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A study to evaluate the vitamin D status in rheumatoid arthritis patients

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Abstract

Introduction: Vitamin D deficiency has been shown to be associated with autoimmune diseases, which is a controversial finding in various previous studies. Moreover there are limited studies done on serum vitamin D and its association with rheumatoid arthritis in Indian population. Therefore the present study was designed to study the level of vitamin D in patients with rheumatoid arthritis and to compare it with healthy controls.

Materials and Methods: The study included 40 patients diagnosed for rheumatoid arthritis (RA) and 40 age and sex matched healthy controls who were willing to participate in the study from the department of orthopaedics and General medicine. After obtaining the written informed consent from all the participants, 2 ml of blood was collected in a plain vacutainer for the estimation of vitamin D by chemi-luminescent method.

Results: The cut off values of 25-OH vitamin D level, being <20ng/ml as deficiency, 29 -30ng/ml as insufficiency and >30ng/ml as normal. There was neither any statistical difference in the vitamin D levels nor in the vitamin D deficiency level (<20ng/ml) between the two groups (p= 0.302), 82.5% of the patients with RA had Vitamin D deficiency and 80% of the controls had vitamin D deficiency.

Conclusion: As vitamin D levels are significantly low in the healthy general population, this could be the reason for no association of vitamin D deficiency and rheumatoid arthritis.

Keywords: Rheumatoid arthritis, Auto-immune disease, Chemi-luminescent method, Vitamin D.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterised by chronic inflammation of the flexible joints and systemic features, leading to progressive disability and early death.(1) Arthritic diseases are the most common disease in the world which includes rheumatoid arthritis, osteoarthritis and gout, affecting around 0.3% -0.75% of the Indian population. Globally the prevalence of rheumatoid arthritis is more in women than in men of the age group 30 -50 years.(2)

Rheumatoid arthritis is a whole body disorder, primarily affecting the synovial joints. Early changes include inflammation and thickening of the synovial membrane. Late changes include destruction of the cartilage along with formation and deposition of pannus in the joint spaces. The inflammation and various symptoms such as fever, loss of weight and weakness is due to the release of a chemical mediator called cytokines. The etiology of the disease could be attributed to genetic and environmental factors; the exact cause is still unknown. Vitamin D could be one of the environmental factors linked to rheumatoid arthritis.(3)

Vitamin D is a steroid hormone involved in bone and calcium homeostasis. This hormone is synthesised in the skin by the action of ultraviolet rays and from dietary sources, but the vitamin D amount is very little in the food, attributing to only 20% of the body requirement.(4) Exposure to ultraviolet B rays results in conversion of 7- dehydrocholestrol, present on the epidermal layer of the skin to vitamin D3, followed by the two step activation process occurring firstly in the liver causing hydroxylation at the 25th position and secondly the kidney causing hydroxylation at the first position, hence forming 1, 25 dihydroxycholecalciferol (calcitriol), the active form of vitamin D.(5) The cut off values of 25-OH vitamin D level being < 20ng/ml is deficiency, 29 -30ng/ml is insufficiency and >30ng/ml is normal.(6)

Vitamin D deficiency has been shown to be associated with autoimmune diseases, such as diabetes mellitus type I and multiple sclerosis. The major biological action of calcitriol are mediated through vitamin D receptors, the detection of these receptors in the monocytes, macrophages and dendritic cells may release 1-alpha hydroxylase enzyme that helps synthesise calcitriol and this could have an autocrine and paracrine effect causing suppression of T cell proliferation, antigen presenting cells and it also regulates the immune cells that produce pro inflammatory proteins called cytokines, hence there could be an association between vitamin D and rheumatoid arthritis disease.(7) Therefore, the present study has been designed to evaluate the status of vitamin D in rheumatoid arthritis patients.

Materials and Methods

The present study is a case control hospital based study performed after the institutional ethical clearance. The study included 40 patients diagnosed for rheumatoid arthritis and 40 age and sex matched

healthy controls who were willing to participate in the study from the department of orthopaedics and General medicine. Patients with chronic illness, obesity, renal dysfunction and liver disorder, malabsorption syndromes like obstructive jaundice and steatorrhea, subjects who have taken vitamin D supplements in the past 6months, subjects with Type- 1 diabetes mellitus and multiple sclerosis and subjects on anticonvulsants and medications to treat AIDS/HIV were excluded.

After obtaining the written informed consent from all the participants, 2 ml of blood was collected in a plain vacutainer for the biochemical analysis. The blood samples in plain tube was left undisturbed for 20 minutes to clot and then centrifuged at 4000 rpm for 10 minutes so as to obtain the serum which was analysed for vitamin D. The total Vitamin D level was estimated using chemi-luminescent method as follows.

1st incubation: By incubating the sample (15 microlitre) with pre-treatment reagent 1 and 2, bound vitamin D (25-OH) is released from the vitamin D binding protein.

2nd incubation: By incubating the pre-treated sample with the ruthenium labelled vitamin D binding protein, a complex between the vitamin D (25- OH) and the ruthenylated vitamin D binding protein is formed.

3rd incubation: After adding of streptavidin coated micro particles and vitamin D labelled with biotin, unbound ruthenium labelled vitamin D binding protein become occupied. A complex consisting of the ruthenylated vitamin D binding protein and the biotinylated vitamin D is formed and becomes bound to the solid phase via interaction of biotin and sreptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Procell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Statistical Analysis: The data was expressed as percentages and mean ± SD. Student t test was used to find the significance of study parameters between the two groups using SPSS versions16.

Results

The details regarding the participants were explained in Table 1. There were 45% of case and 72.5% of controls in the age group 25-40 yrs. 42.5% of case and 25% of controls belonged to the age group 41-55 yrs and 12.5% of case and 2.5% of controls belonged to the age group 56-70 yrs. Among all the participants 15% each of case and controls were males whereas, 85% each of case and controls were females. Among the participants, 82.5% of case and 80% of controls belonged to the group having vitamin D level <20 ng/ml. 10% of case and 15% of controls were belonged to the group having vitamin D level between 20-40 ng/ml and 7.5% of case and 5% of controls were belonged to the group having vitamin D level between 20-40 ng/ml respectively. The mean serum vitamin D level in RA patients was shown in Table 2. On comparison, there was no statistical difference in the mean serum vitamin D levels in the cases and controls (p= 0.379).

Table 1: Demographic Characteristics

Age wise distribution (in yrs)	**n (%)**
• 25-40	Cases: 18 (45%), Controls: 29 (72.5%)
• 41-55	Cases: 17 (42.5%), Controls: 10 (25%)
• 56-70	Cases: 5 (12.5%), Controls: 1 (2.5%)
Gender wise distribution (in yrs)	**n (%)**
• Males	Cases: 6(15%), Controls: 6 (15%)
• Females	Cases: 34(85%), Controls: 34 (85%)
Subgroups based on Vitamin-D level n (%)	
• <20	Cases: 33(82.5%), Controls: 32 (80%)
• 20-30	Cases: 4(10%), Controls: 6 (15%)
• >30	Cases: 3(7.5%), Controls: 2 (5%)

Table 2: Group-wise comparison of age and vitamin D level in the participants. Values are Mean ± SD. N=40 in each group

Parameters	**Cases**	**Controls**	**P value**
Age	44.35 ± 10.05	36.35 ± 9.45	0.0004
Vitamin D	14.14 ± 10.26	12.31 ± 8.07	0.379

Table 3: Comparison of Vitamin D levels between case and controls

Vitamin D level	**Cases (N=33)**	**Controls (N=32)**	**P value**
<20ng/ml	10.34 ± 4.65	9.11 ± 4.94	0.302
20-30 ng/ml	23.76 ± 2.13	23.14 ± 3.05	0.735
>30 ng/ml	43.09 ± 4.59	31.14 ± 0.63	0.040

As there was no statistical difference of vitamin D among cases and control, vitamin D level was compared between various age groups. The mean Vitamin D level (27.79 ± 15.44) was highest in the age group of 56 – 70 years. On comparison of Vitamin D level between group 1 vs 2 and group 1 vs 3 showed a significant difference (p=0.001) respectively (Fig. 1). Comparison of Vitamin D levels between case and controls was shown in table-3. There was no statistical difference in the vitamin D level between case and controls belongs to the group <20ng/ml, group 20-30ng/ml and group >30ng/ml (p=0.302, 0.735 and 0.040 respectively

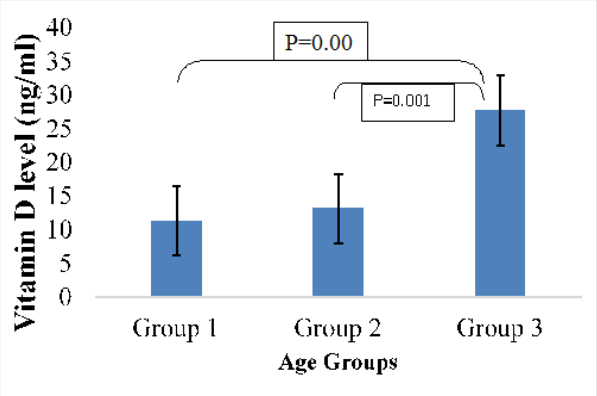

Fig. 1: Comparison of Vitamin D level in different age groups. Values are Mean ± SD. N=40 in each group. P=0.001 between group 1 vs 2 and group 1 vs 3 respectively

Discussion

This study was conducted to evaluate the status of vitamin D levels in patients of rheumatoid arthritis and apparently healthy individuals. The findings of this study indicated that neither the serum vitamin D levels nor the vitamin D deficiency in rheumatoid arthritis patients were significantly different from controls which is similar to a study done by Maurizio Rossini et al involving 1,191RA patients and 1,019 controls. They reported that 55% of RA patients were not on vitamin D supplements, of these 52% patients had vitamin D deficiency (<20 ng/ml) as compared to the controls 58.7%. In a study conducted by Turhanoflu AD et al too showed no significant difference in vitamin D levels between 65 RA cases and controls[8] and a study involving 108 RA cases and 239 healthy volunteers also showed no difference in the mean serum vitamin D levels between the two groups.[9]

In our study vitamin D deficiency has been found to be highly prevalent in the patients with RA as it is with the general population. Decreased vitamin D levels in the general population is seen in many other studies such as, a study in eastern India showed that vitamin D deficiency was present in all age groups, 47.5% of the study population had deficiency and 40% had vitamin D insufficiency. In a study involving 771 healthy subjects, 60% had vitamin D deficiency whereas 20% had sufficient levels[10] and a study done in North West Punjab by Rachna Bachhel et al showed prevalence (90%) of vitamin D deficiency.[11]

The Iowa women's health study by Merlino and colleagues analysed data from a prospective cohort study of 29,368 women aged 56- 69 years. 152 cases of RA were reported; they found that greater intake of vitamin D might be associated with a lower risk of RA.[12] The two cohort studies conducted by Costenbader and colleagues found that vitamin D intake was not associated with the risk of rheumatoid arthritis. The first study included 91,739 women and the second study included 94,650 women in the nurse's health study.[13] A cohort study conducted by Ifigenia Kostuoglou-Athanassiou and colleagues involved 44 patients with RA and 44 controls showed low levels of vitamin D compared with the controls and also found that vitamin D deficiency maybe linked to disease severity.[14]

Different metabolites of vitamin D levels were measured in 102 patients with rheumatoid arthritis and were divided into 3 groups according to their treatment regime by O.S. Als, et al. In all the 3 groups the mean serum 25-OH vitamin D3 levels were low as compared to controls.[15] A pilot study done by S. jagtap et al showed higher mean levels of vitamin D in rheumatoid arthritis patients than controls and there was no association of the incidence of RA with low vitamin D.[16] Calcium and vitamin D levels were measured by H.Kroger et al in 143 women with RA, calcium levels were normal whereas serum vitamin D levels were significantly low in these women. Among these RA patients 16% had vitamin D levels as low as12.5nmol/L which suggested of osteomalacia.[17]

From the results of the present study, we can conclude that, as vitamin D levels are significantly low in the healthy general population, this could be the reason for no association of vitamin D deficiency and rheumatoid arthritis in our study. The limitation of this study is that we did not assess the disease activity score and therefore the association between the disease activity and vitamin D could not be assessed.

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