

## Original Article

# Epigenetic changes of sodium-glucose co-transporter 2 inhibitors in patients with type 2 diabetes mellitus

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## ABSTRACT

**Objectives:** The sodium-glucose co-transporter 2 (SGLT2) inhibitors have been found to possess cardioprotective and renoprotective effects in studies conducted recently. These beneficial effects may be due to the epigenetic modifications caused by the SGLT2 inhibitors. Metformin's effects on DNA methylation have already been reported to produce both hypomethylation and hypermethylation. However, the effects of SGLT2 inhibitors on DNA methylation have not been thoroughly researched. Therefore, the purpose of the study was to ascertain whether type 2 diabetic patients taking SGLT2 inhibitors had changed DNA methylation.

**Materials and Methods:** The patients on metformin and glimepiride treatment with poor glycaemic control were administered SGLT2 inhibitors as an add-on therapy. The blood samples for the determination of DNA methylation were collected before adding the SGLT2 inhibitors, and blood samples to determine DNA methylation were also recollected after 24 weeks of adding SGLT2 inhibitors. A total of 34 patients were analysed upon completion of the study.

**Results:** DNA methylation of the patients receiving metformin and glimepiride treatment was increased from 3.09 as the baseline value to 4.24 when the patients were added with SGLT2 inhibitors as add-on therapy for 24 weeks. The glycaemic parameters, which include mean fasting plasma sugar (mg/dL), postprandial plasma sugar (mg/dL) and haemoglobin A1C (%), were changed from 172.47, 249.09 and 8.37 to 131.47, 191.71 and 7.35 ( $P < 0.001$ ), respectively.

**Conclusion:** DNA methylation was significantly increased after adding 24 weeks of therapy with SGLT2 inhibitor medication to the patients already taking metformin and glimepiride for 24 weeks.

**Keywords:** DNA methylation, Epigenetics, Metformin, Sodium-glucose co-transporter 2 inhibitors, Type 2 diabetes mellitus

## INTRODUCTION

In 2021, the International diabetes federation (IDF) predicted that 537 million people worldwide suffer from diabetes.<sup>[1]</sup> Patients with diabetes may develop microvascular, macrovascular and neurological complications.<sup>[2]</sup> It is commonly acknowledged that particular genetic and epigenetic variables increase the risk of developing diabetes. Major  $\beta$ -cell differentiation-controlling genes are epigenetically controlled; they include the glucagon-like peptide 1 receptor, paired box gene 4 and pancreatic and duodenal homeobox 1.<sup>[3]</sup>

Oral drugs known as sodium-glucose co-transporter 2 (SGLT2) inhibitors are prescribed to treat type 2 diabetes mellitus (T2DM).<sup>[4]</sup> SGLT2 inhibitors (dapagliflozin, canagliflozin, empagliflozin,

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etc.) are gaining popularity due to their insulin-independent mechanism of action and favourable cardiovascular profile.<sup>[5]</sup>

The study of mitotically (and potentially meiotically) heritable alterations in gene expression that is not brought on by variations in DNA sequence is known as epigenetics.<sup>[6]</sup> These changes occur regularly and naturally, but they can also be influenced by several factors such as age, environment/lifestyle, medical condition and medications. The two most important epigenetic regulators are histone post-translational modifications and DNA methylation.<sup>[7]</sup>

Metformin-related effects and changes are one of the most frequently researched epigenetic changes.<sup>[8]</sup> Its effects on DNA methylation have been reported to include both hypomethylation and hypermethylation at various promoter regions of a gene. There is proof that these modifications affect gene expression and the epigenome, which may help explain why metformin has antidiabetic effects alongside its protective effects against ageing, cancer, cardiovascular disease and cognitive decline.<sup>[9]</sup>

A particular class of medications known as sodium-glucose co-transporter inhibitors, which are used to treat patients with type 2 diabetes, has drawn attention because of its beneficial cardiovascular effects. The effects of SGLT2 inhibitors on epigenetics, in particular DNA methylation, are not well understood. Thus, the purpose of this investigation was to find whether SGLT2 inhibitors can trigger DNA methylation.

## MATERIALS AND METHODS

This perspective and the open-level study were carried out at the referral hospital in northern India from November 2021 to December 2022. Eligible patients were enrolled in the study according to inclusion criteria following receipt from the Institutional Ethics Committee (Ref No: IECJNMC/536). To participate in the study and use patient data for research and educational purposes, written informed consent was required. The study's methods adhere to the principles stated in the Declaration of Helsinki 2013.

### Inclusion and exclusion criteria

Patients between the ages of 45–60 years with T2DM and poor glycaemic control on metformin and glimepiride (sulfonylurea) and who were added with SGLT2 inhibitors were incorporated in the study.

Patients on insulin, other injectable antidiabetic agents, corticosteroids, history of type 1 diabetes, diabetic ketoacidosis, liver disease, lung disease, septicaemia and psychotic patients were not included in the study.

### Sample size

The sample size was calculated as the sample size of convenience and the cost of investigation ( $n = 34$ ).

### Study participants

Patients with poor glycaemic control on metformin and glimepiride were added with SGLT2 inhibitors by the treating endocrinologist.

### 5 mC and 5 hmC quantitation

A commercial kit was utilised to extract genomic DNA (GSure<sup>®</sup> Blood DNA Mini extraction Kit-GCC, Cat. No. G4625). DNA's integrity and purity were evaluated by spectrophotometer on a Thermo Scientific<sup>™</sup>  $\mu$ Drop<sup>™</sup> Plate. Using commercial kits, the levels of global DNA methylation and hydroxymethylation were determined from MethylFlash<sup>™</sup> Methylated 5 mC DNA Quantification (Colorimetric) (EpigenTek, Cat.No.- P-1034).

100 ng of purified DNA from each sample was added to an ELISA plate to detect methylated DNA using capturing and detecting antibodies. Absorbance was measured at 450 nm on a microplate reader within 2–15 min.

### Statistical analysis

The Statistical Package for the Social Sciences, version 23, an MS Excel spreadsheet from IBM Corp., was used to code and store the data. When appropriate, data were displayed graphically using box-and-whisker plots, histograms and pie charts for categorical data. An independent sample  $t$ -test was used to compare two groups in group comparisons for continuously dispersed data.  $P < 0.05$  was maintained for statistical significance.

## RESULTS

A total of fifty patients were enrolled in the study. On follow-up till 24 weeks, 16 patients were withdrawn from the study (ten lost to follow-up, four were prescribed with other antidiabetic drugs and two developed urinary tract infections). Therefore, 34 patients who had completed the study were finally analysed.

1. Socio-demographic and baseline parameters of patients with T2DM ( $n = 34$ ) [Table 1]
2. Change in DNA methylation over time of 24 weeks [Figure 1].

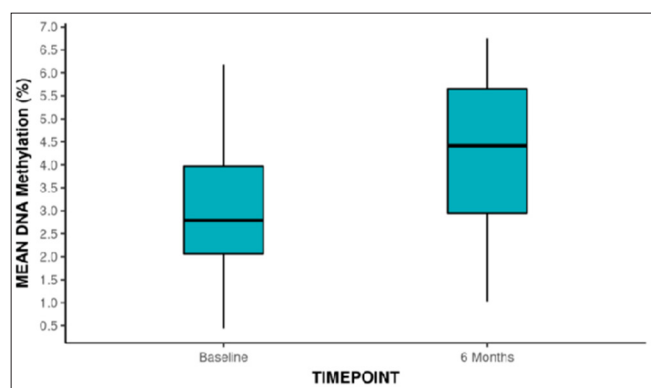
The mean DNA methylation (%) improved from a minimum of 3.09 at the baseline time point to 4.24 at the 24-week time point. This alteration was statistically significant (paired  $t$ -test:  $t = -7.0$ ,  $P \leq 0.001$ ) [Figure 1].

3. Association between DNA methylation (%) and changes in glycaemic parameters over a time of 24 weeks [Figures 2-5].

There was no statistically significant correlation between DNA methylation (%) (24 weeks) with other parameters (body mass index [BMI]:  $P = 0.295$ , fasting plasma sugar [FPS] [mg/dL]:  $P = 0.506$ , postprandial plasma sugar [PPPS] [mg/dL]:  $P = 0.590$ , haemoglobin A1C [HbA1c] [%]:  $P = 0.920$ ), respectively, in Figures 2-5.

Variables	Baseline
Gender	
Male: Female	17: 17
Age	
45-50 years: 51-60 years	10: 24
Body mass index (Kg/m <sup>2</sup> )	24.47±2.26
Fasting Plasma Sugar (mg/dl)	172.47±55.71
Post Prandial Plasma Sugar (mg/dl)	249.09±74.95
HbA1c (%)	8.37±1.22
Serum Urea (mg/dl)	0.96±0.34
Serum Creatinine (mg/dl)	28.53±5.97
Systolic Blood Pressure (mmHg)	132.0588±1.2
Diastolic Blood Pressure (mmHg)	79.6176±1.552

BMI: Body mass index, HbA1c: Haemoglobin A1C, S. Urea: Serum urea, S. Creatinine: Serum creatinine. This table clearly shows the basic health information of the patients as baseline parameters. Body mass index, Fasting plasma glucose, Post prandial plasme glucose, HbA1c, S. Urea, S. Creatinine, Systolic Blood Pressure and Diastolic BP are all values expressed in Mean ± Standard deviation.



**Figure 1:** The observed improvement in DNA methylation levels (mean increase from 3.09 to 4.24) over the study period of 24 weeks. It highlights the statistical significance of this change ( $P < 0.001$ ) and attributes the improvement to the effect of sodium-glucose co-transporter 2 inhibitor therapy, as demonstrated in the paired  $t$ -test ( $t = -7.0$ ). In the Box-and-Whisker plot, the center line of each box indicates the median DNA methylation (%). The top and bottom edges of the box represent the 75<sup>th</sup> and 25<sup>th</sup> percentiles, respectively.

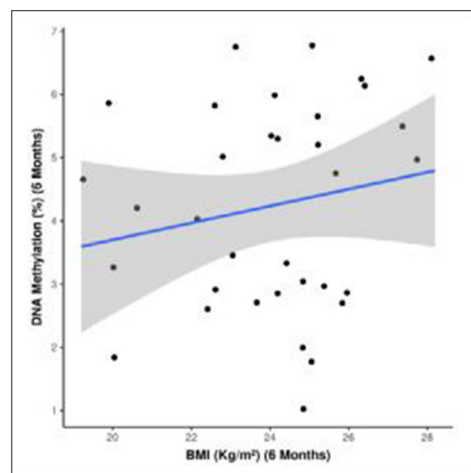
The two variables' overall trend of correlation is shown by the blue trendline. The 95% confidence interval for this trendline is shown by the shaded grey area.

4. Changes in BMI, glycaemic variables and renal function tests over 24 weeks [Table 2].

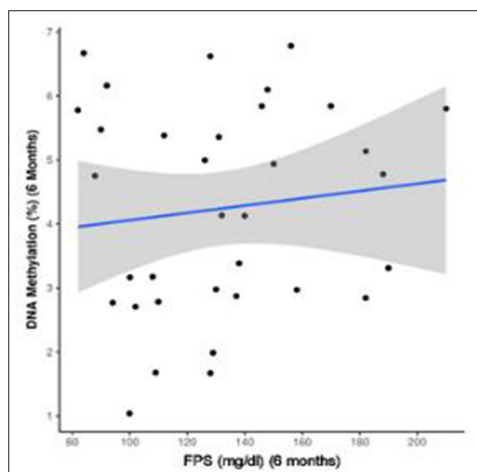
## DISCUSSION

Recently, SGLT2 inhibitors have demonstrated cardioprotective and renoprotective effects. Epigenetic elements such as DNA methylation and histone post-translational changes may be the cause of these protective effects.<sup>[10]</sup>

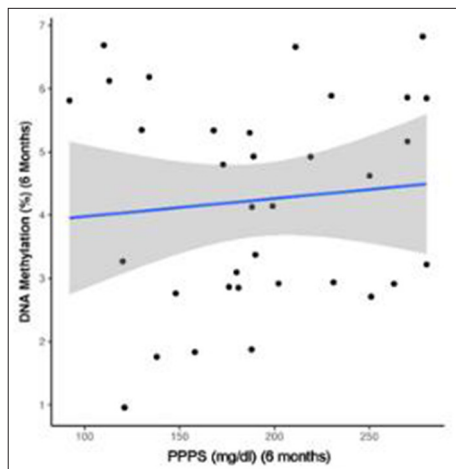
The main finding of this study was the increased mean DNA methylation (%) from a minimum of 3.09 at the baseline



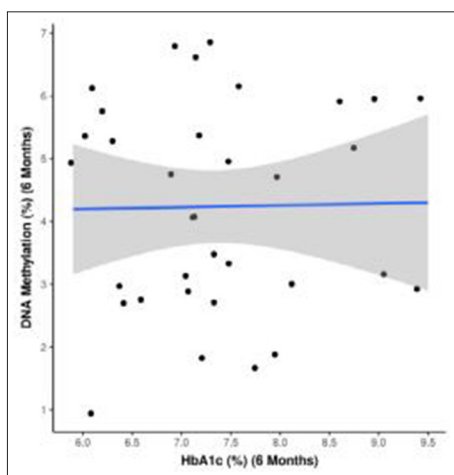
**Figure 2:** The scatter plot illustrates the association between DNA methylation (%) and BMI (kg/m<sup>2</sup>). BMI: Body mass index



**Figure 3:** The scatter plot illustrates the association between DNA methylation (%) and fasting plasma sugar (FPS) (mg/dL).



**Figure 4:** The scatter plot illustrates the correlation between DNA methylation (%) and post-prandial plasma sugar mg/dL.



**Figure 5:** The scatter plot illustrates the correlation between DNA methylation (%) and haemoglobin A1C (%).

timepoint (metformin and glimepiride combination) to a maximum of 4.24 (metformin, glimepiride and SGLT2 inhibitors combination) over 24 weeks, and the mean absolute change observed was +1.15 at the 24-week timepoint.

SGLT2 inhibitors' beneficial effects, such as cardioprotective and renoprotective effects, are not fully explained by their known anti-diabetic mechanism. Scisciola *et al.*, in 2022, have shown that empagliflozin-induced hypermethylation may cause gene silencing effects, and it may be an explanation for its antioxidant and anti-inflammatory effects in cardiac myocytes, resulting in its cardioprotective effects.<sup>[11]</sup> SGLT2 inhibitors elevated plasma and tissue levels of the ketone 3-hydroxybutyric acid, which in turn causes  $\beta$ -hydroxybutyrylation of H3 at Lys9 of the adiponectin gene

in adipocytes irrespective of their acetylation or methylation, which may be the reason behind its renoprotective effects.<sup>[12]</sup>

Genomic and global hypermethylation have been recognised as a cause of oncogenesis, cancer and occurrence of metastasis;<sup>[13]</sup> therefore, it may be suggested that hypermethylation caused by SGLT2 inhibitors may provide protective effects against the cancer.

Hyperglycaemia contributes to cancer development and progress; therefore, SGLT2 inhibitors may protect against cancer by decreasing glucose levels. Diabetes and cancer patients share a common pathophysiological feature: inflammation. Inflammation linked to elevated glucose levels makes the microenvironment more susceptible to tumorigenesis, which increases the risk of malignant tumour formation by altering and maintaining a pathological state in tissues. Glycaemic control with SGLT2 inhibitors and reduced inflammation with DNA hypermethylation have both been linked to favourable cancer outcomes.<sup>[14]</sup>

Specific alterations in the 5-methylcytosine content are a part of ageing and age-related illnesses, which are often characterised by promoter-specific hypermethylation and genome-wide hypomethylation<sup>[15]</sup> so SGLT2 inhibitors are likely to be associated with anti-ageing effects.

One study done by Tseng *et al.*, 2014 showed a significant decrease in DNA methylation in patients with Severe Major Depressive Disorder (MDD) compared to healthy control.<sup>[16]</sup> so there is also some chance that SGLT2 inhibitors are protective against Alzheimer's disease and MDD.

The individuals with a suicide attempt history, however, had global DNA methylation levels that were noticeably greater than those of the controls.<sup>[17]</sup>

In the present study, mean BMI ( $\text{Kg/m}^2$ ) decreased from 24.47 to 24.03 at the 6-month timepoint (absolute change  $-0.44$ ) ( $P < 0.001$ ). These results are similar to the observation obtained from the study in a meta-analysis conducted by Cheong *et al.*, 2022. They have shown that SGLT2 inhibitors reduced the mean BMI ( $\text{Kg/m}^2$ ) in patients by  $-0.71$ .<sup>[18]</sup> It is unclear exactly how SGLT2 inhibitors lower body weight, but new research indicates that two main effects of the drug – calorie loss from excretion of glucose and water loss through osmotic diuresis – are responsible for weight loss.<sup>[19]</sup>

The mean FPS (mg/dL) decreased from 172.47 at the baseline to 131.47, and the mean percent change was  $-21.1\%$  at the 24-week time point.

Mean PPS (mg/dL) decreased from 249.09 at the baseline time point to 191.71 at the 24-week time point. Similar observations were also reported by Bhosle *et al.*, (2022) where the levels of PPS (mg/dL) decreased from 287.72 to 203.71 and 289.23 to 205.44 by empagliflozin and dapagliflozin in 24 weeks, respectively.<sup>[20]</sup>

**Table 2:** Changes in BMI, glycaemic variables (fasting plasma glucose, post-prandial plasma glucose, HbA1c) and renal function tests (Serum urea, Serum creatinine) over 24 weeks.

Variables	Baseline	At 24-weeks	Absolute Change	p-Value
Body mass index (Kg/m <sup>2</sup> )*	24.47±2.26	24.03	-0.44	<0.001
Fasting Plasma Glucose (mg/dl) *	172.47±55.71	131.47	-41.00	<0.001
Postprandial Plasma Glucose (mg/dl)*	249.09±74.95	191.71	-57.38	<0.001
HbA1c (%)*	8.37±1.22	7.35	-1.02	<0.001
Serum Urea (mg/dl)	0.96±0.34	27.88	-0.65	0.502
S. Creatinine (mg/dl)	28.53±5.97	0.85	-0.11	0.161

This table represents the mean values and standard deviations for each parameter at baseline and week 24. The p-values indicate significant improvements in all measured parameters over the 24-week period. \*Statistically significant at  $P < 0.05$ . HbA1c: Glycated hemoglobin

The mean HbA1c (%) decreased from 8.37 at the baseline time point to 7.35 and the total mean absolute change was -1.02 % at the 24-week time point. These results are steady with the identifying of Schork *et al.*, (2019) and Bashier *et al.*, (2017), when they were study was done on SGLT2 inhibitors for 24 weeks in which they found the reduction in HbA1c by 0.8 and 0.9 %, [21,22]

## CONCLUSION

Epigenetics is a fast-emerging science associated with several diseases, including T2D. The frequently prescribed anti-diabetic medication SGLT2 inhibitors can modify a variety of epigenetic mechanisms that may support its core hypoglycaemic activity but may also have off-target effects that may be favourable or possibly detrimental. The activity of numerous distinct epigenetic modifying enzymes can be regulated by SGLT2 inhibitors. In the present study, the use of SGLT2 inhibitors over 6 months has significantly increased the whole blood DNA methylation. DNA hypermethylation caused by SGLT2 inhibitors has cardioprotective effects and renoprotective effects. In addition, it is capable of preventing a plethora of diseases, including cancer, autoimmune disorders, Alzheimer's disease and severe major depression disorders and also delays the ageing process. Further detailed studies are suggested to understand the role of DNA methylation, pleiotropic effects and long-term side effects of SGLT2 inhibitors.

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**Use of artificial intelligence (AI)-assisted technology for manuscript preparation:** The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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