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Methylglyoxal Modified DNA: A Possible Trigger in the Pathophysiology of Diabetes Mellitus

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Abstract

Background: Methylglyoxal (MG), a highly reactive electrophile, is a glycating agent having much higher reactivity than that of glucose. Previous studies have suggested that MG reacts with proteins' amino groups which result in the formation of advanced glycation end products and free amino group of nucleic acids resulting in the formation of DNA–AGEs. Previous studies have been done which showed that the concentration of MG and its derived AGEs increases in patients with diabetes and its complications. However, most studies till date have focused on the glycation reactions between glucose and proteins as glucose is the most abundant sugar in blood and is substantially elevated in diabetes. The aim of the study our aim was to analyze the effects of MG on DNA and to evaluate the impact of glycated DNA in etiopathogenesis of Type 2 Diabetes Mellitus.

Subject and Methods: In this study, DNA was modified by the glycation reaction of MG with lysine in the presence of Cu2+. The modified DNA has been characterized by various physiochemical techniques, like Absorption (UV/Vis) spectroscopy and fluorescence spectroscopic analysis. MG-modified-DNA exhibited extensive damage as revealed by various physico-chemical studies. The antibody analysis was undertaken by direct binding and competitive inhibition ELISA. MG-modified-DNA showed increased hyperchromicity and increased fluorescence intensity. Sera of diabetic patients showed preference for MG-modified-DNA in comparison to native DNA. Inhibition ELISA supported the above findings. MG-modified-DNA emerged as a powerful inhibitor as compared to native DNA.

Results: Our study showed that the structural perturbations in MG–Lys–Cu2+-DNA which implied the generation of new epitopes that might have made the molecule immunogenic and furthermore, it suggested that DNA glycated with MG may trigger an immune response which might result in generation of anti-glycated DNA antibodies (sera autoantibodies) which are preferably bound by glycated-DNA as compared to native DNA in Type 2 DM patients.

Keywords: Glycation end products, Etiopathogenesis, Hyperchromicity, Fluorescence, Spectroscopy.

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Introduction

Diabetes Mellitus (DM), a metabolic disorder is caused by defective insulin secretion, defective insulin action or both. There is persistent hyperglycemia, as the glucose rises in the blood, instead of being taken up by the cells. Thus despite the high glucose levels, the body cells are starved of energy. In Type 1 DM, there is a significant role of genes (mainly HLA expression) and environmental factors whereas Type 2 DM is characterized by insulin resistance mainly caused by interaction of genetic factors and obesity even in presence of normal insulin levels [1]. Its prevalence is increasing at a very fast rate, all over the world, such that by 2040, is has been estimated there would be almost 640 million diabetic adults, the current number being 415 million. In India, which has 18% of the world's total population, there are 69.1 million diabetics present representing 16.65% of the total diabetics in the world [2]. Hyperglycemia is the initiating cause of the various complications of DM such as retinopathy, nephropathy and neuropathy which are irreversible. These complications are produced either through mechanisms which include direct changes in the cellular glucose metabolism, or indirectly by the accumulation of advanced glycated end products (AGEs). AGEs interact with RAGE, Receptor for AGEs and cause oxidative stress to the endothelial membranes of the various organs and increase the pro-inflammatory signaling, finally causing endothelial dysfunction, arterial stiffening and micro-vascular complications (triad) which cause end-organ irreversible damage classically seen in DM [3]. Various studies have suggested that glycation, is a feasible explanation for the association between hyperglycemia and various tissue pathologies in DM [4]. Glycation, also known as "the browning reaction", "the Maillard reaction", or "non-enzymatic glycosylation" was described by Louis–Camille Maillard is a chemical modification of proteins, lipids or nucleic acids. This reaction takes place between carbonyl group of reducing sugars and amino groups of proteins, lipids and nucleic acids. The glycation involves the condensation reaction of the carbonyl group of reducing sugar aldehydes with α-and ε-amino groups of lysine residues. The reversible Schiff base is formed by the nucleophilic addition reaction. After this, molecular rearrangement occurs. It is followed by formation of N-substituted glycosylamine. This forms covalently bonded amadori products (1-amino, 1-deoxy, 2-ketose) such as fructose-lysine which rearranges to form stable ketoamine derivatives [5-7]. These ketoamine compounds rearrange to form irreversible AGEs after oxidation, dehydration, cyclization [8]. Rates of these reactions are quite slow and over the course of days to weeks. Schiff bases and amadori products degrade to form alpha dicarbonyl compounds, such as 3-deoxyglucosone, methylglyoxal (MG), and glyoxal [9]. These compounds react more strongly than their parent sugars with amino groups of proteins to form AGEs [10]. The amino groups of adenine and guanine bases in DNA also undergo glycation and form AGEs [11]. The amino group (exocyclic) of 2'-deoxyguanosine is susceptible to glycation and produces N2-carboyxmethyl, N2-carboxyethyl, N2-(1-carboxy-3-hydroxypropyl) and N2-(1-carboxy-3,4,5-trihydroxy-pentyl) 7 alterations and cyclic dicarbonyl adducts [12-14]. The diastereomers of N2-carboxyethyl-2'-deoxyguanosine (CEdGA, B) are DNA-AGEs formed by glycation of DNA with sugars. These are formed from a variety of glycating agents including glucose, dihydroxyacetone (DHA), or MG [15]. Methylglyoxal (MG) is a universal product of cellular metabolism either under physiological or pathological conditions [16] MG, a dicarbonyl compound is formed during spontaneous degradation of triose phosphates: DHAP (dihydroxyacetone phosphate) and DAP (D-glyceraldehyde 3-phosphate), also from polyol pathway through dihydroxyacetone phosphate pathway [17]. Cigarette smoke and various food items like coffee, beer contain methylglyoxal [18-20]. It is also generated during catabolism of L-threonine and glycine [21]. MG is produced during autoxidation of sugars [22] and ketone bodies [23]. It is also formed during an early stage of the Maillard reaction [24] and oxidative degradation of lipoperoxidation reactions [25]. Also an important source of MG generation is oxidation of acetone by cytochrome P450 [26]. MG is a highly

reactive glycating agent which is highly mutagenic and modifies structure and function of proteins, nucleic acids, lipids and other biomolecules [27]. It has been estimated that 99.7 % of MG which is produced daily gets metabolized by the body. It is detoxified by various mechanisms. Body has effective glutathione-dependent glyoxylase (Glo) system which is present in all the cells for metabolizing it [28]. MG forms the hydroimidazolone adducts (MG-H1) with arginine side chain on proteins. Also, protein glycation by MG is very damaging because arginine residues are more common than lysine residues in functional domains of proteins [29]. MG forms cross linked products with lysine and arginine residues [30]. It has been observed that MG and arginine residues react to form Nı-(4-carboxy-4,6-dimethyl-5,6-di-hydroxy-1,4,5,6-tetra-hydropyrimidine-2-yl) ornithine (THP) [31], argpyrimidine - a major fluorescent product which is present in human serum and cornea [32,33] and MG-derived hydroimidazolone [34]. Most importantly, its concentration rises in Diabetes Mellitus and its complications as it is formed in oxidation of ketone bodies [35,36]. Some studies have shown the concentration of MG was 256nM in healthy controls and 479nM in diabetic patients which suggested that perturbation of proteins in vivo by MG might be of more significance than that of glucose. This could be most probably due to the rapid formation of Schiff bases with the amino acids in presence of MG leading to the rapid production of AGEs [37,38]. MG-derived AGEs may also be increased more in DM because of increased degradation of MG at some sites and Glo1 down regulation [39]. The correlation between hyperglycaemia and pathogenesis of DM complications have always been an important topic of interest in diabetes research yet not understood thoroughly [40]. Studies have also suggested that treatments against MG and MG-derived AGEs are beneficial [41]. The present study was formulated to assess the effects of MG on DNA and to explore the possible role of MG-modified-DNA as a likely trigger for DM initiation/progression of Type 2 DM The present study aims to evaluate the presence of antibodies against native and glycated DNA in the sera of diabetic patients and to see whether it has any possible role in the disease etio-pathogenesis and its complications.

Materials and Methods

Methylglyoxal, highly polymerized calf thymus DNA, p-nitrophenyl phosphate (PNPP), PBS, Tris-buffered saline, Tween-20, TBS-T, lysine, copper sulphate, sodium chloride, sodium carbonate, sodium azide, sodium bicarbonate, anti-human alkaline phosphatase conjugate. Flat bottomed polysorp/maxisorp ELISA modules, dialysis tubing. All other reagents or chemicals were of the highest analytical grade available. Double distilled water was used throughout these studies Collection and processing of blood samples.

Informed consents from patients as well as healthy subjects were obtained before taking blood samples. A total of 50 patients diagnosed as cases of type 2 diabetes mellitus attending the medicine OPD of HAHC Hospital, Jamia hamdard, New Delhi were included in the study. Sera from healthy individuals (n=30) will serve as negative control. The clinical history, examination and other details of the patients were taken. The subjects having any coexisting autoimmune disorder were excluded. Ethical clearance certificate were obtained prior to conduction of the study. 5ml blood will be withdrawn and transferred to plain vials. The blood samples were allowed to clot and sera were separated. To inactivate the complement proteins all the serum samples were heated at 56˚C for 30 min and stored at -20ºC with 0.2% sodium azide as a preservative.

Modification of DNA by methylglyoxal

The modification was carried out by the method was described by Kang [42] with slight modifications incubating 25 µg/ml

of commercially available calf thymus DNA with 40 mM MG, 40 mM lysine and 300 µM Cu^{2}+ in 10 mM sodium phosphate buffer, pH 7.4 containing 150 mM NaCl was incubated at 37°C followed by extensive dialysis against phosphate buffer to remove unbound constituents.

Absorption (UV/Vis) spectroscopy

The absorbance (Abs.) profile of native- (control) and MG modified DNA was recorded on spectrophotometer in the wavelength range of 200-400 nm using quartz cuvette of 1 cm path length. Hyperchromicity was calculated as follows;

$$\% \text{ Hyperchromocity } = \frac{\text{Abs. of modified DNA } - \text{Abs. of control DNA}}{\text{Abs. of modified DNA}} \text{ x } 100$$

Fluorescence measurements

Fluorescence emission was recorded on spectrofluorometer. Fluorescence of native- (control) and MG modified DNA was monitored in the wavelength range of 380-500 nm after excitation at 370 nm. Increase in fluorescence intensity (FI) calculated as follows;

$$\% \text{ Increase in FI } = \frac{\text{FI of modified DNA } - \text{FI of control DNA}}{\text{FI of modified DNA}} \text{ x } 100$$

ELISA procedure

ELISA was carried out on flat bottom polystyrene modules [43]. Briefly, microtitre wells were coated with 100µl of native- and modified calf thymus DNA (2.5 µg/ml in TBS) and incubated for 2 hr. At 37°C and overnight at 4°C. Each sample was coated in duplicate and half of the wells served as control devoid of antigen only. The antigen-coated wells were emptied and washed thrice with TBS-T to remove unbound antigen. Unoccupied sites were blocked with 150 µl of 2% non-fat dry milk (in TBS, pH 7.4) for 4-5 hr at 37°C and then wells were washed once. In direct binding ELISA, sera (1:100 diluted) were directly added into antigen coated wells and incubated for 2 hr at 37°C and overnight at 4°C. The wells were emptied and extensively washed with TBS-T. Anti-immunoglobulin G alkaline phosphatase conjugate was added to each well. The conjugate was incubated at 37°C for 2 hr and then washed four times with TBS-T and three times with distilled water. Para-nitrophenyl phosphate was added and final colour was read at 405 nm on a micro plate reader. The results were expressed as mean of difference of absorbance values in test and control wells (A test – A control)

Competitive Inhibition ELISA

The antigenic specificity of the antibodies was determined by inhibition ELISA [44]. Varying amounts of inhibitors (0-20 µg/ml) were mixed with a constant amount of antibody/antisera. The mixture was incubated at room temperature for 2 hr and overnight at 4°C. The immune complexes thus formed were added to the antigen coated ELISA plates instead of serum and the bound antibodies were assayed as described in direct binding ELISA. The remaining steps were same as in direct binding ELISA. Percent inhibition was calculated using the following formula:

$$\text{Percent inhibition} = \frac{\text{A unhinibited} - \text{A inhibited}}{\text{A unhinibited}} \text{ x } 100$$

Statistical analysis

Data are presented as mean ± SD. Statistical significance of the data was determined by Student's t-test. A value of $p < 0.05$ was considered to be statistically significant.

Results

DNA (25 µg/ml) was incubated at 37ºC with 40 mM MG, 40 mM lysine and 300 µM Cu_2+ in 10 mM sodium phosphate buffer, pH 7.4 containing 150 mM NaCl. Glycation induced structural changes were recorded after 4, 8, 16 and 24 hours. The glycated samples showed progressive hyperchromicity at 260 nm compared to native DNA (**FIG. 1**). The percent hyperchromicity in DNA glycated with 40 mM methylglyoxal was 52.80, 67.07, 69.98 and 71.05 percent respectively (**TABLE 1**). Furthermore, a new peak appeared at 310 nm in the MG-modified-DNA sample. Further incubation did not yield much change in absorbance.

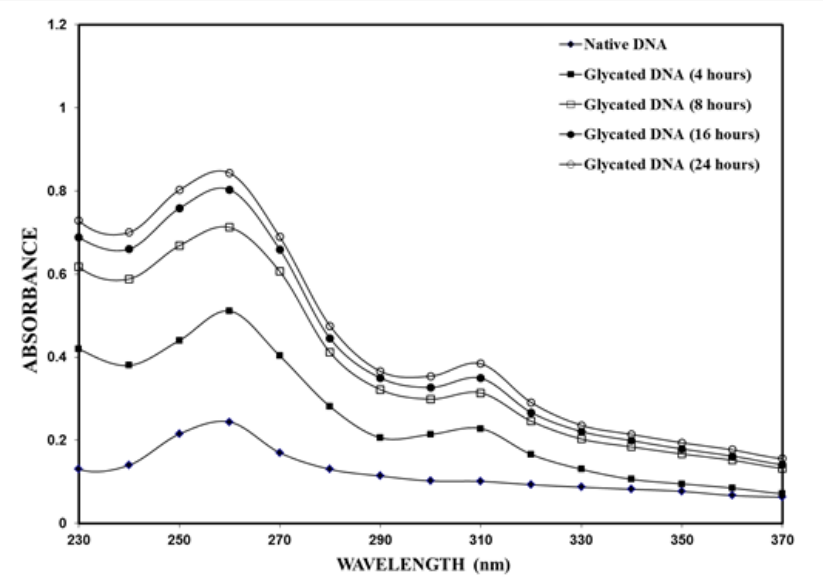

FIG. 1. **Profile of native DNA incubated with 40 mM methylglyoxal for different time intervals.**

TABLE 1. **Effect of time on absorbance and hyperchromicity of calf thymus DNA incubated with 40 mM methylglyoxal.**

INCUBATION TIME (hours)	ABSORBANCE AT 278nm	% HYPERCHROMICITY
0	0.244	-
4	0.517	52.8
8	0.741	67.07
16	0.813	69.98
24	0.843	71.05

Fluorescence of native- and MG-modified-DNA

The possible formation of fluorogenic AGEs in MG-modified-DNA samples was assessed from fluorescence intensity (FI) of samples excited at 370 nm, the wavelength for identifying fluorophoric AGEs. The FI of modified-DNA samples showed increase in FI with increase in time at 440 nm, the emission wavelength (**FIG. 2**). However, native DNA did not show appreciable FI when excited at 370 nm. The FI of glycated DNA samples increased by 69.46%, 84.28%, 90.80 and 91.04% respectively after incubation for 4, 8, 16 and 24 hours (**TABLE 2**). Further experiment was carried out on 24 hour DNA sample glycated with 40 mM MG-modified-DNA.

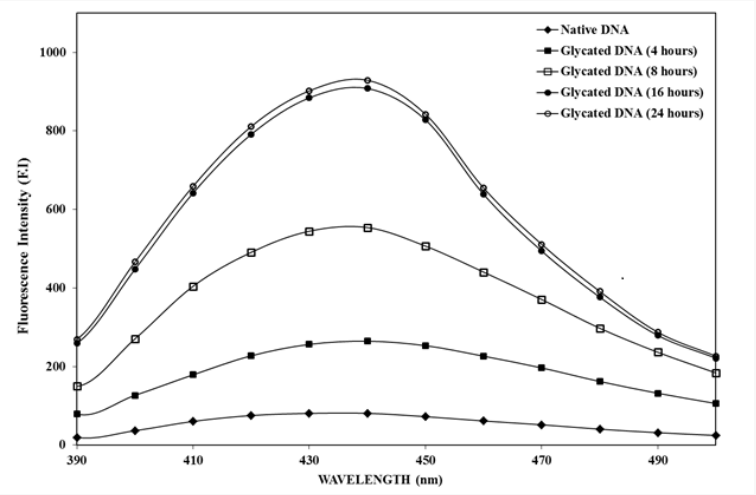

FIG. 2. **Fluorescence emission profile of calf thymus DNA incubated with different concentrations of methylglyoxal. The samples were excited at 370 nm.**

TABLE 2. **Fluorescence characteristics of calf thymus DNA incubated with 40 mM methylglyoxal.**

INCUBATION TIME (hours)	FLUORESCENCE INTENSITY (A.U.)	% INCREASE IN FLUORESCENCE INTENSITY
0	80.81	-
4	264.59	69.46
8	514.1	84.28
16	878.21	90.8
24	908.21	91.04

Enzyme immunoassay of diabetic sera with native- and MG-modified-DNA

Fifty diabetic sera were tested for binding with native DNA and glycated DNA. The sera were diluted to 1:100 and subjected to direct binding ELISA on microtitre wells separately coated with equal amounts of respective antigens (**FIG. 3-5**). Sera (n=30) of apparently healthy subjects served as control. The mean of the data indicated the abundance of anti-glycated DNA auto-antibodies. Sera of normal subjects showed negligible binding with coated antigens. Twenty three DM sera (*labelled) showed significantly ($p<0.05$) higher binding with MG-modified-DNA were selected for further studies. The data are summarized in **TABLE 3**.

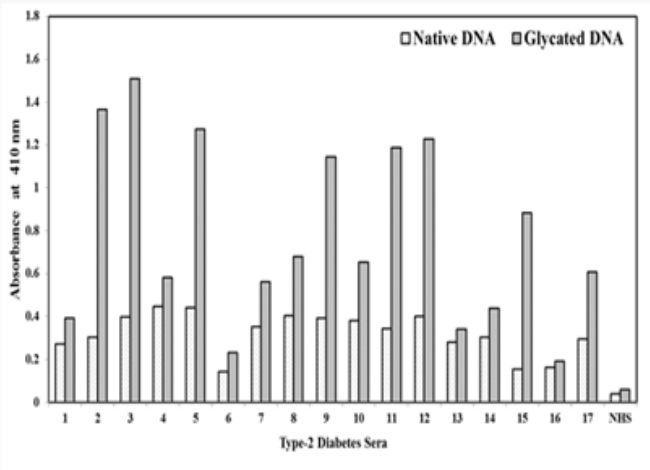

FIG. 3. **Direct binding ELISA of Type-2 Diabetes sera (1-17) with native DNA and glycated-DNA. Pooled normal human sera (NHS) served as control. Microtitre plate was coated with respective antigens.**

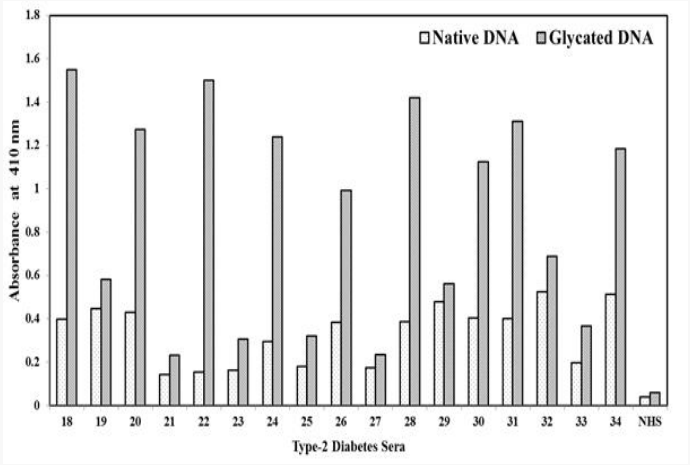

FIG. 4. **Direct binding ELISA of Type-2 Diabetes sera (18-34) with native DNA and glycated-DNA. Pooled normal human sera (NHS) served as control. Microtitre plate was coated with respective antigens.**

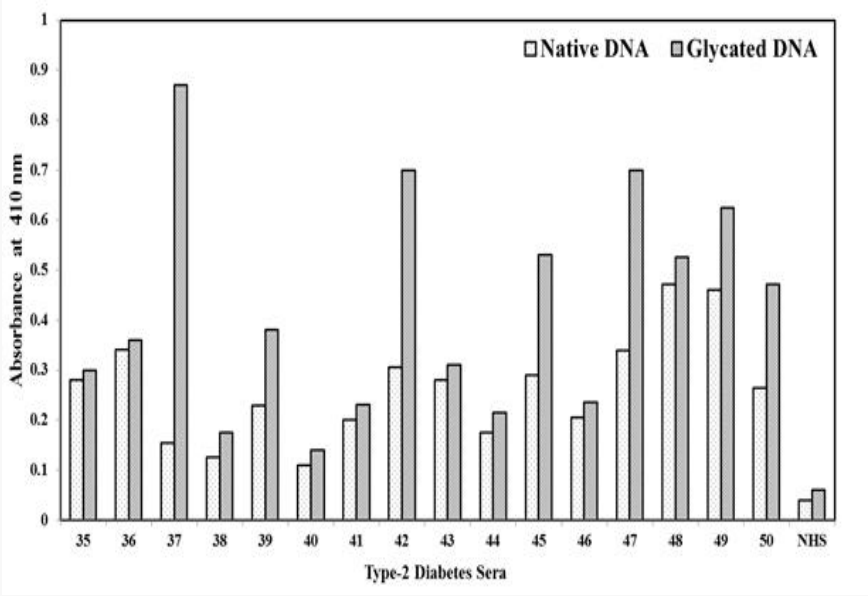

FIG. 5. **Direct binding ELISA of Type-2 Diabetes sera (35-50) with native DNA and glycated-DNA. Pooled normal human sera (NHS) served as control. Microtitre plate was coated with respective antigens.**

TABLE 3. **Direct binding ELISA of type-2 diabetes sera with native DNA and methylglyoxal-modified-DNA**

Type-2 Diabetes sera	Direct binding with	
	Native DNA	Methylglyoxal-modified-DNA
1	0.272	0.393
2	0.303	1.364
3	0.399	1.508
4	0.446	0.583
5	0.441	1.274
6	0.143	0.231
7	0.351	0.562
8	0.405	0.678
9	0.391	1.145
10	0.381	0.654
11	0.345	1.187
12	0.4	1.228
13	0.28	0.341
14	0.303	0.439
15	0.155	0.883
16	0.162	0.192
17	0.295	0.609
18	0.399	1.548
19	0.446	0.583
20	0.431	1.274
21	0.143	0.231
22	0.155	1.5
23	0.162	0.306
24	0.295	1.24
25	0.18	0.32
26	0.385	0.993
27	0.174	0.234
28	0.386	1.421
29	0.478	0.563
30	0.404	1.123
31	0.402	1.312
32	0.523	0.687
33	0.198	0.367
34	0.512	1.184
35	0.28	0.341
36	0.313	0.439
37	0.155	0.823
38	0.136	0.629
39	0.229	0.734
40	0.11	0.18
41	0.144	0.288
42	0.306	1.112
43	0.286	0.632
44	0.16	0.23
45	0.251	0.973
46	0.183	0.265
47	0.339	1.006
48	0.471	0.526
49	0.427	0.884
50	0.265	0.677
Mean ± S.D.	0.304 ± 0.12	0.758 ± 0.42

Competitive Inhibition ELISA of Selected Diabetic Sera by Native DNA and MG-modified-DNA

The binding specificity of selected samples was evaluated by inhibition ELISA using native DNA and MG-modified-DNA as inhibitors. **FIG. 6** showed inhibition profile of diabetic sera number 22. The inhibition of autoantibodies present in Type 2 DM by native DNA was found to be in the range of 26-39%. The MG-modified-DNA showed higher inhibition in the range of 56-76% under identical experimental conditions. However, the mean percent inhibition by native DNA and MG-modified-DNA was 32.35 ± 3.39 and 64.34 ± 6.67 percent respectively (**TABLE 4**). MG-modified-DNA emerged as a more powerful inhibitor as compared to native DNA indicating that the serum auto-antibodies of these diabetic patients have strong specificity for glycated DNA. Inter-comparison of the data by Student's t- test yielded a p-value of less than 0.01 for MG-modified-DNA vs native DNA. This points out that the data is statistically significant.

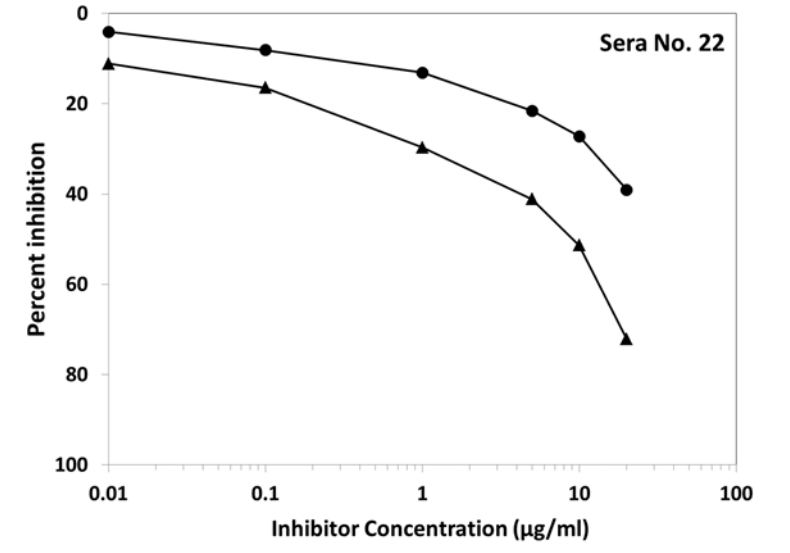

FIG. 6. **Inhibition of type-2 dibetes sera (22) binding by native DNA (—●—) and methylglyoxal-modified-DNA (—▲—). Microtitre wells were coated with native DNA (2.5 µg/ml).**

TABLE 4. **Summary of inhibition ELISA of selected type-2 diabetes sera.**

SLE sera	Maximum percent inhibition at 20 µg/ml	
	Native DNA	**Methylglyoxal-modified-DNA**
2	29.09	60.14
3	32.02	68.11
5	26.47	64.12
9	31.96	57.98
11	30.44	58.09
12	32.94	75.91
15	31.14	63.09
17	33.1	74.16
18	39.01	71.06
20	33.02	59.07
22	36.09	76.14
24	37.04	73.01
26	26.13	54.08
28	33.09	62.01
30	32.1	65.99
31	31.07	59.12
34	30.13	62.64
37	33.11	58.35
39	35.15	73.37
42	39.48	61.21
45	29.58	56.48
47	29.48	65.32
50	32.48	60.48
Mean ± S.D	**32.35 ± 3.39**	**64.34 ± 6.67**

Discussion

Glycation or non-enzymatic glycosylation is the reaction between reducing sugars and amino groups of proteins, lipids and nucleic acids [6]. The dicarbonyl compounds such as MG, glyoxal, 3-deoxyglucosone and short-chain aldehydes including diacetyl and purvaldehyde are formed during glycation. These highly reactive products form AGEs with proteins' amino groups [9,10]. Previous studies have shown that nucleic acids also reacts with sugars as that of proteins in vitro in a similar way [44]. Also, various glycating agents including glucose, MG or dihydroxyacetone (DHA) form a product N2-carboxyethyl-2'-deoxyguanosine (CEdGA,B) which is stable [15]. DNA-AGEs such as CEdG, identified as a main glycation product of DNA is potentially genotoxic which could alter DNA structure and function. Preliminary studies have shown that CEdG destabilizes the N-glycosidic bond between carboxyethylguanine and the sugar-phosphate DNA backbone. Also, it causes depurination and mutation [45-47]. DNA glycation has received minimal attention although characterization of

proteins by glycation has always been the topic of interest. In the present study, physico-chemical techniques revealed extensive damage of DNA on glycation with MG. The UV/Vis spectra done at 260 nm exhibited hyperchromicity could be due to damage to nitrogenous bases which resulted in the exposure of chromophoric groups of MG-modified-DNA with reference to native DNA. The possible reason of hyperchromicity at 260 nm might be destruction of sugar-phosphate backbone by adduction-cum-free radical followed by partial unfolding of double helix which has resulted in more exposure of chromophoric bases [48]. Furthermore, a new peak appeared at 310 nm in the glycated DNA samples. This is attributed to the formation of AGEs in the DNA macromolecule which depicted the higher absorbance which might suggest Cu2+enhanced AGEs formation. Few studies have also reported an extra peak at 330 nm supporting our findings [49]. Fluorogenic AGEs are analysed at the characteristic excitation and emission wave-lengths for AGEs fluorophore, i.e., 370 nm (excitation) and 440 nm (emission) wavelength [50]. In our study, in the case of glycated DNA showed an increase in fluorescence intensity of 91% as compared to its native DNA which did not show any appreciable fluorescence under identical condition. It suggested that fluorescent DNA–AGEs were produced when DNA was glycated by methylglyoxal and lysine in the presence of copper sulphate. Previous studies have revealed that dicarbonyl compounds can react with bases in DNA (purine and pyrimidine) non enzymatically and can alter DNA's structure and conformation. Earlier studies have also shown that glycation of DNA has generates chromophores and fluorophores with properties similar to that of AGEs [51].

Therefore, UV/Vis and fluorescence analysis suggested that DNA glycation by MG has resulted in structural perturbation of DNA which resulted in single strand breaks and base modifications. The present study was undertaken to explore the possible role of MG-modified-DNA in etiopathogenesis of DM in a sub-population. Binding characteristics of serum auto-antibodies to native DNA and MG-modified-DNA was investigated so that some possible role of MG-modified-DNA in Type 2 DM could be searched. Diabetic patients' sera revealed preference for MG-modified-DNA in comparison to native DNA. It might suggest that DNA-AGEs in circulation might have become an effective antigen to attract immunoregulatory response resulting in the generation of anti-glycated DNA-antibodies as demonstrated by high titre of anti-glycated DNA auto-antibodies by direct binding ELISA. Inhibition ELISA supported the above findings. It showed that MG-modified-DNA came out as a powerful inhibitor as compared to native DNA indicating that the serum auto-antibodies have strong specificity for MG-modified-DNA of these Type 2 DM patients. Earlier studies also supported our findings that DNA-AGEs cause depurination and mutation of DNA and its persistence could result or initiate an immune response keeping in mind the immunogenic nature of AGEs [52,53]. Also, it will not be out of context to think that apoptotic DNA might have stayed in diabetic blood for a longer time and resulted in generation of immunogenic DNA-AGEs.

Our study revealed that MG-modified-DNA might be a potential target for circulating autoantibodies in patients with Type 2 DM and this target could be used as a predictive biomarker for controlling the disease and its complications. MG could be a missing link between hyperglycemia and perpetuation of Type 2 DM. All these interesting findings suggested that glycation modifies the structure of DNA to the extent that it becomes immunogenic which might have resulted in generation of neo-antigenic epitopes which are better antigens for antibodies in Type 2 diabetes patients. The need of hour is that to carry out larger multicentric studies to establish the strong association between DNA-AGEs and their autoantibodies for better understanding of pathophysiology of Type 2 DM and hence to discover more effective treatment.

REFERENCES

1. niddk.nih.gov

2. Njølstad PR, Sagen JV, Bjørkhaug L, et al. Permanent neonatal diabetes caused by glucokinase deficiency: inborn error of the glucose-insulin signaling pathway. Diabetes. 2003; 52(11):2854-60.
3. Negre-Salvayre A, Salvayre R, Augé N, et al. Hyperglycemia and glycation in diabetic complications. Antioxid Redox Signal. 2009; 11(12): 3071-109.
4. Dyer DG, Dunn JA, Thorpe SR, et al. Accumulation of Maillard reaction products in skin collagen in diabetes and aging. J Clin Invest. 1993; 91(6):2463-9.
5. Reynolds TM. Chemistry of nonenzymic browning I. The reaction between aldoses and amines. Adv Food Nutr Res 1963; 12: 1-52.
6. Takeuchi M, Yamagishi SI. Involvement of toxic AGEs (TAGE) in the pathogenesis of diabetic vascular complications and Alzheimer's disease. J Alzheimers Dis. 2009; 16(4):845-58.
7. Watkins NG, Thorpe SR, Baynes JW. Glycation of amino groups in protein. Studies on the specificity of modification of RNase by glucose. J Biol Chem. 1985; 260(19):10629-36.
8. Furth AJ. Glycated proteins in diabetes. Br J Biomed Sci. 1997; 54(3): 192-200.
9. Frye EB, Degenhardt TP, Thorpe SR, et al. Role of the Maillard reaction in aging of tissue proteins advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins. J Biol Chem. 1998; 273(30):18714-9.
10. Schmidt AM, Du Yan S, Wautier JL, et al. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circ Res. 1999; 84(5):489-97.
11. Baynes JW. The Maillard hypothesis on aging: time to focus on DNA. Ann N Y Acad Sci. 2002; 959(1):360-7.
12. Knerr T, Severin T. Reaction of glucose with guanosine. Tetrahedron letters. 1993; 34(46):7389-90.
13. Ochs S, Severin T. Reaction of 2′-Deoxyguanosine with Glyceraldehyde. Liebigs Annalen der Chemie. 1994; 1994(8):851-3.
14. Larisch B, Pischetsrieder M, Severin T. Formation of guanosine adducts from L-ascorbic acid under oxidative conditions. Bioorg Med Chem Lett. 1997; 7(20):2681-6.
15. Seidel W, Pischetsrieder M. Immunochemical detection of N2-[1-(1-carboxy) ethyl] guanosine, an advanced glycation end product formed by the reaction of DNA and reducing sugars or L-ascorbic acid in vitro. Biochim Biophys Acta -General Subjects. 1998; 1425(3):478-84.
16. Silva MS, Gomes RA, Ferreira AE, et al. The glyoxalase pathway: the first hundred years… and beyond. Biochem J. 2013; 453(1):1-5.
17. Meyerhof O. On the enzymatic equilibrium reaction between Hexosediphosphorsaure and Dioxyacetonephosphorsaure. Biochem Z. 1934; 271: 89-110.
18. Nagao M, Fujita Y, Wakabayashi K, et al. Mutagens in coffee and other beverages. Environ Health Perspect. 1986; 67: 89-91.
19. Moree-Testa P, Saint-Jalm Y. Determination of α-dicarbonyl compounds in cigarette smoke. J Chromatogr A. 1981; 217: 197-208.
20. Meyerhof O. On the enzymatic equilibrium reaction between Hexosediphosphorsaure and Dioxyacetonephosphorsaure. Biochem Z. 1934; 271: 89-110.

21. Lyles GA, Chalmers J. The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. Biochem Pharmacol.1992; 43(7):1409-14.
22. Ahmed N, Babaei-Jadidi R, Howell SK, et al. Degradation products of proteins damaged by glycation, oxidation and nitration in clinical type 1diabetes. Diabetologia. 2005; 48(8): 1590-603.
23. Aleksandrovskii YA. Antithrombin III, C1 inhibitor, methylglyoxal, and polymorphonuclear leukocytes in the development of vascular complications in diabetes mellitus. Thromb Res. 1992; 67(2): 179-89.
24. Monnier VM, Cerami A. Nonenzymatic browning in vivo: possible process for aging of long-lived proteins. Science. 1981; 211(4481): 491-3.
25. Esterbauer H, Cheeseman KH, et al. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2+ in rat liver microsomes. Biochem J. 1982; 208(1):129-40.
26. Casazza JP, Felver ME, Veech RL. The metabolism of acetone in rat. J Biol Chem. 1984; 259(1): 231-6.
27. Dhar A, Desai K, Liu J, et al. Methylglyoxal, protein binding and biological samples: are we getting the true measure? J Chromatogr. B. 2009; 877(11-12): 1093-100.
28. Rabbani N, Thornalley PJ. Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. Amino acids. 2012; 42(4): 1133-42.
29. Thornalley PJ, Rabbani N. Protein damage in diabetes and uremia—identifying hotspots of proteome damage where minimal modification is amplified to marked pathophysiological effect. Free Radic Res. 2011; 45(1): 89-100.
30. Nagaraj RH, Shipanova IN, Faust FM. Protein cross-linking by the Maillard reaction isolation, characterization, and in vivo detection of a lysine-lysine cross-link derived from methylglyoxal. J Biol Chem. 1996; 271(32): 19338-45.
31. Oya T, Hattori N, Mizuno Y, et al. Methylglyoxal Modification of Protein Chemical and Immunochemical Characterization of Methylglyoxal-Arginine Adducts. J Biol Chem. 1999; 274(26): 18492-502.
32. Shipanova IN, Glomb MA, Nagaraj RH. Protein modification by methylglyoxal: chemical nature and synthetic mechanism of a major fluorescent adduct. Arch Biochem Biophys. 1997; 344(1): 29-36.
33. Shamsi FA, Partal A, Sady C, et al. Immunological evidence for methylglyoxal-derived modifications in vivo determination of antigenic epitopes. J Biol Chem. 1998; 273(12): 6928-36.
34. Kilhovd BK, Giardino I, Torjesen PA, et al. Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. Metabolism. 2003; 52(2): 163-7.
35. Turk Z, Nemet I, Varga-Defteardarović L, et al. Elevated level of methylglyoxal during diabetic ketoacidosis and its recovery phase. Diabetes & metabolism. 2006; 32(2): 176-80.
36. Odani H, Shinzato T, Matsumoto Y, et al. Increase in Three α, β-Dicarbonyl Compound Levels in Human Uremic Plasma: Specificin VivoDetermination of Intermediates in Advanced Maillard Reaction. Biochem Biophys Res Commun. 1999; 256(1): 89-93.
37. McLellan AC, Phillips SA, Thornalley PJ. The assay of methylglyoxal in biological systems by derivatization with 1, 2-diamino-4, 5-dimethoxybenzene. Anal. Biochem. 1992; 206(1): 17-23.
38. Thornalley P. Advanced glycation and the development of diabetic complications. Unifying the involvement of glucose, methylglyoxal and oxidative stress. Endocrinol Metab. 1996; 3: 149-66.
39. Bierhaus A, Fleming T, Stoyanov S, et al. Methylglyoxal modification of Na v 1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. Nat Med. 2012; 18(6): 926-33.

40. Hudson BI, Hofmann MA, Bucciarelli L, et al. Glycation and diabetes: The RAGE connection. Curr Sci. 2002; 25: 1515-21.
41. McLellan AC, Thornalley PJ, Benn J, et al. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. Clin Sci. 1994; 87(1): 21-9.
42. Kang JH. Oxidative damage of DNA induced by methylglyoxal in vitro. Toxicology letters. 2003; 145(2): 181-7.
43. Ali R, Alam K. Evaluation of antibodies against oxygen free radical-modified DNA by ELISA. In Oxidative Stress Biomarkers and Antioxidant Protocols 2002; 186: 171-81.
44. Alam K, Jabeen S. Immunogenicity of mitochondrial DNA modified by hydroxyl radical. Cellular immunology. 2007; 247(1): 12-7.
45. Singh R, Barden A, Mori T, et al. Advanced glycation end-products: a review. Diabetologia. 2001; 44(2): 129-46.
46. Pischetsrieder M, Seidel W, Münch G, et al. N2-(1-carboxyethyl) deoxyguanosine, a nonenzymatic glycation adduct of DNA, induces single-strand breaks and increases mutation frequencies. Biochem Biophys Res Commun. 1999; 264(2): 544-9.
47. Seidel W, Pischetsrieder M. DNA-glycation leads to depurination by the loss of N2-carboxyethylguanine in vitro. Cellular and molecular biology. 1998; 44(7): 1165-70.
48. Pethig R, Szent-Györgyi A. Electronic properties of the casein-methylglyoxal complex. Proceedings of the National Academy of Sciences. 1977; 74(1): 226-8.
49. Schmitt A, Schmitt J, Münch G et al. Characterization of advanced glycation end products for biochemical studies: side chain modifications and fluorescence characteristics. Anal. Biochem. 2005; 338(2): 201-15.
50. Mironova R, Niwa T, Handzhiyski Y, et al. Evidence for non-enzymatic glycosylation of Escherichia coli chromosomal DNA. Molecular microbiology. 2005; 55(6): 1801-11.
51. Bucala R, Model P, Cerami A. Modification of DNA by reducing sugars: a possible mechanism for nucleic acid aging and age-related dysfunction in gene expression. Proceedings of the National Academy of Sciences. 1984; 81(1): 105-9.
52. Turk Z, Ljubic S, Turk N, et al. Detection of autoantibodies against advanced glycation endproducts and AGE-immune complexes in serum of patients with diabetes mellitus. Clin Chim Acta. 2001; 303(1-2): 105-15.
53. Pihoker C, Gilliam LK, Hampe CS, et al. Autoantibodies in diabetes. Diabetes. 2005; 1(54): 52-61.