Original Research Paper

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Pathology

CORRELATION OF PERIPHERAL BLOOD EOSINOPHILIA WITH VITAMIN D DEFICIENCY- REALITY OR MYTH

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ABSTRACT **Introduction:** Vitamin D is an important fat-soluble vitamin. It is required for normal calcium homeostasis. It is involved in various physiological processes like bone mineralization, muscle activity, nerve conduction as well as in cellular proliferation. It also plays an important role in inflammation and immune reactions. Currently immunomodulatory role is in focus. It is suggested that Vit D has got a protective role in allergic disorders like asthma, atopic dermatitis etc, where its deficiency is associated with increased eosinophilia and raised IgE levels. **Methods:** In this retrospective study, total 528 cases without history of allergies or parasitic infections, from a 6-month period were selected who underwent complete blood count (CBC) and serum Vitamin D testing on same day. CBCs were analysed for total leukocyte count, absolute eosinophils count, absolute neutrophils and absolute lymphocyte count. Serum vitamin D were evaluated and stratified into sufficient, insufficient, deficient, severe deficient categories. Standard statistical analysis was using MS excel and Medcalc software. **Results:** Out of 528 cases, 252 were males and 276 were females. Most of the cases were from age group 21-30 years with age range of 2 to 89 yrs. Prevalence of Vitamin D deficiency was only noted in 13.83% cases with preponderance of females. No statistical correlation observed in total leukocytes, eosinophils, neutrophils or lymphocytes among Vit D stratification groups. **Conclusion:** Study showed lower prevalence of 13.83% as compared to other studies. No correlation between eosinophil count and vitamin d deficiency was noted.

KEYWORDS : Vitamin D, Vitamin D Deficiency, Eosinophils, Eosinophilia

INTRODUCTION

Vitamin D is a fat-soluble vitamin. Generally, it is Vitamin D3 or cholecalciferol, most of which is synthesized endogenously in skin from 7 dehydrocholesterol under sun exposure by action of ultraviolet B (UV B) rays. Vitamin D (vit D) is also found in dietary sources like fish and fortified food and supplements.[1,2] Though Indian subcontinent comes under tropical and subtropical areas, prevalence of vitamin deficiency is very much prevalent.[3] The factors attributed contributing despite adequate sun exposure include increased indoor life style, increased skin pigment, cultural habits like Burqa or purdah system, increased pollution and dietary inadequacy as well as avoiding sun exposure due to fear of darkening.[2,4]

Vit D plays a major role in maintaining blood calcium & phosphorous levels where it plays important role in processes bone mineralization and muscle contraction. It is also found important various physiological & pathological conditions like cell proliferation, cell differentiation, inflammation and immune function.[2] Its role as an immunomodulator is under focus in current days. Immunomodulatory effect of Vit D is mainly in the form of anti-inflammatory effects carried through Vit D receptors present in immune system. [5]

Vitamin D deficiency (VDD) is considered as a most common nutrition deficiency and probably most common medical condition globally. [4]

Hiraguchi et al [6] demonstrated that 1,25-(OH) 2 D 3 regulates CXC chemokine receptor type 4 (CXCR4) expression in eosinophils which may be involved in eosinophil recruitment to noninflammatory sites. It modified a migratory function of eosinophils while maintaining viability.

According to the above study, Eosinophils are mainly engaged in allergic responses by acting through effector arm of T-helper type 2 (Th2) cell immunity. Various allergic diseases like asthma, atopic dermatitis and allergic rhinitis have shown association with low serum vit D3 levels. This supports a protective role of Vit D in this allergic disease.

A few studies have noted that lower levels of vit D are associated with an increased blood eosinophil count, but most other studies have reported no significant association. However, no significant correlation between levels of serum Vit D and peripheral blood eosinophils is established yet. [5,7]

Hence in this context, we tried to assess the correlation between serum Vit D and peripheral blood absolute eosinophil count.

MATERIAL & METHODS-

This was a retrospective cross sectional record-based study in which we retrieved the data of patients who were referred to our laboratory from outpatient department of our hospital from period of 6 months (January to June 2022). Patients who underwent complete blood count (CBC) and vitamin D3 - 25(OH) D3 testing on same day were included in the study. Relevant clinical data like clinical history, demographic details like age and sex were recorded from the archives. The patients with known history of asthma, allergies, suspected parasitic infestation, etc were excluded. From complete blood count the following data was analysed including total leukocyte count, absolute eosinophils count, absolute neutrophils and absolute lymphocyte counts. Total 528 cases were selected as per inclusion criteria mentioned.

Peripheral venous samples were collected from the patients for evaluation of serum Vit D3 and complete blood count (CBC) testing. Serum Vit D3 testing was carried out using electrochemiluminescence method using VITROS 5600 analysers (Ortho diagnostics). While CBC was performed using Sysmex XN 3100 Automated 6-part analyser. The values of total leukocyte count and absolute eosinophils, neutrophils and lymphocytes were noted. The cases with eosinophilia were re-evaluated using Wright- Giemsa-stained peripheral blood smears for confirmation.

Eosinophilia was defined as the absolute eosinophil count above or equal to 500 /cmm. [8] Eosinophilia has been arbitrarily stratified into mild (500-1500/cmm), moderate (1500-5000/cmm) and severe (>5000/cmm) eosinophilia, though its practical significance is not clear.

Vitamin D3 or 25 (OH) D3 is the major circulating form of Vitamin D and is considered as the marker of vitamin D status.[2,9] The levels were further categorised as sufficient (>30 ng/ mL), insufficient (20 to 30 ng/mL), deficient (10 to 20 ng/mL) and severely deficient (<10 ng/mL), hypervitaminosis (>100 to 150 ng/ml) and toxic (>150 ng/ml) as per previous studies.

Standard statistical analysis in the form of mean, standard deviation and percentage was performed using MS excel and difference between observed means was calculated using Medcalc statistical calculator with a significant p value of $p<0.05$.

RESULTS-

Total 528 cases satisfied the inclusion criteria. Out of 528 cases, 252 were males and 276 were females. Age range between 2 years to 89 yrs. Most of the cases were from age group 21-30 years. (Figure 1). Range of vit D3 from 8 to 137 ng/ml. (Figure 2) Out of all cases, 313 cases showed sufficient vit D levels including 7 cases of hypervitaminosis, 142 were insufficient, 70 were deficient and 3 were severely deficient. (Figure 3) While none of the case showed toxic vit D levels. Vitamin D deficiency noted mostly in females (68%) as compared to males (32%). With standard statistical analysis, we found no any statistical correlation between mean total leukocytes, mean neutrophils or mean eosinophils among four vitamin d stratification groups. Characteristics of various parameters among vitamin d status stratification are shown in table 1 while table 2 shows comparison with a similar study conducted by Filho et al.

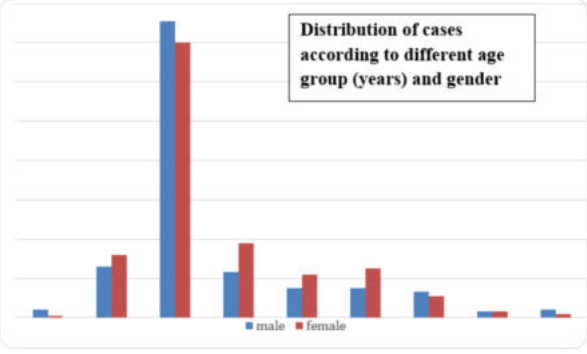

Figure 1- Distribution Of Cases According To Different Age Group (years) And Gender

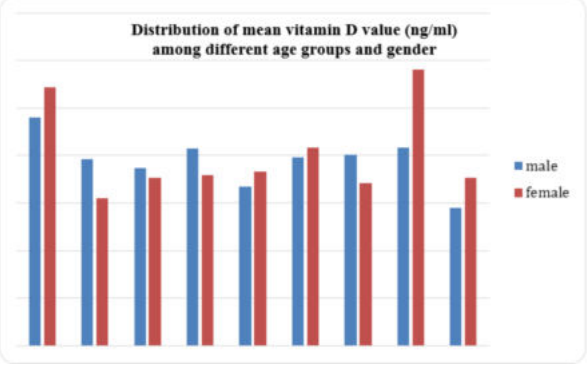

Figure 2- Distribution Of Mean Vitamin D Value Among Different Age Groups (years) And Gender

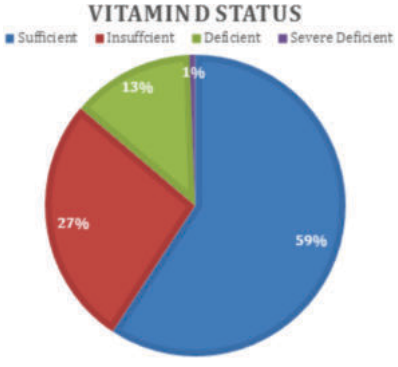

Figure 3- Vitamin D Status Among Study Population

Table 1- Comparison Of Different Parameters With Vitamin D Stratification Groups

	Sufficient (Vit D >30 ng/ml)	Insufficient (Vit D >20 to 30 ng/ml)	Deficient (Vit D >10 to 20 ng/ml)	Severely Deficient (Vit D <10 ng/ml)
No of cases (n)	313	142	70	3
Males (n)	155 (49.52%)	74 (52.11%)	22 (31.43%)	1 (33.33%)
Females (n)	158 (50.48%)	68 (47.89%)	48 (68.57%)	2 (66.67%)
Mean Vit D3 (ng/ml) +/- SD (range)	47.18 +/- 17.19392 (30 to 137)	26.89 +/- 2.964834 (20 to 29.9)	16.33 +/- 2.699 (10 to 19.9)	8.43 +/- 0.750555 (8 to 9.3)
Mean age (years) (range)	33.08 +/- 15.89749 (2 to 89yrs)	29.6 +/- 14.31161 (8 to 81yrs)	27.51 +/- 11.89781 (12-67 yrs)	28 +/- 21.07131 (8-50 yrs)
Mean TLC (cells/lL) +/- SD (range)	7747.54 +/- 2358.35025 (3.05 to 27.57)	7707.77 +/- 2357.162 (3.73 to 16.09)	7816.57 +/- 1827.557 (4.79 to 12.67)	9373.33 +/- 1945.696 (8.24 to 11.62)
Mean AEC (cells/lL) +/- SD (range)	238.21 +/- 206.62541 (0 to 1720)	258.04 +/- 227.9983 (0 to 1323)	207.09 +/- 148.258 (0 to 616)	297.4 +/- 56.29387 (232 to 300.4)
Mean ANC (cells/lL) +/- SD	4400.73 +/- 1564.38542	4430.69 +/- 1678.884	4702.33 +/- 1500.026	4606.67 +/- 2059.216
Mean ALC (cells/lL) +/- SD	2528.76 +/- 1192.7378	2472.43 +/- 847.5368	2299.83 +/- 733.25	3564.27 +/- 596.3108
Cases of eosinophilia n (%)	32 (10.22%)	13 (9.15%)	4 (5.71%)	0

Table 2- Comparison Of Study Parameters With Similar Study By Filho et al

	Filho et al (2018)	Present Study (2022)
Characteristics	N = 669	N=528
Gender		
Male, n (%)	139 (20.8)	252 (47.727%)
Female, n (%)	530 (79.2)	276 (52.272%)
Age, mean ± SD (median)	44.5 ± 19.3 (43)	31.38068 +/- 15.14149
Vitamin D3 (ng/mL), mean ± SD (median)	32.2 ± 11.1 (30.9)	36.93 +/- 18.41918
Severely deficient (≤10 ng/mL), n (%)	11 (1.6)	3 (0.568)
Deficient (>10 ng/mL and ≤20 ng/mL), n (%)	62 (9.3)	70 (13.257)
Insufficient (>20 ng/mL and ≤30 ng/mL), n (%)	238 (35.6)	142 (26.893)
Sufficient (>30 ng/mL), n (%)	358 (53.5)	313 (59.279)
Leukocyte count (cells/lL), mean ± SD	6247.4 ± 1859.9	6040.896 +/- 2292.96
Neutrophil count (cells/lL), mean ± SD	3395.1 ± 1287.1	4449.945 +/- 1588.662
Lymphocyte count (cells/lL), mean ± SD	2190.4 ± 777.3	2489.144 +/- 1057.62
Eosinophil count (cells/lL), mean ± SD	192.6 ± 192.63	239.7538 +/- 205.7339

DISCUSSION

Prevalence of Vitamin deficiency in various hospital-based studies is variable in different populations. Our study showed lowest prevalence of 13.83% among OPD patients which was significantly lower as compared to other studies except Filho et al [5] which showed 10.9% prevalence. In other similar study carried out in OPD patients by Bawaskar et al (2017) [10] showed prevalence of 65.4% while Shukla et al (2016) [11] found 93% cases among health check-up of executives. Pal

et al (2016) [12] found prevalence of 61.2% in orthopaedic OPD & emergency patients. While studies conducted by Ajmani et al., 2016[13], Dasgupta et al., 2012[14]Sharma et al., 2016[15] and Kumar et al., 2015[16] in a population of pregnant females showed prevalence of 37.5%, 42%, 93.5% and 70.5% respectively. Basu et al., 2015[17] & Garg et al., 2014[18] showed prevalence of 52.9% & 96.9% in young children respectively. [2]

Nishant raizada et al [4] Our study found hypervitaminosis D in 319 (1.2%) and vitamin D toxicity in 27 (0.1%) among 26,339 measurements, while Sharma et al. [16] reported hypervitaminosis in 225 (4.7%) and vitamin D toxicity in 151 (2.8%) among 5527 patients as compared to our study where 7 cases out of 528 (1.36%) were of hypervitaminosis and no case of Vit D toxicity was found.

Hypervitaminosis D may be caused due to supplementation overdose or prescription errors. Nishant et al suggested that there is rise in cases of hypervitaminosis over time which may be attributed increased awareness of vitamin d deficiency among the treating doctors.[4]

Vitamin D deficiency (VDD) has an impact not only on skeletal system producing rickets and osteomalacia, but also on other diseases like heart disease, autoimmune disease, infections like tuberculosis, depression, Obesity, Parkinson's disease. [2,19]

Lu et al [20] showed that VDD induces Eo activation to release inflammatory mediators. In a Vit D deficient environment**,** EoL-1 (eosinophil cell line 1) cells produced high levels of the Eosinophilic mediators, including major basic protein (MBP), Eo peroxidase (EPX), Eo cationic protein (ECP) and Eo-derived neurotoxin (EDN), which could be suppressed by the addition of calcitriol to the culture. EoL-1 cells expressed Vit D receptor (VDR), which was up regulated by exposure to calcitriol. They suggested that Vit D deficiency related Eo activation may play an important role in the pathogenesis of the Eo-related chronic inflammation. Vit D may be used as supplements in the treatment of allergic diseases. [20]

In various allergic disorders like bronchial asthma, atopic dermatitis, allergic rhinitis as well as eosinophilic astroenteritis, various studies have shown significant correlation between peripheral blood eosinophilia with low serum Vit D3 levels. Also, the protective roles of vit D supplementation in these diseases is also evaluated. [21,22,23]

In an animal study, Vilalva [24] et al found an elevated count of neutrophils, eosinophils, and basophils associated with reduced vit d concentrations among spring borne calves.

Various studies have been carried out to correlate haematological parameters with VDD which showed variable results. [25 -29] Sim et al [25] demonstrated an association of VDD and a greater risk of anemia, lower mean haemoglobin, and higher usage of erythrocyte-stimulating agents. Ismail et al [26] did not find any correlation between vit d def and haematological parameters like Hb, TLC etc. Kulling et al [19] in their review of role of vit d in haematological malignancies concluded that calcitriol suppresses pro-inflammatory cytokine production, restricts proliferation, and inhibits antibody production in normal lymphocytes. While in malignant cells, it inhibits proliferation, induces apoptosis, promotes differentiation, sensitizes malignant cells to anti-cancer therapies, and enhances cell cycle arrest.

There was no any statistical correlation between mean total leukocytes, mean neutrophils or mean eosinophils among four vitamin d stratification groups. Only mean Lymphocytes among severe deficiency vs deficiency and severe deficiency vs insufficiency groups were found to be statistically significant with p value of p=0.004 & p=0.028 respectively. However no significant correlation among sufficient and deficient groups. Also, the prevalence of eosinophilia among all cases was low (9.28%) while was more or less similar in deficient cases (5.71%).

Thus, our study did not find any significant correlation between serum vitamin D3 and peripheral blood eosinophil count or eosinophilia. Filho et al [5] only showed statistically significant results of mean absolute eosinophil count among Vit D stratification groups with no significant eosinophilia in OPD population without any allergic disorders.

In conclusion, in a hospital based OPD settings our study failed to show any correlation between absolute eosinophil count and serum vit d levels. Hence further studies may be needed to prove or disprove the assumption of peripheral blood eosinophilia among vit d deficient individuals.

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