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Original Article

Serotonin Transporter Gene Polymorphism (5- HTTLPR and VNTR) among Sudanese patients with Irritable Bowel Syndrome (IBS)

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ABSTRACT

Objective: This study was aimed to determine the frequency of serotonin transporter gene (SERT) (5-HTTLPR) and VNTR polymorphism in patients with post infectious irritable bowel syndrome (PI-IBS)

Design: This is a cross sectional study

Setting: The study was carried out in gastroenterology out patient's clinics at Omdurman teaching hospital/Khartoum State, Sudan

Subjects: Fifty-four consecutive IBS patients age range between 18–73 years old, who were referred to gastroenterology out patient clinics, and 50 apparently healthy (age range 19–68 years) were examined and enrolled between April, 2016 to April, 2017.

Intervention: Collection of venous blood samples

Main outcome measures: Determining overall frequency of gene Serotonin Transporter Gene Polymorphism (5-HTTLPR and VNTR) among IBS Sudanese patients following parasitic infectious (PI-IBS)

Results: SERT polymorphisms were found to be significantly increased in IBS patients in comparison with controls (p=.05), as well as family history IBS patients. According to bowel habits; diarrhea predominant (n=18), constipation predominant (n=26), and alternating diarrhea and constipation (n=10) and we were found that the 5-HTTLPR allele L/L genotype occurred with greater frequency in the diarrhea predominant group than in the other two subgroups (p=.05).

Conclusion: The family history is playing major role in presence of IBS. We found that the LS and SS genotypes are significantly correlated with IBS. No relationship was found between IBS and SERT gene polymorphism. The presence of the S/S genotype in IBS patients carries an increased risk of the constipation predominant type of IBS.

KEY WORDS: irritable bowel syndrome, serotonin

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic disease characterized by impaired bowel function and abdomen pain and changes in bowel habit (constipation and/or diarrhea). It has been reported to have an incidence of 20% in developed countries [1]. IBS is a complex, heterogeneous disorder, whose development is widely considered as multifactorial in nature [2, 3]. Persistent low-grade inflammation may plays a role in IBS and it is one of proposed mechanism of IBS through persistent antigenic exposure as in persistent carriage. It is estimated that 7-31% of patients with infectious gastroenteritis go on to develop IBS (post- infectious IBS) [4, 5]. Also a gene-environment has been proposed for IBS, whereby a combination of genetic determinants and environmental factors gives rise to alterations in GI sensation and motor functions Serotonin transporter (SERT) is a protein which reuptakes 5-hydroxytryptamine (5-HT) in synaptic cleft and then reduces the function of 5-HT such as inducing urgency, cramps, diarrhea and vomiting [6, 7].

Serotonin 5-HT acts as a main regulator in gut, it plays an important role in the motility and secretion of the gut, secretion and sensory signaling, and plays also a pivotal role in the control of mood at level of the central

nervous system (CNS) [8, 9]. The magnitude and duration of 5-HT actions depend mostly on 5-HT transporter (SERT), which mediates the extracellular reuptake of 5-HT, thus ensuring its recycling and catabolic breakdown [10, 11].

The lower expression of SERT will indicate higher level of 5-HT, which may be associated with bowel symptoms in IBS patients. The two well investigated polymorphism regions are variable number tandem repeat (VNTR) and serotonin transporter linked polymorphic region (5-HTTLPR).

Up to now, the genes associated with serotonin, inflammation, adrenergic, mucosal barrier, and psychology which may play a role in IBS have been widely examined [12].

One of the first SERT polymorphisms characterized was the variable number of tandem repeats (VNTR) in intron 2 of the gene and consists of 17 base pair (bp) repeats [13]. The promoter region of human SERT gene is located on chromosome 17q11.2, contains a polymorphism, designated as '5-HT transporter length polymorphic region' (5HTTLPR), which consists of a 44-base pair deletion/insertion, resulting in a short (S) and long (L) allele [13, 14]. The implications of this polymorphism in IBS are currently under evaluation since, as compared with LL genotype, SS or LS genotypes are associated with lower levels of SERT mRNA transcripts, and thereby lower levels of SERT expression and lower efficiency of 5-HT reuptake [15, 16]. There were studies supported that the SERT polymorphism or a polymorphism in linkage disequilibrium with the SERT polymorphism might play a role in the development of IBS [7]. A second SERT polymorphism, variable number of tandem repeats (VNTR) STin2, located in intron 2 and consisting of a variable number (usually 9, 10, or 12) of nearly identical 17-bp segments, had been found to be associated with IBS in one study, with the 10/12 genotype more frequent in Chinese patients than in controls [8]. Most authors, however, had found no association between STin2 VNTRs and IBS [7, 17, 18]. So the current study aimed to investigate the association of the 5- HTTLPR and VNTR gene polymorphism among IBS Sudanese patients.

SUBJECTS AND METHODS

Study design

This was analytical cross sectional study was conducted from April, 2016 to April, 2017, 54 consecutive IBS patients age range between 18–73 years old, who were referred to gastroenterology out patients clinics at Omdurman teaching hospital/Khartoum State, and 50 apparently healthy, their age range from 19–68 years old) were included in the study.

Inclusion criteria

Patients recruited in the study according to have had symptoms that fulfilled the Rome III criteria for IBS for at least 6 months before.

Exclusion criteria

Patients with the following conditions were excluded from: severe organic diseases; history of major abdominal surgery; severe psychiatric disorders; organic lesions at colonoscopy, or abdominal ultrasonography; lactose intolerance.

Sampling technique and sample size

A non-probability sampling technique namely convenience sampling method was followed (where samples are selected from the population randomly because they are conveniently available to researchers. The total sample size is 54 Irritable Bowel Syndrome patients (IBS) and 50 control subjects. Five mL of venous blood was collected from each subject, immediately transferred into EDTA tubes (EDTA as anticoagulant) and stored at 4^0C for DNA extraction.

Methods

Insertion/deletion polymorphism (with variants 484 and 528) was analyzed by the polymerase chain reaction (PCR), as described by Heils et al [19]. DNA was extracted from blood sample using salting out method. Samples quality was detected by performing gel electrophoresis. The reaction was performed in 20µl volume using Maxime PCR PreMix Kit (i- Taq): Product Catalog No.25025 (for 20µL rxn, 96tubes) ,iNtRON biotechnology(Made in china - Model number: JY- 02S - Serial number: 0903002D). The PCR master mix for one reaction was prepared as follows: .5 µL of forward primer, 0.5 µL of reverse primer Made in Korea by MacrogenInc – dong Geumchun and 16 µL sterile water to PCR Premix tube and finally 3 µL of DNA total volume 20 µL The mixture amplified in thermo- cycling conditions which were initial denaturation at 95ºC for 10 minutes followed by 40 cycles Denaturation at 95ºC for 60 s, annealing at 59ºC for 60 s and template extension at 72ºC for 60 s. The cycle was repeated 40 times. with a final extension 72ºC for 7 minutes, 10µl of the amplified product was subjected to direct analysis by Gel Electrophoresis in 2%. PCR primers for intron 2 polymorphism were described by Lesch *et al* in 1996; the conditions were modified from those described previously [16]. A deletion /insertion polymorphism (5-HTTLPR) in the promoter region of the SERT gene was typed by PCR using flanking primers (forward) 5- GGCGTTGCCGCTCTGAATGC-3 and (reversed) 5 GAGGGACTGAGCTGGACAACCAC-3. STin 2.9, STin 2.10, and STin 2.12. VNTR polymorphism in intron 2 of the SERT gene was typed by PCR using primers 5-HTT F 5-TGGATTTCCTTCTCTCAGTGATTGG-3 and 5-HTT R 5-TCATGTTC- CTAGTCTTACGCCAGTG-3. Amplification products were resolved by electrophoresis on 2% agarose gels and visualized with ethidium bromide staining. Alleles were designated S (484 bp) and L (528 bp) according to Lesch *et al* [14].

Statistical Analysis

Statistical analysis of the data was conducted with the SPSS version 20.0 statistics package program. In addition to descriptive statistics (e.g., frequency and percentage), chi-square test, were used. The threshold for statistical significance was $p < 0.05$.

Ethical approval

The study was approved by the Ethics Committee of Al- Neelain University. The participants gave written informed consent to participate in the research.

RESULTS

Demographic characteristics

Out of 54 participants, 26 (48.1%) male and 28 (51.9%) female, their age group range between 18-70 years old. The majority of them 25(46.3%) in age group (31-50) years old. Regarding period of illness, about 35.2% of patients infected with IBS for more than 10 years ago. There was significant association between family history and the expression of 5-HTTLPR alleles in IBS patients ($p \geq 0.05$), and in other socio-demographic variables including age group, gender, and period of IBS are shown no significance association (Table 1).

Genotype and allele frequencies

Genotype distributions for the 5-HTTLPR, STin2 VNTR, polymorphisms were checked. VNTR and 5-HTTLPR alleles were present in all patients in the IBS and control groups, and the expression of 5-HTLPR and VNTR gene was significantly more in IBS patients compared to controls (0.05 and 0.006 respectively) figure 1 and table 2.

A total 54 IBS patients (26 had a constipation predominant bowel pattern, 18 had a diarrhea predominant bowel pattern, and the remaining 10 had a bowel pattern alternating between diarrhea and constipation participated in this study. Table 3 summarize Serotonin Gene 5- HTTLPR and VNTR alleles distributions in IBS according to predominant bowel habit However, there is no significant in association of gene 5- HTTLPR alleles in IBS patient according to predominant bowel habit, but S/S genotype occurred with greater frequency in the constipation predominant group than in the other two subgroups. Moreover, in comparison of SERT gene VNTR alleles in IBS patients STin2.12/12 allele is more than two other alleles (50%, 37.1%. 12.9% respectively)

Table 4 summarized the genotype frequencies of 5 HTTLPR and VNTR in IBS patients were infected and non-infected with intestinal parasites. Statistical analysis revealed no significant result regarding (5HTTLPR and VNTR) genotype infective and no infective with intestinal parasites (P=0.107).

DISCUSSION

Number of studies has implicated correlation between IBS and serotonin gene polymorphisms in the differential diagnosis of IBS since they cause significant flares of IBS with acquisition. This is the first study in Sudan, we studied two alleles 5-HTTLPR and VNTR. Findings of this study agree with many similar works [20, 21].

In our study we found that majority of patient in age group 31-50 years old and the frequency of females (51.1%%) more than males (48.9%) since female sex hormone such as estrogen affect the bowel habits, these finding is in agreement with Eltayeb LB *et. al* [21]. About 53.7% of participant had family history of IBS, regarding 5HTTLPR gene we found that family history has a significant correlation with L/S genotype expression ($p \leq 0.05$), which agreed

Saito YA (2011) [12] and Adam B, *et.al* [22]. Studies on families or twins have given support to the hypothesis of a genetic background in IBS and, consequently, intensive efforts are being made to evaluate whether specific polymorphisms of a number of candidate genes are pathogenically associated with IBS. Furthermore, since genetic factors do not act necessarily as risk factors, but they can also impact on clinical phenotypes; great attention is being paid also to possible relationships linking genetic polymorphisms to intermediate phenotypes [23]

Generally 5-HTTLPR subtypes were significantly increased in IBS patients when it was compared with controls, (22.1%) of IBS patients had S/S genotype compare with control (14.4%), , The SERT protein is responsible for reuptake of 5-HT in serotonergic nerves and mucosa of bowel, and is a factor that determines 5-HT activity. In a lymphoblast cell line, s/s genotype at promoter polymorphic site of SERT gene was associated with lower transcriptional efficiency, resulting in lower SERT expression and therefore lower cellular uptake of 5-HT[24, 25]. These studies support and explain our results.

For VNTR there were a significantly associations found (P value 0.006), with the 12/12 genotype more frequent among Sudanese patients than in controls (26%, 19%) respectively, this is apparently different from Jing [25] result who found 11/11 genotype was more frequent. One of the reasons for the variety was the heterogeneous pathogenesis. IBS may be heterogeneous in different populations as it is a syndrome diagnosed using symptom based criteria, its prevalence being variable in different countries with different ethnicity [26,27]. Such heterogeneity in phenotypes may also partly explain the result of genetic studies.

Moreover, our results showed a lack of significant interaction between bowel habits and two genotypes studied 5HTTLPR and VNTR and this is in agreement with the majority of previous studies [28, 29] . while most authors did not find specific links of 5HTTLPR with C-IBS or D-IBS, [30, 31] others have reported significant associations of SS with D-IBS, SS with C-IBS [32], but It is conceivable that the presence of the S/S genotype in IBS patients carries an increased risk of the constipation predominant type of IBS.

Among the variety of factors which might account for the heterogeneity of these observations, the most relevant relies perhaps in the different distribution of 5HTTLPR genotypes among different populations.

In more recent study conducted by M, Mojgan [33], 2017 about SLC6A4 polymorphism they found that SLC6A4 is a possible candidate gene associated with the pathogenesis of IBS-C, in addition Yubing Zhu 2018 *et. al* indicated that the *SERT* insertion/deletion polymorphism may serve as a genetic biomarker of IBS in Asians and Caucasians and it is significantly correlated with the risk of constipation predominant IBS (IBS-C), their result support our finding [34].

CONCLUSION

In conclusion, the present study suggests that the LS and SS genotypes are significantly correlated with IBS symptom severity, although their possible direct causal role remains to be proven. In addition, our result did not found an association of 5HTTLPR/or VNTR with IBS or its clinical presentation in terms of bowel habit predominance.

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Conflict of interest statement: The authors declare that they have no conflict of interest.

Author's contribution:

Entsar Elamin (sample collection and laboratory works)

Nasma Elshaikh (sample collection and laboratory works

Sara Abdelghani (Calibration of machines, preparation Study protocol and SOPs (Standard Operating Procedures)

Mohammed Madani Eltayeb (purchasing reagents)

Hisham Ali Waggiallah (Statistical analysis of Data)

Lienda Bashier Eltayeb (Scientific writing of manuscript)

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Table 1: Association between socio-demographic variables and SERT gene 5-HTTLPR and VNTR polymorphism among IBS patients

Respondents Characteristics	5-HTTLPR N=54(%)				VNTR N=54(%)			
	Allele L/L	Allele L/S	Allele S/S	Total	STin2.10/ 10	STin2.10/ 12	STin2.12/ 12	Total
Age group								
- 18-30	1(1.9%)	2(3.7%)	2(3.7%)	5(9.6%)	0 (0.0%)	2 (3.7%)	3 (5.6%)	5 (9.3%)
- 31-50	3(5.6%)	10(18.5 %)	12(22.2 %)	25(46.3 %)	5 (9.3%)	10(18.5%	10(18.5%	25(46.3 %
- 51-70	6 (11.1%)	9(16.7%)	9(16.9%)	24(44.4 %)	2 (3.7%)	8 (14.8%)	14(25.9%	24(44.4 %
Gender								
Male	6 (11.1%)	9 (16.7%)	11(20.4 %)	26 (48.1)	3 (5.6%)	13(24.1%	10 (18.5)	26(48.1 %
Female	4 (7.4%)	12 (22.2%)	12(22.2 %)	28 (51.9%)	4 (7.4%)	7 (13%)	17(31.5%	28(51.9 %
Period								
1 to less than 5 yrs	4 (7.4%)	6 (11.1%)	8 (14.8%)	18 (33.3%)	4 (4.4%)	5 (9.3%)	9 (16.7%)	18(33.3 %
5 to less than 10 yrs	0 (0.0%)	7 (13.0%)	10(18.5 %)	17 (31.5%)	2 (3.7%)	5 (9.3%)	10 18.5%	17(31.5 %
≥10 yrs	6 (11.1%)	8 (14.8%)	5 (9.3%)	19 (35.2%)	1 (1.9%)	10(18.5%	8 (14.8%)	19(35.2 %
Family history*								
Yes	9(16.7%)*	9 (16.7%)	11(20.4 %)	29 (53.7%)	4 (7.4%)	11(20.4%	14(25.9%	29(53.7 %
No	1 (1.9%)	12 (22.2%)	12(22.2 %)	25 (46.3%)	3 (5.6%)	9(16.7%)	13(24.1%	25 (46.3)

Values are expressed in percentage, L- long allele, S- short allele, yrs- years, *Statistically significant at p<0.05

- Figures in parenthesis indicate percentage
- Not statistically significant at p>0.05

Table 2: Comparison between IBS patients control 5-HTTLPR and VNTR

Respondents	**5-HTTLPR**			
	Allele L/L	**Allele L/S**	**Allele S/S**	**Total**
- IBS patients	10(9.6%)	21(20.2%)	23(22.1%)	54(51.9%)
- Control	20(19.9%)	15(14.4%)	15(14.4%)	50(48.1%)
- P. value	0.05			
VNTR				
	STin2.10/10	**STin2.10/12**	**STin2.12/12**	**Total**
- IBS patients	27 (26.0)	20 (19.2)	7 (6.7)	54 (51.9)
- Control	13 (12.5)	18 (17.3)	19 (18.3)	50 (48.1)
- P. value	0.006			

Table 3: Serotonin Gene 5- HTTLPR and VNTR alleles distributions in IBS according to predominant bowel habit

Genotype HTTLPR	**IBS-D**	**IBS-C**	**IBS-M**	**Total**
L/L	**3 (5.6%)**	4(7.4%)	3 (5.5%)	10 (46.5%)
L/S	6 (11.1%)	8 (14.8%)	7 (13%)	21 (38%)
S/S	9 (16.6%)	14 (25.9%)	0 (0.0%)	23 (15.5%)
Total	18 (33.3%)	26(48.1%)	10(18.5%)	54 (100%)
P value	0.239			
Genotype VNTR				
STin2.10/10	2 (3.7)	5 (9.3)	0	7 (12.9%)
STin2.10/12	8 (14.8)	9 (16.7)	3 (5.6)	20 (37.1%)
STin2.12/12	8 (14.8)	12 (22.2)	7 (13)	27 (50%)
Total	18(33.3)	26(48.1)	10 (18.5	54 (100%)
P value	0.469			

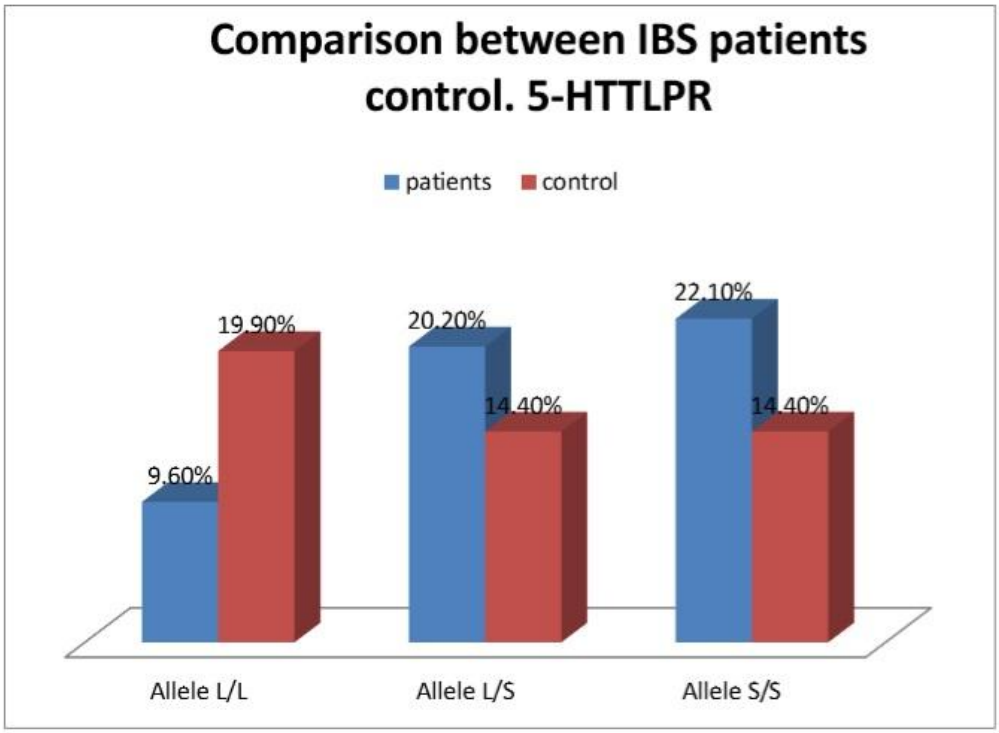

Figure 1 Comparison between IBS patients control. 5-HTTLPR

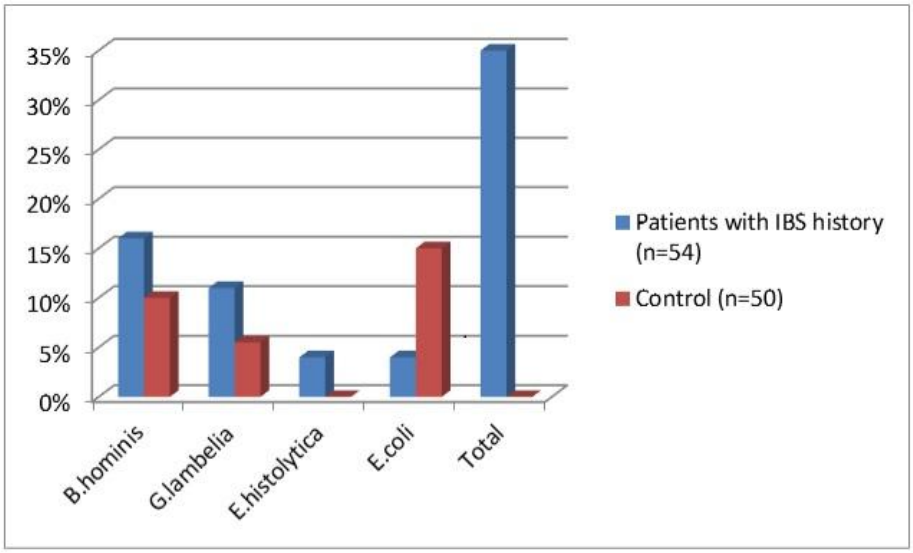

Figure 2: spectrum of intestinal parasites in IBS patients and control