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Original Article

Genotyping of the serotonin transporter gene (SLC6A4) and tumor necrosis factor-alpha in patients with irritable bowel syndrome in Iraq

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ABSTRACT

Irritable bowel syndrome is chronic functional gastrointestinal disturbance characterized by abdominal discomfort or pain improves by defecation, and accompanied by alter in bowel habits such as diarrhea or constipation, which cannot be explained by biochemical, structural, or metabolic abnormalities. The current study was conducted to evaluate the role of serotonin transporter gene and tumor necrosis factor-α G308A genes polymorphism in the Irritable bowel syndrome. We have investigated single nucleotide polymorphisms of tumor necrosis factor-α G308Agene and insertion /deletion polymorphism of serotonin transporter gene in 110 subjects. Sixty-five were Irritable bowel syndrome patients while others were apparently healthy individuals used as controls. The frequencies of tumor necrosis factor-α A308A (7.7 vs 8.88%) genotypes and A allele(13.07 vs 17.78%) were higher in control group than Irritable bowel syndrome patients while serotonin transporter gene S/S genotypes was(47.7 vs 26.67%) and S allele (66.92 vs 47.78%) were higher in patient than control groups. AA genotype with tumor necrosis factor-α G308A allele polymorphism and S/S genotype with serotonin transporter gene are mainly expressed among patients with Irritable bowel syndrome and susceptibility with disease might be prospected.

Key Words: Irritable bowel syndrome, TNF-α, SLC6A4, Allele, Genotype, RFLP.

INTRODUCTION

Irritable bowel syndrome (IBS) is chronic functional gastrointestinal disorder (FGID) characterized by abdominal discomfort or pain improves by defecation, and accompanied by alter in bowel habits such as diarrhea or constipation, which cannot be explained by biochemical, structural, or metabolic abnormalities.[1] The exact pathophysiology of IBS is not well known, however, believed that there are important role for inflammatory reactions in pathogenesis of IBS.[2] There are three clinical subtypes of IBS are encountering in medical practice: IBS with diarrhea (IBS-D), IBS with constipation (IBS-C) and mixed IBS with constipation and diarrhea (IBS-M).[3]

The prevalence of Irritable bowel syndrome ranges from (5% to 15%) of the world population. Generally, using of Manning criteria, selected populations and woman gender commonly get the highest prevalence.[4] The prevalence of IBS is increasing and vary widely in countries in the Asia–Pacific region, particularly in countries with developing economies. As in study in Mumbai and according to Manning criteria-based community the IBS prevalence was 7.4%.[5] Study performed on Japan population revealed that the prevalence of IBS was 6%, that is not larger comparative to other Asian countries. In this study the clear risk factor for IBS was female gender, chiefly the IBS-C. Also, it questions whether a distinct economic condition would cause wider prevalence of IBS within this developed community. Possibly various issues unite to cause IBS rather than certain developed society [6]

The pathogenesis of IBS can be result from dysregulated brain-gut axis, intestinal dysmotility, visceral hypersensitivity, psychological factors and genetic or environmental factors.[7] In addition to these factors, dysregulated intestinal immune function, bacterial infection, and low-grade mucosal inflammation have all been participate in pathogenic mechanisms.[8] Molecules that communicate among cells of the immune system

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are referred to as cytokines. It is significant modulators of inflammatory reactions and immune responses and play an essential role in intestinal inflammation.[2] The genetic polymorphisms in cytokine genes (within promoter or coding regions) affect production of cytokines.[9] Therefore, disease susceptibility and clinical outcome may affected by a genetic predisposition for the low or high production of certain cytokine.[10] For example, Tumor necrosis factor-α (TNF-α) gene polymorphism in the promoter region (G→A substitution at position –308), affect secretion of TNF-α.[11] TNF-α production increases with the presence of the A allele (A/A or G/A).[12]

The main function of TNF is to regulate immune cells. TNF is able to induce sepsis, to induce apoptotic cell death, to induce fever (through production of IL1 and IL6), induce inflammation, cachexia, and to inhibit viral replication and tumorigenesis.[13]

Serotonin (5-hydroxytryptamine), is a neurotransmitter in the enteric nervous system promoting gut motility, visceral sensation and secretion. 5-HT is thought to play a key role in the pathophysiology of IBS.[14] Long and a short alleles (L and S alleles) which generate when insertion or deletion 44 base pair in the promoter region "5-HT transporter linked polymorphic region (5-HTTLPR)". Low transcriptional efficiency with presence of S allele in 5-HTTLPR, which may lead to increased serotonin level in patients with IBS.[15] The present study was conducted to evaluate the role of SLC6A4 and TNF-α G308Agenes polymorphism in the Irritable bowel syndrome

MATERIALS AND METHODS

Subject: The current study was conducted on 65patients (38 female and 27 male) attended to Al-Diwaniya Teaching Hospital, Iraq ,for the period from December 2014 to March 2015, the patients were diagnosed clinically based on Rome Ⅲ Criteria under the supervision of a Physician as having IBS. Another group consist of 45apparently healthy individuals (16 male and 29 female) without any history of systemic disease were clinically considered as healthy also included in this study as a control group. Informed consent was obtained from all study subjects after explanation of the nature and possible consequences of the study.

Genotyping: The genotypes of the SLC6A4 gene were determined by PCR technique, Table (1), and TNF-α G308A gene were identified by PCR-RFLP technique. The PCR products were purified using a AccuPower^{TM} PCR PreMix (Bioneer), then use a UV Transilluminator to visualize the PCR products in an ethidium bromide-stained 1.5% agarose gel. Following, The PCR products were digested with the Nco1 restriction enzymes for TNF-α. Use a UV Transilluminator to visualize the digested PCR products in an ethidium bromide-stained 2.5% agarose gel.

Statistical analysis: The Hardy–Weinberg equilibrium (HWE) assumption was evaluated for both the control and patient groups by compare the observed numbers of each genotype with those expected under the HWE for the estimated allele frequency. Data were presented, summarized and analyzed using two software programs. These were the Statistical Package for Social Science (SPSS) version 22 and Microsoft Office Excel 2010. Logistic regression analysis was used to estimate the 95% confidence intervals (CI) and odds ratios (OR) for the relationship between the alleles or haplotypes, genotypes and the risk of IBS. The results are presented as the mean values ± 1 standard deviation (SD), and a P value of ≤0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Demographic and clinical parameters: Patients with IBS were comparable in age and gender with controls (Tables2 and 3). According to the patient's perception, of 65 patients with IBS, 20 (31%), and 28 (43%) were constipation, and diarrhea predominant subtypes respectively, while 17 (26%) were of alternating type, these classified by using Rome III criteria.

Distribution of serotonin transporter gene, tumor necrosis factor-α G308A Alleles and Genotypes in Patient and Control Groups: Distribution of SLC6A4 polymorphism was detected by PCR technique, TNF-α G308A polymorphism was detected by PCR-RFLP technique, at this locus there're three genotype; for SLC6A4 S/S, S/L and L/L with band sizes 484pb, 484/528pb and 528pb respectively, figure (1),and for TNF-αG308A GG, GA and AA with band sizes 20/97pb, 20/97/117pb and 117pb respectively, figure (2).The frequency distribution of alleles and genotypes of TNF-α G308A, SLC6A4 in control and patients groups are summarized in (Tables 4 and 5).In this study, we genotyped the SLC6A4 and TNF-α genes polymorphisms in IBS patients to determine the relationship between the genotypes/allele types and their clinical features.

The current study revealed that there was no association between IBS and the common TNF-α(-308 G/A) polymorphism, Statistical analysis indicated that TNF-α (-308 G/A) was not a risk

factor for IBS. Czogalla et al.[16] who found that there was no correlation between IBS and TNF-α (-308 G/A) polymorphism, (95% CI=0.87–1.12,OR= 0.98), p-value=1. Also Santhosh et al.[17] who found not significantly different in patients group in comparison with control group, Genotypic frequencies for (GG), (GA) and (AA) in patients group were (91.3%), (4.4%) and (4.4%) respectively, while in control group were (75.0%), (25.0%) and (0%) respectively, P= 0.08, and allelic frequencies in patients group were G allele (93.5%) and A allele (6.5%), while in control group were G allele (87.5%) and A allele (12.5%), P=0.46.

In Mexico, study done by Schmulson et al.[18] who found there was no differences between IBS and controls in the frequency of the high producer TNF-α genotype(AA genotype)(0 vs. 1.1%), intermediate producer TNF-α genotype(GA genotype) (55.4 vs. 43.2 %), or low producer TNF-α genotype (GG genotype) (43.5 vs. 56.8 %), p = 0.296.

The current study revealed that there was correlation between the common SLC6A4 polymorphism and IBS, S/S (deletion/deletion) genotype was related with higher risk of IBS as compare to L/L (insertion /insertion) genotype (p =0.03, OR = 2.507, 95% CI = 1.103 - 5.696). At allelic level, presence of "S" allele was related with a higher risk of IBS as compare to the presence of "L" allele (p=0.005, OR=2.211, 95% CI=1.273-3.840). The result of the present study showing S/S genotype to be a risk factor for IBS is similar to the results of US, Korean and India studies. Moreover, in our study the S/S genotype has obviously suggests an etiology for IBS, as it had an OR of 2.507 and Etiologic Fraction (EF) of 0.432, In contrast, the L/L genotype had rather preventive role as it had Protective Fraction (PF) of 0.415 and low OR (0.355).With the possibility of L allele may be protective, whereas the S allele may increase susceptibility to IBS.

Kumar et al.[19] who found significantly different in patients group in comparison with control group, Patients with IBS more often had S/S genotype [89 (59%) vs. 92 (37%), p < 0.001], S/L genotype [44 (29%) vs. 114 (45%), p < 0.001] and L/L genotype [17 (12%) vs. 46(18%), p>0.05] compared to healthy controls. S/S (deletion/deletion) genotype was connected with higher risk of IBS as compare to L/L genotype (p = 0.003, OR = 2.6, 95% CI = 1.4 – 4.9). presence of "S" allele was connected with a higher risk of IBS as compare to the presence of "L" allele (p < 0.001, OR = 1.9, 95% CI = 1.4 – 2.7).

CONCLUSION

There was no correlation between TNF-α (G/A-308) polymorphism in the pathogenesis of IBS in Iraqi population. The important role of SLC6A4 polymorphism in the pathogenesis of IBS in Iraqi population is discovered, With regard SLC6A4 polymorphism, the S/S genotype is considered as a risk factor for Irritable bowel syndrome.

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Table (1): primers sequence with orientation and the PCR product size.

Type of gene	Sequence		Amplicon Size
TNF-α Gene	F	5 'AGG CAA TAG GTT TTG AGG GCC AT3'	117bp
	R	5 'ACA CTC CCC ATC CTC CCG GCT3'	
*SLC6A4*gene	F	5 'GGC GTT GCC GCT CTG AAT GC3'	528bp
	R	5 'GAG GGA CTG AGC TGG ACA ACC AC3'	484bp

Table (2): The Patient-Control Difference in mean age.

Age (years)	Healthy controls	Patients	P-value
Range	(20 - 78)	(19 - 78)	0.133[NS]
Mean	39.57	41.07	
SD	15.11	15.23	
SE	2.25	1.88	
N	45	65	

SD =Standard deviation, SE= Standard Error.

Table(3):Comparison of Gender Frequency Distribution Between Control and Patient Groups.

	Patients		Healthy controls		P-value
Gender	N.	%	N.	%	(0.021) Significant
Females	38	58.5	16	35.55	
Males	27	41.5	29	64.45	
Total	65	100	45	100	

Table (4): Association Between Disease and *TNF-α* Gene Expression.

Genotype / Allele	Patients (n = 65)		Control (n = 45)		P-value	Odd ratio	95 % Confidence interval	EF	PF
	No.	%	No.	%					
GG	53	81.53	33	73.33	0.351	1.606	0.646 - 3.992	0.232	***
GA	7	10.77	8	17.79	0.397	0.558	0.186 - 1.668	***	0.269
AA	5	7.7	4	8.88	>0.999	0.854	0.216 - 3.373	***	0.086
G	113	86.93	74	82.22	0.344	1.437	0.683 - 3.021	0.183	***
A	17	13.07	16	17.78	0.344	0.695	0.331 - 1.462	***	0.184

OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction

Table (5): Association Between Disease and *SLC6A4 Gene* Expression.

Genotype / Allele	Patients (n = 65)		Control (n = 45)		P-value	Odd ratio	95 % Confidence interval	EF	PF
	No.	%	No.	%					
S/S	31	47.7	12	26.67	0.03	2.507	1.103 - 5.696	0432	***
S/L	25	38.46	19	42.22	0.697	0.855	0.394 - 1.855	***	0.087
L/L	9	13.84	14	31.11	0.034	0.355	0.138 - 0.915	***	0.415
S	87	66.92	43	47.78	0.005	2.211	1.273 - 3.840	0.366	***
L	43	33.08	47	52.22	0.005	0.452	0.260 - 0.785	***	0.366

OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction

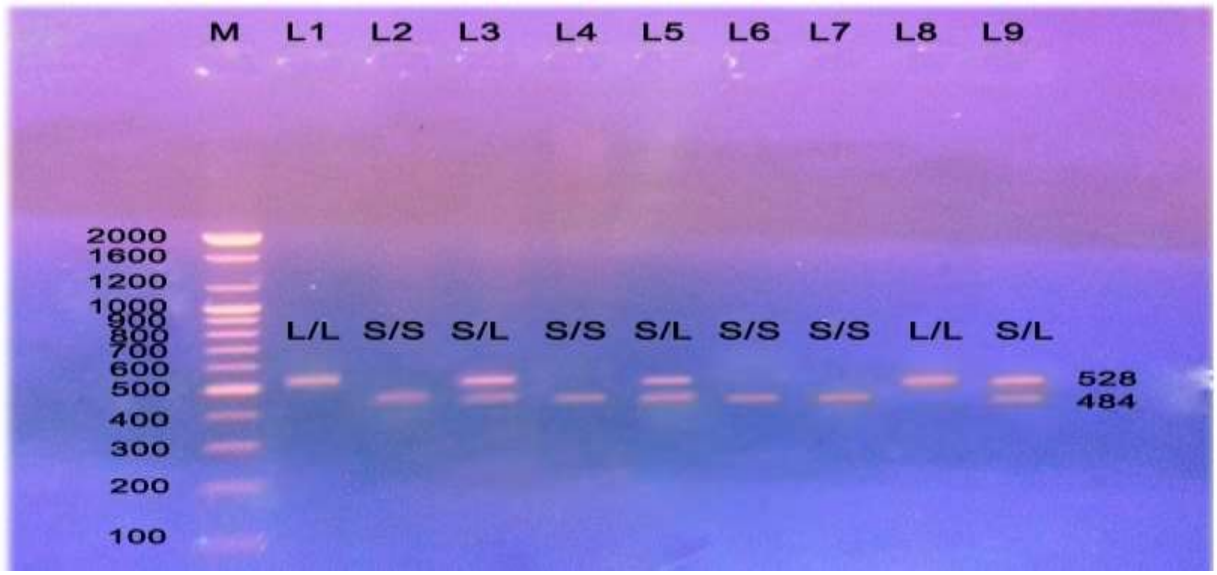

Figure (1): Ethidium bromide-stained Agarose gel of PCR amplified of *SLC6A4* gene. Lane (M):DNA molecular size marker (100bp ladder), Lane 1-6 for IBS patients and Lane 7-9 for control group. Lane 2,4,6,7=S/S genotype (484bp), Lane1,8= L/L genotype (528bp), Lane 3,5,9= S/L genotype (484/528bp).

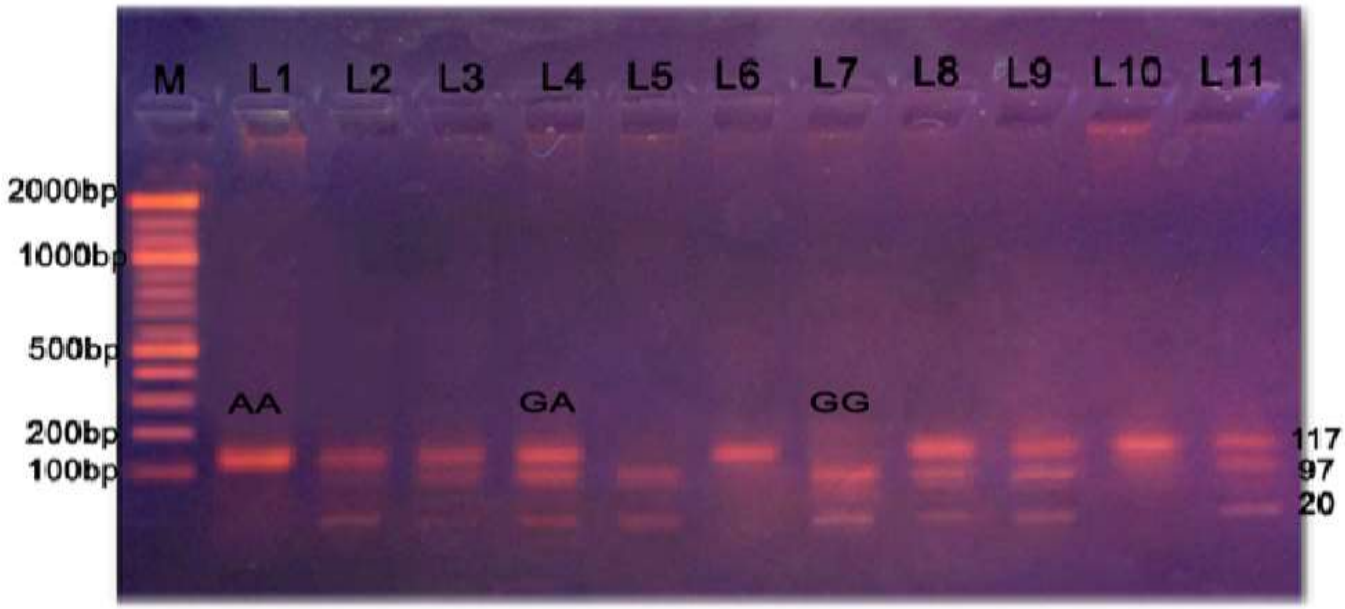

Figure (2):TNF-α G308A electrophoresis after restriction digestion with Nco1. Lane (M): DNA molecular size marker (KAPA Universal Ladder), Lane 1,6,10= AA genotype (117bp), Lane 2,3,4,8,9,11=GA genotype(20/97/117bp), Lane 5,7=GG genotype(20,97bp).

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