Effect of Metformin and Vitamin D Supplementation on Metabolic and Hormonal Profiles in Polycystic Ovary Syndrome Women: A Follow-Up Study

Zainab Nur-Eldeen Aziz[1*], Basil O. Saleh [1]

Abstract

Background: Insulin resistance in association with obesity is implicated in the pathogenesis of polycystic ovarian syndrome and its primary characteristic of hyperandrogenism. Metformin at a dose of (1700-2000) mg/day, with and without lifestyle, is associated with a beneficial enhancement in BMI and menstrual cycles. We aimed to determine the role of free testosterone: dihydrotestosterone ratio, and sex hormone binding globulin in the evaluation of the efficacy of metformin and vitamin D in the treatment of polycystic ovary syndrome. **Methods:** This cross-sectional study included 50 infertile women who were diagnosed with PCOS with an age range of (18-40 years). They were classified into Group I: Twenty-five women treated with metformin 850 mg/twice daily and followed for 8 weeks; Group II: Twenty-five women treated with vitamin D3 50.000 IU/wk for the same period. Serum investigations included measurements of prolactin, 25 hydroxyvitamin D, LH, FSH, free testosterone, DHT and SHBG by ELISA technique. **Results:** This study revealed that the mean values of BMI, HOMA-IR, prolactin and free testosterone: dihydrotestosterone ratio were significantly improved (by lowering) after treatment with metformin and vitamin D. Moreover, Polycystic Ovary Syndrome women treated with metformin revealed significant negative association between HOMA-IR with SHBG (r= -0.593, p=0.009), also significant positive association between HOMA-IR with BMI (r= 0.631, p=0.003). **Conclusion:** Serum measurements of free testosterone: dihydrotestosterone ratio, along with SHBG, are good biochemical markers that reflect the enhancement of insulin resistance and BMI in Polycystic Ovary Syndrome women post metformin treatment or vitamin D supplementation.

Keywords. Polycystic Ovary Syndrome (PCOS), prolactin, androgen hormone, HOMA-IR, metformin, vitamin D

Significance | Positive Impacts of Metformin and Vitamin D Supplementation on Polycystic ovary syndrome (PCOS) Women

*Correspondence. Zainab Nur-Eldeen Aziz, Department of Biochemistry, College of Medicine, University of Baghdad, Iraq.
E-mail: zainabnureldeen79@gmail.com

Editor Mahfoudh A.M. Abdulghani And accepted by the Editorial Board Jan 22, 2024 (received for review Dec 18, 2023)

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder that primarily affects women within the reproductive age range of 15-44 years (Williams et al., 2020; Zhao et al., 2021). It is widely recognized as the most common condition of its kind, with a global prevalence ranging from 5-20% (Williams et al., 2020; Zhao et al., 2021). Concerning the Rotterdam criteria, females meet a minimum of two out of three criteria for the identification of PCOS. These criteria include the presence of infrequent or absent ovulation, observable physical and chemical indications of excessive androgen levels, and the detection of polycystic ovaries through ultrasound imaging (Deswal et al., 2020; Williams et al., 2020).

Author Affiliation.
[1] Department of Biochemistry, College of Medicine, University of Baghdad, Iraq.
E-mail: basil_omsal@comed.uobaghdad.edu.iq, basil_omsal@yahoo.com

Please cite this article.
Zainab Nur-Eldeen Aziz, Basil O. Saleh, (2024). Effect of Metformin and Vitamin D Supplementation on Metabolic and Hormonal Profiles in Polycystic Ovary Syndrome Women: A Follow-Up Study, Journal of Angiotherapy, 8(1), 1-06, 9438

PCOS exhibits a diverse array of characteristics, encompassing metabolic, endocrine, reproductive, and psycho-social symptoms (Deswal et al., 2020; Zhao et al., 2021). Insulin resistance and hyperandrogenism, two prominent characteristics of the syndrome, exhibit a strong interconnection that impacts not only the reproductive capabilities but also the metabolic composition of individuals with PCOS, irrespective of their body mass index (BMI) (Armanini et al., 2022; Pasquali, 2018).

Hyperandrogenism (HA) is intricately linked with a suboptimal metabolic profile. Women diagnosed with PCOS who exhibit elevated levels of androgens are predisposed to an increased susceptibility to insulin resistance when juxtaposed with PCOS individuals presenting with androgen levels within the normal range. Elevated peripheral enzymatic conversion of testosterone into dihydrotestosterone (DHT), the biologically active androgen variant, has been linked to an unfavorable metabolic configuration in PCOS (Shah et al., 2019).

Metformin, a potent insulin sensitizer, is the preeminent pharmaceutical agent for managing PCOS owing to its remarkable therapeutic effectiveness and impeccable safety profile. Metformin, a pharmacological compound commonly prescribed at a daily dosage ranging from 1700 to 2000 mg, has been extensively studied with lifestyle modifications. This combined approach has demonstrated a notable positive impact on various physiological parameters, including BMI and menstrual cycles (Singh et al., 2023; Witchel et al., 2019).

Recently, adequate serum levels of vitamin D "25-hydroxyvitamin D" (≥30 ng/ml) should be required in women with PCOS. Otherwise, vitamin D deficiency may aggravate insulin resistance and hyperandrogenism (Gokosmanoglu et al., 2020; Morgante et al., 2022). So, oral vitamin D supplementation could help treat ovulation dysfunction in PCOS by promoting follicular development and improving menstruation (Mohan et al., 2023; Zhuang et al., 2019).

The present investigation was meticulously crafted to delve into the intricate interplay between the proportion of free testosterone to dihydrotestosterone and the esteemed sex hormone binding globulin. Its primary objective was to assess the effectiveness of metformin and vitamin D as therapeutic agents in managing multifaceted polycystic ovary syndrome.

Materials and methods

Study design

The cross-sectional investigation was performed at the Department of Biochemistry, College of Medicine/University of Baghdad, and Kamal Al-Samarraei Hospital for infertility management and In Vitro Fertilization from September 2022 to June 2023. The research encompassed a cohort of 50 women who had been previously diagnosed with PCOS and were experiencing infertility. The age range of the participants spanned from 18 to 40 years, and they had not undergone any form of fertility treatment for a minimum duration of two months. Ethical approval was duly acquired from all female participants involved in the research. A Gynecologist diagnosed the individual following thorough assessments involving physical, biochemical, and gynecological examinations. The diagnosis was further supported by ultrasound imaging by the Rotterdam Consensus. PCOS is characterized by the presence of at least two out of three specific criteria: irregular or absent ovulation, excessive androgen levels, and the presence of polycystic ovaries (defined as having 12 or more follicles measuring 2-9 mm in diameter and an ovarian volume exceeding 10 mL in at least one ovary) (Rotterdam, 2004). The entities were categorized into two distinct subgroups; **Group I**: included 25 women who were treated with metformin 850 mg/twice daily and followed for 8 weeks, and **Group II:** Twenty-five women who were treated with vitamin D3 50.000 IU/wk for the same period.

Exclusion Criteria and Ethical Considerations

This research excluded women with any type of cancer, acute and chronic illness, DM, chronic liver disease, pregnant women, smokers, endocrine disorders, and chronic renal failure. Informed consent was gained from each included adult woman. Ethical approval of this research was gained from the Scientific Committee of the Department of Biochemistry, College of Medicine/ University of Baghdad, along with that gained from the Ministry of Health, Iraq.

Blood Biochemical and hormone analsyis

Blood samples were withdrawn from each included woman of the two studied groups after (10-12) hours of overnight fasting state in the follicular phase between the second and seventh day of the menstrual cycle before starting their designed treatment between 08:00 and 10:00 hr. A.M. from an antecubital vein after 5 minutes of rest in the supine position. The blood samples were separated by centrifugation at 3000 rpm. for 5 minutes to obtain serum after remaining clot at room temperature for (10-15) minutes. The resulting serum samples were aliquot, frozen, and maintained at (-20 °C) for two months till the day of measurement of fasting serum glucose-insulin (The electrochemiluminescence immunoassay "ECLIA" is intended for use on Auto analyzer by Cobas e 411, c 111 Roche Switzerland company immunoassay analyzers), 25 hydroxyvitamin D, luteinizing hormone (LH), free testosterone, dihydrotestosterone, (competitive enzyme immunoassay technique by Cloud-Clone USA and ELK Biotechnology Chinese companies), prolactin (PRL), follicle-stimulating hormone (FSH), and sex-hormone binding globulin (SHBG) (sandwich enzyme immunoassay technique by ELK Biotechnology Chinese company) for the in vitro determination of human serum and plasma.

ELISA

ELISA reader by HumaReader HS by Human Diagnostics German company, Spectrophotometer by Cecil CE 72000 France company, Centrifuge by Hettich Universal German company, Shakers by Kahn Techno-laboratory Italian company, Printer by Epson UK company, Micropipettes by Gilson France company, Deep freeze by Sanyo Japanese company.

Questionnaire

The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was identified using the following equation: HOMA-IR = [insulin concentration (measured in milliunits per liter) multiplied by glucose concentration (measured in milligrams per deciliter)] divided by 405 (Lewandowski et al., 2019). The weight and height of the women under consideration were carefully assessed. Subsequently, their BMI was identified using the following equation: BMI (Kg/m2) = Weight (Kg)/height (m2) (Organization, 2004). A transvaginal ultrasound scan was performed at Kamal Al-Samarraei Hospital, specializing in infertility management and in vitro fertilization (IVF). The purpose of the scan was to assess both ovaries using a 6.5 MHz transducer to determine the overall count of early antral follicles.

Statistical analysis

The data underwent analysis utilizing the Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft Office 2010. The data was subjected to measurement of descriptive statistics, encompassing range, mean, and standard deviation, in order to provide a comprehensive portrayal. The groups were compared utilizing the paired sample t-test, a statistical method commonly used to analyze a single variable with two periods within a single group. The level of interdependence among continuous variables was quantified using Pearson's association coefficient (r). The statistical significance of the results was identified based on a p-value threshold of 0.05 or lower.

Results

The results of this follow-up research for PCOS women showed that 18 of the women who were treated with metformin and 13 of the women who were treated with vitamin D supplement completed the 8-week follow-up of their treatment.

The comparison of demographic, biochemical and hormonal parameters before and after metformin treatment was demonstrated in table (1), PCOS women after metformin treatment compared with before showed significantly lower mean values of BMI (27.56 ± 5.30 kg/m² *vs.* 30.76 ± 6.40; $p<0.001$), HOMA-IR (2.84 ± 1.25 *vs.* 4.37 ± 1.19; $p < 0.001$), LH (4.94 ± 0.49 mIU/ml *vs.* 6.44 ± 0.72; $p < 0.001$), FSH (5.47 ± 0.63 mIU/ml *vs.* 6.08 ± 0.76; $p < 0.001$), prolactin (19.97 ± 1.63 ng/ml *vs.* 27.38 ± 1.34; $p < 0.001$) and Free testosterone / DHT proportion (0.86 ± 0.06 *vs.* 0.72 ± 0.12; $p < 0.001$). On the other hand, there were significantly higher mean values of 25 hydroxyvitamin D (13.63 ± 1.64 ng/ml *vs.* 12.35 ± 1.65; $p=0.01$) and SHBG levels (17.64± 4.66 ng/ml *vs.* 14.68 ± 4.67; $p < 0.001$) after treatment compared with before.

The comparison of demographic, biochemical, and hormonal parameters before and after vitamin D treatment was demonstrated in table (2), PCOS women after vitamin D treatment in comparison with before showed significantly lower mean values of BMI (24.75 ± 3.28 kg/m² *vs.* 28.62 ± 6.15; $p=0.007$), HOMA-IR (2.88 ± 1.72 *vs.* 4.17 ± 1.69; $p < 0.001$), LH (5.29 ± 0.58 mIU/ml *vs.* 6.33 ± 0.80; $p < 0.001$), FSH (5.42 ± 0.66 mIU/ml *vs.* 5.99 ± 0.65; $p=0.002$), prolactin (16.44 ± 2.75 ng/ml *vs.* 22.05 ± 2.99; $p < 0.001$) and Free testosterone / DHT ratio (0.89 ± 0.09 *vs.* 0.72 ± 0.12; $p < 0.001$).

In addition, there were significantly higher 25 hydroxyvitamin D levels (18.20 ± 1.93 ng/ml *vs.* 12.26 ± 1.61; $p < 0.001$) after treatment compared with before treatment. At the same time, there were no significant changes in SHBG levels ($p=0.196$).

Table (3) shows no significant differences in the mean values of BMI, HOMA-IR, LH, FSH, and Free testosterone / DHT ratio. At the same time, there were significant differences in the mean values of 25 hydroxyvitamin D, prolactin, and SHBG between the two groups after metformin and vitamin D supplementation.

PCOS women treated with metformin revealed a significant negative association between HOMA-IR with SHBG (r= -0.593, $p=0.009$) and SHBG with BMI (r= -0.880, $p<0.001$), as well as a significant positive association between HOMA-IR and BMI (r= 0.631, $p=0.003$). PCOS women treated with vitamin D also revealed a significant negative association between SHBG and BMI (r= -0.976, $p <0.001$).

Discussion

We determiend the positive effects of metformin and vitamin D supplementation on metabolic and hormonal parameters in women diagnosed with Polycystic Ovary Syndrome (PCOS). Our study showed that PCOS women exhibit higher levels of BMI, HOMA-IR, LH, FSH, prolactin, and free testosterone/dihydrotestosterone ratio, alongside lower levels of 25 hydroxyvitamins and SHBG before treatment. On the contrary, prior to the administration of metformin and vitamin D treatments, PCOS women exhibited diminished levels of 25 hydroxyvitamins and SHBG. The recent study highlighted elevated BMI and free testosterone levels in PCOS groups (Saleh et al. 2015). Similarly, a prevalent deficiencies or inadequacies was found in 25 hydroxyvitamins among women with PCOS in 2018, aligning with the outcomes of this current research (Alawad, 2018). The average magnitudes of BMI were significantly more significant in the PCOS group, while serum levels of vitamin D3

Table 1. The comparison of demographic, biochemical, and hormonal parameters before and after metformin treatment in Group 1 patients. BMI: Body mass index, LH: Luteinizing hormone; FSH: Follicle stimulating hormone; DHT: Dihydrotestosterone; SHBG: Sex hormone binding globulin; Ŧ: paired sample t-test, S: Significant (p less than or equal to 0.05).

Parameter	Before metformin treatment (n=25)	After metformin treatment (n=18)	p-value
BMI (kg/m²)	30.76 ± 6.40	27.56 ± 5.30	< 0.001 Ŧ S
HOMA-IR	4.37 ± 1.19	2.84 ± 1.25	< 0.001 Ŧ S
25 hydroxyvitamin D (ng/ml)	12.35 ± 1.65	13.63 ± 1.64	0.01 Ŧ S
LH (mIU/ml)	6.44 ± 0.72	4.94 ± 0.49	< 0.001 Ŧ S
FSH (mIU/ml)	6.08 ± 0.76	5.47 ± 0.63	< 0.001 Ŧ S
Prolactin (ng/ml)	27.38 ± 1.34	19.97 ± 1.63	< 0.001 Ŧ S
Free testosterone/ DHT ratio	0.86 ± 0.06	0.72 ± 0.12	< 0.001 Ŧ S
SHBG (ng/ml)	14.68 ± 4.67	17.64 ± 4.66	< 0.001 Ŧ S

Table 2. The comparison of demographic, biochemical, and hormonal parameters before and after vitamin D treatment in Group 2 patients. BMI: Body mass index, LH: Luteinizing hormone; FSH: Follicle stimulating hormone; DHT: Dihydrotestosterone; SHBG: Sex hormone binding globulin; Ŧ: paired sample t-test, S: Significant (p less than or equal to 0.05), NS: Not significant (p higher than 0.05).

Parameter	Before vitamin D treatment (n=25)	After vitamin D treatment (n=13)	p-value
BMI (kg/m²)	28.62 ± 6.15	24.75 ± 3.28	0.007 Ŧ S
HOMA-IR	4.17 ± 1.69	2.88 ± 1.72	< 0.001 Ŧ S
25 hydroxyvitamin D (ng/ml)	12.26 ± 1.61	18.20 ± 1.93	< 0.001 Ŧ S
LH (mIU/ml)	6.33 ± 0.80	5.29 ± 0.58	< 0.001 Ŧ S
FSH (mIU/ml)	5.99 ± 0.65	5.42 ± 0.66	0.002 Ŧ S
Prolactin (ng/ml)	22.05 ± 2.99	16.44 ± 2.75	< 0.001 Ŧ S
Free testosterone/ DHT ratio	0.89 ± 0.09	0.72 ± 0.12	< 0.001 ŦS
SHBG (ng/ml)	16.43 ± 5.06	20.23 ± 4.56	0.196 Ŧ NS

Table 3. The comparison of demographic, biochemical, and hormonal parameters between the two studied groups after the treatment. BMI: Body mass index, LH: Luteinizing hormone; FSH: Follicle stimulating hormone; DHT: Dihydrotestosterone; SHBG: Sex hormone binding globulin; Ŧ: paired sample t-test, S: Significant (p less than or equal to 0.05), NS: Not significant (p higher than 0.05)

Parameters	After metformin treatment (n=18)	After vitamin D treatment (n=13)
BMI (kg/m²) [NS]	27.56 ± 5.30	24.75 ± 3.28
HOMA-IR [NS]	2.84 ± 1.25	2.88 ± 1.72
25 hydroxyvitamin D (ng/ml) [S]	13.63 ± 1.64	18.20 ± 1.93
LH (mIU/ml) [NS]	4.94 ± 0.49	5.29 ± 0.58
FSH (mIU/ml) [NS]	5.47 ± 0.63	5.42 ± 0.66
Prolactin (ng/ml) [S]	19.97 ± 1.63	16.44 ± 2.75
Free testosterone/ DHT ratio [NS]	0.72 ± 0.12	0.72 ± 0.12
SHBG (ng/ml) [S]	17.64 ± 4.66	20.23 ± 4.56

have decreased significantly in the PCOS group (Qasim et al., 2022) significant in the PCOS group, while serum levels of vitamin D3 have decreased significantly in the PCOS group (Qasim et al., 2022). They also revealed that women who suffer from PCOS were more prone to lack vitamin D levels than those without PCOS, resulting in obesity contributing to vitamin D deficiency risk.

Table 1 shows the comparison of demographic, biochemical, and hormonal parameters before and after metformin treatment. The results indicated a significant improvement in PCOS women after metformin intervention, with a notable reduction in BMI and HOMA-IR, accompanied by a substantial increase in 25 hydroxyvitamin D levels. Researchers demonstrates statistically significant enhancements in fasting insulin, metabolic management, and weight outcomes (specifically, BMI and HOMA-IR reduction) with metformin treatment in in 2019 across all BMI categories for women with PCOS, aligning cohesively with the outcomes of the present research (Teede et al., 2019). Metformin use with and without lifestyle variation in PCOS women resulted in significant and beneficial effects on BMI (Witchel *et al.* 2019). It has also been reported that therapeutic intervention with metformin in women with PCOS improved several effects on reducing weight, reduction of BMI, and, in turn, HOMA-IR (Lashen, 2010), which was also consistent with this research. Moreover, it has been demonstrated that several impacts were stated as related to metformin (therapeutic intervention with metformin) in PCOS patients involving restoring ovulation and improved ovarian function (Lashen, 2010). Their results were consistent with this research.

After metformin treatment, PCOS women also improved significantly lower levels of free testosterone/ dihydrotestosterone ratio compared with before treatment (Shah et al., 2019). On the other hand, there were significantly higher levels of SHBG. Metformin could decrease the hyperandrogenic symptoms and signs of PCOS patients by decreasing the androgen levels (Williams *et al.* 2020). A statistically significant difference post-treatment with metformin for testosterone for all participants were found in recent research (Teede et al., 2019). Several effects related to metformin therapeutic intervention in PCOS women, such as reducing circulating androgen levels with confirmed increased serum levels of SHBG was reported before (Lashen, 2010). Upon these findings, their results were consistent with our results.

Women with a PCOS diagnosis who received metformin treatment showed a significant correlation between HOMA-IR and BMI. This connection is likely linked to the relationship between the expression of glucose transporter 4 (GLUT4) and the level of insulin resistance (IR). Consequently, the noticeable increase in peripheral glucose utilization following metformin intervention primarily results from the stimulation of GLUT4 expression and its enhanced translocation to the plasma membrane (Herman et al., 2022).

Metformin increases insulin sensitivity by potentially increasing the activity of the insulin receptor's tyrosine kinase, aiding in glycogen synthesis, and elevating the involvement of GLUT4. Additionally, it activates adenosine 5-monophosphate-activated protein kinase (AMPK), potentially inducing the liberation of glucagon-like peptide-1, leading to increased insulin excretion and reduced glucose concentration, as supported by a study by Herman et al. in 2022. In the context of Vitamin D supplementation, an improved insulin resistance was observed which showed decreased BMI, and HOMA-IR values in individuals with PCOS (Williams et al., 2020). Another study by Xue et al. in 2017 found no significant alterations in HOMA-IR with vitamin D administration in PCOS females. Vitamin D influences insulin synthesis, enhances insulin receptor expression, suppresses pro-inflammatory cytokines, and improves glucose metabolism, potentially benefiting PCOS management (Angellotti et al., 2018, Menichini & Facchinetti, 2019). Treatment in PCOS women showed improved LH, FSH, prolactin, and free testosterone/dihydrotestosterone ratio, with Vitamin D receptors (VDRs) detected in reproductive cells, suggesting a role in modulating the female reproductive cycle (Alomda et al., 2019, Mohan et al., 2023). Elevated androgen levels may be linked to reduced plasma vitamin D levels (Hamdi et al., 2018; Menichini & Facchinetti, 2019). Our results determine the incorporation of vitamin D supplementation for PCOS individuals and recommend the need for future research, clinical focus, and larger randomized controlled trials, acknowledging variations in findings based on diagnostic criteria and geographic locations. It emphasizes consulting healthcare professionals for personalized advice and treatment.

Conclusion

In conclusion, we have determined the serum measurements of free testosterone: dihydrotestosterone ratio and SHBG as good biochemical markers to reflect the enhancement of insulin resistance and BMI in PCOS women post metformin treatment or vitamin D supplementation.

Author contribution

Z.N.E.Z collected dat from PCOS study group, collected blood samples, conducted practical measurements, and contributed to the introduction, subjects, methods, and results sections. B.O.S. performed statistical analysis, study design, and contributed to the discussion and conclusion sections.

Acknowledgment

The authors are greatful to Dr. Ansam Zeki Thaker, Kamal Al-Samarraei Hospital, Department of Biochemistry, College of Medicine, University of Baghdad, Kamal Al-Samarraei Hospital for Infertility Management and In Vitro Fertilization, Dr. Salem Abed Mohammed, Chemist Wiaam Hazem for PCOS diagnosis and treatment.

Competing financial interests

The authors have no conflict of interest.

References

Alawad, Z. M. (2018). Level of follicular fluid vitamin D and embryo quality in a sample of Iraqi women undergoing IVF. Journal of the Faculty of Medicine Baghdad, 60(4), 215–221.

Alomda, F. A. E.-A., Hashish, M. A. E.-L., El-Naggar, W. E.-S., & Mosallam, A. M. A. E.-R. (2019). The effect of vitamin D supplementation on the androgenic profile in patient with polycystic ovary syndrome. The Egyptian Journal of Hospital Medicine, 75(3), 2461–2466.

Angellotti, E., D'Alessio, D., Dawson-Hughes, B., Nelson, J., Cohen, R. M., Gastaldelli, A., & Pittas, A. G. (2018). Vitamin D supplementation in patients with type 2 diabetes: the vitamin D for established type 2 diabetes (DDM2) study. Journal of the Endocrine Society, 2(4), 310–321.

Armanini, D., Boscaro, M., Bordin, L., & Sabbadin, C. (2022). Controversies in the pathogenesis, diagnosis and treatment of PCOS: focus on insulin resistance, inflammation, and hyperandrogenism. International Journal of Molecular Sciences, 23(8), 4110.

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.

Gokosmanoglu, F., Onmez, A., & Ergenç, H. (2020). The relationship between Vitamin D deficiency and polycystic ovary syndrome. African Health Sciences, 20(4), 1880–1886.

Hamdi, R. A., Abdul-Qahar, Z. H., Kadhum, E. J., & Alsaeed, F. A. (2018). Assessment of Serum Vitamin D Levels in Women with Polycystic Ovary Syndrome. Journal of the Faculty of Medicine Baghdad, 60(2), 93–97.

Herman, R., Kravos, N. A., Jensterle, M., Janež, A., & Dolžan, V. (2022). Metformin and insulin resistance: A review of the underlying mechanisms behind changes in GLUT4-mediated glucose transport. International Journal of Molecular Sciences, 23(3), 1264.

Lashen, H. (2010). Role of metformin in the management of polycystic ovary syndrome. Therapeutic Advances in Endocrinology and Metabolism, 1(3), 117–128.

Lewandowski, K. C., Skowrońska-Jóźwiak, E., Łukasiak, K., Gałuszko, K., Dukowicz, A., Cedro, M., & Lewiński, A. (2019). How much insulin resistance in polycystic ovary syndrome? Comparison of HOMA-IR and insulin resistance (Belfiore) index models. Archives of Medical Science, 15(3), 613–618.

Menichini, D., & Facchinetti, F. (2019). Effects of vitamin D supplementation in women with polycystic ovary syndrome: a review. Gynecological Endocrinology.

Mohan, A., Haider, R., Fakhor, H., Hina, F., Kumar, V., Jawed, A., Majumder, K., Ayaz, A., Lal, P. M., & Tejwaney, U. (2023). Vitamin D and polycystic ovary syndrome (PCOS): A review. Annals of Medicine and Surgery, 85(7), 3506–3511.

Morgante, G., Darino, I., Spanò, A., Luisi, S., Luddi, A., Piomboni, P., Governini, L., & De Leo, V. (2022). PCOS physiopathology and vitamin D deficiency: biological insights and perspectives for treatment. Journal of Clinical Medicine, 11(15), 4509.

Organization, W. H. (2004). International Statistical Classification of Diseases and related health problems: Alphabetical index (Vol. 3). World Health Organization.

Pasquali, R. (2018). Contemporary approaches to the management of polycystic ovary syndrome. Therapeutic Advances in Endocrinology and Metabolism, 9(4), 123–134.

Qasim, M. N., Kadhem, H. K., & EL-Yassin, H. D. (2022). CORRELATION BETWEEN VITAMIN D3 DEFICIENCY AND SERUM LEPTIN LEVELS IN A PATIENT WITH POLYCYSTIC OVARY SYNDROME. Biochemical & Cellular Archives, 22(2).

Rotterdam, E. (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril, 81(1), 19–25.

Saleh, B. O., Ibraheem, W. F., & Ameen, N. S. (2015). The role of anti-Mullerian hormone and inhibin B in the assessment of metformin therapy in women with polycystic ovarian syndrome. Saudi Medical Journal, 36(5), 562.

Shah, S., Banu, H., Sultana, T., Akhtar, N., Begum, A., Zamila, B. M., Fariduddin, M., & Hasanat, M. A. (2019). Increased ratio of total testosterone to dihydrotestosterone may predict an adverse metabolic outcome in polycystic ovary syndrome. Journal of Endocrinology and Metabolism, 9(6), 186–192.

Singh, S., Pal, N., Shubham, S., Sarma, D. K., Verma, V., Marotta, F., & Kumar, M. (2023). Polycystic ovary syndrome: etiology, current management, and future therapeutics. Journal of Clinical Medicine, 12(4), 1454.

Teede, H., Tassone, E. C., Piltonen, T., Malhotra, J., Mol, B. W., Peña, A., Witchel, S. F., Joham, A., McAllister, V., & Romualdi, D. (2019). Effect of the combined oral contraceptive pill and/or metformin in the management of polycystic ovary syndrome: A systematic review with meta-analyses. Clinical Endocrinology, 91(4), 479–489.

Williams, A., Babu, J. R., Wadsworth, D. D., Burnett, D., & Geetha, T. (2020). The effects of vitamin D on metabolic profiles in women with polycystic ovary syndrome: a systematic review. Hormone and Metabolic Research, 52(07), 485–491.

Witchel, S. F., Oberfield, S. E., & Peña, A. S. (2019). Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls. Journal of the Endocrine Society, 3(8), 1545–1573.

Xue, Y., Xu, P., Xue, K., Duan, X., Cao, J., Luan, T., Li, Q., & Gu, L. (2017). Effect of vitamin D on biochemical parameters in polycystic ovary syndrome women: a meta-analysis. Archives of Gynecology and Obstetrics, 295, 487–496.

Zhao, J.-F., Li, B.-X., & Zhang, Q. (2021). Vitamin D improves levels of hormonal, oxidative stress and inflammatory parameters in polycystic ovary syndrome: a meta-analysis study. Ann. Palliat. Med, 10(1), 169–183.

Zhuang, L., Wei, C. U. I., Jianxiang, C., & Zhang, Y. (2019). Efficacy of vitamin D combined with metformin and clomiphene in the treatment of patients with polycystic ovary syndrome combined with infertility. Iranian Journal of Public Health, 48(10), 1802.