ORIGINAL ARTICLE

Association between Serum 25 (OH) Vitamin D Concentrations and Inflammatory Bowel Diseases (IBDs) Activity

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SUMMARY

Inflammatory bowel diseases (IBDs) are immune mediated diseases affecting the gastrointestinal tract. Several environmental factors in concert with genetic susceptibilities can trigger IBDs. Recently, one of the important environmental factors contributing to the development of autoimmune diseases is vitamin D (VitD) deficiency. Furthermore, some new evidence points to VitD deficiency and its receptor dysfunction as an underlying factor for the emergence experimental IBDs. The aim of the current study was to evaluate the correlation between serum 25(OH)D concentrations and IBD activity in patients with ulcerative colitis or Crohn's disease.

Sixty patients with confirmed diagnosis of IBD were recruited for a cross sectional study. Most of the identified confounders affecting serum VitD concentrations were excluded. Disease activity was assessed using validated questionnaires, including Truelove for Ulcerative Colitis and Crohn Disease Activity Index (CDAI) for Crohn disease. Serum 25(OH)D concentrations were determined by chemiluminescent assay. Serum 25(OH)D≤10 (ng/ml) was considered as VitD deficiency and 11≤25(OH)D<29(ng/ml) as VitD insufficiency.

Mean serum 25(OH)D value was 13.1 ± 11.1(ng/ml) in IBD patients. Almost 95% of patients were vitamin D insufficient or deficient. Forty one percent of IBD patients had active disease. VitD deficiency was not associated with IBD activity (p=0.23). However, VitD deficiency was significantly associated with a history of IBD related intestinal surgery (p=0.001). In conclusion, this cross-sectional prospective study suggested that there is no association between vitamin D deficiency and disease activity in a relatively small number of IBD patients in a short period of time.

KEY WORDS:
IBD; 25(OH)D; IBD; Ulcerative Colitis; Chron's disease; disease activity index; vitamin D; Autoimmunity

INTRODUCTION

The influence of various environmental factors on development of autoimmune diseases like rheumatoid arthritis (RA), inflammatory bowel diseases (IBDs), and multiple sclerosis (MS) has generally been proved [1]. However, predisposing factors contributing to a breakdown in tolerance and subsequently leading to immunity imbalance, is not completely discovered in autoimmune diseases [1-2]. Recently, the discovery of immunomodulatory functions of VitD, has led to the hypothesis that VitD and its receptors can play an extenuating role in occurrence and progression of autoimmune diseases [1-3].

The identification of the intracellular receptors for 1,25(OH)D on the skeletal muscles, intestinal and kidney cells in addition to the ability of calcium and phosphorus homeostasis, has encouraged researchers to review more about the other capabilities of VitD, especially its immunomodulatory effects [1, 4].

IBDs are autoimmune, chronic, and relapsing diseases with unknown etiology. There are two classic forms of IBD: Ulcerative colitis (UC) and Crohn's disease (CD) [5]. The familial aggregation of IBD is a hallmark of the influence of genetic susceptibility and environmental risk factors like nutritional habits. Besides, tobacco, infection, medications like NSAIDs, retinoic acid and oral contraceptives are other important environmental risk factors [5]. Accumulating evidence for the ability of intestinal cells to express VitD metabolic and catabolic hydroxylases as well as VitD receptors [6-8], in addition to the results of epidemiologic studies revealing that VitD from sunlight exposure is less in areas where IBD is more prevalent [9], have helped the formation of the theory that VitD play a crucial role in the pathogenesis of IBDs. Cantona *et al.*[10] showed that 1,25(OH)D can improve IBD in mouse models. Ulitsky *et al.* [11] suggested that any condition which interrupts the interaction between 1,25(OH)D and VitD receptors at the level of gut mucosa, can increase the risk of IBD. Interestingly, VitD deficiency in animal species and probably in humans, predisposes them to mycobacterial infection which implies a potential role in the pathogenesis

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of CD [12]. On the other hand, there is strong evidence which supports the idea that VitD deficiency is the outcome of IBD. For instance, anorexia, food intolerance, mal-absorption, reduction of outdoor activities, corticosteroid therapy, bowel surgery, lack of sex hormones and circulating cytokines, which are the consequences of gastrointestinal involvement, may lead to VitD deficiency [9-10].

According to the above information, it is assumed that VitD may also correlate with IBD activity. This hypothesis has recently triggered a host of studies with contradictory results [11,13-14]. The current experiment was designed to investigate the correlation between IBDs disease activity and VitD serum concentrations in the northeast of Iran.

MATERIALS AND METHODS

This study was conducted in Mashhad in the north-east of Iran, located at 36.20º latitude and 59.35º east longitude. Iran is a four-season country with a maximum amount of sunshine in summer.

Sixty out of seventy two patients with confirmed diagnosis of IBD and committed inclusion criteria were recruited consecutively in this study in the summer. Diagnosis of IBD was made based on the endoscopic, histologic, and radiologic findings. Patients who were diagnosed to have identified confounders interfering with the VitD serum values were excluded. These confounding factors included renal failure, liver disease, pregnancy, lactation, medications like anticonvulsants and VitD supplements and prominent malabsorption (albumin< 2mg/dl, Cholesterol<100 mg/dl, BMI≤ 18.5kg/m^2).

25(OH)D serum concentrations were measured by a commercial radioimmunoassay kit (Bio Source Europe, Nivelles, Belgium). The kit's sensitivity was 0.6 ng/ml. Intra- and inter-assay coefficient of variation (CV) were 6.1-7.9% and 7.1-8.2% respectively.

Different cut off points for VitD deficiency or insufficiency were defined according to aforementioned epidemiological studies [15-16]. We adopted below definitions for suboptimal serum VitD values based on Moradzade *et al.* [15] research in our country:
25(OH)D ≤10 (ng/ml): VitD deficiency.
11≤25(OH)D≤29(ng/ml):VitD insufficiency.
25(OH)D ≥30 (ng/ml): VitD sufficiency.
Demographic information, disease duration, number of hospitalization, IBD-related surgeries, involved segments of intestine, and drug history during the year before the study, were recorded for each patient in a questionnaire. Crohn's Disease Activity Index (CDAI) and Truelove index for UC questioners were also completed for each patient to determine disease activity. According to Truelove index, UC patients were divided into two groups of inactive (remission or mild disease) and active (moderate to severe disease). According to CDAI scoring system, CD patients are also divided into two groups; active group (CDAI <150) and (CDAI≥ 150) inactive group.

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences. All participants signed a written consent prior to their enrollment for this survey.

Statistical analysis:
The statistical analysis was performed using the SPSS 11.5 program (SPSS Inc., Chicago, IL, USA). Values are reported as mean ± SD for normally distributed variables and median with inter-quartile range (IQR) for others.

We used Pearson correlation, chi-square and t-student tests as appropriate. In cases that quantitative variables were not normally distributed, we applied non-parametric tests like Mann-Whitney and Spearman correlation coefficient test.

RESULTS

Sixty IBD patients (34 UC, 26 CD) were enrolled in this study. The mean duration of disease was 2.8±2 years (0.3–10 years).The mean concentration of serum 25(OH)D was 13.1±11.3ng/ml. The main demographics of patients are mentioned in table I. The mean score of CDAI in CD patients was 154± 117. Table I shows the number and percentage of active disease in each group.

In this study, 95% of IBD patients were VitD deficient. Serum vitamin D concentrations of UC and CD patients did not relate significantly to patients' gender (Table II).

Serum vitamin D concentrations in active disease were lower than inactive disease (11.5±7.2 vs. 14.33±13.5 ng/ml), but this was not statistically significant (Table III).

Frequency of VitD deficiency was 60% in active disease group and 45.7% in inactive disease group. However, there was not a significant difference between serum VitD status in active and inactive groups of IBD (Table IV). Considering separately, in patients with UC (P=0.1) and CD (P=0.68), serum VitD status did not have significant difference between active and inactive diseases conditions (chi-square test). History of surgery in CD (34.6%) patients was more common than UC (2.9%) patients (P=0.001) (chi-square test).

The survey revealed that serum VitD concentrations were significantly lower in IBD patients with a history of intestinal surgery compared with those without it (7.36±3.03 vs. 14.32±12.08, P=0.001)(t-test).

Other variables such as glucocorticoids (P=0.18,r_s=-0.17), number of hospitalizations (P=0.94,r_s=0.009), age (P=0.12,r_s=0.17), and duration of diseases (P=0.76,r_s=-0.04) did not correlate with serum VitD levels (Spearman correlation coefficient test).

Although lower serum VitD values were detected in patients with UC, who had intestinal involvement after splenic flexure (12.9±7.2 nmol/ml) compared with those who suffered from left colon (15.7±16.8 nmol/ml) and rectosigmoid involvements (17.7±13.6 nmol/l), we found no statistical difference among them (P=0.76 One-Way ANOVA). In CD patients, VitD serum levels in patients with only small

Table I: Demographics of IBD patients

IBD	Age (years)	Male n (%)	Female n (%)	Surgical treatment n (%)	Active disease n (%)
UC	30±11	10(29.4)	24(70.6)	1(2.9)	13(52)†
CD	34±18	7(26.9)	19(73.1)	9(34.6)	12(48)‡
Total	32±13	17(28.3)	43(71.7)	10(16.6)	25(41.6)

†According to truelove criteria
‡ According to CDAI criteria

Table II: Vitamin D serum values in IBD patients according to gender

IBD subtypes	Vit D serum values(ng/ml) Mean ±SD		P value*
	Male	female	
UC	16±7.6	14.86±15.4	0.81
CD	11.27±8.03	10±6.99	0.59
Total	14.29±7.87	12.7±12.5	0.19

*Mann-Whitney test

Table III: Vitamin D serum values in two groups of active and inactive IBD

Disease subtypes	Disease activity status				P value* Mann-Whitney test
	inactive		active		
	VitD(ng/ml) Mean ±SD	Number	VitD(ng/ml) Mean ±SD	Number	
UC	11.25 ± 7.74	13	17.68 ± 15.78	21	0.182
CD	11.8 ± 0.8	12	9.3 ± 7.4	14	0.389
Total IBD	11.5 ± 7.2	25	14.33 ± 13.5	35	0.736

Table IV: Frequency of each definition for vitamin D status in active and inactive groups of IBD

VitD status	IBD groups				P value (chi-square test)
	Active		inactive		
	Percentage	Number	Percentage	Number	
Deficiency	10	60	16	45.7	
Insufficiency	15	40	16	45.7	0.238
sufficiency	0	0	3	8.6	
Total	25	100	35	100	

intestinal involvement (5.3±3.7 nmol/l) was lower than those with large intestinal involvement alone (9.4±6.6 nmol/l) or patchy involvements of both small and large intestine (12.4±7.7 nmol/l). However, there was no statistical difference in VitD concentrations among these groups (P=0.26 One-Way ANOVA). In general, serum VitD values did not correlate with the sites of intestinal involvement (P=0.5 One-way ANOVA) in IBD.

DISCUSSION

As the main result, the current study showed that despite the high prevalence of vitamin D deficiency in IBD patients, serum VitD concentrations were not associated with IBD disease activity in a short period of time.

Low serum values of VitD in IBD patients has been demonstrated in several studies [2-4,10,17-18]. While, there is very limited data on the correlation between IBD activity and VitD status [9, 11, 13-14], Matary et al. reported in their study that serum values of vitamin D were significantly lower in 60 children with newly diagnosed IBD compared with healthy controls, but the activity of IBD for both CD and UC did not correlate with vitamin D values in these children [13].

On the other hand, Ulitsky *et al.*, in their study on 504 IBD patients (403 CD and 101 UC patients), reported a correlation between vitamin D concentrations and disease activity only in CD patients according to Harvey Bradshaw index [11]. In another study, which compared 34 patients with CD and 34 age and sex matched controls; VitD levels were significantly lower in CD patients. In addition, severity of disease, which was assessed by the Harvey Bradshaw score, correlated with serum 25(OH)D levels [14]. In the aformentioned study, Crohn's severity was evaluated by Bradshaw score that consists of clinical parameters [14]. Tajika *et al.* demonstrated that 25(OH)D levels were correlated with CD duration and activity (with CDAI) in 33 CD patients in Japan [19].

These contradictions may stem from study populations and genetic susceptibilities, duration of disease, scoring systems by which disease activity was assessed, and nutritional habits that could affect VitD serum levels at a point of time in such cross-sectional studies. In the current study, IBD patients, whether in active or inactive stages of the disease, showed high frequency of VitD hypovitaminosis; (60% and 45.7% respectively). In total, 95% of these patients were VitD deficient. Considering the fact that all samplings were conducted in summer in a city with a latitude and east

longitude of 36.20º and 59.35 º respectively, which is usually sunny during the year, reveals the importance of VitD deficiency in IBD.

In our study, the mean serum 25(OH)D level in 60 IBD patients was 13.1±11.3(ng/ml) and it was lower compared with VitD level of those suffering from other diseases such as: diabetes (32.4±21.6 ng/ml)[20], early post-menopause (17.1 ±11.3ng/ml) [21], osteoarthritis (23.8±18.8ng/ml), and healthy controls (34.5. ±29.6 ng/ml) [22] in our region. According to the results of an epidemiological study released by Moradzadeh *et al.*, prevalence of moderate to severe vitamin D deficiency (25(OH)D<25 nmol/L) was 35.4% in females and 35.8% in males in Mashhad [16]. As stated previously, the frequency and severity of VitD deficiency is higher in IBD patients compared with healthy population and those with some other diseases. Nevertheless, intestinal involvement and malnutrition in IBD may be an important reason for VitD deficiency, regardless of its role in the pathogenesis of IBD [23-25]. We found a significant relationship between IBD-related surgical history and vitamin D deficiency. We could not, however, clearly conclude whether this correlation is due to the resection of vitamin D absorptive segments of the intestine or to the severity of the disease.

Strengths and limitations

Some strong points of this study were: using strict exclusion criteria to enroll patients without confounding factors on VitD serum values. Sampling was performed in the summer in all patients. In addition, we used a clinical and laboratory scoring system for the evaluation of disease activities.

However, this study was not without limitations. The number of patients, for instance, did not permit a power calculation on the correlation between vitamin D and disease activity in IBD patients. The limited number of patients may be a consequence of the study design. As we all know, most of the IBD patients usually receive VitD supplements or have liver disfunction. Therefore, a small number of patients could be enrolled in this study. Moreover, IBD is less prevalent in Asian people compared with other ethnicities. This may also justify some differences like female sex preponderance in our study in comparison with other geographic regions. Likewise, some epidemiologic studies in Iran showed a small female dominance [26-27].

Another limitation is that in intestinal diseases, it is difficult to conclude whether vitamin D deficiency is a primary predisposing factor or a consequence of malabsorption.
Moreover, colonoscopic evaluation combined with truelove or CDAI criteria may be a better indicator of disease activity in such diseases. Besides, this cross-sectional study could not reveal the exact influence of VitD on disease status.

To this end, we note that most of the definitions for low vitamin D values are according to the body requirements for calcium, phosphorus, and skeletal homeostasis. In other words, the exact amount of vitamin D essentional for supporting the immune system is not clear. Therefore, future studies can determine the serum cut-off point for vitamin D to suppress autoimmunity and those definitions may change the results of current studies about vitamin D deficiencies and autoimmune diseases.

In conclusion, this cross-sectional prospective study suggested that there is no association between vitamin D deficiency and disease activity in IBD in a short period of time. However, future prospective cohort studies with larger study populations should be undertaken to explore the exact correlation between VitD and IBDs disease activity.

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