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Inflammatory Bowel Disease: The Association of Inflammatory Cytokine Gene Polymorphisms

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Additional information is available at the end of the chapter

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Abstract

The frequencies of alleles and genotypes of *TNF- α* , *TNF- β* , and *IL-10* genes were examined in Saudi subjects including IBD patients (UC and CD) and matched controls. Venous blood samples were collected from IBD patients and healthy control subjects, and genomic DNA was extracted using commercially available kit (Qiagen, CA, USA). In order to detect *TNF- α* (-308G/A), *TNF- β* (+252A/G), *IL-10* (-1082G/A), (-819C/T), and (-592C/A) polymorphisms, the *TNF- α* , *TNF- β* , and *IL-10* genes were amplified using an amplification refractory mutation systems PCR methodology. Analysis of data showed that the frequencies of alleles and genotype of *TNF- α* (-308G/A), *TNF- β* (+252A/G), and *IL-10* (-1082G/A), (-819C/T), and (-592C/A) polymorphisms differ between IBD patients and control subjects. Our study clearly indicated that the *TNF- α* (-308G/A), *TNF- β* (+252A/G), and *IL-10* (-1082 G/A) polymorphisms are associated significantly with the risk of IBD susceptibility while other two, *IL-10*-819C/T and *IL-10*-592C/A, polymorphisms are not associated with IBD in Saudi population. However, well-designed epidemiological as well as genetic association studies with large sample size among different ethnicities should be performed in order to have better understanding of this relationship.

Keywords: tumor necrosis factor, interleukin-10, polymorphism, inflammatory bowel disease, Saudis, Crohn's disease, ulcerative colitis

1. Introduction

The inflammatory bowel diseases (IBDs), encompassing Crohn's disease (CD, OMIM 266600) and ulcerative colitis (UC, OMIM 191390), are chronic inflammatory disorders of the gastrointestinal tract. The incidence and prevalence of IBD have been increasing with time in

different regions around the world, indicating its emergence as a global disease [1–5]. Available literature indicates that IBD is a complex and multifactorial disease though the exact etiology is still not clear. However, it has been suggested that immune dysregulation caused by genetic and/or environmental factors plays an important role in the etiology of IBD [6–8]. IBD appears to be caused by overly aggressive T-cell responses directed against environmental factors and/or a subset of commensal bacteria/pathogens that inhabit the distal ileum and colon of genetically susceptible hosts. Patients with long-lasting IBD, both UC and CD, have been at increased risk of developing colorectal cancer, and CD patients are at increased risk of small intestine cancer [9].

The incidence of IBD is higher in North American and European populations compared with those in Asian and African, reflecting the role of both environmental and genetic factors. The rising prevalence of various autoimmune and inflammatory conditions in developed countries has been attributed to hygiene hypothesis, and they are thought to result from the lack of early exposure to select microbial agents due to stringent sanitation conditions [10]. The changes in dietary and intestinal microbial milieu have been suggested to play a key pathogenic role in the etiology of IBD, though the exact environmental factors responsible for changing IBD prevalence are not clearly defined [11]. Intriguingly, the characteristics of Western and Asian IBD patients differ in epidemiology, phenotype, and genetic susceptibility [12–15] highlighting ethnic variations. Various epidemiological and population-based studies have indicated that genetic factors contribute to the pathogenesis of IBD [16–18].

According to Jump and Levine [19], cytokines act as key signal in the intestinal immune system and participate in the disruption of the physiological inflammation of the gut. They are produced mainly by immune cells as small peptide proteins and facilitate communication between cells, by stimulating the proliferation of antigen-specific effector cells, and mediate the local and systemic inflammation in an autocrine, paracrine, and endocrine pathways [20]. A critical role is played by innate immune system in IBD pathology, and several cytokines secreted by activated dendritic cells (DC) and macrophages actively regulate the inflammatory response in IBD.

The production of cytokines can be affected by genetic polymorphisms within the coding and promoter regions of cytokine genes [21, 22]. Therefore, a genetic predisposition for the high or low production of a particular cytokine may affect disease susceptibility and clinical outcome [23, 24].

The IBD is believed to be caused by immunogenic responses against environmental factors and/or microbes inhabiting distal ileum and colon of genetically susceptible hosts. Inflammatory response in IBD is an important feature and proinflammatory cytokine; tumor necrosis factor- α (TNF) has been indicated to play a key role in the initiation and propagation of IBD. Increased expressions of TNF- α have been reported in peripheral phagocytes and intestinal tissues of IBD patients. High levels of TNF- α have also been documented in the serum of IBD patients [25–27]. Moreover, monoclonal antibodies against TNF- α have been effectively used to decrease inflammation in IBD [28]. Variations in levels/expression of TNF due to its genetic polymorphism have been linked with pathogenic role of this cytokine in various autoimmune and inflammatory diseases and thus have been regarded to be an appropriate target for management of diseases by interfering with the inflammatory responses.

In view of the important immunoregulatory roles of TNF- α and TNF- β , they are considered as subject of interest for studies in IBD. TNF- α is produced mainly by monocytes and activated macrophages while TNF- β is produced mainly by activated T cells. Both *TNF- α* (OMIM 191160) and *TNF- β* (MIM153440) genes are located on chromosome 6 within the MHC III region and show close linkage to the HLA class I (*HLA-B*) and class II (*HLA-DR*) genes. It has been shown by various studies on monozygotic twins and first-degree relatives that 60% of variation in the production capacity of TNF- α is genetically determined [29]. A number of polymorphisms within the promoter region of *TNF- α* and the intron 1 polymorphism of *TNF- β* , in particular have been associated with variations in the serum levels of TNF- α [30, 31]. One of the best described single nucleotide polymorphisms (SNPs) is located at nucleotide position -308 within the *TNF- α* promoter region (rs1800629) and affects a consensus sequence for a binding site of transcription factor AP-2 [32]. TNF- α (-308) promoter polymorphism leads to a less common allele-A (allele 2), which has been associated with increased TNF- α production in vitro [33, 34] and higher rate of TNF- α transcription than wild-type GG genotype [35, 36]. This polymorphism has been linked to increased susceptibility to several chronic metabolic degenerative, inflammatory and autoimmune diseases [37–41].

Of interest, G/A polymorphism at nucleotide position -308 within the human TNF- α promoter region is associated with elevated TNF levels, disease susceptibility, and poor prognosis in several diseases [42–45]. Adenine at position -308 makes the TNF- α promoter a much more powerful transcription activator than guanine [42].

TNF- β +252A/G (rs909253) polymorphism affects a phorbol ester-responsive element. The presence of G at +252 position refers to the less frequent mutant allele known as TNF- β * 1 (allele-1), which is associated with higher TNF- α and TNF- β production [42, 46].

TNF- β resembles to TNF- α in terms of several biological activities including apoptosis and gives rise to a similar proinflammatory response and has been shown to play a critical role in pathogenesis of many diseases. TNF- β has also been shown to contribute to the susceptibility of several inflammatory/autoimmune diseases. Association of TNF- β +252 A/G polymorphism has been reported with various autoimmune disorders including Gravis' disease [47] idiopathic membranous glomerulonephritis, IgA nephropathy, insulin-dependent diabetes mellitus [48], myasthenia gravis [49], asthma diathesis [50], SLE with nephritis [51], systemic sclerosis [52], plaque psoriasis [53], rheumatoid arthritis [54], and type 1 diabetes [55]. Recently, TNF- β +252 A/G polymorphism is reported to be associated with both susceptibility to and mortality from sepsis [56].

A few studies have been undertaken to determine the association of TNF- α polymorphisms and IBD in different parts of the world [57–59]. The results of these studies on association of TNF- α polymorphism with IBD are not consistent, and variations have been reported [60]. These variations might be due to genetic differences in populations or systemic variations in the ancestry of IBD patients and control subjects involved in the studies [27]. Moreover, differences have been found in the characteristics, epidemiology, phenotype, and genetic susceptibility to IBD in Western and Asian populations [15, 16]. Therefore, studies involving these unique features in different ethnic populations will help not only identifying the pathophysiology but also understanding the etiology of IBD.

No research has been done on the association between TNF- β polymorphism and IBD. TNF- α and TNF- β are closely related cytokines, and both are involved in the expression of TNF- α and in a suggested mechanism for autoimmune/inflammatory diseases; therefore, the joint analysis of polymorphisms in *TNF- α* and *TNF- β* genes will provide further insight into the pathogenesis of IBD and help in developing effective therapeutic agents. Saudi population is ideal for such genetic association studies because of the fact that it is a closed and isolated society with quite high rate of consanguinity. So, we studied and evaluated the possible association of alleles and genotypes of TNF- α (-308G/A) and TNF- β (+252A/G) polymorphisms with the susceptibility risk to IBD in this population.

On the other hand, interleukin-10 (IL-10) is an anti-inflammatory cytokine and can inhibit the synthesis of proinflammatory cytokines, such as interferon- γ , IL-2, IL-3, and tumor necrosis factor- α (TNF- α), produced by macrophages and regulatory T cells [61]. IL-10 is responsible for various functions. It shifts the Th1/Th2 balance by downregulating the Th1 responses and by suppression of proinflammatory cytokines [62]. Several studies have shown that serum IL-10 levels are significantly lower in IBD patients than in normal controls, suggesting that altered IL-10 levels may be involved in the pathogenesis of IBD and may be an IBD biomarker. IL-10 is capable of depressing the activated immune system. It has been reported that IL-10 knockout mice develop colitis when they are kept in unsterile environment [63], and the inflammation is reduced after administration of IL-10 in vivo and in vitro models [64]. Moreover, the production of IL-10 has been found to be impaired in severe cases of IBD [65, 66].

IL-10 suppresses CD4⁺ T helper, Th1, clones (which secrete IL-2, interferon- γ , and TNF- α) and promotes the immunomodulatory T helper, Th2, clones (which secrete IL-4, IL-10, and IL-13). The secretion of cytokines is responsible for regulating the balance between Th1 and Th2 cells which is critical for immunoregulation. In case of reduced capacity of T cells to produce IL-10 in response to a stimulus, Th1 responses continue with the breakdown of peripheral tolerance and are potential to develop autoimmunity [67].

IL-10 is a multifunctional cytokine mainly produced by immune cells, such as T cells, monocytes, appropriately stimulated macrophages, some subsets of dendritic cells (DCs), and B cells [68]. Non-immune cell sources of IL-10 also exist, including keratinocytes, epithelial cells, and some tumor cells [69, 70]. The human *IL-10* gene is located on chromosome 1q32.1 and contains five exons. Recently, IL-10 has been identified as an important player in the development of immunological and inflammatory responses involving in the pathogenesis of various diseases including IBD [71–73].

Several single nucleotide polymorphisms (SNPs) have been reported in the proximal and distal regions of the *IL-10* gene, out of which three promoter polymorphisms (rs18000896-1082A/G, rs1800871-819T/C, and rs1800872-592A/C) are involved in IL-10 transcription rate and directly affect its production level and expression [74–77]. The -1082G, -819C, and -592C (GCC) alleles have been associated with elevated levels of IL-10 production [78], while ACC and ATA haplotypes show intermediate and low *IL-10* gene transcription, respectively [79]. These IL-10 gene polymorphisms are reported to be associated with susceptibility/development to various inflammatory disorders [40, 80–82]. However, data are limited and inconsistent and therefore do not allow drawing unequivocal conclusions.

Studies on the *IL-10* promoter polymorphisms and IBD susceptibility have also been inconsistent [71, 83–89]. Some studies have found an associations between *IL-10* polymorphism and IBD [71, 86, 87, 90], whereas other studies were unable to find any association between IBD and the *IL-10* promoter polymorphisms [83, 84, 88, 91]. In this study, we evaluated the association of five polymorphism in *IL-10*, *TNF- α* , and *TNF- β* genes with susceptibility risk of IBD in Saudi patients.

2. Methods

2.1. Subjects

Study groups consisted of 379 Saudi subjects including 179 IBD patients and 200 age- and sex-matched healthy controls visiting Gastroenterology Clinic of Prince Sultan Military Medical City (PSMMC), Riyadh. IBD patients included 20 cases of familial forms and 159 cases of sporadic forms. Of these patients, 95 were diagnosed to suffer with CD (57 men, 38 women) aged 17–65 years (mean age 32 years), while 84 patients with UC (34 men, 50 women) aged 22–68 years (mean age 34 years). Control group consisted of 120 men and 80 women matched for age and ethnicity (Saudi). Control subjects were screened for any history of IBD, diabetes, rheumatoid arthritis, systemic lupus erythematosus, or other autoimmune/inflammatory diseases and excluded if found positive. The diagnoses of CD and UC were based on conventional endoscopic, radiological, and histological criteria [92]. Demographic and clinical data were collected and used for exclusion and inclusion as described elsewhere [93]. This study was approved by the research and ethical committee of PSMMC, and written informed consent was obtained from all subjects to participate in this study.

2.2. Polymerase chain reaction (PCR) amplification

Venus blood (3 ml) was collected from all the participants, and genomic DNA was extracted using a commercially available kit (Qiagen, CA, USA). To detect polymorphisms at position -308 and intron 1 +252 of the *TNF- α* and *TNF- β* genes, respectively, and at position -592, -819 and -1082 of *IL-10* gene, the amplification of *TNF- α* , *TNF- β* , and *IL-10* genes was performed using an amplification refractory mutation systems PCR methodology described elsewhere [39, 93]. PCR amplification was carried out in PuReTaq Ready-to-Go PCR Beads (GE Healthcare, Buckinghamshire, UK) as described earlier [82]. The allele and genotype frequencies of all 5 polymorphisms were evaluated in IBD patients and controls. Hardy-Weinberg equilibrium was determined using Hardy-Weinberg Equilibrium Calculator for 2 Alleles. (<http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2alleles.html>)

2.3. Statistical analysis

The difference between the frequency distribution of various alleles and genotypes in patients and controls was analyzed by Fisher's exact test using the CalcFisher software (<http://www.jstatsoft.org/v08/i21/paper>), and the *P*-values ≤ 0.05 were considered as significant. The odd ratio interpreted as *relative risk* (RR) indicated the strength of the association of disease with

respect to a particular allele/genotype and was calculated according to the method of Woolf as outlined by Schallreuter et al. [94]. The RR was calculated using the following formula only for those alleles and genotype, which were increased or decreased in IBD patients as compared to normal Saudis.

$$RR = \frac{a \times d}{b \times c} \quad (1)$$

where “a” indicates number of patients expressing the allele or genotype, “b” number of patients without allele or genotype expression, “c” number of controls expressing the allele or genotype, and “d” number of controls without allele or genotype expression.

The etiologic fraction (EF) is the hypothetical genetic component of the disease. Values >0.00–0.99 are significant. EF is calculated for positive associations where value of RR is >1 using the following formula [95]:

$$EF = \frac{(RR - 1)f}{RR}, \text{ where } f = \frac{a}{a + c} \quad (2)$$

Preventive fraction (PF) shows the hypothetical protective effect of one allele/genotype for a disease. PF is calculated for negative associations where RR is <1 using following formula [95]. Values >0.00–0.99 indicate the protective effect of an allele/genotype against the manifestation of disease.

$$PF = \frac{(1 - RR)f}{RR(1 - f) + f}, \text{ where } f = \frac{a}{a + c} \quad (3)$$

3. Results

The representative gel pictures of amplification of different genotypes for TNF- α (-308G/A) and TNF- β (+252A/G) are shown in **Figures 1** and **2**.

Allelic frequencies and genotype distributions of TNF- α (-308G/A) and TNF- β (+252A/G) polymorphisms were different in patients and controls. The allele frequencies of both patients and

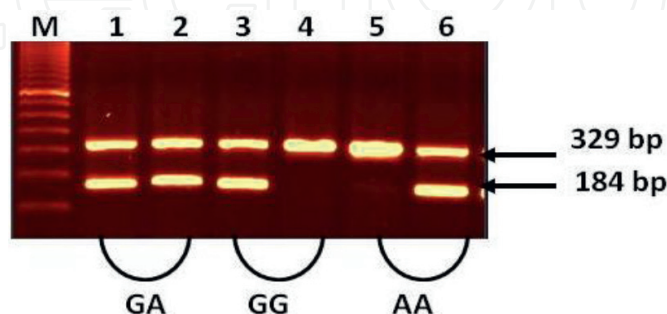


Figure 1. Shows the amplification of TNF- α (-308G/A) alleles (G and A). Lane M: 100-bp DNA marker, lanes 1 and 3: amplification of allele G, lanes 2 and 6: amplification of allele A, 184-bp band for target DNA, 329-bp band for internal control.

controls were in Hardy-Weinberg equilibrium. The frequencies of genotype GA and allele A were significantly higher, while those of genotypes (GG and AA) and allele G of TNF- α (-308G/A) were lower in IBD patients as compared to controls (**Table 1**). Allele A and genotype GA were susceptible to the IBD ($P < 0.001$), while allele G and genotype GG were protective against IBD ($P < 0.001$) in Saudi patients.

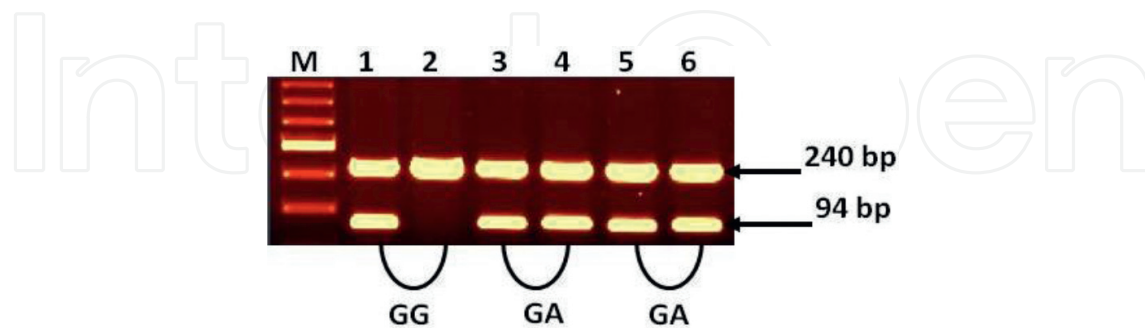


Figure 2. Shows the amplification of TNF- β (+252A/G) alleles (A and G). Lane M: 100-bp DNA marker, lanes 1, 3, and 5: amplification of allele G, lanes 4 and 6: amplification of allele A, 94-bp band for target DNA, 240-bp band for internal control.

Genotype/allele	IBD (n = 179)		Control (n = 200)		P-value	RR	EF*/PF
	n	%	n	%			
GG	5	2.79	110	55	0.00001*	0.235	0.123
GA	173	96.65	76	38	0.0001*	47.044	0.080*
AA	1	0.56	14	7	0.0009*	0.075	0.449
G-allele	183	51.12	296	74	0.0001*	0.367	0.397
A-allele	175	48.88	104	26	0.0001*	2.722	0.396*

EF = Etiologic fraction, PF = preventive fraction.

♣Statistically significant.

*data for EF

Table 1. Genotype and allele frequencies of TNF- α (-308G/A) polymorphism in IBD patients and matched controls.

Because of the fact that two forms of IBD are characterized by different clinical pictures, it is reasonable to perform genetic association studies on homogenous group of patients; therefore, the genotyping results were stratified into UC and CD. However, similar association with TNF- α (-308G/A) polymorphism was noticed in the two groups. The genotype GA and allele A were significantly associated with CD and UC susceptibility in our population (**Table 2** and **Figure 1**). Allele A and genotype GA were susceptible to the UC and CD ($P_s < 0.01$), while allele G and genotype GG were protective ($P_s < 0.01$) in Saudi patients with UC and CD.

The association of TNF- α (-308G/A) polymorphism with UC, CD, or IBD in various ethnic populations worldwide has been summarized in **Table 3**. The association is not consistent, and

Genotype/allele	CD (95) n (%)	UC (84) n (%)	Control (200) n (%)
GG	3 (3.16)*	2 (2.38)*	110 (55)
GA	91 (95.79)*	82 (97.62)*	76 (38)
AA	1 (1.05)*	0 (0.0)*	14 (7)
G-allele	97 (51.05)*	86 (51.19)*	296 (74)
A-allele	93 (48.95)*	82 (48.81)*	104 (26)

*P value <0.05 compared to the frequency in controls.

Table 2. Genotype and allele frequencies of TNF- α (-308G/A) polymorphism in UC and CD patients.

ethnic variations are evident in the type or/and degree of association of TNF- α (-308G/A) polymorphism and IBD, UC, or CD susceptibility/severity or response to therapy.

On the other hand, studies on TNF- β gene polymorphism showed that the frequency of GG at position +252 of intron 1 was significantly higher in IBD as compared to controls, while the frequency of GA genotype was also higher in patient group but the difference was not statistically significant. The difference in the distribution of allele A and allele G was also not statistically significant in IBD and control groups albeit the frequency of mutant allele G is higher in IBD patients (Table 4).

The stratification of TNF- β gene polymorphism results for IBD patients into UC and CD showed that the distribution of genotypes GG and GA was different in UC as compared to controls indicating that the genotype GG is susceptible and GA protective only for UC but not for CD as almost similar distribution of genotypes and allele frequencies of TNF- β -intron 1 +252 polymorphism was found among the CD and controls (Table 5 and Figure 2).

The frequency distribution of alleles and genotypes of both TNF- α and- β polymorphisms is not affected by gender or type of IBD (familial or sporadic) (Tables 6 and 7).

The representative gel pictures of amplification of different genotypes for IL-10 G (-1082)A, IL-10 C (-819)T, and IL-10 C (-592)A are shown in Figures 3–5.

The results of three promoter polymorphism of IL-10 gene are summarized in Tables 8–12. The genotype GG of IL10 (-1082) was significantly higher ($P = 0.02$) in IBD patients (15.08%) than control group (7.50%). Contrarily, the genotype AA was found to be significantly lower ($P = 0.02$) in IBD patients (9.50%) as compared to controls (17.50%). On the other hand, the heterozygous GA genotype was almost same in patients and controls ($P = 0.99$) (Table 8).

Upon stratification of genotyping results into CD and UC, we noticed that frequency of genotypes and alleles of IL-10 G (-1082)A differed significantly between CD patients and controls. Frequencies of genotype GG and allele G were higher in CD patients while those of genotype AA and allele A lower in CD patients as compared to controls. On the other hand, no significant different was found in the frequencies of alleles and genotypes between UC and controls (Table 9).

The frequency of -819 CC genotype was 33.52% in the IBD patients compared to 41.50% in controls, while CT was 38.12% in IBD patients as compared to 48.50% in controls. The frequency of homozygous TT genotype was similar in both IBD and control samples (10.61 vs. 10.00%). The frequencies of all genotypes of IL-10 (819C/T) polymorphism did not differ

Ethnicity/population	Type of association with IBD	Reference
American	UC susceptibility	[96]
*Asian	Associated with UC	[59]
*Asian	Associated with UC and CD	[58]
*Asian	Associated with UC susceptibility	[97]
Belgian	No association with CD treatment	[98]
Belgian	Associated with CD behavior	[99]
Brazilian	Associated with severity of CD	[60]
Canadian	No association with CD	[27]
Canadian	No association with IBD	[100]
*Caucasians	Better response to TNF blockers	[101]
Czech	Associated with IBD	[102]
Dutch	No association with IBD	[103]
English	IBD susceptibility	[104]
*European	No association with UC	[97]
*European	Associated with UC and CD	[58]
German	No association with IBD	[105]
Han Chinese	Association with UC susceptibility	[106]
Han Chinese	Association with UC susceptibility	[57]
Hungarian	IBD susceptibility	[107]
Indian	No association with IBD	[108]
Iranian	No association with IBD	[109]
Iranian	No association with IBD	[110]
Irish	No association with IBD	[83]
Israeli	No association with granulomas in CD	[111]
Italian	Associated with therapy	[112]
Japanese	UC susceptibility	[113]
Korean	Association with CD susceptibility	[114]
Korean	Association with CD susceptibility	[115]
Mexican-Mestizo	UC susceptibility	[116]
Portuguese	Pathological profiles of CD	[117]
Russian	UC susceptibility	[118]
Saudis	IBD susceptibility	[93]
Spanish	No association with CD	[114]
Spanish	No association with UC	[119]
Turkish	No association with IBD susceptibility	[120]
Turkish	Association with UC susceptibility	[121]

*meta-analysis

Table 3. Association of TNF- α 308G/A polymorphism in UC, CD, or IBD in various ethnic populations worldwide.

Genotype/allele	IBD (n = 179)		Control (n = 200)		P-value	RR	EF*/PF
	n	%	n	%			
GG	39	21.79	28	14	0.05*	1.711	0.241*
GA	120	67.04	148	74	0.14	0.714	0.151
AA	20	11.17	24	12	0.87	0.922	0.037
G-allele	198	55.31	204	51	0.24	1.189	0.078*
A-allele	160	44.69	196	49	0.24	0.841	0.078

EF = Etiologic fraction, PF = preventive fraction.

*Statistically significant.

*data for EF

Table 4. Genotype and allele frequencies of TNF-β (+252A/G) polymorphism in IBD patients and matched controls.

Genotype/allele	CD (n = 95) n (%)	UC (n = 84) n (%)	Control (n = 200) n (%)
GG	16 (16.84)	23 (27.38)*	28 (14)
GA	69 (72.63)	51 (60.72)*	148 (74)
AA	10 (10.53)	10 (11.90)	24 (12)
G-allele	101 (53.16)	97 (57.74)	204 (51)
A-allele	89 (46.84)	71 (42.26)	196 (49)

*P value <0.05 compared to the frequency in controls.

Table 5. Genotype and allele frequencies of TNF-β (+252A/G) polymorphism in CD and UC patients.

Genotype/allele	Male (n = 89)		Female (n = 90)		P-value
	n	%	n	%	
GG	23	25.84	16	17.78	0.209
GA	57	64.05	63	70.00	0.429
AA	9	10.11	11	12.22	0.813
G-allele	103	57.87	95	52.78	0.340
A-allele	75	42.13	85	47.22	0.340

N = number of subjects.

Table 6. Genotype and allele frequencies of TNF-β (+252A/G) polymorphism in IBD male and female patients.

Genotype/allele	Familial (n = 22)		Sporadic (n = 157)		P-value
	n	%	n	%	
GG	4	18.18	35	22.29	0.780
GA	15	68.18	105	66.88	1.000
AA	3	13.64	17	10.83	0.717
G-allele	23	52.27	175	55.73	0.746
A-allele	21	47.73	139	44.27	0.746

Table 7. Genotypes and alleles of TNF- β (+252A/G) polymorphism in familial and sporadic IBD patients.

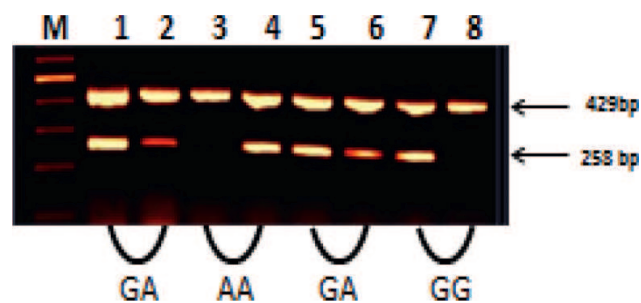


Figure 3. Shows the amplification of IL-10-1082G/A genotypes (GG, GA, and AA). Lane M: 100-bp DNA marker, lanes 1, 5, and 7: amplification of allele G, lanes 2, 4, and 6: amplification of allele A, 258-bp band for target DNA, 429-bp band for internal control.

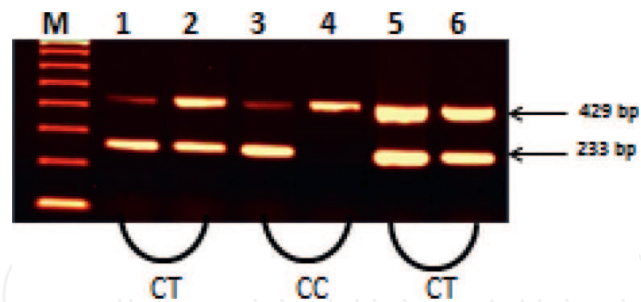


Figure 4. Shows the amplification of IL-10-819C/T genotypes (CT and CC). Lane M: 100-bp DNA marker, lanes 1, 3, and 5: amplification of allele C, lanes 2 and 6: amplification of allele T, 233-bp band for target DNA, 429-bp band for internal control.

significantly in patients and control groups. The allelic frequencies were also not different in patient and control groups (Table 10).

Upon stratification of subjects in to CD and UC, no significant difference was found in distribution of alleles and genotypes between patients and controls (Table 11).

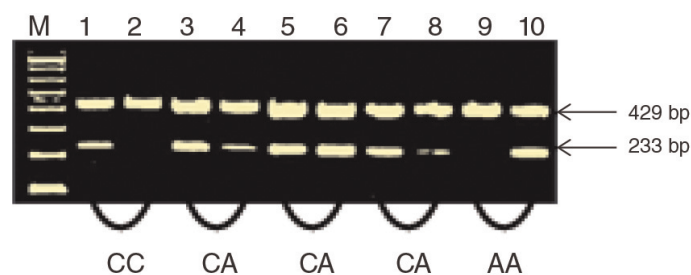


Figure 5. Shows the amplification of IL-10-592C/A genotypes (CC, CA, and AA). Lane M: 100-bp DNA marker, lanes 1, 3, 5, and 7: amplification of allele C, lanes 4, 6, 8, and 10: amplification of allele A, 233-bp band for target DNA, 429-bp band for internal control.

Genotype/allele	IBD (n = 179)		Control (n = 200)		P-value	RR	EF*/PF
	n	%	n	%			
GG	27	15.08	15	7.50	0.02*	2.19	0.347*
GA	135	75.42	150	75.00	0.99	1.02	0.009*
AA	17	9.50	35	17.50	0.02*	0.49	0.253
G-allele	189	52.79	180	45.00	0.03*	1.36	0.135*
A-allele	169	47.21	220	55.00	0.03*	0.73	0.138

EF = Etiologic fraction, PF = Preventive fraction.
*Statistically significant.
*data for EF.

Table 8. Genotype and allele frequencies of (-1082G/A) IL-10 variants in IBD and matched controls.

Genotype/allele	CD (95) n (%)	UC (84) n (%)	Control (200) n (%)
GG	17 (17.90)*	10 (11.90)	15 (7.5)
GA	73 (76.84)	62 (73.81)	150 (75.0)
AA	5 (5.26)*	12 (14.29)	35 (17.50)
G-allele	107 (56.32)*	82 (48.81)	180 (45.0)
A-allele	83 (43.68)*	86 (51.19)	220 (55.0)

*P value <0.05 compared to the frequency in controls.

Table 9. Genotype and allele frequencies of (-1082G/A) IL-10 variants polymorphism in UC and CD patients.

Similarly, the frequencies of alleles and genotypes of IL-10(-592C/A) polymorphism were not significantly different in IBD patient and controls (**Table 12**).

Upon stratification of subjects into CD and UC, no significant difference was found in distribution of IL-10(-592C/A) alleles and genotypes between patients and controls (**Table 13**)

Genotype/allele	IBD (n = 179)		Control (n = 200)		P-value	RR	EF*/PF
	n	%	n	%			
CC	60	33.52	83	41.50	0.11	0.91	0.039
CT	100	55.87	97	48.50	0.18	1.34	0.128*
TT	19	10.61	20	10.00	0.86	1.06	0.027*
C-allele	220	61.45	263	65.75	0.22	0.83	0.085
T-allele	138	38.55	137	34.25	0.22	1.20	0.084*

*data for EF

Table 10. Genotype and allele frequencies of (819C/T) IL-10 variants in IBD and matched controls.

Genotype/Allele	CD (95) n (%)	UC (84) n (%)	Control (200) n (%)
CC	31 (32.63)	29 (34.52)	83 (41.50)
CT	53 (55.79)	47 (55.95)	97 (48.50)
TT	11 (11.58)	8 (9.53)	20 (10.00)
C-allele	115 (60.53)	105 (62.5)	263 (65.75)
T-allele	75 (39.47)	63 (37.5)	137 (34.25)

Table 11. Genotype and allele frequencies of (819C/T) IL-10 variants polymorphism in UC and CD patients.

Genotype/allele	IBD (n = 179)		Control (n = 200)		P-value	RR	EF*/PF
	n	%	n	%			
CC	60	33.52	83	41.50	0.11	0.91	0.039
CA	100	55.87	97	48.50	0.18	1.34	0.128*
AA	19	10.61	20	10.00	0.86	1.06	0.027*
C-allele	220	61.45	263	65.75	0.22	0.83	0.085
A-allele	138	38.55	137	34.25	0.22	1.20	0.084*

*data for EF

Table 12. Genotype and allele frequencies of (-592C/A) IL-10 variants in IBD and matched controls.

The association of IL-10 promoter polymorphism with UC, CD, or IBD in various ethnic population worldwide has been summarized in **Table 14**. The association is not consistent, and ethnic variations are evident in the type polymorphism and IBD, UC, or CD susceptibility.

Genotype/allele	CD (95) n (%)	UC (84) n (%)	Control (200) n (%)
CC	31 (32.63)	29 (34.52)	83 (41.50)
CA	53 (55.79)	47 (55.95)	97 (48.50)
AA	11 (11.58)	8 (9.53)	20 (10.00)
C-allele	115 (60.53)	105 (62.5)	263 (65.75)
T-allele	75 (39.47)	63 (37.5)	137 (34.25)

Table 13. Genotype and allele frequencies of (592C/A) IL-10 variants polymorphism in UC and CD patients.

Ethnicity/population	IL-10 polymorphism	Type of association with IBD	Reference
Australian	1082 G/A, 592 C/A	CD susceptibility	[84]
Canadian	1082 G/A, 819 C/T, 592 C/A	No association with IBD	[100]
Canadian	819 C/T	Associated with CD	[87]
*Caucasian	1082 G/A, 819 C/T	Associated with IBD susceptibility	[89]
*Caucasian	592 C/A	No association with IBD	[89]
Caucasian	1082 G/A	Associated with IBD	[38]
*Mixed	819 C/T, 519 C/A	Associated with UC	[88]
*Mixed	1082 G/A	No association with CD or UC	[88]
Mixed	1082 G/A, 819 C/T, 592 C/A	Associated with CD phenotype	[73]
*Mixed	1082 G/A	Associated with CD, No association with UC	[122]
New Zealand population	1082 G/A	Associated with CD,	[72]
Indian	1082 G/A, 819 C/T, 592 C/A	No association with IBD	[123]
Mexican	1082 G/A, 592 C/A	Associated with IBD susceptibility	[90]
Danish	1082 G/A, 819 C/T, 592 C/A	No association with IBD	[124]
Tunisian	Promoter polymorphism	Associated with CD	[125]
Turkish	1082 G/A	No association with IBD	[120]
Spanish	1082 G/A, 819 C/T, 592 C/A	No association with UC or CD	[126]
Spanish	1082 G/A	Associated with CD	[71]
Hungarian	1082 G/A	No association with CD	[127]
Korean	Promoter polymorphism	No association with IBD	[114]
German	1082 G/A, 592 C/A	No association with IBD	[91]
Italian	1082 G/A	Associated with UC	[86]
Italian	819 C/T	No association with UC	[86]

*Meta-analysis.

Table 14. Association of IL-10 promoter polymorphisms in UC, CD, or IBD in various ethnic populations worldwide.

4. Discussion

TNF- α being a key cytokine in the inflammatory response of IBD plays an important role in the digestive and systemic manifestations of the disease. Available literature on the TNF- α (-308G/A) polymorphism shows its importance in the pathogenesis of CD and UC [58–60]. From the outgoing results, it is clear that allele A and genotype GA of TNF- α (-308G/A) polymorphism are associated with IBD susceptibility in Saudi population. Our results are in accordance with the earlier reports from other populations. This polymorphism has been shown to affect the UC and CD susceptibility in Asians and Europeans. The allele A of TNF- α (-308G/A) is associated with UC susceptibility in Japanese and Han Chinese patients [57, 106, 113]. The genotype GA is a risk factor for UC in Asians, whereas homozygous genotype AA is risk for both UC and CD in European patients [58]. A meta-analysis besides supporting the association of TNF- α (308G/A) polymorphism with IBD in Asians suggested that genetic polymorphisms vary in Asians from Caucasians [59].

On the other hand, some reports support the association of TNF- α (-308G/A) polymorphism with the severity of IBD. TNF- α (-308G/A) polymorphism is reported to be significantly associated with the severity of CD and/or UC in Irish [83], Czech [102], Italian [112], Caucasian patients from New Zealand [128], and Brazilian patients [60]. However, it is not clear whether it is directly involved in pathophysiology of IBD or serve merely as markers in Linkage disequilibrium with susceptibility genes [83].

Moreover, the carriers of allele A are at greater risk of pancolitis and more likely to require bowel resection in UC and CD [102, 112]. The CD patients with allele A were reported to be more resistant to steroids compared with non-carriers. The CRP levels in UC and CD patients carrying allele A were found to be higher and reported to modify the disease phenotype, influence its activity, and lead to a more intense inflammatory response [112].

The increased inflammation, higher levels of C-reactive protein (CRP), TNF- α , and interleukin-1 β have been associated with the A-containing genotypes of TNF- α (-308G/A) polymorphism in the active phase of IBD [98, 99, 107, 129]. The higher frequency of allele A of TNF- α (308G/A) was found in anti-neutrophil cytoplasmic antibodies (ANCA)-positive than ANCA-negative IBD patients, which may have influences on the susceptibility IBD or the behavior of IBD [114].

On the other hand, homozygous genotype AA of TNF- α (-308) has been associated with susceptibility to CD in Portuguese patients, and it has been suggested that TNF- α (-308G/A) polymorphism is responsible for displaying distinct clinicopathological profiles in Portuguese CD patients [117].

However, contrary reports are also available in the literature. The lower frequency of allele A of TNF- α (-308G/A) has been reported in North European Caucasian and Korean patients with CD or UC as compared to healthy controls [103]. Although the frequency of allele G was reported to be slightly higher in Iranian Azeri Turkish IBD patients, it did not reach statistical significance [110]. Further, no association of TNF- α (-308G/A) polymorphism with IBD susceptibility was found in Australian [82], Brazilian [60, 130], Canadian [100], Chinese [106, 131],

Czech [132], French [133], Indian [108], Korean [115], Newfoundland [27], Spanish [126], and Turkish [120] populations. The reason for these differences in the TNF- α genetic associations with IBD etiology might be the variations in sample size, genotyping methods, and/or ethnicity itself as frequencies of alleles and genotypes of TNF- α (-308G/A) also vary in different ethnic healthy populations worldwide [39] (**Table 3**).

Our genotyping results for TNF- β (+252A/G) polymorphism showed that genotype GG was significantly associated with IBD susceptibility. Our results also indicated that genotype GA was slightly lower in IBD patient than the controls, but the difference did not reach statistical significance. However, when the results were stratified into CD and UC, it became evident that this polymorphism was associated only with UC but not with CD in Saudi population (**Table 4, Figure 2**). In contrast, some earlier reports suggested that the TNF- β (+252) polymorphism is not associated with CD or UC in Chinese, French, Korean, and Spanish patients [57, 106, 115, 119, 133]. It is possible that the TNF- β (+252A/G) polymorphism may be indirectly associated with IBD as it has been suggested to influence the expression/production of TNF- α [42].

Muro et al. [134] reported that the inflammatory response in IBD is effected by the changes in TNF- α and TNF- β levels and IBD patients are commonly treated with TNF- α inhibitors. Moreover, TNF- α gene polymorphisms are reported to affect the gene expression level of TNF- α , and a particular TNF- α genotype may influence the response of IBD patients treated with TNF- α inhibitors as mutated allele A of TNF- α (-308) and allele G of TNF- β (+252) polymorphisms have been associated with greater TNF- α transcription [35, 36, 42, 135].

Our study on Saudi IBD patients suggested a significant association between allele and genotype frequency of TNF- α (-308G/A) and TNF- β (+252A/G) polymorphisms and IBD susceptibility in Saudi population. It is evident from outgoing discussion that ethnicity plays a very important role in genetic association of TNF- α and TNF- β polymorphism with IBD. It is also inferred that the both the polymorphism may have synergistic effect on the susceptibility and may work in tandem to influence the etiology of IBD in Saudi population. The outcome of present study will not only help in the prognosis of IBD in Saudi population but also provide guideline for the treatment with anti-TNF therapy as individuals with different genotypes of TNF- α (-308G/A) respond differently to anti-TNF- α treatment [136, 137]. However, further studies are required involving other ethnic populations to strengthen these findings.

The genotyping results for IL-10-1082 G/A polymorphism indicated that genotype -1082GG and allele G are susceptible to IBD (RR = 2.19, EF = 0.347, RR = 1.36, EF = 0.135, respectively), while genotype AA and allele A are resistant to IBD (RR = 0.49, PF = 0.0.253, RR = 0.73, PF = 0.138, respectively). Upon stratification of genotyping results into CD and UC, we noticed that genotype GG and allele G of -1082 G/A polymorphism were associated with CD susceptibility, while genotype AA and allele A might be protective for CD. On the other hand, no significant association was found either with alleles or genotypes of -1082 G/A polymorphism and UC in Saudi patients. These results are in accordance with the various reports, which also indicted an association of IL-10-1082G/A polymorphism susceptibility to IBD [89, 122]. A meta-analysis including 18 case-control studies provided evidence for the association between IL-10-1082G/A polymorphism and susceptibility of CD [122]. Another met-analysis including 15 studies

demonstrated clear association between the IL-10-1082G/A polymorphisms and the risk of IBD [89]. The allele G of -1082G/A polymorphism has been associated with the IBD, and higher serum levels of IL-10 concentration have been reported in IBD patients than in the controls [72, 90]. Earlier studies with CD patients also indicated that IL-10-1082 G/A polymorphisms contribute to susceptibility to CD [71] and -1082G allele was significantly increased in patients with CD than controls [73] while A allele of the IL-10-1082G/A was associated with decreased IL-10 production in CD patients and controls [78].

Contrary to these, some studies reported that there are no significant differences in the allele and genotype frequencies of the IL-10-1082G/A polymorphism between IBD patients and controls in various populations [88, 91, 120]. Klein et al. [91] reported that IL-10-1082G/A polymorphism is not demonstrably involved in the predisposition of IBD in German cohort. Similarly, no association between Turkish IBD patients and IL-10-1082G/A was found [120]. A meta-analysis by Zou et al. [88] observed that IL-10-1082G/A polymorphism is not associated with IBD. These data provide evidence that the effect of IL-10 gene polymorphisms on cytokine production differs in CD, UC patients, and controls in various populations.

Further, our results indicated that IL-10-1082G/A polymorphism is not associated with UC susceptibility in Saudi patients. These are in accordance with earlier reports indicating no association of IL-10-1082G/A polymorphisms with susceptibility of UC [88, 122]. Mendoza et al. [138] reported that IL-1082G allele is not associated with the phenotype of UC patients in Madrid's Spanish population.

On the other hand, IL-10-1082 G/A polymorphism has been reported to influence susceptibility to UC [38, 86, 126]. A gender effect has been reported, with women of AG/AA genotypes of IL-10-1082 G/A, having a higher risk of developing UC at a younger age and is related to the lower IL-10 production associated with the -1082A allele and to the IL10 downregulating effect of estrogens [86]. A mild influence of -1082 G allele in UC appearance has also been reported by Castro-Santos et al. [126]. In a stratified analysis, a highly significant association between the -1082 AA genotype and the steroid dependency was observed in IBD, and it was suggested that carriage of the -1082 AA genotype (low producer) is a relevant risk factor for developing steroid-dependent IBD. Tagore et al. [38] suggested that individuals genetically predisposed to produce less IL-10 are at a higher risk of developing IBD, and the frequency of the high IL-10 producer allele (-1082 G) is decreased in the whole IBD group and in the UC patients compared with normal.

The two other polymorphisms of IL-10 gene (IL-10-819C/T and IL-10-592C/A) are not associated with the susceptibility of IBD as the frequency distribution of genotypes and alleles of these two polymorphisms did not differ significantly between controls and IBD patient groups. The stratification of our results in to CD and UC patients also indicated that IL-10-819C/T and IL-10-592C/A polymorphisms are associated with neither with CD nor UC susceptibility in our patients. Similarly, Castro-Santos et al. [126] did not find any association between IL-10 (-812 C/T and -592 C/A) polymorphisms and UC or CD susceptibility. A recent meta-analysis demonstrated no significant association between the -592C/A polymorphism and IBD, CD, or UC, but a clear association with IL-10-819C/T polymorphism [89], while several other report showed that these polymorphisms are associated with IBD risk [72, 87, 88, 90, 139].

These differences in the association of IL-10-819C/T and IL-10-592C/A polymorphisms with CD or UC can be attributed to ethnic variations.

The exact mechanism by which IL-10 affects the susceptibility/pathogenesis of IBD is far from clear. It participates in the regulation of the immune response at several levels [69]. IL-10 regulates the inflammatory response, by inhibiting proinflammatory Th1 cytokines production [140].

IL-10 cytokine downregulates the expression of major histocompatibility complex (MHC) of class I and II molecules [141, 142]. It also has potent stimulatory effects on B lymphocytes, resulting in increased production of immunoglobulin and DNA replication [141]. The immune-stimulating effects of IL-10 have also been reported. IL-10 is shown to induce activated B cells to secrete large amounts of IgG, IgA, and IgM and in combination with IL-4 which results in the secretion of four immunoglobulin isotypes. The increased levels of IL-10 play a role in the amplification of humoral responses in some diseases [141].

Sanchez-Munoz et al. [143] suggested that the intestinal inflammation in IBD is controlled by a complex interplay of innate and adaptive immune mechanisms. Cytokines determine T-cell differentiation of Th1, Th2, T regulatory, and Th17 cells in IBD, and cytokines levels regulate the development, recurrence, and exacerbation of the inflammatory process in IBD. The dysregulation of T cells, or an over-production of effector T cells, results in the development and exacerbation of IBD [144]. Thus, the antigen-presenting cells (APCs), Th1, Th2, T regulatory cells, and Th17 and their cytokine products play an important role in the etiology of IBD [8]. These cellular interactions are modulated by pro- or anti-inflammatory cytokines (such as TNF- α , INF- γ , IL-1, IL-6, IL-4, IL-5, IL10, TGF- β , IL-13, IL-12, IL-18, IL-23) [145]. Although many common responses in IBD are mediated by cytokines, how cytokines determine the nature of the immune response in IBD may be quite different among different IBD forms [146].

A highly significant increase in IL-10 mRNA levels in T lymphocytes and in IL-10-positive cells in the colons of UC patients has been reported by Melgar et al. [147]. Moreover, IL-10 production by regulatory T cells has also been implicated as important factor in IBD [148]. Another regulatory B cells subtype called Bregs may also take part in UC etiology by producing IL-10 [149]. The significance of IL-10 produced by B cells has been indicated in IBD patients and animal models also [150, 151]. The Bregs can be responsible for the suppression and/or recovery from acquired immune-mediated inflammations by IL-10 and TGF- β 1 in IBD [143, 149]. However, the exact mechanism is still far from clear and needs to be investigated.

5. Conclusion

Our study dealing with the five polymorphisms of proinflammatory and anti-inflammatory cytokine genes in Saudi IBD patients clearly indicates that the TNF- α (-308G/A), TNF- β (+252A/G), and IL-10 (-1082 G/A) polymorphisms are associated significantly with the risk of IBD susceptibility while other two, IL-10-819C/T and IL-10-592C/A, polymorphisms are not associated with IBD in Saudi population. However, due to several limitations in the present

study, it is suggested that well-designed epidemiological as well as genetic association studies with large sample size among different ethnicities should be performed in order to have better understanding of this relationship.

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