International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

TCF7L2 rs12255372 (G/T) Emergence as a Prospective Gene for Type 2 Diabetes Mellitus Predisposition

Shubhra Chowdhry[1], Gyanendra Kumar Sonkar[2], Roshan Alam[1], Satyendra Kumar Sonkar[3], Mohd. Mustufa Khan[4], Saba Khan[1]*

[1]*Department of Biochemistry, Integral Institute of Medical Sciences & Research, Integral University, Lucknow, 226026, Uttar Pradesh, India*

[2]*Department of Biochemistry, King George's Medical University, Lucknow, 226003, Uttar Pradesh, India*

[3]*Department of Medicine, King George's Medical University, Lucknow, 226003, Uttar Pradesh, India*

[4]*Department of Basic Medical Sciences, Integral Institute of Medical Sciences & Research, Integral University, Lucknow, 226026, Uttar Pradesh, India.*

#Contributed equally

Abstract

There is a chance that Transcription Factor 7 like 2 (TCF7L2) variants could lead to type 2 diabetes mellitus (T2DM) but the results are debatable. In this study, we sought to ascertain the correlation between the TCF7L2 rs12255372 variation and the likelihood of T2DM in the North Indian cohort. For this study, 154 patients and 154 healthy controls between 25-75 years were enrolled. Fasting and postprandial blood sugar levels, glycated haemoglobin (HbA1c), lipid profile, liver function test (LFT) and kidney function test (KFT) were among the biochemical tests carried out. Commercially available kits were used to measure the levels of TCF7L2 in the blood. To genotype samples, PCR-RFLP was utilized. The overall mean of the biochemical profile showed that people with T2DM had considerably higher results than healthy controls ($p< 0.001$). When compared to healthy individuals, T2DM was shown to have significantly reduced levels of circulatory TCF7L2 ($p=0.045$). The genotypes GG and GT but also GG and TT were positively related with T2DM patients ($p=0.0389$ and $p=0.0305$, respectively). The prevalence of the "T" allele of rs12255372 (G/T) polymorphism was substantially greater in cases (30.51%) compared to controls (20.77%). The current investigation verified a considerable linkage between increased susceptibility of T2DM in the North Indian community and the TCF7L2 gene rs12255372 (G/T) polymorphism. It was discovered that the circulatory TCF7L2 levels in T2DM subjects are significantly impacted by the GT genotype of rs12255372.

Keywords: TCF7L2, T2DM, Polymorphism, Genotype

Full length article **Corresponding Author, E-mail: ksaba@iul.ac.in*

1. Introduction

T2DM affects more than 422 million individuals globally, and genetics can be somewhat to blame for this. Prevalence has been rising more swiftly in middle- and low-income countries compared to high-income nations. Renal illness, heart attacks, strokes, blindness, and lower limb amputation are all significantly exacerbated by diabetes. Between 2000 and 2019, the mortality rates for diabetics according to age rose by 3%. An estimated 2 million people died from diabetes and renal disease in 2019. A proper nutrition, regular exercise, preserving a healthy body weight, and quitting smoking are all ways to avoid or prolong the onset of T2DM. Diet, exercise, medication, and routine monitoring and management for complications can all help treat diabetes and delay or prevent its effects [1]. Since more than 20 years ago, it has been thought that finding the "suspect" genes will enable us to comprehend the basic aetiology of this prevalent as well as significant condition. Finally, common gene mutations are reliably linked to T2DM, and they are also translating this genetic data into fresh biological findings. The aetiology of T2DM is often polygenic, although recent research has revealed certain variations like PPARG Pro12Ala and KCNJ11 E23K that result in a monogenic form of diabetes [2]. In addition to candidate gene techniques, several hypothesis from genome-

wide analyses are carried out in T2DM cases to identify the prevalent variations that occur in the general population. In one among these studies, the Wnt pathway, also known as the APC/β-catenin/TCF pathway, was implicated that might behave as a crucial factor in the pathogenesis of T2DM [3]. The High mobility group box TCF7L2 that is produced by the TCF7L2 gene is a component of the β -catenin signalling cascade [4]. This transcription factor is regulated by β -catenin and aids in the transcription of genes such as PPAR-γ, Myc, Cyclin D1, etc. It may influence β-cell activity by altering the β-cell response to sugars or by altering the function or production of incretins [5].

The TCF7L2 gene contains numerous intronic single nucleotide polymorphisms (SNPs), many of which have been linked to various Indian, European, African, American, and Asian cohorts of the world. However, there is a significant variation in allele frequencies among these populations due to their ethnic heritage. In order to understand the importance of circulatory levels of TCF7L2 and other biochemical parameters in patients of North India with T2DM and controls, this study was set out to analyse the frequency distribution of the rs12255372 gene polymorphism of TCF7L2.

2. Materials and methods

Three hundred and eight unrelated individuals attending the outpatient department of IIMS&R, Integral University, Lucknow (India) and King George's Medical University, Lucknow (India), participated in this study. 154 healthy individuals who had not been clinically or lab-tested to have T2DM or any associated conditions made up the control group. 154 people who met the World Health Organization (WHO) criteria for T2DM (random blood glucose > 200 mg/dl and/or fasting plasma glucose > 126 mg/dl) made up the patient group. For every participant laboratory results comprising of HbA1c, fasting blood glucose, high-density lipoprotein cholesterol, total cholesterol, triglyceride levels, low-density lipoprotein cholesterol, LFT, and KFT were analysed and the level of obesity as determined by body mass index (BMI; defined as weight (kg)/height (m^2) was also evaluated. Individuals in the patient and control groups signed informed permission forms before participating in the study, and the Institutional Ethics Committee authorized the methods.

2.1.1 DNA isolation and Genotyping

To extract DNA from whole-blood samples, Qiagen's genomic DNA extraction kit was utilized. Using Nanodrop 2000 (Thermoscientific) spectrophotometer, the DNA concentration was assessed upon isolation. Purity of extracted DNA was verified by agarose gel electrophoresis which was carried out using 10 µg/ml ethidium bromide and viewed using the Gel Documentation system (Bio-Rad, Gel Doc XR+, Universal Hood II). After performing a polymerase chain reaction (PCR) for genotyping, restriction endonucleases were used for enzymatic digestion (RFLP). The following primers were used to genotype the rs12255372 polymorphism:

Forward: 5′- GGCTCCTATCTCTCCTCAATCT -3′,

Reverse: 5′ - GCCTAAGTAATGGGTCGATGTT -3′

Gradient PCR was used for setting the annealing temperature (Tm). The best band was achieved at 54º C. A final reaction volume of 20 µl was made for the Polymerase Chain Reaction (PCR), which contained 100 ng of genomic DNA and 5 pmol of each primer, and a premixed PCR master mix consisting of a reaction buffer made up of Tris Hcl - 10mM at pH 8.3, KCl - 50mM, MgCl2 - 2mM a catalyst, dNTPs in a concentration of 200µM each, Taq polymerase in a concentration of 1.25 U and to make up the volume 4µl of nuclease free water was added. A 5 min initial denaturation at 95°C was followed by 34 cycles of 30 s of denaturation at 95°C, 30 s of annealing at 54°C, 30 s of extension at 72°C, and a final extension step of 9 min at 72°C comprised the PCR reaction. On a 2% agarose gel with 10 µg/ml ethidium bromide, PCR products were confirmed and then exposed to UV light for visualisation. After PCR, the results were treated with Thermo Scientific's RsaI restriction endonuclease at 37°C for an overnight digestion. A 2.5% agarose gel was used to separate the restriction fragments of the PCR products. Each run incorporated a 50 bp DNA ladder.

2.1.2 Quantification of serum TCF7L2 levels

TCF7L2 serum levels were measured using an ELISA kit that is widely accessible in the market (Krishgen Biosystems). The test was carried out in duplicates in accordance with the manufacturer's methodology. TCF7L2 had a CV of <18% between assays and a CV of <15% within assays. Human TCF7L2 typically has a minimum detectable dosage (sensitivity) of less than 1.0 ng/ml; the normal calibration spectrum was 1.5 ng/ml - 24 ng/ml, and its sensitivity).

2.1.3 Estimation of Biochemical parameters

Laboratory investigations included fasting blood sugar, post parandial blood sugar, HbA1c, LFT, KFT, lipid profile. These were estimated by fully automatic autoanalyzer.

2.2 Statistical analysis

Version 20.0 of SPSS was used for all of the statistical analysis (Armonk, NY, USA). ANOVA or an unpaired t-test were used to compare all of the phenotypic data. Values were presented as mean ± SD (Standard Deviation). The genotypic and allelic patterns were examined by ANOVA test along with 95% confidence intervals (CI). Hardy-Weinberg equilibrium was assessed for TCF7L2 rs12255372 genotypes. p -values of 0.05 or below were considered statistically significant for all the data investigated.

3. Results and Discussions

3.1 Clinical traits of T2DM patients and controls

This case control study included 308 participants in total (154 T2DM and 154 healthy controls). Table 1 provides the biochemical traits of both patients and controls. Average values for anthropometric variables, such as with respect to SBP, DBP, and BMI, T2DM cases had substantially higher values at (p=0.0001*), (p=0.0488*), and (p=0.0001*),

respectively. Similar to this, T2DM sufferers had significantly higher levels of biochemical variables as blood sugar, HbA1c, total cholesterol, triglycerides, HDL, S.ALT, S.AST, and potassium than healthy controls ($p<0.001$). In comparison to healthy controls, there was a substantial increase in LDL and BUN levels in T2DM cases ($p=0.0075^*$ and $p=0.0032^*$, respectively). When compared to healthy controls, the levels of circulatory TCF7L2 were observed to be considerably lower in people with T2DM ($p=0.0454^*$).

3.2 Genotype and Allele Frequencies

Fig. 1 shows TCF7L2 polymorphism at rs12255372 which was discovered by using the RsaI restriction enzyme to break down the 634 bp PCR-amplified product, which was then run through a 2.5% agarose gel electrophoresis. The GG homozygote wild type had a fragment of 634 bp (absence of RsaI restriction site). The three 634bp, 358bp, and 276bp segments were present in the GT heterozygous. For the TT mutant homozygote, fragments of 358bp and 276 bp were found. Analysis was done using a 50bp DNA ladder. The genotypic distribution complied with Hardy-Weinberg equilibrium in both the cases and control groups. Table 2 displays the genotypic and allelic patterns of the TCF7L2 gene polymorphism at position rs12255372 in T2DM patients and healthy controls. In comparison to controls (20.77%), diabetic participants (30.51%) had a substantially greater frequency of the "T" allele of rs12255372 (G/T), with an allelic odds ratio of 0.59 (95% confidence interval [CI], 0.41 - 0.86; $p = 0.0059^*$). The percentage of the GG, GT and TT genotypes of rs12255372 were 48.05%, 42.85%, 9.09% in T2DM cases and 62.33%, 33.76%, 3.89% in healthy controls respectively. The allele frequencies of the G and T were 69.48% and 30.51% in T2DM and 79.22% and 20.77% in healthy controls respectively. Significant correlations were found between T2DM patients and the homozygous GG and heterozygous GT genotypes (OR: 0.60; CI: 0.37 – 0.97; $p= 0.0389^*$). Also, significant association was seen between homozygous GG and heterozygous TT genotype with T2DM patients (OR: 0.33; CI: 0.12 - 0.90; $p= 0.0305^*$). When compared to healthy individuals, the frequencies of the G and T alleles of the rs12255372 demonstrated a significant correlation with T2DM patients (OR: 0.59; CI: 0.41 - 0.86; $p= 0.0059^*$). Additionally, the dominant genotype (GG vs. GT+ TT) was examined, and it was discovered that T2DM cases and healthy controls differed significantly (OR: 0.55; CI: 0.35 - 0.88; $p= 0.0120^*$). However, there was no discernible difference between T2DM cases and healthy controls for the recessive genotype (GG+GT vs. TT) (OR: 0.40; CI: 0.15 - 1.08; p= 0.0721).

3.3 Influence of Different Genotypes on Clinical Features

Based on the genotypes for rs12255372(G/T), Table 3 compares the clinical and biochemical parameters of the T2DM participants. T2DM patients with the TT genotype of the rs12255372 (G/T) polymorphism exhibited greater fasting blood sugar levels (mean ± SD, 190.14 ± 45.98 mg/dl) than the GG carriers (mean ± SD, 190.14 45.98 mg/dl; p = 0.5918) but statistical significance was not attained. Similarly, the 2 hr, post parandial sugar, HbA1c, Total cholesterol, triglycerides, LDL, HDL, serum bilirubin, BUN and potassium were also higher in T2DM cases carrying TT genotype but were statistically insignificant. Serum creatinine levels were higher and statistically significant in T2DM cases carrying TT genotype (mean ± SD, 0.95 ± 0.17 mg/dl; $p = 0.0408^*$). However, serum ALT and serum AST levels were lower in T2DM patients with TT genotype. None of the further biochemical measurements, such as serum ALP and sodium, revealed any discernible variations among the three genotypes in T2DM participants. The rs12255372 polymorphism did, however, significantly affect the level of TCF7L2 in the blood ($p=0.0451^*$). In comparison to the GG and TT genotypes, the serum TCF7L2 level was considerably higher in the GT genotype (mean ± SD, 2.81 ± 1.33 ng/dl). Men were shown to have a larger percentage of TT genotypes (5.19%) than women (0.64%) in our study of the SNP rs12255372 of TCF7L2 gene but statistical significance was not seen (Table 4). The occurrence of T allele was also higher in males (21.84%) as compared to females (19.60%). However statistical significance was not achieved. T2DM is one of the top 10 causes of morbidity and mortality. With a rising prevalence, T2DM and its consequences are regarded as one of the dangerous health concerns of the 21st century [6]. A new line of inquiry into diabetes was opened by Grant et al. from an Icelandic case-control research suggesting that hereditary factors may considerably contribute to the development of T2DM [7]. The probability of occurrence of T2DM in the Russian and Iranian populations was found to be significantly correlated with the TCF7L2 rs12255372 gene polymorphism [8-9]. Furthermore, through numerous genome-wide association analyses, these findings were validated in various ethnic groups. It is yet unknown how the TCF7L2 polymorphism contributes to the development of T2DM. The intronic regions are where all of the TCF7L2 SNPs that have been found so far are situated. It is crucial to elucidate how the expression of TCF7L2 gene is affected by intronic changes. Accordingly, Srinivasan et al. discovered that in rs12255372 the activity of TCF7L2 mRNA in human pancreatic islets was considerably higher in T allele carriers, and this was linked to decreased insulin secretion and increased hepatic glucose generation rates. [10]. Additionally, it has a secondary function via altering GLP-1 levels because TCF7L2 controls the transcription of its gene. In the current investigation, we looked into any potential relationships between the TCF7L2 gene (rs12255372 SNP) and circulatory concentrations of TCF7L2 and other clinical indicators in people with T2DM in North India. Data from the current investigation showed that, compared to diabetic patients, healthy controls had higher occurrence of the (GG) wild genotype as well as the (G) allele. When compared to healthy controls, diabetic patients had elevated rates of the (T) allele, (GT), and (TT) genotypes. Diabetes patients had higher rates of the (T) allele, heterozygous genotypes (GT), and homozygous genotypes (TT) compared to healthy controls. Additionally, there was a substantial variation in genotypic trend and allelic prevalence between the two study groups. These results are in accordance with study in Moroccan population (11) in which a significant association was found between TCF7L2 rs12255372 gene polymorphism and T2DM [11]. A research on a Kurdish population that demonstrated a statistically substantial correlation between the presence of the T allele and T2DM provided additional support for our findings [12].

Table 1: Clinical and Biochemical characteristics of Case (T2DM) and Control Groups

Parameters	Case (n=154)	Control(n=154)	p-value
AGE (years)	52.00 ± 9.97	53.32 ± 4.06	0.27
BMI ((kg/m^2))	28.52 ± 3.19	21.79 ± 1.89	< 0.0001*
SBP (mmHg)	132.05 ± 8.45	120.23 ± 8.91	< 0.0001*
DBP (mmHg)	80.03 ± 7.30	78.06 ± 6.23	0.0488*
FBS (mg/dl)	182.19 ± 31.08	80.02 ± 6.17	< 0.0001*
PPBS (mg/dl)	249.97 ± 35.21	112.54 ± 10.70	< 0.0001*
HbA1c (%)	7.65 ± 0.97	4.89 ± 1.23	< 0.0001*
Total Cholesterol (mg/dl)	135.07 ± 25.98	107.68 ± 11.27	< 0.0001*
Triglyceride (mg/dl)	121.56 ± 23.97	90.50 ± 11.69	< 0.0001*
HDL(mg/dl)	37.46 ± 9.01	46.76 ± 5.81	< 0.0001*
LDL (mg/dl)	81.11 ± 10.42	77.31 ± 8.69	0.0075*
Serum Creatinine (mg/dl)	0.83 ± 0.18	0.82 ± 0.20	0.73
BUN (mg/dl)	13.93 ± 3.07	12.70 ± 2.49	0.0032*
S. Bilirubin (mg/dl)	0.70 ± 0.255	0.66 ± 0.25	0.27
S.ALT (mg/dl)	32.10 ± 10.22	23.16 ± 6.43	< 0.0001*
S.AST (mg/dl)	30.83 ± 10.64	20.97 ± 6.12	< 0.0001*
S.ALP (mg/dl)	71.05 ± 25.56	68.78 ± 16.90	0.48
K^+ (mEq/L)	4.14 ± 0.43	3.78 ± 0.16	< 0.0001*
Na^+ (mEq/L)	137.77 ± 2.35	137.52 ± 2.10	0.42
TCF7L2 (ng/ml)	2.3 ± 0.91	4.18 ± 1.85	0.0454*

Values are expressed as Mean ± Standard Deviation; *Significant considered as P<0.05.
FBS: Fasting Blood Sugar, PPBS: Post-Prandial Blood Sugar, HbA1c: Glycated Haemoglobin, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, BMI: Body Mass Index, TC: Total Cholesterol, TG: Triglyceride, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein

Table 2: TCF7L2 rs12255372 Gene Polymorphism in T2DM Cases and Control Groups Based On Genotypes and Allele Distribution

rs12255372 Polymorphism	Case n (%)	Controls n (%)	OR (95% CI)	p-value
Co dominant				
GG	74 (48.05)	96 (62.33)	1.00	-
GT	66 (42.85)	52 (33.76)	0.60 (0.37 – 0.97)	0.0389*
TT	14 (9.09)	6 (3.89)	0.33 (0.12 - 0.90)	0.0305*
Dominant				
GG	74 (48.05)	96 (62.33)	1.00	-
GT + TT	80 (51.95)	58 (37.66)	0.55 (0.35 - 0.88)	0.0120*
Recessive				
GG + GT	140 (90.90)	148 (96.10)	1.00	-
TT	14 (9.09)	6 (3.89)	0.40 (0.15 - 1.08)	0.0721
Alleles				
G	214 (69.48)	244 (79.22)	1.00	-
T	94 (30.51)	64 (20.77)	0.59 (0.41 - 0.86)	0.0059*

Number (n) and Percentage (%)

OR: Odd Ratio; CI: Confidence Interval; Significant considered as p<0.05.

Table 3: Clinical characteristics of T2DM cases based on rs12255372 genotypes of the TCF7L2 gene

GENOTYPES → VARIABLES ↓	GG (n=74)	GT (n=66)	TT (n=14)	p- VALUE
FBS (mg/dl)	180.60 ± 31.92	182.28 ± 28.21	190.14 ± 45.98	0.5918
PPBS (mg/dl)	249.56 ± 34.25	248.5 ± 32.63	259.07 ± 50.78	0.5917
HbA1c (%)	7.71± 0.58	7.50 ± 1.07	8.04 ± 1.81	0.1512
TC (mg/dl)	134.08 ± 24.06	133.09 ± 26.34	135.5 ± 34.85	0.9002
TG (mg/dl)	120.43 ± 22.85	120.54 ± 23.14	132.35 ± 31.85	0.2109
LDL (mg/dl)	81.12 ± 9.80	80.06 ± 11.24	86 ± 8.66	0.1537
HDL (mg/dl)	38.56 ± 9.49	37.28 ± 9.13	39.11 ± 8.80	0.1633
ALT (U/L)	32.66 ± 10.99	32.55 ± 9.45	27.07 ± 8.59	0.1543
AST (U/L)	30.86 ± 11.72	31.04 ± 9.62	29.64 ± 9.90	0.9051
ALP (U/L)	65.81 ± 19.81	75.96 ± 29.43	75.57 ± 29.26	0.0693
S.BILIRUBIN (mg/dl)	0.70 ± 0.27	0.69 ± 0.23	0.74 ± 0.23	0.7999
BUN (mg/dl)	13.46 ± 2.91	14.15 ± 3.06	15.34 ± 3.55	0.0802
S. CREATININE (mg/dl)	0.83 ± 0.17	0.81 ± 0.18	0.95 ± 0.17	0.0408*
Na+ (mEq/L)	137.59 ± 2.69	137.96 ± 1.93	137.85 ± 2.24	0.6436
K+ ((mEq/L)	4.09 ± 0.47	4.185 ± 0.35	4.19 ± 0.50	0.4562
TCF7L2 (ng/ml)	1.34 ± 1.41	2.81 ± 1.33	1.23 ± 0.61	0.0451*

Values are expressed as Mean ± Standard Deviation; *Significant considered as p<0.05.

Table 4: Genotype and Allele frequency distribution of SNP rs12255372 of TCF7L2 gene in Genders of T2DM cases

rs12255372 Polymorphism	**Males N (%) (M)**	**Females N (%) (F)**	**OR (95% CI)**	**p-value**
Co dominant				
GG	66 (42.85)	32 (20.77)	1.00	-
GT	29 (18.83)	18 (11.6)	1.28 (0.62 – 2.64)	0.5037
TT	8 (5.19)	1 (0.64)	0.25 (0.03 – 2.15)	0.2104
Dominant				
GG	66 (42.85)	32 (20.77)	1.00	-
GT + TT	37 (35.9)	19 (18.44)	1.05 (0.52- 2.12)	0.8715
Recessive				
GG + GT	95 (92.2)	50 (48.54)	1.00	-
TT	8 (5.19)	1 (0.64)	0.23 (0.02 – 1.95)	0.1811
Alleles				
G	161 (78.15)	82 (80.39)	1.00	-
T	45 (21.84)	20 (19.60)	0.87 (0.48 – 1.57)	0.6509

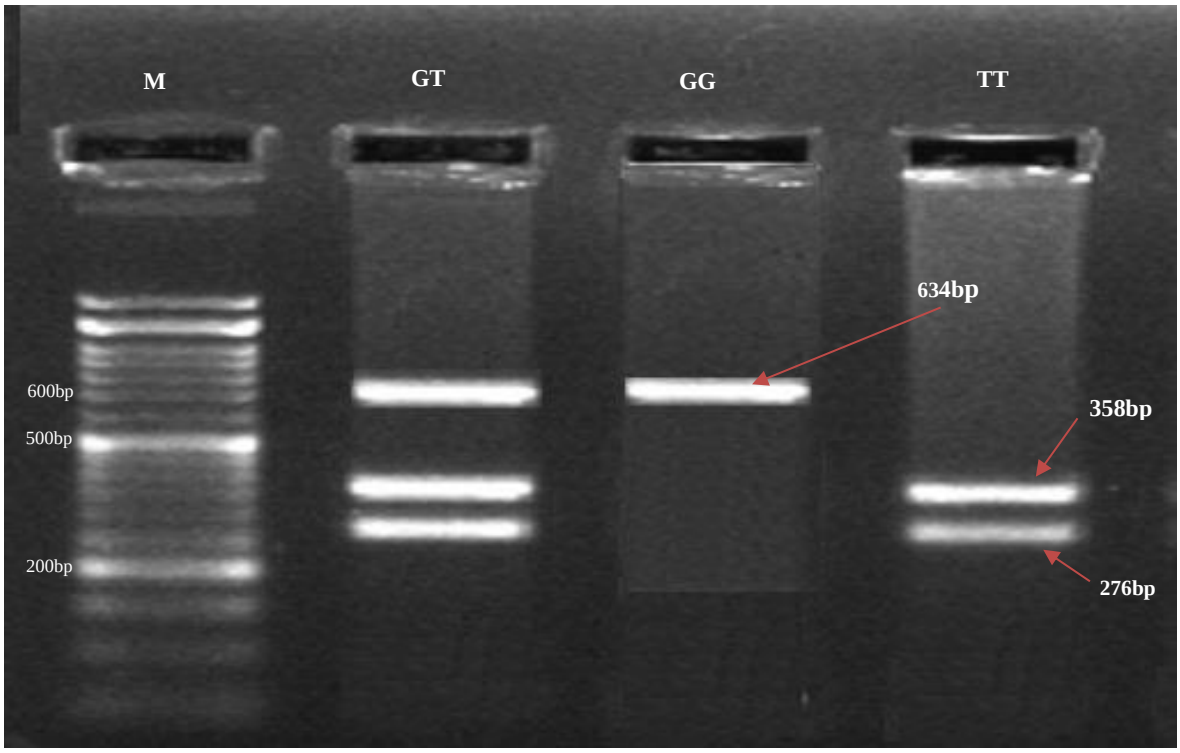

Fig. 1: TCF7L2 gene rs12255372 genotyping outcome.
M: Marker, GT genotype: 634/358/276bp, GG genotype: 634bp, and TT genotype:358/276bp.

TCF7L2 gene rs12255372 G>T polymorphism and the emergence of T2DM were found to be statistically significantly associated in the Egyptian population [13]. Both Hassan et al. and Zhou et al. reached the precise result that there is a substantial correlation in between the TCF7L2 rs12255372 G>T polymorphism and T2DM predisposition [14-15]. The TCF7L2 rs12255372 G>T and T2DM had a favourable relationship, among people from various cultural backgrounds [16]. An additional support for this came from a meta-analysis by Xia and Ma, who discovered a marked decline in heterogeneities in both Caucasians and Asians, indicating that there may be a racial component to the genetic relationship between the TCF7L2 gene and T2DM. [17]. However, additional investigations that were duplicated across different racial and ethnic groups revealed a meager connection between TCF7L2 rs12255372 gene polymorphism and T2DM. These studies included those carried out in Iran [18] and in the United States [19]. Similar investigations carried out in the Turkish community by Vural [20] and in the Balinese people by Saraswati et al. [21] also produced the same results. Additional evidence supporting these conclusions came from a study conducted among the Egyptian population, where the G allele represented 48.3% of controls and 60.8% of diabetic patients, whereas the T allele represented 51.7% of healthy controls and 39.2% of diabetic patients and did not appear to be related with T2DM. [22]. According to Palizban et al. the TCF7L2 protein is essential for the Wnt signalling pathway because it controls adipocyte differentiation and adipogenesis. As a result, impaired Wnt signalling is linked to a slight elevation in TCF7L2 activity in adipose tissue, which causes unfavourable dyslipidemia [23]. Our study, which demonstrated a fairly notable elevation in blood total cholesterol, triglycerides, and LDL-C in TT genotype contrasted to GT and GG genotypes as well as in T allele versus G allele, supports this. The TCF7L2 gene may be a significant locus for the genetic vulnerability to T2DM, as per the consistency of relationship demonstrated by numerous replication studies, although the biological basis for this link is yet unknown. Furthermore, the analysed genetic variations are found in the introns rather than the coding areas. However, this might still have an impact on how proteins behave and/or how alternatively spliced variants are expressed. A potential limitation of our study is the comparatively small number of cases and control subjects, as a result, estimations of the connection have rather high confidence risks, especially for homozygous subjects.

4. Conclusions

By our study we can conclude that, in the studied North Indian population TCF7L2 was associated with T2DM, and the T allele and TT genotype of rs12255372 (G/T) were both associated with a significant probability of developing the disease. Considerable correlations were found between the GG and TT as well as GG and GT genotypes.

Additionally, when compared to healthy individuals, the T allele frequencies of rs12255372 are considerably higher in T2DM cases also it was found that the GT genotype of rs12255372 significantly affects the levels of TCF7L2 in the blood.

Acknowledgement

We are thankful to all those without whom the completion of this research work would have been a difficult task to complete. We are thankful to the Biochemistry and Medicine department of K.G.M.U. and IIMS&R, Lucknow for their help and support. The manuscript number allotted by the university is IU/R&D/2023-MCN0001828.

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