Association of Variant rs7903146(c/t) Single Nucleotide Polymorphism of Transcription Factor 7-like 2 Gene with Newly Detected Hyperglycemia in Pregnancy

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Abstract

Background: Transcription factor 7-like 2 (*TCF7L2*) gene has a significant role in hyperglycemia in pregnancy (HIP) risk. The current study was planned with the aim to evaluate the association of single nucleotide polymorphism (SNP) rs7903146 in patients of newly detected HIP among Indian population of northern region. **Methods:** This study was an observational case control study done among newly detected HIP (The World Health Organization (WHO) criteria, 2013) and healthy pregnant females without diabetes. Participants from both the group were genotyped for rs7903146 (C/T) variant of *TCF7L2* gene using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique. **Results:** A total of 71 cases of newly detected HIP were included in the study, out of which 25 (35.2%) of them were of first-time detected diabetes mellitus in pregnancy (DIP) and 46 (64.7%) were of gestational diabetes (GDM) and 100 were pregnant females without diabetes in third trimester were enrolled as controls. Average age of participants in the case group was 28.7 ± 4.0 years and the control group were 26.5 ± 3.6 years (*P* value 0.09). The wild homozygous CC genotype, heterozygous CT genotype and homozygous TT genotype were present in 39.4%, 53.5%, 7.1% of case group vs 53%, 43% and 4% of control group, respectively. No significant association of rs7903146(C/T) SNP of TCF7L2 gene in HIP (CC/CT, CC/TT *P* value 0.15, 0.38, respectively) in our population was found. There was no significant difference in the distribution of genotypes between DIP and GDM. **Conclusion:** This study shows no evidence of association of rs7903146(C/T) SNP of *TCF7L2* gene with newly detected HIP in our population.

Keywords: Gestational diabetes mellitus, hyperglycemia, pregnancy

INTRODUCTION

Hyperglycemia, that is, first detected during pregnancy, is classified as either GDM or diabetes mellitus in pregnancy (DIP).[1] Diabetes mellitus (DM) in pregnancy differs from GDM in the fact that the hyperglycemia is more severe and does not resolve after pregnancy as it does with GDM. According to International Diabetes Federation (IDF), 15.8% (20.4 million) of live births were affected with hyperglycemia in pregnancy (HIP), in 2019, across the world.[2] The majority (86.8%) of these cases were seen in low- and middle-income countries, where access to antenatal care is limited. It is projected that by 2030, this figure will reach 18.0 million marks.[2] Nearly 83.6% of the total HIP cases were due to GDM, 7.9% were due to diabetes detected prior to pregnancy and 8.5% were due to diabetes first detected in pregnancy.[2] The prevalence of GDM varies from 9.8% to 25.5% throughout the world as the difference in diagnostic criteria used, ethnicity, body mass index (BMI), screening policy was observed. South East Asia region has the highest prevalence of HIP at 27% against 11% in North America and Caribbean,[3,4] 7.5% in the Middle East and North Africa (MENA) region.[2,4]

Hyperglycemia is detrimental to both maternal and fetal health, and this effect is observed even with mildly elevated

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glucose.[4] The adverse effects can be short-term (preterm delivery, macrosomia, neonatal hypoglycemia, shoulder dystocia, birth injury, hypocalcemia, hyperbilirubinemia, need for neonatal intensive care unit (ICU), preeclampsia)[5,6] or long-term (eightfold more risk of developing diabetes or prediabetes in children born to women with GDM in adulthood).[7] Women with GDM have seven times more risk of developing type 2 DM (T2DM) in the future.[8,9] It can be of polygenic inheritance similar to T2DM. Insulin resistance and insulin insufficiency, which are characteristic features of GDM, are partly shown as hereditary in a study in twins.[10]

Among the various genes studied so far in relation to GDM, transcription factor 7-like 2 (*TCF7L2*) gene related to insulin secretion has been widely studied among various ethnic groups. *TCF7L2* gene belongs to the high-mobility group-box family and has a transcription factor role in the wingless-related integration (Wnt) signaling pathway.[11] The *TCF7L2* protein has been found to be important for blood glucose homeostasis.[12] TCF7L2 has multiple single nucleotide polymorphisms (SNPs), eight of which are rs790314611, rs1225537216, rs790169518, rs29048728, rs1119620528, rs1119621832, rs1224332625, and rs4506565, respectively. The SNPs rs7903146 C/T, rs12255372 G/T and rs7901695 T/C are strongly related to GDM risk.[13] The SNP rs7903146 has been most widely researched and significant correlation between T allele of SNP rs7903146 of *TCF7L2* and HIP risk has been found. A few studies on this have been done so far in India and current the study was planned with the aim to evaluate the association of SNP rs7903146 in patients of newly detected HIP among Indian population of northern region.

MATERIAL AND METHODS

This study was an observational case control study done from January 2019 to August 2020 in the Department of Endocrinology and Metabolism, Institute of Medical Sciences and SS Hospital and Department of Molecular and Human Genetics, Banaras Hindu University, Varanasi (Uttar Pradesh [UP]). Participants included in the case group were patients of newly detected HIP (The World Health Organization [WHO] criteria, 2013)[1] and in control group were healthy pregnant females without diabetes in their third trimester. Those who refused to give consent were excluded. The sample size was calculated for matched case control study with ratio of cases to controls 1:1 using formula for difference in proportion. The power of the study was 80%. However, due to SARS-CoV-2 pandemic equal number of cases and control could not be enrolled. As per antenatal care program at our center, all pregnant women in both cases and control underwent 75 g oral glucose tolerance test (OGTT) in second trimester after 20 weeks of gestation for GDM screening. All those who were at high risk for GDM underwent early OGTT in the first trimester or repeated assessment in the third trimester. A detailed clinical history was taken from the patients regarding the risk factors for DIP, symptoms of diabetes, poor fetal outcomes in previous pregnancies. Clinical examination was

done on all the participants and relevant biochemical tests were also done. Participants from both the group were genotyped for rs7903146 (C/T) variant of the *TCF7L2* gene in DNA obtained from the peripheral blood using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique. PCR amplification of rs7903146 (C/T) variant of *TCF7L2* gene mutation region was done using previously described forward primer (5′-GAG AGC TAA GCA CTT TTT AGG TA-3′) and a reverse primer (5′-CTG ACA TTG ACT AAG TTA CTT GC-3′). The PCR reaction was set in a total reaction volume of 20 μL containing 50 ng genomic DNA, 5 pmol each primer (Sigma-Aldrich, St Louis, MO), 10 μL 2X DreamTaq PCR Master Mix (Fermentas, Hanover, MD) containing DreamTaq DNA Polymerase, 2X DreamTaq buffer, deoxynucleotide triphosphate (dNTPs) and 4 mmol/L MgCl2. PCR thermal cycling was done on Applied Biosystems Veriti 96-well Thermal Cycler. Initial denaturation was done at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, and annealing at 58°C for 40 s, and extension at 72°C for 40 s. This was followed by a final extension step of 10 min at 72°C. The 113 bp fragment generated was restriction digested with two units of RsaI (Fermentas) at 37°C for 8 h. The digested products were then separated on 3% agarose gel along with 100 bp DNA ladder (Fermentas). In the presence of C/T transition, the 113 bp fragment was not cleaved, while in the presence of C allele it generated two sub fragments of 91 and 22 bp [Figure 1]. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25 software (SPSS Inc, Chicago, USA). Chi-square test and independent sample *t*-test were used as the test of significance. A *P* value ≤ 0.05 was considered as statistically significant. Binary logistic regression analysis was used to assess and determine the strength of association between the patients of newly detected HIP and the underlying risk factors.

RESULTS

A total of 71 cases of newly detected HIP were included in the study, out of which 25 (35.2%) were of first-time detected DIP, 46 (64.7%) were of GDM and 100 were age-matched pregnant females without diabetes in third trimester enrolled as controls. Demographic profile and baseline parameters
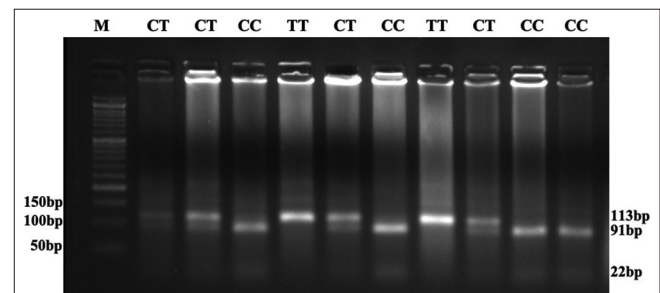
Figure 1: Representative gel picture showing different genotypes of TCF7L2 gene polymorphism. Lane 1 is 50 bp DNA ladder; Lane 2, 3, 6, and 9 showing CT genotype; Lane 4, 7, 10, and 11 showing CC genotype; and Lane 5 and 8 showing TT genotype

of the patient have been tabulated in Table 1. Average gravidity among the case group was 2.9 ± 1.3 pregnancies and control group was 2.3 ± 1.4 pregnancies. Maximum number of patients of HIP were detected in the second trimester (47.8%), followed by the third trimester (29.5%) and the first trimester (22.5%). Distribution of risk factors for HIP has been tabulated in Table 2. We found that previous history of GDM (*P* = 0.032) and family history of GDM (*P* = 0.01) was significantly more in newly detected DIP than GDM group. A total of 29.6% participants in the case group and 16% in the control group patients had other medical disorders in addition to HIP [Table 3]. In the present study, we assessed rs7903146(C/T) SNP of *TCF7L2* gene in patients of hyperglycaemia first detected in pregnancy using PCR-RFLP. Wild homozygous CC genotype, heterozygous CT genotype and homozygous TT genotype were present in 39.4%, 53.5%, 7.1% of the case group versus 53%, 43% and 4% of the control group, respectively [Table 4]. The statistical analysis showed no significant association of rs7903146(C/T) SNP of *TCF7L2* gene in HIP (CC/CT, CC/TT *P* value 0.15, 0.38, respectively) in our population. There was also no significant difference in distribution of genotypes among DIP and GDM in our population.

Discussion

The present study was planned to assess the clinical spectrum and the association of rs7903146 *TCF7L2* in our population of newly detected HIP in eastern UP. Among 71 cases, 64.7% were of GDM and 35.2% were of newly detected diabetes in pregnancy which is higher than other studies. In a study from Brazil, 94.7% were GDM and only 5.3% were of newly detected DIP cases.[14] Categorizing first-time detected hyperglycaemia patients into these two groups is important as approach to management differs. DIP patients require more aggressive therapy and monitoring and post-delivery also they require closer follow-up for residual diabetes.[1,5,15] None of the patients were symptomatic for hyperglycaemia. As expected, prevalence of conventional risk factors for hyperglycaemia were significantly more in case group as compared to control group. We also observed that all cases of HbsAg carrier state belonged to GDM group. Peng *et al.*[16] found that maternal HBsAg carrier (OR 1.47, 95% confidence interval [CI] 1.06–

Table 1: Demographic profile, baseline clinical and biochemical parameters of participant				
Parameter	Newly detected DIM (*n*=25)	GDM (*n*=46)	Total cases (HIP) (*n*=71)	Control (*n*=100)	*P* value (between case and control group)
Age group (years)					
18-20	–	–	0 (0.0%)	6 (6.0%)	
21-30	19 (76.0%)	33 (71.7%)	52 (73.2%)	78 (78.0%)	
31-40	6 (24.0%)	13 (28.3%)	19 (26.8%)	16 (16.0%)	
Education					
Illiterate	0 (0.0%)	2 (4.3%)	2 (2.8%)	0 (0.0%)	0.009
Primary	3 (12.0%)	0 (0.0%)	3 (4.2%)	20 (20%)	
Middle to higher secondary	10 (40.0%)	10 (21.7%)	20 (28.2%)	37 (37.0%)	
Graduate and above	12 (48.0%)	34 (74.7%)	46 (64.8%)	43 (43.0%)	
Gravidity					
Primi	4 (16.0%)	9 (19.6%)	13 (18.3%)	37 (37.0%)	0.008
Multi	21 (84.0%)	37 (80.4%)	58 (81.7%)	63 (63.0%)	
Average POG (weeks)	18.7±7.9	23.1±10.0	21.5±9.5	36.1±1.9	0.000
First trimester	8 (32%)	8 (17.4%)	16 (22.5%)	0	
Second trimester	12 (48%)	22 (47.8%)	34 (47.8%)	0	
Third trimester	5 ((20%)	16 (34.7%)	21 (29.5%)	100 (100%)	
Acanthosis nigricans	14 (56.0%)	22 (47.8%)	36 (50.7%)	15 (15.0%)	0.001
Systolic BP Mean±SD (mm Hg)	122.40±13.4	119.2±14.9	120.4±14.4	112.5±11.3	0.000
Diastolic BP Mean±SD (mm Hg)	77.92±8.5	75.67±7.07	76.5±7.6	69.7±9.1	0.000
Hb (g/dL)	11.53±1.4	10.95±1.31	11.2±1.4	10.1±1.4	0.094
TLC (cells/mm³)	9085.20±2472.19	9230.8±2482.6	9179.5±2462.2	11292±15999.6	0.814
Platelet count (/mm³)	221200±79875.4	230434.7±82187.3	227183.1±80929.2	256968±108806.8	0.649
Creatinine (mg/dL)	0.62±0.1	0.63±0.2	0.62±0.1	0.70±0.2	0.767
T3 (ng/dL)	169.57±49.8	154.95±38.2	160.1±42.8	181.6±77.7	0.172
T4 (µg/dL)	11.79±1.97	11.29±2.06	11.4±2.0	11.10±3.7	0.325
TSH (mIU/mL)	2.07±1.43	2.60±2.04)	2.4±1.8	3.18±2.5	0.252
OGTT					
0 h BG	145.96±43.08)	117.1±34.4	117.1±34.4	84.59±6.8	0.000
1 h BG	264.64±57.48)	183.04±33.26)	211.7±58.1	147.6±21.5	0.000
2 h BG	237.88±54.83)	153.63±28.06)	183.3±56.3	115.9±18.9	0.000

POG period of gestation, TSH thyroid stimulating hormone

Table 2: distribution of risk factors for hyperglycemia in pregnancy among case and control groups

Risk factor	Newly detected diabetes in pregnancy (n=25)	GDM (n=46)	Total cases (HIP) (n=71)	Control (n=100)	P value (between case and control group)	OR (CI)
Previous history of GDM	7 (28.0%)	4 (8.7%)	11 (15.5%)	0 (0.0%)	0.000	0.00
Previous history of premature baby	3 (12.0%)	3 (6.5%)	6 (8.5%)	2 (2.0%)	0.049	0.221 (0.043-1.129)
Family history of GDM	5 (20.0%)	1 (2.2%)	6 (8.5%)	0 (0.0%)	0.003	0.00
Family history of type 2 DM	13 (52.0%)	19 (41.3%)	32 (45.1%)	26 (26.0%)	0.009	0.428 (0.224-0.818)
Current twin pregnancy	0	1 (2.1%)	1 (1.4%)	3 (3%)	0.09	
Unexplained pregnancy loss						
Abortion	10 (40.0%	12 (26.1%	22 (31.0%)	21 (21.0%)	0.001	
IUD	1 (4.0%)	7 (15.2%)	8 (11.3%)	0 (0.0%)		
Still birth	1 (4.0%)	0 (0.0%)	1 (1.4%)	3 (3.0%)		
Congenital anomalies	2 (8.0%)	2 (4.3%)	4 (5.6%)	5 (5.0%)	0.855	
Neonatal deaths	3 (12.0%)	4 (8.7%)	7 (9.8%)	13 (13.0%)	0.529	
Previous history of macrosomic baby	2 (8%)	5 (10.8%)	7 (9.8%)	5 (5%)	0.049	0.455 (0.251-1.827

IUD intrauterine death

Table 3: Distribution of other medical disorders among case and control group

	Newly detected diabetes in pregnancy (n=25)	GDM (n=46)	Total cases (HIP) (n=71)	Control n=100	P value (between case and control group)
Total participants with medical disorders	12 (48%)	9 (19.5%)	21 (29.6%)	16 (16.0%)	0.034
Primary hypothyroidism	7 (28%)	3 (6.5%)	10 (14.1%)	7 (7.0%)	0.127
Hypertension/pregnancy induced hypertension	6 (24%)	1 (2.1%)	7 (9.9%)	2 (2.0%)	0.023
HbsAg carrier	–	5 (10.8%)	5 (7.0%)	0 (0.0%)	0.007
Depression	1 (4%)	1 (2.1%)	2 (2.8%)	0 (0.0%)	0.091
Genitourinary TB	0	1 (2.1%)	1 (1.4%)	0 (0.0%)	0.234
Cholestasis of pregnancy	0	1 (2.1%)	1 (1.4%)	0 (0.0%)	0.234
Seizures	0	0	0 (0.0%)	1 (1.0%)	0.398
Graves` disease	0	0	0 (0.0%)	4 (4.0%)	0.088
Rheumatic heart disease	0	0	0 (0.0%)	2 (2.0%)	0.231

TB tuberculosis

Table 4: Distribution of rs7903146 (C/T) SNP of *TCF7L2* gene among case and control group

rs ID	Genotype	Newly detected diabetes in pregnancy (n=25)	GDM (n=46)	Total cases HIP (n=71)	Controls (n=100)	Odds ratio	95% CI	P value (between case and control group)
rs7903146 (C/T)	CC	9 (36%)	19 (41.3%)	28 (39.4%)	53 (53%)	1	–	–
	CT	14 (56%)	24 (52.1%)	38 (53.5%)	43 (43%)	1.6	0.8-3.14	0.15
	TT	2 (8%)	3 (6.5%)	05 (7.1%)	04 (4%)	2.36	0.58-9.5	0.38
Allele	C	32 (62%)	62 (67.4%)	94 (66.1%)	149 (74.5%)	1	0.93-2.3	–
	T	18 (36%)	30 (32.6%)	48 (33.8%)	51 (25.5%)	1.4		0.12

2.03) was an independent risk factor for GDM in multivariable logistical regression model.

TCF7L2 so far has strongest effect on T2DM risk yet described.[17] The SNP rs7903146 has been most widely researched and has strongest association so far with T2DM and GDM. C>T transition results in either CT heterozygous or TT homozygous risk genotypes. In a metanalysis of 22 studies (included 1 from India), covering 8 TCF7L2 SNPs and involving 5,573 cases and 13,266 controls found SNPs rs7903146 C>T, rs12255372 G>T and rs7901695 T>C impart strongest GDM risk.[18] These studies included used varied criteria for GDM- ADA, WHO, OGTT. In Caucasians and other races, all these SNPs were found to have a significant association with GDM risk, but in Asians, only SNP rs7903146 showed a significant association. T allele of SNP rs7903146 conferred increased risk of GDM with OR 1.36 (95% CI: 1.17–1.57).[18] In another metanalysis by Lin of 16 studies involving 4,853 cases and 10,631 controls, a significant association between the T-allele of rs7903146 and GDM risk was observed under all genetic models, dominant model (OR = 1.44, 95% CI = 1.19–1.74), recessive model (OR = 1.35, 95% CI = 1.08–1.70), heterozygous model (OR = 1.31, 95% CI = 1.12–1.53), homozygous model (OR = 1.67, 95% CI = 1.31–2.12), and allele model (OR = 1.31, 95% CI = 1.12–1.53) across all

ethnicity. Asians with homozygous TT allele of rs7903146 polymorphism had the highest risk of GDM (OR = 2.08).[19] The literature from India is limited and shows conflicting reports.

The TCF7L2 protein is one of the four mammalian transcription factors in T cells (T cell factor [TCF]) and is encoded by gene located on chromosome 10 q25, its size is 215.9 Kb. It has role in Wnt pathway which affects cell proliferation and differentiation. Its expression on pancreatic β-cells highlights its role in insulin secretion regulation, β-cell survival and modulation of incretin action. The risk T-allele of rs7903146 is associated with decreased insulin content, impaired glucose and other secretagogues stimulated insulin secretion in beta cells, and perturbed proinsulin processing.[12,20,21] *TCF7L2* gene polymorphisms are also associated with increased hepatic glucose production and reduced hepatic insulin sensitivity, and hepatic glucose metabolism via the gluconeogenesis pathway in human.[22]

We observed in our study that the wild type homozygous CC, heterozygous CT, and genotype TT were present in 39.4%, 53.5%, 7.1% cases, and 53%, 43%, 4% controls, respectively. This distribution of genotypes was in accordance to Hardy Weinberg equilibrium. Though CT and TT genotypes were more common in cases group but this association was not significant (CC/CT *P* 0.15, CC/TT *P* 0.38). The statistical analysis failed to show any significant association of rs7903146(C/T) SNP of *TCF7L2* gene in first time detected HIP patients ($P > 0.05$) in our region. There was also no significant difference between newly detected DIP and GDM groups. These finding imply different genetic constitution of our population and low risk allele frequency of rs7903146, which is approximately 0.02 in East Asians.[23] In a study by Yadav *et al*.[24] involving T2DM, T1DM and GDM patients, distribution of genotype TT was 10.5% in T2DM, 38.85% in T1DM and all GDM participants had CC genotype. In a similar study in south Indian population, Thomas *et al*.[25] analyzed three SNP variants of *TCF7L2* gene: rs4506565, rs7903146 and rs12255372 and found no significant association with rs7903146 variant. However, they found significant association of variant rs4506565 (OR) 3.75 (CI = 0.75–18.53, *P* = 0.08) with GDM risk in their population. Ye D *et al*. studied Chinese Han population for 18 SNPs including rs7903146 of TCFL2 gene and found rs290487 genetic variation (OR = 2.686 per each C allele, *P* = 0.002) as independent risk factors of GDM in multivariate analysis.[26] In a study by Gorczyca-Siudak from Poland on 50 women with glucose tolerance disorders diagnosed for the first time during the current pregnancy, no correlation was found between rs7903146 polymorphism of the *TCF7L2* gene and GDM.[27] In a study from Bangladesh, Hasan observed that in women <25 years, frequency of GDM was significantly higher in those with CT/TT genotype of rs7903146 of *TCFL2* gene than those with CC (*P* = 0.03), but not in women ≥25 years (60% GDM in both groups). Also, women with BMI <25 kg/m² had higher frequency of GDM in those with CT/TT genotype than those with CC (*P* = 0.047), but not in women having BMI ≥25 kg/m². These findings suggests that polymorphism of *TCF7L2* rs7903146 confer increased risk of GDM in mothers at younger age and lean BMI.[28] Since pre-pregnancy BMI was not available, so corelation of *TCF7L2* rs7903146 in GDM with pre-pregnancy BMI was not evaluated. Another limitation was heterogeneity in the genetic population studied. In addition to SNP in *TCF7L2* rs7903146, multiple polymorphisms in *TCF7L2* and other genes influence HIP, genetic matching of the cases and controls on the basis of the other polymorphisms was not possible. Also due to SARS-CoV-2 pandemic equal number of participants could not be included.

Conclusion

There is no evidence of association of rs7903146 (C/T) SNP of *TCF7L2* gene with newly detected HIP in our population and hence this variant might not confer increased risk for newly detected HIP in our population. Large-scale studies for identification of other susceptibility gene variants are required in our population.

Statement of Ethics

We state that subjects had given their written informed consent and that the study protocol was approved by the institute's committee on human research and complies the guidelines for human studies in accordance with world medical association declaration of Helsinki. The study protocol was reviewed and approved by the ethical committee of Institute of Medical Sciences, BHU, Varanasi, UP, India (ECR/Bhu/Inst/UP/2014/Re-registration-2017 dt 31.01.2017) letter no. Dean/2019/EC/1049 on 18/01/2019. Written informed consent was obtained from the participants (or their parent/legal guardian/next of kin) to participate in the study.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Conflicts of interest

There are no conflicts of interest.

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