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Study of Vitamin D and Calcium Levels in Patient with Rosacea

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ABSTRACT

Background: Rosacea is a chronic skin condition that primarily affects the central face, and is often characterized by flare-ups and remissions. The increase in serum vitamin D levels is associated with rosacea. Rosacea may be happened due to high level of vitamin D that increases level of cathelicidin, which affect inflammatory process and vascular response. In the other hand Ca level does not have relationship to the cause of rosacea.

Objective: To investigate the relationship between serum vitamin D and calcium levels in patients with rosacea and analyze the association of vitamin D with clinical features.

Patient and methods: This prospective case control study was carried out on thirty patients with rosacea and twenty age and gender matched healthy controls. All enrolled participants were subjected to a full history taking, general and detailed dermatological examination.

Results: Rosacea group showed significantly higher mean vitamin D level when compared to control group (25.5±5.3 versus 17.7±5.2) ($p<0.001$). Rosacea cases had 17 with optimal vitamin D levels and 13 with mild to moderate deficiency, while control group cases had 4 with optimal vitamin D level and 16 with mild to moderate deficient vitamin D levels. There was significant ($p=0.010$) difference between cases and controls regarding vitamin D status. Total and ionized calcium levels did not differ significantly ($p= 0.662$ and 0.888 respectively) between cases and control groups.

Conclusion: Increased vitamin D levels may lead to the development of rosacea. New findings can be increased the understanding of pathogenesis of rosacea and may lead to development of new treatment options for rosacea.

Keywords: Calcium, Rosacea, Vitamin D.

INTRODUCTION

Rosacea (L. rosaceus, rosy) is a chronic inflammatory and vascular skin condition that affects around 10% of the population [(1)]. In persons with darker complexion, masking of face redness by skin pigment, protective effects of melanin against UV radiation (an aggravating factor for rosacea), or hereditary predisposition to rosacea appear to be the most important factors contributing to the lower probability of diagnosis [(2, 3)]. Microbes, UV radiation, diet, extremes of temperature, (skin) barrier disruption, psychosocial stress, and hormones are all potential triggers for an increased innate immune response and/or neurovascular dysregulation that increase rosacea symptoms[(4-5)].

Vitamin D (also known as "calciferol") is a fat-soluble vitamin that can be found naturally in a few foods, added to others, or purchased as a dietary supplement. When ultraviolet (UV) rays from sunshine impact the skin and induce vitamin D production, it is also generated endogenously. Vitamin D received from the sun, meals, and supplements is physiologically inactive and must be activated in the body by two hydroxylations. Vitamin D is converted to 25-hydroxyvitamin D (25(OH)D), commonly known as "calcidiol," via the first hydroxylation, which takes place in the liver. The second hydroxylation, which takes place largely in the kidney, results in the physiologically active 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), commonly known as "calcitriol" [(6)].

Cathelicidin LL-37 is an important effector molecule of innate immunity in the skin. Ultraviolet B (UV-B) irradiation and microbial component increase vitamin D3 and toll like receptor 2 expression in keratinocytes leading to an increase of cathelicidin production. The production of cathelicidin, an antimicrobial peptide (AMP), is strongly increased in rosacea. [(7)].

In our study, we aimed to investigate the relationship between serum vitamin D levels in patients with rosacea and analyze the association of vitamin D with clinical features.

PATIENTS AND METHODS

30 patients suffering from rosacea aged 20 – 50 years old and 20 apparently normal subjects matched for age and sex were chosen as control, from June 2019 to the end of February 2020.

The rosacea patients were collected from the outpatient Dermatology and Andrology Clinic, Menoufia University Hospital at Menoufia Faculty of Medicine. The laboratory work was carried out in Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University.

Patients with acne vulgaris, seborrheic dermatitis, pustular folliculitis, and systemic lupus erythematosus were excluded from our study. Also, presence of any systemic chronic inflammatory or autoimmune disorders were considered as exclusion criteria.

Ethical approval:

Informed consent according to ethics was obtained from all participants (including cases and controls) after being informed about the aim and process of the study as well as applicable objectives. The study

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design was approved by the Ethical Committee of the Faculty of Medicine, Menoufia University and followed the tents of the Declaration of Helsinki.

The enrolled rosacea patients were subjected to the following: detailed history taking, complete general examination, detailed dermatological examination with determination of the distribution, clinical variants, the extent of the rosacea, and assessment of the disease severity by clinical score of rosacea.

The presence of one or more of the following main characteristics in a central facial distribution was used to diagnose rosacea: flushing (transient erythema), non-transient erythema, papules and pustules, and telangiectasia. We identified the following rosacea subtypes based on physical findings: erythematotelangiectatic, papulopustular, ocular, and phymatous.

Because there are so many different types of rosacea, the National Rosacea Society put together a team of rosacea specialists to create a subtype categorization system to help with diagnosis and therapy [8]. The consensus committee of the National Rosacea Society identified four categories and one variation, with severity ranging from mild to moderate to severe.

Blood samples:

A venous blood sample was taken from all studied groups and quantitative determination of 25-(OH) D was performed. Final measurement of vitamin D concentration was performed by the automatic Elisa reader. According to standards' concentration and the corresponding to the OD values, the standard curve linear regression equation was calculated out, and then the OD values of the sample on the regression equation were applied to calculate the corresponding sample's concentration.

Laboratory investigation:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human 25-dihydroxy vitamin D（25-OH-D) in samples. Add 25-dihydroxy vitamin D（25-OH-D) to monoclonal antibody enzyme well, which is pre-coated with human 25-dihydroxy vitamin D（25-OH-D) monoclonal antibody, incubation; then, add 25-dihydroxy vitamin D（25-OH-D) antibodies labeled with biotin, and combined with enzyme-linked immune-sorbent assay subjects using commercially available ELISA kits. Quantitative determination of 25-(OH) D level was performed using ELISA kits supplied from Sun Red Bio (Shanghai-China).

Statistical Analysis:

The results were statistically analyzed by SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Statistics were calculated in terms of number and percentage for qualitative data. Mean, standard deviation (SD) was used for quantitative continues group. Chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. Correlation analysis was used to assess the strength of association between two quantitative variables. The ROC curve (receiver operating characteristic) was constructed to evaluate the sensitivity and specificity for quantitative diagnostic measures.

RESULTS

The difference between control and rosacea group was insignificant as regard age and gender (Table 1).

Table (1): Demographic data of the studied population

		Control (N=20)		Rosacea (N=30)		P
Age (years)	Mean±SD	33.4	±4.7	34.1	±5.7	0.651
Males	N, %	2	10%	6	20%	0.345
Females	N, %	18	90%	24	80%	

SD: standard deviation

Rosacea group showed significantly higher mean vitamin D level when compared to control group. There was significant difference between cases and controls regarding vitamin D status. Total and ionized calcium levels did not differ significantly between cases and control groups (Table 2).

Table (2): Comparison of vitamin D and calcium levels between all studied groups

			Control (N=20)		Rosacea (N=30)		P
25 (OH) D (ng/mL)		Mean±SD	17.7	±5.2	25.5	±5.3	<0.001
Vitamin D level	Optimal level	N, %	4	20%	17	56.7%	0.010
	Mild to moderate deficiency	N, %	16	80%	13	43.3%	
Total calcium (mg/dL)		Mean±SD	8.9	±1.1	9	±1	0.741
Ionized calcium (mg/dL)		Mean±SD	4.6	±0.4	4.7	±0.5	0.458

SD: standard deviation

(T-test is not suitable to compare 25 (OH) D because samples are not normally distributed. Use another test, e.g. Mann-Whitney test)

Vitamin D showed good AUC at cut off point of 19.6. Total calcium showed failed AUC at cut off value of 8.9. Ionized calcium showed failed AUC at cut off value of 4.7. Combination of studied markers increased validity of each separated

marker (AUC=850) (Table 3).

Table (3): ROC curve for validity of vitamin D and calcium levels for discrimination between rosacea cases and control groups

	25 (OH) D	Total calcium	Ionized calcium	Vit D+Ca+iCa
AUC	0.847	0.528	0.571	0.850
Cut off point	19.6	8.9	4.7	-
Sensitivity (%)	80	56.7	53.3	80
Specificity (%)	75	45	60	65
PPV (%)	82.8	60.7	66.7	77.4
NPV (%)	71.4	40.9	46.1	68.4
Accuracy (%)	78	52	56	74

AUC: Area under the curve, PPV: Positive predictive value, NPV: Negative predictive value

Moderate flushing and non-transient erythema higher frequency were significantly associated with optimal vitamin D level. Higher score was significantly associated with optimal vitamin D level. Otherwise, no significant differences were found regarding clinical data according to vitamin D status in all studied cases (Table 4).

Table (4): Comparison of clinical data according to vitamin D status in all studied cases

			Optimal level N=17		Mild to moderate deficiency N=13		P
Flushing	Mild	N, %	9	52.9%	12	92.3%	**0.020**
	Moderate	N, %	8	47.1%	1	7.7%	
Non transient erythema	Mild	N, %	5	29.4%	9	69.2%	**0.030**
	Moderate	N, %	12	70.6%	4	30.8%	
papules pustules	Absent	N, %	2	11.8%	2	15.4%	0.651
	Mild	N, %	6	35.3%	7	53.8%	
	Moderate	N, %	8	47.1%	3	23.1%	
	Severe	N, %	1	5.9%	1	7.7%	
Telangiectasia	Mild	N, %	6	35.3%	6	46.2%	0.547
	Moderate	N, %	11	64.7%	7	53.8%	
Burning	Mild	N, %	8	47.1%	8	61.5%	0.431
	Moderate	N, %	9	52.9%	5	38.5%	
Plaques	Absent	N, %	3	17.6%	2	15.4%	0.325
	Mild	N, %	4	23.5%	2	15.4%	
	Moderate	N, %	7	41.2%	9	69.2%	
	Severe	N, %	3	17.6%	0	0.0%	
Dry appearance	Mild	N, %	17	100.0%	13	100.0%	1
Edema	Absent	N, %	0	0.0%	2	15.4%	0.325
	Mild	N, %	4	23.5%	2	15.4%	
	Moderate	N, %	13	76.5%	9	69.2%	
Ocular manifestation	Absent	N, %	3	17.6%	2	15.4%	0.869
	Mild	N, %	14	82.4%	11	84.6%	
Phymatous manifestation	Absent	N, %	6	35.3%	9	69.2%	0.139
	Mild	N, %	1	5.9%	0	0.0%	
	Moderate	N, %	10	58.8%	4	30.8%	
Granulomatous manifestation	Absent	N, %	3	17.6%	4	30.8%	0.361
	Mild	N, %	7	41.2%	7	53.8%	
	Moderate	N, %	7	41.2%	2	15.4%	
Score		Mean±SD	15.5	±3.2	12.9	±3.4	**0.044**

SD: standard deviation

No significant associations were found regarding total calcium level according to different studied parameters in rosacea group (Table 5).

Table (5): Comparison of total calcium level according to different studied parameters in rosacea group

	Total calcium	p

		N	mean	±	SD	
Gender	Male	6	9.2	±	0.6	0.528
	Female	24	8.9	±	1.1	
Family history	Negative	11	8.6	±	1.1	0.054
	Positive	19	9.3	±	0.8	
Smoking	Negative	26	9.0	±	1.1	0.724
	Positive	4	9.2	±	0.3	
Relation to sun exposure	Less	14	9.3	±	0.8	0.171
	Excess	16	8.8	±	1.1	
Relation to diet	Less	7	9.1	±	1.1	0.822
	Excess	23	9.0	±	1.0	
Relation to psychological stress	Less	7	9.1	±	0.8	0.826
	Excess	23	9.0	±	1.1	
Onset	gradual	19	8.8	±	1.1	0.199
	sudden	11	9.3	±	0.8	
Course	Stationary	21	9.0	±	1.1	0.800
	Progressive	9	9.1	±	0.6	
Scar	Absent	25	8.9	±	1.0	0.316
	Present	5	9.4	±	1.0	

SD: standard deviation

Total and ionized calcium showed significant positive correlation in rosacea group. Otherwise, no significant correlation of vitamin D and calcium with other parameters in rosacea group was found. Clinical score showed significant positive correlation with 25 (OH) D level (Table 6).

Table (6): Correlations of vitamin D and calcium with other parameters in rosacea group

	25 (OH) D		Total calcium		Ionized calcium	
	r	p	r	p	r	p
Age	0.204	0.279	0.043	0.823	0.19	0.315
BMI	0.022	0.909	0.154	0.416	0.125	0.509
Duration	0.082	0.667	0.049	0.798	-0.087	0.649
25 (OH) D	-	-	0.128	0.499	0.011	0.955
Total calcium	-	-	-	-	0.518	0.003
Score	0.510	0.004	0.075	0.695	0.062	0.745

DISCUSSION

A prospective case control study was conducted. Participants were divided into 2 groups, group I (Cases) includes 30 patients. Patient and control group were age, sex and BMI matched. Rosacea group showed significantly higher mean vitamin D level when compared to control group. That rosacea group was significantly associated to sun exposure, diet habits and stress.

Regarding sex in our study, there was female predominance by (80%) compared to (20%) of male patients with rosacea. This agreed with **Crawford *et al.* [9], Jansen and Plewig [10] and Kyriakis *et al.* [11]** who showed that most studies reported the disease to be more common in women, but to develop into phymatous stages is more frequently in men.

Watson *et al.* [12] showed that symptoms present in various combinations and degrees of severity, often fluctuating between periods of exacerbation and remission, and the present study, showed that the mean disease duration was 3.1 ±0.8 months. Disease onset was gradual in 63.3%, while was acute in 36.7%. Disease course was stationary in 70%, while was progressive in 30%.

Also, our study showed that BMI, family history and smoking did not differ significantly between cases and control groups. In contrary with our results, **McAleer *et al.* [13]**, showed that family history of rosacea was found in up to 30% of patients in some case-series and in 11% of those in the above-mentioned study from Ireland. In the study from Estonia, a family history of rosacea was associated with an odds ratio (OR) for the disease of 4.31 (95% confidence interval (95% CI), 2.34-7.92) [14]. On the other hand, a report of rosacea in only one of two monozygotic female twins suggests a crucial role for environmental factors [15].

Our study showed that rosacea group was significantly associated to sun exposure, diet habits and stress, which are in accordance with the results shown by **Schauber[16] and Schauber and Gallo[17]** in the pathogenesis of rosacea, UV irradiation increases the level of vitamin D17. Subsequently, vitamin D increases the cathelicidin peptides and LL-37. The cathelicidin peptide and LL-37 induces the expression

of proinflammatory cytokines in keratinocytes, chemotaxis of adaptive immune cells and angiogenesis [18].

In our study, moderate flushing and non-transient erythema higher frequency were significantly associated with optimal vitamin D level. Higher score was significantly associated with optimal vitamin D level. Otherwise, no significant differences were found regarding clinical data according to vitamin D status in all studied cases.

Yamasaki *et al.* [18] showed that patients with rosacea express abnormally high levels of cathelicidin in the LL-37 peptide. Based on this evidences and our preliminary results, increased vitamin D levels may lead to the development of rosacea and we can speculate that this effect can arise through the cathelicidin peptides. In our study, serum vitamin D mean levels were low in both patient and control groups. But patients with rosacea have relatively high serum vitamin D levels compared to control groups.

Rosacea cases had 17 with optimal vitamin D level and 13 with mild to moderate deficiency, while control group cases had 4 with optimal vitamin D level and 16 with mild to moderate deficient vitamin D level. There was significant difference between cases and controls regarding vitamin D status.

Gombart *et al.* [19] found a vitamin D response element in the promoter of the cathelicidin gene. In addition, it was found that activation of the inflammatory signal system passing through TLR2 augmented the action of 1α-hydroxylase(CYP27B1), an enzyme converting 25(OH)D3 into the activated form 1,25(OH)2D3[20]. In consideration of the high recurrence rate in rosacea, the authors hypothesized that the systemic vitamin D level might be associated with the cathelicidin level in the skin and body. In this context, one study reveals that serum vitamin D levels are elevated in patients with rosacea compared to control group [21].

In the other hand, **Schauber J *et al.*** [7] reported that dysfunction of cathelicidin is related with the pathogenesis of several cutaneous diseases including atopic dermatitis, psoriasis and rosacea and **Xu *et al.*** [22] showed in the study on Chinese patients with vitiligo that there was no association between vitamin D levels and onset of vitiligo, however, they demonstrated that 25(OH)D deficiency may be associated with autoimmune disorders.

In our study, vitamin D and total calcium failed to discriminate between stationary and progressive rosacea cases. No significant differences were found regarding rosacea features according to vitamin D status in all studied cases. Excess sun exposure was significantly associated with higher vitamin D level. Higher frequency of sun exposure, hyperglycemic diet, psychological stress, higher level of vitamin D were suggested to be independent risk predictors for rosacea development in uni- and multivariable analyses.

Our study showed that vitamin D level was significantly higher in rosacea cases when compared to the control group and can be used for the diagnosis of rosacea cases at cut off point of 19.6 with 80% sensitivity 75% specificity, 82.8% PPV, 71.4%, NPV and 78% accuracy. Vitamin D, total and ionized calcium had no association with demographic, risk factors or rosacea features. Rosacea patients have significantly higher mean vitamin D level when compared to control group. Rosacea cases had 34 with highly optimal vitamin D level and 26 with mild to moderate deficiency, while control group cases had 5 with optimal vitamin D level and 25 with mild to moderate deficient vitamin D level. There was significant difference between cases and controls regarding vitamin D status.

These data suggests that increased vitamin D levels may lead to the development of rosacea. The new findings can increase the understanding of pathogenesis of rosacea and may lead to development of new treatment options for rosacea.

Limitations of the study were: Firstly, relatively small sample size. Secondly, it is well known that every disorder has many risk factors (genetic, environmental, racial...etc.), but we tried to fix the race (Egyptians) with the age and sex categories. Thirdly, no follow up period. Fourthly the blood samples were taken from the patients in the winter period (November-February) and the mean age and BMI were high.

CONCLUSION

The result of our study suggests that rosacea may happen due to high level of vitamin D that increase level of calcitonin, which affect inflammatory process and vascular response. In other hand Ca level does not have relationship to rosacea cause. These findings increases the understanding of pathogenesis of rosacea and leads to new treatment options for rosacea. Block the expression of cathelicidin via the vitamin D pathway may help to control rosacea.

Larger series of studies in different centers, which will support these results are needed. Further studies are needed to prove the involvement of vitamin D and calcium in rosacea and its relation with cathelicidin level.

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