



Journal Homepage: - www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/5127
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/5127>



RESEARCH ARTICLE

CORRELATION OF VITAMIN D DEFICIENCY AND SUB-CLINICAL OSTEOMALACIA IN AXIAL SPONDYLOARTHROPATHY.

***Parminder Viridi¹, Baljeet Singh², Maninder Kaur³ and Baldish Singh Oberoi⁴.**

1. Senior Resident, Department of Orthopedics, Sri Guru Ram Dass institute of medical sciences and research, Amritsar, India.
2. Professor, Department of Orthopedics, Sri Guru Ram Dass institute of medical sciences and research, Amritsar, India.
3. Senior resident, Medicine Department, Sri Guru Ram Dass Institute of Medical Sciences, Amritsar, India.
4. Graduate N.K.P. Salve institute of medical sciences, India.

Manuscript Info

Manuscript History

Received: 10 June 2017
 Final Accepted: 12 July 2017
 Published: August 2017

Key words:-

Axial spondyloarthritis (axSpA);
 Vitamin D deficiency; Osteomalacia;
 Bone turnover biomarkers.

Abstract

Introduction: Recently vitamin D deficiency has been linked to autoimmune, inflammatory and carcinogenic diseases. In autoimmune diseases, vitamin D deficiency might be associated with risk of both susceptibility and severity of the bone mineral density.

Objective: Our study aims at finding vitamin D deficiency in axial spondyloarthritis and its effect on bone integrity.

Material: We compared 95 axial spondyloarthritis patients and 75 control patients regarding Vit-D levels, bone markers, CRP, ALP, PTH, BMD and sunshine exposure.

Conclusion: We conclude significant correlation between vitamin D deficiency and axial spondyloarthritis and significantly lower femoral neck T and Z scores in BMD than controls.

Copy Right, IJAR, 2017,. All rights reserved.

Introduction:-

Osteomalacia occurs due to lack of active metabolite of Vitamin D in serum leading to mineralization and bone matrix defects. It is frequently found in South East Asia despite adequate sun exposure and in adults starts insidiously as back pain later spreading to the arms and ribs [1]. These symptoms are very common in our community and difficult to differentiate in most of spondyloarthritis patients due to assumption of these symptoms due to joint disease. In various autoimmune diseases condition the deficiency of vitamin D might be associated with risk of both susceptibility and severity of the bone mineral density (BMD) due to significant consequence of innate and acquired immunity [2]. However, the potential immunomodulatory role of vitamin D in spondyloarthritis (SpA) has not been discussed widely, especially the interconnection between SpA and osteomalacia.

Spondyloarthritis is a chronic inflammatory disorder, typically a disease of young men (age 25-45) involving the sacroiliac (SI) joints and the axial skeleton. Clinical feature of Spondyloarthritis includes the inflammatory back pain and stiffness of the spine. Moreover, fatigue in AS patients is associated with increased pain, stiffness and decreased functional capacity. Glucocorticoids are not commonly used to treat this group of patients, as this increases chances to development of systemic osteoporosis. However, osteoporosis has been reported as an early event in ankylosing spondylitis (AS) whilst it cannot be related only to the spine ankylosis and immobilization [3,4]. Another

Corresponding Author:- Parminder Viridi.

Address:- Senior Resident, Department of Orthopedics, Sri Guru Ram Dass institute of medical sciences and research, Amritsar, India.

study also states that AS is associated with lower vitamin D concentration of serum and negatively affect disease activity, but statistically no significant differences in ALP, Ca, P, OC and PTH levels [5].

The role of BMD in SpA remains of great interest with evidence of an increased risk on bone fragility and fracture. The character of the disease process itself and effects on lifestyle factors such as sun light exposure, hormones or a diet deficient in vitamin D remains uncertain. The study was to examine those factors on skeletal integrity in axial spondyloarthritis (axSpA) patients associated with sub-clinical osteomalacia.

Material and methods:-

Patients:-

Between March 2014 to March 2016, 95 axSpA patients from orthopedics clinic and 74 healthy individuals from the same area of Government medical college Patiala, India were recruited. All axSpA patients met the Assessment of Spondyloarthritis International Society (ASAS) 2009 criteria [6] and ASAS axSpA 2013 criteria [7]. Patients younger than 18 or older than 65 years, inflammatory bowel disease (IBD), psoriasis, a preceding symptomatic infection of urogenital or gastrointestinal tract and Bechet's disease or familial Mediterranean fever, pregnancy and postmenopausal women, chronic renal insufficiency, renal tubular acidosis, hypophosphatemia, osteomalacia patients with tumor induced, who had taken drugs for one year that may affect bone metabolism and vitamin D metabolism such as thyroxine, corticosteroids, calcium, vitamin D, bisphosphonates, and anticonvulsants were excluded. Written informed consents were obtained from all participants.

Laboratory assessment:-

Disease activity was assessed by using AS-endorsed disease activity score (ASDAS). 25-hydroxyvitamin D (25OHvitD), parathyroid hormone (PTH) and bone turnover biomarkers procollagen type 1 N-terminal peptide(PINP) and osteocalcin (OC), and bone resorption marker serum C telopeptides of type I collagen (sCTX) were assessed at the Department of Clinical Biochemistry in the same hospital by using radioimmunoassay. Four groups were identified according to vitamin D levels, normal ≥ 30 ng/ml, insufficiency 20–30 ng/ml, deficiency 10–20 ng/ml, severe deficiency ≤ 10 ng/ml were set up. The alkaline phosphatase (ALP), serum adjusted calcium (Ca), phosphorus (P), C-reactive protein (CRP) were also measured. Radiographs of the pelvis (anterior- posterior view) and MRI /CT scan of sacroiliac joints were obtained from all axSpA patients.

Bone Mineral Density (BMD) measurement:-

BMD of total proximal femur and lumbar spine were measured by using DEXA (Hologic QDR-4500A dual-energy X-ray). According to World Health Organization (WHO) classification and April 2014 National Osteoporosis Foundation (NOF) released Clinician's guidelines for the prevention and treatment of osteoporosis, measurement results for non-menopausal women and young men (Age<50 years) using Z Score represent, postmenopausal women or elderly men (age ≥ 50 years) using the T-Score represent. Osteopenia was defined as a T-score (or Z-score) between -1 and -2.5 and osteoporosis as a T-score (or Z score) ≤ -2.5 [12].

Questionnaires:-

Recruitment questionnaire ascertained average daily sun exposure, dairy consumption, countryside or city living, smoking or nonsmoking, indoor or outdoor working environment, calcium and vitamin D tablet consumption.

Statistical analysis:-

Results were expressed as mean \pm SD or median (range) for normally distributed and non-normally distributed data, respectively. Independent samples t test, Mann Whitney U test, and Chi-Square test were used to compare differences in characteristics between groups. Pearson's and Spearman's correlation coefficients were used as appropriate to analyze the relationship between vitamin D, BMD, clinical measures of disease activity, and bone turnover biomarkers for parametric and nonparametric data respectively. P values ≤ 0.05 were considered statistically significant.

Results:-

Findings among patients and control subjects:-

Patients and controls were well matched for age, gender and daily sun exposure time in two groups. 71(83%) of axSpA patients were HLAB27 positive with a wide variety of disease duration (median date: 3.0, 0.25-20 years). The disease activity ASDAS-CRP were with median scores 2.28, (0.11~5.95), and CRP with median value 5.2, (0.2-

99.1) mg/L. The bone resorption biomarker sCTX, formation markers OC and ALP in axSpA patients were significantly higher than controls (Table 1). Though no significant difference was observed between the two groups, there were higher adjusted serum calcium and raised PTH in axSpA patients than control (Table 1).

Table 1:- Characteristics of the axSpA and healthy controls study population. axSpA: Axial Spondyloarthritis; BMD: Bone Mineral Density; PTH: Parathyroid Hormone; ALP: Alkaline Phosphatase; PINP: N-amino terminal propeptide of type I collagen; CTX: CrossLaps of type I collagen cross-linked C-telopeptide; OC: Osteocalcin; LS: Lumbar Spine

Vitamin D levels in axSpA patients and controls: Vitamin D levels in axSpA patients were significantly lower

variables	AxSpA patients{n=95}	Control {n=75}	P value
Age (years)	29 (18~55)	29.5 (18~52)	0.487
Gender (male) (n%)	69 (73)	50 (68)	0.474
BMI (kg/m ²)	21.71 ± 3.27	22.89 ± 2.96	0.009
	21.15 (15.62~30.55)	22.77 (17.01~31.14)	
Sun exposure time (n%)	3 (1~4)	3 (1~4)	
5 minutes/daily	11 (11.6)	6 (8)	0.813
10~30 minutes/daily	29 (30.5)	25 (35)	
30~60 minutes/daily	24 (25.3)	16 (22)	
>1 hour /daily	31 (32.6)	25(35)	
25OHD (ng/ml)	25.1 ± 10.1	34.9 ± 12.6	0.00
PTH (pg/ml)	27.4 (3~101)	25.0 (3~76.6)	0.129
Adjusted Calcium (mmol/L)	2.26 ± 0.12	2.22 ± 0.23	0.2
Phosphate (mmol/L)	1.20 ± 0.19	1.21 ± 0.15	0.888
sCTX (ng/ml)	0.57 ± 0.20 (0.55, 0.13~0.98)	0.43 ± 0.19 (0.37, 0.15~1.13)	0.00
OC (ng/ml)	22.65 (7~51)	18.43 (10~45)	0.018
ALP (U/L)	76.5 (39~165)	63 (32~145)	0.011
PINP(ug/L)	50.92 (21.2~139.2)	47.86 (22.3~99.9)	0.154
Femoral BMD T-score	-1.10 ± 0.90	-0.52 ± 0.89	0.00
Femoral BMD Z-score	-0.93 ± 0.95	-0.33 ± 0.93	0.00

than the control group ($p < 0.001$). However, there were no significant differences with serum calcium, phosphorus and PTH between two groups (Table-2).

25(OH)D	Normal	inefficiency	Deficiency	Serious deficiency	p
axSpA n,%	26 (28%)	34 (36%)	30 (32%)	4 (4%)	0
Controls n,%	45 (61%)	20 (27%)	8 (11%)	1 (1%)	0

Table 2:- Vitamin D Levels in axSpA patients and Controls. According to vitamin D level, normal ≥ 30 ng/ml, insufficiency 20–30 ng/ml, deficiency 10–20 ng/ml, Severe deficiency ≤ 10 ng/ml. p value < 0.05 statistically significant.

Femoral BMD T-scores in axSpA patients and controls:-

There were higher proportions of osteopenia and osteoporosis in axSpA patients than controls. The femoral neck T and Z scores in axSpA patients were significantly lower than control (Table 3).

Femoral BMD T scores	Normal	Osteopenia	osteoporosis	p
axSpA n, %	44 (51%)	34 (40%)	8(9%)	0
Control n, %	51 (74%)	18(26%)	0	

Table 3:- Femoral BMD T score in axSpA patients and Controls. Normal: T-score ≥ -1.0 ; Osteopenia: T-score between -1.0 and -2.5 ; Osteoporosis: T-score ≤ -2.5 .

Correlation between femoral BMD T-scores and other variables in axSpA patients:

The femoral BMD T-scores were inversely correlated to the disease duration, ASDAS-CRP, spinal stiffness and ALP (Table 4).

	Femoral BMD Z-score A						Lumbar BMD Z-score B					
	ASDA S	CRP	ESR	ALP	sCTX	Spinal stiff ness	ASD AS	CRP	ESR	ALP	sCTX	Spinal stiff ness
r	-0.267	-0.299	-0.446	-0.332	-0.26	0.415	-0.312	-0.295	-0.504	-0.242	-0.29	-0.235
p	0.015	0.006	0.008	0.039	0.017	0	0.01	0.015	0.005	0.046	0.017	0.054

Table 4:- Correlation between femoral BMD Z-scores, Lumbar BMD Z scores and other variables in axSpA patients. A: The correlation between femoral BMDZ-scores and ASDAS, CRP, ESR, ALP, sCTX, Spinal stiffness; B: The correlation between lumbar BMD Z-scores and ASDAS, CRP, ESR, ALP, sCTX, BMD, spinal stiffness, back pain; r: correlation coefficient.

Correlation between vitamin D and other disease variables: In axSpA patients vitamin D levels were inversely related to the disease duration (Figure 1A) and positively correlated to daily sunlight exposure time and calcium levels (Figures 1B and 1C).

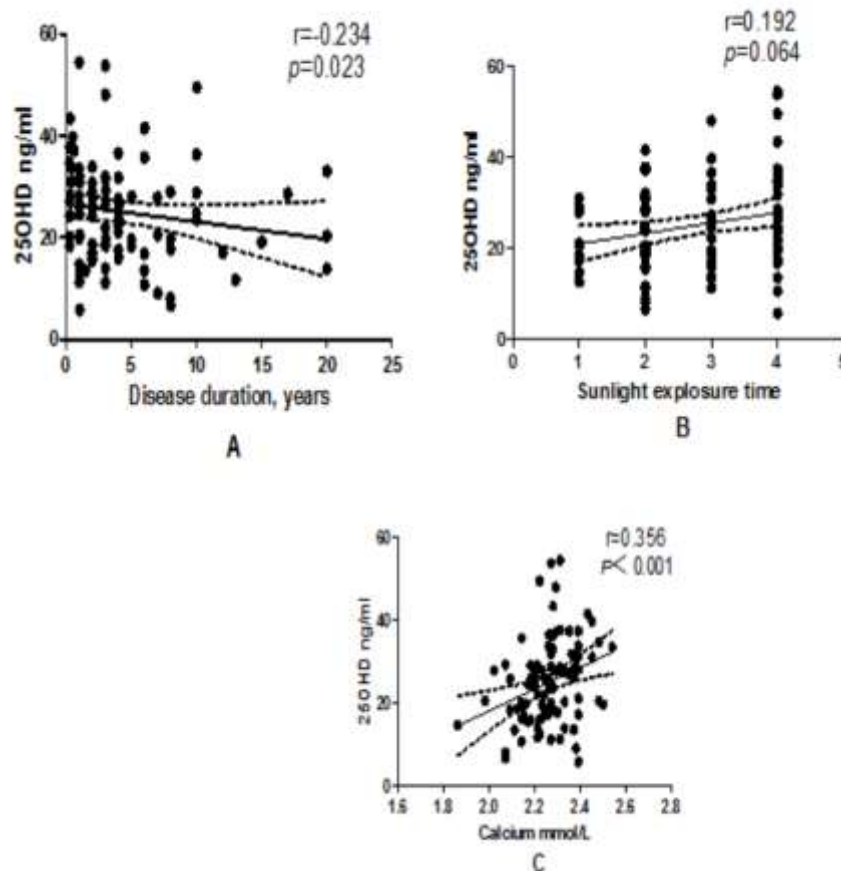


Figure 1:- Correlation between vitamin D and other disease variables. 1. A: The 25(OH)D levels were inversely related to disease duration. 1. B: and positively related to daily sunlight exposure time. 1. C: calcium levels Daily sunlight exposure time 1 (5 minutes/ daily), 2 (10~30 minutes/daily), 3 (30~60 minutes/daily), 4(1 hour/ daily).

Correlations between serum ALP and disease activity, bone biomarkers, BMD in axSpA patients: The Spearman Rank correlation test demonstrated that ALP levels significantly correlated with ASDAS-CRP ($r=0.348$; $p=0.022$), OC ($r=0.351$; $p=0.021$), sCTX ($r=0.308$; $p=0.04$), however, inversely related to the femoral BMD T-scores ($r=-0.379$; $p=0.016$) in axSpA patients (Table 5).

variables	r- coefficient	p-value
ASDAS	0.348	0.022
OC	0.351	0.021
sCTX	0.308	0.04
Femoral BMD Z score	-0.379	0.016

Table 5:- Correlation between serum ALP and disease activity, bone biomarkers, BMD in axSpA patients. ASAS-endorsed disease activity score (ASDAS), osteocalcin (OC), serum C-telopeptides of type collagen (sCTX) were significantly positively correlated whereas femoral BMD Z-score was negatively correlated in axSpA patients.

Discussion:-

Vitamin D deficiency is not only a problem in India but also in countries like Pakistan, China, middle-East and Africa. It is relatively less common in Japan, USA, Canada and South-east Asia [8]. There was a disbelief that Vitamin D (Vit D) deficiency is uncommon in India, however from the data available in the published literature, Vit D deficiency is very common in India in all the age groups and both sex across the country [8]. Furthermore, other cross-sectional studies also describe vitamin D deficiency is prevalent across many urban Indian residents [9]. Evidence also showed that patients with vitamin D deficiency may have an increased risk of immune-mediated inflammatory diseases including AS [10], with further inflammatory activity in SpA patients indirectly linked with vitamin D deficiency and predisposition to the development of osteoporosis, which can be considered as early phase of osteomalacia. Furthermore, some studies also revealed that low plasma levels of serum 25(OH)D were presented in AS patients when compared to healthy control [11,12]. Our study showed 25(OH)D was significantly lower in axSpA patients (25.06 ± 10.06 ng/ml) compared to borderline to the normal level (34.90 ± 12.57 ng/ml) in the control group, consistent with the previous study of Baskan et al. [13]. In our axSpA patients, serum 25(OH)D levels found normal levels in 26 (28%), insufficient in 34 (36%), and deficient in 30 (32%) and severely deficient in 4 (4%) compared with a distribution in healthy controls of 45 (61%), 20 (27%), 8 (11%) and 1 (1%) respectively. Taken together, data suggests that Vitamin D insufficiency is present in axSpA patients from north Indian population.

Recent bone biopsy studies from Germany have shown that as 25(OH)D levels falling below 30 ng/ml suggests rising prevalence of osteomalacia [14]. Furthermore, hypovitaminosis D can lead to hypocalcemia and then elevated PTH level. However, in the early phases of osteomalacia biochemicals such as calcium, phosphate and soluble phosphatase are released in serum because of a low 25(OH)D level, may be lacking due to compensatory changes conversely the PTH may be lifted and it lead to decalcification of the skeleton. AS patients are associated with low vitamin D in serum concentrations are linked with higher disease activity [15]. Although vitamin D concentrations were not associated with higher disease activity in our study, vitamin D levels were significantly lower in axSpA patients. Furthermore, AS patients with osteoporosis it has been shown that the levels of PTH are significantly elevated [13]. In our study axSpA patients serum level of PTH was elevated but not significantly different when compared with healthy control. However, vitamin D levels were inversely related to the disease duration and levels of PTH (Figure 1), which may be due to alteration in vitamin D metabolism and increased bone resorption of prolonged duration. The hypovitaminosis D complicated by secondary hyperparathyroidism is associated with significantly decreased bone mineral density.

The accurate method of detecting osteopenia and osteoporosis may be provided by the measurement of BMD at the femoral neck. Despite the most solid analytic test for osteomalacia is the bone biopsy, Cosman et al. have demonstrated, two decades ago, that significant correlations between histological estimation BMD of the spine and proximal femur in patients with different metabolic bone diseases including osteomalacia [16]. Intriguingly, in our axSpA patients the frequencies of osteopenia and osteoporosis were significantly higher than controls (40% vs 26%, and 9% vs 0) respectively. Consistently, Karberg et al. found that the proportion of osteoporotic patients varies according to the disease duration of AS. In patients with disease duration <5 years, 11% and 15% were osteoporotic at the hip; in patients with disease duration >10 years, 29% were osteoporotic at the hip is assessed by DXA scan. The previous study showed that serum bone formation marker OC and resorption markers sCTX seem to be valuable markers to detect bone loss in AS patients [17]. Interestingly in our axSpA patients serum level of OC and sCTX

was significantly increased when compared to healthy control. Besides BMD T/Z-score has significant differences between axSpA patients and healthy control group, furthermore, axSpA patients femoral and lumbar BMD Z-scores were negatively correlated with active inflammatory marker such as CRP, ESR and disease activity ASDAS, bone turnover marker sCTX and OC (Table 4). Consistently, the acute phase reactant ESR and CRP was significantly elevated and inversely correlated with vitamin D levels in AS patients [13], disease activity ASDAS was negatively correlated with BMD because of vitamin D receptor gene may contribute to BMD differences in patients with AS, as gene polymorphisms are also linked to inflammatory activity [18]. Thus, osteoporotic risk may be significantly increased with raised serum levels of bone turnover markers and low levels of vitamin D. Previous study also has shown that the relationship between vitamin D with CRP and ESR, were significantly negatively correlated [19]. In addition, axSpA patients femoral BMD T/Z-score was negatively correlated with ALP, spinal stiffness and BMI. Furthermore, previous study found that serum alkaline phosphates is a sensitive screening tool for the diagnosis of osteomalacia. Hence, these parameters may specify the reasons of early bone loss in axSpA patients and potential immunomodulatory role of vitamin D to prevent osteomalacia in axSpA during active disease condition. In our observational study, axSpA patients had shown lower 25(OH)D levels below 30 ng/ml and higher bone turnover biomarkers than control and net effects of 25(OH)D on bone structure BMD in axSpA are due to vitamin D deficiency and disease activities.

Plasma ALP activity appears to be a sensitive single test in the past which acceptably predicts the presence or absence of histological osteomalacia [20]. We found that the ALP levels were significantly correlated with ASDAS-CRP and bone turnover biomarkers. So increased serum ALP levels were associated with high disease activity, low BMD and higher structural damage scores in axSpA patients. Serum ALP levels does not only reflect the disease activity but also associated with BMD. Our finding is consistent with an earlier result. Serum ALP levels may be a useful marker for predicting bone-related complications such as osteomalacia in axSpA patients. Interestingly, our current study also found that the plasma levels of vitamin D in the axSpA group were significantly lower than control group. Bone resorption biomarker sCTX, bone formation markers OC and ALP were significantly higher in axSpA group than controls. Furthermore, in axSpA patients vitamin D levels were inversely related to the disease duration and positively correlated to daily sunlight exposure time and calcium levels. Consistently, ALP levels significantly correlated with ASDAS-CRP, OC, sCTX, inversely related to the femoral BMD T scores in axSpA patients. Taken together, our data indicate that low Vitamin D levels may lead to subclinical osteomalacia in axSpA patients which is associated with increased PTH and ALP levels, higher bone turnover biomarkers sCTX and OC, low BMD. This evidence shows that axSpA patients may be associated with high rate of development of subclinical osteomalacia.

Limitations:-

Limitations of this study are the small number of patients of axSpA. Although, all recruited cases in our study were diagnosed cases of axSpA without any infection and other bone diseases history. We did not perform bone biopsy to confirm the chemical osteomalacia. Low levels of vitamin D can also be found in other conditions including acute myocardial infarction, heart failure, chronic kidney disease, diabetes and infections. However, these conditions are unlikely to be developed in axSpA patients and no evidence was found in our cases.

Conclusion:-

There were significantly lower vitamin D levels in Indian axSpA patients compared to a control group. axSpA patients with low vitamin D, increased PTH and serum ALP levels, higher bone turnover biomarkers sCTX and OC, low BMD indicated that subclinical biochemical osteomalacia is associated with most axSpA patients.

References:-

1. Saghafi M, Azarian A, Hashemzadeh K, Sahebari M, Rezaieyazdi Z (2013) Bone densitometry in patients with osteomalacia: is it valuable? Clinical cases in mineral and bone metabolism: the official journal of the Italian Society of Osteoporosis, Mineral Metabolism, and Skeletal Diseases 10:180-182.
2. Amarasekara DS, Yu J, Rho J (2015) Bone Loss Triggered by the Cytokine Network in Inflammatory Autoimmune Diseases. J Immunol Res 2015: 832127.
3. Will R, Palmer R, Bhalla AK, Ring F, Calin A (1989) Osteoporosis in early ankylosing spondylitis: a primary pathological event? Lancet 2:1483-1485.

4. Roux C (2011) Osteoporosis in inflammatory joint diseases. *Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 22: 421-433.
5. Durmus B, Altay Z, Baysal O, Ersoy Y (2012) Does vitamin D affect disease severity in patients with ankylosing spondylitis? *Chin Med J (Engl)* 125: 2511-2515
6. Sieper J, van der Heijde D, Landewe R, Brandt J, Burgos-Vargas R, et al. (2009) New criteria for inflammatory back pain in patients with chronic back pain: a real patient exercise by experts from the Assessment of Spondyloarthritis international Society (ASAS). *Ann rheum dis* 68:784-788.
7. van den Berg R, de Hooze M, Rudwaleit M, Sieper J, van Gaalen F, et al. (2013) ASAS modification of the Berlin algorithm for diagnosing axial Spondyloarthritis: results from the SPondyloArthritis Caught Early (SPACE)-cohort and from the Assessment of Spondyloarthritis international Society (ASAS)-cohort. *Ann rheum dis* 72: 1646-1653.
8. Harinarayan CV, Joshi SR. Vitamin D status in India-Its implications and Remedial Measures. *J Assoc Physicians India* 2009;57:40-48.
9. Marwaha RK, Sripathy G. Vitamin D and Bone mineral density of healthy school children in northern India. *Indian J Med Res* 2008;127:239-244.
10. Zhao S, Duffield SJ, Moots RJ, Goodson NJ (2014) Systematic review of association between vitamin D levels and susceptibility and disease activity of ankylosing spondylitis. *Rheumatology (Oxford, England)* 53:1595-1603.
11. Lange U, Teichmann J, Strunk J, Muller-Ladner U, Schmidt KL (2005) Association of 1.25 vitamin D3 deficiency, disease activity and low bone mass in ankylosing spondylitis. *Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 16:1999-2004.
12. Will R, Palmer R, Bhalla AK, Ring F, Calin A (1989) Osteoporosis in early ankylosing spondylitis: a primary pathological event? *Lancet* 2:1483-1485.
13. Mermerci Baskan B, Pekin Dogan Y, Sivas F, Bodur H, Ozoran K (2010) The relation between osteoporosis and vitamin D levels and disease activity in ankylosing spondylitis. *Rheumatol int* 30: 375-381.
14. Priemel M, von Demarsh C, Klatte TO, Kessler S, Schlie J, et al. (2010) Bone mineralization defects and vitamin D deficiency: histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients. *Journal of bone and mineral research. J Bone Miner Res* 25: 305-312.
15. Erten S, Kucuksahin O, Sahin A, Altunoglu A, Akyol M, et al. (2013) Decreased plasma vitamin D levels in patients with undifferentiated spondyloarthritis and ankylosing spondylitis. *Intern med (Tokyo, Japan)* 52: 339-344.
16. 1. 16 Cosman F, Schnitzer MB, McCann PD, Parisien MV, Dempster DW, et al. (1992) Relationships between quantitative histological measurements and noninvasive assessments of bone mass. *Bone* 13: 237-242.
17. Arends S, Spoorenberg A, Bruyn GA, Houtman PM, Leijnsma MK, et al. (2011) The relation between bone mineral density, bone turnover markers, and vitamin D status in ankylosing spondylitis patients with active disease: a cross-sectional analysis. *Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 22: 1431-1439.
18. Borman P, Bodur H, Bingol N, Bingol S, Bostan EE (2001) Bone mineral density and bone turnover markers in a group of male ankylosing spondylitis patients: relationship to disease activity. *J Clin Rheumatol* 7:315-321.
19. Lange U, Jung O, Teichmann J, Neeck G (2001) Relationship between disease activity and serum levels of vitamin D metabolites and parathyroid hormone in ankylosing spondylitis. *Osteoporos* 12:1031-1035.
20. Peach H, Compston JE, Vedi S, Horton LW (1982) Value of plasma calcium, phosphate, and alkaline phosphatase measurements in the diagnosis of histological osteomalacia. *J Clin Pathol* 35: 625-630.