful in this situation for comparing particular parameters to others. The more effective a region is for discriminating, the closer it is to one (the ideal test).

Results

The results of our research and statistical analysis showed that there are no statistically significant differences between PCOS women and control group women in terms of total cholesterol, TG, HDL, and LDL levels (180.7±35.68, 149.5±53.31, 40.16±13.68 and 104.6±38.27 vs 170.7±20.65, 129.7±42.48, 45.42±8.678 and 101.4±23.12) mg/dL respectively, At a probability level (P= 0.1115, 0.0513, 0.0333 and 0.6428) respectively. While through the study's findings and statistical findings, it was discovered that there is have increased significantly VLDL among PCOS

Women compared to the healthy control group (35.98±9.834 vs 24.14±7.567) mg/dL accordingly, at a degree of probability (P≤0.0001) like what is seen in Figure and in the (table1).

Table 1: Mean ±SD for Parameters in PCOS Patients and Healthy Controls

Parameter	Healthy Controls			PCOS Patients			p-value
	Mean	SD	SEM	Mean	SD	SEM	
CAPS ng/mL	7.384	1.359	0.2148	14.04	2.047	0.2643	<0.0001
T.Cho. mg/dL	170.7	20.65	3.264	180.7	35.68	4.607	0.1115
TG mg/dL	129.7	42.48	6.717	149.5	53.31	6.883	0.0513
HDL mg/dL	45.42	8.678	1.372	40.16	13.78	1.779	0.0333
LDL mg/dL	101.4	23.12	3.655	104.6	38.27	4.941	0.6428
VLDL mg/dL	24.14	7.567	1.196	35.98	9.834	1.270	<0.0001

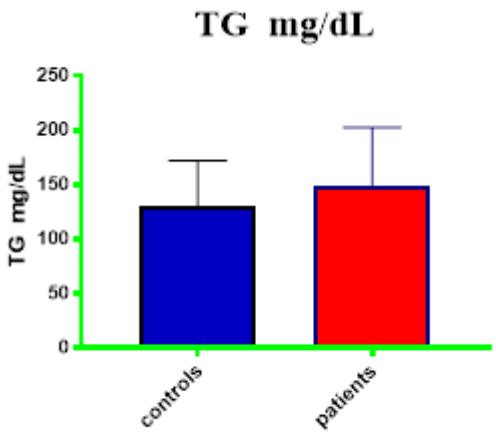

Fig. : Mean+ S.D for TG. in Control and Patients

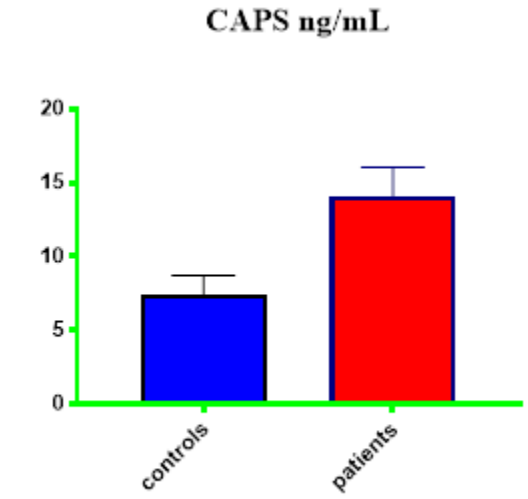

Fig. : Mean+ S.D for CAPS in Control and Patients

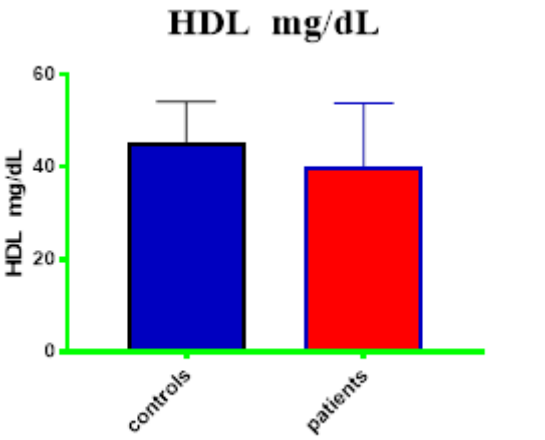

Fig. : Mean+ S.D for HDL in Control and Patients

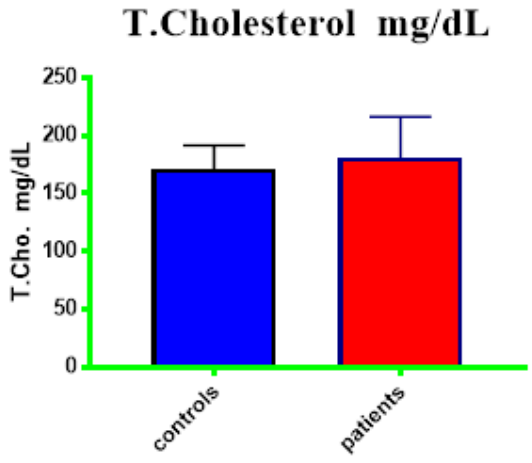

Fig. : Mean+ S.D for T.Cho. in Control and Patients

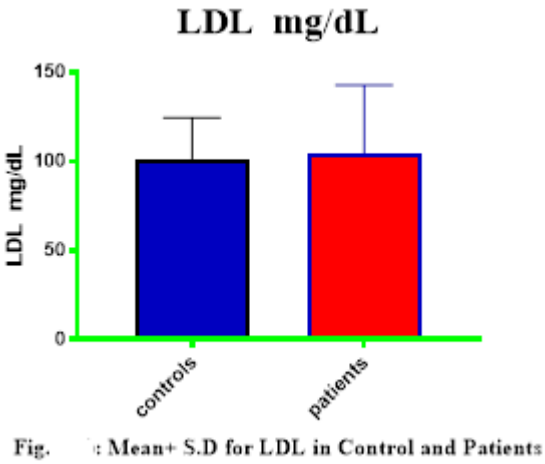

Fig. : Mean+ S.D for LDL in Control and Patients

These findings support our findings, as they demonstrate the prevalence of dyslipidemia in PCOS. regardless of BMI, in addition to documented changes in HDL cholesterol and triglycerides.

For the purpose of effectively reducing All PCOS-afflicted women are assessed for dyslipidemia in order to reduce their risk of cardiovascular disease. Our results were in line with

research by Kiranmayi Dontho the autosomal parameters of more than 80% of PCOS-afflicted women were abnormal, and more than 70% of these women also had lipid abnormalities[21]. These findings support our findings from [22] and [23].

Correlation of CAPS level with Lipid Profile in PCOS Patients

As shown in table 2 showed a Cholesterol, TG, LDL, VLDL give weak positive correlation relationship with values (0.218, 0.156, 0.184, 0.0273 and 0.430) mg/dL respectively, as demonstrated in (table2).

Table (2) The Correlation of CAPS with all Studied Parameters

Correlation of CAPS with all Studied Parameters		
Parameter	**r (CAPSng/mL)**	**p-value**
CAPS ng/mL	1.000	0.000
TG	0.184	0.066
Cholesterol	0.156	0.122
HDL	-0.128	0.205
VLDL	0.430	<0.0001
LDL	0.0273	0.787

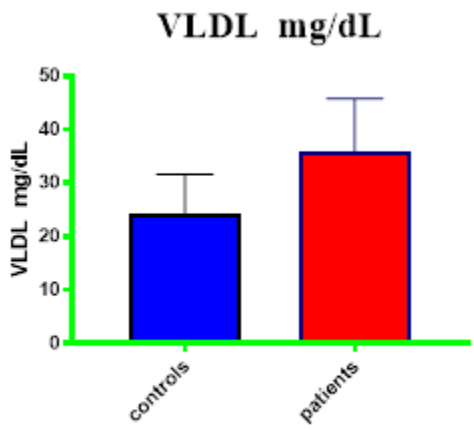

Fig. : Mean+ S.D for VLDL in Control and Patients

Receiver Operating Characteristic Curve Analysis

Table (3) shows the result of ROC curve analysis showing which biomarkers were most effective at distinguishing patients with PCOS from the control group were CAPS [AUC = 0.9967 p < 0.0001, 95% Confidence Interval (CI): 0.9905 to 1.003 and SE: 0.003122], figure.

While VLDL value, a crucial parameter, has a value of [AUC = 0.8321 p < 0.0001, 95% Confidence Interval (CI): 0.7507 to 0.9134 and SE: 0.04151].

While the HDL value is an average parameter with a value of HDL [AUC = 0.6196, P= 0.0435, 95% Confidence Interval (CI): 0.5102 to 0.729 and SE: 0.05582] figure, as well TG [AUC = 0.6056 p = 0.0745, 95% Confidence Interval (CI): 0.493 to 0.7183 and SE: 0.05747], figure.

T. cholesterol and LDL were among the factors with the lowest predictive validity for PCOS.

Cholesterol [AUC = 0.5694, P = 0.2414, 95% Confidence Interval (CI): 0.4581to 0.6806 and SE: 0.05676], LDL [AUC = 0.5006, p = 0.9916, 95% Confidence Interval (CI): 0.3873 to 0.614 and SE: 0.05784] figures.

(Table 3) Standard of ROC Curves for Tested Variables in PCOS Patients

Parameter	AUC	Std. Error	95% confidence interval	P-value
FGFR2 pg/mL	1	0	1 to 1	<0.0001
CAPS ng/mL	0.9967	0.003122	0.9905 to 1.003	<0.0001
F.S.G mg/dL	0.7919	0.04486	0.7039 to 0.8798	<0.0001
FSI µIU/mL	0.951	0.02188	0.9081 to 0.9939	<0.0001
HOMA-IR	0.9838	0.009676	0.9648 to 1.003	<0.0001
HOMA-β٪	0.591	0.06257	0.4684 to 0.7137	0.1242
T.Cho. mg/dL	0.5694	0.05676	0.4581 to 0.6806	0.2414
TG mg/dL	0.6056	0.05747	0.493 to 0.7183	0.0745
HDL mg/dL	0.6196	0.05582	0.5102 to 0.729	0.0435
LDL mg/dL	0.5006	0.05784	0.3873 to 0.614	0.9916
VLDL mg/dL	0.8321	0.04151	0.7507 to 0.9134	<0.0001
T.Proteins g/dL	0.5996	0.0563	0.4892 to 0.7099	0.0926
Albumin g/dL	0.9806	0.009936	0.9612 to 1	<0.0001
Globulin g/dL	0.7648	0.04771	0.6713 to 0.8583	<0.0001
ALB/GLB	0.9996	0.0007116	0.9982 to 1.001	<0.0001
Ca Ions mg/dL	0.9413	0.0223	0.8975 to 0.985	<0.0001
FSH mIU/mL	0.5869	0.05685	0.4754 to 0.6983	0.1438
LH mIU/mL	0.9665	0.01574	0.9356 to 0.9973	<0.0001
PRL ng/mL	0.9217	0.02872	0.8654 to 0.978	<0.0001
TSH mIU/mL	0.7333	0.04974	0.6359 to 0.8308	<0.0001
AMH ng/mL	0.8363	0.03899	0.7598 to 0.9127	<0.0001

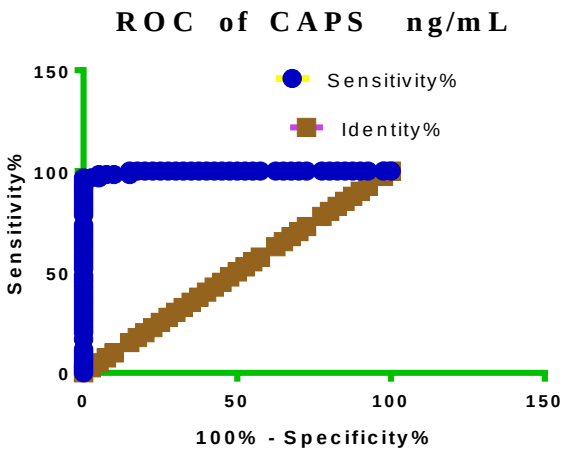

Fig: Area under Curve of CAPS in PCOS Patients

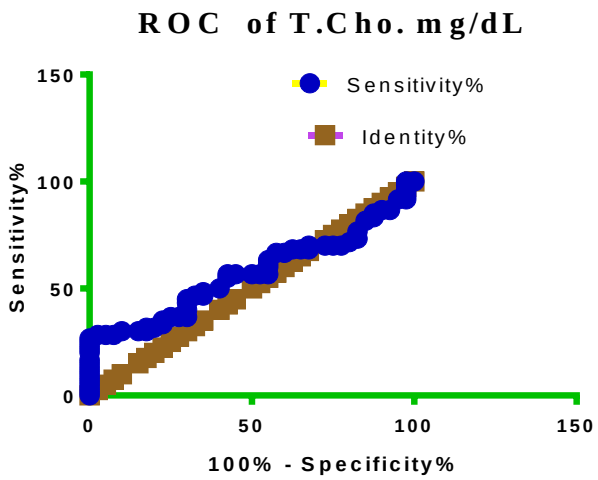

Fig: Area under Curve of T.Cho. in PCOS Patients

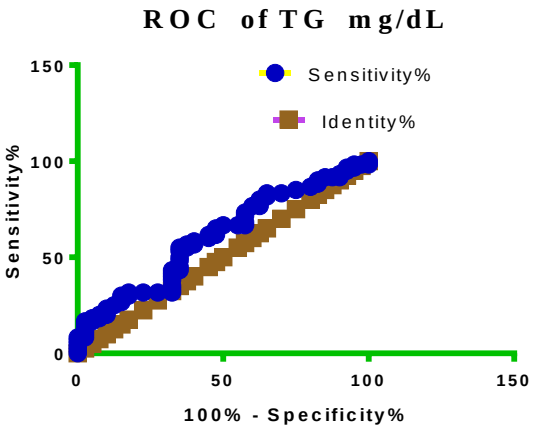

Fig: Area under Curve of TG in PCOS Patients

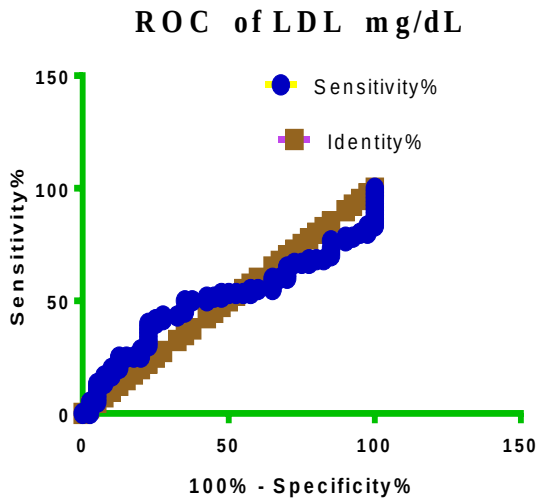

Figure: Area under Curve of LDL in PCOS Patients

DISCUSSION

PCOS is a common endocrine condition that affects up to 18% of women globally who are of reproductive age.[24]. Calcyphosine has been discovered in some mammals, including humans. [25], dog, rabbit, and bovine Band sponge[26], but not present in the mouse and the other five rodents. [27]. It has been shown that when thyrotropin stimulates the dog thyroid cells, calcyphosin a is a primary a cyclic AMP (cAMP)-dependent substrate for protein kinase A that has been phosphorylated. Thus, cAMP and calcium-phosphatidylinositol cascades may cross-signal with the help of cytophosine to control cellular proliferation and differentiation.[28].

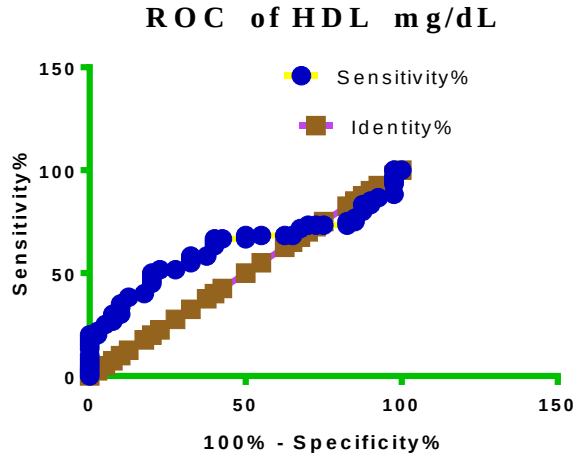

Fig: Area under Curve of HDL in PCOS Patients

Our results concurred with those of research by Hussein Kadhem; however, this study was the first to document an increase in CAPS in iron-overloaded b-TM patients. These

characteristics contribute to the patients' impaired Ca homeostasis [29]. These findings support our findings, as they demonstrate the prevalence of dyslipidemia in PCOS, regardless of BMI, in addition to confirmed changes in HDL cholesterol and triglycerides. All PCOS-afflicted women are assessed for dyslipidemia in order to successfully lower their risk of cardiovascular disease. [30].

CONCLUSION

In conclusion, the current study discovered that PCOS patients have high serum levels of Calcyphosin a, it could be the cause of all of the issues they experience as the disease progresses. These findings can be used as potential biomarkers and predictors of polycystic ovaries as well as in the development of new therapies for polycystic ovaries disease.

REFERENCES

Z. Shojaeian, R. Sadeghi, and R. L. Roudsari, "Calcium and Vitamin D supplementation effects on metabolic factors, menstrual cycles and follicular responses in women with polycystic ovary syndrome: A systematic review and meta-analysis," Casp. J. Intern. Med., vol. 10, no. 4, pp. 359–369, 2019, doi: 10.22088/cjim.10.4.359.

Miri. F et al., "Investigation of the prevalence of polycystic ovary syndrome in Iranian women," vol. 17, no. 115, pp. 11–21, 2014.

S. Bansal, M. Goyal, C. Sharma, and S. Shekhar, "Letrozole versus clomiphene citrate for ovulation induction in anovulatory women with polycystic ovarian syndrome: A randomized controlled trial," Int. J. Gynecol. Obstet., vol. 152, no. 3, pp. 345–350, 2021, doi: 10.1002/ijgo.13375.

S. A. Amer, J. Smith, A. Mahran, P. Fox, and A. Fakis, "Double-blind randomized controlled trial of letrozole versus clomiphene citrate in subfertile women with polycystic ovarian syndrome," Hum. Reprod., vol. 32, no. 8, pp. 1631–1638, 2017, doi: 10.1093/humrep/dex227.

S. Franik, S. M. Eltrop, J. A. M. Kremer, L. Kiesel, and C. Farquhar, "Aromatase inhibitors (letrozole) for subfertile women with polycystic ovary syndrome," Cochrane Database Syst. Rev., vol. 2018, no. 5, 2018, doi: 10.1002/14651858.CD010287.pub3.

E. Diamanti-Kandarakis et al., "Serum concentrations of carboxylated osteocalcin are increased and associated with several components of the polycystic ovarian syndrome," J. Bone Miner. Metab., vol. 29, no. 2, pp. 201–206, 2011, doi: 10.1007/s00774-010-0211-2.

T. Mahmoudi, H. Gourabi, M. Ashrafi, R. S. Yazdi, and Z. Ezabadi, "Calciotropic hormones, insulin resistance, and the polycystic ovary syndrome," Fertil. Steril., vol. 93, no. 4, pp. 1208–1214, 2010, doi: 10.1016/j.fertnstert.2008.11.031.

C. Karadağ, T. Yoldemir, and D. G. Yavuz, "Effects of vitamin D supplementation on insulin sensitivity and androgen levels in vitamin-D-deficient polycystic ovary syndrome patients," J. Obstet. Gynaecol. Res., vol. 44, no. 2, pp. 270–277, 2018, doi: 10.1111/jog.13516.

H. El Housni, A. Radulescu, R. Lecocq, J. E. Dumont, and D. Christophe, "Cloning and sequence analysis of human calcyphosine complementary DNA," Biochim. Biophys. Acta - Gene Struct. Expr., vol. 1352, no. 3, pp. 249–252, 1997, doi: 10.1016/S0167-4781(97)00073-0.

J. Grimwood et al., "The DNA sequence and biology of human chromosome 19," Nature, vol. 428, no. 6982, pp. 529–535, 2004, doi: 10.1038/nature02399.

W. Shao, Q. Wang, F. Wang, Y. Jiang, M. Xu, and J. Xu, "Abnormal expression of calcyphosine is associated with poor prognosis and cell biology function in colorectal cancer," Onco. Targets. Ther., vol. 9, pp. 477–487, 2016, doi: 10.2147/OTT.S92226.

S. M. Ghazi and F. S. Farhan, "Evaluation of the hormonal and biochemical changes in obese and non-obese polycystic ovary syndrome (PCOS) Iraqi women before and after vitamin D supplementation," Int. J. Drug Deliv. Technol., vol. 9, no. 3, pp. 132–136, 2019, doi: 10.25258/ijddt.v9i3.2.

M. A. Garza-Garza, A. E. Aguilar-Melgar, S. A. Dávila-Garza, P. Galache-Vega, and R. Santos-Haliscak, "Correlation of vitamin D values with those of anti-Müllerian hormone in infertile women," Ginecol. Obstet. Mex., vol. 88, no. 12, pp. 833–838, 2020.

E. Waldmann, "Lipidtherapie bei Diabetikern — ein Update," MMW - Fortschritte der Medizin, vol. 162, no. 8, pp. 38–42, 2020, doi: 10.1007/s15006-020-0427-6.

G. S. Conway, R. Agrawal, D. J. Betteridge, and H. S. Jacobs, "Risk factors for coronary artery disease in lean and obese women with the polycystic ovary syndrome," Clin. Endocrinol. (Oxf)., vol. 37, no. 2, pp. 119–125, 1992, doi: 10.1111/j.1365-2265.1992.tb02295.x.

S. W. Coppack et al., "Adipose tissue metabolism in obesity: Lipase action in vivo before and after a mixed meal," Metabolism, vol. 41, no. 3, pp. 264–272, 1992, doi: 10.1016/0026-0495(92)90269-G.

D. B. Savage, K. F. Petersen, and G. I. Shulman, "Disordered lipid metabolism and the pathogenesis of insulin resistance," Physiol. Rev., vol. 87, no. 2, pp. 507–520, 2007, doi: 10.1152/physrev.00024.2006.

J. Holte, T. Bergh, C. Berne, and H. Lithell, "Serum lipoprotein lipid profile in women with the polycystic ovary syndrome: Relation to anthropometric, endocrine and metabolic variables," Clin. Endocrinol. (Oxf)., vol. 41, no. 4, pp. 463–471, 1994, doi: 10.1111/j.1365-2265.1994.tb02577.x.

E. Ferrannini, E. J. Barrett, S. Bevilacqua, and R. A. DeFronzo, "Effect of fatty acids on glucose production and utilization in man," J. Clin. Invest., vol. 72, no. 5, pp. 1737–1747, 1983, doi: 10.1172/JCI111133.

G. M. Reaven and Y. D. I. Chen, "Role of abnormal free fatty acid metabolism in the development of non-insulin-dependent diabetes mellitus," Am. J. Med., vol. 85, no. 5 SUPPL. 1, pp. 106–112, 1988, doi: 10.1016/0002-9343(88)90402-0.

D. Kiranmayee, K. Kavya, Y. Himabindu, M. Sriharibabu, G. Madhuri, and S. Venu, "Correlations between anthropometry and lipid profile in women with PCOS," J. Hum. Reprod. Sci., vol. 10, no. 3, pp. 167–172, 2017, doi: 10.4103/jhrs.JHRS_108_16.

X. Qu and R. Donnelly, "Sex hormone-binding globulin (Shbg) as an early biomarker and therapeutic target in polycystic ovary syndrome," Int. J. Mol. Sci., vol. 21, no. 21, pp. 1–17, 2020, doi: 10.3390/ijms21218191.

S. Robinson, S. -P Chan, S. Spacey, V. Anyaoku, D. G. Johnston, and S. Franks, "Postprandial thermogenesis is reduced in polycystic ovary syndrome and is associated with increased insulin resistance," Clin. Endocrinol. (Oxf)., vol. 36, no. 6, pp. 537–543, 1992, doi: 10.1111/j.1365-2265.1992.tb02262.x.

F. R. Tehrani, M. Simbar, M. Tohidi, F. Hosseinpanah, and F. Azizi, "The prevalence of polycystic ovary syndrome in a community sample of Iranian population: Iranian PCOS prevalence study," Reprod. Biol. Endocrinol., vol. 9, no. 1, p. 39, 2011, doi: 10.1186/1477-7827-9-39.

F. Li, D. Zhu, Y. Yang, K. Wu, and S. Zhao, "Overexpression of calcyphosine is associated with poor prognosis in esophageal squamous cell carcinoma," Oncol. Lett., vol. 14, no. 5, pp. 6231–6237, 2017, doi: 10.3892/ol.2017.6973.

H. J. Yuasa, A. Nakatomi, T. Suzuki, and M. Yazawa, "Genomic structure of the sponge, Halichondria okadai calcyphosine gene," Gene, vol. 298, no. 1, pp. 21–27, 2002, doi: 10.1016/S0378-1119(02)00920-4.

S. Clément, J. E. Dumont, and S. Schurmans, "Loss of calcyphosine gene expression in mouse and other rodents," Biochem. Biophys. Res. Commun., vol. 232, no. 2, pp. 407–413, 1997, doi: 10.1006/bbrc.1997.6297.

A. Lefort et al., "Cloning and sequencing of a calcium-binding protein regulated by cyclic AMP in the thyroid," EMBO J., vol. 8, no. 1, pp. 111–116, 1989, doi: 10.1002/j.1460-2075.1989.tb03354.x.

H. K. Al-Hakeim and Z. H. Alhillawi, "Effect of serum fibroblast growth factor receptor 2 and CAPS proteins on calcium status in β-

thalassaemia major patients who are free from overt inflammation," Growth Factors, vol. 36, no. 3–4, pp. 178–185, 2018, doi: 10.1080/08977194.2018.1520707.

E. Diamanti-Kandarakis and A. Dunaif, "Insulin resistance and the polycystic ovary syndrome revisited: An update on mechanisms and implications," Endocr. Rev., vol. 33, no. 6, pp. 981–1030, 2012, doi: 10.1210/er.2011-1034.