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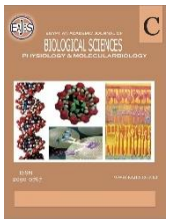

Measurement Visfatin Level and Its Genetic Polymorphisms (rs61330082) in Women with Polycystic Ovary Syndrome With and Without Insulin Resistance

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ABSTRACT

Bachground: Polycystic ovary syndrome (PCOS) is a common condition that often leads to metabolic complications such as insulin resistance (IR) and obesity. Visfatin is a peptide that plays a role in endocrine, autocrine, and paracrine functions. **Methods**: Samples were collected from 90 women aged 16 to 40 years, divided into two groups. The PCOS group comprised 60 samples, with 30 women who had insulin resistance and 30 women who did not. The healthy group included 30 samples. The visfatin levels for all participants and performed genotyping for specific regions of the visfatin (rs1330082) gene using Real-time PCR were measured. The statistical significance is defined as $p \leq 0.05$ for all comparisons. **Results:** The results showed that women with PCOS and insulin resistance had significantly higher levels of serum visfatin compared to other studies (6.640±0.3886, p <0.0001). However, there was no significant difference regarding the frequency of visfatin rs1330082 allele between the study groups. **Conclusion:** The research indicates that measuring serum visfatin levels could be a helpful tool for predicting insulin resistance in women with PCOS. However, we did not find any significant correlation between visfatin allele frequencies (rs1330082) and susceptibility to PCOS when compared to healthy controls.

INTRODUCTION

Polycystic ovary syndrome (PCOS), also known as hyperandrogenic anovulation or Stein–Leventhal syndrome, is a multifactorial and polygenic endocrine disorder, affecting women of reproductive age worldwide (Deans, 2019). In 1935 Irving F. Stein and Michael L. Leventhal described a symptom complex due to anovulation (Stein and Leventhal,1935). It is a chronic endocrine disorder characterized by menstrual dysfunction, infertility, hirsutism, acne, and obesity, with varying presentation (Mohammad and Seghinsara *et al*.,2017).

Based on the NIH 1990 guide and Rotterdam 2003 criteria, the global prevalence rate of PCOS is in the range of 4-21% (Zhang *et al*.,2019). In 2019, the MENA region had 6,647,566 prevalent cases of PCOS among women, with an age-standardised point prevalence of 2079.7 per 100,000. This represents a 37.9% increase since 1990 (Motlagh *et al.*, 2022).

PCOS has been recognized as a chronic metabolic condition beyond a merely reproductive disorder (El Hayek *et al.*,2016). PCOS has metabolic characteristics that include prominent defects in insulin action and β-cell function, defects that confer a substantially increased risk for glucose intolerance and type 2 diabetes (Sam and Dunaif.,2003).

Obesity is a common finding in women with PCOS and between 40–80% (Sam, 2007). In addition, women with PCOS have a higher risk of developing cardiovascular diseases, asthma, high cholesterol, and high blood pressure (Juber *et al*.,2023) and increase in BMI, Triglyceride, VLDL and Sugar(Abd-alkareem and Omear.,2020). In addition, the risk factors for chronic diseases and PCOS are shared, including systemic inflammation (Xu *et al*.,2022), and hormonal factors (Dalibalta *et al*.,2022).

Apart from environmental factors, many candidate genes are involved in the etiology of PCOS, Alteration in the metabolic pathway due to a defect in the gene leads to the progression of PCOS and ovary dysfunction (Ajmal *et al*.,2019). Genetic studies can aid in identifying the underlying cause of PCOS development. Genome-wide association studies (GWAS) and related genetic studies have changed the scenario for diagnosing and treating this reproductive and metabolic condition known as PCOS (Nautiyal *et al*.,2022). Genetic factors also contribute to the etiology of PCOS, in addition to environmental variables. Candidate genes, SNPs polymorphisms, or any nucleotide alteration that affects a gene's transcriptional activity can be the cause of PCOS (DIAMANTI-KANDARAKIS.,1997). Visfatin is an adipokine identified in 2004 (Fukuhara *et al.*,2005), consists of 11 exons and 10 introns spanning 34.7-kb and is located on chromosome 7q22.2, It has a molecular weight of 52 KDa and its gene encodes 491 aminoacids (Saddi-Rosa *et al*.,2010). Visfatin is a newly discovered adipokine present in various fat tissues like subcutaneous, visceral, perivascular, and epicardial (Cheng *et al.*,2008; Wang *et al*.,2009). Serum visfatin levels have a strong correlation with body mass index (BMI) or percentage of body fat (Berndt *et al.*,2005). PCOS is associated with the dysfunctional secretion of adipokines, promoting inflammation (Manneras-Holm *et al.*,2011). Studies have shown that visfatin gene expression or serum levels are higher in women with PCOS compared to matched controls (Plati *et al.*,2010; Seow *et al.*,2011). while other Studies have found no significant difference in serum visfatin levels between women with PCOS and controls (Güdücü *et al*.,2012, Farshchian *et al*.,2014). The aim of the study was to assess serum visfatin levels and visfatin rs13330082 polymorphism in PCOS patients with and without insulin resistance in comparison to healthy women.

MATERIALS AND METHODS

A population study was conducted in Salah al-Din Governorate, Iraq between February and September 2022. The study included 90 women participants aged between 16 to 40 years. The participants were divided into two groups: the PCOS patients group consisting of 60 samples and the healthy women group consisting of 30 samples. The patient group was further divided into two subgroups: the first subgroup included 30 women with insulin resistance, and the second subgroup consisted of 30 women without insulin resistance. Every participant provided written, fully informed permission for the study. The study's design was approved by the ethics committee of the College of Science at Tikrit University in Iraq.

Measurements of Biochemical Parameters:

After an overnight fast, 5 ml of morning venous blood samples were collected from women who were in the follicular phase of their menstrual cycle. Hormone and biochemistry analyses were performed on the samples. The collected plasma was stored at -70°C for further testing. The Central Laboratory of Tikrit University in Iraq utilized a BioTek EL 800 analyzer to measure the serum visfatin level by ELISA. Fasting glucose, insulin levels, and 2-hour blood glucose and insulin levels following glucose overload (OGTT) were measured. Insulin resistance (IR) indices (HOMA, QUICKI, Matsuda) were calculated using the standard formula based on the test results.

DNA Genotyping: Genomic DNA was extracted from whole blood using the Genaid

kit approach (Taiwan). Primers and probes were supplied by Macrogen (Korea) as listed in Table 1. The specific region of visfatin gene rs1330082 was amplified using the 20 µl reaction mixtures indicated in Table 2 for real-time PCR. The real-time PCR was performed using the ABI Prism 7700 Sequence Detection System (SDS, PE Biosystems) with the following cycling conditions: The activation enzyme was heated to 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 second.

Table 1. Primer and Probe sequence of visfatin gene rs1330082.

Primer	**Sequence**	**Reference**
F	GACTACAGGCACTATATTGACCAGAC	This study
R	AGAAAGTTTCACCCTTGCTCAT	
Probes	**Sequence**	
FAM (wild)	CCAAAGCTCTGGGATTACCT-BHQ	
HEXA (mutant)	CCAAAGCTCTGGGATTACCG-BHQ	

Table 2. Reaction Component and volume for RT PCR**.**

Component	**Volume**	**Final concentration**
Template	1 pg-1µg	As required
Forward primer (10µM)	0.4µl	0.2µM
Reverse primer(10µM)	0.4µl	0.2µM
Probe(10µM)	0.4µl	0.2µM
2 × *PerfectStar* ™ ll Probe q PCR SuperMix	10µl	1×
Passive Reference Dye (50×) (optional)	0.4µl	1×
Nuclease-free Water	Variable	-
Total volume	20 µl	-

Statistical Analysis:

Biochemical and hormonal studies were evaluated using the t-test. The chi-square test was utilized to determine if the genotype frequencies conformed to the assumptions of Hardy-Weinberg equilibrium (HWE). Statistical tests were conducted using the SPSS statistical software package for social sciences. The significance level was set at $p < 0.05$.

RESULTS

A total of 60 PCOS and 30 non-PCOS patients were included in the study sample. Table 3 displays the BMI characteristics of women under 3 categories: non-PCOS (healthy group), PCOS (insulin resistance group), and PCOS (noninsulin resistance group). As a result of matching, PCOS and non-PCOS patients differ significantly in BMI at the probability level of $P<0.001$, the BMI (mean± S. E.) k/m2 for those with PCOS reached (29.88±0.7792) kg\ m2 compared to the healthy women group (24.99±0.5076) kg\m2. In the present study, it was observed that there was a significant difference in the body mass index (BMI) between the healthy group and the group of patients, which was further divided into two subgroups based on the presence or absence of insulin resistance associated with polycystic ovary syndrome.

Table 3. Represents the value of the body mass index (BMI) in PCOS (B = PCOS with insulin resistance, and C= PCOS without insulin resistance) compared to the control group, and the value.

Study groups	**BMI (mean± SE) k/m2**
PCOS Group (60)	29.88±0.7792
Healthy group (30)	24.99±0.5076
P value <0.0001	
Study groups	**BMI (mean± SE) k/m2**
PCOS B (30)	33.45±1.005
PCOS C (30)	26.32±0.7650
Healthy group (30)	24.99±0.5076
P value <0.0001	
Study groups	**BMI (mean± SE) k/m2**
PCOS B (30)	33.45±1.005
PCOS C (30)	26.32±0.7650
P value <0.0001	

The (mean ± SE) mg/dl of visfatin Hormone was significantly higher (5.820±0.2523, p-value <0.0172) in the women with PCOS compared to the healthy women group. Also, the ANOVA test for the (mean ± SE) mg/dl shows a higher statically significant difference among the three study groups: the control group (4.850±0.2532), PCOS with insulin resistance (6.640±0.3886), and PCOS without insulin resistance (5.000±0.2481) (p-value <0.0001). Table 4 presents the outcomes of the correlational analysis.

Table 4. The concentration of the visfatin in POCS women ((B = PCOS with insulin resistance, and C= PCOS without insulin resistance) compared to the healthy control group, and the value (p).

Study groups	**visfatin Hormone (Mean ±SE) ng\dl)**	**P value**
PCOS Group (60)	5.820±0.2523	<0.0172
Healthy group (30)	4.850±0.2532	
Study groups	**visfatin Hormone (Mean ±SE) ng\dl)**	**P value**
PCOS B (30)	6.640±0.3886	<0.0001
PCOS C (30)	5.000±0.2481	
Healthy group (30)	4.850±0.2532	

The study investigated the pathogenic role of the visfatin gene, specifically SNP rs1330082. It also explored the relationship between different genetic variants of rs.2414096 and PCOS susceptibility. Table 5, presents the genotyping and allelic results between PCOS and healthy groups. The results show that the PCOS group had 66 (A) alleles and 54 (G) alleles, whereas the healthy women group had 32 (A) alleles and 28 (G) alleles. However, there was no significant difference between the two groups, with a p-value of 0.2357. In the study, we identified two genotypes (AA and GA), with the AA genotype serving as the reference group. Out of the PCOS group, 6 individuals had AA genotypes while only 2 individuals in the healthy women group had AA genotypes. However, our genotyping results did not indicate any significant difference between the two groups.

Table 5. Allelic and Genotypic distribution with statistical analyses of visfatin gene (rs1330082) in the PCOS group and healthy group**.**

Genotypes	Contro	Patient	OR (95% CI)	p-Valu
AA	2	6	1 (ref.)	
AG	28	54	0.6429 (0.1260 to 2.82	0.6004
GG	0	0	0.3846 (0.0059 to 25.20	0.6543
Alleles	**Contro**	**Patient**	**OR (95% CI)**	**p-Valu**
A	23	66	1 (ref.)	
G	28	54	0.6721 (0.3558 to 1.31	0.2357

Table 6, presents the genotype and allele details of two subgroups of Polycystic Ovary Syndrome (PCOS) - B for PCOS with insulin resistance and C for PCOS without insulin resistance. The data shows that the number of (A) alleles was 32 in the B group and 34 in the C group, respectively. This difference was not statistically significant ($p < 0.3871$). Similarly, the (G) allele count was 28 in the B group and 26 in the C group with no significant difference ($p < 0.2243$). Two genotypes were identified - AA and AG - with 2 and 28 genotypes respectively in the B group. In the C group, the AA and AG genotypes were 4 and 26, respectively. The genotyping results did not show any significant differences between the two groups.

Table 6. Allelic and Genotypic distribution with statistical analyses of visfatin gene (rs1330082) in the PCOS group (B = PCOS with insulin resistance, and C= PCOS without insulin resistance) and healthy group.

Genotypes	Control	B	C	OR (95% CI)	p-Value
AA	2	2	-	1 (ref.)	
		-	4		
AG	28	28	-	1(0.1487 to 6.725)	>0.9999
		--	26	0.4643 (0.08393 to 2.160	0.3894
GG	0	0	-	1(0.0136 to 73.2695)	1
		-	0	0.5556 (0.0082 to 37.5655	0.7845
Alleles	**Contro**	**B**	**C**	**OR (95% CI)**	**p-Value**
A	23	32	-	1 (ref.)	
		-	34		
G	28	28	-	0.7188 (0.3415 to 1.484)	0.3871
		--	26	0.6282 (0.2961 to 1.299)	0.2243

DISCUSSION

PCOS is closely linked to metabolic disorders such as obesity and insulin resistance (IR) (Gilbert *et al*.,2018). Compared to women without PCOS, those with PCOS have a significantly higher prevalence of obesity and overweight, as opposed to being at an optimum weight or being lean. Also, there was a significant difference between insulin-resistant vs. non-insulin-resistant PCOS patients. Studies have found that women with Polycystic Ovary Syndrome (PCOS) are often overweight or obese (Gowri *et al*.,2018; Saboor Aftab *et al*.,2013). A common characteristic of PCOS is a higher body mass index (BMI), which is primarily linked to insulin resistance (IR) and elevated insulin levels (Ganie *et al.*,2019). It is difficult to determine if obesity causes PCOS or vice versa (Makhija *et al*.,2023). PCOS is closely associated with metabolic dysfunctions that arise due to complex mechanisms (Sanchez,2020). Abnormal plasma glucose values may be due to IR, which is commonly found in PCOS, as reported by several studies (Deswal *et al*.,2020; Nathet *et al*.,2019).This study's results show an increase in the level of

visfatin among PCOS with insulin resistance. One interesting finding is no big difference in visfatin level between the health group and PCOS noninsulin resistance group. Visfatin, an adipocytokine, is an insulin resistance (IR) marker in diabetes since PCOS and diabetes share insulin resistance as an etiological factor (Ali and Nori,2022). This finding is consistent with that of Moustafa, and Al-Hakeim who suggest that There was an association between visfatin, and polycystic ovary syndrome-related insulin resistance (Moustafa and Al-Hakeim,2022). This also accords with our earlier observations, which showed a strong positive correlation of visfatin with insulin resistance, followed by PCOS cases irrespective of BMI (Ali and Nori,2022).

The latest research using different omics approaches revealed SNPs in adipokines and their receptors as PCOS-susceptibility markers, and epigenetic changes conferred from mother to child modulating adipokine expression, increasingly becoming a focus of interest, have already substantiated the importance of adipokines in PCOS (Pei *et al*.,2021; Vazquez-Martinez *et al*.,2019). in our study, we did not find any link between the frequency of different visfatin alleles (rs1330082) and the susceptibility of women to PCOS, as compared to healthy control women. Additionally, we observed that certain genotypes were absent from the genetic data. This can be attributed to several factors, including the small sample size or the fact that the samples were collected from a population that shares genetic similarities. It is common for genetic data to have missing genotypes. For studies that employ low-density SNPs, the usual approach for dealing with missing genotypes is to eliminate observations with missing genotypes from the analysis (Wu *et al*.,2006). The -1535C>T polymorphism (rs1330082) is situated on the promoter region of visfatin. It is just one of the SNP of the complex genetic loci of PCOS and insulin resistance. The absence of any association with PCOS might be due to the fact that rs1330082 is only a single SNP among several genetic loci associated with PCOS and insulin resistance. However, several reports have suggested that the -1535C>T polymorphism might provide a useful marker for predicting the development of obesity in children (Ooi *et al.*,2016), increased serum triglyceride, and HDL-cholesterol levels (Tokunaga *et al*.,2008) in coronary artery disease (Wang *et al*.,2011). Several questions remain unanswered at present.

Conclusions

The purpose of this study was to investigate whether visfatin has an impact on the vulnerability of PCOS. Our findings suggest that measuring serum visfatin levels could serve as a useful predictive marker for women with PCOS who are insulin-resistant. However, we did not find any significant association between visfatin allele frequencies (rs1330082) and susceptibility to PCOS when compared to healthy controls. The main limitation of this study is its small sample size, which may have affected the accuracy of the results. Further research on visfatin is needed to establish a more conclusive understanding of its role in PCOS.

REFERENCES

Abd-alkareem, M. A. A., & Omear, H. A. (2020). INSR and PLIN Polymorphism in Women with Poly Cystic Ovary Syndrome (PCOS) and Its Correlation with Insulin Resistance: Polycystic ovary syndrome. *International Journal of Medical Sciences*, *3*(3), 93-110.

Ajmal, N., Khan, S. Z., & Shaikh, R. (2019). Polycystic ovary syndrome (PCOS) and genetic predisposition: A review article. *European Journal of Obstetrics & Gynecology and Reproductive Biology: X*, *3*, 100060.

Ali, A. I., & Nori, W. (2022). Correlation of Serum Visfatin Level in Non-obese Women with Polycystic Ovary Syndrome and Matched Control. *Reproductive Sciences*, *29*(11), 3285-3293.

Berndt, J., Kloting, N., Kralisch, S., Kovacs, P., Fasshauer, M., Schon, M. R., ...

& Bluher, M. (2005). Plasma visfatin concentrations and fat depot–specific mRNA expression in humans. *Diabetes*, *54*(10), 2911-2916.

Cheng, K. H., Chu, C. S., Lee, K. T., Lin, T. H., Hsieh, C. C., Chiu, C. C., ... & Lai, W. T. (2008). Adipocytokines and proinflammatory mediators from abdominal and epicardial adipose tissue in patients with coronary artery disease. *International Journal of Obesity*, *32*(2), 268-274.

Dalibalta, S., Abukhaled, Y., & Samara, F. (2022). Factors influencing the prevalence of polycystic ovary syndrome (PCOS) in the United Arab Emirates. *Reviews on Environmental Health*, *37*(3), 311-319.

Deans, R. (2019). Polycystic ovary syndrome in adolescence. *Medical Sciences*, *7*(10), 101.

Deswal, R., Narwal, V., Dang, A., & Pundir, C. S. (2020). The prevalence of polycystic ovary syndrome: a brief systematic review. *Journal of Human Reproductive Sciences*, *13*(4), 261.

Diamanti-Kandarakis, E. V. A. N. T. H. I. A. (1997). The Polycystic Ovary Syndrome Pathogenesis, Metabolic Implications, and Therapeutic Approach. *Annals of the New York Academy of Sciences*, *816*(1), 177-193.

El Hayek, S., Bitar, L., Hamdar, L. H., Mirza, F. G., & Daoud, G. (2016). Poly cystic ovarian syndrome: an updated overview. *Frontiers in Physiology*, *7*, 124.

Farshchian, F., Tehrani, F. R., Amirrasouli, H., Pour, H. R., Hedayati, M., Kazerouni, F., & Soltani, A. (2014). Visfatin and resistin serum levels in normal-weight and obese women with polycystic ovary syndrome. *International Journal of Endocrinology and Metabolism*, *12*(3).

Fukuhara, A., Matsuda, M., Nishizawa, M., Segawa, K., Tanaka, M., Kishimoto, K., ... & Shimomura, I. (2005). Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science*, *307*(5708), 426-430.

Ganie, M. A., Vasudevan, V., Wani, I. A., Baba, M. S., Arif, T., & Rashid, A. (2019). Epidemiology, pathogenesis, genetics & management of polycystic ovary syndrome in India. *The Indian Journal of Medical Research*, *150*(4), 333.

Gilbert, E. W., Tay, C. T., Hiam, D. S., Teede, H. J., & Moran, L. J. (2018). Comorbidities and complications of polycystic ovary syndrome: an overview of systematic reviews. *Clinical Endocrinology*, *89*(6), 683-699.

Gowri, B. V., Chandravathi, P. L., Sindhu, P. S., & Naidu, K. S. (2015). Correlation of skin changes with hormonal changes in polycystic ovarian syndrome: A cross-sectional study clinical study. *Indian Journal of Dermatology*, *60*(4), 419.

Güdücü, N., İşçi, H., Görmüş, U., Yiğiter, A. B., & Dünder, I. (2012). Serum visfatin levels in women with polycystic ovary syndrome. *Gynecological Endocrinology*, *28*(8), 619-623.

Juber, N. F., Abdulle, A., AlJunaibi, A., AlNaeemi, A., Ahmad, A., Leinberger-Jabari, A., ... & Ali, R. (2023). Association between self-reported polycystic ovary syndrome with chronic diseases among emiratis: a cross-sectional analysis from the UAE healthy future study. *International Journal of Women's Health*, 289-298.

Makhija, N., Tayade, S., Toshniwal, S., & Tilva, H. (2023). Clinico-Metabolic Profile in Lean Versus Obese

Polycystic Ovarian Syndrome Women. *Cureus*, *15*(4).

Mannerås-Holm, L., Leonhardt, H., Kullberg, J., Jennische, E., Odén, A., Holm, G., ... & Lönn, M. (2011). Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. *The Journal of Clinical Endocrinology & Metabolism*, *96*(2), E304-E311.

Mohammad, M. B., & Seghinsara, A. M. (2017). Polycystic ovary syndrome (PCOS), diagnostic criteria, and AMH. *Asian Pacific Journal of Cancer Prevention: APJCP*, *18*(1), 17.

Motlagh Asghari, K., Nejadghaderi, S. A., Alizadeh, M., Sanaie, S., Sullman, M. J., Kolahi, A. A., ... & Safiri, S. (2022). Burden of polycystic ovary syndrome in the Middle East and North Africa region, 1990–2019. *Scientific Reports*, *12*(1), 7039.

Moustafa, S. R., & Al-Hakeim, H. K. (2020). The impact of serum visfatin, and resistin levels on insulin resistance in patients with polycystic ovary syndrome. *Zanco Journal of Medical Sciences,* 24(1), 80-88.

Nath, C. K., Barman, B., Das, A., Rajkhowa, P., Baruah, P., Baruah, M., & Baruah, A. (2019). Prolactin and thyroid stimulating hormone affecting the pattern of LH/FSH secretion in patients with polycystic ovary syndrome: A hospital-based study from North East India. *Journal of Family Medicine and Primary Care*, *8*(1), 256.

Nautiyal, H., Imam, S. S., Alshehri, S., Ghoneim, M. M., Afzal, M., Alzarea, S. I., ... & Kazmi, I. (2022). Polycystic ovarian syndrome: a complex disease with a genetics approach. *Biomedicines*, *10*(3), 540.

Ooi, D. S. Q., Ong, S. G., Heng, C. K., Loke, K. Y., & Lee, Y. S. (2016). In-vitro function of upstream visfatin polymorphisms that are associated with adverse cardiometabolic parameters in obese children. *BMC Genomics*, *17*, 1-7.

Pei, C. Z., Jin, L., & Baek, K. H. (2021). Pathogenetic analysis of polycystic ovary syndrome from the perspective of omics. *Biomedicine & Pharmacotherapy*, *142*, 112031.

Plati, E., Kouskouni, E., Malamitsi-Puchner, A., Boutsikou, M., Kaparos, G., & Baka, S. (2010). Visfatin and leptin levels in women with polycystic ovaries undergoing ovarian stimulation. *Fertility and Sterility*, *94*(4), 1451-1456.

Saboor Aftab, S. A., Kumar, S., & Barber, T. M. (2013). The role of obesity and type 2 diabetes mellitus in the development of male obesity-associated secondary hypogonadism. *Clinical Endocrinology*, *78*(3), 330-337.

Saddi-Rosa, P., Oliveira, C. S., Giuffrida, F. M., & Reis, A. F. (2010). Visfatin, glucose metabolism and vascular disease: a review of evidence. *Diabetology & Metabolic Syndrome*, *2*, 1-6.

Sam, S. (2007). Obesity and polycystic ovary syndrome. *Obesity Management*, *3*(2), 69-73.

Sam, S., & Dunaif, A. (2003). Polycystic ovary syndrome: syndrome XX?. *Trends in Endocrinology & Metabolism*, 14(8), 365-370.

Sanchez, N. (2020). Women with polycystic ovary syndrome: a marginalized population in the United States. *Health & Social Work*, *45*(1), 40-46.

Seow, K. M., Hwang, J. L., Wang, P. H., Ho, L. T., & Juan, C. C. (2011). Expression of visfatin mRNA in peripheral blood mononuclear cells is not correlated with visfatin mRNA in omental adipose tissue in women with polycystic ovary syndrome. *Human Reproduction*, *26*(10), 2869-2873.

Stein, I. F., & Leventhal, M. L. (1935). Amenorrhea associated with bilateral polycystic ovaries. *American Journal of Obstetrics and Gynecology*, *29*(2), 181-191.

Tokunaga, A., Miura, A., Okauchi, Y., Segawa, K., Fukuhara, A., Okita, K., ... & Yamagata, K. (2008). The-1535 promoter variant of the visfatin gene is associated with serum triglyceride and HDL-cholesterol levels in Japanese subjects. *Endocrine Journal*, *55*(1), 205-212.

Vázquez-Martínez, E. R., Gómez-Viais, Y. I., García-Gómez, E., Reyes-Mayoral, C., Reyes-Muñoz, E., Camacho-Arroyo, I., & Cerbón, M. (2019). DNA methylation in the pathogenesis of polycystic ovary syndrome. *Reproduction*, *158*(1), R27-R40.

Wang, L. S., Yan, J. J., Tang, N. P., Zhu, J., Wang, Y. S., Wang, Q. M., ... & Huang, J. (2011). A polymorphism in the visfatin gene promoter is related to decreased plasma levels of inflammatory markers in patients with coronary artery disease. *Molecular Biology Reports*, 38, 819-825.

Wang, P., Xu, T. Y., Guan, Y. F., Su, D. F., Fan, G. R., & Miao, C. Y. (2009). Perivascular adipose tissue-derived visfatin is a vascular smooth muscle cell growth factor: role of nicotinamide mononucleotide. *Cardiovascular Research*, *81*(2), 370-380.

Wu, S. P., Leng, L., Feng, Z., Liu, N., Zhao, H., McDonald, C., ... & Bucala, R. (2006). Macrophage migration inhibitory factor promoter polymorphisms and the clinical expression of scleroderma. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *54*(11), 3661-3669.

Xu, Y., Zhou, Z. Y., Pan, J. X., & Huang, H. F. (2022). Associations between asthma and polycystic ovary syndrome: current perspectives. *Frontiers in Endocrinology*, *13*, 936948.

Zhang, J., Sun, Z., Jiang, S., Bai, X., Ma, C., Peng, Q., ... & Zhang, H. (2019). Probiotic Bifidobacterium lactis V9 regulates the secretion of sex hormones in polycystic ovary syndrome patients through the gut-brain axis. *Msystems*, *4*(2), 10-1128.