RESEARCH ARTICLE

Association of Inflammatory Bowel Disease and Tumor Necrosis Factor-863 C/A Polymorphism in Iraq

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Received: 05th January, 2023; Revised: 28th January, 2023; Accepted: 12th March, 2023; Available Online: 25th March, 2023

ABSTRACT

Background: The underlying causes of inflammatory bowel disease (IBD) are unknown, but they are thought to be a combination of genetics, environmental factors, abnormal immune responses, and disruption of the gut microbiota. This study aims to investigate the effect of tumor necrosis factor-alpha (TNF-α) -863 C/A(rs 1800630) single nucleotide polymorphism (SNP) in inflammatory bowel patients and it is relation with the patient's clinical characteristics.

Methods: The study was conducted on 74 blood samples from patients of IBD including 47 patients with ulcerative colitis (UC) and 27 patients with Crohn's disease (CD) in addition to 20 blood samples apparently healthy individuals, TNF-α-863 C>A genotype was screened by PCR and Sanger sequencing techniques.

Results: The results showed that the homozygous CC genotype frequency was the higher genotype frequency in 45/60 (75%) for IBD patients with less than 50 years ages compared with 7/14 (50%) the IBD patients with more than 50 years, significant high association OD (CI): 2.75 (1.38–4.08), The allelic C frequency in ulcerative colitis (UC) patients was (0.83) and significantly higher than the A allele frequency (0.17) and it may act as risk factor inflammatory disease. The homozygous CC genotype of the TNF-α-863 gene was 9/27 (70.37%) in CD patients compared with 6/20 (30%) in the control group with high significant differences ($p \leq 0.01$, OR=1.00). Significant differences also applied for the heterozygous CA genotype in -863 SNP,it was 8/27 (29.62%) compared with the control group 14/20 (70%) the odds ratio (2.62), while the homozygous AA genotype frequency showed no significant association with CD (p -1.00).

Conclusion: The frequency of homozygous CC genotype of the TNF-α-863 gene was higer in CD patients than in the control group with significant differences. Significant differences also applied for the heterozygous CA genotype while the three genotypes (CC, CA and AA) of the TNF-α-863 gene showed non-significant differences in ulcerative patients in comparison with the control.

Keywords: Tumour necrosis factor α, Ulcerative colitis, Crohn's disease, Inflammatory bowel disease.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.1.12

How to cite this article: Hussian SE, Mahood WS. Association of Inflammatory Bowel Disease and Tumor Necrosis Factor-863 C/A Polymorphism in Iraq. International Journal of Drug Delivery Technology. 2023;13(1):81-85.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The intestinal epithelium is an amazing mixture of specialized cellular constituents, precise preservation, skeletal organization, and regeneration mechanisms that assure barrier function and absorption. Flaws in one or more of these constituents can have serious consequences for organisms, especially when they appear as IBD.[1] IBD is a disease of unknown cause resulting from an abnormal immune response to patients' intestinal microflora. IBD is usually classified into Crohn's disease (CD), which can involve any part of the gastrointestinal tract from the mouth to the anus, the leaped lesions, and ulcerative colitis (UC), which is restricted to the colonic mucosa.[2] Genetic readiness is a suggestion for IBD, and predispose patients are more exposed to the risk of malignancy when exposed to environmental factors that increase the risk of infection.[3,4]

One cellular kinetics is TNF-α that plays an important role in the manifestation of intestinal infections and is produced by different types of cells, including macrophages, monocytes, and neutrophils. These are all examples of macrophage cells.[5] The TNF-α gene is located on chromosome 6 in the class III region of the major histocompatibility between the (HLA-B and HLA-DR genes), *TNF*- α gene is a strong positional and functional candidate for IBD susceptibility.

Two types of outer membrane-bound receptors bind to TNF-α on target cells, including TNFR1 and TNFR2, activates cell survival and pro-inflammatory NF-B and MAP kinases. Single nucleotide variants (-863,-857, and -1031 SNPs) were discovered to be associated with the TNF gene's

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transcription start site and are known to play a role in TNF- α regulation and transcription efficiency, so many researchers have studied possible nucleotide variations in these sites for their possible effect in the regulation of TNF- α and its relationship with the predisposition to many autoimmune diseases.[7] Also the −308G/A TNF-α promotor polymorphism has been associated many times with the the development of autoimmune diseases, however some discrepant results have been recorded. The aim of this study is to investigate the effect of tumor TNF-α -863(C>A) single nucleotide polymorphism (SNP) in inflammatory bowel patients and it is related with characteristic clinical patients.

MATERIALS AND METHODS

Subjects

A total of 94 Iraqi subjects, including 74 IBD patients visiting the Gastroenterology and Hepatology Center at City Hospital of Medicine in Baghdad, in addition to 20 blood samples that were withdrawn from healthy people as control group, and the samples were kept in EDTA tube by refrigeration until use at 20°C, TNF- α (-863) C>A SNP polymorphism was detected by Sanger sequencing technique in all samples (94). These were classified into 95 patients IBD (UC =47 and CD=27) (32 men and 42 women), with the average onset of the disease occurring in the second decade (range, 12–74 years). Data obtained from each patient included age at diagnosis, disease location, disease characteristics, and extraintestinal location, which were used to group the patients.

DNA Extraction

DNA Genomic was extracted using the Relia Prep Blood DNA miniprep system from Promega company (USA country). DNA was eluted and stored at −20°C for further PCR procedure.

Polymerase Chain Reaction (PCR)

PCR was carried out using A 809 bp DNA fragment was amplified, using the forward primer 5′-GCTTCAGGGATATGTGATGG-3′ and reverse primer 5′-CTTCTGTCTCGGTTTCTTCTC -3′. PCR amplification was performed by applied PCR Biosystems 2720 thermal cycler(Singapore). The total volume of PCR amplification reaction 25, (including, 12.5 µL master Mix, 1-µL of each primer, 7.5 µL nuclease-free water (ddH_2O), and 3 µL of genomic DNA). The PCR reaction involved the following steps: an initial denaturation of 95°C for 5 minutes then 30 cycles of (denaturation: 95°C for 30 seconds, anneling 60°C for 15 seconds and extension 72°C for 45 seconds; and a final extension step at 72°C for 10 minutes.

Table 1: Clinical characteristics of patients with inflammatory bowel disease

Characteristics	*IBD Patients*	*Percentage (%)*	*Chi-Square (χ^2)*
Total No.	74	100	--
Average Age 50			
Less 50 than	60	81	28.594 **
More than 50 or equal	14	19	
Sex			
Male	32	43	1.351 NS
Female	42	57	
Disease			
CD	27	36	5.405 *
UC	47	64	

* ($p \leq 0.05$), **($p \leq 0.01$), NS: Non-Significant.

RESULTS

Characteristics of Patients

A total 74 patients in addition to 20 apparently healthy individuales have participated in this study. The results showed 81% of patients were less than 50 years and 57% of the patients were female. The results showed that 64% of patients had UC while 36% were with CD (Table 1).

High significance ($p \leq 0.01$) for more than 50 years, and this keep with the study by,[8] also agree with studies by[9] The current study data showed that 64% of patients with UC disease while 36.48% of patients have CD. A Significant difference ($p \leq 0.05$) was found in this study. Our results keep with while inconsistent with the study.[10,11]

The Amplification of *TNF*- α Gene and DNA Sequences Analysis

Our previous Iraqi study refers to the important role of TNF-α -1031 gene polymorphisms in the promoter region may be contributing to the occurrence of inflammatory bowel disease in the Iraqi population, especially in CD patients.[12] The present study was aimed to investigate the *TNF*-α -863 C/A genotypes and their alleles frequencies in the promoter region In IBD patients. This study may be the first Iraqi study that highlighted the TNF-α -863 (rs 1800630) SNP polymorphisms. The PCR products were showed narrow DNA bands (973 bp) referred to clear bands of PCR product on gel electrophoresis Figure 1 and the results were confirmed by Sanger sequencing technique and analyze the fast file results with NCBI references (rs 1800630) by Bioedit Program, Figure 2 refer to the sequences analysis of TNF-α in gene promoter region. .

Table 2 showed the genotype and allele frequencies of TNF- α (-863) C>A in IBD patients and control groups.

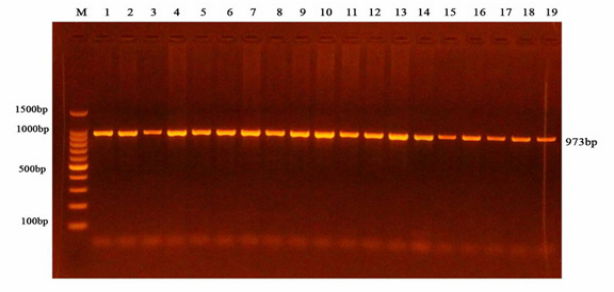

Figure 1: Amplification of TNF- α gene promotor fragment, fractionated samples on 1.5% agarose gel electrophoresis stained with Ethidium Bromide M: 100bp ladder marker, Lanes (1-19) PCR products of IBD2 patients with samples band size 973bp.

The homozygous CC genotype frequencies of *TNF*-α -863 C>A in inflammatory bowel patients were appeared in 52/74 (70.27%) compared with the 14/20 (70%) in control group, no significant differences were found (P=0.966,OR=1.00), while the heterogeneous CA genotype in inflammatory bowel patients was 20/72 (27.02%) compared to with the 6/20 (30%) in control group, no significant difference (=0.437), the AA genotype in IBD, was apear in 2/74 (2.70%). Also, without significant association. The results of the current study in contrast to a previous study,[13] reported that a high *TNF*- α −863 AA genotype was associated with an increased risk of IBD. Similarly, the combined effect of the *TNF*- α polymorphism in the haplotype analysis showed an additional increase in the risk of IBD. The C allelic frequency in the present study was higher (0.84) than the A allele (0.16).

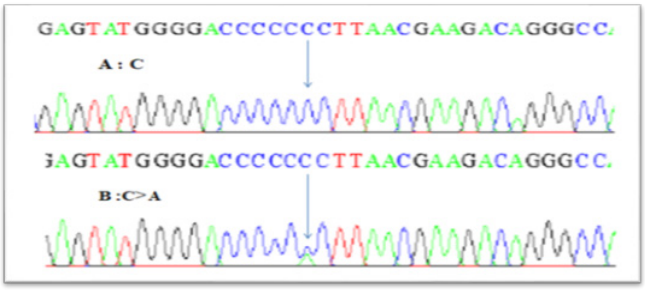

Figure 2: Sequences analysis of TNF-α gene promoter, the representative sequences analysis is shown for single nucleotide polymorphism - 863(C>A) rs 1800630, A: Wild type CC, B: Heterozygous C>A, (blue- Cytosine,green- Adenine , red-Thiamine and black- Guanine).

Frequency of TNF- α(-863)C>A SNP Depend on the Age and Gender of IBD Patients

Table 3 indicate to the TNF- α-863 (C>A) genotype frequencies depend on age of IBD patients,the results showed that the homozygous CC genotype frequency was found in 45/60 (75%) for IBD patients with less than 50 years ages compared with 7/14 (50%) the IBD patients with more than 50 years, high significant association ($p \leq 0.01$, OR:1), significant differences also it applied for the heterogeneous CA genotype frequency in -863 (C>A) SNP, it was 14/60 (23.33%) in IBD patients with less than 50 years compared with 6/14 (42.85%) IBD patients with more than 50 years age, the OD(CI)=2.75 (1.38-4.08), while the homozygous AA genotype frequency showed no significant association with the age of IBD patients.

Table 4 indicate to the TNF- α -863 (C>A) genotype frequencies depend on the gender of IBD patients, the homozygous CC genotype frequency was found in 21/32 (65.62%) and 31/42 (73.80%) in male and female respectively with significant association ($p \leq 0.05$, OR:1) while the heterozygous CA genotype frequency showed no significant differences, similar results were obtained for AA genotype frequencies. According to the low of Hardy Weinberg the allelic Frequencies of C allele were slightly superior in female than males (0.86 and 0.81), respectively.

The Association of *TNF-α* -863 (C>A) SNP Polymorphisms with UC

The results of the current study showed that the three genotypes (CC,CA and AA) at locus (-863) showed non-significant differences in ulcerative patients in comparison with the control groupe, although highy percentage 70.21% of homozygous CC

Table 2: The genotype and allele frequencies of *TNF*- α (-863) C>A in IBD patients and control groups

Genotype	*IBD patients No.(%)*	*Control No.(%)*	*p-value*	*Chi-square*	*O.R.(CI)*
Total No. (%)	74 (100%)	20 (100)			
CC	52 (70.27)	14 (70)	0.966	0.164 NS	1.00
CA	20 (27.02)	6 (30)	0.437	0.774 NS	1.11 (0.45-1.78)
AA	2 (2.70)	0 (0%)	0.502	0.603 NS	0
Allele Frequency					
C	0.84	0.85	-	- -	
A	0.16	0.15	-	- -	

NS: Non-Significant, OR = Odd Ratio, CI = Confidence Interval.

Table 3: The genotype and allele frequencies of the TNF-α (-863) C/A age of patients

Genotypes	*IBD patients less than 50 years*	*IBD patients more than 50 years*	*p-value*	*Chi-square*	*O.R. (CI)*
Total No. (%)	60 (81)	14 (19)			
CC	45 (75)	7 (50)	0.0078	8.893 **	1.00
CA	14 (23.33)	6 (42.85)	0.0096	7.012 **	2.75 (1.38-4.08)
AA	1 (1.66)	1 (7.14)	0.085	3.116 NS	
Allele Frequency					
C	0.87	0.71	-	- -	
A	0.13	0.29	-	- -	

**($p \leq 0.01$).
OR = Odd Ratio , CI = Confidence Interval

Table 4: The genotype and allele frequences of the TNF–α(-863)C>A depend on gender of patients

Genotypes	*Male No.(%)*	*Female No.(%)*	*p-Value*	*Chi-Square*	*O.R. (CI)*
Total No. (%) 4(100%)	32 (43)	42(57)			
CC	21(65.62)	31(73.80)	0.0415	4.92 *	1.00 (CI)
CA	10(31.25)	10(23.80)	0.092	2.07 NS	0.67 (0.29-0.96)
AA	1(3.12)	1(2.38)	0.477	0.661 NS	0.67 (0.27-0.94)
Allele Frequency					
C	0.81	0.86	-	-	-
A	0.19	0.14	-	-	-

*($p \leq 0.05$), OR = Odd Ratio , CI = Confidence Interval.

genotype was compared to 25.53 and the 4.25% for CA and AA genotypes, respectively Table 5. This results is consistent with the results of Naderi and colleagues 2014, they did not find significant relation for all of the genotype and allele frequencies in stimulation of phagocytic or phagocytic cells during the immune response leads to the secretion of a large amount of TNF-α, which plays an important role in the pathogenesis of IBD and the ability to produce different amounts of TNF-α appears to have its basis genetically determined.[14] Single nucleotide variants (-857, -863, -1031 SNPs) were found to be related to the transcription start site of a gene TNF is well known to have a role in the TNF-α gene and its efficient replication, so many studies have been done to investigate nucleotide variations at these sites for possible influence in regulating expression of TNF-α and its relationship with sensitivity to several autoimmune diseases.

A previous study[15] found a significant association between the *TNF*-alpha gene and colorectal cancer, but the higher incidence was associated with an increased risk of colon cancer.

The Frequency of *TNF- α*-863 C>A SNP in Patients with the CD

Table 6 showed that the homozygous CC genotype -863 SNP of the TNF- α gene 9/27 (70.37%) compared with the control group 6/20 (30%) with high significant differences ($p \leq 0.01$, OR=1.00). Significant differences also applied for the heterozygous CA genotype in -863 SNP,it was 8/27 (29.62%) compared with the control group 14/20 (70%) the odds ratio (2.62), while the homozygous AA genotype frequency showed no significant association with Crhon's disease (*p*- 1.00), the results of this study agree with,[15] they referred to that *TNF-α* (-863) polymorphism was positively associated with CD and may influence not only susceptibility to CD, but also the site of the disease. The frequencies of the TNF-α (-863A) SNP were found to have a slight increase in the Asian population and not in the Caucasian population in CD patients, while the rest of the countries did not appear to be association between the TNF-α (-863A) SNP and CD.[16]

Table 5: Genotype and allele frequencies of the TNF- α(-863) C>A in UC patients and control groups

Genotypes	*UC Patients No. (%)*	*Control No. (%)*	*p-Value*	*Chi-Square*	*O.R. (CI)*
Total No. (%)	47 (100)	20(100)			
CC	33(70.21)	14(70)	0.952	0.077 NS	1.00
CA	12(25.53)	6(30)	0.098	1.261 NS	1.17 (0.62-2.36)
AA	2(4.25)	0(0)	0.155	1.083 NS	0
Allele Frequency					
C	0.83	0.85	-	-	-
A	0.17	0.15	-	-	-

**($p \leq 0.01$), OR = Odd Ratio, CI = Confidence Interval.

Table 6: The genotype and allele frequences in -863 C>A polymorphism of the TNF- α gene in CD patients and control groups

Genotypes	*CD Patients No.(%)*	*Control No.(%)*	*p-Value*	*Chi-Square*	*OR. (CI)*
Total No. (%)	27 (100)	20 (100)			
CC	9 (70.37)	6 (30)	0.0006	10.63 **	1.00
CA	8 (29.62)	14 (70)	0.0006	10.63 **	2.62 (0.96-4.05)
AA	0 (0)	0 (0)	1.00	0.00 NS	0.00
Allele Frequency					
C	0.48	0.65	-	-	-
A	0.52	0.35	-	-	-

**($p \leq 0.01$), OR = Odd Ratio , CI = Confidence Interval.

CONCLUSION

The frequency of the homozygous CC genotype of the TNF- α -863 gene was higer in CD patients compared with the control group with high significant differences ($p \leq 0.01$, OR=1.00). Significant differences also applied for the heterozygous CA genotype while the three genotypes (CC, CA and AA) of the TNF- α -863 gene showed non-significant differences in ulcerative patients in comparison with the control.[18,19].

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