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25 (OH) vitamin D level in Crohn's disease: association with sun exposure & disease activity

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***Background & objectives*: Western studies show that up to 65 per cent of patients with Crohn's disease have low serum 25-hydroxy vitamin D concentrations, and 45 per cent of these patients have metabolic bone disease. No data are available from India or from any country with comparable climatic conditions or ethnicity. We carried out this study to measure the serum 25 (OH) vitamin D levels of Crohn's disease patients and compare with matched controls and to assess the consequences of low 25 (OH) vitamin D levels on bone and mineral metabolism in these patients.**

***Methods*: Adult patients with Crohn's disease were compared with age and sex matched patients diagnosed to have irritable bowel syndrome. Serum 25 (OH) vitamin D, the effect of disease characteristics, sunlight exposure and milk consumption on 25 (OH) vitamin D level, and the consequences of low 25 (OH) vitamin D level on bone and mineral metabolism were assessed.**

***Results*: Thirty four patients with Crohn's disease (M:F, 24:10, age 39.2 ± 12.9 yr) and 34 controls (M:F, 24:10, age 38.9 ± 13.4 yr) were studied. 25 (OH) vitamin D levels were significantly lower in patients with Crohn's disease as compared to controls (Crohn's disease vs controls: 16.3 ± 10.8 vs 22.8 ± 11.9 ng/ml; *P*<0.05). The severity of disease activity as assessed by the Harvey Bradshaw score correlated negatively (Correlation coefficient -0.484, significance *P*<0.004), and the duration of sunlight exposure correlated positively (Correlation coefficient 0.327, significance *P*=0.007) with the serum 25 (OH) vitamin D level.**

***Interpretation & conclusions*: Serum 25 (OH) vitamin D levels were significantly lower among patients with Crohn's disease as compared to age and sex matched controls. Further, 25 (OH) vitamin D levels in patients with Crohn's disease were lower in those with severe disease activity and less sun exposure. Further studies need to be done to correlate low 25 (OH) vitamin D level with bone density and assess the effect of vitamin D supplementation in these patients.**

Key words Crohn's disease - Harvey Bradshaw score - osteomalacia - renal threshold phosphate concentration - sun exposure - 25 (OH) vitamin D

Crohn's disease can lead to metabolic bone disease and an increased incidence of fractures. Low bone mineral density has been found in approximately 30 per cent of these patients[1]. In the Manitoba IBD database, the incidence of fractures among patients with inflammatory bowel disease was 40 per cent greater than that of the general population[2]. Questionnaires issued to members of the Danish Crohn's and Colitis association

showed a relative fracture risk of 2.5 in female patients with Crohn's disease[3]. The main pathophysiological mechanisms include impaired absorption of calcium and vitamin D, treatment with steroids and the effect of chronic inflammatory disease on bone.

Vitamin D insufficiency is common in patients with inflammatory bowel disease[4,5] and seen in up to 65 per cent of patients with Crohn's disease[6]. Metabolic bone disease was present in 45 per cent of patients with low levels of serum 25 (OH) vitamin D[7]. Activity of the disease and nutritional status have been shown to correlate with serum 25 (OH) vitamin D level[8]. Vitamin D deficiency causes decreased intestinal calcium absorption, which leads to decreased ionized serum calcium. This enhances the synthesis and secretion of parathyroid hormone, which normalizes the serum calcium by bone resorption, and causes phosphaturia and hypophosphataemia. The consequences of vitamin D deficiency are osteomalacia and parathormone induced bone resorption. Calcium and vitamin D supplementation have been shown to increase lumbar spine bone mineral density in osteoporotic patients with inflammatory bowel disease[9] and reduce the incidence of fractures in post-menopausal women and elderly men. Information on vitamin D status among patients with Crohn's disease in India or in places with comparable climatic conditions and ethnicity in not available. Hence, this study was undertaken to compare serum 25 (OH) vitamin D levels in patients with Crohn's disease and matched controls, to evaluate disease characteristics that correlate with low 25 (OH) vitamin D level, and the consequence of low 25 (OH) vitamin D level on parameters of bone and mineral metabolism.

Material & Methods

Subjects: Consecutive patients with Crohn's disease, above the age of 18 yr, managed by the Department of Gastroenterology, Christian Medical College Hospital, Vellore, India, were recruited for the study between August 2004 and April 2007. Controls were age (± 2 yr) and sex matched patients diagnosed to have irritable bowel syndrome (IBS), by Rome III criteria[10], attending the outpatient clinic of the same department. After a Crohn's disease patient was recruited, the next age/sex matched IBS patient attending the out patient department, was recruited. Patients with irritable bowel syndrome were chosen as controls as they were presumed to have normal gastrointestinal absorptive functions. Diagnosis of Crohn's disease was made on the basis of endoscopic, histological and radiological findings in the appropriate clinical setting[11-16]. Patients in whom the diagnosis was equivocal or in whom tuberculosis could not be confidently ruled out were excluded from the study. Patients who received vitamin D or calcium supplements in the previous 6 months, patients with significant renal, hepatic, thyroid disease, and pregnant women were also excluded. Body mass index, milk consumption, sunlight exposure and dietary habits were recorded. The quantity of sunlight exposure was calculated by multiplying the number of hours spent outdoors by the percentage of body surface area exposed[17].

Laboratory tests: All subjects had serum levels of vitamin D, calcium, phosphorus and alkaline phosphatase measured in the fasting state; 24 h urinary excretions of calcium, phosphate and creatinine were also measured. Subjects were labelled as hypocalciuric if 24 h urinary calcium excretion was < 100 mg and hypercalciuric if 24 h urinary calcium excretion was more than 4 mg /kg body weight[18]. Those in between were considered normocalciuric. Serum and urine calcium were estimated by the colorimetric method using o-cresolphthalein complexone (Sigma-Aldrich, USA) in an alkaline medium[19]. Phosphorus levels in serum and urine were estimated by the reaction with ammonium molybdate (Sigma-Aldrich, USA) and sulphuric acid, and measuring absorbance at 340 nm, of an unreduced complex of phosphomolybdate[20]. Alkaline phosphatase activity was measured by estimating the liberation of phosphate and formation of 4-nitro phenoxide from p-nitrophenol phosphate (Sisco Research Laboratories, Mumbai)[21]. Indices of Crohn's disease activity (erythrocyte sedimentation rate, albumin and C-reactive protein) and the Harvey Bradshaw score[22] were measured in the patient group.

Serum 25 (OH) vitamin D levels were estimated by radioimmunoassay (DiaSorin 25-OH D assay, Stillwater, Minnesota, USA). Inter-assay coefficients of variation were 11 per cent for control with low 25 (OH) vitamin D level, and 20 per cent for control serum with high 25 (OH) vitamin D level. Intra-assay coefficients of variation ranged between 1 and 13 per cent with an average of 4.5 per cent. The test was known to have a 0.8 per cent cross reactivity with vitamin D2 and D3, and an 11 per cent cross reactivity with 1, 25 $(OH)_2$ D2 and 1, 25 $(OH)_2$ D3.

Renal threshold phosphate concentration($TmPO_4$ /GFR): Fractional excretion of phosphate ($FePO_4$) was calculated by the following formula: $FePO_4$ = phosphate clearance/creatinine clearance. The renal

threshold phosphate concentration was estimated by plotting the values of serum phosphate concentration and the fractional excretion of phosphate ($FePO_4$) on the nomogram devised by Walton and Bijvoet[23]. Patients were classified as being hypocalciuric, normocalciuric or hypercalciuric based on the 24 h urinary calcium excretion.

Informed consent was obtained before the start of the study and the study was approved by the Institutional Review Board of Christian Medical College.

Statistical analysis: Based on data obtained from a previous study, 17 patients and 17 controls were required to detect a difference of 9.8 ng/ml with a mean standard deviation of 10.15 in serum 25 (OH) vitamin D, with an alpha value of 0.05 and a power of 80 per cent[24]. All statistical analyses were done using the SPSS 11.0 software. Mann-Whitney test was used to compare means of serum 25 (OH) vitamin D level between patients and controls. Chi square test was used to compare the proportion of patients who were (*i*) vitamin D deficient (*ii*) vitamin D insufficient and (*iii*) vitamin D sufficient, in the patient and control groups respectively. Spearman correlation was used to study the association of vitamin D level and patients' disease characteristics. A multiple linear regression model was used to define variables that significantly affected the serum 25 (OH) vitamin D level, among patients with Crohn's disease. $P<0.05$ was considered significant.

Results

A total of 38 patients with Crohn's disease were eligible for the study. One patient refused to be enrolled and three did not give tests as advised. Thus there were 34 patients with Crohn's disease and an equal number of sex and age matched controls included in the study. This was no significant difference in baseline characteristics of patients and controls (Table I).

Three patients were asymptomatic at the time of recruitment. Of the remaining 31, 7 had intermittent and 24 had continuous symptoms. Gastroduodenal involvement was seen in 6 patients, jejunal involvement in 15 and distal ileal involvement in 26. Isolated colonic involvement was seen in 5 patients.

Patients with Crohn's disease had significantly lower body mass index (18.5±3.8 vs 20.8 ± 3.3, $P<0.01$), haemoglobin (11.2 ± 2.2 vs 13.0 ± 1.2 g%, $P=<0.001$), serum albumin (3.3 ± 0.8 vs 4.3 ± 0.3 g%, $P=<0.001$), and undertook less physical activity (19.7±18.9 vs 32.6 ± 17.0 h/wk, $P<0.01$) compared to the controls.

Table I. Baseline characteristics

	Patients (n=34)	Controls (n=34)
Male / Female	24 / 10	24 / 10
Age (yr)	39.2 ± 12.9	38.9 ± 13.4
Milk intake (ml/day)	128.2 ±113.5	123.4 ± 139.6
Smoking (yes / no)	6 / 28	5 / 29
Alcohol (yes / no)	0 / 34	3 / 31
Diet (veg / non-veg)	4 / 30	5 / 29
Income per month (Rs/month) (<1000 / 1000-5000 / > 5000)	5 / 12 / 17	3 / 14 / 17

Values for age and milk intake are mean ± SD

The serum 25 (OH) vitamin D level in Crohn's disease group was significantly lower than controls (Crohn's disease vs controls: 16.25 ± 10.8 vs 22.78 ± 11.9 ng/ml; $P<0.05$). Subjects having 25 (OH) vitamin D level less than 20 ng/ml were labelled as vitamin D deficient, those between 20-32 ng/ml as vitamin D insufficient and those above 32 ng/ml as having adequate level of vitamin D[25]. Among the patients, 27 (79%) were deficient, 4 (12%) were insufficient and 3 (9%) were normal. Among controls 17 (50%) were deficient, 10 (29%) were insufficient and 7 (21%) were normal ($P<0.05$).

Among patients and controls, in bivariate correlation, the quantum of sun exposure (number of hours in the sun per week x body surface area exposed) correlated positively (correlation coefficient 0.327, $P<0.007$) with the serum 25 (OH) vitamin D level. Other variables like age, body mass index, quantity of milk intake, physical activity, smoking and alcohol use did not correlate with the serum 25 (OH) vitamin D level.

The effect of various disease characteristics like severity of disease [assessed by Harvey Bradshaw score C-reactive protein (CRP) and ESR], duration of illness, site of bowel involvement and therapy received, on serum vitamin D levels was evaluated. Disease activity as assessed by Harvey Bradshaw score correlated negatively with the serum 25 (OH) vitamin D level (correlation coefficient –0.484, $P<0.004$). Serum 25 (OH) vitamin D levels were comparable to controls in patients with mild disease but were significantly lower in patients with moderate and severe Crohn's disease (Fig.).

Lower vitamin D levels were seen in the 15 patients who had jejunal involvement, compared to 15 patients who did not (12.6 ± 7.3 vs 20.4 ± 13.6 ng/ml; $P<0.074$, not significant). All 23 patients with a prior diagnosis of Crohn's disease were on amino salycilic

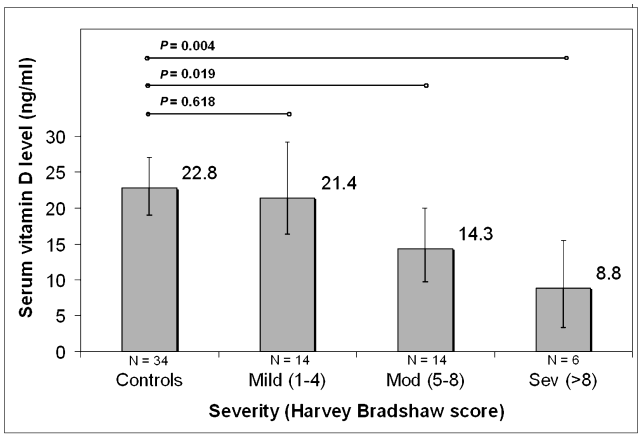

Fig. Correlation between Crohn's disease activity and serum vitamin D level.

acid (ASA) compounds, eight were on steroids, with 4 of the 8 also on immunosuppressant (azathioprine/ 6-mercaptopurine). There was no correlation between the type of drug or the cumulative dose and the serum 25 (OH) vitamin D level.

Multiple linear regression model constructed with the variables that had a significant correlation with serum 25 (OH) vitamin D level, showed that disease severity and quantum of sunlight exposure were the factors that affected the serum 25 (OH) vitamin D level. Calculations yielded the following regression equation (R^2 0.394, P<0.001). Serum 25 (OH) vitamin D level = 18.792 (constant) – (β1 x 0.956) + (β2 x 2.492). β1 = Harvey Bradshaw score, β2 = quantum of sunlight exposure.

The levels of serum calcium, phosphorus, alkaline phosphatase, 24 h urinary calcium excretion and the renal phosphate threshold concentration (TmPO4/ GFR) were measured to study the effect of low 25 (OH) vitamin D level, on bone and mineral metabolism. There was no significant difference between the patient and control groups with regard to any of these parameters. The proportion of patients who were hypo-, normo- and hypercalciuric were similar in the patient and control groups (Table II).

Table II. Consequence of low vitamin D on bone and mineral metabolism

	Subjects	N	Mean	SD
Serum 25 (OH) vitamin D level	Patients	34	16.3*	10.8
	Controls	34	22.3	11.9
Corrected calcium[a] (mg/dl)	Patients	33	9.33	0.56
	Controls	34	9.09	0.43
Phosphorus (mg/dl)	Patients	33	4.02	0.58
	Controls	34	4.11	0.52
Alkaline phosphatase (U/l)	Patients	34	93.97	25.65
	Controls	32	90.06	25.35
24 h urinary calcium (mg)	Patients	33	147.03	91.41
	Controls	32	140.03	93.75
Urinary Ca excretion hypo/normo/hyper	Patients	33	12/14/7	
	Controls	32	13/14/5	
$TmPO_4/GFR$[b] (mg/dl)	Patients	31	3.98	0.97
	Controls	33	4.37	0.98

[a] Corrected calcium = Measured calcium + [(4 – serum albumin) x 0.8]

[b] $TmPO_4/GFR$ = Renal phosphate threshold concentration

*P<0.05 compared to controls

Discussion

Metabolic bone disease is a significant problem in patients with Crohn's disease[1,5,7]. The main pathophysiological mechanisms involved include impaired absorption of calcium and vitamin D, treatment with steroids and the effect of the chronic inflammatory process on bone[1]. There are no data on vitamin D levels in Crohn's disease patients in the tropical countries where sun exposure and skin pigmentation are different from the West.

Our study showed significantly lower vitamin D levels in patients with Crohn's disease as compared to age and sex matched controls. After controlling for other factors affecting vitamin D, the duration of sun exposure and the severity of disease emerged as the factors affecting serum vitamin D level. There was no correlation between vitamin D and milk intake. This is probably because milk unless fortified with vitamin D is not a rich source of vitamin D. Prior studies from south India have shown a high prevalence of hypovitaminosis D in the population[26]. Fifty per cent of controls also had low vitamin D level suggesting a need for fortification of milk with vitamin D in developing countries[27]. It will probably become more relevant with changing lifestyles which may lead to a reduction in the duration of sun exposure.

The mean renal phosphate threshold concentration, a reflection of the secondary hyperparathyroidism was lower among patients with Crohn's disease compared to controls but did not reach statistical significance. Parathyroid hormone level would have given a better index of the impact of the low vitamin D on bone and mineral metabolism. It will be worthwhile to correlate vitamin D deficiency with bone density and assess the therapeutic effect of vitamin D supplementation.

In conclusion, the serum vitamin D levels were significantly lower in patients with Crohn's disease

when compared with matched IBS controls. The degree of deficiency correlated with the severity of disease activity.

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