Determination of Vitamin D Serum Levels and Status of the C3435T Polymorphism of Multidrug Resistance 1 Gene in Southeastern Iranian Patients with Ulcerative Colitis

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Received: 30 May 2015
Accepted: 28 Jul. 2015

ABSTRACT

BACKGROUND

Ulcerative colitis (UC) is a multi-factorial autoimmune disease. P-glycoprotein is encoded by the multidrug resistance 1 (MDR1) gene. The C3435T polymorphism in the MDR1 gene is correlated with low P-glycoprotein expression. Additionally, vitamin D has regulatory effects on the immune system. The aim of our study was to determine the association between the C3435T MDR1 polymorphism and UC and to detect the vitamin D serum levels in patients with UC.

METHODS

One hundred healthy controls and 85 patients with UC were evaluated. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the C3435T MDR1 polymorphisms. Serum levels of vitamin D were measured by Enzyme-linked immunosorbent assay (ELISA). The research was performed in Kerman, Iran, from 2011 to 2013.

RESULTS

We could not find any association between the C3435T MDR1 polymorphism and susceptibility to UC. There was a significant decrease in serum levels of vitamin D in patients with UC compared with healthy controls ($p<0.001$).

CONCLUSION

Controversies regarding the association between the C3435T MDR1 polymorphism with UC have been reported in different populations. The difference between our results and others may be attributed to the heterogeneity of the Iranian population and the sample size. Additionally, our data indicated that UC might be correlated with vitamin D insufficiency. Therefore, the administration of vitamin D might be suggested as a valuable treatment for patients with UC.

KEYWORDS
Vitamin D, MDR1 gene polymorphism, Ulcerative colitis

Please cite this paper as:
Mohammadi M, Zahedi MJ, Nikpoor AR, Nazem M, Khazaeli P, Hayatbakhsh MM. Determination of Vitamin D Serum Levels and Status of the C3435T Polymorphism of Multidrug Resistance 1 Gene in Southeastern Iranian Patients with Ulcerative Colitis. *Middle East J Dig Dis* 2015;7:246-53.

INTRODUCTION

Inflammatory bowel disease (IBD) is classified to ulcerative colitis (UC) and Crohn's disease (CD). Its etiology is still unknown. Inappropriate activation of the mucosal immune system induced by intestinal bacterial flora and also environmental and genetic factors may participate in IBD susceptibility and clinical phenotype.[1,2] Genetic factors are an important issues in the development of UC as evidenced by observations focused on the familial aggregation of the disease and on non-identical and identical twins.[3] However, there have been contradictory results in the genetic studies, as UC may or may not be associated with candidate gene polymorphisms.[4-7] P-glycoprotein is a trans-membrane efflux pump that extrudes a variety of drugs and toxins from cells and is encoded by the multidrug resistant 1 (MDR1) gene.[8] P-glycoprotein has been detected in the small intestine and colon of the human. The MDR1 gene is located on the long arm of chromosome.[9] Fifty mutations have been identified so far in this gene, some of which can affect its function and expression.[10] The MDR1 gene is a potential candidate for studying the pathogenesis of IBD. Moreover, response to treatment in patients with IBD might be associated with MDR1 gene in both functional and genetic levels.[11] Farrell and colleagues[12] showed that response failure to treatment with glucocorticoids in patients with IBD might be related in part to higher expression of the MDR-1 gene. One well known single nucleotide polymorphisms (SNPs) in the human MDR1 gene is the C to T transformation at position 3435 of exon 26 which is associated with decreased expression and function of intestinal P glycoprotein; however, there is still controversy about the effects of this polymorphism on IBD.[13,14] On the other hand, many studies have shown the role of vitamin D in the regulation of calcium and other bone-building processes. Vitamin D has an important role in the immune system for regulating T cell-mediated responses.[15] This vitamin has also shown inhibitory effects on the production of inflammatory cytokines such as IL-2 and IL-12.[16] Additionally, there is evidence about the role of vitamin D in the pathogenesis and treatment of IBD.[17-19] Vitamin D deficiency is more common in patients with IBD (especially CD), and correlates with several factors such as malabsorption-related surgeries, mucosal disease, reduced daily exercise, low intake of vitamin D in the diet and reduced exposure to sunlight.[18] Results of one study showed that low bone mass density (BMD) was associated with IBD. 12-14% of patients with IBD had osteoporosis and 22-77% had osteopenia.[20] Glucocorticoids use is the most well known risk factor for osteoporosis in IBD. However reduced BMD is reported in patients with IBD with no history of steroid use.[21] Smoking, low body mass index, reduced daily excersice, older age, malnutrition, and low level of vitamin D are reported to be other risk factors for reduced BMD in IBD.[20-22]

By employing this knowledge, and because the Iranian population is genetically heterogeneous and there is no published data from the Kerman population in southeast Iran, we aimed to study the association between the C3435T MDR1 polymorphism and UC for the first time. Moreover, the determination of the serum levels of vitamin D in patients with UC and controls was another objective in our the present study.

MATERIALS AND METHODS

Patients and controls

This case-control study was designed to determine the association of the C3435T gene polymorphism with the MDR1 gene. This research was performed in Kerman, Iran, from 2011 to 2013 and was approved by Ethics Committee of Kerman University of Medical Sciences. Informed consent to take part in the research was obtained from the participants. The approval number is K/89/50. One hundred and eighty five subjects including 100 sex- and age-matched healthy controls (41 women and 59 men, mean age 38.19 ± 12.24 years) from the Kerman Blood Transfusion Center and 85 patients (47 women and 38 men, mean age 37.79 ± 15.79 years) with UC were enrolled in our study. The patients with UC were diagnosed according to

the protocol of the American Gastroenterology Association (18).

Genotyping

Genomic DNA was extracted from 5 mL of whole blood by using a routine salting out method.[23] The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to detect the single nucleotide polymorphism, C3435T, in the MDR1 gene using 5-ACT CTT GTT TTC AGC TGC TTG-3' as the forward primer and 5'-AGA GAC TTA CAT TAG GCA GTG ACT C-3' as the reverse primer.[24] The PCR amplifications were performed based on the following conditions: initial denaturation at 94°C for 5 minutes followed by 33 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 20 seconds, an extension at 72°C for 30 s, and a final extension at 72°C for 5 minutes. Five µL of the PCR product (231 bp in size) were digested at 37°C for 4 hours by 5 U of allele-specific restriction endonucleases MboI resulting in the following fragments:163, 68 bp in wild type homozygotes (C/C genotype), no digestion (231 bp) in the polymorphic homozygotes (T/T genotype), and 231, 163, 68 bp in the heterozygotes (CT genotype). The restriction fragments were separated by electrophoresis on 2% agarose gels containing ethidium bromide and visualized by UV light.

Enzyme-linked immunosorbent assay (ELISA)

Sera from all the patients and controls were separated from the whole blood and kept in -80°C. In order to measure vitamin D levels in the serum, the competitive ELISA technique (DLD Diagnostika kit, Germany) was performed according to the manufacturer instructions.

Statistical analysis

The genotype and allotype frequencies deviations from the Hardy-Weinberg equilibrium were analyzed for all individuals. Statistical analyses such as logistic regression, independent t test, Chi-squared, Fisher's exact test, and descriptive statistics were performed using the SPSS software version 17.0. p values less than 0.05 were considered as statistically significant.

RESULTS

Taking the clinical history and determining the status of the disease was done by a gastroenterologist for all patients. Demographic, clinical, and paraclinical data of the patients are summarized in table 1.

The genotype and allotype frequencies for all individuals did not deviate significantly from the Hardy-Weinberg expectations. In the patients, the genotype frequencies were 14.1% CC, 50.6% CT, and 35.3% TT whereas in the controls, the genotype frequencies were 18% CC, 51% CT, and 31% TT (table 2).The results of our study suggested no association between C3435T MDR1 gene polymorphism and UC.

Additionally, after measuring the serum levels of vitamin D in the patients and the control groups, a significant difference was observed between the two groups. Serum levels of vitamin D in the controls were considerably higher than those in the patients ($p<0.0001$, table 3). Moreover, there was no significant correlation between the C3435T MDR1 gene polymorphism and serum levels of vitamin D in the patients with IBD and healthy controls (data not shown).

DISCUSSION

P-glycoprotein is produced by MDR1 gene and is found in the epithelium of the ileum. Due to the location, function, and high level of P-glycoprotein expression, it is believed that this pump excretes toxins into the bile, urine, and the bowel and can act as a barrier to prevent the accumulation of toxins in the body.[25] Several studies have focused on the relationship between the C3435T MDR1 polymorphism and susceptibility to IBD, but the results are controversial.[26-38] We could not find any association between the C3435T MDR1 polymorphism and UC in the current study of the population in southeast Iran. Our finding of no association between the C3435T MDR1 gene polymorphism and UC

Table 1: Demographic, clinical, and paraclinical characteristics of the patients with ulcerative colitis

Variables			UC patients (%)
Demographic and clinical characterizations	Gender	Male	38 (44.7%)
		Female	47 (55.3%)
	Age	year±SD, (range)	37.79±15.79, (14-84)
	Disease duration	year±SD	3.44±3.07
	Bowel movements	Mild	45 (52.9%)
		Moderate	28 (32.9%)
		Severe	12 (14.1%)
	Immunosuppressive drugs	Cytotoxic and steroidal	14 (16.5%)
		ASA	40 (47.1%)
		Others	31 (36.4%)
	Anemia	Mild	41 (48.2%)
		Moderate	35 (41.2%)
		Severe	9 (10.6%)
	Blood in stool	Mild	39 (45.9%)
		Moderate	26 (30.6%)
		Severe	20 (23.5%)
	Tachycardia	Mild	72 (84.7%)
		Severe	13 (15.3%)
	Age at diagnosis	year±SD, (range)	34.72±15.49, (11-82)
	Appendectomy		3 (3.5%)
	Oral contraceptive consumption (female)		10 (21.27%)
	Cigarette smoking		5 (5.9%)
	Opium consumption		14 (16.5%)
	Family history of disease		8 (9.4%)
	Endoscopic criteria	Mild	44 (51.8%)
		Moderate	25 (29.4%)
		Severe	16 (18.8%)
	Erythrocyte sedimentation rate (ESR)	< 25 mm/hr	58 (68.2%)
		> 25 mm/hr	27 (31.8%)
Total			85

is in agreement with earlier studies from Greece[26], Iran[27], Poland[28], Caucasians in the UK and Germany[29], Spain[30] and a sample of the population of non-Jewish and white Ashkenazi Jewish.[31] Additionally, our results are similar to the findings of a meta-analysis done by Wang and colleagues in 2014.[32] Some reports have demonstrated an association between the C3435T MDR1 gene polymorphism and UC, but our results are inconsistent with their findings.[33-38] Significant associations were observed between the C3435T MDR1 gene polymorphism and UC in an ethnic Iranian group from Tehran[33], Slovenian Caucasian[34], and German 35and Scottish white.[36] Additionally, a significant association was reported between the forenamed gene polymorphism and UC after performing a meta-analysis in 2006 by Onnie and co-workers[37] and Annese and colleagues.[38] Inconsistency between our results and the published articles from others might be due to the heterogeneity of the populations, the effect of other known/unknown polymorphisms on the disease, environmental interactions, the uncertainty in the diagnosis of UC, and also the disparity in the number of samples.

Table 2: Genotype and allotype frequencies for C3435T polymorphism in the MDR1 gene

			UC patients	Controls	*p*‡	OR (CI 95%)
C3435T C>T Genotype	Co-dominant	CC (%)	12 (14.1%)	18 (18%)	0.710	1.0 (reference)
		CT (%)	43 (50.6%)	51 (51%)		1.265 (0.548- 2.917)
		TT (%)	30 (35.3%)	31 (31%)		1.452 (0.598- 3.522)
		HWE p	0.58	0.70		---
	Dominant	CC	12(14.1%)	18 (18%)	0.550	1.0 (reference)
		T/T-C/T	73 (85.9%)	82(82%)		1.335(0.6025- 2.959)
	Recessive	C/T-C/C	55 (64.7%)	69 (69%)	0.638	1.0 (reference)
		T/T	30 (35.3%)	31 (31%)		1.214 (0.6567 - 2.245)
	Over-dominant	C/T	43 (50.6%)	51 (51%)	1.00	1.0 (reference)
		T/T-C/C	42 (49.4%)	49 (49%)		1.017 (0.5700 - 1.813)
	Allele	C (%)	67 (39.4%)	87 (43.5%)	0.459	1.0 (reference)
		T (%)	103 (60.6%)	113(56.5%)		1.184 (0.781- 1.794)

Abbreviations: HWE:Hardy-Weinberg equilibrium, OR: Odds Ratio, CI95%: Confidence Interval 95%
‡pvalue for genotype and allele analysis

Table 3: Comparison of vitamin D serum levels between the patients with UC and controls

Groups	Mean vitamin D level (ng/mL ± SD)	*p* value	OR	95% CI
UC patients (n=85)	20.82 ± 11.47	<0.001	1.056	1.02 – 1.08
Controls (n=100)	28.43± 12.39			

Vitamin D can reduce the risk of disease-related immune mechanisms by reducing the proliferation and differentiation of T-helper cells which, in turn, causes a decrease in inflammatory cytokine production, including interferon-gamma (IFN-γ), interleukin -2 (IL-2), and interleukin-5 (IL-5).[39] In other words, a decrease in vitamin D levels is correlated with an increase in inflammatory cytokine production. The result of our study showed that lower serum levels of vitamin D were associated with UC. Vitamin D deficiency is associated with several diseases such as UC and Crohn's disease. Recent published data have shown that the disease activity in patients with UC was significantly increased in vitamin D-deficient patients in comparison with the patients with UC who had normal vitamin D levels (p= 0.04).[40] In a cohort study, vitamin D deficiency was reported to be common in IBD and was associated with more disease activity in CD but not UC.[41] Anathakrishnan and co-workers[42] reported an association between vitamin D deficiency and increased risk of surgery in CD and hospital admissions in both CD and UC. However, El-Matary and colleagues[43] and Levin and co-workers[44] did not find a correlation between vitamin D levels and disease activity in patients with IBS. Additionally, a high prevalence of vitamin D deficiency has been reported in patients with IBD in several studies. Levin and colleagues[44] reported vitamin D insufficiency in 38% and deficiency in 19% of children with IBD in a cohort study including 70 patients with CD and 5 patients with UC. Anathakrishnan and co-workers[42] reported vitamin D insufficiency in 28% and deficiency in 32% of a large population with IBD including 1763 patients with CD and 1454 patients with UC. Fu and colleagues[45] reported low level of vitamin D in sera of 39% of the entire cohort, which was more frequent in 43% of patients with CD and 37% of patients with UC. Pappa and others[46] reported vitamin D insufficiency in 31% of patients with CD and 28% of patients with UC. Leslie and colleagues[47] reported low level of vitamin D in serum of 88% of patients with IBD including 56 patients with CD and 45 patients with UC.

Several interventional studies showed the effect of vitamin D supplementation on CD activity. Miheller and others[48] reported a decrease in CD activity index in patients treated with alfacalcidiol.

Jorgensen and colleagues[49] showed lower rates of relapse in patients with CD treated with vitamin D3 compared with placebo. Yang and co-workers[50] showed a reduction in CD activity index and improved quality of life in patients with CD treated with vitamin D supplementation. As we mentioned earlier, vitamin D deficiency is common in patients with IBD. Several risk factors have been reported for vitamin D deficiency in IBD. Lower levels of vitamin D have been associated with smoking and with duration of disease.[51] Administration of oral corticosteroids within three months of diagnosis of UC are more prevalent in patients with vitamin D deficiency (<20 ng/ml).[52] Additionally, an IBD cohort study showed a significant prevalence of vitamin D deficiency in patients who took long term glucocorticoid.[53] Moreover, an interesting study among populations from geographical area with lower exposure to ultraviolet B (UVB) light showed an association between increasing incidence of IBD and lower levels of vitamin D.[54]

By employing this knowledge, it appears that further analyses will be needed to verify the clinical efficacy of vitamin D in patients with UC and to answer whether vitamin D has a role in the prevention of UC and modulation of its severity. In conclusion, the result of the present study shows the lack of association between the C3435T MDR1 gene polymorphism and UC. Additionally, our results show that lower serum levels of vitamin D is associated with UC.

ACKNOWLEDGEMENTS

Special acknowledgement should be dedicated to the staff of Kerman Blood Transfusion Centre who helped us with blood collection from healthy volunteers. This research was financially supported by Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.

Sources of Funding:

Special thanks should be dedicated to the staff members of Kerman Blood Transfusion Centre who helped us with blood collection from healthy volunteers. This research was financially supported by Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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