STUDY THE ASSOCIATION BETWEEN VISFATIN LEVELS AND INSULIN RESISTANCE IN POLYCYSTIC OVARIAN SYNDROME OF IRAQI WOMEN

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ABSTRACT

Background: Polycystic ovarian syndrome is a common inflammatory endocrine disorder that affects women of reproductive age and considered risk factor for infertility. Visfatin is an adipocytokine known as pre-B cell colony-enhancing factor expressed in a variety of tissues including adiposities, lymphocytes, bone marrow, liver, and muscle. Visfatin binds to insulin receptor and exhibits insulin-mimetic actions; therefore, it stimulates glucose uptake in adipocytes and muscle cells and suppresses glucose release from hepatocytes. It is now believed that visfatin action can be endocrine, paracrine, and autocrine as well. These autocrine effects of visfatin may play an important role in regulating insulin sensitivity in the liver. **Aim**: This study aims to investigate serum visfatin level among Iraqi women with PCOS and control to detect any relation as a predicator for PCOS pathogenesis and its association with age, BMI and insulin resistance. **Materials and Methods:** This study is a case-control study involves (120 women) with age ranged between (20-40) years and their BMI between (20-35) kg/m². Sixty samples with PCOS patients and another 60 are non-PCOS women as a control group collected during Dec., 2021 to April, 2022 from child-bearing age at the reproductive fertility consultant of gynecological and obstetric teaching hospital, Babylon Health Directorate, Babylon- Iraq. The ethical agreement was obtained from a committee at college of medicine, university of Kerbala / Kerbala – Iraq. The Rotterdam criteria were applied to select the PCOS patients. Controller group have regular menstruation, with normal ovaries as they were detected by the gynecologist. Five ml of blood was drawn and but in gel tube to measure serum (Endothelin-1, visfatin, LH, FSH, free testosterone levels) in cycle day two (CD2). Lipid profile (total cholesterol TC, high density lipoprotein HDL-C, low density lipoprotein cholesterol, LDL-C, very low density lipoprotein VLDL and triglyceride TG) were determined. Insulin, HOMA-IR and blood glucose were also determined. **Results:** The mean levels of biomarkers among infertility groups in women with PCOS shown that all parameters were significantly differed among the groups (p values <0.05). Level of LH ; free testosterone ; TG ; insulin and LH/FSH ratio were shown a positively significant increasing with secondary infertility cases, while FSH ; HDL-C and fasting blood glucose were decreased significantly in the same group. Visfatin was shown diagnostic points for predicting PCOS cases compared to control group. Positive relation between visfatin and LH, free testosterone, TG and negative relation with HDL-C, fasting blood glucose, HOMA-IR. The efficiency of the predicting value was assessed using receiver operating characteristic (ROC) curve. **Conclusion:** According to the observed data we can conclude that visfatin may be pre indicator diagnostic markers for PCOS and associated with abdominal obesity and insulin resistance as well as obesity and has effect on development of insulin resistance, also increase in high age.

KEYWORDS: Visfatin, PCOS, Insulin Resistance, Lipid Profile, Endothelin-1, ROC.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common gynecological disease in women at reproductive age and is also called hyperandrogenic anovulation, which is characterized by endocrine and metabolic disturbances with features of multiple hormonal imbalances that produce short and long term consequences on women health as well as chronic inflammation **(Al-Lami, *et. al.,* 2020 and Seyyed Abootorabi *et al.,* 2018)**. It is now categorized as the most common pathology of the endocrine system in females at reproductive age with typical clinical features includes hirsutism, irregular menses, chronic anovulation, and infertility. The persistent hyper-androgenism is associated with impaired hypothalamic–pituitary feedback, LH hypersecretion, premature granulosa cell luteinization, aberrant oocyte maturation, and premature arrest of activated primary follicles **(Palomba, 2017)** .The pathogenesis of PCOS is due to a combination of genetic, environmental, and endocrine factors, which are the main causes of female

ovulatory infertility **(Szczuko *et al*., 2017).** The most common risk factors for the progress of PCOS include family history of PCOS, fast food diet habits, lack of physical exercise, body mass index and waist circumference **(Begum *et al*., 2017).** Women with PCOS are more likely to develop many metabolic and reproductive health complications that include dyslipidemia, abnormal glucose level, insulin resistance, miscarriage, gestational diabetes, mood disorders, hypertensive disorders, preeclampsia, pre-diabetes, type 2 diabetes, hormonal imbalance, obesity, obstructive sleep apnea, cardiovascular disease, stroke, chronic kidney disease, renal failure, breast cancer, endometrium cancer and others **(Al-Tu'ma, *et. al.,* 2015 and Escobar-Morreale, 2018).**

The diagnosis of PCOS is dependent on two of the following three findings: intermittent or absent menstrual cycles, high circulating levels of testosterone (T) or hirsutism **(Gorsic *et al*., 2019)** and ultra sonographic findings of the ovary (12 or more small follicles that are between two and nine mm in diameter in both ovaries) **(Kadri *et. al.,* 2021).** Other conditions that produce similar symptoms include thyroid disease, hyper-prolactinemia and hyperlipidemia **(Trent and Gordon, 2020).** Stein-Leventhal first described polycystic ovaries syndrome as infertility, menorrhagia, hirsutism, enlarged ovaries, amenorrhoea, chronic an ovulation and obesity **(Escobar-Morreale, 2018).** The National Institutes of Health suggested hyper-androgenism and oligo-anovulation as the two criteria that are needed to diagnose PCOS which is the first formal attempt to classify PCOS **(Anagnostis *et. al.,* 2018 and Jungari *et al.,* 2020).**

Visfatin, termed of pre-B-cell colony-enhancing factor or nicotinamide phosphor ribosyl transferas, is an adipocytokine that is predominantly produced in visceral adipose tissue **(Shi *et. al*., 2016).** Visfatin is highly preserved across animal evolution. It has a molecular weight of 52 KD and its gene encodes 491 amino acids. It is identical to pre-B cell colony-enhancing factor (PBEF), described in 1994 as a cytokine produced by lymphocytes, acting on lymphocyte maturation and inflammatory regulation. Visfatin functions as an immune modulatory cytokine involved in the inflammatory responses, and are a factor associated with obesity, inflammation and insulin resistance **(Sun *et. al.,* 2017).** Visfatin serves important roles in the induction of insulin resistance **(Hosseinzadeh-Attar *et. al.,* 2016).** Furthermore, visfatin functions as the rate-limiting enzyme of NAD^+ coenzyme biosynthesis from nicotinamide **(Stromsdorfer *et. al.,* 2016).** The presented work aimed to investigate the level of serum visfatin among Iraqi women with PCOS and to see if it has any role as a predicator for PCOS pathogenesis, and also to see its association with age, BMI and insulin resistance.

MATERIALS AND METHOD

This case-control study was conducted on (120 women) with age ranged between (20-40) years and body mass index, BMI (20-35) kg/m². They obtained from reproductive fertility consultant of gynecological and obstetric teaching hospital, Babylon health directorate / Iraq during the duration from Dec., 2021 to April, 2022 and they divided into 60 PCOS patients and 60 non-PCOS women as apparently control group. The ethical agreement was approved by specified committee at College of Medicine, University of Kerbala Iraq. The Rotterdam criteria was presumed to patients with any 2 of 3 items can be recognized in diagnosis: oligomenorrhea or amenorrhea, increase androgen levels, ovarian volume > 10mL on U/S, and follicles ≥12 with diameter 2-9 mm **(Park *et al.,* 2022).** Controller group have regular menstruation, with normal ovaries as they were detected by the gynecologist. The PCOS patients were also sub-classify into three subgroups (A, B and C) according to infertility (normal which no infertility, primary infertility was 40, secondary infertility was 20). Five ml of blood was drawn from each women and put it in gel tube to measure serum endothelin-1, ET-1, insulin and visfatin level by ELISA method, LH, FSH and free testosterone in cycle day two (CD2) by mini vidas. Lipid profile (total cholesterol, TC, high density lipoprotein-cholesterol, HDL-C, low density lipoprotein-cholesterol, LDL-C, very low density lipoprotein-cholesterol, VLDL-C and triglyceride, TG were determined by colorimetric assay using spectrophotometer, while blood glucose was determined by automated biochemical analyzer, HOMA-IR was finally evaluated according to the formula: **(HOMA-IR = (Fasting Insulin Conc. x Fasting Glucose Conc.) / 405).**

RESULTS AND DISCUSSION

Polycystic ovary syndrome (PCOS) was hypothesized to result from functional ovarian hyperandrogenism (FOH) due to dys-regulation of androgen secretion. Various studies have been performed in sera of Iraqi women with PCOS including phenotypes, biomarkers and gene polymorphisms and receptor studies which indicate that the main pathophysiological components of PCOS are gonadotropic dysfunction and insulin resistance, which are often associated with high body mass index (BMI) **(Al-Faris, *et. al.,* 2017 ; Krynytska, *et al*., 2018 ; Al-Lami, *et. al.,* 2020 and Al- Quraishy, et. al., 2022).** The PCOS was associated with irregular gonadotropin secretion, increased steroid hormone secretion and frequency, and a high LH/FSH ratio, which leads to an increase in androgen synthesis and prevents normal follicle development (**Rosenfield, *et al*., 2016**). Table-1 indicates the mean ± SD levels of biomarkers among apparently control groups in women with PCOS. The mean ± SD levels of LH, free testosterone, TG, insulin, and LH/FSH ratio were shown a positively significant increasing with secondary infertility cases. While FSH, HDL-C and fasting blood sugar, FBG were decreased significantly in the same group. On the other hand, total

cholesterol, VLDL-C, LDL-C, ET-1 and visfatin levels were significantly increased in infertility cases. The most common cause of female infertility is ovulation disorders, and the most common non-ovulatory cause is polycystic ovary syndrome (PCOS). The PCOS is a complex hormonal and metabolic disorder characterized by oligomenorrhea or amenorrhea, hyper-androgenism, and infertility (**Zhou, *et al*., 2017**).

Table 1: Mean ± SD levels of biomarkers in women with PCOS as compared to control group.

Biomarkers	Study Groups		P Value
	Control N = 60	**Patients N = 60**	
LH mIU/m	4.87±1.88	9.03±7.25	**<0.001[S]**
FSH mIU/m	6.71±2.06	5.42±2.96	**0.002[S]**
Free Testost. (pg/ml)	0.96±0.35	1.81±0.78	**<0.001[S]**
TC mg/dl	148.58±37.46	173.01±53.07	**0.017[S]**
TGmg/dl	108.87±22.58	145.47±66.24	**<0.001[S]**
HDL-C, mg/dl	44.17±7.78	37.22±9.40	**<0.001[S]**
VLDL-C, mg/dl	21.75±4.47	29.19±13.51	**0.002[S]**
LDL-C, mg/dl	82.17±41.89	106.53±47.43	**0.009[S]**
FBG, mg/dl	95.69±8.28	91.77±26.60	**0.004[S]**
ET-1, ng/ml	54.37±6.31	92.31±62.49	**<0.001[S]**
Visfatin, ng/ml	10.48±3.15	25.11±43.33	**<0.001[S]**
HOMA-IR	3.46±1.54	3.35±3.79	**0.006[S]**
LH/FSH	0.74±0.22	1.73±0.87	**<0.001[S]**
Student ANOVA was [S] significant, [NS] non-significant **LH, luteinizing hormone, FSH, Follicular stimulating hormone, ; TC: Total cholesterol ; TG: Triglyceride ; HDL-: High density lipoprotein –cholesterol ; LDL-C: Low density lipoprotein – cholesterol ; FBG: Fasting blood glucose ; ET-1: Endothelin-1 and HOMA-IR: Homeostatic model assessment for insulin resistance.**			

An increase in gonadotropin-releasing hormone up regulates transcription of the LH β-subunit through the FSH β-subunit, which leads to an increase in the LH/FSH ratio in PCOS patients (**Park, *et al*., 2016**). Experimental exhibited ovarian changes, such as an increase in the number of cystic follicles and increased granulosa cell degeneration, with thin granulosa cell walls and a thicker surrounding layer of theca cells suggesting that infertility is associated with dyslipidemia in PCOS. Abnormal lipid metabolism can promote the pathophysiology of hyperandrogenism, insulin resistance, oxidative stress, and infertility in PCOS (**Franks, *et al*., 2008**).

In case of primary and secondary infertility, lipid disorders are hypothesized to play a role in female reproduction. The synthesis of steroid hormones in reproductive tissues occurs in thecal and granulosa cells, and utilizes cholesterol as the substrate for steroidogenesis. The lipoproteins HDL-C and LDL-C play important roles in the transport of cholesterol and other fatty substances to ovarian tissue (**Huang, *et al*., 2019**). Altered endometrial lipid levels may impair endometrial receptivity and early embryo implantation (**Li, *et al*., 2019**).

Many studies have reported associations between serum lipids and reproductive outcomes in sub-fertile women. In one prospective cohort study that included couples attempting pregnancy, increased serum free cholesterol concentrations in both men and women led to reduced fecundity (**Schisterman, *et al*., 2014**). In couples with prior pregnancy loss, higher serum TC and TG were associated with less spontaneous pregnancy (**Pugh, *et al*., 2017**). Another study in women undergoing *in vitro* fertilization (IVF) suggests that HDL-C within the follicular fluid may play protective roles in the health of the human oocyte by reducing oocyte fragmentation. It has also been reported that follicular fluid HDL-C had an antioxidative function and was associated with normal oocyte fertilization (**Nagy, *et al*., 2019**).

There is a clear positive relationship between level of visfatin level and infertility in women. Normal visfatin level is needed for normal reproductive function in women; impaired level is associated with increased incidence of PCOs and endometriosis which reduce fertility in women. the positive correlation between serum visfitin, BMI, and insulin contributes significantly to the reproductive, neuroendocrine abnormalities associated. High visfatin level increase incidence of women's infertility, visfatin level increase in PCOS when associated with insulin resistance or diabetes mellitus (**Hussein Mohamed Hussein, *et al*., 2018**).

Visfatin functions as an immune modulatory cytokine involved in the inflammatory responses, and are a factor associated with obesity, inflammation and insulin resistance (**Sun *et. al.,* 2017**). Visfatin induces the production of inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α in human leukocytes. Its plasma level increases during chronic

inflammatory conditions such as obesity. Visfatin serves important roles in the induction of insulin resistance **(Hosseinzadeh-Attar *et. al.,* 2016).**

Visfatin is a cytokine with a physiological effect in reducing the level of plasma glucose **(Asia, 2017).** The observed data indicated that serum visfatin levels was positively weakly related to the endothelin-1; free testosterone and luteinizing hormone (LH) levels (p <0.001), while only FBG and VLDL-C were negatively significant correlated to the level of visfatin as presented in (Table-1). Visfatin binds to the insulin receptor at a site differ from that of insulin and causes hypoglycemia by stimulating glucose utilization in adipocytes and myosytis and reducing glucose release from liver**,** and its levels observed has a role in pathogenesis of PCOS and it correlated positively and it deregulated by obesity. The observed data was agreed with another study which revealed that serum visfatin levels were significantly positively correlated with PCOS with other clinical and biochemical phenotype as well as cardio-metabolic factors observed as TC, LDL-C, FBG and insulin **(Nearmeen *et. al.,* 2018).**

Table-2: Correlation coefficients between mean levels of Visfatin and biomarkers among women with PCOS.

Biomarkers	**Visfatin, ng/ml**	
	Correlation coefficient (r)	*P Value*
LH, (mIU/ml)	0.3	**<0.001 [S]**
FSH, (mIU/ml)	-0.1	0.577[NS]
Free Testost. (pg/ml)	0.4	**<0.001[S]**
TC, (mg/dl)	0.1	0.260[NS]
TG, (mg/dl)	0.4	**<0.001[S]**
HDL-C, (mg/dl)	-0.4	**<0.001[S]**
VLDL-C, (mg/dl)	0.3	**0.001[S]**
LDL-C, (mg/dl)	0.1	0.163[NS]
FBS, (mg/dl)	-0.2	**0.016[S]**
ET-1, (ng/ml)	0.6	**<0.001[S]**
HOMA-IR	- 0.1	0.228[NS]
LH/FSH	0.1	0.621[NS]
p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant ; FSH: Follicle-Stimulating Hormone; LH: Luteinizing Hormone **Free Testost.: Free Testosterone; TC: Total cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; VLDL: Very Low Density Lipoprotein; LDL: Low Density Lipoprotein; FBS: Fasting Blood Sugar; ET-1:Endothelin-1; HOMA-IR: Insulin Resistance.**		

Another study conducted by Kowalsk demonstrated a positive correlation with markers of hyperandrogenism, suggesting that obesity may deregulate visfatin expression and that other factors may be involved in this process **(Kowalska *et. al.,* 2007).** Plasma concentration of visfatin observed increases along with rising insulin resistance and body mass index as shown in table-1 which shown the mean ± SD differences of the ET-1 and visfatin levels in PCOS patient as compared to control based on the age groups. Visfatin is involved in local ovarian energy metabolism as a key enzyme in nicotinamide adenine dinucleotide (NAD) biosynthesis and may thus affect follicular development **(Reverchon, 2013).** There are contradictory reports on the levels and expression of visfatin in PCOS subjects. More studies tend to favor a higher level of ovarian visfatin in PCOS **(Nejabati, 2020).**

Robust connection between visfatin with hormones and insulin resistance (HOMA-IR) parameters in PCOS pathophysiology were determined**.** Serum visfatin levels were found to be strongly correlated with free testosterone levels suggesting a possible role of visfatin in the pathogenesis of PCOS**.** There were significant

positive correlation between BMI, weight, total cholesterol, TG and serum visfatin levels, table-2 which agreed with others **(Noura, 2020).** Hyperlipidemia may have relation with increase visfatin hormone levels and high lipids level in blood **(Mahmood and Alkanaani, 2019).**

Visfatin levels were correlated positively with body mass index as indicated in table-3. This result was agreed with others which indicated that there was a statistically significant correlation between serum visfatin level and BMI (p<0.04) and indicated that serum visfatin was higher in obese and overweight patients as compared with controls **(Noura, 2020).**

Table-3: Correlation between visfatin levels with various BMI (kg/m^2) groups studied in PCOS patients.

Visfatin	Normal weight		Over-weight		Obese	
	PCOS Patients	Control	PCOS Patients	Control	PCOS Patients	Control
Mean ± SD of BMI, kg/m^2	14.21 ± 5.2	10.10 ± 2.4	20.7844 ± 4.0	9.813 ± 2.8	34.60 ± 5.82	11.41 ± 3.78
Correlation Coefficient (r)	-0.2		-0.1		0.3	
P value	0.06[S]		0.05[S]		0.04[S]	

Associated factors of alteration biomarkers levels with PCOS cases as compared to control group was indicated in table-4 in which the odds ratio (OR) with 95% confidence intervals (CI) for potential biomarkers were evaluated for their association with PCOS patients on multivariate conditional logistic regression analysis (Table-4). Obesity, insulin resistance, and dyslipidemia are PCOS-related morbidities and were found to be correlated with the LH/FSH ratio (**Aug, *et al.,* 2020**). Also, IR plays an important role in the development of PCOS, in which the insulin response increases resulting in hyperinsulinemia as a unique feature in PCOS (**Qi Liu et al., 2019**).
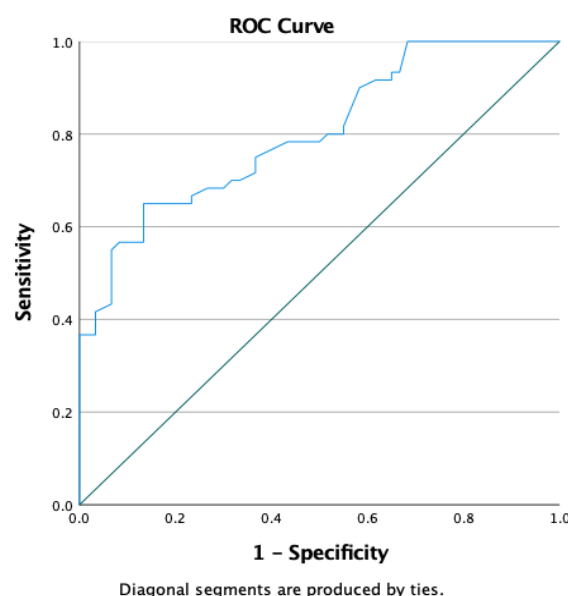
Table-4: Associated factors of Dependent Variable in PCOS Patients Compared to control group.

Study Groups (PCOS/ Healthy control)		
Biomarkers	OR (95% CI)	p value
LH, (mIU/ml)	3.130 (1.537 - 6.372)	0.002 [S]
FSH, (mIU/ml)	0.230 (0.093 - 0.566)	0.001 [S]
Free Testost. (pg/ml)	46.700 (1.156 – 1. 886)	0.042 [S]
ET-1, ng/ml	1.079 (0.907 - 1.284)	0.390 [NS]
Visfatin, (ng/ml)	1.36(1.208 – 1.532)	<0.001[S]
HOMA-IR	0.995 (0.878-1.129)	0.941[NS]
LH/FSH ratio	1915.829 (102.301 – 378.346)	<0.001[S]
p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant ; FSH: Follicle-Stimulating Hormone; LH: Luteinizing Hormone F.T: Free Testosterone; FBS: Fasting Blood Sugar; ET-1:Endothelin-1; HOMA-IR: Insulin Resistance.		

It was found that serum levels of LH, free testosterone and ET-1 ratio were to be dependent risk factors for PCOS cases since they were shown an associated with higher odds of PCOS outcome. For LH (OR: 3.130; 95% CI: 1.537 - 6.372), for free testosterone (OR: 46.7; 95% CI: 1.156 – 1.886), For ET- 1 (OR: 1.079; 95% CI: (0.907 - 1.284) and for LH/FSH ratio (OR: 1915.82; 95% CI: (102.301 – 378.346), while FSH and HOMA-IR were shown an associated with lower odds of outcome in PCOS patients. The results showed relatively good sensitivity and specificity. For VISFATIN the AUP was 0.851 while for Endotheline-1was 0.85.

Results of the receiver operating curve (ROC) and AUC analysis for the visfatin and Endotheline-1 levels as possible diagnostic parameters for prediction PCOS. Patients are indicated that endotheline-1 was shown more appropriate optimal diagnostic points for predicting PCOS cases compared to control group (sensitivity = 85%, specificity = 80%) at a level = 55.975 ng/dl. Youden's J statistics of the parameters in Figures (1) confirm these results.

Numerous studies have reported that endothelial dysfunction is commonly associated with PCOS (**Diamanti-Kandarakis, *et al*., 2006**).

Diagonal segments are produced by ties.

Fig. 1: ROC curves for visfatin in PCOS patients to analyze the optimal diagnostic points for predicting cases as compared to control group.

CONCLUSION

According to the observed data, we can conclude.
1. Visfatin may be predicator for PCOS, increase in women with PCOS.

2. Visfatin was associated with abdominal obesity and insulin resistance as well as obesity, but not with metabolic syndrome and pre-diabetes mellitus.
3. Visfatin may has effect on development of insulin resistance

REFERENCES

1. Al-Lami, HB; Al-Tu'ma, FJ and Al-Safi, W.G. (2020). Association between anti-Müllerian hormone and other biomarkers with ovarian function in polycystic ovarian syndrome of Iraqi women. J of Contemp Med Sci|, 6(4): 168–175.
2. Al-Faris, N.N.; Al-Tu'ma, F.J. and Al-Safi, Wasan Ghazi (2017). Assessments of C-Reactive Protein and its Correlation with Total Antioxidant Capacity in Woman with Poly Cystic Ovary Syndrome. Iraqi. Natl. J. of Chemistry, 7(1): 44-56.
3. Asia Ali Hamza aMajid Khadum Hussein aAli Abdulzahra AhmedLambertini, L., Saul, S.R., Copperman, A.B., Hammerstad, S.S., Yi, Z., Zhang,W., Tomer, Y. and Kase, N.(2017). Intrauterine reprogramming of thepolycystic ovary syndrome: evidence from a pilot study of cord bloodglobal methylation analysis. Frontiers in endocrinology, 8.352.
4. Al-Quraishy, A.M.; Ali, H. A., Al-Tu'ma, M. M. K. and Al-Tu'ma, FJ. (2022). A Novel Study for the Detection of Programmed Cell Death-1 Gene Variants used in Consequences of PCOS in Iraqi Women. *WJPMR, 8(9): 01-06.*
5. Aug, Saadia Z. (2020). Follicle Stimulating Hormone (LH: FSH) Ratio in Polycystic Ovary Syndrome (PCOS) - Obese vs. Non- Obese Women. Med Arch, 74(4): 289-293 and Australia, 209: S3-S8.
6. Anagnostis, P., Tarlatzis, B.C. and Kauffman, R.P. (2018). Polycystic ovarian syndrome (PCOS): Long-term metabolic consequences. Metabolism, 86: 33-43.
7. Al-Tu'ma, FJ ; Farhan, NH and Al-Safi, WG (2015). Association between fat mass and obesity Gene polymorphism (rs 9939609) with PCOS women in Iraqi population. IJPPR. Human, 5(1): 62 -72.
8. Begum, G., Shariff, A., Ayman, G., Mohammad, B., Housam, R. and Khaled, N., (2017). Assessment of risk factors for development of polycystic ovarian syndrome. Diabetes, 1: 2.
9. Escobar-Morreale, H.F. (2018). Polycystic ovary syndrome: definition, aetiology,diagnosis and treatment. Nature Reviews Endocrinology, 14(5): 270. Evaluation and treatment of polycystic ovary syndrome-part 1. Endocrine Practice, 21(11): 1291-1300.
10. Gorsic LK, Dapas M, Legro RS, Hayes MG, Urbanek M. (2019). Functional genetic variation in the anti-Mullerian hormone pathway in women with polycystic ovary syndrome. J Clin Endocrinol Metab, 104: 2855–2874.
11. Hosseinzadeh-Attar MJ, Golpaie A, Foroughi M, *et al* (Aug. 2016). The relationship between visfatin and serum concentrations of C-reactive protein, interleukin 6 in patients with metabolic syndrome. Journal of Endocrinological Investigation, 39(8): 917-922.
12. Jungari, M.L., Nair, P., Gode, S. and Jaiswal, A. (2020). PCOS: Clinical Picture of PCOS Patients in a Peri Urban Tertiary Care Hospital of Central. India.Journal of Critical Reviews, 7(8): 1076-1080.
13. Kadri, S., Izhar, S., Azeem, N., Rashid, S. and Gudaro, Z.N. (2021). Transabdominal versus transvaginal ultrasound in assessment of polycysticovary. The Professional Medical Journal, 28(2): 202-207.
14. Kowalska, M. Straczkowski, *et al*. (2007). Serum visfatin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome Hum. Reprod, 22(7): 1824-1829.
15. Hussein Mohamed Hussein, *et al*. (2018). Role of leptin and visfatin in infertility in obese and non obese women. Sohag Medical Journal, 22(1).
16. Huang Q, Liu Y, Yang Z, Xie Y, Mo Z. (2019). The Effects of Cholesterol Metabolism on Follicular Development and Ovarian Function. Curr Mol Med, 19(10): 719–30.
17. Kowalska, M. Straczkowski, *et al.,* (2007). Serum visfatin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome Hum. Reprod, 22(7): 1824-1829.
18. Krynytska IY, Marushchak MI (July 2018). The indices of nitrogen (II) oxide system in experimental hepatopulmonary syndrome. Ukr Biochem J, 90(5): 91-7.
19. Li J, Gao Y, Guan L, Zhang H, Chen P, Gong X, *et al (2019).*. Lipid Profiling of Peri-implantation Endometrium in Patients With Premature Progesterone Rise in the Late Follicular Phase. J Clin Endocrinol Metab, 104(11): 5555–65.
20. Lambertini, L., Saul, S.R., Copperman, A.B., Hammerstad, S.S., Yi, Z., Zhang,W., Tomer, Y. and Kase, N. (2017). Intrauterine reprogramming of the polycystic ovary syndrome: evidence from a pilot study of cord blood global methylation analysis. Frontiers in endocrinology, 8: 352.
21. Nearmeen M. Rashad, M.N. Ayman Abd-Elrahman, et al., (2018). Serum visfatin as predictive marker of cardiometabolic risk in women with polycystic ovary syndrome, Middle East Fertility Society Journal, 23(4): 335-341.
22. Nejabati HR, Samadi N, Shahnazi V, Mihanfar A, Fattahi A, Latifi Z, Bahrami-Asl Z, Roshangar L, Nouri M. (2020). Nicotinamide and its metabolite N1-Methylnicotinamide alleviate endocrine and metabolic abnormalities in adipose and ovarian tissues in rat model of Polycystic Ovary Syndrome. Chem Biol Interact, 1; 324: 109093.
23. Nagy RA, van Montfoort APA, Groen H, Homminga I, Andrei D, Mistry RH, *et al.* (2019). Anti-oxidative function of follicular fluid HDL and

outcomes of modified natural cycle-IVF. Sci Rep, 9(1): 12817.

24. Palomba S, Daolio J, La Sala GB. (2017). Oocyte competence in women with polycystic ovary syndrome. Trends Endocrinol Metab, 28(3): 186–198.

25. Park KS, Gang W, Kim P-W, Yang C, Jun P, Jung S-Y, Kwon O, Lee JM, Lee HJ, Lee SJ. (2022). Efficacy and safety of acupuncture on oligomenorrhea due to polycystic ovary syndrome: An international multicenter, pilot randomized controlled trial. Medicine, 101(7): e28674-e.

26. Pugh SJ, Schisterman EF, Browne RW, Lynch AM, Mumford SL, Perkins NJ, *et al.* (2017). Preconception maternal lipoprotein levels in relation to fecundability. Hum Reprod, 32(5): 1055–63.

27. Reverchon M, Cornuau M, Cloix L, Ramé C, Guerif F, Royère D, *et al* (2013). Visfatin is Expressed in Human Granulosa Cells: Regulation by Metformin Through AMPK/SIRT1 Pathways and its Role in Steroidogenesis. Mol Hum Reprod, 19: 313–26.

28. Rosenfield RL and Ehrmann,DA (Oct. 2016). The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited Endocr. Rev, 37(5): 467-520.

29. Schisterman EF, Mumford SL, Browne RW, Barr DB, Chen Z, Louis GMB. (2014). Lipid concentrations and couple fecundity: the LIFE study. J Clin Endocrinol Metab., 99(8) : 2786–94.

30. Seyyed Abootorabi, M., Ayremlou, P., Behroozi-Lak, T. and Nourisaeidlou, S., (2018). The effect of vitamin D supplementation on insulin resistance, visceral fat and adiponectin in vitamin D deficient women with polycystic ovary syndrome: a randomized placebo-controlled trial. *Gynecological Endocrinology*, *34*(6): 489-494.

31. Shi KL, Qian JY, Qi L, Mao DB, Chen Y, Zhu Y, Guo XG. (2016). Atorvastatin antagonizes the visfatin-induced expression of inflammatory mediators via the upregulation of NF-κB activation in HCAECs. *Oncol Lett,* 12: 1438–1444.

32. Stromsdorfer KL, Yamaguchi S, Yoon MJ, Moseley AC, Franczyk MP, Kelly SC, Qi N, Imai S, Yoshino J.(2016). NAMPT-Mediated NAD$^+$ Biosynthesis in Adipocytes Regulates Adipose Tissue Function and Multi-organ Insulin Sensitivity in Mice. Cell Rep, 16(7): 1851-60.

33. Sun L, Chen S, Gao H, Ren L and Song G (2017): Visfatin induces the apoptosis of endothelial progenitor cells via the induction of pro-inflammatory mediators through the NF-κB pathway. Int J Mol Med, 40(637): 646, .syndrome. Fertility and sterility, 106(1): 615.

34. Szczuko, M., Malarczyk, I. and Zapalowska-Chwyc, M. (2017). Improvement inanthropometric parameters after rational dietary intervention in women with polycystic ovary syndrome as the best method to support treatment Roczniki Państwowego Zakładu Higieny, 68(4): 409-417.

35. Zhou J, Yang L, Yu J, Wang Y, Liu Z. (2017). Efficacy of acupuncture on menstrual frequency in women with polycystic ovary syndrome: Protocol for a randomized, controlled trial. Medicine. 96(47).

36. Trent, M. and Gordon, C.M. (2020). Diagnosis and management of polycystic vary syndrome in adolescents. Pediatrics, 145(Supplement 2): S210-S218.