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Research Article

ASSESSMENT OF VITAMIN D AND CALCIUM LEVELS IN WOMEN WITH PCOS: AN OBSERVATIONAL STUDY

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ABSTRACT

Objectives: Polycystic ovarian syndrome (PCOS) is a prevalent endocrinal disorder in the women of reproductive age group. The present study aims to investigate the correlation between Vitamin D and calcium levels in PCOS subjects as well as to establish the correlation between body mass index, hyperandrogenism, and metabolic syndrome in various phenotypes of PCOS.

Methods: In the present prospective and observational study, a total of 80 patients diagnosed with PCOS were recruited. The recruited patients were divided in the following two groups - study group (60 women diagnosed with PCOS based on Rotterdam criteria); and control group (20 women without PCOS recruited from outpatient department and infertility clinics). Baseline characteristics, Vitamin D level, and serum calcium levels were measured in the two groups and then compared.

Results: The mean age of patients in both the groups was found to be comparable (study group: 26.45±5.95 years versus control group: 28.40±6.65 years; p=0.221). The difference of mean 25-Hydroxy Vitamin D3 level was found to be statistically highly significant (study group 14.71 [±9.12] ng/mL versus control group was 22.47 [±6.71] ng/mL; p=0.0008). The difference of mean serum calcium level was also found to be statistically highly significant (study group 9.14 [±0.50] mg/mL, and control group 9.74 [±0.45] mg/mL; p<0.0001).

Conclusion: Women with PCOS have a significantly lower serum 25-hydroxyvitamin D and calcium levels as compared to controls. There is need for routine Vitamin D3 and calcium screening in all patients of PCOS for better insight into its role in the syndrome. Screening and correction of Vitamin D3 and calcium deficiency may prevent PCOS and its manifestations.

Keywords: Vitamin D, Polycystic ovarian syndrome, Calcium.

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INTRODUCTION

A gynecological disease complex called polycystic ovarian syndrome (PCOS) affects 3–26% of women [1]. Stein and Leventhal first described the disease as a triad of oligomenorrhea or anovulation, hyperandrogenism and hirsutism in 1935 [2]. It is also regarded as a metabolic disorder since PCOS frequently has characteristics similar to the primary components of the metabolic syndrome, such as obesity, glucose intolerance, atherogenic dyslipidemia, and hypertension [3]. Among PCOS patients, the prevalence of metabolic syndrome ranges from 10% to 43% [4]. Pregnant women with this condition are more likely to have perinatal mortality, fetal macrosomia, pre-eclampsia, gestational diabetes mellitus, and small-for-gestational-age newborns [5]. Type 2 diabetes mellitus, endometrial cancer, and cardiovascular disease are long-term health hazards [6,7].

The underlying mechanisms of PCOS are still poorly understood, which makes it difficult for gynecologists and endocrinologists to diagnose and treat patients [8]. In addition, PCOS manifests as a variety of phenotypes, thus necessitating individualized treatment [5,9]. The causative roles of many implicated factors have been analyzed to explore the underlying mechanisms of PCOS. One such factor is Vitamin D, which has been linked to insulin or glucose metabolism and has been shown in PCOS patients to have an inverse relationship with metabolic abnormalities. However, it is unclear whether or not women with and without PCOS have different levels of serum Vitamin D [10].

In the current study, to decide whether to implement screening programs or supplement Vitamin D and calcium for PCOS patients, the correlation between their levels in these subjects in the local community was studied. In addition, in various PCOS phenotypes, the correlation between body mass index (BMI), hyperandrogenism, and metabolic syndrome was assessed.

Aims and objectives

The aims are as follows:

1. To study levels of 25 hydroxyvitamin D and calcium in women with PCOS
2. To study the correlation between serum 25 hydroxyvitamin D concentration and calcium in normal women and women with PCOS
3. To study prevalence of metabolic syndrome in various phenotypes of PCOS.

METHODS

This study was a prospective and observational study.

Source of data

Patients enrolled in the study were recruited from the out-patient department and infertility clinic of Obstetrics and Gynaecology, Rajindra hospital, Government Medical College, Patiala from a period from January 2019 to June 2020.

Inclusion criteria

The following criteria were included in the study:

1. Women aged 18–45 years
2. Women who had been diagnosed with PCOS based on Rotterdam criteria [11]. These criteria included 2 of 3:
   a. Different clinical manifestations of oligo and/or anovulation
   b. Clinical and/or biochemical evidence of hyperandrogenism including hirsutism, acne, and elevated testosterone

c. Polycystic ovaries in ultrasound, meaning presence of 12 or more follicles measuring 2–9 mm in diameter in each ovary and/or ovarian volume more than 10 cm^3.

Exclusion criteria

The following criteria were excluded from the study:

1. Women with renal, liver or chronic diseases
2. Women who had a history of hormonal treatment in the past 3 months before the study
3. Women who were not willing to participate in study
4. Women who were on medication affecting serum calcium levels.

Patients with clinically suspected PCOS were further cross-examined before recruitment into the study. Detailed clinical history regarding onset of symptoms was taken. Written informed consent was obtained from all the subjects before the study. A total of 60 patients with PCOS based on Rotterdam criteria were taken as cases. 20 women without PCOS were taken as controls.

Study group

60 women diagnosed with PCOS.

Control group

20 women without PCOS.

Under all aseptic conditions, blood sample was collected in plain vial by venepuncture. The levels of Vitamin D and calcium were measured. The reference range of Vitamin D was 15–60 ng/mL and the reference range of calcium was 8.6–10.2 mg/dl.

The results of observations of individual patients were pooled and analyzed. Statistical analysis was performed using statistical program for social sciences software version 20.0 Chicago, Illinois, USA.

Ethical clearance

The ethical clearance was obtained from the institutional ethics committee before conducting the study (Reference No. GMC/OBGY/231/2019).

RESULTS

Baseline characteristics

The mean age of patients in both the groups was found to be comparable (study group: 26.45±5.95 years versus control group: 28.40±6.65 years; p=0.221) (Table 1). The distribution of patients according to socioeconomic strata was also comparable (p=0.472). In the study group, 46 patients (76.66%) had irregular menstrual cycle (i.e., oligomenorrhea, amenorrhea, or heavy menstrual bleeding) while in the control group, three patients (15%) had irregular menstrual cycle (p<0.0001 (HS). The patients were compared on the basis of dermatological findings. The comparison showed that there was highly significant difference (p<0.0001) between the groups for hirsutism, acne, acanthosis nigricans, and androgenetic alopecia. Significant difference was noted for acrochordon (p=0.013) and seborrhea (p=0.052), while no significant difference was found for striae (p=0.112) (Table 1).

PCOS phenotypes and metabolic syndrome

The components of metabolic syndrome were studied in the study group and control group: Waist circumference (36.18±4.56 inches vs. 33.70±2.45 inches; p=0.023), systolic blood pressure (131.08±13.93 mm Hg vs. 127.10±8.59 mm Hg; p=0.233), diastolic blood pressure (84.83±11.69 mm Hg vs. 82.60±9.64 mm Hg; p=0.44), high-density lipoprotein (HDL) (36.83±9.51 mg/dl vs. 51.80±6.08 mg/dl; p<0.0001), triglycerides (171.38±27.59 mg/dl vs. 145.60±14.85 mg/dl; p=0.0002), and fasting blood sugar (103.41±33.40 mg/dl vs. 87±19.91 mg/dl; p<0.041). Overall, a diagnosis of metabolic syndrome was established among 29 patients of PCOS (48.33%) using the NCEP-ATP III criteria.

According to phenotype of PCOS in the study group, 27 (45%) patients belonged to Phenotype A (hyperandrogenism + ovulatory dysfunction + polycystic ovarian morphology), 19 (31.66%) patients belonged to Phenotype B (hyperandrogenism + ovulatory dysfunction), and 14 (23.33%) patients belonged to Phenotype C (hyperandrogenism + polycysticovarian morphology). Metabolic syndrome was diagnosed among 13 (21.66%) patients of phenotype A, 9 (15%) patients of phenotype B, and 7 (11.66%) patients of phenotype C (Table 2).

Levels of 25-Hydroxy Vitamin D3 and calcium

Level of 25-Hydroxy Vitamin D3 was described in terms of deficient (<20 ng/mL), insufficient (20–30 ng/mL), and sufficient (30–100 ng/mL) categories. In the study group, 48 (80%) belonged to the deficient category, 9 (15%) belonged to the insufficient category, and 3 (5%) belonged to the sufficient category. In the control group, 6 (30%) belonged to the deficient category, 11 (55%) belonged to the insufficient category, and 3 (15%) belonged to the sufficient category. The mean 25-Hydroxy Vitamin D3 level in the study group was 14.71 (±9.12) ng/mL. The mean 25-Hydroxy Vitamin D3 level in the control group was 22.47 (±6.71) ng/mL. The difference was found to be statistically highly significant (p=0.0008) (Table 3).

The analysis of serum levels of calcium revealed that in the study group, 31 (51.66%) subjects had <9 mg/dl calcium levels, 24 (40%) subjects had 9–10 mg/dl calcium levels, and 5 (8.33%) subjects had >10–12 mg/dl calcium levels. In the control group, 3 (15%) subjects had <9 mg/dl calcium levels, 11 (55%) subjects had 9–10 mg/dl calcium levels, and 6 (30%) subjects had >10–12 mg/dl calcium levels. The mean serum calcium level in the study group was 9.14 (±0.50) mg/mL, while it was 9.74 (±0.45) mg/ml in the control group. The difference was found to be statistically highly significant (p<0.0001) (Table 3).

DISCUSSION

PCOS is characterized byhyperandrogenism, chronic anovulation, and abnormal development of ovarian follicles. In addition, insulin resistance and central obesity are common features found in PCOS patients [12].

In the present study, the mean age of patients in both the groups was found to be comparable (study group: 26.45±5.95 years vs. control group: 28.40±6.65 years; p=0.221). The finding was in agreement with the study conducted by Firouzabadi *et al*. who reported the mean ages of the patients in study and control group as 28.46±4.16 and 27.96±4.07, respectively [13]. PCOS patients belonged more to the middle (71.66%) and lower (16.66%) socioeconomic status as compared to the upper socioeconomic status (11.66%). However, similar patient distribution was noted in the control group too. Gowri and Ramana also found that diagnosis of PCOS was associated with lower socioeconomic status [14]. Irregular menstrual cycle (i.e., oligomenorrhea, amenorrhea, or heavy menstrual bleeding) (76.66%) is a common finding among the PCOS patients. This finding is also supported by Gowri and Ramana who reported 82% of PCOS patients experienced irregular bleeding [14]. Thys-Jacobs *et al.* (1999) reported that calcium and Vitamin D supplementation can improve menstrual disturbances in PCOS patients in long-term period [15].

Dermatological examination shows that PCOS have more hirsutism (91.66%), acne (73.33%), acanthosis nigricans (51.66%), androgenetic alopecia (48.33%), acrochordon (25%), and seborrhea (16.66%) as compared to control group (Table 1) that was negatively correlated with Vitamin D levels with similar results shown in various studies [16-18].

In the present study, metabolic syndrome was established among 29 patients of PCOS (48.33%) using the NCEP-ATP III criteria [19]. Some components of metabolic syndrome were more common in the PCOS patients than in the control group: Increased waist circumference, decreased HDL, increased triglycerides, and increased fasting blood sugar. The mean BMI of the study group was 33.69±6.76 kg/m^2 while it was 29.54±5.42 kg/m^2 in the control

Table 1: Baseline characteristics of the patients

Parameters	Study group (n=60) (%)	Control group (n=20) (%)	p-value (Sig.)
Age	26.45±5.95	28.40±6.65	0.221 (NS)
Socio-economic status			
Upper	7 (11.66)	2 (10)	0.839 (NS)
Middle	43 (71.66)	16 (80)	0.465 (NS)
Lower	10 (16.66)	2 (10)	0.472 (NS)
Body mass index (kg/m^2)	33.69±6.76	29.54±5.42	0.015 (S)
Menstrual cycle parameters			
Regular menstrual cycle	14 (23.33)	17 (85)	<0.0001 (HS)
Irregular menstrual cycle	46 (76.66)	3 (15)	<0.0001 (HS)
Dermatological findings			
Hirsutism (FG score≥8)	55 (91.66)	1 (5)	<0.0001 (HS)
Acne	44 (73.33)	4 (20)	<0.0001 (HS)
Acanthosis nigricans	31 (51.66)	0 (0)	<0.0001 (HS)
Androgenetic alopecia	29 (48.33)	0 (0)	<0.0001 (HS)
Acrochordon	15 (25)	0 (0)	0.013 (S)
Seborrhea	10 (16.66)	0 (0)	0.052 (S)
Striae	7 (11.66)	0 (0)	0.112 (NS)
Metabolic syndrome components			
Waist circumference (inches)	36.18±4.56	33.70±2.45	0.023 (S)
Systolic blood pressure (mmHg)	131.08±13.93	127.10±8.59	0.233 (NS)
Diastolic blood pressure (mmHg)	84.83±11.69	82.60±9.64	0.44 (NS)
HDL (mg/dl)	36.83±9.51	51.80±6.08	<0.0001 (HS)
TG (mg/dl)	171.38±27.59	145.60±14.85	0.0002 (HS)
FBS (mg/dl)	103.41±33.40	87±19.91	<0.041 (S)

HDL: High-density lipoprotein, TG: Triglyceride, FBS: Fasting blood sugar

Table 2: Prevalence of metabolic syndrome with distribution of patients according to PCOS phenotype

Metabolic syndrome	PCOS phenotype				Total (%)
	A (%)	B (%)	C (%)	D	
Yes	13 (21.66)	9 (15)	7 (11.66)	0	29 (48.33)
No	14 (23.33)	10 (16.66)	7 (11.66)	0	31 (51.66)
Total	27 (45)	19 (31.66)	14 (23.3%)	0	60 (100)

Table 3: Comparison of 25-hydroxy Vitamin D3 and serum calcium levels in subjects of study and control group

Category (levels)	Study group (n=60) (%)	Control group (n=20) (%)
25-Hydroxy Vitamin D3 levels		
Deficient (<20 ng/ml)	48 (80)	6 (30)
Insufficient (20–30 ng/ml)	9 (15)	11 (55)
Sufficient (30–100 ng/ml)	3 (5)	3 (15)
Mean±SD (ng/ml)	14.71 (±9.12)	22.47 (±6.71)
Range (ng/ml)	2.17–45.67	10.53–34.98
p-value	0.0008 HS)	
Calcium levels		
<9 mg/dl	31 (51.66)	3 (15)
9–10 mg/dl	24 (40)	11 (55)
>10–12 mg/dl	5 (8.33)	6 (30)
Mean±SD (mg/dl)	9.14 (±0.50)	9.74 (±0.45)
Range (mg/dl)	8.60–10.2	8.80–10.2
p-value	<0.0001 (HS)	

group which was statistically significant. The present study found majority of the patients (80%) had deficient 25-(OH) Vitamin D3 levels <20 ng/ml. Furthermore, majority of the PCOS patients (51.66%) had <9 mg/dl serum calcium levels, the result similar to previous literature [20,21]. Similar results were found by Wehr *et al*. who demonstrated that 72.8% of the subjects were Vitamin D deficient [16]. Obesity is a well-recognized risk factor of Vitamin D deficiency. An inverse correlation between BMI and serum 25-(OH) Vitamin D3concentrations in PCOS women was demonstrated in this study and previous reports [22,23]. In obese individuals, a higher proportion of Vitamin D, which is fat soluble, is sequestered in adipose tissues; and hence, bioavailability of the vitamin is lowered. Alternatively, obese subjects may tend to spend less time outdoors exposed to sunlight, leading to insufficient Vitamin D biosynthesis in skin. Dietary preference and Vitamin D metabolism may also be different between obese and non-obese individuals. It is conceivable that the prevalence of Vitamin D deficiency in PCOS women in our study could be related to obesity [24].

Serum 25-(OH) Vitamin D3concentration is widely accepted as the functional indicator of Vitamin D status in the body. It has been proposed as the possible missing link between insulin resistance and PCOS [25,26].

Several limitations deserve note. The duration of the study was short with small sample size. Another limitation was the region-specific nature of the research data. Hence, the results cannot be generalized to other population groups.

Further studies with a larger sample size and longer duration are, therefore, warranted. Patient enrollment should be extended beyond the outpatient department so as to improve external validity in the general population and different settings. The studies with a multicentric patient enrollment will help in the generalization of data to larger populations.

CONCLUSION

This study demonstrates that women with PCOS have a significantly lower serum 25-hydroxyvitamin D and calcium levels as compared to controls. Low Vitamin D levels in PCOS women were found to be associated with metabolic and hormonal disturbance through impact on lipid profile, insulin release, and metabolic function. The accumulating data shows negative correlation between Vitamin D3 and calcium levels with PCOS; hence, it is speculated that improvement of Vitamin D3 and calcium levels at a younger age can contribute to prevention of PCOS. There is need for routine Vitamin D3 and calcium screening in all patients of PCOS for better insight into its role in the syndrome. Screening and correction of Vitamin D3 and calcium deficiency may prevent PCOS and its manifestations.

AUTHORS CONTRIBUTION

S: Data collection, manuscript drafting, PK: Conceptualization and manuscript drafting, RG: Manuscript revision, data curation and supervision, MK: Statistical analysis and manuscript revision, SA: Supervision and critical revision of manuscript.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest.

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REFERENCES

1. El Hayek S, Bitar L, Hamdar LH, Mirza FG, Daoud G. Poly cystic ovarian syndrome: An updated overview. Front Physiol 2016;7:124. doi: 10.3389/fphys.2016.00124, PMID 27092084
2. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obste Gynecol 1935;29:181-91. doi: 10.1016/S0002-9378(15)30642-6
3. Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech 2009;2:231-7. doi: 10.1242/dmm.001180, PMID 19407331
4. Mandrelle K, Kamath MS, Bondu DJ, Chandy A, Aleyamma T, George K. Prevalence of metabolic syndrome in women with polycystic ovary syndrome attending an infertility clinic in a tertiary care hospital in south India. J Hum Reprod Sci 2012;5:26-31. doi: 10.4103/0974-1208.97791, PMID 22870011
5. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril 2016;106:6-15. doi: 10.1016/j.fertnstert.2016.05.003, PMID 27233760
6. Brady C, Mousa SS, Mousa SA. Polycystic ovary syndrome and its impact on women's quality of life: More than just an endocrine disorder. Drug Healthc Patient Saf 2009;1:9-15. doi: 10.2147/dhps.s4388, PMID 21701605
7. Palomba S, Santagni S, Falbo A, La Sala GB. Complications and challenges associated with polycystic ovary syndrome: Current perspectives. Int J Womens Health 2015;7:745-63. doi: 10.2147/IJWH.S70314, PMID 26261426
8. Ni CM, Huang WL, Jiang YM, Xu J, Duan R, Zhu YL, *et al*. Improving the accuracy and efficacy of diagnosing polycystic ovary syndrome by integrating metabolomics with clinical characteristics: Study protocol for a randomized controlled trial. Trials 2020;21:169. doi: 10.1186/s13063-020-4060-6, PMID 32046752
9. Huang CC, Tien YJ, Chen MJ, Chen CH, Ho HN, Yang YS. Symptom patterns and phenotypic subgrouping of women with polycystic ovary syndrome: Association between endocrine characteristics and metabolic aberrations. Hum Reprod 2015;30:937-46. doi: 10.1093/humrep/dev010, PMID 25662806
10. Kim JJ, Choi YM, Chae SJ, Hwang KR, Yoon SH, Kim MJ, *et al*. Vitamin D deficiency in women with polycystic ovary syndrome. Clin Exp Reprod Med 2014;41:80-5. doi: 10.5653/cerm.2014.41.2.80, PMID 25045632
11. Witchel SF, Oberfield SE, Peña AS. Polycystic ovary syndrome: Pathophysiology, presentation, and treatment with emphasis on adolescent girls. J Endocr Soc 2019;3:1545-73. doi: 10.1210/js.2019-00078, PMID 31384717
12. Firouzabadi RD, Aflatoonian A, Modarresi S, Sekhavat L, MohammadTaheri S. Therapeutic effects of calcium and Vitamin D supplementation in women with PCOS. Complement Ther Clin Pract 2012;18:85-8. doi: 10.1016/j.ctcp.2012.01.005, PMID 22500844
13. Gowri C, Ramana GV. Impact of socio-economic status on poly cystic ovarian syndrome (A study at Anantapur district, Andra Pradesh). East Afr Scholars J Med Sci 2020;3:21-5
14. Shojaeian Z, Sadeghi R, Roudsari RL. Calcium and Vitamin D supplementation effects on metabolic factors, menstrual cycles and follicular responses in women with polycystic ovary syndrome: A systematic review and meta-analysis. Caspian J Intern Med 2019;10:359-69. doi: 10.22088/cjim.10.4.359, PMID 31814932
15. Thys-Jacobs S, Donovan D, Papadopoulos A, Sarrel P, Bilezikian JP. Vitamin D and calcium dysregulation in the polycystic ovarian syndrome. Steroids. 1999;64:430-5. doi: 10.1016/s0039-128x(99)00012-4. PMID: 10433180.
16. Yildizhan R, Kurdoglu M, Adali E, Kolusari A, Yildizhan B, Sahin HG, *et al*. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. Arch Gynecol Obstet 2009;280:559-63. doi: 10.1007/s00404-009-0958-7, PMID 19214546
17. Slyper AH, Kashmer L, Huang WM, Re'em Y. Acanthosis nigricans, Vitamin D, and insulin resistance in obese children and adolescents. J Pediatr Endocrinol Metab 2014;27:1107-11. doi: 10.1515/jpem-2013-0465, PMID 25010776
18. Mazloomi S, Sharifi F, Hajihosseini R, Kalantari S, Mazloomzadeh S. Association between hypoadiponectinemia and low serum concentrations of calcium and Vitamin D in women with polycystic ovary syndrome. ISRN Endocrinol 2012;2012:949427. doi: 10.5402/2012/949427, PMID 22363895
19. Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber TR, *et al*. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. Eur J Endocrinol 2009;161:575-82. doi: 10.1530/EJE-09-0432, PMID 19628650
20. Subashree I, Valvekar UR, Prasad G. Study of serum calcium and Vitamin D levels with hormonal profile along with biochemical profile in women with polycystic ovary syndrome. Int J Reprod Contracept Obstet Gynecol 2017;6:4075-80. doi: 10.18203/2320-1770.ijrcog20174065
21. Mazloomi S, Sharifi F, Hajihosseini R, Kalantari S, Mazloomzadeh S. Association between hypoadiponectinemia and low serum concentrations of calcium and Vitamin D in women with polycystic ovary syndrome. ISRN Endocrinol 2012;2012:949427. doi: 10.5402/2012/949427, PMID 22363895
22. Lagunova Z, Porojnicu AC, Lindberg F, Hexeberg S, Moan J. The dependency of Vitamin D status on body mass index, gender, age and season. Anticancer Res 2009;29:3713-20. doi: 10.14341/2071-8713-4886, PMID 19667169
23. Kotsa K, Yavropoulou MP, Anastasiou O, Yovos JG. Role of Vitamin D treatment in glucose metabolism in polycystic ovary syndrome. Fertil Steril 2009;92:1053-8. doi: 10.1016/j.fertnstert.2008.07.1757, PMID 18930208
24. Thacher TD, Clarke BL. Vitamin D insufficiency. Mayo Clin Proc 2011;86:50-60. doi: 10.4065/mcp.2010.0567, PMID 21193656
25. 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;25:15-28. doi: 10.1515/hmbci-2015-0051, PMID 26943610
26. Toosi P, Azizan Z, Yavari H, Fakhim TH, Amini SH, Enamzade R. Serum 25-hydroxy Vitamin D levels in patients with acne vulgaris and its association with disease severity. Clin Cases Miner Bone Metab 2015;12:238-42. doi: 10.11138/ccmbm/2015.12.3.238