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ORIGINAL ARTICLE

Genetic Variants of Pre-microRNAs $A_{-499}G$(rs3746444) and $T_{-196a2}C$(rs11614913) with Ulcerative Colitis (UC) and Investigated with Thiopurine-S-Methyltransferase (TPMT) Activity

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SUMMARY

Background: **Abnormal expression and different splicing of miRNAs are involved in several human inflammatory disorders. It has been suggested that gene variants of miRNAs may be associated with increased risk of ulcerative colitis (UC). We aimed to evaluate the association of two SNPs (miRNA-$A_{-499}G$(rs3746444) and miRNA-$T_{-196a2}C$(rs11614913)) with the risk of UC and monitor their effect on thiopurine-S-methyltransferase (TPMT) activity in Kurdish population of Iran.**

Methods: **This case-control study was performed on 210 UC patients and 212 healthy individuals. Genotyping assay was performed using PCR-RFLP and the TPMT-activity was measured via non-extraction-HPLC method.**

Results: **We found that the existence of GG genotypes and G allele of miRNA-$A_{-499}G$ SNPs significantly increased the risk of UC by 1.76 and 1.32 times, respectively. The distribution of GG genotype (23.8% vs. 16%, $\chi^2 = 4.2$, $p = 0.041$) and G allele (46.4% vs. 39.4%, $\chi^2 = 4$, $p = 0.046$) of miRNA-$A_{-499}G$, were significantly higher in UC patients compared to control group. Our results indicate that miRNA SNPs (miRNA-$T_{-196a2}C$ and miRNA-$A_{-499}G$) have no significant effect on TPMT activity of studied population.**

Conclusions: **Our results, for the first time, demonstrate that the GG genotype and G allele of miRNA-$A_{-499}G$ significantly increase the risk of UC. However, miRNA SNPs showed no significant effect on TPMT activity in studied population.**

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KEY WORDS

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of disorders with unknown etiology [1]; therefore, genetic investigation may give us a new insight regarding its mechanisms, course and treatment. Crohn's disease (CD) and ulcerative colitis (UC) are two complex disorders of IBD which are reflected by a wide variation in clinical practice [2,3]. In recent decades, the incidence of inflammatory bowel diseases has been estimated up to 120 - 200/100,000 persons for UC and 50 - 200/ 100,000 persons for CD [4]. The pathophysiology of such diseases is not clear, but it seems that association between genetic, epigenetic, infectious, physiological, and immunological factors play a crucial role in the development of UC [5]. Unexpected results from animal models with intestinal inflammation indicate that dysregulated responses of the immune system to intraluminal antigens of bacterial origin in genetically predisposed persons lead to ulcerative colitis and Crohn's disease [6].

Gazouli and colleagues reported that CD and UC are associated with different gene expressions which are involved in immune function, inflammation, and tissue remodeling. Cytokines, growth factors, inflammatory mediators, extracellular matrix proteins, antimicrobial factors and cell cycle regulators are of this kind [7]. MicroRNAs are a novel group of small, single stranded, conserved, and non-coding RNAs (sncRNAS), which are present in all species, are responsible for regulation of gene expression and a variety of biological processes including cell proliferation, cell death, stress resistance, and differentiation of intestinal epithelial cells [8,9]. MiRNAs consist of approximately (18 - 25) nucleotides and play an important role in post-transcriptional regulation of gene expression and are able to affect the stability and translation of mRNAs. Recently, miRNAs have been considered as biomarkers of specific diseases [10,11]. They are usually transcribed by RNA polymerase II and III in order to form long primary RNA transcripts (pri-miRNAs) in the nucleus. Then, they undergo a series of cleavage events to form mature micro RNA in the cytoplasm [12,13]. Drosha and Dicer, two members of the ribonuclease (RNase) III endonuclease protein family are involved in miRNAs processing [14]. The nuclear RNase III is named Drosha which cleaves primary miRNAs (pri-miRNAs) to release hairpin-shaped pre-miRNAs and is subsequently cut by cytoplasmic RNase III, known as Dicer, in order to form mature miRNAs [15]. Recently the role of miRNA in triggering and progression of some disorders such as cancer and autoimmune diseases have been studied; however, less work has been done on UC [16-17]. Several SNPs have been reported within pre-miRNA sequences that may affect the miRNA expression and function [18,19]. Polymorphic sites of miRNA C$_{-196a2}$T (rs11614913) and miRNA A$_{-499}$G (rs3746444) is located in the pre-miRNA region and might be associated with some inflammatory diseases. Previous studies have shown that A$_{-499}$G SNP might be linked to susceptibility to UC, and miRNA A$_{-499}$G and miRNA C$_{-196a2}$T SNPs may be associated with pathophysiological features of UC [20]. Tian reported that miRNA C$_{-196a2}$T SNP might affect mature miRNA expression and target mRNA-binding activity and significantly associates with cancer survival [21]. However, roles of miRNA C$_{-196a2}$T and miRNA A$_{-499}$G SNPs in determination the risk of UC are poorly understood.

In the last decade, in order to reduce and remission the IBD disease immunomodulatory, drugs have been prescribed by the physicians [2]. One group of immunosuppressant drugs are thiopurines which are widely used as first-line treatment for IBD. Although thiopurines are effective and safe, a significant number of patients are suffering from their side effects or show poor response to them [22]. Metabolic inactivation by xanthine oxidase (XO), or by thiopurine-S-methyltransferase (TPMT) leads to a decrease in the toxicity of thiopurines [23]. TPMT (EC 2.1.1.67) is a cytosolic enzyme found in many body cells. Bahrehmand et al., have reported no frequency for deficient, 2.8% for low, and 97.2% for normal activity of TPMT in western population of Iran [2,3], which is different compared to the results of other studies, and there is no known specific target for microRNA. In this study, we aimed to assess whether miRNA A$_{-499}$G and miRNA C$_{-196a2}$T SNPs could affect TPMT expression and consequently its activity. The association between mentioned SNPs in pre-miRNAs and the risk of UC in population of Kermanshah province, Iran was another reason for our study.

MATERIALS AND METHODS

Patients

Our study was pre-approved by the ethical committee of Kermanshah University of Medical Sciences. This is a case-control study with convenience sampling and definite time period. Ten milliliters peripheral blood in ethylene diamine tetra acetic acid (EDTA) (0.5 mM) was obtained from 210 unrelated UC patients and 212 unrelated healthy individuals as control group in Mahdieh clinic of Kermanshah Medical University in the period from February to June 2015 (given that in this study region the majority of IBD patients have UC, the study was performed only on UC patients). Personal and family histories of healthy and patient individuals were unremarkable. The procedures of the study were approved by the Helsinki research ethics committee of the Iranian authority ministry of health according to the World Medical Association Declaration of Helsinki

[24], and a written consent form was obtained from each participant.

DNA extraction and genotyping of miRNA A$_{-499}$G and miRNA C$_{-196a2}$T SNPs

Genomic DNA was extracted from 5 mL peripheral blood using the phenol chloroform extraction method [25]. Genotyping of the C$_{-196a2}$T and A$_{-499}$G miRNA genes was determined by PCR-based RFLP assays using A$_{-499}$G forward
5′-CAAAGTCTTCACTTCCCTGCCA- 3′ and reverse
5'-GATGTTTAACTCCTCTCCACGTGATC-3′
and C$_{-196a2}$T forward
5′-CCCCTTCCCTTCTCCTCCAGATA-3′ and reverse
5′-CGAAAACCGACTGATGTAACTCCG-3 primers. In brief, the PCR reaction for A$_{-499}$G consisted of DNA denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 61.2°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. After amplification, the product (146 bp) was digested with the restriction enzyme BclI. Wild-type AA genotype showed a 146 bp product, whereas homozygous mutant GG genotype showed two fragments (120 and 26 bp) and heterozygous AG genotype showed three fragments (146, 120, and 26 bp) [26] (Figure 1). The PCR reaction for C$_{-196a2}$T consisted of DNA denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 62.1°C for 1 minute and 72°C for 1 minute, and 5 minutes final extension at 72°C. After amplification, the products (149 bp) were digested with the restriction enzyme MspI. Accordingly, wild-type CC genotype showed a 149 bp product; homozygous mutant TT genotype showed two fragments (125 and 24 bp) and heterozygous CT genotype showed three fragments (149, 125, and 24 bp) [27] (Figure 2).

TPMT activity

TPMT activity was measured in whole blood by using a non-extraction high performance liquid chromatography (HPLC) system (Agilent Technologies 1200 Series, Agilent Corp., Germany) using an EC 250/4.6 Nucleodur 5 µm C18 column (Macherey-Nagel, Düren, Germany), as previously described [28].

Statistical analyses

Statistical significance was assumed at $p < 0.05$. SPSS statistical software (SPSS for Windows 16; SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The allelic frequencies were calculated by the gene counting method. The χ^2 test was used to verify the agreement of the observed genotype frequencies with those expected according to the Hardy-Weinberg equilibrium. The miRNA A$_{-499}$G and miRNA C$_{-196a2}$T genotypes and allele frequencies in patients were compared to control samples using the χ^2 test. Odds ratios (OR) were calculated as estimates of relative risk for disease and 95% of confidence intervals (CI) were obtained by SPSS logistic regression. The interaction between miRNA A$_{-499}$G and miRNA C$_{-196a2}$T polymorphism was determined using a logistic regression model. The correlation of TPMT activity with the miRNA A$_{-499}$G and miRNA C$_{-196a2}$T polymorphisms between studied groups were calculated using linear regression and unpaired t test (Pearson's). A two-tailed Student's *t*-test, analysis of variance (ANOVA), and nonparametric independent-sample Mann-Whitney analyses were used to compare quantitative data.

RESULTS

Details of the clinical, laboratory, and demographic characteristics of the patient and control groups are summarized in Table 1. There were no significant differences between age, BMI, gender, and TPMT activity in mU/L of two groups, while Hb concentration in UC patients was significantly lower than control group (13.8 ± 1.85 vs. 14.6 ± 1.73, $p \leq 0.001$). Details of other parameters have been described previously [2].

The frequency of miRNA A$_{-499}$G and C$_{-196a2}$T genotypes and alleles in UC patients and the control group are shown in Tables 2 and 3, respectively. The distribution of GG genotypes miRNA A$_{-499}$G in UC patients was monitored to be significantly higher than control subjects by 23.8% versus 16% ($\chi^2 = 4.2$, df = 1, $p = 0.041$) (Table 2). The age and gender adjusted OR indicated that GG genotypes of miRNA A$_{-499}$G gene mutations significantly increased the risk of UC by 1.76-fold (1.01 - 1.75, $p = 0.041$) (Table 2). As it is shown in Table 2, the frequency of the miRNA A$_{-499}$G polymorphism, G allele was considerably increased in UC patients compared to control subjects by 46.4% versus 39.4%, and increased the risk of IBD 1.32-fold (1.002 - 1.32, $p = 0.046$).

The overall distribution of the miRNA C$_{-196a2}$T genotypes and alleles in UC patients was not significantly different from that of the control group ($\chi^2 = 0.46$, df = 2, p=0.7) ($\chi^2 = 0.21$, df = 1, $p = 0.6$) (Table 3). The relationship of miRNA A$_{-499}$G (AG + GG vs. AA) and miRNA C$_{-196a2}$T (CT + TT vs. CC) genotypes with Hb concentration and TPMT activities in mU/L analyzed are shown in Tables 4 and 5, respectively. We inferred from Tables 4 and 5 that UC patients with one or two copies of mutant alleles of both A$_{-499}$G (AG + GG vs. AA) and C$_{-196a2}$T (CT + TT vs. CC) had lower Hb g/dL ($p < 0.001$) concentration compared to the control group with the mentioned genotypes. In addition, we analyzed logistic regression and the interaction between miRNA C$_{-196a2}$T and miRNA A$_{-499}$G alleles in UC patients shown in Table 6. Our results indicate that there was not significant association between miRNA C$_{-196a2}$T, T alleles and miRNA A$_{-499}$G, G alleles in this study.

Table 1. The demographic characteristics in Ulcerative colitis (UC) patients and control group.

Values	UC patients (n = 210)	Control subjects (n = 212)	p
Age (years)	35.9 ± 13.2	34 ± 14.2	0.58
Gender (M/F)	86/129	96/116	0.27
Hb (g/dL)	13.8 ± 1.8	14.6 ± 1.73	< 0.001
TPMT activity (mU/L)	1.09 ± 20.4	1.1 ± 20.6	0.2
BMI (Kg/m^2)	24 ± 3.97	24.1 ± 4.51	0.87

BMI - Body Mass Index, TPMT - thiopurine methyltransferase, UC - ulcerative colitis.

Table 2. Odds ratio and distribution of miRNA $A_{-499}G$ genotypes and alleles in patients with UC and control subjects.

	UC patients (n = 210)	Control subjects (n = 212)
miRNA $A_{-499}G$ genotypes		
A/A	65 (31%)	78 (36.8%)
A/G	95 (45.2%) (χ^2 = 0.36, df = 1, p = 0.55) OR = 1.14 (0.74 - 1.76), p = 0.55	100 (47.2%)
G/G	50 (23.8%) (χ^2 = 4.2, df = 1, p = 0.041) OR = 1.76 (101 - 1.75), p = 0.041	34 (16%)
G/G + A/G	145 (69%) (χ^2 = 1.6, df = 1, p = 0.2) OR = 1.3 (0.93 - 1.4), p = 0.2	134 (63.2%)
	(χ^2 = 4.35, df = 2, p = 0.11)	
	UC patients	Control group
miRNA $A_{-499}G$ alleles		
A	n = 225 (53.6%)	n = 256 (60%)
G	n = 195 (46.4%)	n = 168 (40%)
	(χ^2 = 4, df = 1, p = 0.046) OR = 1.32 (1.002 - 1.32, p = 0.046)	

miRNA - micro RNA, OR - odds ratio (an estimated relative risk for disease that was calculated and 95% confidence interval was obtained by using χ^2 regression binary logistic analysis).

Table 3. Odds ratio and distribution of miRNA$C_{196a2}T$ genotypes and alleles in patients with UC and control subjects.

	UC patients (n = 210)	Control subjects (n = 212)
miRNA $C_{-196a2}T$ genotypes		
C/C	44 (21%)	39 (18.4%)
C/T	83 (39.5%)	88 (41.5%)
T/T	83 (39.5%)	85 (40.1%)
	(χ^2 = 0.46, df = 2, p = 0.7)	
	UC patients OR (95% confidential interval)	Control group
miRNA $A_{-499}G$ alleles		
C	n = 225 (53.6%)	n = 256 (60%)
T	n = 195 (46.4%)	n = 168 (40%)
	(χ^2 = 0.21, df = 1, p = 0. 6) OR = 0.94 (0.8 - 1.1, p = 0. 6)	

miRNA - micro RNA, OR - odds ratio is an estimate relative risk for disease that was calculated and 95% confidence interval was obtained by using χ^2 regression binary logistic analysis.

Table 4. Comparison of Hb concentrations, TPMT activities (mU/L) between dominant models of miRNA A$_{-499}$G in UC patients with control subjects.

	UC patients (n = 56)	Control subjects (n = 77)	p-values
miRNA A$_{-499}$G genotypes	A/A	A/A	
Hb (g/dL)	14.02 ± 1.9	14.6 ± 1.6	0.25
TPMT activity (mU/L)	111 ± 16.8	112 ± 19.5	0.73
	A/G+G/G	A/G+G/G	
Hb (g/dL)	13.7 ± 1.8	14.5 ± 1.7	< 0.001
TPMT activity (mU/L)	107 ± 21.4	111 ± 19.9	0.16

Table 5. Comparison of Hb concentrations and TPMT activities (mU/L) between dominant models of miRNA C-$_{196a2}$T in UC patients and control subjects.

	UC patients (n = 56)	Control subjects (n = 77)	p-values
miRNA C-$_{196a2}$T genotypes	C/C	C/C	
Hb (g/dL)	13.5 ± 1.7	14.5 ± 1.8	0.01
TPMT activity (mU/L)	108 ± 16.8	111 ± 15	0.37
	C/T+T/T	C/T+T/T	
Hb (g/dL)	13.8 ± 1.8	14.6 ± 1.7	< 0.001
TPMT activity (mU/L)	109 ± 20.4	111 ± 20.6	0.24

Tables 6. Carrier odds ratios interaction between miRNAC$_{196a2}$T allele and miRNA A$_{499}$G allele in UC patients compared with control group.

C$_{196a2}$T A	A$_{499}$G C	UC patients	Control group	OR (95%CI)
-	-	n = 9 (4.3%)	n = 9 (4.4%)	Reference values
+	-	n = 56 (26.7%)	n = 61 (29.9%)	0.92 (0.34 - 2.5, p = 0.6) (χ^2 = 0.03, df = 1, p = 0.96)
-	+	n = 35 (16.7%)	n = 22 (10.8%)	1.6 (0.74 - 2.2, p = 0.39) (χ^2 = 0.73, df = 1, p = 0.39)
+	+	n = 110 (52.4%)	n = 112 (54.9%)	0.98 (0.7 - 1.4, p = 0.7) (χ^2 = 0.07, df = 1, p = 0.97)

Overall distribution: (χ^2 = 3.1, df = 1, p = 0.37).

DISCUSSION

Relapsing inflammatory disorders of the gastrointestinal tract with diarrhea, abdominal pain, rectal bleeding, and malnutrition clinical symptoms are known as IBD [29]. Complex interaction between genetics, immune response, and environmental factors are the main causes of this disease. Pekow et al., cited that alteration of gene expression occurs in Crohn's disease (CD) and ulcerative colitis (UC), the two main subtypes of IBD [30]. mRNA translation can be regulated by miRNAs binding to the 3′ untranslated regions (UTRs) of messenger RNAs. So, it seems logical that miRNA SNPs may affect gene expression. One of the key mechanisms in the incidence of cancer is gene deregulation [31]. UC and CD increase the risk of developing colorectal cancer [32]. The present study focused on the role of two SNPs of pre-miRNAs including miRNA A$_{-499}$G and miRNA C$_{-196a2}$T in susceptibility to UC and their effects on TPMT activity. Because there is no study concerning the role of the two SNPs of miRNA on TPMT activity, our paper for the first time indicated that there is no sig-

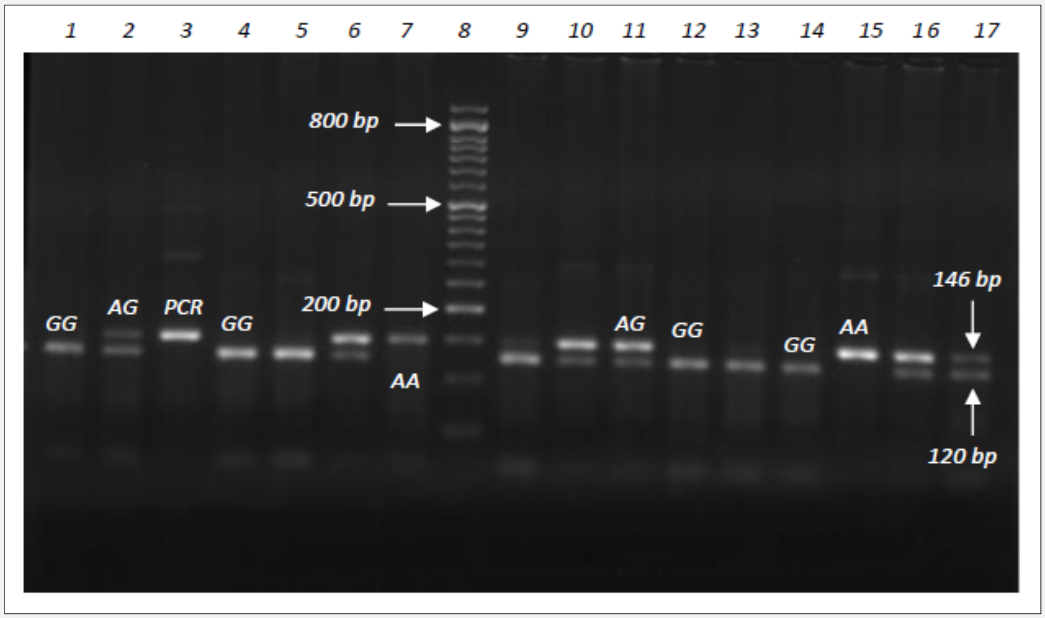

Figure 1. Agarose gel electrophoresis (2.5%) patterns for miRNA $A_{-499}G$ (rs3746444) alleles analyzed by PCR-RFLP.

Lane 8: 50 bp DNA ladder; Lane 3: undigested PCR product; Lanes 2, 6, 10, 11, 16, and 17: heterozygous mutant (AG); Lanes 7 and 8: homozygous wild-type (AA); Lanes 1, 4, 5, 9, 12, 13, and 14: homozygous mutant (GG).

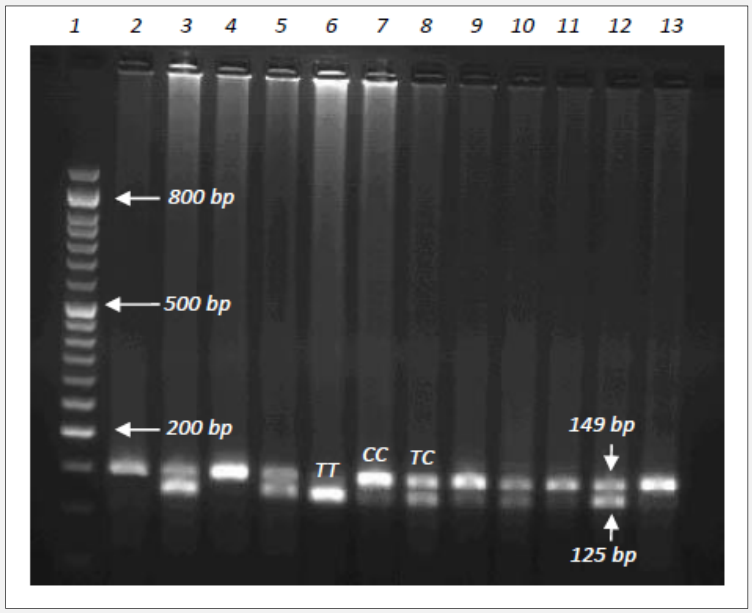

Figure 2. Agarose gel electrophoresis (2.5%) patterns for miRNA-196a2 C>T (rs11614913) alleles analyzed by PCR-RFLP.

Lane L*: 50 bp DNA ladder;* Lane 1*: undigested PCR product;* Lanes 3, 5, 8, 10, and 12*: heterozygous mutant (CT);* Lanes 2, 4, 7, 9, 11, and 13 *homozygous wild-type (CC);* Lane 6*: homozygous mutant (TT).*

nificant association between miRNA A$_{-499}$G and miRNA C$_{-196a2}$T polymorphisms with TPMT activity in western population of Iran. We found that the distribution of GG genotypes and G alleles of miRNA A$_{-499}$G were higher in UC patients and increased the risk of UC.

There are no adequate studies concerning the effect of miRNA A$_{-499}$G polymorphism on UC disease. One case control study including 267 CD patients, 207 UC patients, and 298 matched healthy controls indicated no significant association between the MIR122, MIR499, MIR146A, MIR196A2, and MIR124A SNPs with IBD susceptibility. But they demonstrated that MIR196A2 polymorphism has considerable role in early age at onset in both UC and DC [33]. Their results showed a trend with the case control study performed by Zhu et al. in Chinese population, which indicated the MIR-196A2 SNP may affect the IBD progression [34]. Raisch et al. reported that in a Japanese cohort study consisting of 170 UC patients and 403 healthy controls, pre-miR-499, pre-miR-196a2, and pre-miR-146a SNPs were in correlation with IBD [35]. Susceptibility to UC has been reported to be higher in carriers of AG genotype. Also, both miRNA A$_{-499}$G and miRNA C$_{-196a2}$T SNPs may affect the pathophysiological features of UC in Japanese population [20]. Our study shows no direct association between C$_{-196a2}$T SNPs with increased risk of IBD. Race, nationality, inadequate sample size, genetic heterogeneity, and gene-environmental interactions may be tangible reasons for debatable reports. Our results demonstrated that neither miRNA C$_{-196a2}$T genotypes nor allele polymorphisms were significantly different between UC patients compared with healthy control from population in western Iran. Inconsistent with our finding, among 468 patients from China, CC genotype of miRNA C$_{-196a2}$T polymorphism was significantly associated with increased risk of UC [34]. Gazouli reported no considerable differences in the genotype or allele of miRNA C$_{-196a2}$T distributions among CD patients and control subjects, while TT genotype and T allele showed a protective role against UC in Greek population [7].

CONCLUSION

Our results demonstrated, for the first time, that the GG genotype and G allele of miRNA A$_{-499}$G significantly increased the risk of UC, although there was no significant effect from miRNA SNPs on TPMT activity in this study. Additional analysis with larger samples is needed to prove the possible contribution of miRNA A$_{-499}$G and miRNA C$_{-196a2}$T SNPs with progression of UC in Kermanshah population.

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Declaration of Interest:
There is no conflict of interest.

References:

1. Podolsky DK. Inflammatory bowel disease. N Engl J Med. 1991; 325(14):1008-16 (PMID: 1886623).
2. Bahrehmand F, Kiani A, Vaisi-Raygani A, et al. Pharmacogenetics of drug metabolizing enzyme: thiopurine methyl transferase phenotypes and multidrug resistance 1 gene polymorphism in inflammatory bowel disease. Cell Mol Biol (Noisy-le-Grand, France). 2016;62(7):102 (PMID: 27453281).
3. Bahrehmand F, Vaisi-Raygani A, KianiA, et al. Whole-Blood Thiopurine S-Methyltransferase Genotype and Phenotype Concordance in Iranian Kurdish Ulcerative Colitis (UC) Patients. Clin Lab. 2017;63(5):947-54 (PMID: 28627831).
4. Cosnes J, Gower–Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011;140(6):1785-94 (PMID: 21530745).
5. Vahedi H, Merat S, Momtahen S, et al. Epidemiologic characteristics of 500 patients with inflammatory bowel disease in Iran studied from 2004 through 2007. Arch Iran Med. 2009;12(5):454-60 (PMID: 19722766).
6. Bamias G, Nyce MR, Sarah A, Cominelli F. New concepts in the pathophysiology of inflammatory bowel disease. Ann Intern Med. 2005;143(12):895-904 (PMID: 16365470).
7. Gazouli M, Papaconstantinou I, Stamatis K, et al. Association study of genetic variants in miRNAs in patients with inflammatory bowel disease: preliminary results. Dig Dis Sci. 2013;58(8): 2324-8 (PMID: 23543085).
8. Wei B, Pei G. microRNAs: critical regulators in Th17 cells and players in diseases. Cell Mol Immunol. 2010;7(3):175-81 (PMID: 20364159).
9. Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. Cell. 2003;113(6):673-6 (PMID: 12809598).
10. Chatzikyriakidou A, Voulgari PV, Georgiou I, Drosos AA. miRNAs and related polymorphisms in rheumatoid arthritis susceptibility. Autoimmun Rev. 2012;11(9):636-41 (PMID: 17011485).
11. Kloosterman WP, Plasterk RH. The diverse functions of micro RNAs in animal development and disease. Dev Cell. 2006;11(4): 441-50 (PMID: 17011485).
12. Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004;23(20):4051-60 (PMID: 153 72072).

13. MacFarlane LA, R Murphy P. MicroRNA: biogenesis, function and role in cancer. Curr Genomics. 2010;11(7):537-61 (PMID: 21532838).

14. Gregory RI, Chendrimada TP, Shiekhattar R. MicroRNA biogenesis: isolation and characterization of the microprocessor complex. Methods Mol Biol. 2006;342:33-47 (PMID: 16957365).

15. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev. 2004;18(24):3016-27 (PMID: 15574589).

16. Paraskevi A, Theodoropoulos G, Papaconstantinou I, Mantzaris G, Nikiteas N, Gazouli M. Circulating MicroRNA in inflammatory bowel disease. J Crohns Colitis. 2012;6(9):900-4 (PMID: 223 86737).

17. Kanaan Z, Rai SN, Eichenberger MR, et al. Differential Micro RNA expression tracks neoplastic progression in inflammatory bowel disease-associated colorectal cancer. Hum Mutat. 2012; 33(3):551-60 (PMID: 22241525).

18. Dai ZJ, Shao YP, Wang XJ, et al. Five common functional polymorphisms in microRNAs (rs2910164, rs2292832, rs11614913, rs3746444, rs895819) and the susceptibility to breast cancer: evidence from 8361 cancer cases and 8504 controls. Curr Pharm Des. 2015;21(11):1455-63 (PMID: 25483824).

19. Park YS, Jeon YJ, Lee BE, et al. Association of the miR-146aC> G, miR-196a2C> T, and miR-499A> G polymorphisms with moyamoya disease in the Korean population. Neurosci Lett. 2012;521(1):71-5 (PMID: 22659075 19293314).

20. Okubo M, Tahara T, Shibata T, Yamashita H, Nakamura M, Yoshioka D, et al. Association study of common genetic variants in pre-microRNAs in patients with ulcerative colitis. J Clin Immunol. 2011;31(1):69-73 (PMID:22659075).

21. Tian T, Shu Y, Chen J, et al. A functional genetic variant in micro RNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. Cancer Epidemiol Biomarkers Prev. 2009; 18(4):1183-7 (PMID: 19293314 22659075).

22. Goel RM, Blaker P, Mentzer A, Fong SC, Marinaki AM, Sanderson JD. Optimizing the use of thiopurines in inflammatory bowel disease. Ther Adv Chronic Dis. 2015;6(3):138-46 *(*PMID: 25954 498).

23. Stocco G, Crews KR, Evans WE. Genetic polymorphism of inosine-triphosphate-pyrophosphatase influences mercaptopurine metabolism and toxicity during treatment of acute lymphoblastic leukemia individualized for thiopurine-S-methyl-transferase status. Expert Opin Drug Saf. 2010;9(1):23-37 (PMID: 20021291).

24. Association WM. Declaration of Helsinki. Ethical principles for medical research involving human subjects. 2009.

25. Asefi M, Vaisi-Raygani A, Bahrehmand F, et al. Paraoxonase 1 (PON1) 55 polymorphism, lipid profiles and psoriasis. Br J Dermatol. 2012;167(6):1279-86 (PMID: 22835076).

26. Chu YH, Hsieh MJ, Chiou HL, et al. MicroRNA gene polymorphisms and environmental factors increase patient susceptibility to hepatocellular carcinoma. PLoS One. 2014;9(2):e89930 (PMID: 24587132).

27. Song ZS, Wu Y, Zhao HG, et al. Association between the rs11614913 variant of miRNA-196a-2 and the risk of epithelial ovarian cancer. Oncol Lett. 2016;11(1):194-200 (PMID: 26870 188).

28. Bahrehmand F, Kiani A, Vaisi-Raygani A, et al. TPMT phenotyping & activity with a non-extraction liquid chromatography in IBD. Acta Med Iran. 2017;55(6):360-367.

29. Abraham C, Cho J. Interleukin-23/Th17 pathways and inflammatory bowel disease. Inflamm Bowel Dis. 2009;15(7):1090-100 (PMID: 19253307).

30. Pekow JR, Kwon JH. MicroRNAs in inflammatory bowel disease. Inflamm Bowel Dis. 2012;18(1):187-93 (PMID: 21425211).

31. Landi D, Gemignani F, Naccarati A, et al. Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis. 2008;29(3):579-84 (PMID: 18192692).

32. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physioy. 2004;287(1):G7-G17 (PMID: 15194558).

33. Ciccacci C, Politi C, Biancone L, et al. Polymorphisms in MIR 122, MIR196A2, and MIR124A Genes are Associated with Clinical Phenotypes in Inflammatory Bowel Diseases. Mol Diagn Ther. 2017 Feb;21(1):107-14 (PMID: 27718165).

34. Zhu M, Li D, Jin M, Li M. Association between microRNA polymorphisms and the risk of inflammatory bowel disease. Mol Med Rep. 2016;13(6):5297-308 (PMID: 27109937).

35. Raisch J, Darfeuille-Michaud A, Nguyen H. Role of microRNAs in the immune system, inflammation and cancer. World J Gastroenterol. 2013;19(20):2985-96 (PMID: 23716978).