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Original Research Article

Role of Tumor Necrosis Factor Alpha α and Interleukin 10 Gene Polymorphisms in Irritable Bowel Syndrome Patients

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ABSTRACT

Aims: The current study conducted to determine the frequency of the IL-10 (-1082G/A) and TNF-α (-308G/A) polymorphisms in subjects with IBS in Sudan.
Methods: After giving written consent, a total of 71 patients with symptoms of IBS according to the Rome III criteria and 40 controls were enrolled. DNA was extracted from peripheral blood leucocytes of subjects using salting out method. Polymorphisms were determined by PCR-RFLP method. SPSS software program was used for statistical analysis.
Results: Patients with irritable bowel syndrome had significantly reduced frequencies of the high producer genotype for interleukin 10 than controls (21% v 32%; p=0.003). there was no significant difference in the frequency of the genotypes or the alleles of the IL-10 (-1082G/A) and the TNF-α (-308G/A) polymorphisms between IBS and the controls, however it was demonstrated that the high producer (AA) TNF α genotype was more prevalent in IBS patients compared to healthy controls (8.5% VS *zero).* IBS patients were classified as IBS-D (53.5 %), IBS-C (18.3 %) and IBS-A/M (28.2 %) and there were no statistical differences IBS subgroups, were found among these IBS subgroups 57% of patients had family history of IBS.
Conclusions: The frequency of the IL-10 (-1082G/A) and TNF-α (-308G/A) genotypes was similar in IBS and controls. However, there was a greater frequency of the low producer of IL-10 in those subjects with IBS-D, suggesting a genetic predisposition to abnormal immune regulation due to a lower anti-inflammatory cytokines IL10 in this subgroup.
Key word: IL-10, TNF α,IBS, polymorphism.

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal tract, which is characterized by recurrent episodes of abdominal pain and discomfort with changes in frequency or consistency of the stool in the absence of an organic etiology. it affects approximately 12% world population and it represents an important socioeconomic concern. [1] The condition is heterogeneous, exhibiting variability in the frequency of symptoms reported within and between males and females. [2] IBS is clinically classified into different subgroups: IBS with constipation predominance (IBS-C), IBS with diarrhea predominance (IBS-D), mixed or alternating IBS (IBS-M). [3]

Prevalence of Irritable bowel syndrome ranges from (5% to 15%) of the

world population. [4] The prevalence of IBS is increasing and varies widely in countries in the Asia–Pacific region, particularly in countries with developing economies.

The exact pathophysiology of IBS is not well known, however, believed that there are important role for inflammatory reactions in pathogenesis of IBS. [5] The pathogenesis of IBS can be result from dysregulated brain-gut axis, intestinal dysmotility, visceral hypersensitivity, dietary, and intestinal microbiota, there is also a familial tendency to IBS and, although this could be psychosocial in origin, but a genetic influence is a possibility. [6] In addition to these factors, dysregulated intestinal immune function, bacterial infection, and low-grademucosal inflammation have all been participating in pathogenic mechanisms. [7] Cytokines is under genetic control and are significant modulators of inflammatory reactions and immune responses and play an essential role in intestinal inflammation. [5] The genetic polymorphisms in cytokine genes (within promoter or coding regions) affect production of cytokines. [8] Therefore, disease susceptibility and clinical outcome may affected by a genetic predisposition for the low or high production of certain cytokine. [9] We study Tumor necrosis factor-α (TNF-α) gene polymorphism in the promoter region (G→Asubstitution at position –308), and IL 10 gene polymorphisms in promoter region (G→A substitution at position –1028).

The predisposition to produce high or low levels of a specific cytokine could be related to the presence of the polymorphisms that encode each of their gene expressions, as has been suggested for the immuno regulatory IL-10 and the pro-inflammatory TNF-α cytokines. [10,11] These polymorphisms could then affect the susceptibility for developing IBS, as well as its phenotypic expressions (IBS-D; constipation-predominant IBS: IBS-C; and alternating/mixed IBS: IBS-A/M), at least in a subgroup of patients in whom immune activation would be a supposed factor. Nevertheless, according to a recent systematic review of the literature, the data on the genetic variants of the cytokines in IBS and controls are inconsistent. [12, 13] On the other hand, no studies on poly-morphisms in IBS have been conducted in Sudan to date.

Given the importance of understanding potential etiologic agents in IBS and to overcome some of the problems of individual studies, therefore, our aim was to explore the role of the IL-10 and TNFα polymorphisms among Sudanese IBS patients comparing with control group. Our hypothesis was that the subjects with IBS, when compared with the controls, should present with a greater frequency of both the low producer IL-10 polymorphism and the high producer TNF-α polymorphism.

MATERIALS AND METHODS

A case control study with patients who met the diagnostic criteria (Rome III criteria) for IBS with following inclusion criteria: Both sexes male and female, aged between 18- 75 years old, confirmed as IBS patients with professional gastroenterologist, and have informed consent, study was conducted during (2014-2016) in Khartoum state.

Study subjects

Blood samples were obtained, after informed consent, from 71 patients fulfilling the Rome III criteria for IBS attending the Gastroenterology Out-Patients Clinic. Patients were of all bowel habit subtypes divided into, IBS Diarrhea predominant IBS-D, IBS Constipation predominant IBS-C, those subjects that did not fit the IBS-D or IBS-C criteria were regarded as IBS-A/M (an alternating pattern). A total of 40 controls were used for comparison. The study was approved by the local ethics committee.

Polymorphism determination

A venous blood sample was collected from the subjects that had signed informed consent statements to participate in the study. Leukocyte DNA was extracted through the salting out technique using the

BDTract™ Genomic DNA Isolation Kit (Maxim Biotech Inc). Cytokine genotyping was done through the Restriction-Fragment Length Polymorphisms (RFLP). The polymorphisms of the IL-10 promoter regions at positions -1082*G and -1082*A, and the TNF-α promoter regions at positions -308*G and -308*A were analyzed through the polymerase chain reaction (PCR) using specific oligonucleotides. The amplified DNA products were separated through electrophoresis in 2 % agarose gel and stained with ethidium bromide. The gel was visualized under ultraviolet (UV) transillumination with a marker that had a molecular weight of 100 base pairs (bp), and they were photographed. The genotypes were expressed as high or low producers for the homozygotes and as intermediate producers for the heterozygotes. [14] It should be mentioned that the genotyping was blinded; there was no knowledge of the clinical characteristics of the subjects; in other words, it was not known whether they presented with IBS or were controls.

Statistical analysis

Allele frequencies for patients and controls were compared by calculation of the odds ratio (OR) and 95% confidence intervals (95% CI). Genotypes were calculated for each individual, with those homozygous for the high producer allele classed as "high producer", heterozygotes as "intermediate producer", and those homozygous for the low producer allele as "low producer" genotype, respectively.15 19 Genotype frequencies were then compared by χ^2 analysis.

Table (1) Single-nucleotide polymorphisms genotyped in IL-10, and TNF-α gene:

Gene	location	SNP & SNP name	Sequence of primer	Method	PCR product	Restriction enzyme
TNF α	CH 6	1082 G/A	F 5 CTC GCC GCAACC CAA CTG GC-3, R 5 TCT TAC CTA TCC CTA CTT CC-3.	PCR+RE	143 bp).	*MnlI*
IL10	CH 1	308 G/A	F 5 AGG CAA TAG GTT TTG AGG GCC AT3, R 5ACACTC CCC ATC CTC CCG GCT-3.	PCR+RE	117-bp	*NcoI*

PCR = polymerase chain reaction; RE = restriction enzyme

RESUTS

One hundred and eleven volunteers participated in the study. Of that total, 71 fit the IBS criteria and 45 were regarded as controls. Table I shows the epidemiologic and clinical characteristics of the groups. There were no statistically significant differences in age, sex, between the IBS subjects and the controls.

Further, the subjects with IBS were classified as IBS-D (53.5 %), IBS-C(18.3 %) and IBS-A/M(28.2 %) and there were no statistical differences IBS subgroups, were found among these IBS subgroups figure -1. In figure -2; 57% of patients had family history of IBS. Genotype and allele frequencies for TNF-α are shown in Table (1). Homozygous high producer (AA) was low (8.5%). The heterozygous genotype (G/A) was more prevalent in IBS patients compared with controls (45.1% versus 37.5%, *P*=0.08).

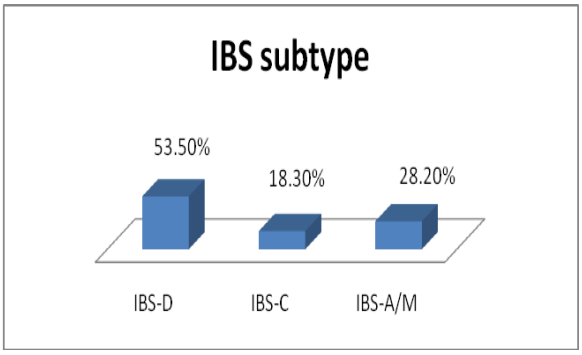

Figure 1: Distribution of IBS subtypes according to predominate bowel habit

Table (2) shows genotype and allele frequencies for the IL-10 G-1082 G/A SNPS. The homozygous low producer genotype (A/A) was more frequently distributed in patients than controls (46.5%% versus 40%). followed by the intermediate producer and no difference was found in the frequency of the genotypes (*P*=0.8). Likewise, frequencies of the A allele (low IL-10 production) were comparable between IBS patients and

controls (48% versus 47%, *P*=0.71; OR 1.07, 95% CI 0.76 - 1.50).

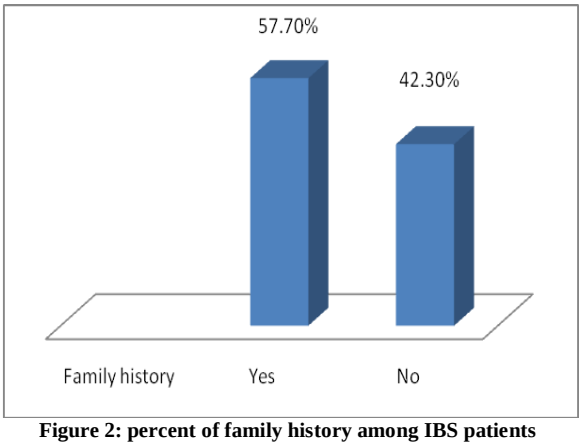

Figure 2: percent of family history among IBS patients

Table 2: TNF-α G-308A genotype and allele distribution in IBS patients (all subtypes) and controls

Genotype frequency	Patients	Control
AA	6(8.5%)	0(0%)
GG	33(46.5%)	25(62.5%)
A/G	32(45.1%)	15(37.5%)
Total	71(100%)	40(100%)
Allele frequency		
A+ (A/A or A/G)	38(53.5%)	53(47.7%)
(G/G)	33(46.5%)	58(52.3%)

*P value 0.083

Table 3: IL-10 G-1082A genotype and allele distribution in IBS patients and controls

Genotype frequency	Patients N (%)	Control
AA	33(46.5%)	16(40%)
GG	11(15.5%)	7(17.5%)
G/A	27(38.0%)	17(42.5%)
Total	71(100%)	40(100%)
Allele frequency		
G+ (G/G or G/A)	38(53.5%)	62(55.8%)
G- (A/A)	33(46.5%)	49(44.1%)

*P value 0.803

DISCUSSION

In present study in Sudanese IBS patients there were no significant differences was found in the frequency of the genotypes of the TNF-α (-308G/A) polymorphisms in the comparison between the subjects that fit the Rome III criteria for IBS and the controls (P value =0.80), however it was demonstrated that the high producer (AA) TNF α genotype was more prevalent in IBS patients compared to healthy controls (8.5% *VS* zero*)*, although homozygous high producers genotype were rare in IBs groups and absent in control, the heterozygous genotype, which is also associated with a high TNF-α production genotype, was present in 45.3% of patients *VS* 37.5% of controls, this result is in agreement with Max Schmulson et al, [15] who found the intermediate producer (heterozyous genotype) TNF-α polymorphism in position -308 was more frequent in IBS, [15] whereas no differences in the TNF-α polymorphisms were found in IBS *vs.* controls in the South Korean, [16] Indian [17] and Iranian [18] studies.

With regard IL-10 cytokines, the results demonstrated no association between IL-10 (1028) genotypes between IBS and control, (p value 0.803); although high producer genotype (-1082 G/G) was present in 15.5% of patients and 17.5% of control subjects. These findings are in agreement with the observations by Gonsalkorale et al., who showed a significant reduction in the high producer IL-10 genotype frequency in IBS patients compared to controls (21% versus 32%). [10] When comparing these and our data, it is important to recognize that genotype frequencies vary according to ethnicity. [19] For instance, a recent study showed that the frequency of the high producer IL-10 genotype is much higher in the Irish population (34%) than in Africans (9.5%) or Singapore Chinese (0%). [20] Although the study by Gonsalkorale *et al* [15] provided no information on the ethnic origin of patients and controls, this may well explain the disparity between their study and ours. Also lack of differences could be due to the population variations, themselves, or to the fact that our sample size did not enable a definite conclusion to be reached. All these data point to the absence or decrease of IL-10 production in patients seems to be logical, since IBS patients have an inflammatory processes and IL- 10 is related to regulation of inflammation.

On the other hand lower prevalence of the high producer genotype (GG) in IBS; suggests that high production of IL-10 may have some protective role or, conversely, that individuals predisposed to produce lower amounts of this cytokine might be more likely to develop IBS. A genetic predisposition to lower anti-inflammatory cytokine production could mean that control of the inflammatory response may be

compromised in some individuals and may help to explain why gastrointestinal infections, can sometimes lead to continuing problems. It is possible that an inflammatory process is perpetuated by failure of down regulation secondary to an inadequate anti-inflammatory cytokine response.

A high incidence of A allele positive subjects, both homozygous (- 1082*A/A) and heterozygotes (-1082*G/A), has been observed which code a low and intermediate production of IL-10, respectively. Van derVeek et al, [11] however, did not confirm this data, having observed regular levels of IL-10 genotypes and alleles at position -1082. The IL-10 gene does have a number of different polymorphisms but the site selected in this study (-1082) is known to influence IL-10 production in lymphocytes.

When we compare patient subgroups based on post-infectious symptom onset or predominant bowel habit. Patient numbers in these subgroups were small and therefore these results should be interpreted with caution. Our data indicated that the proportion of individuals' positive for the higher producer TNF- α was relatively large in IBS patients with a diarrhea predominant bowel habit (26.8%) compared to patients with constipation (15.2%) or alternating bowel habits (12.7%). These are potentially interesting results, as several studies indicated that TNF-α is associated with the occurrence of diarrhea. For instance, TNF-α is an important mediator of distal colonic secretion [21] and stool TNF- α concentrations are elevated in IBD. [22]

Also low and intermediate producer of IL-10 (AA and A/G) was more frequent in those subjects with IBS-D (25.4%, 22.5% respectively). There were no differences in the TNF-α and IL 10 genotypes among the IBS subgroups in IBS population. These result come in agreement with Max Schmulson *et. al*, [15] who observed a greater frequency in the low producer IL-10 polymorphism in IBS-D subgroup.

These findings, which are suggestive of a genetic predisposition to an abnormal immune regulation in subjects with IBS-D, concur with other data in the literature. Recently in the same population in Mexico by Max Schmulson *et. al*, they found that the presence of low serum levels of IL-10 was an independent predictive factor for IBS. [23] Furthermore, women with IBS-D presented with lower IL-10 levels compared with those with IBS-C and IBS-A/M. Other data in the literature also suggest immune regulation alterations, especially in IBS-D. For example, higher IL-10 levels derived from mononuclear cells from peripheral blood in IBS-D, when compared with controls, have been reported, even though no differences were reported in the comparison with the IBS-C or IBS-A/M subgroups. [24] Gecse K *et.al* [25] and others reported other factors have also been found, such as an increase in the serine proteases in stools in IBS-D, when compared with IBS-C and IBS-A/M. These proteases can trigger and increase in cellular permeability, which has also been found to be associated with an increase in defecation frequency, as well as with IBS-D itself. [25] Thus our findings are indicated that a genetic predisposition to produce lower levels of the anti-inflammatory IL-10 cytokine in subjects with IBS-D, we can't able to determine whether the greater frequency of the low producer IL-10 genotype would result in lower levels of this serum cytokine, lower expression in the colon mucosa, or in a predisposition to low grade inflammation in the colon mucosa, as has been described in patients with IBS. In the first study conducted in the United States regarding this, Chang *et al.* [26] evaluated both serum cytokines and their level of expression in the colon mucosa, finding only a lower expression of IL-10 mRNA in the mucosa, with no differences in its cellularity or in the serum levels of the cytokines. [26] Given the above, the relation of the abnormalities in the immune regulation alterations due to the serum cytokines and the low grade inflammation in the colon mucosa are still to be determined. [16]

Regarding IBS in family history; our study found that about 57% of patients had family history of IBS, these finding is in agreement with WhorwellPJ et al who found up to 33% of patients with IBS had a family history of IBS. [27] Aggregation of IBS symptoms has been described in families, suggesting a possible contribution by genetic factors to the pathogenesis of this disorder. Studies on monozygotic and dizygotic twins, however, do not support a key role for genetic factors in IBS, and the predominant influences appear to be environmental. [28,29] In recent years a number of biological molecules have emerged as potential genetic markers for IBS. These include molecules involved in serotonin metabolism, [30] and factors controlling production of pro and anti-inflammatory cytokines. [31] Imbalances in the genetically controlled pro- and anti-inflammatory cytokine production may promote the low-grade mucosal inflammation observed in some patients with IBS.

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