European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 08, Issue 2, 2021

TO STUDY THE EFFECT OF MONO DRUG THERAPY IN TCF7L2 GENE ASSOCIATED TYPE 2 DIABETES MELLITUS.

Ksheerasagar Vinay Kumar[1], Dr. Anil Kumar[2], Dr. Pranjal Pankaj[3], * Dr. Nilam Nigam[4]

[1]Ph.D Scholar, Department of Pharmacology, Rama Medical College Hospital & Research Centre, Faculty of Medical Sciences, Rama University, GT Road, Mandhana, Kanpur, Uttar Pradesh.

[2]Assistant Professor, Biotechnology, Rama Engineering College, Rama University, Kanpur, Uttar Pradesh.

[3]Professor, Department of General Medicine, Rama Medical College Hospital & Research Centre, Faculty of Medical Sciences, Rama University, GT Road, Mandhana, Kanpur, Uttarpradesh.

[4]Professor, Department of Pharmacology, Rama Medical College Hospital & Research Centre, Faculty of Medical Sciences, Rama University, GT Road, Mandhana, Kanpur, Uttarpradesh.

Corresponding Author
Dr. Nilam Nigam
Professor, Department of Pharmacology, Rama Medical College, Hospital & Research Centre, Rama University, Kanpur, Uttarpradesh
Email id – drnilamn@gmail.com

ABSTRACT

Background: Type 2 diabetes (T2D), is also known as adult-onset diabetes, it is characterized by raised in blood glucose levels, insulin resistance, and relative lack of insulin. Most of the cases of diabetes is due to gene polymorphism, the transcription factor-7-like 2 (TCF7L2) gene has been found as the most important gene to cause type 2 diabetes mellitus (T2DM). The main aim of the study is to study the effect of mono drug therapy in TCF7L2 gene associated type 2 diabetes mellitus.

Material and Methods: Prospective, randomised study. Group A- Metformin and Group B - Glimepride.

Results: TCF7L2 gene is associated with Type 2 diabetes mellitus. Genetic investigation was done in all 20 patients three genotypes have been identified CC, CT and TT genotypes in both the groups. Baseline values are higher in TT genotype when compared with CT & CC genotypes. In all group there is a significant improvement in blood glucose levels were as a controlled lipid profile was observed in CC genotype of Group A but no significant was observed in CT, TT and in group B genotypes.

Conclusion: Patients HbA1c ranges from 6.5 to 7.5 % prescribed with mono drug therapy of metformin and Glimepride shows significant improvement in blood glucose

levels in all three genotypes but no significant was observed in CT, TT and Glimepride treated groups. As a result metformin is the better drug of choice in CC genotype associated type 2 diabetes mellitus when compared with Glimepride.

Keywords: TCF7L2 gene, metformin, Glimepride

INTRODUCTION:

Diabetes mellitus is a metabolic disorder leading to hyperglycemias. It is classified as T1DM, T2DM, GDM and MODY. [1] In the year 1980 the prevalence of diabetes mellitus is 0.67% but significantly increases to 11.6% in 2010. It is estimated to be 642 millions in 2040. [2] Proinsulin is converted to Insulin and gets secreted from pancreatic beta cell. If the concentration of proinsulin is increased, insulin also gets increased. This increased insulin may cause insulin resistance than to diabetes mellitus. [3] There are different risk to cause T2DM among them genetic factor is the one of important factor. Transcription factor 7 like 2 genes (TCF7L2) is located on chromosome 10. [4]This gene involves in WNT signalling pathway as a result it causes reduction in insulin, incretin, glucagon like peptide and glucagon like peptide 1 secretions. [5] As TCF7L2 gene causes apoptosis of beta cells leading to disturbance in production of proinsulin as a result it causes decrease in insulin formation. [4] The risk allele of TCF7L2 gene are rs1225537 and rs7903146 they causes increase in production of proinsulin and proinsulin to insulin ratio but T allele causes impaired proinsulin. [6] This is the important mechanism to cause type 2 diabetes mellitus. The main aim of the study is to study the effect of mono drug therapy in TCF7L2 gene associated type 2 diabetes mellitus.

MATERIALS AND METHODS:

A present study is a randomized, prospective; open-label study was conducted in the Department of pharmacology in association with the Department of general medicine& Central research laboratory in Rama Medical College Hospital & Research Centre, Kanpur in 2017. The study was carried out after taking approval from the institutional ethical committee. Patients were enrolled in the study as per inclusion and exclusion criteria.

Inclusion criteria.

- Newly diagnosed type 2 diabetes mellitus patients.
- Patients of both sexes.
- Age – 30 years to 70 years.

- Glycosylated haemoglobin (HbA1c) > 7.6% - 9.0 %.
- Fasting plasma glucose > 126 mg/dl (7.0 mmol/L)
- 2-hours plasma glucose > 200mg/dl (11.1 mmol/L)
- Patients ready to give an informed consent form**.**

Exclusion Criteria

- Type 1 diabetes mellitus
- Patients already diagnosed with diabetes mellitus and on treatment
- Pregnancy
- Smokers and alcohol.
- Patients who are not ready to give informed consent form.
- Patients having other diseases.

Patient Categorization:

- Patients under inclusion criteria underwent routine investigation of blood glucose levels (HbA1c, fasting blood glucose levels & postprandial blood glucose levels) , Lipid profile (Total Cholesterol, Triglycerides, HDL –C, LDL – C & VLDL – C) &for DNA isolation to detect TCF7L2 gene polymorphism. A total of 48 patients were detected as TCF7L2 gene polymorphism was analysed.

Biochemical tests:

Estimation of blood glucose levels is done by glucose oxidase and peroxidase enzymatic methods; estimation of HbA1c was done by ion exchange resin method

Estimation of Lipid profiles, Triglycerides estimation was done by Tinder Methods, HDL – C estimation is done by Phosphotungstic acid methods.

Genomic DNA was extracted from peripheral blood cells by using Qiagen Kit (Procedure and standard protocol was given in Qiagen kit). Genotyping was done by PCR- restriction fragment length polymorphism methods. TCF7L2 gene were analysed for the SNP rs7903146 sequence located on chromosome 10q25.2–q25.

- Forward primer: 5’-GAACAATTAGAGAGCTAAGCACTTTTTAGAAAC-3’
- Reverse Primer: 5’-AGATGAAATGTAGCAGTGAAGTGC-3’.

The PCR involved 38 cycles of 94ºC for 30s for 62º C for 30s and 67ºC for 30s. Then the PCR products were digested overnight at 67ºC with Rsa1 (restriction endonuclease), electrophoreses on 2.5% Agarose gel and strained with ethium bromide.

As per the treatment plan patients were selected according to the range of HbA1c levels > 7.6 to 9.0 %. Patients were divided into two groups Group A & Group B 3 genotypes were observed in each group. All the patients under went follow up for 6 Months.

- Group - A Patients were prescribed with Metformin
- Group - B Patients were prescribed with Glimepride

Follow Up:

All the patients had been briefed about symptoms of hypoglycaemia. Patients underwent follow up for 6 months and routine investigations of HbA1c, fasting blood glucose, postprandial blood glucose, and lipid profile was done and noted down (after treatment). The total study period for 6 months.

Statical Analysis:

The SPSS windows version 21 to analyse the results. The percentage changes were determined after tabulating the value in data, student 't' test were used to analyses the data. Statical significant value less than 0.05.

RESULTS:

The present study is to study the effect of monodrug therapy in TCF7L2 gene associated type 2 diabetes mellitus is carried out in department of pharmacology in association with department of medicine and central research laboratory. Total of 20 patients were included in the study underwent route investigation of HbA1c, FBS, PPBS, Triglycerides and HDL levels also genetic testing were conducted. Three genotypes have been identified CC, CT and TT genotypes in both the groups. In all group there is a significant improvement in blood glucose levels were as a controlled lipid profile was observed in CC genotype of Group A but no significant was observed in CT, TT and in group B genotypes.

Table 1.Baseline values with 6 months finding in group A

		Baseline	6 Months
HbA1C	CC	6.96±0.23	5.41±0.24
	CT	7.18±0.31	5.91±0.38
	TT	7.20±0.51	6.13±0.64
FBS	CC	143.00±7.34	120.80±5.40
	CT	148.16±9.13	130.16±15.34
	TT	149.33±9.16	133.33±10.06
PPBS	CC	239.80±1.16	211.20±6.22
	CT	245.83±12.17	224.00±10.35

	TT	252.33±23.45	233.33±29.14
TG	CC	147.80±9.80	135.20±3.66
	CT	150.00±8.09	140.66±8.06
	TT	138.66±6.42	133.66±20.42
HDL	CC	36.60±8.87	46.60±6.46
	CT	34.33±6.65	39.66±9.24
	TT	35.66±7.37	40.00±10.39

Table 2.Mean difference after comparing baseline values with 6 Months finding in group A

		Mean difference	P value
HbA1C	CC	1.55±0.28	0.001
	CT	1.26±0.12	0.001
	TT	1.06±0.15	0.001
FBS	CC	22.20±4.02	0.001
	CT	18.00±12.7	0.018
	TT	16.00±5.29	0.035
PPBS	CC	28.60±6.22	0.001
	CT	21.83±10.64	0.004
	TT	19.00±6.24	0.034
TG	CC	12.60±9.04	0.036
	CT	9.33±9.60	0.063
	TT	2.00±14.00	0.828
HDL	CC	10.00±2.91	0.002
	CT	5.33±10.30	0.261
	TT	4.33±3.51	0.166

Fig No 01: Graphical representation of mean difference in Group A

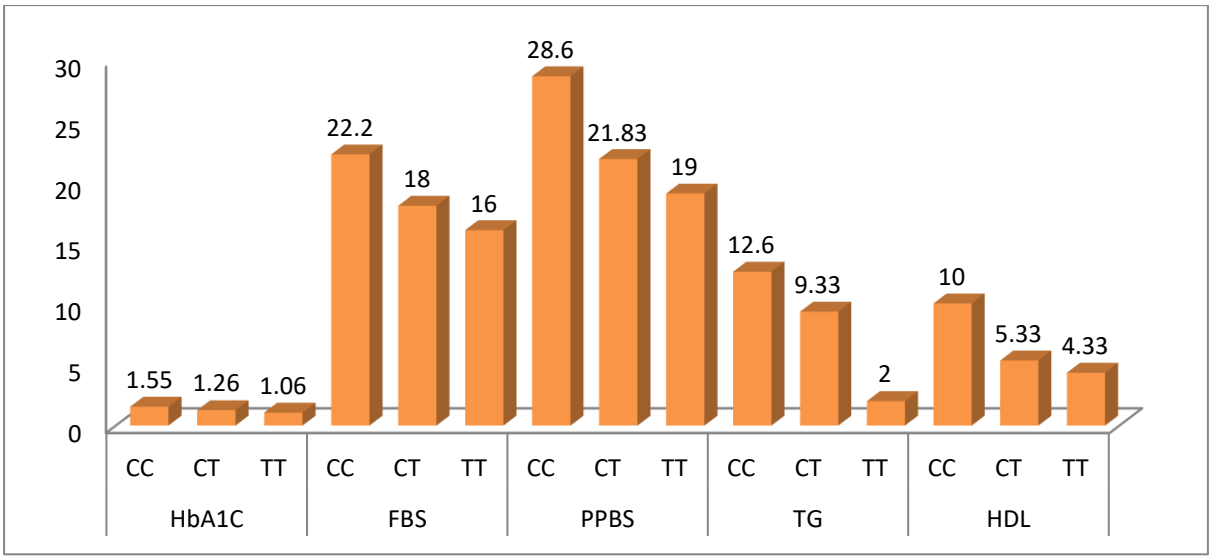


Table 3. Baseline values with 6 months finding in group B.

		Baseline	6 Months
HbA1C	CC	6.92±0.50	5.75±0.38
	CT	7.20±0.36	6.20±0.20
	TT	7.25±0.26	6.20±0.51
FBS	CC	141.20±20	122.40±13.44
	CT	146.40±9.20	127.60±6.98
	TT	150.50±11.12	135.00±18.40
PPBS	CC	236.00±11.66	211.40±15.51
	CT	240.00±10.58	221.60±11.28
	TT	247.00±15.09	230.00±14.14
TG	CC	144.80±11.81	137.60±5.89
	CT	150.80±23.34	144.40±15.19
	TT	136.25±13.07	131.00±12.27
HDL	CC	41.80±7.08	48.80±12.45
	CT	150.80±23.34	44.50±13.25
	TT	39.80±8.69	42.75±7.97

Table 4.mean difference after comparing baseline values with 6 Months finding in group B

		Mean difference	P value
HbA1c	CC	1.17±0.24	0.001
	CT	1.00±0.18	0.001
	TT	1.05±0.66	0.051
FBS	CC	18.80±3.21	0.001
	CT	15.50±3.41	0.003
	TT	18.80±7.42	0.005
PPBS	CC	23.60±4.56	0.001
	CT	18.40±3.84	0.001
	TT	17.00±3.46	0.002
TG	CC	7.20±7.79	0.108
	CT	6.40±13.44	0.347
	TT	5.25±18.39	0.608
HDL	CC	7.00±11.87	0.258
	CT	5.60±14.90	0.448
	TT	3.25±14.40	0.682

Fig N0 02: Graphical representation of mean difference in Group B

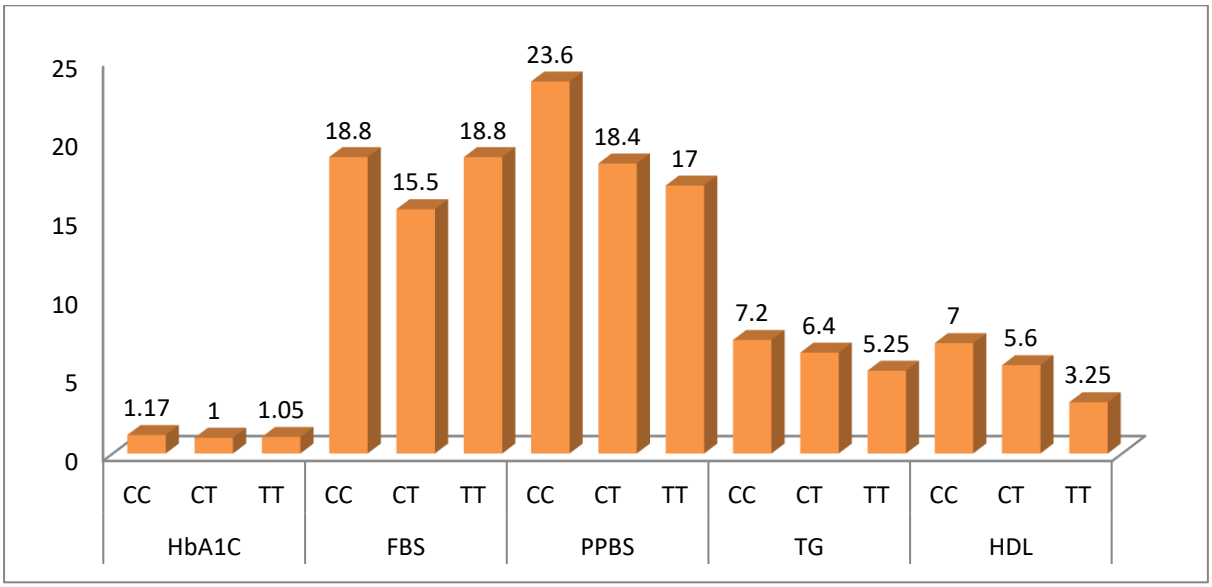
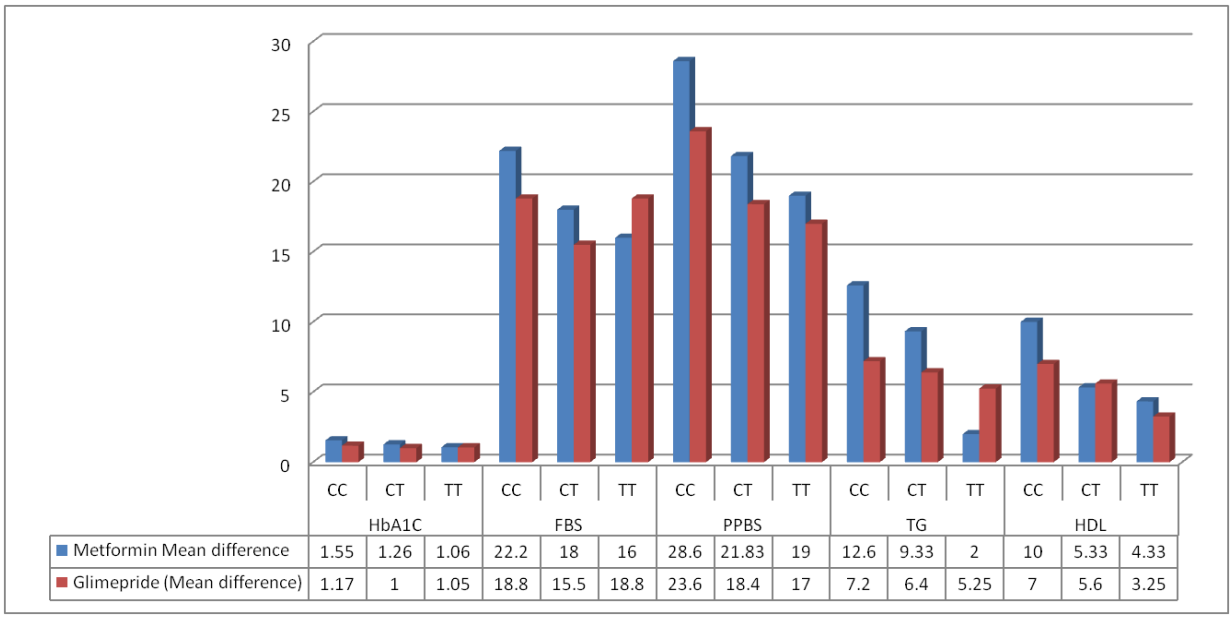


Table No 05: mean difference of both group A and Group B after comparing baseline with 6 Months.

		Metformin (Mean difference)	Glimepride (Mean difference)
HbA1c	CC	1.55±0.28	1.17±0.24
	CT	1.26±0.12	1.00±0.18
	TT	1.06±0.15	1.05±0.66
FBS	CC	22.20±4.02	18.80±3.21
	CT	18.00±12.7	15.50±3.41
	TT	16.00±5.29	18.80±7.42
PPBS	CC	28.60±6.22	23.60±4.56
	CT	21.83±10.64	18.40±3.84
	TT	19.00±6.24	17.00±3.46
TG	CC	12.60±9.04	7.20±7.79
	CT	9.33±9.60	6.40±13.44
	TT	2.00±14.00	5.25±18.39
HDL	CC	10.00±2.91	7.00±11.87
	CT	5.33±10.30	5.60±14.90

	TT	4.33±3.51	3.25±14.40

Fig No 03: Graphical representation of mean difference of group A and Group B



Discussion:

The present study was conducted to evaluate the effect of mono drug therapy in TCF7L2 gene associated type 2 diabetes mellitus.. A study was conducted on 20 newly diagnosed type 2 diabetes mellitus patients attending Rama hospital. In this study patients were treated based on HbA1c levels (more than 6.5 to 7.5%), after 6 months of treatment Statical analysis was done. Genetic testing was also done on all 20 patients three genotypes were observed CC, CT, and TT genotypes. In group A Significant mean difference was observed in HbA1c (CC 1.55%, CT 1.26% & TT 1.06 %), fasting blood glucose levels (CC 22.20 mg/dl (p 0.001), CT 18.00 (p 0.018) mg/dl & TT 16.00(p 0.035) mg/dl, Post prandial blood glucose (CC 28.60 mg/dl (p 0.001), CT 21.83 mg/dl (p 0.004) & TT 19.00 mg/dl (p 0.034)), triglycerides (CC 12.60 mg/dl (p 0.036), CT 9.33 mg/dl (p 0.063) & TT 2.00 mg/dl (p 0.828) & HDL (CC 10.00 mg/dl (p 0.002), CT 5.33 (p 0.261) & TT 4.33 (p 1.66). Metformin recommended as a first line drug and is the commonly used drug in the treatment of type 2 diabetes (T2D) based on its efficacy, safety, low cost, and extensive use [1]. However, there is a considerable inter individual variability in the therapeutic response to metformin. Our study coincide with the study of **Tanja Dujic, et al (2019)** [7] diabetes risk T allele is associated with lower insulin

resistance and better glycemic response in newly diagnosed patients within the first year of metformin treatment. In group B Significant mean difference was observed in HbA1c (CC 1.17 (p 0.001) CT 1.00% (p 0.001) & TT 1.05 % (p 0.051)), fasting blood glucose levels (CC 18.80 mg/dl (p 0.005), CT 15.05 mg/dl (p 0.003) & TT 18.80 mg/dl (p 0.005)), Post prandial blood glucose (CC 23.60 mg/dl (p 0.001), CT 18.40 mg/dl (p 0.001) & TT 17.00 mg/dl (p 0.002)), triglycerides (CC 7.20 mg/dl (p 0.108), CT 6.40 mg/dl (p 0.063) & TT 5.25 mg/dl (p 0.608) & HDL (CC 7.00 mg/dl (p 0..258), CT 5.60 (p 0.448) & TT 3.25 (p 0.682). Z. Schroner et al (2010) [8] study conducted on 87 patients failed to achieve glycemic control by metformin monotherapy, 6 months sulfonylurea's is added with metformin controls HbA1c and FBS in TCF7L2 gene polymorphism. After comparing the mean difference between metformin and Glimepride, metformin shows higher in mean reduction in blood glucose, triglycerides and HDL when compared with that of Glimepride in all genotypes

Conclusion:

The present study the effect of mono drug therapy in TCF7L2 gene polymorphism associated type 2 diabetes. TCF7L2 gene is associated to causes type 2 diabetes mellitus among them TT geno type is a risk allele. Patients HbA1c ranges from 6.5 to 7.5 % prescribed with mono drug therapy of metformin and Glimepride shows significant improvement in blood glucose levels in all three genotypes but no significant was observed in CT, TT and Glimepride treated groups. As a result metformin is the better drug of choice in CC genotype associated type 2 diabetes mellitus when compared with Glimepride.

References:

1. Xu Y, Wang He et al. Prevalence and control of diabetes in Chinese adults. JAMA. 2013; 310(9):948-959.
2. Ogurtsova K, da Rocha Fernandez JD, Huang et al. IDF diabetes atlas: global estimates for the prevalence of diabetes for 2014 and 2040. Diabetes Res Clin Pract. 2017; 128:40-80.
3. Mykk€anen L, Heffner SM, Hales CN, et al. The relation of proinsulin, insulin, and proinsulin-to-insulin ratio to insulin sensitivity and acute insulin response in normoglycemic subjects. Diabetes. 1997; 46(12):1990–1995.
4. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J,

Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulches JR, Kong A, Stefansson K: Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet. 2006; 38:320 –323.

5. da Silva Xavier G, Loder MK, McDonald A, et al. TCF7L2 regulates late events in insulin secretion from pancreatic islet beta-cells. Diabetes. 2009; 58(4):894–905.
6. Shah M, Varghese RT, Miles JM, et al. TCF7L2 genotype and a-cell function in humans without diabetes. Diabetes. 2016; 65(2):371–380.
7. Tanja Dujic, et al. Effects of TCF7L2 rs7903146 variant on metformin response in patients with type 2 diabetes. Bosn J Basic Med Sci. 2019;19(4):368-374.
8. Z. Schroner, M. Javorsky, R. Tkacova, L. Klimcakova et al. Effect of sulphonylurea treatment on glycaemic control is related to TCF7L2 genotype in patients with type 2 diabetes. Journal of pharmacology and therapeutics. 2011;13(1):89-91.