

Normal or Arthritic: Is 25-hydroxy Vitamin D status significant?

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ABSTRACT

Background: Vitamin D is required for proper skeletal development and function. However, the status of vitamin D in healthy subjects and those suffering from arthritis in Nepal is largely unknown.

Objective: This study measured vitamin D level in healthy and arthritic individuals of Western Nepal.

Methods: Vitamin D level in healthy and arthritic subjects were measured by using LIASION 25-hydroxy Vitamin D assay, a direct competitive chemiluminescence immunoassay (CLIA).

Results: Our result suggested that most of the subjects, irrespective of age and disease condition, have subnormal/normal level of vitamin D (≥ 16 ng/mL). Also, the data suggested that serum vitamin D level is significantly higher in males than in females. Moreover, the vitamin D level is higher in healthy individuals when compared with those suffering from arthritis. However, vitamin D level in normal subjects and arthritic patients could not be correlated.

Conclusion: Vitamin D level is higher in normal subjects compared to arthritic individuals. However, the level could not be correlated suggesting need of a pilot study to determine vitamin D level and its association with arthritis in Nepalese.

Keywords

Arthritis, Nepalese, Vitamin D.

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INTRODUCTION

Vitamin D (25-hydroxy-Vitamin D), the sum of 25(OH)D₃ and 25(OH)D₂ is recognized as a pro-hormone which has multiple roles in maintaining optimal musculoskeletal health. Vitamin D₃ (cholecalciferol) and Vitamin D₂ (ergocalciferol) are the most abundant forms of Vitamin D in the body. Vitamin D₃ is synthesized in the skin from 7-dehydrocholesterol in response to sunlight while Vitamin D₂ is best obtained from food sources like vegetables, yeast, and fish. Both forms of vitamin D undergo hydroxylation reactions to yield 25-hydroxyvitamin D [25(OH)D] in the liver and 1,25-dihydroxyvitamin D [1,25(OH)₂D] in the kidney. Although 1,25(OH)₂D is the biologically active metabolite of vitamin D, circulating

25(OH) Vitamin D generally represents the best marker of vitamin D status in body¹. Serum vitamin D level <16 ng/ml, 16 - 20 ng/ml, and >20 ng/ml were considered to be deficient, subnormal, and normal respectively².

Globally, rheumatic and musculoskeletal disorders are the major cause of disability^{3,4}. Of the reported disorders, arthritis is the principle cause for the disability^{5,6}. The 2010 - 2012 National Health Interview Survey conducted in the USA predicted that around 26% of adults suffer from arthritis by 2040⁷.

Arthritis advances with increasing age, thereby elderly populations were mostly affected. It was found that self-reported arthritis was highly prevalent among elders from the Caribbean and Latin America and older Mexican

Americans⁵. It was estimated that 23.8% Mexicans and 55.6% Havanans have arthritis⁵. Similarly, in the Asian countries increase in the proportion of aged population⁸ suggested an increase in arthritis cases.

A meta-analysis found that low level of vitamin D in rheumatoid arthritis (RA) patients⁹. Similarly, low level of serum vitamin D has been found in arthritis patients from Mexico² or Italy/Estonia¹⁰. However, the status of vitamin D and its association with arthritis in Nepalese, in particular those from Western region are unknown. Herein, we analysed vitamin D levels in healthy subjects and arthritis patients, and determined for its association with the disorder.

METHODS

Subjects

Patients were examined to rule out arthritis based on signs, symptoms and physical examination according to criteria described previously^{2,11}. Five ml of venous blood sample were collected from 90 arthritic patients attending outpatient department of Paschimanchal Community Hospital, Pokhara-9, Kaski and private clinics in Pokhara. Blood specimen was kept in serum separating tubes and allowed for complete clot formation prior to centrifugation. Serum samples free of fibrin, red blood cells, or other particulate matter was prepared by repeated centrifugation and used in the assay. Any serum specimens that were delayed more than 24 hours for testing were stored at 2-8°C and were analyzed on following day. Similarly, control samples were obtained from 83 healthy volunteer subjects that do not show any symptoms of joint involvement. The study protocol was evaluated and approved from the Board of Paschimanchal Community Hospital, Pokhara-9, Kaski.

Measurement of Vitamin D

Vitamin D status in healthy and arthritic subjects was determined by measuring total serum 25-hydroxy Vitamin D level using LIASION 25 OH Vitamin D Total assay (DiaSorin, Stillwater, MN, USA). The assay is an antibody-based, fully automated, direct competitive two-step chemiluminescent immunoassay (CLIA). In the closed automated system, during the first incubation, 25-OH-D in sample is dissociated from its binding protein and allowed to bind to the specific antibody on the solid phase for 10 minutes. Then the tracer (25-OH-vitamin D linked to an isoluminol derivate) is added automatically and incubated for a further 10 minutes. Finally, the unbound material is removed with a washing cycle and the starter reagents are added to initiate a chemiluminescent reaction which

is measured by a photomultiplier as relative light units (RLU) that is inversely proportional to the concentration of 25-OH vitamin D present in the sample.

Statistical analysis

All the statistical analyses were done using GraphPad Prism version 5.0 (GraphPad software). Datasets on age distribution between arthritic and normal subjects were analyzed by analysis of variance (ANOVA); while those of the level of vitamin D were analyzed by t-test. The correlation coefficients (r) between the groups were determined by Spearman test. *P*-values less than 0.05 were considered significant.

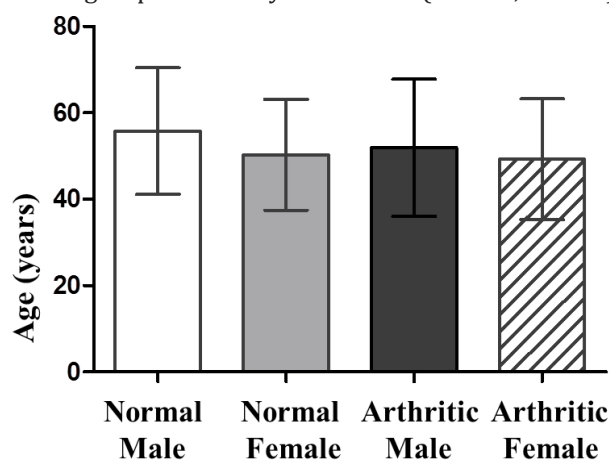
RESULTS

Distribution of age in arthritic and normal subjects

This study recruited 173 subjects of which 91 were males and 82 were females. Of 91 males, 42 were normal and 49 were arthritic. Similarly, of 82 females, 41 were normal and the remaining half was arthritic.

The mean age of normal males and normal females were 55.75 ±14.65 years (range 32 - 75 years) and 50.29 ±12.82 years (range 30 - 77 years), respectively. Similarly, the mean age of arthritic males and arthritic females were 51.91 ±15.85 years (range 30 - 83 years) and 49.22 ±14.02 years (range 32 - 79 years). As shown in Fig 1, the age of the subjects in the normal and arthritic group were similarly distributed (ANOVA, *P*=0.61).

Fig 1: Age wise distribution of study subjects. Histogram showed mean± standard deviation (SD) age of normal/arthritic male and female subjects. The age of the subjects in each group is similarly distributed (ANOVA; *P*=0.61)



Vitamin D level in normal and arthritic subjects

Vitamin D level in normal/arthritic males and females of age below 50 and above 50 were determined (Table 1).

We found that vitamin D level in normal males of age <50 years (Average = 22.62; range 12.44 - 29.36) were significantly lower than those in normal males of age >50 years (Average = 26.06; range 18.98 - 36.39) (t-test, $P=0.04$) (Table 1).

Similarly, vitamin D level in normal females of age <50 years (Average = 21.24; range 2.36 - 28.12) were significantly lower than those in normal females of age >50 years (Average = 27.69; range 10.15 - 38.95) (t-test, $P=0.02$) (Table 1).

It was found that vitamin D level in arthritic males of age <50 years (Average = 24.74; range 16.59 - 34.37) were significantly higher than those in arthritic males of age >50 years (Average = 19.45; range 3.0 - 44.62) (t-test, $P=0.01$) (Table 1).

On the other hand, vitamin D level in arthritic females of age <50 years (Average = 16.18; range 3.97 - 37.15) were indifferent from those in arthritic females of age >50 years (Average = 15.48; range 3.00 - 26.91) (t-test, $P=0.95$) (Table 1).

Table 1: Serum vitamin D level in normal and arthritic males/females aged below 50 years or above 50 years.

Disease status	Sex (M/F)	Serum vitamin D level (ng/ml)		P-value (t-test)
		< 50 years	> 50 years	
Normal	M	22.62	26.06	0.04*
	F	21.24	27.69	0.02*
Arthritic	M	24.74	19.45	0.01*
	F	16.18	15.48	0.95

Data indicated average vitamin D level in each group. M, males; F, Females

*Statistically significant using student's t-test ($P < 0.05$)

Comparison between vitamin D levels in normal and arthritic subjects

Further, we compared vitamin D levels in normal and arthritic subjects of age <50 years or >50 years. It was found that arthritic males and normal males of age <50 years have comparable level of vitamin D (Fig 2a) (t-test, $P= 0.45$). In contrast, normal males of age >50 years had significantly higher vitamin D levels compared with arthritic males of age >50 years (Fig 2b) (t-test, $P=0.01$).

Moreover, normal females had significantly higher level of vitamin D compared with arthritic females of age <50 years (Fig 2c) (t-test, $P=0.01$), or age >50 years (Fig 2d) (t-test, $P=0.0002$).

Further, we determined the correlation between vitamin

D levels in normal and arthritic subjects within the same age group. However, it was found that vitamin D levels in normal and arthritic subjects of age <50 years, or >50 years could not be correlated as determined by Spearman correlation test (Fig 3).

Fig 2: Comparison of vitamin D levels in normal and arthritic subjects. The serum vitamin D level from each subjects were measured. Scatter plot show mean \pm SD from each group. Data is statistically analyzed by student t-test (ns, $P>0.05$; *, $P<0.05$)

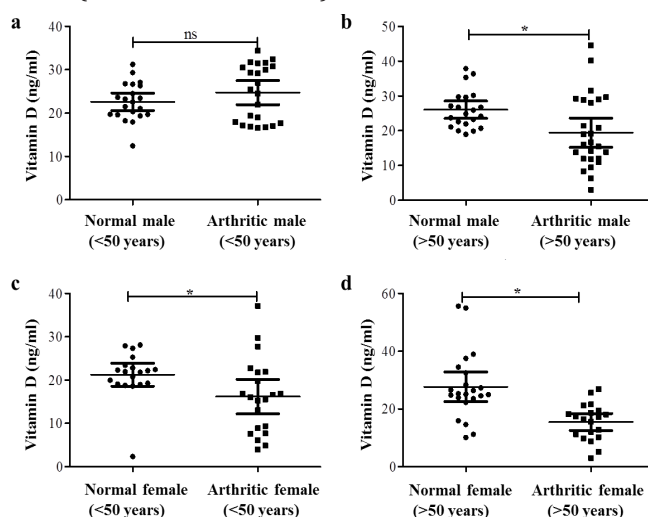
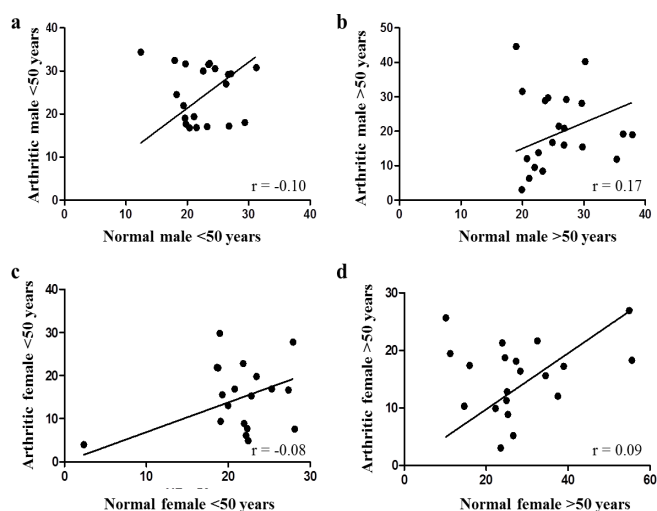


Fig 3: Scatter plot and correlation coefficient of vitamin D level in normal and arthritic subjects of indicated sex and age groups (r, correlation coefficient)



DISCUSSION

Subjects with serum vitamin D level below 16 ng/ml were considered as deficient; those above 20 ng/ml were considered as vitamin D sufficient while those in-between were insufficient². Our study found vitamin D as low as 3 ng/ml despite of gender and disease status.

Moreover, this study found that males (normal/arthritis) and normal females have in average sufficient vitamin D. However, arthritic females were deficient in vitamin D.

In general, our data suggested that vitamin D is lower in arthritic subjects compared to normal subjects. This is in concordance with previous observations wherein RA patients have significantly low level of vitamin D than healthy individuals^{9,10}.

Also, our data suggested that age factors could not be correlated with vitamin D levels in arthritic subjects suggesting that vitamin D level might be independent of age. However, more robust analysis is needed before reaching the conclusion.

Association between vitamin D levels and RA has been previously observed. Some group of investigators found negative association between serum vitamin D level and RA disease^{9,10}. In contrast, we could not find any correlation between vitamin D levels in normal and arthritic subjects. This is similar to previous observation which did not find any association between vitamin D and RA¹².

Serum vitamin D level varies among arthritis patients from different locations within the same region. It was found that RA patients from North Europe (Estonia) had significantly lower level of vitamin D than those from South Europe (Italy)¹⁰. Therefore, it would be of great interest to determine the status of vitamin D in people from different regions of Nepal, and find its correlation with arthritis.

Conflict of Interest

No conflict of interest to disclose.

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