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Original Article

Impact of Serotonin Transporter Gene Polymorphism on Gut Motility in Patients With Type 2 Diabetes Mellitus

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Background/Aims
The pathogenesis of gastrointestinal (GI) symptoms in patients with type 2 diabetes mellitus (T2DM) is yet to be delineated clearly. Serotonin, a monoamine neurotransmitter, resides primarily in the gut and plays a vital role in GI system. However, no study has been documented the role of serotonin and serotonin transporter gene (SLC6A4) polymorphism in the development of GI symptoms in T2DM patients.

Methods
Three hundred diabetes patients attending diabetes clinic at Postgraduate Institute of Medical Education and Research, Chandigarh, and matched healthy controls were enrolled for this study. Plasma from collected blood sample was used for serotonin measurement by enzyme-linked immunosorbent assay method and buffy coat was used for isolation of DNA by phenol chloroform method. Serotonin transporter gene polymorphism was analyzed by polymerase chain reaction method.

Results
The frequency of short allele (S) and SS genotype was significantly higher in patients with T2DM than controls and was associated with increased risk of T2DM. The frequency of LS genotype showed an association with protection from the disease. Regarding GI symptoms, 78.2% of patients with constipation showed LL and LS genotypes, and 97.7% of patients with diarrhea had SS genotype. The patients without GI symptoms did not show any association of gut motility with genotype. Furthermore, serotonin was significantly higher in diabetic patients who belonged to SS genotype compared to LS or LL genotype and who presented with diarrhea.

Conclusion
SS genotypes are prone to develop diarrhea because of faster gut motility resulting from higher serotonin levels as compared to LS and LL genotype in T2DM patients.
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Key Words
Alleles; Diabetes mellitus, type 2; Polymorphism, genetic; SLC6A4 protein, human; Serotonin plasma membrane transport proteins

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Introduction

The main function of the gastrointestinal (GI) tract is to supply the body with nutrients, electrolytes, and water. This is possible because of the combination of 5 distinguished functions such as motility, secretion, digestion, absorption, and storage.[1] The coordinated actions of various gut muscles facilitate food to progress along the digestive tract making sure that the absorption of the important nutrients could take place. Hence, transit time is an important determinant of the physical well being.

Gut motility can be influenced by type 2 diabetes mellitus (T2DM). The abnormal blood glucose level may modulate the intestinal function through alteration of metabolic and signalling pathways.[2] The commonly encountered symptoms in approximately 75% of individuals with diabetes mellitus include bloating, nausea, diarrhea and/or constipation depending on the duration of altered glucose homeostasis.[3]

The regulation of intestinal muscle activity which is the main contributing factor in gut motility, is carried out by various neurotransmitters. Bernhard in 1859 identified the significance of neurohumoral control of gut motility by demonstrating paralytic ileus and hunger contraction in the intestine of experimental animal model.[4,5] Serotonin, the most often considered monoamine neurotransmitter in the central nervous system, plays a vital role in the function of the GI system.[6] The major site of serotonin synthesis, storage, and release in the blood is the enterochromaffin cells of the intestinal mucosa.[7,8] Since this break-through, many studies have anticipated that the release of serotonin from the GI tract may play an important role in the control of gut motility.[9-12] Heredia et al[12] documented that antagonists of serotonin receptors can attenuate the momentum of the gut content by inhibiting the peristalsis. Later on Keating and Spencer[13] demonstrated that exogenous administration of serotonin could accelerate the gut motility. Within the synapse, serotonin can interact with both pre- and post-synaptic receptors. Immediately after that serotonin undergoes reuptake process which determines the extent, duration, and spatial domain of receptor activation. The reuptake is carried out by transporter proteins which is found in the plasma membrane of serotonergic neurons and thus acts as a carrier of serotonin molecules across the membrane.

The gene (SLC6A4) for serotonin transporter (SERT or 5HTT) is a member of the neurotransmitter sodium transporter family of transporters, located on chromosome 17q11.2. This gene has numerous polymorphisms including a functional polymorphism consisting of a 44-base pair insertion/deletion in the 5' promoter region (serotonin transporter linked polymorphic region [5HTTLPR]). This includes long (L) and short (S) alleles that influence the rate of serotonin transcription. The S allele was found to have lower transcriptional efficiency compared with L allele.[14] One study has demonstrated a strong association of S allele of HTTLPR with type 2 diabetes.[15] This polymorphism has been studied in few other conditions such as irritable bowel syndrome (IBS),[16] pain severity in idiopathic trigeminal neuralgia patients,[17] and systemic lupus erythematosus.[18] However, there is no study to report the association between SERT gene polymorphism and gut motility as well as the occurrence of various GI symptoms in T2DM patients. Therefore, this study would deal with these aspects of SERT gene polymorphism in T2DM individuals.

Materials and Methods

This study was conducted in the Department of Gastroenterology and Endocrinology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. Ethical approval (INT/IEC/2015/635 dated 20th October 2015) was taken from the institute ethics committee of PGIMER prior to enrollment of the participants. Study design has been described in details in a previous publication.[19,20] In brief, 300 T2DM patients and 200 healthy controls were enrolled for this study. Informed consents were taken from all the study participants. Diabetes mellitus was diagnosed in subjects according to the American Diabetes Association criteria. Only those patients with glycated hemoglobin percentage > 6.5% and were on insulin or metformin along with sulfonylurea were enrolled. If a patient developed diarrhea following metformin administration, the patient was excluded from this study. Patients with any of the following history were excluded from this study: prior gastric surgery, peptic ulcer and therapy with prokinetics, intestinal pseudo-obstruction, receiving broad spectrum antibiotic or proton pump inhibitor during the month preceding the test, receiving the drugs known to interfere with GI motility such as anticholinergic or antidepressants, and patients showing features suggestive of autonomic neuropathy. Two hundred age and sex matched healthy individuals without diabetes and GI disorders were enrolled as controls. Controls were not relatives of the enrolled patients. All patients and controls used to reside in the local region of study institute and they used to practice similar cultural behaviour including diet habits.

Five milliliters of blood sample was collected in an EDTA vial.

Plasma was used for serotonin estimation by commercially available enzyme-linked immunosorbent assay kit following manufacturer's instructions (DLD Gesellschaft für Diagnostika und medizinische Geräte mbH, Hamburg, Germany). In brief, the assay was based on the competitive enzyme immune assay method. The intensity of the signal measured at 450 nm was inversely proportional to the concentration of serotonin in the sample. Buffy coat was used for gene polymorphism study. Genomic DNA was isolated using the phenol chloroform method. DNA was quantified by spectrophotometric method and purity was checked by calculating the ratio of absorbance at 260 nm and 280 nm. Polymorphism for SERT gene was analyzed by polymerase chain reaction (PCR) using the following set of primers:

SERT- F: 5' GGC GTT GCC GCT CTG AAT GC 3' and SERT-R: 5'GAG GGA CTG AGC TGG ACA ACC AC 3'

PCR conditions include an initial denaturation step at 95℃ for 4 minutes, followed by 35 cycles of denaturation at 94℃ for 1 minute, annealing at 66.3℃ for 30 seconds, and extension at 72℃ for 2 minutes with a final extension of 8 minutes at 72℃. Initially the amplified DNA (10 μL) was electrophoresed in a 1.5% agarose gel containing 0.5 μg/mL ethidium bromide in Tris-borate/EDTA buffer. Bands were visualized by ultraviolet transillumination. However, since 572 bp and 528 bp could not be distinguished on 1.5% agarose gel, 6% acrylamide gel was used to distinguish these bands. PCR amplification revealed 528 bp product for S allele (homozygous) or 572 bp product for L allele (homozygous), and if both bands appeared then the individual was heterozygous with S/L genotype.

The gut motility (orocecal transit time [OCTT]) was measured by non-invasive lactulose breath test following the standard protocol described by Rana et al.[21]

Statistical Methods

Statistical analysis was performed by Statistical Package for Social Sciences version 16.0 (IBM Corp, Bengalore, India). Data were expressed as mean ± SEM and percentage. Parameters with continuous numbers were compared between cases and controls using independent *t* test and categorical values were compared using chi-square test. Odds ratio was calculated by logistic regression method. Comparison between different genotypes was analyzed by independent *t* test. *P*-values less than 0.05 was considered significant.

Table 1. Characteristics of Study Participants

Participants profile	T2DM (n = 300)	Control (n = 200)
Participant (male)	142 (47.3%)	96 (48.0%)
Age (average [range], yr)	54.6 (30-70)	55.4 (28-72)
Patients having diarrhea	43 (14.4%)	0 (0.0%)
Patients having constipation	43 (59.6%)	0 (0.0%)
Patients without any GI problem	78 (26.0%)	200 (100.0%)

GI, gastrointestinal; T2DM, type 2 diabetes mellitus.

Results

The current study included 300 T2DM patients and 200 age and sex matched healthy controls. Out of these 300 patients, 142 (47.3%) were male and 158 (52.7%) female. In controls, there were 96 (48.0%) males and 104 (52.0%) females. Demographic profile and biochemical parameters have been described in detail in our previous publications.[19,20] However, same salient features are presented here in Table 1. The mean age of diabetes patients was 54.6 ± 0.67 years and that of controls was 55.4 ± 0.74 years. Con-

Table 2. Genotype and Allele Frequencies of Study Participants

A. Genotype Frequencies of Serotonin Transporter Polymorphism in Patients With Type 2 Diabetes Mellitus and Controls

T2DM (n = 300)	Controls (n = 200)	OR (95% CI)	*P*-value
LL = 68 (22.7%)	LL = 40 (20.0%)		
LS = 111 (37.0%)	LS = 122 (61.0%)	0.54 (0.34-0.85)	< 0.05
SS = 121 (40.3%)	SS = 38 (19.0%)	1.87 (1.10-3.20)	< 0.05

T2DM, type 2 diabetes mellitus; L, long allele; S, short allele.

B. Allele Frequencies of Serotonin Transporter Polymorphism in Patients With Type 2 Diabetes Mellitus and Controls

T2DM (n = 600)	Controls (n = 400)	OR (95% CI)	*P*-value
L = 247 (41.2%)	L = 202 (50.5%)		
S = 353 (58.8%)	S = 198 (49.5%)	1.45 (1.13-1.88)	0.002

T2DM, type 2 diabetes mellitus; L, long allele; S, short allele.

sidering their residence in the same region and following similar cultural habits, it could be assumed that all participants were from same ethnic background.

Serotonin Transporter Gene SLC6A4 Polymorphism

SERT gene polymorphism cases and controls in this study followed the Hardy-Weinberg equilibrium. Genotype and allele frequencies of SERT gene polymorphism are presented in Table 2. Representative gel picture for SERT gene polymorphism on 6% acrylamide gel is shown in Figure 1.

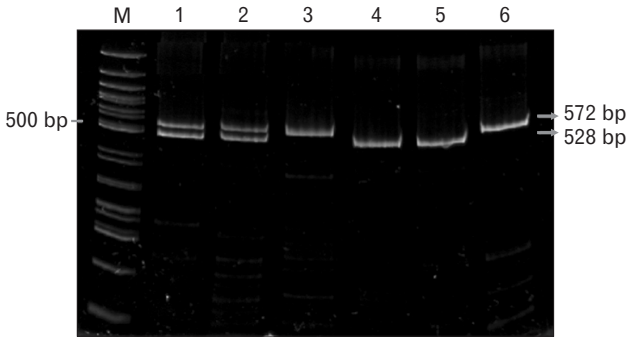

Figure 1. Serotonin transporter gene polymorphism by polymerase chain reaction represented on 6% acrylamide gel. M represents 50 bp marker. Lane 1 and 2: LS genotype (572 bp and 528 bp). Lane 3 and 6: LL genotype (572 bp). Lane 4 and 5: SS genotype (528 bp). L, long allele; S, short allele.

Gut Motility and Serotonin Transporter Gene Polymorphism in Patients With Type 2 Diabetes Mellitus and Controls

In the present study, the gut motility (OCTT) was measured by the non-invasive lactulose breath test. In patients with T2DM, OCTT was significantly faster in individuals with SS genotype (113.9 ± 4.4 minutes) as compared to LS (137.4 ± 4.3 minutes) and LL genotype (160.9 ± 7.1 minutes). Similarly, in healthy controls, OCTT was significantly shorter in SS genotype (93.4 ± 3.2 minutes) as compared to LS (99.1 ± 2.0 minutes) and LL genotype (110.2 ± 3.1 minutes) (Fig. 2).

Hypomotility and Hypermotility, and Serotonin Transporter Gene Polymorphism in Patients With Type 2 Diabetes Mellitus

Patients with T2DM were divided into 2 groups on the basis of their gut motility: (1) hypo motility: OCTT > 90 minutes and (2) hypermotility: OCTT ≤ 90 minutes. The gut motility of LL genotype was significantly prolonged (181.8 ± 6.1 minutes) compared to individuals with LS (154.7 ± 3.7 minutes) and SS genotype (128.1 ± 1.3 minutes). However, in the hypermotility group, there was no significant difference among the 3 genotypes (Fig. 3).

Gastrointestinal Symptoms, Gut Motility, and Serotonin Transporter Gene Polymorphism in Patients With Type 2 Diabetes Mellitus and Controls

Relations between GI symptoms, gut motility, and genotype of SERT polymorphism are presented in Table 3. It was observed

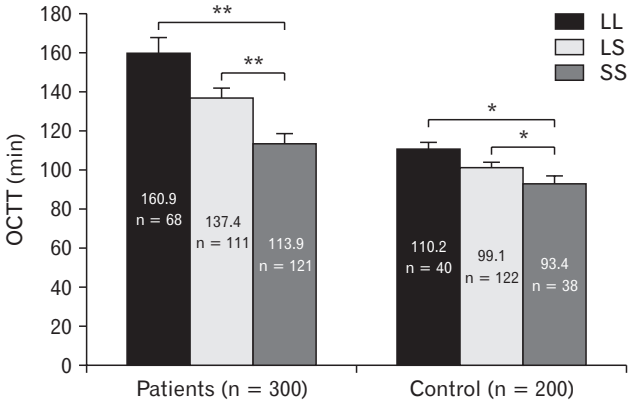

Figure 2. Gut motility (orocecal transit time [OCTT] in minutes) and serotonin transporter gene polymorphism in patients with type 2 diabetes mellitus and controls. Results are expressed as mean ± SEM. *$P < 0.05$, **$P < 0.001$. L, long allele; S, short allele.

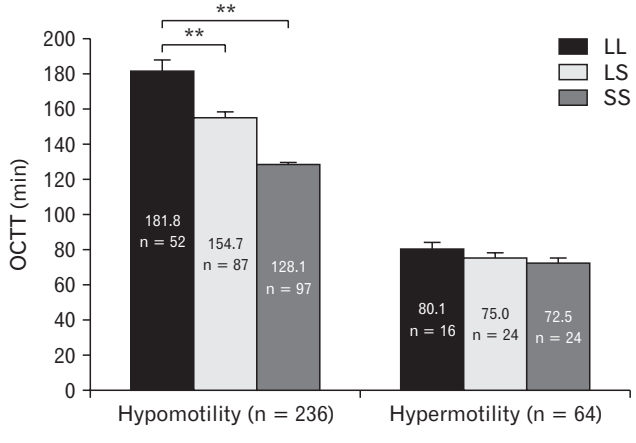

Figure 3. Hypomotility and hypermotility, and serotonin transporter gene polymorphism in type 2 diabetes mellitus patients. Results are expressed as mean ± SEM. **$P < 0.001$. OCTT, orocecal transit time; L, long allele; S, short allele.

Table 3. Gastrointestinal Symptoms, Gut Motility, and Serotonin Transporter Polymorphism in Type 2 Diabetes Mellitus Patients

GI symptoms Motility (min)	LL (n = 68)	LS (n = 111)	SS (n = 121)	*P*-value	
				LL vs SS	LS vs SS
Constipation (n = 179)	54 (79.4%)	86 (77.4%)	39 (32.2%)	< 0.001	< 0.001
OCTT	162.9 ± 5.1	157.3 ± 5.4	116.4 ± 5.4	< 0.001	< 0.001
Diarrhea (n = 43)	1 (1.5%)	0 (0.0%)	42 (34.8%)	< 0.001	< 0.001
OCTT	90.0 ± 0.0		75.9 ± 2.8	NS	
No GI symptom (n = 78)	13 (19.1%)	25 (22.6%)	40 (33.0%)	NS	NS
OCTT	121.7 ± 2.8	120.4 ± 4.3	114.9 ± 1.8	NS	NS

GI, gastrointestinal; L, long allele; S, short allele; OCTT, orocecal transit time, NS, not significant.
Values are expressed as n (%) or mean ± SEM.

that a greater number of diabetes patients (78.2%) with constipation belonged to LL and LS genotype. Moreover, OCTT was significantly delayed ($P < 0.001$) in diabetic patients with constipation (162.9 ± 5.1 minutes) who had LL genotype and LS genotype (157.3 ± 5.4 minutes) in comparison to patients with SS genotype (116.4 ± 5.4 minutes). On the other hand, a greater number of diabetic patients with diarrhea (97.7%) belonged to SS genotype and OCTT was faster (75.9 ± 2.8 minutes) compared to patients with LL genotype (90.0 ± 0.0 minutes). The patients without any GI symptoms did not show significant trend in relation to genotype with gut motility.

Serotonin Level and Serotonin Transporter Gene Polymorphism in Type 2 Diabetes Mellitus Patients and Control

Serotonin level was significantly higher in diabetic individuals (267.2 ± 2.2 ng/mL) as compared to controls (140.8 ± 2.1 ng/mL). Moreover, serotonin levels were found to be significantly higher ($P < 0.001$) in patients with SS genotype (240.9 ± 3.5 ng/mL) as compared to patients with LL genotype (141.4 ± 3.65 ng/mL) and LS genotype (165.3 ± 3.9 ng/mL). Moreover, serotonin levels were significantly higher in patients with diarrhea (230.5 ± 5.9 ng/mL) as compared to diabetic patients with constipation (116.4 ± 1.5 ng/mL), patients without GI symptoms (137.1 ± 2.5 ng/mL), and healthy controls (140.8 ± 2.1 ng/mL).

Discussion

Serotonin has long been known to play a role in GI neurotransmission. Serotonin mediates its effect by binding to a specific receptor.[22] The magnitude and duration of the biological actions exerted by serotonin depend on the SERT that mediates serotonin reuptake, recycling, and catabolic breakdown. Abnormalities in serotonin reuptake can alter enteric serotonergic signalling leading to gut dysfunction. Hence, any kind of polymorphism in SERT may lead to GI dysmotility. In the present study, frequency of S allele and SS genotype was significantly higher in patients with T2DM than in controls, and was associated with increased disease risk. Moreover, the frequency of LS genotype was higher in controls than in diabetic patients, and was associated with protection from the disease. These findings were in agreement with the study conducted by Iordanidou et al[15] who also documented a strong association between S allele and 5HTTLPR polymorphism in T2DM. In 2012, Wilhelm et al[23] documented an association of this genotype with psychological distress in patients with T2DM. Moreover, Dujic et al[24] reported that S allele of SERT gene was associated with increased GI intolerance to metformin. On the contrary, studies conducted on Pakistani and Mexican populations revealed no significant association between 5HTTLPR polymorphism and the development of T2DM.[25,26]

It was observed in the present study that gut motility was significantly faster in individuals with SS genotype compared to LS and LL genotype. Though SERT gene polymorphism has a role in both patients and controls, the prevalence of SS genotype is greater in T2DM patients as compared to controls giving rise to more phenotypic effect in patients compared to controls. Only in one study, the association of colonic transit was compared with the SERT gene polymorphism in IBS patients where colonic transit was measured using the scintigraphic method.[27] They also observed that there was more slowing of transit in long homozygous (LL genotype) than heterozygous (LS genotype) patients, but colon transit did not differ between short homozygous (SS genotype) and heterozygous patients.

The present study revealed that a greater number of T2DM patients with constipation had LL or LS genotype and significantly delayed OCTT. On the other hand, a greater number of T2DM

patients with diarrhea had rapid OCTT. Patients without any GI problems did not show any such trend in relation to genotype although OCTT had delaying trend in LL genotype. These findings could not be compared with previous studies since this is the first study to look into the association of SERT gene polymorphism with gut motility and the most common GI symptoms such as constipation and diarrhea altogether in T2DM patients. However, some studies have observed the association of SERT gene polymorphism with the presence of GI symptoms such as constipation and diarrhea in other diseases. Two studies[28,29] reported that SS and LS genotypes were risk factors for constipation- and diarrhea-predominant IBS, respectively. On the contrary Li et al[30] in 2007 documented that LL genotype was higher in constipation predominant IBS, and Li et al[31] in 2015 showed that SS genotype was a risk factor for constipation in cancer patients. These contradictory findings may be explained by difference in race, ethnicity, and difference in food habits as well as different mechanisms involved in different disease processes.

Due to the significance of serotonin as an intracellular signalling molecule in intrinsic and extrinsic gut reflexes, it was determined whether serotonin level was altered in T2DM patients as compared to healthy controls. The levels of serotonin were found to be increased in T2DM patients. Increase in serotonin levels has been documented earlier in patients with celiac disease[32] and in patients with IBS compared to controls.[33] On the contrary, the level of serotonin was found to be decreased in patients with functional dyspepsia[34] and in patients with active Crohn's disease.[35] On the other hand, Coates et al[36] did not find any change in serotonin levels in IBS patients.

In context with polymorphism, the levels of serotonin in the present study were higher in SS genotypes than in individuals with LL genotypes which was in accord with a previous study conducted by Kumar et al[37] on Indian populations with IBS. In contrast to this, studies conducted by Mohammadi et al,[38] Petrovic et al,[39] and Pivac et al[40] could not find any association between serotonin levels and SERT gene polymorphism in IBS patients, post-traumatic stress disorder patients, and healthy individuals, respectively. However, there is no study which compares the levels of serotonin with SERT gene polymorphism in T2DM patients.

In this study, the level of serotonin in patients with diarrhea was significantly higher as compared to diabetic patients with constipation, patients without GI symptoms, and healthy controls. However, no study is available to compare these findings in T2DM patients. Atkinson et al[41] documented similar findings in IBS patients. Interestingly, Costedio et al[42] and Sjölund et al[43] reported opposite findings where serotonin levels were higher in patients with constipation which may be explained by receptor desensitization.

Conclusions

In brief, the present study revealed that SS genotype was predominant in T2DM patients. Moreover, SS genotype was associated with faster gut motility, higher serotonin levels, and diarrhea. From this study it may be concluded that SS genotypes are prone to develop diarrhea because of faster gut motility resulting from higher serotonin levels. Therefore, this polymorphism study would help T2DM patients in identifying the genotype they belong to and thus help in developing appropriate life styles to avoid GI symptoms which is an important cause of poor quality of life in T2DM patients.

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Conflicts of interest: None.

Author contributions: Aastha Malik: performed tests, and collected and analyzed data; Sarama Saha: wrote manuscript and analyzed data; Rajesh K Morya: performed tests; and Sanjay K Bhadada and Satya V Rana: designed research, and collected and analyzed data.

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