Vitamin D Receptor Level in Biopsy Specimen of Patients with Ulcerative Colitis: Results from a Center in Western Anatolia

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We have no conflict of interest.

Abstract: Background: Ulcerative colitis (UC) is a chronic, inflammatory bowel diseases characterized by uncontrolled inflammatory condition of the colon and rectal mucosa marked by recurrent periods of remission and exacerbation. Vitamin D receptor (VDR) is a member of the steroid receptor family that mediates the effects of vitamin D by regulating transcription of multiple cellular genes. We aimed to evaluate vitamin d receptor level in biopsy specimen of patients with UC in this study.

Methods: VDR levels were retrospectively studied in colon biopsy specimens of UC patients. The Spearman's rho correlation analysis, The Kolmogorov–Smirnov, Mann Whitney U, and chi-square tests were used for statistical analysis. The p values below 0.05 were considered statistically significant.

Results: Study included 112 UC patients (65 male and 47 female) and 30 controls (19 female and 11 male) who had normal results in biopsy examinations carried out due to various reasons. VDR levels of UC patients were statistically lower than control subjects, and was not associated with duration of the disease and place of involvement.

Conclusions: VDR is an important receptor in the pathogenesis of UC, and optimizing vitamin D levels could have a therapeutic role in UC.

Keywords: Ulcerative colitis■Vitamin D receptor■Biopsy specimen

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INTRODUCTION

Ulcerative colitis (UC) is one of the two major forms of inflammatory bowel diseases; and the other is Crohn's disease (CD). UC is a chronic, uncontrolled inflammatory condition of the bowel with colonic and rectal mucosal involvement. In clinical setting, UC shows recurrent periods of remission and exacerbation. The pathogenesis of inflammatory bowel diseases (IBD) is not fully well understood. Complex combinations of genetic, environmental, infectious and immune factors are thought to have a role in the pathogenesis of UC.[1]

Vitamin D deficiency is detected in 16-95% in patients with IBD including even the patients newly diagnosed.[2] These studies suggesting that vitamin D may have a significant role in maintaining the function of intestinal epithelial barrier.[3]

Vitamin D receptor (VDR) is a kind of the steroid receptors. VDR regulates transcription of multiple cellular genes by the effect of vitamin D. The well-known physiological action of the VDR is the regulation of calcium and phosphorus uptake and transport. These are key steps in controlling bone formation. But VDR has more important actions involved in the control of immune functions. $1,25(OH)_2$ D functions mainly via the nuclear vitamin D receptor (VDR). VDR and retinoid X receptor compose a heterodimer compound. This compound binds to the vitamin D response element (VDRE) and recruits co-activators and enzymes with histone acetylation activity.[4,5] VDR regulates genes that play role in bone matrix formation, anti-proliferation and genes associated with anti-inflammation.[6] In US, it is estimated that approximately 436,000 adult to have CD, and 512,000 to have UC.[7]

The association between UC and vitamin D is not clear. There are numerous studies showing the relationship between CD and low level of serum vitamin D.[2] However, the association between UC and vitamin D is studied very rare.[8]

We aimed to determine VDR levels by histopathological examination in colon biopsy specimens obtained from patients with UC. In the present study, VDR levels were retrospectively studied in colon biopsy specimens of UC patients.

METHODS

Study included 112 patients (65 male and 47 female, age: 50 ± 15 years) with confirmed UC diagnoses based on clinical, endoscopic, radiologic and histopathological examinations and 30 controls (19 female and 11 male, age: 54 ± 11 years) who had normal results in biopsy examinations carried out due to various reasons. Subjects with known malabsorption syndrome, chronic liver disease,

subjects being supplemented with vitamin D and calcium, and subjects in pregnancy and gestation periods were excluded. We have got informed consent from patients. Local ethic committee approved our study.

Immunohistochemistry

The immunostaining was carried out at the room temperature using DAKO Autostainer Universal Staining System (Autostainer Link 48 DAKO, Glostrup, Denmark). At the first step, sections obtained from selected paraffin embedded blocks in 4-μm thickness were put on positively charged slides. Then, all the sections were deparaffinized in xylene and dehydrated through a graded series of ethanol solution. At the third stage, antigen retrieval was performed at 96 °C (10 mM/L citrate buffer, pH 6) for 40 min in a thermostatic bath (PT Link). The sections were incubated with Anti-GC (cat. no: HPA001526, Atlas Antibodies, Stockholm, Sweden) for 60 min at the room temperature. Positive and negative controls were added for antibody. A streptavidin-biotin enhanced immunoperoxidase technique (K8000 Envision Flex, DAKO, Glostrup, Denmark) in an automated system was used to show immunoreactions. The sections were incubated with DAB and counterstained lightly with haematoxylin to demonstrate binding. Finally, the sections were dehydrated and mounted onto the slides. The positively immunostained slights were used as positive controls. Normal rabbit serum IgG was used to replace primary antibody as a negative control.

Evaluation of immunostaining

Sections were reviewed by pathologists blinded to the clinicopathological data of the patients' and follow-up information. Immunostaining was scored in relation with the staining intensity and the percentage of the positively stained cells according to Table 1 reproduced from Zhigang et al.[9] (Figure 1). This study was carried out in a region where Mediterranean climate prevails and sunny all year round.

Table 1. Evaluation of the immunostaining scoring.[8]

	Intensity expression (A)	Staining density (B)	Overall Score (AxB)
0	No staining	No staining	Negative expression
+1	Mildly intense	<25% of the sample	1–2 weak expression
+2	Moderately intense	25-50% of the sample	3–6 moderate expression
+3	Severely intense	>50% of the sample	7–9 strong expression

Statistical analysis

Statistical evaluations were performed using SPSS statistical software (Ver. 16.0). (Chicago, SPSS Inc. USA) The Kolmogorov-Smirnov test was used to assess the normality of numeric variables. For the numeric variables that were normally distributed, comparison between two groups was made by independent sample *t*-test and descriptive statistics are presented as mean ± standard deviation. For the numeric variables that were not normally distributed, comparison between two groups was made by Mann–Whitney *U* test and descriptive statistics are presented as median (25-75 percentiles). To analyze the categorical data, a chi-square test was used and descriptive statistics are presented as frequency (%). The Spearman's rho correlation analysis was used to determine the correlation between the numeric variable. The p values below 0.05 were considered statistically significant.

RESULTS

UC patients, examined biopsy specimens had been obtained from, 74 were in active and 38 in remission period. VDR levels of patient and control groups were given in Table 2. VDR levels of UC patients were statistically lower than those of the control subjects. VDR level did not change significantly between patient groups in remission and active periods. VDR level was not associated with duration of the disease and place of involvement (distal, left colon, pancolitis).

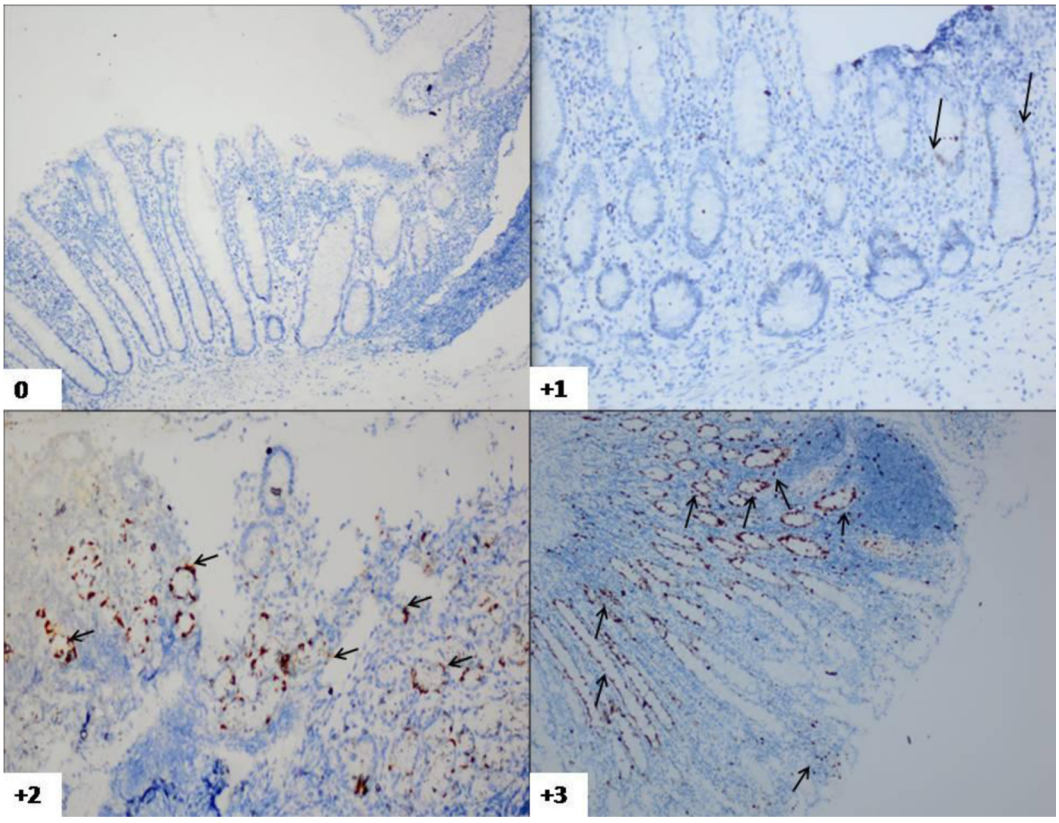
There was no correlation among CRP, sedimentation rate, duration of disease and VDR ($p > 0.05$). The difference of VDR levels were statistically insignificant in patients on steroid treatment.

DISCUSSION

VDR level was significantly low in UC patient. The serum concentration of 25(OH) D did not significantly differ in comparison to normal controls in children and adolescents with IBD, reported by Veit et al.[10] Association between disease severity and vitamin D should be discussed. The information available is generally limited to children age group.

The correlation between vitamin D levels and the duration of the disease is not clear. Some authors reported that longer duration of the disease is suggested as a risk factor for vitamin D deficiency. Nonetheless some others suggested disease duration and serum 25 (OH) D concentrations positively correlated. Data on the clinical association of the vitamin D deficiency with the disease

Figure 1. Immunostaining was scored (as described by Zhigang) based on the intensity of staining and the percentage of cells that stained positively. The intensity of expression was graded on a scale of 0–3+ with 3 being the highest expression observed (0, no staining; 1+, mildly intense; 2+, moderately intense; 3+, severely intense).



activity in IBD patients is conflicting.[11,12] In cross-sectional studies of IBD cohorts, neither el-Matary et al.[13] nor Levin et al.[14] could demonstrated a correlation between vitamin D levels and disease activity.

In patients with CD, vitamin D deficiency was reported in association with lower health-related quality of life and increased disease activity in a retrospective study by Ulitsky et al.[15] but not in UC patients.

Vitamin D deficient patients found more likely to have increased disease activity than patients with normal vitamin D levels reported by Blak et al. Vitamin D deficiency was common, and was associated with increased disease activity, a relapsing disease course and higher inflammatory activity.[16,17] No correlation was found between the duration of disease and VDR levels in our study.

Table 2. VDR levels of patient and control groups.

	Patient group n:112	Control group n: 30	p
Negative	63	3	0.0001
+1	39	7	0.0001
+2	10	15	0.0001
+3	–	5	0.0001

VDR polymorphisms and 25 (OH) D level are significantly correlated with UC risk and severity in a study.[18] Four genetic polymorphisms (*Apa*I, *Bsm*I, *Fok*I, *Taq*I) in *VDR* have been widely evaluated to determine their association with IBD. One meta analysis show that, BsmI and FokI polymorphisms might not be genetic markers for IBD. However, subgroup analyses of the ApaI polymorphism revealed a significant association with CD subtype, while those of the TaqI polymorphism revealed a significant association with the UC subtype, especially in Caucasians.[19] Genetic make-ups of our patients were not known.

VDR is widely expressed in various human tissues that immune cells, keratinocytes, pancreatic beta-cells, cardiac myocytes, central nervous system, renal tubules, and the intestine may be mentioned among them.[2]

VDR-knockout mouse studies showed that loss of VDR expression resulted in inflammation reactions in the irritated gastrointestinal tract. This may cause increased susceptibility to mucosal barrier damage resulting increased risk of IBD.[20,21]

Over a 22 year follow-up, higher plasma 25(OH) D levels were associated with lower risk of CD, but not reduction in risk of UC. Negative correlation between vitamin D intake and the risk for UC was reported. Increase of daily intake of vitamin D from dietary sources and supplement was associated with a 10% relative risk reduction in development of UC per 100 IU increment.[22]

Therapeutic feature of VDR agonist was pointed out in animal models.[23] But it has not been possible yet to reach a conclusion to use them in treatment of the disease.[24,25]

Wada et al.[26] found lower vitamin D receptor level in patients with UC who developed colon cancer compared to the ones who did not develop. It has been implied that there could be an association between low level VDR and colon cancer development. Similar to the present study, Wada et al.[25] couldn't find an association with clinical stage. Also, another study reported that inflammation was negatively correlate with colonic vitamin D receptor expression particularly in patients with Crohn's disease from the IBD cohort.[27] Colon cancer development was not observed in our patients.

In this study, the components of the vitamin D metabolic pathway complex, such as parathyroid hormone (PTH), 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D were not exhaustively evaluated. Increased adiposity was associated with vitamin D deficiency in some studies.[13,14] One of the limitations of our study was that DEXA measurements and body mass index were not evaluated in our patients.

Detection of Vit D deficiency at the same time with elevated erythrocyte sedimentation rate suggests that presence of inflammation in IBD patients is a risk factor for vitamin D deficiency.[1] In our study, there was no significant association between CRP, sedimentation rate and VDR levels.

Vitamin D substitution is recommended in patients with inflammatory bowel disease. Specific guidelines are lacking.[28]

CONCLUSION

We conclude that VDR is an important receptor in the pathogenesis of UC. In addition, optimizing vitamin D levels could have a therapeutic role in UC.

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