Original Research Article

TCF7L2 gene variant rs7903146 affects the risk of Type 2 Diabetes by modulating incretin secretion

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Abstract

Background: Type2 diabetes mellitus (T2DM) is a highly inheritable disease. Transcription factor 7-like 2 (TCF7L2) gene regulates the expression of glucagon-like peptide 1 (GLP-1) in L cells of small intestine. GLP1 plays a critical role in blood glucose homeostasis by stimulating postprandial insulin secretion and increasing insulin sensitivity.
Aim of the study: TCF7L2 gene variants may affect the susceptibility to Type 2 diabetes by altering GLP-1 levels.
Materials and methods: This case-control study was conducted with 90 newly diagnosed patients with Type2 diabetes mellitus as cases and 90 age and sex-matched healthy volunteers as controls. TCF7L2 rs7903146 genotyping was done and we also estimated Fasting and postprandial GLP -1 level, Fasting and Postprandial insulin level and calculated HOMA-IR in both cases and controls.
Results: Out study showed that T+ genotype, lower fasting GLP-1 level and lower postprandial GLP-1 levels were more observed among cases as compared to controls. Low mean GLP 1 activity, high Mean HOMA-IR, low postprandial insulin, low percentage rise in insulin were observed among T+ genotype than among T- genotypic individuals.
Conclusion: Hence, the study concludes that T+ genotype causes a decrease in GLP-1 levels, which in turn by decreasing postprandial insulin levels and by increasing insulin resistance increases the risk of Type2 diabetes.

Key words

Gliptins, GLP-1, Incretins, Polymorphism, Type 2 diabetes, TCF7L2, rs7903146.

Introduction

Type 2 diabetes is a complex metabolic disorder with both genetic and environmental factors such as food habits and lifestyle contributing to its pathogenesis [1]. Due to its complex etiology, the progress of discovery of genetic components for Type 2 diabetes had been very slow until the advent of high throughput genome-wide association (GWA) studies [2, 3]. Most individuals with T2DM suffer serious complications of chronic hyperglycemia, involved in nephropathy, neuropathy, retinopathy, and accelerated development of cardiovascular disease. When the microvascular complications are related to the degree of fasting hyperglycemia [4], macrovascular complication like atherosclerosis is related to postprandial glycemic level [5]. One of the regulators of the postprandial glucose level is GLP-1. The importance of gastrointestinal hormones to augment insulin secretion following nutrient ingestion, termed the incretin effect, has generated renewed interest in recent years [6, 7, 8]. Of the many putative insulin-stimulatory factors released by the gut, glucagon-like peptide 1 (GLP-1) has received considerable attention because of its potency to lower blood glucose in persons with diabetes and the potential of therapeutics based on the GLP-1 signaling system [7, 8]. It is produced in the L cells of gut and brain, and it stimulates insulin secretion and lowers blood glucose in both normal subjects and patients with NIDDM [9-13]. Remarkably, GLP-1 infusion also lowered blood glucose in patients with tissues [14, 15, 16], actions that would also contribute to lowering blood glucose in vivo. The precursor of GLP-1 is preproglucagon, which is also the precursor of glucagon of α cells of the pancreas. In L cells of small intestine, preproglucagon is getting processed to form GLP-1, GLP-2, and glicentin. Tissue-specific difference in processing preproglucagon is explained by a tissue-specific expression of an enhancer TCF7L2 (Transcription factor 7 - like 2) Transcription factor 7-like 2 (TCF7L2) gene spans a 215,863 bases region on chromosome 10q25.317 (114700201–114916063, NCBI build 36.2 [17], and its product is a high-mobility box-containing transcription factor that has a role in activating many genes downstream of the Wnt signaling pathway [18, 19, 20, 21]. The bipartite transcription factor, cat/TCF7L2, activates many genes downstream of the Wnt signaling cascade [15]. One of the genes transcriptionally regulated by cat/TCF7L2 is proglucagon, which encodes the insulinotropic hormone glucagon-like peptide 1 (GLP-1) [20, 21]. The tissue-specific expression of GLP-1 is explained by the fact that Glycogen synthase β binds to TCF7L2 regulator sequence, which is in euchromatin region only in gut cells followed by expression of the transcription factor 7L2 which helps in transcription and processing of preproglucagon to form GLP-1[22]. There is now growing evidence that based on the role of TCF7L2 in intestinal cells [22]. Grant, et al. [23] proposed that variants of TCF7L2 may alter levels of glucagon-like peptide 1, which influences insulin secretion [6, 7, 8] from the β cells of the pancreas or insulin sensitivity [24, 25, 26]. Thus, one hypothesis is that TCF7L2 might influence the risk of Type 2 diabetes by influencing insulin secretion and action, by altering GLP-1 level. Indeed, several studies have reported reduced insulin secretion after an oral glucose tolerance test (OGTT) in subjects with TCF7L2 variants, using ratios between insulin and glucose levels such as insulinogenic index [24, 25, 26]. Reduced insulin secretion in response to oral compared with intravenous glucose has also been reported among subjects with TCF7L2 variants [27, 28], consistent with alterations in the incretin system. However, several studies have shown no change in insulin sensitivity [25, 27], whereas other studies have shown increased [24, 30] or decreased [28, 29] insulin sensitivity. The present study was undertaken to clarify the effect

of TCF7L2 on insulin secretion and sensitivity and in turn on the risk of Type 2 diabetes.

Materials and methods

The study sample comprised 90 south Indian newly diagnosed Diabetic patients (163 male, 18 female) of mean age 50.34 ± 9.84 years.

Inclusion criterion [31] were,

- Fasting plasma glucose ≥ 126 mg/dL or
- Postprandial plasma glucose ≥ 200mg/dL or
- Random plasma glucose ≥ 200mg/dL

Exclusion criteria

- Secondary causes of diabetes like hyperthyroidism, acromegaly, pheochromocytoma were excluded
- Known diabetic patients on treatment were excluded to avoid the interference of glucose toxicity with insulin secretion [32].

Control Subjects: Controls were recruited from people attending the master health check. Age and Sex were matched. Height, weight, waist circumference and hip circumference were measured, and blood samples were collected by Venipuncture after overnight fasting in both a plain tube and the tube with sodium fluoride and potassium oxalate. A plain tube was centrifuged at 2000 rpm for 10 minutes and serum was aliquoted in two tubes and one was used for estimation of fasting insulin levels and the other for GLP-1 levels into which a DPPIV inhibitor [33] was added. Fluoride and oxalate tubes were centrifuged at 2000 rpm for twenty minutes to get the buffy coat for DNA extraction and the plasma was used for fasting plasma glucose estimation. Postprandial blood samples were collected 2 hours after giving 100 g of glucose dissolved in 75 mL of water in plain and sodium fluoride-potassium oxalate tubes. The plain tube was centrifuged at 2000 rpm for 10 minutes and serum was aliquoted in two tubes and one was used for estimation of postprandial insulin and the other for GLP-1 levels into which a DPPIV inhibitor was added. Fluoride, oxalate tube was centrifuged at 2000 rpm for ten minutes and the plasma was used for postprandial plasma glucose estimation.

- Fasting and postprandial plasma glucose were measured by glucose oxidase peroxide method with an autoanalyzer (XL 300) and manufacturers Agent kits.
- Fasting and postprandial Insulin were measured using a sandwich immunoassay.
- Fasting and postprandial bioactive GLP-1 (7-36) levels were measured using a sandwich immunoassay.
- Fasting insulin and glucose levels were used to measure HOMA-IR using a formula,

$$\text{HOMA-IR} = \frac{\text{Fasting insulin(mIU/L) X Fasting plasma glucose (mM)}}{22.5}$$

- % rise in insulin was calculated.
- To remove the effect of confounding factors of insulin resistance [34], Body Mass Index and Waist Hip Ratio were calculated and compared.

TCF-7L2 Polymorphism Screening

DNA was extracted from buffy coat by spin column based Himedia HiPer™ gel extraction kit method. The extracted DNA was checked in 1% agarose gel with a high molecular weight ladder, quantitated based on absorbance at 260 nm and was used for Real-Time PCR. 20 ng of genomic DNA will be amplified in each assay in the presence of specific probes for each SNP variant labeled with a fluorescent dye at the 5' end and a quencher molecule at the 3' end (designed by Applied Biosystems' "assays-by-design" service). Allele C was VIC labeled and T was FAM labeled. PCR was carried out in 1 µl reaction volumes with 1× Absolute™ PCR mix (ABgene) and 1× probe mix (Applied Biosystems). An initial denaturation at 95°C for 10 minutes to be followed by 40 cycles of PCR, with 15 seconds denaturation at 95°C, and 1-minute annealing/extension at 60°C. Following 40 cycles of PCR, fluorescence will be measured for each probe on a Pherastar plate reader and

compared with an internal control ROX dye standard. **Figure - 1** shows the extracted DNA samples run on 1% agarose gel.

Figure - 2 shows the amplification plot of a batch of samples. **Figure - 3** and **Figure - 4** shows the allelic discrimination graph of two plates or Real-Time PCR.

Figure – 1: Extracted DNA (lane 2 to 8) was tested on 1% agarose gel using 1kb ladder (lane 1) Ladder shows 10000, 8000, 7000,6000, 5000, 4000, 3000, 2000 and 1000 bp fragments.

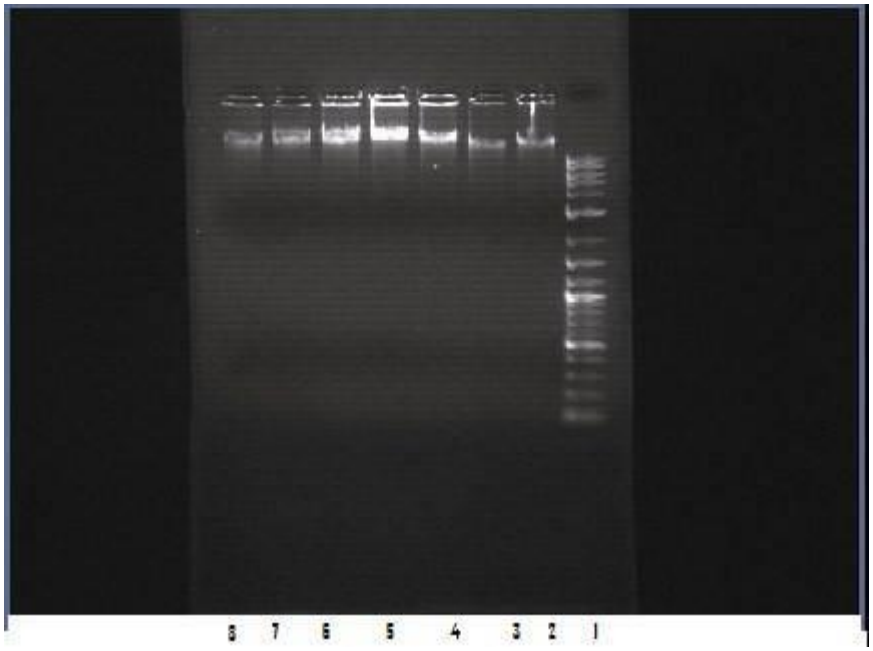
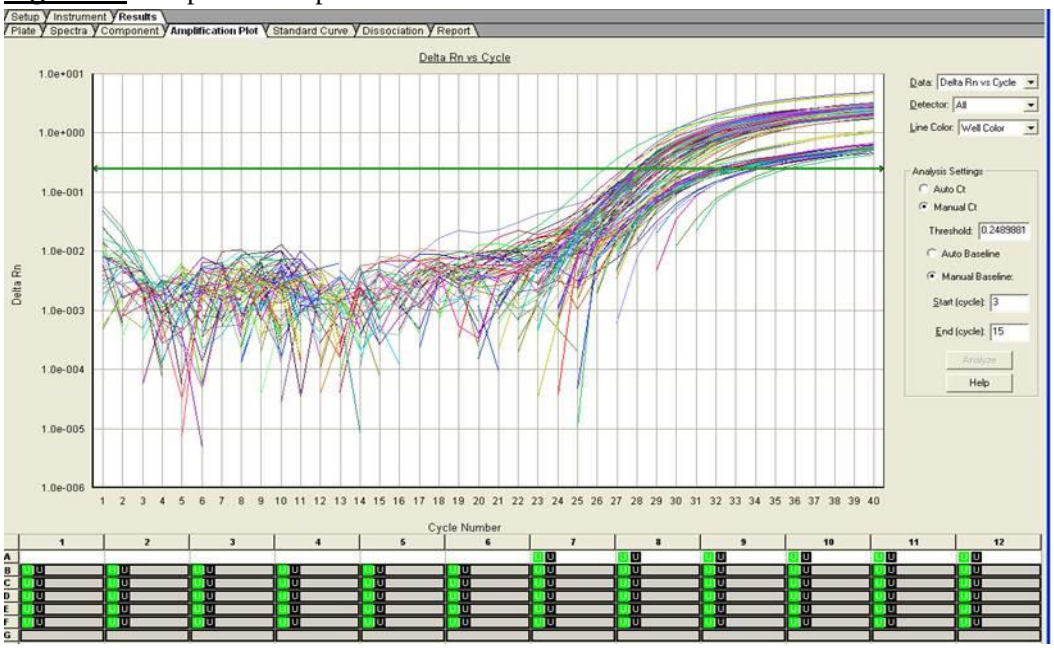


Figure - 2: Amplification plot of Real-Time PCR.



Statistical Analysis

Fasting and postprandial glucose, insulin, GLP-1 levels were compared between control and cases by Student t-test. $p<0.05$ was considered significant.GLP-1 levels for both cases and controls were entered into a Microsoft Excel Spread Sheet. True positive and False positive rates for specific cut-off values were calculated.

ROC curve was plotted and the area under the curve, 95% confidence intervals SE was calculated using Accu ROC Software Version 2.4. GLP-1 levels among the three genotypes were compared by Exact F test. Mean HOMA-IR, fasting insulin, postprandial insulin, percentage rise in insulin among the various genotypes were compared using ANOVA. Logistic regression analysis was performed to evaluate the interaction between TCF7L2 variation and Type 2 diabetes. Independent variables included in the analysis were age (quantitative), sex (male/female), BMI, WHR. The analysis was executed by the SAS Statistical program Version 6.10 for Macintosh.

Figure - 3: Allelic Discrimination Graph of Real Time PCR – Plate 1.

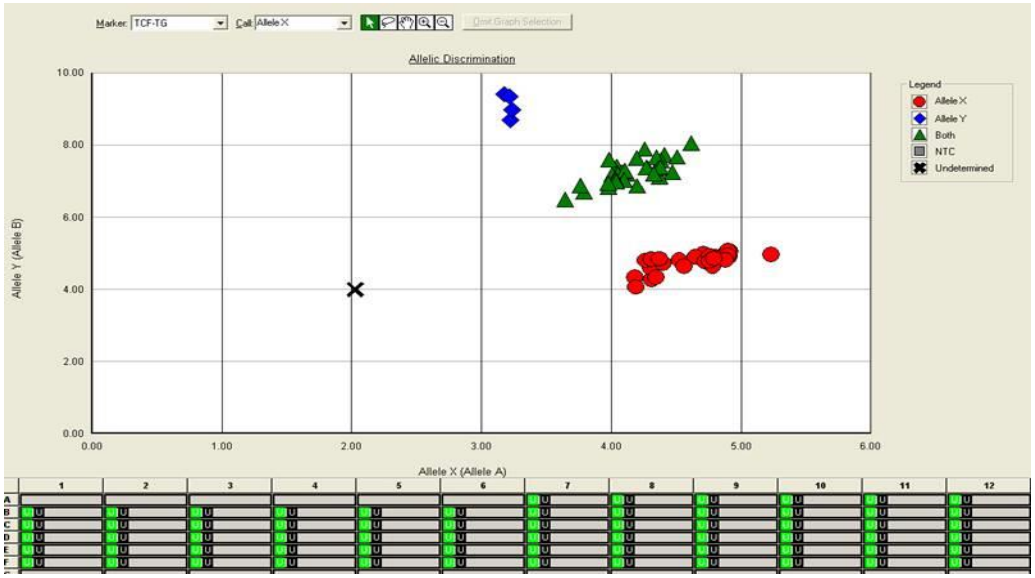
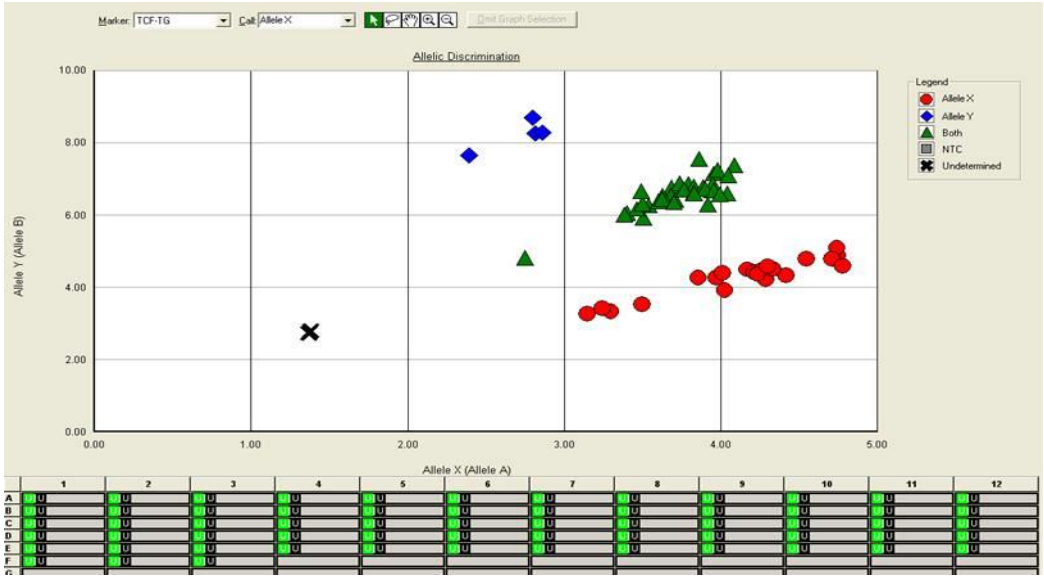


Figure - 4: Allelic Discrimination Graph of Real Time PCR – Plate 2.



Results

Table - 1 shows Age, Sex, BMI, WHR, fasting and postprandial glucose, insulin and GLP-1 levels among patients and control subjects. Since all the confounding factors were matched there were no significant differences between the two groups. Significant differences could be observed with respect to fasting and postprandial glucose, insulin, and GLP-1 levels.

Table - 1: Characteristics of patients with type 2 diabetes and of control subjects.

Variables	Case	Control	P value
Age	54.02±9.276	54.97 ± 8.227	p=0.48
Sex male	52	52	P = 1.0
Female	46	46	
BMI	25.2411 ±3.67	25.04 ± 2.93	P=0.72
WHR	0.9354 ± 0.07	0.9391 ± 0.05	P = 0.68
Fasting Glucose (mg/dL)	146± 23	86± 14	P = 0.001
Postprandial Glucose (mg/dL)	210 ±48	127 ±29	P = 0.001
Fasting Insulin (mIU/L)	10.104 ±1.8879	6.88 ± 1.1	P= 0.001
Postprandial Insulin (mIU/L)	20.364 ±1.967	15.099 ±1.469	P= 0.001
Fasting GLP-1 (pM)	24.186 ±2.208	26.545 ±3.092	P=0.001
Postprandial GLP-1 (pM)	27.022 ±3.71	41.801 ±29.35	P = 0.001

Table - 2: Genotype distribution and Allele frequencies of human TCF7L2 gene.

Genotype	Control	Case	P value
TT	2 (2.8%)	15(16.7%)	Chi sq =12.15
TC	38 (39.4%)	43(47.8%)	P = 0.002
CC	52 (57.7%)	32(35.6%)	

Genotype	Control	Case	P value
T+*	38(42.2%)	58(64.5%)	Chi sq = 7.88
T-*	52(57.7%)	32(35.5%)	P = .004

T+ → TT + TC & T- → CC.

Table – 3: Comparison of glp-1 levels among cases and controls.

Variable	Case	Control	P value
Fasting GLP-1 (pM)	24.19 ± 2.21	26.55 ± 3.1	t = 5.64, p = 0.001
Postprandial GLP-1 (pM)	27.02 ± 3.1	41.8 ± 29.35	t = 4.73, p = 0.001

Table - 2 shows Genotype distribution and Allele frequencies of human TCF7L2 gene in patients with Type 2 diabetes and control subjects. The Allele frequencies were CC = 84, TC =79 and TT = 17. This was found to be in Hardy Weinberg equilibrium. Chi-square value was 3.84, P value was .37. Diabetic patients had significantly higher frequency of T+ genotype (TT and TC) than controls (.65 versus .42; P=.004). Even when the individual genotypes were considered, TT genotype was more common among cases than controls (0.17 versus 0.03, p =.002) and TC genotype more common among cases than controls (0.48 versus 0.39, p=0.002), whereas CC was more common among controls (0.58 versus 0.36, p=0.002).

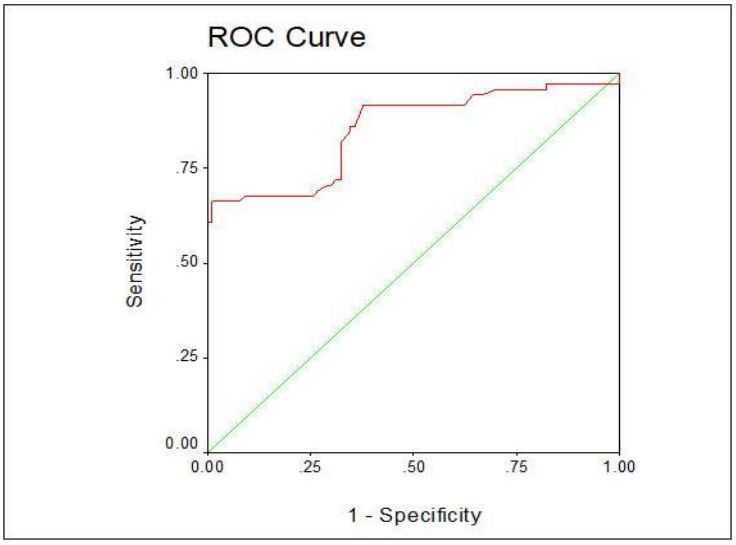
Table - 3 shows the comparison of GLP-1 level among cases and controls. Significantly lower plasma fasting GLP-1 level (24.19 pM versus 26.55pM, P < 0.001) and postprandial GLP-1 levels (27.02 pM versus 41.8pM, P < 0.001) was observed in Type 2 diabetic patients as compared to healthy controls.

Table - 4 shows the true positive and False positive rates for the various GLP-1 cut off values aimed at distinguishing cases from controls. Receiver Operating Characteristics curve analysis had detected the cut off value of GLP-1 as 27.8pM with a sensitivity of 0.789 and specificity 0f 0.678.

Table - 4: True positive and false positive rates for various GLP-1 cut off values.

GLP1 (pM)	Sensitivity	1 - Specificity
25.000	.958	.700
25.150	.944	.667
25.250	.944	.644
25.400	.915	.622
25.650	.915	.600
25.850	.915	.589
25.950	.915	.578
26.050	.915	.556
26.125	.915	.522
26.175	.915	.511
26.250	.915	.500
26.350	.915	.489
26.420	.915	.467
26.445	.915	.456
26.455	.915	.444
26.480	.915	.433
26.650	.915	.389
26.850	.915	.378
26.950	.859	.356
27.050	.859	.344
27.200	.845	.344
27.450	.817	.322
27.850	.789	.322

Figure - 5: Receiver operating characteristics curve for GLP-1 as a predictive biomarker.



Area under the ROC curve (**Figure - 5**) was 0.576 (95% CI was 0.546 to 0.606).

Table - 5 shows the difference in GLP-1 level between the various genotypes. The level was significantly lower among T+ genotype individuals when compared to T- genotype individuals among both cases and controls.

Table - 5: Correlation between phenotype (glp-1 level) and genotype.

Group	Analyte	Genotype	Mean	Std. Deviation	One-way ANOVA
Case	**Fasting GLP1 (pM)**	CC	26.147	1.5168	F=100.84 P=0.001
		TC	23.926	1.1431	
		TT	20.747	.5780	
	Postprandial GLP1(pM)	CC	30.738	2.5689	F=121.03 P=0.001
		TC	26.116	1.6422	
		TT	21.691	.6666	
Control	**Fasting GLP1 (pM)**	CC	28.363	1.8458	F=58.96 P=0.001
		TC	24.557	2.0049	
		TT	17.100	.0000	
	Postprandial GLP1 (pM)	CC	51.195	35.7734	F=5.76 P=0.001
		TC	29.661	3.7689	
		TT	19.200	.1414	

Table - 6: Correlation between genotype and insulin levels and sensitivity.

Group	Variable	Genotype	Mean	Std. Deviation	One-way ANOVA
Case	**HOMA - IR**	CC	2.9963	.25395	F=208.41 P=0.001
		TC	4.1691	.46898	
		TT	5.9825	.75579	
	Post prandial insulin (mIU/L)	CC	21.816	1.1121	F=74.70 P=0.001
		TC	20.388	1.2423	
		TT	17.200	1.2972	
	% Rise in insulin	CC	164.00	15.659	F=474.16 P=0.001
		TC	97.30	12.943	
		TT	31.05	14.964	
Control	**HOMA - IR**	CC	1.1915	.14660	F=125.16 P=0.001
		TC	1.6744	.14569	
		TT	2.2377	.05046	
	Postprandial insulin (mIU/L)	AA	15.622	1.5671	F=11.36 P=0.001
		AB	14.550	.7739	
		BB	12.050	.0707	
	% Rise in insulin	AA	154.84	33.890	F=58.00 P=0.001
		AB	86.40	19.476	
		BB	31.00	2.782	

Table - 7: Correlation between GLP-1 levels and insulin levels and sensitivity.

		HOMA - IR	% Rise in insulin
Postprandial GLP1	**Pearson Correlation**	-.899	.896
	Sig. (2-tailed)	.000	.000
	N	161	161

Table - 6 shows that among both cases and controls, HOMA-IR was high among T+ genotypic individuals when compared to T- individuals, Postprandial insulin % rise in insulin are low among T+ genotypic individuals when compared to T- individuals.

Table - 8: Univariate analysis to find odds ratio between genotype and type 2 diabetes susceptibility.

GENOTYPE	CASES(n)	CONTROLS(n)	Pearson chi square
TT	15	2	$\chi 2$=7.88 P=0.005
TC	43	36	OR = 1.2 to 2.3
CC	32	52	

Table - 7 shows the correlation between GLP-1 level on one hand and the insulin levels and HOMA – IR on the other hand. The negative correlation coefficient for HOMA –IR and Positive correlation coefficient for percentage rise in postprandial insulin indicates that high GLP-1 levels were associated with low HOMA-IR, high percentage rise in insulin.

Table - 8 shows the Odds ratio calculation on Univariate analysis to evaluate the risk of Type 2 diabetes among the various genotypes. Odds ratio was 2.0$\pm$ 0.4 .The age-, sex, BMI, WHR adjusted odds ratio between the genotypes for developing type 2 diabetes was 1.6 (1.2 – 2.3).

Discussion

Genetic factors in combination with several environmental risk factors are involved in the predisposition to Type 2 Diabetes mellitus. The susceptibility to Type 2 Diabetes is complex and recently significance is given to the role of incretins particularly GLP-1 in glycemic control, with the advent of therapeutics aimed at increasing GLP-1 levels, because GLP-1 is found to increase insulin release in response to food in the gut. Some studies have proven that GLP-1 increases insulin sensitivity [26, 27, 28] but some studies defer the concept [25]. Another interesting aspect of GLP-1 synthesis is that it shares the same precursor as that of glucagon which is preproglucagon. Preproglucagon gets differentially processed in the two tissues to give rise to two different products – GLP-1 in L cells of small intestine and glucagon in alpha cells of the pancreas. This differential processing is explained by the tissue-specific expression of enhancer TCF7L2 [22] with a known polymorphic site in intron 6 [32]. Normally, the rs7903146 site is occupied by C, if that is replaced by T, it affects the posttranscriptional modification of TCF7L2 mRNA [33]. To analyze, the phenotypic effect of this polymorphism, we analyzed fasting and postprandial GLP-1 levels among the various genotypes. The mean GLP 1 activity among T+ genotypic individuals is lower than individuals with T- genotype, suggesting us that T polymorphism affects the GLP- 1 levels. Simultaneously, to re-establish the fact that GLP-1 decrease can result in Type 2 diabetes, we compared GLP-1 levels among cases and controls, and we found GLP-1 level to be low among cases than among controls. So, the conclusion is TCF7L2 gene variation by altering the post-transcriptional modification of preproglucagon gene, decreases GLP-1 levels and that increases the risk of Type 2 diabetes. We measured fasting and postprandial insulin, calculated HOMA- IR, as an index of insulin sensitivity. We found that postprandial insulin levels were low and HOMA index was high among cases when compared to controls, proving the fact that Type 2 diabetes is characterized by not only insulin resistance but also an insulin secretory defect. To identify the role of GLP-1 in glycemic control, we correlated GLP-1 levels with HOMA-IR, postprandial insulin and percentage rise in insulin. A positive correlation coefficient with a significant p-value for % rise in postprandial insulin suggests that a decrease in GLP-1 level causes a decrease in insulin secretion. A negative correlation coefficient with a significant p-value for HOMA-IR suggests that a decrease in GLP-1 level causes an increase in HOMA-IR or an increase in insulin resistance. Thus, GLP-1 has got a role in both insulin secretion and in sensitizing the cells to insulin activity. When an attempt was made to set up a cut off value, Receiver Operating Characteristics curve analysis has detected the cutoff the value of GLP-1 as 27.8pM with a sensitivity of 0.789

and specificity 0f 0.678.Thus, Genotypes TT and TC cause a decrease in fasting and postprandial GLP-1 levels, which in turn by decreasing insulin levels and by increasing insulin resistance increase the risk of Type 2 diabetes.

Conclusion

We have examined the association of the TCF7L2 genotypes and phenotypes with Type 2 diabetes and have found a significant association of T variant and low GLP-1 level among Type 2 diabetic individuals. Thus, the low GLP-1 levels and the T genotype may be an independent risk factor for Type 2 diabetes.GLP-1 level can be used as a parameter for assessing Type 2 diabetes risk.TCF7L2 genotype polymorphism detection and GLP-1 levels measurement can be used to customise treatment of Type 2 diabetes with gliptins which increase GLP-1 levels by inhibiting Dipeptidyl Peptidase IV, an enzyme which metabolizes GLP-1.

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