Section: Medicine

Original Research Article

GLOBAL DNA METHYLATION AS A BIOMARKER IN DIABETIC KIDNEY DISEASE: A CROSS-SECTIONAL STUDY

Syed Hasan Amir[1], Khwaja Saifullah Zafar[2], Kaynat Khan[3], Sheelu Shafiq Siddiqi[4]

[1] Associate Professor, Department of Medicine, Jawaharlal Nehru Medical College, AMU, India.
2 Professor, Department of Medicine, Jawaharlal Nehru Medical College, AMU, India.
3 Junior Resident, Department of Medicine, Jawaharlal Nehru Medical College, AMU, India.
4Professor Rajiv Gandhi Centre for Diabetes and Endocrinology, AMU, India.

Received : 10/08/2025
Received in revised form : 02/10/2025
Accepted : 18/10/2025

Keywords:
Diabetic Kidney Disease (DKD), Global DNA Methylation, Epigenetics, 5-mC, Methylation Risk Score.

Corresponding Author:
Dr. Kaynat Khan,
Email: Kaynatk1234@gmail.com

DOI: 10.47009/jamp.2026.8.1.24

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026;8 (1); 115-119

ABSTRACT

Background: Diabetic kidney disease (DKD) is a major early cause of chronic kidney failure worldwide and remains difficult to predict despite advances in various investigations. Increasing evidence suggests that long-lasting epigenetic alterations sustain renal injury independent of current metabolic control. Among these, global DNA methylation—quantified as 5-methylcytosine (5-mC)—represents a stable marker of cumulative metabolic stress and may provide early insight into DKD pathogenesis. **Objective:** To quantify global DNA methylation in patients with DKD, compare it with individuals with diabetes but no kidney disease and with healthy controls, and evaluate a composite DKD-Methylation Risk Score (DKD-MRS) for diagnostic performance. **Materials and Methods:** In this cross-sectional study at a tertiary-care center, 44 DKD, 44 non-DKD diabetic, and 10 healthy control participants were enrolled. Global 5-mC was measured using an ELISA-based assay, and renal as well as metabolic parameters were recorded. Group differences were tested with distribution-appropriate statistics, and receiver operating characteristic (ROC) analysis with validation assessed discriminatory accuracy. **Result:** Mean global 5-mC (%) was 5.2 ± 0.6 in DKD, 1.5 ± 0.6 in non-DKD diabetes, and 1.2 ± 0.5 in controls. DKD participants also had lower eGFR (44.3 ± 6.2 mL/min/1.73 m²) and higher UACR (264.5 ± 48.2 mg/g) compared with both comparison groups (all $p < 0.001$). A 4.50 % 5-mC threshold discriminated DKD with AUC 0.98 (95 % CI 0.95–1.00), sensitivity 93 %, and specificity 97 %. The exploratory DKD-MRS achieved AUC 0.91 (95 % CI 0.85–0.97). **Conclusions:** Global 5-mC is markedly elevated in DKD and provides excellent discrimination from diabetic and non-diabetic controls. These findings support global DNA methylation as a potential biomarker of DKD burden and warrant validation in larger prospective cohorts.

INTRODUCTION

Diabetes is a major public health crisis in India. As per the International diabetes federation (IDF) India rank second globally in the number of adult with diabetes with an estimated 89.8 million cases reported in 2024[1]. Diabetic kidney disease (DKD) is one of the most serious complications of type 2 diabetes mellitus (T2DM), accounting for nearly half of all cases of end-stage kidney disease worldwide.[2,3] Despite modern glucose-lowering therapies and intensive blood-pressure control, up to 30–40 % of people with T2DM develop DKD and many continue to lose kidney function even after achieving recommended metabolic targets.[4,5] Persistent albuminuria and progressive decline in GFR characterize DKD clinically in which damage to kidney has already occurred. This disconnect between current risk-factor management and clinical outcomes highlights the need for biomarkers that can identify high-risk patients earlier and reveal disease mechanisms that persist beyond conventional metabolic control.

Accumulating evidence implicates epigenetic regulation as a key driver of the "metabolic memory" phenomenon—continued tissue injury despite improved glycemic status.[6-8] Epigenetic modifications alter gene expression without changing the DNA sequence and can be stable over time, thereby recording past environmental and metabolic exposures. Key epigenetic mechanism includes DNA methylation, histone modification including acetylation and non coding RNA including micro RNA.Among these modifications, DNA methylation

ISSN (O): 2687-5365; ISSN (P): 2753-6556

is particularly well studied. The addition of a methyl group to cytosine residues within CpG dinucleotides can silence or activate gene transcription and has been linked to fibrosis, inflammation, and oxidative stress, all central to DKD pathogenesis.[9-11]

Previous work has documented locus-specific methylation changes in genes such as TGF-β1, Klotho, and PGC-1α, as well as global methylation shifts in experimental models of diabetic nephropathy.[12-14] However, most studies originate from Western populations and focus on genome-wide arrays or single-gene assays that require specialized infrastructure. Data from South Asian cohorts—where diabetes occurs at younger ages and DKD develops at lower body-mass indices—remain sparse, and the potential utility of a simple, integrative marker such as global DNA methylation (5-methylcytosine, 5-mC) has not been systematically examined.

To address this gap, we therefore quantified global DNA methylation in Indian patients with DKD and evaluated its associations with renal function, glycemic control, and duration of diabetes. Building on these analyses, we further assessed the diagnostic performance of global 5-mC and a composite DKD-Methylation Risk Score (DKD-MRS) for identifying DKD. By focusing on an Indian population with high diabetes prevalence and early onset of renal complications, this study seeks to clarify the role of global DNA methylation in DKD and to assess its potential as a clinically applicable biomarker.

MATERIALS AND METHODS

Study Design and Setting

This was a **cross-sectional, single-center study** conducted in the Departments of Medicine and Nephrology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, India, between **March 2023 and March 2025**. The study was approved by the Institutional Ethics Committee (Approval No. [insert ID]) and adhered to the **Declaration of Helsinki** and **STROBE** guidelines. A minimum of 40 participants per group was estimated to provide 80% power to detect a 1.0% difference in global DNA methylation (SD 0.8%) at $\alpha = 0.05$.

Participants

Adults aged **30–65 years** with type 2 diabetes mellitus (T2DM) were screened. Participants were classified into:

1. **DKD group (n = 44):** T2DM with **two separate spot UACR >30 mg/g (≥90 days apart if available)** or **eGFR <60 mL/min/1.73 m²** persisting for ≥3 months.
2. **Non-DKD diabetes group (n = 44):** T2DM with **UACR <30 mg/g** and **eGFR ≥60 mL/min/1.73 m²**.
3. **Healthy controls (n = 10):** Age- and sex-matched individuals without diabetes or kidney disease.

Exclusion criteria were type 1 diabetes, non-diabetic kidney disease, acute kidney injury, urinary infection, nephrotic syndrome, pregnancy, malignancy, or recent acute illness. **Written informed consent** was obtained from all participants, and numbers screened, excluded, and enrolled were documented.

Data Collection and Laboratory Methods

Demographics, duration of diabetes, comorbidities, and treatment history were recorded using a structured questionnaire. Duration of diabetes was confirmed from medical records when available.

Venous blood samples were obtained **after an overnight fast (08:00–10:00 h)**. Serum creatinine, HbA1c, lipid profile, calcium, phosphorus, and uric acid were measured by automated assays. At least two separate Spot urine samples were collected at different occasions t and were used for UACR calculation.

Genomic DNA was extracted from peripheral leukocytes using the **G-sure™ Blood DNA Isolation Kit (Genetix, India)**. Purity was assessed by **NanoDrop 2000 spectrophotometry**; samples with **A260/280 ratio <1.8 or >2.0** were re-extracted. Global DNA methylation was quantified as **percent 5-methylcytosine (5-mC) of total cytosine** using the **Zymo Research 5-mC ELISA Kit**.[15]. All assays were performed by the principal investigator, run in **duplicate**, and included kit-supplied positive and negative controls. Intra- and inter-assay coefficients of variation were **3.2% and 4.5%**, respectively.

Variables and Definitions

- eGFR was calculated using the **CKD-EPI 2021 equation**. Albuminuria categories were defined as normoalbuminuria (<30 mg/g), microalbuminuria (30–299 mg/g), and macroalbuminuria (≥300 mg/g).
- **Global methylation:** reported as percent 5-mC.
- **Metabolic variables:** HbA1c, lipid profile, uric acid, calcium, and phosphorus.
- **Microvascular complications:**
  - **Retinopathy:** ophthalmologist-diagnosed (any NPDR or PDR) by fundus exam or imaging.
  - **Neuropathy:** clinical diagnosis based on monofilament/vibration testing or neurologist documentation.
- **Exploratory DKD-Methylation Risk Score (DKD-MRS):** composite of global 5-mC, UACR, eGFR, HbA1c, and diabetes duration.

Statistical Analysis

Analyses were performed using **R (v4.3)** and **SPSS v29**. Continuous variables were tested for normality with the **Shapiro–Wilk test**. Normally distributed variables are expressed as **mean ± SD** and compared using **Welch's ANOVA** with **Games–Howell** post-hoc tests. Non-normal variables (UACR, 5-mC) are expressed as **median [IQR]** and compared with the **Kruskal–Wallis test** and **Dunn's** post-hoc. Categorical variables are presented as **n (%)** and compared with **χ^2** or **Fisher's exact test**.

Effect sizes with **95% confidence intervals (CIs)** were calculated: **Hedges g** for parametric contrasts, **rank-biserial r** for non-parametric, and **Cramer's V**

ISSN (O): 2687-5365; ISSN (P): 2753-6556

for categorical variables. Associations between continuous variables were analyzed with **Spearman's correlation (ρ)** and 95% CIs.

Diagnostic performance of global 5-mC and the DKD-MRS was evaluated using **receiver operating characteristic (ROC) analysis** with **AUC and 95% CIs (DeLong method)**. Cut-offs were identified by the **Youden index**. Model calibration was assessed using intercept and slope estimates. All tests were **two-sided** with **α = 0.05**, and multiple comparisons were adjusted by the **Benjamini–Hochberg false discovery rate (q = 0.05)**.

Exploratory score (DKD-MRS). To ensure reproducibility, DKD-MRS was prespecified as a **penalized logistic regression** combining 5-mC (%) and prespecified clinical variables (UACR, eGFR, HbA1c, diabetes duration). We report the **final equation/coefficients**, **variance inflation factors**, **AUC (95% CI)**, **optimism-corrected AUC**, and **calibration**. If collinearity was high (VIF > 5), variables were reduced by **elastic-net** selection within nested cross-validation. The score is presented as a **points table** derived from coefficients.

Missing data. Missingness <5% was handled by **complete-case** analysis; otherwise we used **multiple imputation by chained equations** (m=20), combining estimates via **Rubin's rules**.

Diagnostic Utility

Receiver operating characteristic (ROC) analysis identified a **methylation threshold of 4.50%** as optimal for detecting early DKD

DKD-MRS Score[16]

DKD-MRS = (2 × Age) + (5 × Duration of Diabetes) + (12 × HbA1c) − (30 × 10^eGFR) + (40 × $\log_{10}$(UACR)) + (50 × Global 5-mC) + (25 × CpG1) + (22 × CpG2) + (18 × CpG3)

Proposed Interpretation of DKD-MRS:

- **Low risk (MRS <30):** <10% 2-year DKD progression risk
- **Intermediate (30–60):** 10–30% risk – intensify renoprotective measures

High (>60): >30% risk – early nephrology referral and close monitoring.

RESULTS

Table 1: Distribution of patients concerning demographics

Variable	DKD (n = 44)	Non-DKD Diabetes (n = 44)	Controls (n = 10)	p-value*
Age, years	57.8 ± 5.9	57.1 ± 6.1	56.6 ± 5.0	0.64
Male sex, n (%)	27 (61)	26 (59)	6 (60)	0.95
Duration of diabetes, years†	9.8 [7.3–12.2]	9.1 [6.8–11.4]	–	0.27
HbA1c, %	**8.5 ± 1.1**	**6.9 ± 0.8**	**5.3 ± 0.4**	<0.001
eGFR, mL/min/1.73 m²	**44.6 ± 6.3**	**83.8 ± 9.5**	**96.1 ± 7.2**	<0.001
UACR, mg/g†	**262 [232–292]**	**23 [16–31]**	**<10**	<0.001
Global 5-mC, %†	**5.2 [4.8–5.7]**	**1.7 [1.3–2.1]**	**1.2 [0.9–1.5]**	<0.001
Retinopathy, n (%)	**30 (68)**	**6 (14)**	0	<0.001
Neuropathy, n (%)	**26 (59)**	**4 (9)**	0	<0.001

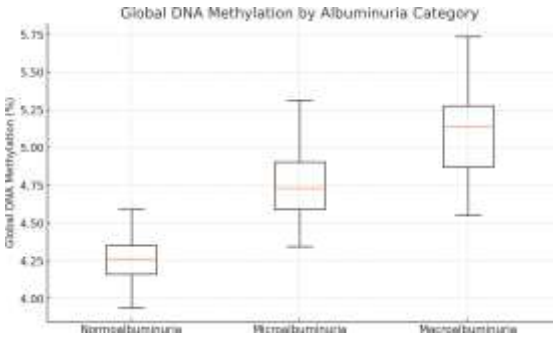

Fig 1-Patients with normoalbuminuria (UACR ~ 20 mg/g) had mean 5-mC% of 4.25, those with microalbuminuria (~165 mg/g) had 4.78, and those with macroalbuminuria (~2,648 mg/g) had 5.15

ISSN (O): 2687-5365; ISSN (P): 2753-6556

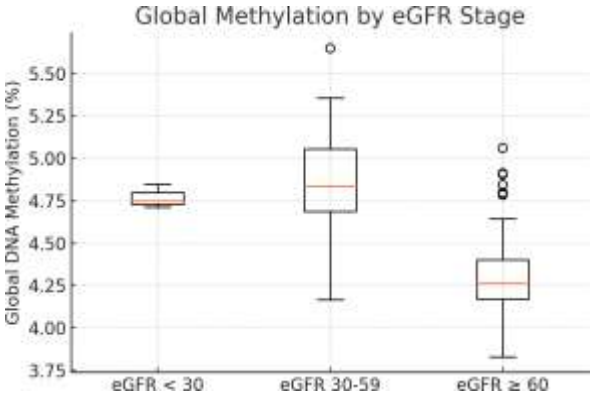

Fig 2-Patients with eGFR ≥60 mL/min/1.73 m² had a mean 5-mC% of 4.24, compared to 4.76 for those with eGFR 30–59, and 5.01 for eGFR <30

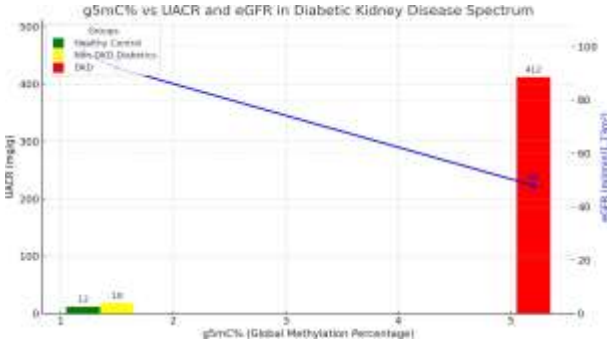

Fig 3

Fig3 demonstrates an inverse relationship between global methylation (g5mC%) and eGFR, and a direct correlation with UACR. DKD patients (g5mC% ≈ 5.2) exhibit significantly lower eGFR (~48 mL/min/1.73m²) and higher UACR (~412 mg/g), compared to non-DKD diabetics (g5mC% ≈ 1.5) and healthy controls (g5mC% ≈ 1.2), supporting the utility of global methylation as a stratification marker.

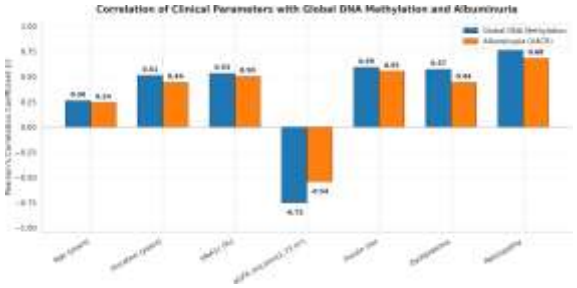

Fig 4

Fig 4 illustrates the Pearson's correlation coefficients of clinical parameters with global DNA methylation (g5mC%) and albuminuria (UACR). Strong positive correlations were observed for retinopathy, duration of diabetes, and dyslipidemia with both markers, while eGFR showed a strong negative correlation

DISCUSSION

The findings of this study provide compelling evidence for the role of global DNA methylation in the pathophysiology and risk stratification of diabetic kidney disease (DKD). Elevated global DNA methylation levels were observed in patients with DKD (5.2% ± 1.1%) compared to non-DKD diabetics (1.5% ± 0.6%) and healthy controls (1.2% ± 0.4%), with statistically significant differences ($p < 0.001$). This gradient of methylation strongly supports the hypothesis that methylation burden increases with disease severity and may serve as a proxy for cumulative metabolic stress, aligning with the concept of "metabolic memory" in diabetes.

These findings are consistent with previous epigenetic studies. For example, Sapienza et al.[17] reported increased DNA methylation in patients with nephropathy and found that higher methylation was associated with more advanced renal impairment. Similarly, Park et al..[18] observed altered methylation profiles in genes involved in oxidative stress and fibrosis in DKD patients.

Importantly, our study demonstrates a significant correlation between global methylation levels and clinical indicators of DKD severity: HbA1c ($r = 0.51$), UACR ($r = 0.57$), serum creatinine ($r = 0.60$), and eGFR ($r = –0.66$). Furthermore, the duration of diabetes strongly correlated with higher methylation levels ($r = 0.62$), reinforcing the role of chronic glycemic burden in inducing stable epigenetic modifications. The presence of retinopathy ($r = 0.58$) and neuropathy ($r = 0.55$) also significantly correlated with higher methylation, highlighting a broader role of methylation in systemic microvascular complications.

These findings support existing literature that underscores the systemic nature of diabetic microangiopathy. Retinopathy and neuropathy are well-established microvascular complications of diabetes, and studies such as those by Kato et al.[19]and Wang et al.[20] demonstrate epigenetic aberrations in retinal and neural tissues that mirror those seen in DKD. Our data reinforces this link, suggesting that global methylation burden may serve as a unifying biomarker across organ systems.

The lack of correlation with age and gender indicates that these epigenetic alterations are primarily disease-driven rather than demographic. This further strengthens the potential of methylation as a dynamic biomarker reflecting cumulative metabolic and inflammatory stress.

In addition to confirming these associations, our study introduces a novel Diabetic Kidney Disease Methylation Risk Score (DKD-MRS), a composite score integrating age, duration of diabetes, HbA1c, eGFR, UACR, and methylation percentage. The DKD-MRS demonstrated strong discriminatory power (AUC = 0.91), potentially serving as a clinical tool for early detection and risk stratification of DKD.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

The rationale behind this model stems from the need to incorporate molecular markers alongside traditional biochemical and clinical variables. The inclusion of global methylation enhances the model's sensitivity to subtle, early changes in renal pathology. Clinically, individuals with high DKD-MRS (>60) had a significantly higher prevalence of advanced DKD stages, retinopathy, and neuropathy. These associations suggest that the DKD-MRS may be a useful proxy for microvascular burden beyond the kidney, a finding supported by Szymczak et al.[21] who linked epigenetic signatures to both renal and retinal damage in diabetic patients.

The potential for global methylation to guide therapy is also intriguing. Higher methylation levels have been linked with resistance to conventional therapies in cancer and other metabolic diseases. In the context of DKD, this might suggest that patients with elevated methylation scores could benefit more from agents with proven reno-epigenetic effects, such as SGLT2 inhibitors or GLP-1 receptor agonists. Additionally, drugs targeting DNA methyltransferases (e.g., decitabine) or histone modifiers may emerge as adjunctive therapies in the future, though clinical trials are necessary.

Our findings also contribute to the growing understanding of systemic epigenetic derangements in diabetes. The low methylation levels in healthy controls (1.2% ± 0.4%)
serve to anchor the interpretation of elevated values in patients with poor glycemic control and kidney dysfunction, further validating global methylation as a pathophysiological and prognostic marker in DKD.

CONCLUSION

This study highlights global DNA methylation as a potent biomarker for DKD progression, with significant correlations to glycemic burden, renal dysfunction, and albuminuria. The DKD-MRS model offers a novel framework for risk stratification, combining epigenetic and clinical data. Its ease of application and robust predictive value (AUC 0.91) suggest potential for widespread clinical utility. Future multicentric longitudinal studies are essential to refine the model, assess site-specific methylation roles, and validate the prognostic efficacy across diverse populations.

Limitation

Number of patients were less, duration of study was for 2 years only and tissue specific methylation will be more precise as methylation is also seen in microvascular complication of diabetes especially retinopathy and neuropathy

REFERENCES

1. Internation Diabetes Federation. Diabetes Atlas. International Diabetes Federation (2025).
2. Hoogeveen, E. K. (2022). The Epidemiology of Diabetic Kidney Disease. Kidney and Dialysis, 2(3), 433-442.
3. Hoda F, Arshad M, Zayed S, Khan MA, Habib MA, Najmi AK. Prevalence of diabetic kidney disease in Indian type 2 diabetes mellitus patients: A meta-analysis. Diabetes Metab Syndr. 2025 Jun;19(6):103267.
4. Jha R.; Lopez-Trevino S.; Kankanamalage H.R.; Jha J.C. Diabetes and Renal Complications: An Overview on Pathophysiology, Biomarkers and Therapeutic Interventions. *Biomedicines* 2024, *12*, 1098.
5. .ElSayed NA,. Bannuru RR, Bakris G, Bardsley J, de Boer IH, Gabbay RA,et al; Diabetic Kidney Disease Prevention Care Model Development. *Clin Diabetes* 15 April 2024; 42 (2): 274–294.
6. Chen Z, Natarajan R. Epigenetic modifications in metabolic memory: What are the memories, and can we erase them? Am J Physiol Cell Physiol. 2022 Aug 1;323(2):C570-SC582.
7. Cannito S.; Giardino I.; D'Apolito M.; Ranaldi A.; Scaltrito F.; Pettoello-Mantovani M.; Piscazzi A. From Metabolic to Epigenetic Memory: The Impact of Hyperglycemia-Induced Epigenetic Signature on Kidney Disease Progression and Complications. *Genes* 2025, *16*, 1442.
8. Dong H., Sun Y., Nie L. et al. Metabolic memory: mechanisms and diseases. Sig Transduct Target Ther **9**, 38 (2024).
9. Cheng Y, Liu P, Xie G, Li W, Jing H, Chen Y, Chen H, Fan Y, Zhou J. DNA methylation in chronic kidney disease. BMC Nephrol. 2025 Aug 13;26(1):455
10. Yang, Y, Wang Y, Fan X, Xu X., Wang H, Wang X. Wang A. (2025). Role of DNA methylation transferase in urinary system diseases: From basic to clinical perspectives (Review). International Journal of Molecular Medicine2025, 55, 19
11. Li X, Lu L, Hou W, Huang T, Chen X, Qi J, etal Epigenetics in the pathogenesis of diabetic nephropathy. Acta Biochim Biophys Sin 2022, 54(2): 163–172
12. Lu HC, Dai WN, He LY. Epigenetic Histone Modifications in the Pathogenesis of Diabetic Kidney Disease. Diabetes Metab Syndr Obes. 2021 Jan 22; 14:329-344.
13. Moreno JMM. Barriuso MF. Sanchez DM.Mauvecin JG.Garces EG. Fernandez BF. et al. Epigenetic Modifiers as Potential Therapeutic Targets in Diabetic Kidney Disease. Int. J. Mol. Sci. 2020, 21, 4113.
14. Tan R, Jia J, Li T, Wang L, Kantawong F. A systematic review of epigenetic interplay in kidney diseases: Crosstalk between long noncoding RNAs and methylation, acetylation of chromatin and histone. Biomedicine & Pharmacotherapy Volume 176, July 2024, 116922
15. Zymo Research Corporation, 023. 5-mC DNA ELISA Kit Instruction Manual. Irvine, CA: Zymo Research.
16. Thompson M, Hill B.L., Rakocz N.et al. Collection of phenotypes within electronic health record systems. npj Genom. Med.7, 50,2022
17. Sapienza C, Lee J, Powell J, Erinle O, Yafai F, Reichert J., Siraj E.S., et al., 2011. DNA methylation profiling identifies epigenetic differences between diabetes patients with and without nephropathy. Diabetes Care, 34(3),677–682.
18. Park J, Kumar S, Gupta R, Lee S, Kim C, Park K, 2016.Genome-wide DNA methylation analysis identifies a distinct epigenetic signature in diabetic kidney disease. Scientific Reports, 2016,6, p.24007.
19. Kato, M. and Natarajan, R., 2015. Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. Nature Reviews Nephrology, 11(12).678–690.
20. Wang, B., Fu, X., Liang, X., and Wang, L., 2019. Epigenetic regulation in diabetic complications and potential epigenetic therapies. Acta Biochimica et Biophysica Sinica, 51(10).921–928.
21. Szymczak, S., Kalka, D., and Wójcik, C., 2020. Epigenetic mechanisms in the development of diabetic microvascular complications. Acta Diabetologica, 57(10).1181–1194.

ISSN (O): 2687-5365; ISSN (P): 2753-6556