
| RESEARCH ARTICLE

Role of SGLT2 Inhibitors in Diabetes Management: Focus on HbA1c Levels, Weight Loss and Genetic Variation

Prabhsimran Kaur¹, Sushil Kotru², Leena Tuteja³, Abhilash Ludhiadch⁴ and Anjana Munshi⁵ ✉

¹⁴⁵Department of Human Genetics and Molecular Medicine, Central University of Punjab, Bathinda-151401, India.

²³EEDOC Department, Max Superspeciality Hospital, Bathinda

Corresponding Author: Anjana Munshi, **E-mail:** anjana.munshi@cup.edu.in

| ABSTRACT

Sodium Glucose Co-transporters-2 (SGLT2) inhibitors are the recent addition to treatment strategies for Type 2 Diabetes Mellitus (T2DM). It is a non-insulin dependent anti-diabetic therapeutic approach that eliminates plasma glucose by urination. The study was carried out with the aim of evaluating the effect of SGLT2 inhibitors on HbA1c levels and weight loss in responders and non-responders. In addition, the role of two significant variants, SLC5A2 (rs9934336) and UGT1A9 (rs72551330), affecting the inter-individual variation in response to SGLT2 inhibitors was evaluated in the study population. 200 confirmed T2DM patients on SGLT2 inhibitors were enrolled for the study. Patients with decreased HbA1c levels and body weight were categorized as responders, whereas the ones who did not show a significant decrease in these two parameters after treatment were categorised as non-responders. Association of HbA1c levels and weight loss before as well as after treatment with responders and non-responders was evaluated. Patients were screened for two significant variants, SLC5A2 (rs9934336) and UGT1A9 (rs72551330), affecting the inter-individual variation in response to SGLT2 inhibitors by Sanger Sequencing. A significant difference in HbA1c levels and weight was found in responders and non-responders before and after the treatment. However, both of the variants, SLC5A2 (rs9934336) and UGT1A9 (rs72551330), were not found to be significantly associated with the drug response. In conclusion, SGLT2 inhibitors reduced HbA1c levels and weight effectively in responders. However, the targeted gene variants need not to be involved in genetic testing before prescribing this class of drugs to T2DM patients from Malwa region of Punjab. Highlights:

1. Treatment of Type 2 diabetes mellitus (T2DM) with Sodium Glucose co-transporter-2 (SGLT2) inhibitors is an insulin-independent method of reducing blood glucose levels by lowering renal tubular glucose reabsorption.
2. Significant decrease in HbA1c levels and weight loss in responders was observed after the treatment with SGLT2 inhibitors.
3. Pharmacogenetic analysis was carried out for two gene variants, SLC5A2 (rs9934336) and UGT1A9 (rs72551330), reported to be involved in inter-individual response to SGLT2 inhibitors.
4. None of the tested variants were found to be significantly associated with inter-individual response to SGLT2 inhibitors.
5. Pharmacogenetic testing for the two most commonly reported variants is not required for the T2DM patients on SGLT2 inhibitors from the Malwa region of Punjab.

| KEYWORDS

SGLT2 inhibitors; diabetes; HbA1c; weight loss; genetic variation.

| ARTICLE INFORMATION

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1. Introduction

Diabetes Mellitus (DM), deemed as a "Pandemic", affects more than 10% of the global population (Singer et al., 2022). It is counted among the ten leading causes of mortality worldwide (Lin et al., 2020). India has been referred to as the "Diabetic capital of the world", and it is anticipated that there will be an alarming 80 million diabetics in India by 2030 (Pandey & Sharma, 2018).

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DM is characterized by prolonged hyperglycemia resulting in long term complications. Broadly, DM is classified into two major types-Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM). Various microvascular complications and macrovascular complications are reported in patients affected by the disease. Non-modifiable risk factors associated with the disease include a family history of diabetes and age, whereas modifiable risk factors include BMI (Body mass index), physical inactivity, blood pressure, smoking, alcohol, diet, and stress are modifiable.

In spite of the advancements in therapeutic strategies for T2DM, the disease is increasing at an alarming rate. Metformin is the first line pharmacologic therapy given to patients with diabetes. If HbA1C persists beyond the permissible limit, then one or two second line agents like Sulfonylureas (SU), Thiazolidinediones (TZD), Glucagon-like peptide-1 (GLP-1) analogs, DPP-4 inhibitors, basal insulin and Sodium-Glucose co-transporters-2 (SGLT2) inhibitors are added. These second line agents are prescribed based on the disease stage, patients' glycemic status and type of the drug. If the level of HbA1c still remains higher, a dual combination is recommended. In case the levels for Hb1Ac are still above the permissible limit after 3 months of dual therapy, then a third agent is added. Since many of these second line agents have been associated with adverse effects, a novel class of SGLT2 inhibitors has been developed with minimum side effects.

SGLT2 inhibition is an insulin independent approach to reduce the blood glucose levels by decreasing renal tubular glucose reabsorption (Hsia et al., 2017). Canagliflozin, dapagliflozin, empagliflozin, ertugliflozin and remogliflozin are the currently used SGLT2 inhibitors. SGLT2 inhibitors have favourable effects on body weight loss, regulation of lipid profile, maintaining blood pressure, and regulating HbA1c levels, in addition to reducing the risk of hypoglycemia (Tentolouris et al., 2019). It has also been observed that SGLT2 inhibitors have impressive cardioprotective and renoprotective effects (Tentolouris et al., 2019). However, some adverse effects, like urinary tract infection, are found to be associated with SGLT2 inhibitors (Scheen, 2019). An inter-individual difference in SGLT2 inhibitors response has been reported in diabetic patients in terms of glucose-lowering potency, anti-hyperglycemic results and adverse effects (Klen & Dolžan, 2021). In addition, the genetic architecture of individuals is associated with SGLT2 inhibitors' response. The inter-individual response to SGLT2 inhibitors has been reported to be influenced by mainly variation in genes involved in their pharmacokinetics (PK) and pharmacodynamics (PD) (Klen & Dolžan, 2021). However, pharmacogenetic/pharmacogenomic studies on SGLT2 inhibitors are very limited.

The aim of the present study was to investigate the role of SGLT2 inhibitors in controlling hyperglycemia, focusing on HbA1c and weight reduction. In addition, two genetic variants, SLC5A2 (rs9934336) and UGT1A9 (rs72551330), involved in PD and PK of the SGLT2 inhibitors, reported by previous studies, were also evaluated for their association with response to SGLT2 inhibitors in T2DM patients from Malwa region of Punjab.

1.1 Ethics Approval

The institutional Ethics Committee of the Central University of Punjab, Bathinda, approved the study on 26 December 2018 (Approval Ref. No. CUPB/IEC/2018/01).

2. Materials and Methods

2.1 Patients Recruitment:

Two hundred newly recruited T2DM patients on SGLT2 inhibitors (on monotherapy or combination therapy) evaluated at the MEDOC Department of Max Superspeciality Hospital, Bathinda (PB, India) were included in the study. The sample size was calculated using Open Epi software. The SGLT2 inhibitors prescribed to the patients included Empagliflozin (10mg) and Dapagliflozin (10mg). The patients belonged to the Malwa region of Punjab and were recruited between January 2019-August 2020. All the patients were recruited with their written informed consent. 5 ml of the blood was collected from each patient, out of which 3 ml of the blood was used for carrying out laboratory tests and 2 ml was used for DNA isolation. T2DM was confirmed by qualified endocrinologists based on the HbA1c (glycated Haemoglobin) levels as per American Diabetes Association (ADA) guidelines. According to ADA guidelines, an HbA1c level of 5.6% or less is considered non-diabetic, 5.7% to 6.4% as prediabetes, and 6.5% or more as diabetes (Association, 2020). Patients with HbA1c of more than 6.5% between the age of 30 years to 70 years and eGFR > 45ml/min/1.73m² on the standard of care were included in the study. Patients with major skeletal disorders, hepatic, cancerous diseases and neurological diseases were excluded from the study. All the recruited patients were interviewed personally, and information about their demographic details was collected in a specially designed Performa.

2.1.1 Follow-up

The follow-up of the patients was carried out with the help of the endocrinologist during their subsequent hospital visits and by telephonic interviews at an interval of 3, 6, 9, 12, 15, 18, 21 and 24 months. The Bio-Rad D-10 Haemoglobin A1c program was used for the determination of HbA1c percent using whole blood by ion-exchange chromatography. Based on the HbA1c levels and body weight changes, the patients on SGLT2 inhibitors were defined into two groups by the endocrinologist, i.e., responders and non-responders. Patients showing at least a 1% decrease in HbA1c levels and/or at least a 3 kg decrease in weight were considered

as responders or having good outcomes, whereas patients with uncontrolled levels of these parameters were categorized as non-responders or with bad outcomes.

2.2 DNA Isolation and Genotyping

SLC5A2 SNP, rs9934336 G/A (intron 1) and UGT1A9 SNP, rs72551330 T/A (missense variant) are reported by most of the studies to be associated with inter-individual response to SGLT2 inhibitors (Table 1) (Francke et al., 2015a; Hoeben et al., 2016). SLC5A2 is the receptor gene encoding sodium glucose cotransporter protein, and UGT1A9 is a metabolising gene involved in the metabolism of SGLT2 inhibitors in humans. Amplification of the specific DNA sequences bearing these SNPs was carried out by Polymerase chain reaction (PCR). PCR products were sequenced by Sanger Sequencing. Whole exome sequencing was carried out in one responder and one non-responder.

Table 1. List of gene variants selected to be screened by Sanger Sequencing

S. No.	Genes	rsIDs studied	Role in PK or PD	References
1.	SLC5A2	rs9934336	PD	(Drexel et al., 2019a; Enigk et al., 2011a)
2.	UGT1A9	rs72551330	PK	(Francke et al., 2015a; Hoeben et al., 2016)

2.3 Statistical analysis

Association of the risk factors like BMI, lifestyle, family history of diabetes, other associated diseases, alcohol consumption, diet pattern and background area (rural/urban) with responders and non-responders was evaluated by carrying out students' t-tests. Association of HbA1c levels before and after treatment with responders and non-responders were found by carrying out the Mann Whitney test since the data was skewed. This association was confirmed by Multiple logistic regression (MLR) analysis by controlling the confounding of all the risk factors stepwise. The independent variables were defined as the following dummy variables: HbA1c (0 for healthy, 1 for pre-diabetic and 2 for diabetic); background (0 for rural and 1 urban); lifestyle (0 for sedentary, 1 for moderately active and 2 for active); BMI (0 for healthy, 1 for overweight and 2 for obese); family history of diabetes (0 for no family history of diabetes and 1 for family history of diabetes); associated diseases like hypertension, CAD, thyroid, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy (0 for absence of the disease and 1 for presence of the disease); alcohol consumption (0 for non-alcoholics and 1 for alcoholics). All the statistical analysis was carried out using SPSS [Version: 28.0.1.1 (15)]. Statistical significance was defined as $p < 0.05$.

3. Results

The current study included 200 T2DM patients on SGLT2 inhibitors from the Malwa region of Punjab. The demographic data of the patients has been given in Table 2. The mean age of the patients was 53.43 years, and the gender distribution was 105:95 (Male: Female). Follow-up was carried out for all the patients based on HbA1c levels and weight. One hundred and twenty-three (61.5%) patients were found to be responders, whereas 77 (38.5%) were non-responders. The mean age of responders was 53.43 years, and of non-responders was 55.15 years. 53.66% of responders and 44.15% of non-responders were from rural whereas 46.34% of responders and 55.84% of non-responders were from urban backgrounds. 35.75% of responders and 35.06% of non-responders had a sedentary lifestyle. Obesity was found in 42.27 % of the responders and 35.06% of non-responders. As far as family history of diabetes is concerned, 71.54% of responders and 68.86% of non-responders had at least one of the family members suffering from diabetes. 27.64% of responders were alcoholics, whereas 27.27% of non-responders were alcohol consumers. 34.14% of the responders and 28.57% of non-responders were following a vegetarian diet. Hypertension was found in 46.34% of responders and 45.45% of non-responders. Other associated diseases, like coronary artery disease (CAD), was reported in 11.36% of responders and 9.6% of the non-responders. 7.31% of the responders and 7.7 % of the non-responders had associated thyroid disease. Out of the three microvascular complications associated with T2DM, Diabetic nephropathy was found to be the most common complication in the study population. 10.56% of the responders and 10.36% of the non-responders were observed to be affected with diabetic nephropathy.

No significant difference was observed in any of the risk factors in responders and non-responders. However, the non-responders had higher levels of HbA1c before as well as after the treatment. A significant difference was observed in HbA1c levels before and after treatment between responders and non-responders (Table 3). In addition, the responders had significant weight loss after the treatment (Table 4). The association was confirmed by MLR after adjusting all other confounding risk factors stepwise. The adjusted p-value for the two variables was found to be ≤ 0.001

Two SNPs, rs9934336 (SLC5A2) and rs72551330 (UGT1A9), previously reported to be associated with drug response in various studies, were screened in the study population by Sanger Sequencing. For rs9936334 (SLC5A2), all the patients were observed to have normal homozygous genotype, whereas, for rs72551330 (UGT1A9), only one of the non-responders was found to bear the

altered AA homozygous genotype. All other non-responders and responders were reported to bear wildtype homozygous genotypes. Therefore, we did not find any significant variation in these genes to be associated with inter-individual response to SGLT2 inhibitors. We also subjected one responder and one non-responder to WES. However, these were found to be homozygous normal for the tested variants as well as other variants of other genes reported to be involved in the pharmacogenetics of SGLT2 inhibitors.

Table 2: Risk Factors in Responders and Non-responders in T2DM Patients

Characteristic	Responders	Non-responders	p-value
Mean Age	53.29 years	55.09 years	
Male: Female	64:59 (123)	41:36 (77)	
<ul style="list-style-type: none"> • Rural • Urban 	66 (53.66%) 57 (46.34%)	34 (44.15%) 43 (55.84%)	0.191 0.192
Lifestyle			
<ul style="list-style-type: none"> • Sedentary • Moderate • Active 	44 (35.75%) 52 (42.25%) 27 (21.95%)	27 (35.06%) 31 (40.25%) 18 (23.37%)	0.921 0.785 0.815
BMI			
<ul style="list-style-type: none"> • Healthy • Overweight • Obese 	26 (21.15%) 45 (36.56%) 52 (42.27%)	24 (31.16%) 26 (33.76%) 27 (35.06%)	0.112 0.687 0.311
Family history of T2DM	88 (71.54%)	53 (68.85%)	0.685
Alcoholic	34 (27.64%)	21 (27.27%)	0.954
Diet Pattern			
<ul style="list-style-type: none"> • Veg 	42 (34.14%)	22 (28.57%)	0.412
Hypertension	57 (46.34%)	35 (45.45%)	0.902
CAD	14 (11.36%)	7 (9.6%)	0.695
Thyroid Disease	9 (7.31%)	6 (7.7%)	0.9188
Nephropathy	13 (10.56%)	8 (10.36%)	0.964
Retinopathy	12 (9.7%)	2 (2.59%)	0.055
Neuropathy	8 (6.56%)	6 (7.5%)	0.799

Table 3: HbA1c levels in responders and non-responders before and after the treatment in T2DM patients

HbA1c	Responders	Non-responders	P-value
Levels before Treatment	9.27 ± 1.879	8.61±1.489	0.001
Levels after Treatment	8.455 ± 1.712	9.768 ± 1.64	0.0001
P-value	0.001	0.001	

Table 4: Weight in responders and non-responders before and after the treatment in T2DM patients

Weight	Responders	Non-responders	P-value
Levels before Treatment	81.39 ± 13.49	76.40 ± 14.82	0.001
Levels after Treatment	79.05 ± 13.09	77.15 ± 15.27	0.001
P-value	0.001	0.001	

Table 5: Gene variants influencing the response to SGLT2 inhibitors

Gene	SNP	Clinical significance	References
SLC5A2	rs9934336	During the oral glucose tolerance test, the G-allele was nominally linked to higher 30-min plasma glucose, 120-min insulin concentrations, and AUC120-min(glucose) values.	(Enigk et al., 2011b)
		Decreased HbA1c levels were significantly related to the variant rs9934336.	(Drexel et al., 2019b)
		Under the dominant genetic mode, SLC5A2 rs9934336 was significantly associated with higher fasting blood sugar levels and HbA1c levels.	(Klen et al., 2020)
UGT1A9	rs72551330	In comparison to the wild type, UGT1A9*3 carriers had higher plasma canagliflozin exposure (C _{max,ss} , 11%; AUC _{ss} , 45%).	(Francke et al., 2015b; Hoeben et al., 2016)
UGT2B4	UGT2B4*2	Participants with the UGT2B4*2 genotype had higher plasma canagliflozin exposure (C _{max,ss} , 21%; AUC _{t,ss} , 18%) than UGT2B4*2 noncarriers.	(Francke et al., 2015b)
PNPLA3	rs738409 (C>G)	The liver PDFF reduction was greater in the dapagliflozin plus omega-3 carboxylic acids treatment group (CG/GG genotype) and less in the dapagliflozin alone treatment group.	(Eriksson et al., 2018a)
WFS1	rs10010131	Bodyweight loss with DAPA + ExQW was associated with the SNP variant rs10010131 A allele, lower baseline adiposity (BMI), and lower baseline insulin secretion (IGI)	(Pereira et al., 2018a)

4. Discussion

As per the reports of the World Health Organization (WHO), diabetes led to 1.6 million deaths globally in 2019, and this number is estimated to increase to 592 million by the year 2035 (Tao et al., 2015). Diet and exercise are recommended initially for T2DM patients. Patients are prescribed oral antidiabetic drugs based on their clinical profile if diet and exercise prove insufficient for glycemic control.

SGLT2 inhibitors are anti-diabetic drugs with a distinguishable mode of action that reduce blood glucose independent of insulin. This treatment strategy holds many benefits, including weight loss, cardiovascular and reno-protective effects and prevention of hypoglycemia as compared to other oral anti-diabetic drugs.

Based on the number of patients reporting in the study hospital (Max Superspeciality Hospital, Bathinda, Punjab), T2DM seems to be quite prevalent in the Malwa region of Punjab. A total of 200 T2DM patients were recruited for the study. The mean age of the diabetic patients was 53.43 years which is consistent with the mean age (53 years) of the T2DM patients reported in a study carried out by Yoo et al. (2019) (Yoo et al., 2019).

Studies have reported that SGLT2 inhibitors administration is associated with a decrease in weight upto 1.5-2 kgs. Body weight loss in response to SGLT2 administration is attributed to two major consequences of SGLT2 inhibition: caloric loss through the excretion of glucose (upto 60g/day) and water loss via osmotic diuresis. Additionally, a series of metabolic processes resulting in lipolysis and lipid oxidation are triggered by a rise in glucagon concentration following lowered insulin and blood sugar levels (figure 1) (Janež & Fioretto, 2021; Pereira & Eriksson, 2019). Furthermore, SGLT2 inhibitors elevate glucose excretion via urine by reducing the renal threshold for glucose and reabsorption of glucose. SGLT2 inhibitors are reported to lower the HbA1c levels by 0.6-1% by this mechanism. The shift in metabolism to gluconeogenesis and ketosis plays a significant role in kidney and heart protection. Moreover, in renal tubular cells, SGLT2 inhibitors can decrease glucotoxicity by decreasing mitochondrial dysfunction and inflammation. They also lower renal hypoxia by minimizing the oxygen requirements and tubular energy (figure 1) (Fonseca-Correa & Correa-Rotter, 2021; Saisho, 2020).

In the current study, no significant difference was observed between the demographic profile of the responders and non-responders. However, a significant difference in HbA1c levels and weight was observed in responders before as well as after the treatment with SGLT2 inhibitors. The responders showed a significant decrease in HbA1c levels and weight after treatment. This is consistent with previous studies reporting the efficiency of SGLT2 inhibitors in reducing body weight and HbA1c levels (Bashier et al., 2017; Tamez-Perez et al., 2017).

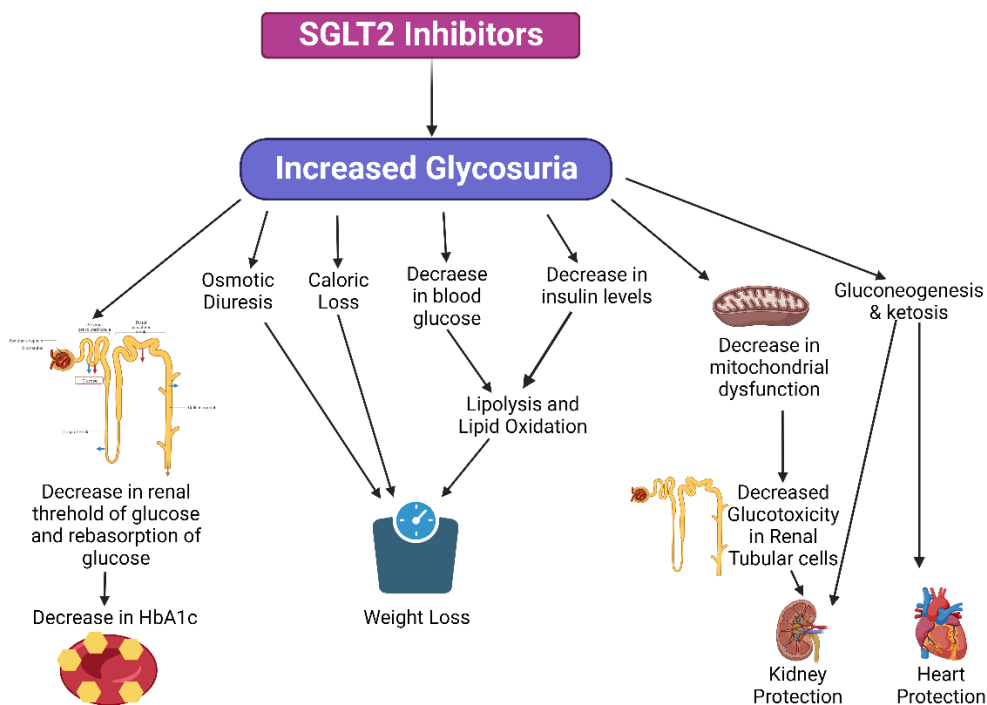


Figure 1. Mechanism by which SGLT2 inhibitors led to increased glycosuria and decreased HbA1c levels and body weight. Increased glycosuria leads to protective cardio-renal effects.

Different patients have been reported to respond to SGLT2 inhibitors in different ways. The inter-individual variation in response to these drugs has also been attributed to genetic variation as well. Very few pharmacogenetic/genomic studies have been carried out on SGLT2 inhibitors evaluating the effect of genetic alterations on drug response. Table 5 enlists the pharmacogenetic studies

carried out on SGLT2 inhibitors to date. The SLC5A2 gene is situated on chromosome 16p11. 2. The gene encodes SGLT2, which is responsible for the reabsorption of glucose from urine. SNPs in SLC5A2, including rs9934336 G>A, rs3116150 G>A, rs3813008 G>A, rs11646054 G>C, and rs3116149 G>A, have been reported to affect the PD of SGLT2 inhibitors (Kaur et al., 2021). According to Enigk et al. (2011), the G-allele of rs9934336 (SLC5A2) was found to have a nominal association with higher 30-min plasma glucose and 120-min insulin concentration and area under the curve (AUC) 120-min during oral glucose tolerance test. UGT1A9 is the enzyme that primarily breaks down SGLT2 inhibitors, and certain UGT1A9 SNPs have been found to alter the enzyme's metabolic activity for some substrates (Naagaard et al., 2022). A significant SNP in UGT1A9 gene rs72551330 T>C has been reported to affect the PK of SGLT2 inhibitors. Francke et al. (2015) reported that UGT1A9*3 carriers exhibited increased exposure to plasma canagliflozin as compared to wild type (Francke et al., 2015a; Hoeven et al., 2016). Studies have also reported the role of rs100010131 of the WFS1 gene and rs738409 of the PNPLA3 gene in the pharmacogenetics of SGLT2 inhibitors, as summed up in Table 5 (Eriksson et al., 2018b; Pereira et al., 2018b).

Personalised medicine, on account of different genetic makeup, holds the potential to ameliorate the high-cost and hit & trail method of drug prescription in various complex disorders, including T2DM. Two significant variants, rs9934336 (SLC5A2) and rs72551330 (UGT1A9), reported previously to be involved in PD and PK of the SGLT2 inhibitors, respectively, did not show any significant association with drug response in the studied population. The results of our study are in accordance with the previous study carried out by Zimdahl et al. (2017) in Tubingen Family and Ordelheide et al. (2017), who also couldn't observe the variation in the SLC5A2 gene in affecting inter-individual response to SGLT2 inhibitors. Zimdahl et al. (2017) observed in a cross-sectional study that none of the SNPs in the SLC5A2 gene had any significant effect on any of the metabolic traits, including body fat, HbA1c, plasma glucose or systolic blood pressure insulin sensitivity/resistance, insulin release. Furthermore, SNPs tested in the study were not found to have any relevant influence on response to SGLT2 inhibitor treatment in terms of HbA1c and weight loss (Zimdahl et al., 2017). Another study by Ordelheide et al. (2017) observed that the SNPs tested in the SLC5A2 gene were not significantly associated with plasma glucagon concentrations at any time (Ordelheide et al., 2017).

The current study was the first in India to find the therapeutic outcome of SGLT2 inhibitors in responders in comparison with non-responders focusing on HbA1c levels and weight loss. In addition, this study is the first study from India evaluating the association of genetic variation with inter-individual response to SGLT2 inhibitors in the population from the Malwa region of Punjab. The limitation of the study is that only 200 patients could be enrolled for the current study.

Future studies with larger cohort sizes can frame a better understanding of the pharmacogenetics of SGLT2 inhibitors.

5. Conclusion

The Use of SGLT2 inhibitors is an insulin-independent approach against hyperglycaemia for the treatment of diabetic patients. The current study was carried out with the objective to evaluate the effect of SGLT2 inhibitors in responders as well as non-responders on their HbA1c levels and weight loss. Further, two significant variants, SLC5A2 (rs9934336) and UGT1A9 (rs72551330), previously reported to be associated with inter-individual variation in response to SGLT2 inhibitors were also evaluated to find out their association with the drug response. We found a significant decrease in HbA1c levels and weight in responders after the treatment with SGLT2 inhibitors adding to the reported action of these drugs in reducing the HbA1c levels and weight. However, we could not establish the role of two variants, SLC5A2 (rs9934336) and UGT1A9 (rs72551330), with inter-individual response to SGLT2 inhibitors in the Malwa region of Punjab, although 38.5 % of the patients were found to be non-responders. Therefore, there might be other factors affecting the inter-individual response to SGLT2 inhibitors which remain to be explored by future studies.

Nevertheless, the current study is in consensus with previous existing studies, which also observed no association of variations in studied genes to be significantly associated with response to SGLT2 inhibitors. Our study was the first of its kind in the studied population as well as India evaluating the role of SGLT2 inhibitors in diabetes management in context with HbA1c levels and weight loss. Therefore, the current study concludes that no pharmacogenetic testing for these two genes is required before prescribing SGLT2 inhibitors to T2DM patients from this region. However, the pharmacogenetics of SGLT2 inhibitors has not been explored at length. Therefore, future studies should be carried out focusing on all the genes involved in PK and PD of SGLT2 inhibitors. Future research involving a larger cohort size might help to provide a clearer picture of the pharmacogenetics of SGLT2 inhibitors.

Statements & Declaration:

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Conflicts of Interest: All the authors declare that they have no conflicts of interest

Data and Material Availability: The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval: The institutional committee of the Central University of Punjab, Bathinda, has approved the study.

Research involving human participants and/ or animals: The research study involves human blood samples

Consent to Publication: Written informed consent was taken from all the participants included in the study.

Consent to Participate: Written informed consent was taken from all the participants included in the study

Author's Contribution: The research study was designed by PK, SK, LT and AM. Sample collection was done by PK with the help of SK and LT. Experimental work was carried out by PK. Statistical Analysis was carried out by PK and AL. Compilation of the results and manuscript was prepared by PK and AM. The manuscript was critically revised and edited by AM and SK.

“Compliance with Ethical Standards”

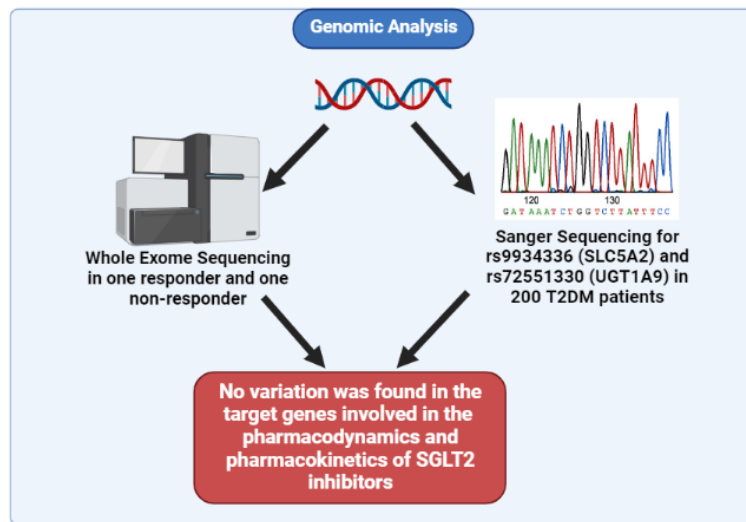
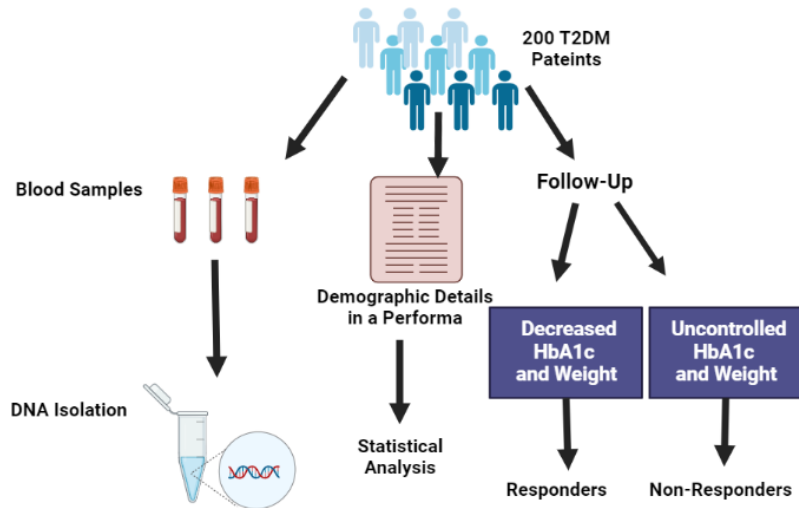
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Appendix

Graphical Abstract



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