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Original Article

The effect of glycemic variability on DNA damage in children with type 1 diabetes mellitus

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ABSTRACT

Objective: The aim of this study was to determine the extent of DNA damage in pediatric patients with type 1 diabetes and the influence of glycemic variability on DNA damage.

Method: The study involved 50 patients under the age of 18 with type 1 diabetes and 21 healthy control individuals. The Medtronic iProTM2 Enlite Glucose Sensor® was implanted, and continuous glucose monitoring metrics were calculated, including standard deviation, glucose management indicator, coefficient of variation, time in range, time below range, and time above range. Blood samples were also taken to assess DNA damage and HbA1c levels.

Results: The mean age of children with type 1 diabetes was 13.69±2.99 years, and the male-to-female ratio was 30:20. DNA damage was found to be similar in patients with type 1 DM and in a healthy control group. However, among children with type 1 diabetes mellitus, head length, a measure of undamaged DNA, was significantly higher in patients with good glycemic control (HbA1c≤7.5%) than in those with poor glycemic control (HbA1c>7.5%). A positive correlation was observed between DNA damage parameters and % coefficient of variation, a marker of glycemic variability.

Conclusion: The correlation between the coefficient of variation and DNA damage demonstrates the critical importance of maintaining consistent glycemic management in diabetes.

Keywords: DNA damage, glycemic variability, type 1 diabetes

INTRODUCTION

Type 1 diabetes mellitus is one of the most common chronic systemic diseases in childhood and is a risk factor for long-term vascular complications.[1] There is a significant correlation between hyperglycemia and the risk of micro- and macrovascular complications in type 1 diabetes (T1DM). The hemoglobin A1c (HbA1c) is a surrogate marker for glycemia and reflects average blood glucose levels. Therefore, it is utilized as a target for metabolic control.[1-3] However, it is essential to note that HbA1c

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should not be used as the sole indicator of glycemic control and may not accurately reflect short-term changes in blood glucose levels.[4,5]

Individuals with similar HbA1c values may have different glycemic variability (GV), which may contribute to diabetes-related complications that are not related to the degree of HbA1c.[6,7] Compared to HbA1c, continuous glucose monitoring (CGM) provides information on daily and inter-day blood glucose fluctuations and the extent of these fluctuations.[8] Although various CGM metrics have been reported, utilizing all these indices in daily clinical practice is impractical. A report has been published on the standardization of CGM metrics, and ten metrics have been chosen for use in clinical practice.[8] The most useful metrics in clinical practice are number of days CGM worn (recommend 14 days), percentage of time CGM is active, mean glucose, glucose management indicator (GMI), glycemic variability (%CV, target ≤36%), time above range (TAR, % of readings >181 mg/dL), time in range (TIR, % of readings between 70–180 mg/dL), time below range (TBR, % of readings below 69 mg/dL).

All changes in the molecular integrity of DNA caused by endogenous and exogenous factors are referred to as ‘DNA damage’. In patients with diabetes, there is an increase in the generation of reactive oxygen species and oxidative stress. Hyperglycemia has been identified as a major cause of increased oxidative stress leading to DNA damage.[9-11] Another causative factor that induces oxidative stress was found to be GV.[11-16]

Although there is a sufficient number of publications investigating both the relationship between oxidative stress and GV, and the link between hyperglycemia and DNA damage, no publication directly explores the relationship between GV and DNA damage.

Comet assay, also called the Single Cell Gel Electrophoresis Method (SCGE), is a fast, reliable, and quantitative method that can determine various types of DNA damage in cells.[17,18] DNAs are subjected to electrophoresis to move rapidly towards the anode. The migration speed increases according to the number of chain breaks in the DNA. This migration is like a comet. The amount of DNA damage is determined by the comet’s tail length and the DNA density in the tail length. The longer the tail length, the more damaged the DNA.[17,18]

To the best of our knowledge, no research has been conducted to examine the association between glycemic variability and DNA damage in pediatric patients diagnosed with type 1 diabetes. This study aimed to investigate the relationship between DNA damage and CGM indices, particularly glycemic variability.

PATIENTS AND METHODS

Subjects

The study included 62 patients (24 males and 38 females) with T1DM who were being followed at the Department of Pediatric Endocrinology, Pamukkale University Faculty of Medicine. T1DM was initially diagnosed using the diagnostic criteria published by the International Society for Pediatric and Adolescent Diabetes (ISPAD 2022).[2] Twentyone age and gender-matched healthy controls were also enrolled.

The following criteria were used to exclude individuals from the study group: (i) children with acute infection and fever; (ii) children with type 1 diabetes in partial remission; (iii) children treated with any medication (except insulin) in the previous ten days; (iv) children who had been treated with oral vitamins in the previous month. The “partial remission” period was defined as insulin reduced to ≤ 0.5 IU/ kg per day and HbA1c <7%. Demographic and clinical data (duration of diabetes, diabetes treatment, and the presence of microvascular complications) were collected from medical records. Those with serum low-density lipoprotein (LDL) ≥100 mg/dL and/or triglycerides ≥150 mg/dL were considered to have dyslipidemia.[19]

The research group was separated into subgroups for a more extensive statistical analysis. According to coefficient of variation (CV) % values; those with CV<36% were considered stable, while those with CV≥36% were unstable. Patients with time in range (TIR) values above 70% were considered in the ideal target range, while those below 70% were considered to be in the suboptimal target range. Individuals with a mean HbA1c ≤ 7.5% were considered to have good metabolic control, whereas those with a value greater than > 7.5% were considered to have poor metabolic control. The Institutional Ethical Committee approved based on the Declaration of Helsinki’s principles (number:60116787-020/59514). Each study subject provided informed consent.

CGM metrics

The Medtronic iProTM2, Enlite Glucose Sensor® was implanted, and continuous glucose monitoring (CGM) metrics were calculated, including standard deviation (SD), glucose management indicator (GMI), coefficient of variation (CV), time in range (TIR), time below range (TBR), and time above range (TAR). The participants wore the “Medtronic iProTM2, Enlite Glucose Sensor®” for seven days. TIR was defined as the proportion of time spent in the target range (70–180 mg/dL), TBR as the proportion of time spent below 70 mg/dL, and TAR as the proportion of time spent above >180 mg/dL. The CV % was

calculated as the SD of the glucose level divided by the mean glucose level.[8] CV% was considered as an indicator of glycemic variability.[8]

Blood samples

Blood samples were taken on the same day and immediately transported to the laboratories.

The HbA1c level was determined using the high-pressure liquid chromatography method (Tosoh G8 Bioscience, Inc., Tokyo, Japan).

Blood collection and lymphocyte isolation

All participants had their peripheral venous blood drawn into a 10-mL vacutainer tube containing K3EDTA, and lymphocytes were isolated using Histopaque-1077. Blood was diluted 1:1 with phosphate-buffered saline (PBS) and placed into the Leucosep tube directly. After that, it was centrifuged for 15 minutes at 800g and room temperature. Buffy coats were removed and washed with PBS twice.

Cell cryopreservation prior to comet assay

The cell suspension was centrifuged at 200g for 5 minutes, and the pellet was resuspended at 3105 cells/mL in freezing media containing 10% DMSO, 40% RPMI, and 50% fetal calf serum, as reported by Visvardis et al.[20] The cell suspension was transferred in aliquots of 2106 cells to plastic freezing vials. The vials were placed in a Cryo 1°C freezing container, then immediately into a -80°C freezer to achieve a cooling rate of 21°C/min, and then kept at -80°C.

Comet assay

The assay was carried out according to the protocol described by Nandhakumar et al.[18] To summarize; the vials were collected and immersed in a 37°C water bath until all ice was melted. The thawed cells were transferred immediately to conical centrifuge tubes containing 15 mL of pre-chilled thawing medium composed of 50% fetal calf serum, 40% RPMI, and 10% dextrose. To perform the comet experiment, cells were centrifuged at 200g for 10 minutes at 4°C, and the pellet was resuspended in ice-cold PBS pH 7.3.

The comet assay was done in alkaline conditions using a modification of Singh et al.[17] Cells were suspended in 1% low melting point agarose in PBS, pH 7.4, and pipetted 100 µl onto a frosted glass microscope slide pre-coated with 1% average melting point agarose. After 10 minutes on ice, the agarose was allowed to set, and the slide was placed in a lysis solution (2.5 M NaCl, 100 mM Na2 EDTA, 10 mM Tris, NaOH to pH 10.0, and 1% Triton X-100) at 4°C for 1 hour to remove cellular proteins.

After placing slides in an electrophoresis tank, they were allowed to soak for 30 minutes in an alkaline buffer (0.3 M NaOH and 1 mM Na EDTA) to unwind DNA strands and reveal alkali labile spots (alkali unwinding) before electrophoresis. After 30 minutes, electrophoresis at 25 V, 300 mA for 30 minutes at the same temperature was done. The slides were carefully removed from the electrophoresis buffer and put on a staining tray at the end of the 30 minutes. The slides were washed three times with the neutralizing buffer for five minutes each (0.4 M Tris-HCl, pH 7.5). The slides were then observed using the fluorescence staining process. Each slide was coated with 50 mL of ethidium bromide stain and covered with a clean coverslip. Before examining the slides, the excess pigment was wiped off the back and edges of the slides. A fluorescent microscope with an excitation filter of 515–560 nm, a barrier filter of 590 nm, and a magnification of 20 was utilized to visualize ethidium bromide-stained slides. To limit the likelihood of cellular DNA damage, all stages, starting with lymphocyte isolation, were performed under yellow light. Microscopically, slides were analyzed using Comet IV Computer Software (Perceptive Instruments, United Kingdom)

Statistical methods

Statistical Package for Social Sciences (SPSS) version 20.0 software was used for statistical analyses. Data were examined for normality. For continuous variables, Data with normal distribution were expressed as mean ± SD, as a median and interquartile range of non-normal distribution. Categorical variables were expressed as frequencies and proportions.

Mann-Whitney's U-test assessed differences in measured parameters between control and patient groups. The significance level was considered as $p< 0.05$. Correlation analysis was performed by using Spearman's correlation coefficient.

RESULTS

Twelve children were excluded from the trial because they had removed the CGM sensor due to local side effects (pruritus, discomfort) or due to sensor incompatibility or inadequate sensor data (<70%). The remaining 50 patients had a mean duration of diabetes of 4.39 ± 2.39 years and an HbA1c of 9.2 ±2.1%. Table 1 summarizes the clinical characteristics of all patients. None of the patients had micro- or macrovascular complications. Fourteen patients (29.8%) had dyslipidemia. Among those with dyslipidemia, 64.3% (n=9) had elevated triglycerides, 21.4% (n=3) had elevated LDL, and 14.3% (n=2) had elevated LDL and triglycerides together.

Table 1. Clinical and laboratory findings of the entire group

	Control (n=21)	T1 DM (n=50)	*p*
Age (year)	13.16±3.78	13.69±2.99	0.57
Female/Male	20/11	30/20	0.33
Prepubertal/Pubertal	6/15	14/36	0.96
Weight SDS	-0.25±1.50	0.02±1.20	0.40
Height SDS	-0.02±1.27	-0.04±1.09	0.92
BMI SDS	-0.28±1.24	1.19±5.66	0.43
DNA damage parameters			
Head length (µm)	30.18±2.22	30.43±3.10	0.74
Tail length (µm)	36.07±7.03	32.52±9.79	0.14
Tail intensity (%)	25.10±10.63	20.78±11.56	0.15
Tail moment (µm)	4.89±2.99	3.92±3.55	0.28
Tail migration (µm)	21.02±7.67	17.34±9.93	0.24

BMI: body mass index, SDS: standard deviation score, T1 DM: type 1 diabetes.

Table 2. Comparison of DNA damage parameters among subgroups divided according to metabolic control, coefficient variation %, and time in range %

	HbA1c ≤% 7.5 (n=11)	HbA1c > %7.5 (n=39)	*p*
Head length (µm)	32.28±2.43	29.88±3.09	**0.02**
Tail length (µm)	31.12±7.24	32.94±10.47	0.59
Tail intensity (%)	16.86±9.18	21.95±12.05	0.20
Tail moment (µm)	3.19±2.48	4.13±3.81	0.44
Tail migration (µm)	15.18±7.78	17.99±10.49	0.41
	CV % <36 (n=14)	**CV % ≥36 (n=36)**	
Head length (µm)	30.65±3.11	30.35±3.14	0.77
Tail length (µm)	30.10±9.52	33.42±9.86	0.30
Tail intensity (%)	17.48±12.70	22.01±11.05	0.23
Tail moment (µm)	3.23±4.23	4.17±3.29	0.42
Tail migration (µm)	14.66±9.74	18.34±9.95	0.25
	TIR > %70(n=11)	**TIR < % 70 (n=39)**	
Head length (µm)	30.88±3.16	30.30±3.11	0.59
Tail length (µm)	33.21±13.95	32.32±8.41	0.79
Tail intensity (%)	20.88±17.08	20.76±9.67	0.97
Tail moment (µm)	4.58±5.69	3.72±2.7	0.48
Tail migration (µm)	17.58±14.66	17.27±8.31	0.93

HbA1c: hemoglobin A1c, CV: coefficient variation, TIR: time in range.

Comet assay parameters indicating DNA damage did not differ between the diabetes and control groups (Table 1). However, when DNA damage was compared between diabetes subgroups, head length, an indicator of intact DNA, was found to be higher in the good metabolic control group than in the poor metabolic control group (Table 2). There was no difference between the subgroups that were separated according to CV % and TIR % (Table 2). DNA damage did not differ between those with hyperlipidemia and those without it.

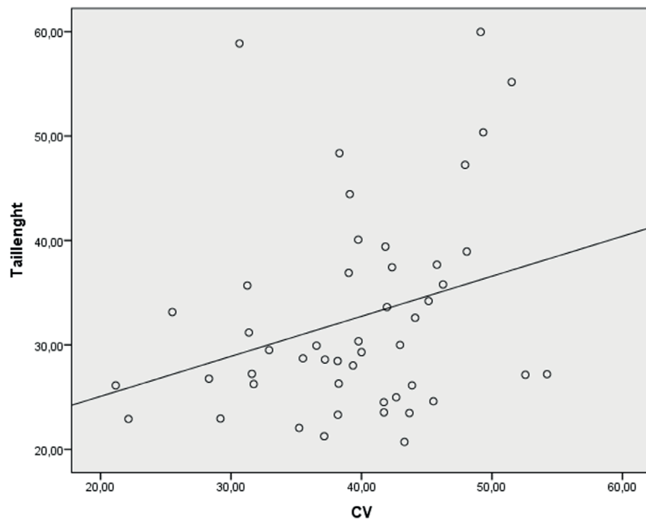

Figure 1. The correlation between coefficient variation (CV) % and tail length

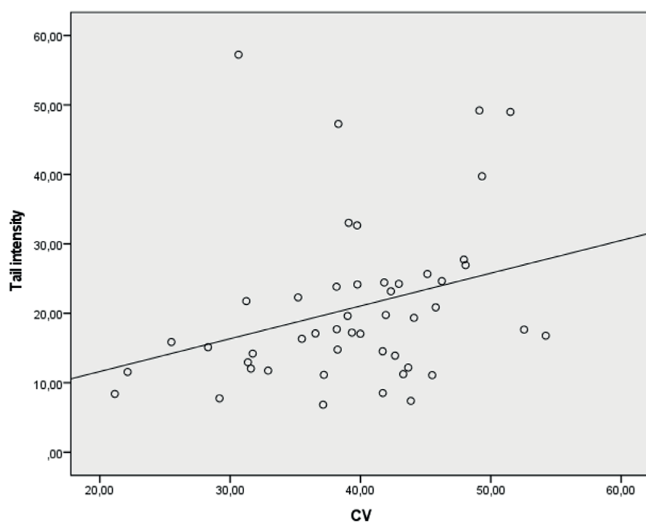

Figure 2. The correlation between coefficient variation (CV) % and tail intensity

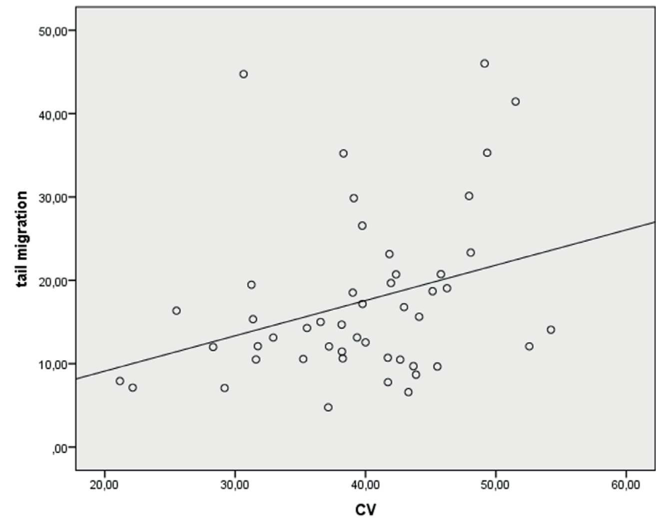

Figure 3. The correlation between coefficient variation (CV) % and tail migration

CV%, a marker of glycemic variability, was positively correlated with tail length, tail density, and tail migration, which are indicators of DNA damage (r= 0.29, p=0.04; r= 0.30, p=0.03; r=0.32, p=0.02, respectively) (Figure 1, 2, 3 and Table 3).

DNA damage parameters did not correlate with age, diabetes duration, HbA1c %, or serum lipid levels.

DISCUSSION

This study aimed to investigate the association between GV and DNA damage in young patients with type 1 diabetes. Although there was no difference between individuals with stable CV% and those without in terms of DNA damage parameters, we observed a positive correlation between the CV% and DNA damage parameters, including tail length, intensity, and migration.

In subgroup comparisons based on HbA1c, a marker of hyperglycemia, the only difference among the DNA damage parameters was in head length. There was no correlation between Hba1c or mean glucose values and DNA damage. These findings suggest that glycemic variability, rather than hyperglycemia, maybe a more potent factor in inducing DNA damage.

It is well known that hyperglycemia causes oxidative stress, which promotes the development of diabetic vascular complications. Glycemic variability also stimulates oxidative stress.[11,12] It has even been reported that glucose fluctuations have a more

Table 3. Correlation between DNA damage parameters and CGMS data in patients with type 1 DM

		Mean glucose	HbA1c%	TIR %	TAR %	TBR%	GMI%	SD	CV%
Head length	r*	0.00	-0.1	0.07	-0.03	-0.12	0.00	-0.04	-0.10
	p	0.98	0.48	0.62	0.83	0.39	0.97	0.77	0.48
Tail length	r*	-0.12	0.09	0.04	-0.09	0.18	-0.12	0.03	0.29
	p	0.39	0.95	0.77	0.53	0.20	0.39	0.79	**0.04**
Tail intensity	r	-0.12	0.22	0.02	-0.07	0.19	-0.12	0.06	0.30
	p	0.40	0.88	0.88	0.61	0.17	0.40	0.68	**0.03**
Tail moment	r	-0.14	0.05	0.08	-0.11	0.13	-0.14	-0.00	0.25
	p	0.33	0.73	0.57	0.42	0.35	0.33	0.99	**0.08**
Tail migration	r	-0.11	0.01	0.01	-0.07	0.21	-0.12	0.05	0.32
	p	0.42	0.92	0.89	0.60	0.15	0.41	0.70	**0.02**

HbA1c: hemoglobin A1c, CV: coefficient variation, GMI: glucose management indicator, SD: Standard deviation, TIR: time in range, TAR: time above range, TBR: time below range.

significant effect on inducing oxidative stress than continuous, sustained chronic hyperglycemia.[13,21]

It is unclear why glucose variability is a greater driver of oxidative damage than chronic hyperglycemia. One of the possible explanations is based on the tumor suppressor gene p53. Enhanced oxidative stress activates p53 phosphorylation.[22] Liu et al. reported that high levels of glucose activate the tumor suppressor gene p53 in human endothelial cells. The study demonstrated that activation of p53 leads to an increase in the expression of other genes that are involved in apoptosis.[23] This process persists even after achieving normoglycemia, called as “metabolic memory”.[23,24] Schisano et al. reported that fluctuations in glucose induce a higher transcriptional activity of p53 than constant hyperglycemia.[25]

Several studies have reported a significant increase in DNA damage in patients with diabetes.[9-11,16,26-28] These studies have investigated the association between oxidative stress, hyperglycemia, and DNA damage in diabetes mellitus, but they were limited to adult populations, with most participants having type 2 diabetes.[9,11,16,27-30] However, only a few clinical trials have studied DNA damage in children with T1DM.[10,26] DNA damage was found to be increased in children with T1DM, and the enhanced oxidative stress resulting from hyperglycemia has been shown to cause DNA damage.[10,26] No reports have been conducted on children regarding DNA damage and glycemic variability. Therefore, our study contributes to the literature in this field, and more research is needed in this area.

In the current study, we did not find any difference in DNA damage between the control group and the group of diabetic patients. Consistent with our research, some studies did not report increased DNA damage among diabetic patients compared to healthy controls.[29-31] Varvarovská et al.[31] demonstrated increased oxidative stress but unchanged DNA damage in children with T1DM compared to healthy controls. On the other hand, DNA repair capacity was increased. The authors attributed this to increased stimulation of DNA repair capacity due to the stimulation of oxidative stress.

Anderson et al.[30] offered the following possible explanation for this situation; the damage must exceed a certain level to trigger DNA repair mechanisms. Regular exposure to oxidative damage in people with diabetes may keep DNA repair mechanisms dynamic. In healthy people, unstimulated lymphocytes may not be competent in DNA repair and, therefore, accumulate small amounts of DNA damage.[30] Aging may be another explanation. Numerous studies have examined the association between DNA damage and aging. They found that aging was related to a lower ability for DNA repair.[32,33]

In our study, among children with T1DM, we didn’t find a correlation between DNA damage and HbA1c %. On the contrary, we found a shorter head length in the poor metabolic control group compared to the good metabolic control group, which is associated with greater DNA damage. Although most of these studies have shown an association between HbA1c, hyperglycemia, and DNA damage[10,11,16,27], there is also one study that did not report an association between HbA1c, duration of diabetes, complications, and DNA damage.[9]

In summary, unaltered DNA damage in children with T1DM may be explained by increased DNA repair mechanisms due to

chronic stress, shorter duration of disease, or young age-related high repair mechanisms.

Study limitations

The main limitation of this study is the short duration of the CGM wearing time and the small number of the study population. Due to economic issues, patients received only one CGM sensor (for seven days). Second, although participation in the study was offered to all patients, those who were already using CGM technology declined to enter the study because they refused to wear a device other than their CGM device. Additionally, some individuals with good metabolic control did not want to wear a CGM because they already had good blood glucose regulation and did not want to put extra effort into the study. Unfortunately, this condition may be viewed as a selection bias, which may have influenced our understanding of the link between HbA1c and CGM measures. These associations may be valid in patients with good metabolic control and low CV%.

CONCLUSION

In conclusion, we found that glycemic variability, assessed by CV%, is associated with DNA damage in children with type 1 diabetes, even when DNA damage is not enhanced compared to healthy controls.

Ethical approval

This study has been approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval date 02.07.2019, number 60116787-020/59514). Written informed consent was obtained from the participants.

Author contribution

Surgical and Medical Practices: GG; Concept: GG, SAA; Design: SAA; Data Collection or Processing: GG, BÖ; Analysis or Interpretation: ÖKE, MTA, EKT, VK; Literature Search: GG, SAA, EKT; Writing: SAA. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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