International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Ansari A et al. Int J Reprod Contracept Obstet Gynecol. 2023 Mar;12(3):629-633
www.ijrcog.org

pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20230527

Original Research Article

Serum 25-OH vitamin D and insulin resistance in women with polycystic ovary syndrome

Arshi Ansari*, Savita Chandra

Department of Obstetrics and Gynecology, Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India

Received: 21 December 2022
Revised: 07 February 2023
Accepted: 08 February 2023

***Correspondence:**
Dr. Arshi Ansari,
E-mail: doc.arshiansari@gmail.com

ABSTRACT

Background: This was a prospective case control study conducted in the department of obstetrics and gynecology, Era's Lucknow Medical College and Hospital from November 2018 to March 2020. Aim was to find whether there was a link between serum 25-OH vitamin D levels, and insulin resistance in women with polycystic ovarian syndrome (PCOS).
Methods: 30 newly diagnosed cases of PCOS and 30 women without PCOS were enrolled. Their clinical details as well as BMI and waist hip ratio were noted. The biochemical parameters which were measured included fasting glucose, fasting insulin, serum 25-OH vitamin D. The insulin resistance was calculated.
Results: 50% of the PCOS had insulin resistance and also deficient /or insufficient vitamin D levels, versus 16.7% in the control group. This association was statistically highly significant with p value as 0.00016. No statistical significance was found with regard to the mean serum 25-OH vitamin D level. 53.4%, of the PCOS women had hirsutism versus 3.3% in the control group and was statistically significant, p value <0.0001. 20% of PCOS women had acne in contrast to 3.3% in the control group which was statistically significant with p value 0.0443.
Conclusions: Hirsutism and acne were important clinical signs of PCOS. There was a negative correlation between insulin resistance and serum 25-OH vitamin D levels. Thus, vitamin D may be an important link in the genesis of PCOS. Larger randomised case control studies need to be done to understand PCOS.

Keywords: Fasting glucose, Fasting insulin, Insulin resistance, PCOS, Vitamin D

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age, with prevalence up to 10% depending on which diagnostic criteria are used.[1] The aetiopathogenesis of this syndrome has been attributed to various dysfunctions such as ovulatory dysfunction, hyperandrogenism, metabolic disturbances, impaired glucose intolerance, insulin resistance compensatory hyperinsulinemia, and adipose tissue dysfunction.[2,3] There are deeper complexities to be understood, for instance, obese women affected by PCOS have insulin resistance; however, a substantial number of lean women affected by PCOS have insulin resistance as well, independent of obesity.[4,5] Yet another complexity is the role of vitamin D deficiency in the pathogenesis of PCOS and this opens a new dimension in the understanding of PCOS.

Vitamin D deficiency has been proposed as the possible missing link between insulin resistance and PCOS.[6] Several observational studies have demonstrated that vitamin D deficiency is related to type II diabetes mellitus, renin-angiotensin system, endothelial function, blood pressure or chronic inflammation.[7-10] Therefore, vitamin D deficiency may play a key role in the development of PCOS. Vitamin D levels, metabolic and hormonal dysfunctions in women affected by PCOS, have been

investigated, but there continues to be lack of convincing evidence. Hence, more studies are needed to understand the link between vitamin D levels, insulin resistance and PCOS. With this in mind the study was planned.

METHODS

This prospective case control study was conducted at Era's Lucknow Medical College and Hospital, Lucknow, India during the period from November 2018 to March 2020. The study was approved by the Ethics Committee of the institution. Written and informed consent was obtained from all the 60 study participants in the age group of 15-44 years. The experimental group and the control group each comprised of 30 participants.

The criteria used for defining deficiency, insufficiency, sufficiency of 25-OH vitamin D were as follow:

Serum levels of 25-OH vitamin D <20 ng/ml was considered as deficiency, 21-29 ng/ml as insufficiency, and 30-100 ng/ml as normal/sufficiency.[11]

Insulin resistance was calculated by using the homeostatic model assessment-insulin resistance (HOMA- IR).

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting Glucose x Fasting Insulin}}{405}$$

Insulin resistance was defined as HOMA-IR>2.512.

Inclusion criteria for the participants

The age of the participants in both the groups was within 15 to 44 years.

The control group comprised of women with no clinical history and signs suggestive of PCOS.

The experimental group comprised of women newly suspected of PCOS. Clinical diagnosis of PCOS was made based on any two of the three Rotterdam's criteria i.e. oligomenorrhea, signs of hyperandrogenism (acne/hirsutism), and polycystic ovaries on ultrasonography.

Exclusion criteria

Those clinically suspected of hypothyroidism, congenital adrenal hyperplasia, Cushing's syndrome, and androgen secreting tumour were excluded.

The history and clinical examination details, weight and height, waist and hip circumference of each participant were recorded on a pre-prepared proforma. The body mass index (BMI) and the waist hip ratio (W/H) of each subject was calculated by using the standard formulas. The W/H ratio ≥0.85 was considered above normal.

The enrolled subjects were advised to report the following morning in a fasting state, and 5 ml of blood was collected from the antecubital vein, of which 3 ml was put in a plain vial and 2 ml was put in a sodium fluoride vial. The vials were labelled bearing the registration number of the enrolled subject.

The blood samples were then subjected to centrifugation at 3500 rpm for 10 min to separate the serum. Serum fasting glucose level was measured using commercial kits available for fully automated HumaStar 600 Biochemistry Analyzer (Germany). Serum fasting insulin level and serum 25-OH vitamin D level was measured by chemiluminescence immunoassay (CLIA) method.

The observations were tabulated and the results subjected to statistical analysis. The p value of <0.05 was considered to be statistically significant.

RESULTS

In this study, it was found that all the cases of PCOS were below the age of 30 years. The mean age of PCOS women was 22±3.92 years and of the controls was 30.63±7.42 years, the difference was found to be statistically significant with p value <0.0001 (Table 1).

Table 1: The mean of various parameters in the experimental and control group.

Parameters	Cases (n=30) Mean±SD	Controls (n=30) Mean±SD	P value
Age in years	22±3.92	30.63±7.42	<0.0001
BMI (kg/m^2)	24.34±5.89	24.10±4.21	0.85
Waist-hip ratio	0.87±0.08	0.87±0.05	0.97
Fasting glucose (mg/dl)	88.1±6.47	89.7±8.69	0.42
Fasting insulin (µIU/ml)	16.44±11.51	9.70±6.14	0.006
Insulin resistance	3.54±2.4	2.2±1.55	0.013
Serum 25-OH vitamin D (ng/ml)	22.62±14.98	21.88±14.84	0.83

P value of <0.05 was considered to be as statistically significant

The mean BMI for the women with PCOS and those in the Control Group was 24.34±5.89 and 24.10±4.21 kg/m^2 respectively; while the W/H ratio, was 0.87±0.08 and 0.87±0.05 respectively, the difference between the cases and controls did not reach statistical significance (Table 1).

Though the mean fasting glucose level among the PCOS women was found to be lower than the controls, the difference did not reach statistical significance.

The mean fasting insulin for the PCOS women was 16.44±11.51 µIU/ml higher than for the control group which was 9.70±6.14 µIU/ml, the difference was statistically significant with p value 0.006 (Table 1).

When the insulin resistance was calculated by HOMA-IR, it was observed that the mean insulin resistance was 3.54±2.4 in the PCOS women and 2.2±1.55 in the control group which was found to be statistically significant with p value 0.013 (Table 1).

No statistical significance was found with regard to the mean serum 25-OH vitamin D level between the two groups (Table 1).

Table 2: Distribution of the subjects based on hirsutism.

Hirsutism	Cases (n=30)	Controls (n=30)	Total
Absent	14 (46.6%)	29 (96.7%)	43
Present	16 (53.4%)	1 (3.3%)	17
Total	30	30	60

***Chi-square value: 18.4679; p value:<0.0001**
Odds ratio:0.0302 (0.0036, 0.2510)

It is seen that 16 women with PCOS i.e. 53.4%, had hirsutism in contrast to only one woman i.e. 3.3% in the control group. In this study hirsutism was found to be statistically significant with p value <0.0001 (Table 2).

Table 3: Distribution of subjects based on acne.

Acne	Cases (n=30)	Controls (n=30)	Total
Absent	24 (80%)	29 (96.7%)	53
Present	6 (20%)	1 (3.3%)	7
Total	30	30	60

***Chi-square value: 4.0431; p value:0.0443**
Odds ratio:0.1379 (0.0155, 1.2263)

Table 3 shows that 6 of the 30 women with PCOS had acne i.e. 20%, in contrast to only one woman in the control group (3.3%) which was statistically significant with p value 0.0443.

In the experimental group, 15 of the 30 women (50%) had insulin resistance and also and the deficient/or insufficient vitamin D levels, whereas 5 of the 30 women (16.7%) in the control group had insulin resistance plus deficient/or insufficient vitamin D levels. This association was statistically highly significant with p value as 0.00016 (Table 4). Hence, the results of this study show the negative correlation between serum 25-OH vitamin D and insulin resistance.

Table 4: Serum 25-OH vitamin D and insulin resistance correlation.

Serum 25OH vitamin D level	Cases (n=30)		Controls (n=30)	
	Without insulin resistance	**With insulin resistance**	**Without insulin resistance**	**With insulin resistance**
<20 ng/ml (deficiency)	1 (3.3%)	14 (46.7%)	15 (50%)	3 (10%)
21-29ng/ml (insufficiency)	6 (20%)	1 (3.3%)	4 (13.3%)	2 (6.7%)
30-100 ng/ml (sufficiency)	3 (10%)	5 (16.7%)	6 (20%)	0
Chi-square	13.5054		2.4000	
Probability	0.0012		0.3012	

DISCUSSION

This prospective case-control study was conducted at Era's Lucknow Medical College, Lucknow, India from November 2018 to March 2020. A total of 60 women were enrolled of which 30 subjects were in the experimental group and 30 in the control group.

In our study it was found that all the women diagnosed as PCOS were ≤30 years of age. This observation was statistically significant (p<0.0001) (Table 1). The mean age in this study for PCOS women was 22±3.9 years which was very close to the mean age of 23.26±3.56 years reported by Yadav et al in 2019.[13]

However, earlier in 2017, Kumar et al in their study found the mean age of PCOS cases to be 28.6±6.3 years while Gul et al in 2018, found the mean age to be 26.7±7.54 years, higher than the mean age of this study.[14,15] This difference could be due to multiple factors explained as follows:

Firstly, delay in establishing the diagnosis of PCOS and in the process, often times, several years pass by. This delay in diagnosis could be accounted for at different levels of health care process; some at the level of the patient herself, while others at the level of the health care provider, the type of health care facility and that lab facilities. For instance, how early the woman reported to a health facility was itself dependent on various factors such as her own self perception of health, her psychological state, literacy, socio- economic status, her family background, the out-of-pocket expenditure that could be incurred; these factors in turn would determine the type of health facility to which she reported. Thereafter, the knowledge and effort made by the health functionaries to establish the diagnosis of PCOS, and the laboratory/imaging facilities available at

the specific health facility would impact how early/late the diagnosis was established. Thus, variations in these factors could explain the differences in the mean age of PCOS women in different studies.

In this study, 47.2% of the PCOS cases had normal BMI while 52.8% had BMI above normal, the difference was not statistically significant. Elkholy et al, and Kumar et al found a similar trend.[16,17] In contrast in 2018, Gul et al found that PCOS subjects had higher BMI and the difference reached statistical significance (p value <0.01).[15]

The observations in this study showed that 76.7% had increased waist-hip ratio (Table 1) with the mean as 0.87±0.08. Yadav et al in their study reported the mean waist-hip ratio as 0.87±0.05 which was close to our study.[13] Interestingly, the association of PCOS and the waist hip ratio reached statistical significance in the study by Yadav et al unlike our study wherein statistical significance was not reached. This could be explained by the difference in total number of cases which was 60 in this study versus 400 in their study.

In this study 53.4% of PCOS women had hirsutism versus 3.3% in the control group which reached statistical significance with p value <0.0001 (Table 2). In 2019, Yadav et al also found a similar trend and their results also reached statistical significance.[13]

In our study 20% of the PCOS cases had acne in contrast to 3.3% in the control group or expressed another way, acne was seen about 6 times more in PCOS subjects as compared to the control group. This was statistically significant with p value 0.0443 (Table 3). Similarly, Yadav et al found acne in 32% of the PCOS subjects higher than our results.[13] Thus, acne is an important clinical sign for PCOS.

In this study the mean fasting insulin level in the experimental group was 16.44±11.51 µIU/ml and in the control group was 9.70±6.14 µIU/ml (Table 1). Serum fasting insulin levels in PCOS cases were found to be significantly higher than the serum insulin levels in the control group and the difference was statistically significant, with p value 0.006 (Table 1). Our results were closely comparable to those of Saha et al who reported the mean fasting insulin level in women with PCOS as 18.20±0.76 µIU/ml; statistical significance was also reached in their study with p value <0.001.[18]

It was found in this study that 66.7% of the PCOS women had insulin resistance in contrast to the control group where only 16.7% had insulin resistance which was statistically significant with p value<0.001 (Table 4). Similarly, Saha et al also reported that PCOS women had insulin resistance stating that HOMA-IR was significantly higher in PCOS women, which reached statistical significance with p value <0.001.[18]

The mechanism for insulin resistance is complex and not fully understood. Many components may be involved such as insulin receptors, insulin receptor signalling, various regulatory enzymes, excessive serine phosphorylation, serine/threonine kinase ratio or alteration in the key regulatory enzyme i.e. P450c17. Recent studies strongly suggest that insulin is acting through its own receptor, rather than the IGF-I receptor, to augment not only ovarian and adrenal steroidogenesis but also pituitary LH release. These complex mechanisms are also involved in hyperandrogenism. It's possible that a single primary defect, yet unknown, may initiate both insulin resistance and hyperandrogenism which progressively later manifests as the entity PCOS.

Vitamin D deficiency has also been suggested to play a role in insulin resistance and in the pathogenesis of PCOS.

In our study, 50% of women with PCOS had both vitamin D deficiency/insufficiency and insulin resistance. However, in comparison with the control group, only 16.7% of the women had unsatisfactory vitamin D and insulin resistance. This observation was found to be statistically significant with p value 0.0001 (Table. 4). Thus, there is a negative correlation between 25-OH vitamin D and insulin resistance (Figure 1). Saha et al found negative correlation of vitamin D status with insulin resistance and androgen status in PCOS cases.[19]

Kumar et al also found significant association between vitamin D and insulin resistance.[17] Patra et al also did a multiple regression analysis and concluded that serum 25OH vitamin D levels were the best predictor of insulin resistance.[18]

He et al showed that lower serum vitamin D levels were related to metabolic and hormonal disorders in women with PCOS.[20] Specifically, PCOS patients with vitamin D deficiency were more likely to have increased levels of fasting glucose and homeostatic model assessment-insulin resistance index (HOMA-IR)) compared to those without vitamin D deficiency.

On the contrary, Ardabili et al evaluated the fasting serum insulin and glucose levels and the insulin sensitivity and homeostasis model assessment of insulin resistance after giving three oral doses of vitamin D of 50,000 IU for 2 months.[21] They found that vitamin D supplementation had no effect on insulin sensitivity/insulin resistance in women with PCOS and vitamin D deficiency. Wehr et al showed significant negative correlation with IR and positive correlation with insulin sensitivity in women with PCOS.[22]

CONCLUSION

While our study showed that there was a negative correlation between insulin resistance and serum 25-OH vitamin D levels, there are in this regard, conflicting results in the scientific literature. Larger randomised case control

studies need to be done to understand the complexity and relationship of insulin resistance, vitamin D, and PCOS.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat Rev Endocrinol. 2011;7(4):219-31.
2. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012;97(1):28-38.
3. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2010;16(4):347-63.
4. Ketel IJ, Serne EH, Ijzerman RG, Korsen TJM, Twisk JW, Hompes PGA, et al. Insulin-induced capillary recruitment is impaired in both lean and obese women with PCOS. Hum Reprod. 2011;26(11):3130-7.
5. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes. 1989;38(9):1165-74.
6. Nandi A, Sinha N, Ong E, Sonmez H, Poretsky L. Is there a role for vitamin D in human reproduction? Horm Mol Biol Clin Investig. 2016;25(1):15-28.
7. Ferder M, Inserra F, Manucha W, Ferder L. The world pandemic of vitamin D deficiency could possibly be explained by cellular inflammatory response activity induced by the renin-angiotensin system. Am J Physiol. 2013;304:C1027-39.
8. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with type 2 diabetes mellitus and low vitamin D levels. Diabetic Med. 2008;25:320-5.
9. Vimaleswaran KS, Cavadino A, Berry DJ, Jorde R, Dieffenbach AK, Lu C, et al. Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study. Lancet Diabetes Endocrinol. 2014;2:719-29.
10. Chagas CE, Borges MC, Martini LA, Rogero MM. Focus on vitamin D, inflammation and type 2 diabetes. Nutrients. 2012;4:52-67.
11. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Handley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911-30.
12. Esmaeilzadeh S, Tahmasbpour E, Gholinezhad-Chari M. Hyperhomocysteinemia, insulin resistance and body mass index in Iranian young women with polycystic ovary syndrome. Middle East Fertil Soc J. 2017;22(2):149-55.
13. Yadav S, Tarware R. (2019). Waist hip ratio: an anatomical predictive marker of risk of PCOS. Int J Reprod Contracept Obstet Gynecol. 2019;8:1630.
14. Kumar A, Barki S, Raghav V, Chaturvedi A, Kumar KH. Correlation of vitamin D with metabolic parameters in polycystic ovarian syndrome. J Fam Med Prim Care. 2017;6(1):115.
15. Gul OO, Ulutas F, Cander S, Ersoy C. Serum 25-Hydroxy vitamin D levels and insulin resistance in polycystic ovary syndrome. InEndocrine Abstracts. 2018;56:EP143.
16. Elkholy SS, Mostafa RA, Riad AA, AbouZaghla HM. Assessment of vitamin D levels in women with polycystic ovarian syndrome. Egypt J Hosp Med. 2018;70(4):594-600.
17. Kumar AN, Naidu JN, Satyanarayana U, Anitha M, Ramalingam K. Association of insulin resistance and serum 25-OH vitamin-D in Indian women with polycystic ovary syndrome. Int J Clin Biochem Res. 2015;2(1):22-6.
18. Saha PK, Pal S, Mukhopadhyay T. Association of vitamin D status with polycystic ovary syndrome in relation to insulin resistance and serum total testosterone. Int J Res Rev. 2019;6(6):11-7.
19. Patra SK, Nasrat H, Goswami B, Jain A. Vitamin D as a predictor of insulin resistance in polycystic ovarian syndrome. Diabetes Metab Syndr. 2012;6(3):146-9.
20. HE C, Lin Z, Robb SW, Ezeamama AE. Serum vitamin D levels and polycystic ovary syndrome: a systematic review and meta-analysis. Nutrients. 2015;7(6):4555-77.
21. Ardabili HR, Gargari BP, Farzadi L. Vitamin D supplementation has no effect on insulin resistance assessment in women with polycystic ovary syndrome and vitamin D deficiency. Nutr Res. 2012;32(3):195-201.
22. Wehr E, Trummer O, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Vitamin D-associated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. Eur J Endocrinol. 2011;164(5):741.

Cite this article as: Ansari A, Chandra S. Serum 25-OH vitamin D and insulin resistance in women with polycystic ovary syndrome. Int J Reprod Contracept Obstet Gynecol 2023;12:629-33.