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Assessment of Serum Malondialdehyde (MDA) and Urinary 8-hydroxydeoxyguanosine (8-OHdG) in Egyptian Children with Type I Diabetes Mellitus and Factors affecting.

Abeer M Nour El Din Abd El Baky[1]*, Nagwa Abdallah Ismail[1], Maha MA Abo-Hashesh[1], Manal E Kandil[1], Inas A Rasheed[2], Eman H Thabet[2] and Dalia El-Lebedy[2]

[1]Department of Pediatrics, National Research Centre (NRC), Egypt.
[2]Department of Clinical Pathology, National Research Centre (NRC), Egypt

ABSTRACT

Hyperglycemia generates oxidative stress. Malondialdehyde (MDA) is one of the indicators of oxidative stress. 8-hydroxydeoxyguanosine (8-OHdG) is a sensitive, stable, and integral marker of oxidative damage. This study estimated serum MDA level and urinary 8hydroxydeoxyguanosine (8-OHdG) in type 1 DM Egyptian children and to correlate their values with severity of type 1 DM. Material and Methods :Our study included 132 children with type 1 DM and 50 children ages matched healthy . Clinical examination and evaluation of lipid profile , glycosylated hemoglobin , serum malondialdehyde (MDA) and urinary8-hydroxydeoxyguanosine were done for all subjects . Results :MDA and 8-OHdG were significantly higher in diabetic children than control . MDA was significantly positively correlated with urinary level of 8-OHdG in diabetic children . 8-OHdG showed a negative correlation with age. Conclusion, the gained results support that oxidative stress in Type 1 diabetic may start early in disease course. Therefore, MDA and urinary 8-OHdG can be a beneficial marker of oxidative stress assessment and glycemic control should be intensified to prevent diabetic complications. Using antioxidant medication could help in delaying diabetic complications.

Keywords : Type 1 Diabetes Mellitus , Malondialdehyde (MDA), 8hydroxydeoxyguanosine (8-OHdG) , children

Corresponding author

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INTRODUCTION

Hyperglycemia generates oxidative stress, which is exacerbated by metabolic stress. Free oxygen radicals generation in DM lead to accumulation of malondialdehyde (MDA) by peroxidative breakdown of phospholipids [1]. It is one of the indicators of oxidative stress.

Nuclear and mitochondrial DNA is the most serious target for oxidative attack by free radicals. 8-hydroxydeoxyguanosine (8-OHdG) is produced by the oxidation of deoxyguanosine, which can induce mutations [2,3]. In autoxidation process, glucose may undergo metal-catalyzed autoxidation to produce reactive carbonyl precursors of advanced glycation end products_(AGEs). Free radicals are created in this process, which can cause DNA oxidation, lipid peroxidation, and other cellular lesions [4]. 8-OHdG is a sensitive, stable, and integral marker of oxidative damage in cellular DNA. Biomonitoring has demonstrated that 8-OHdG can be excreted in the urine. As 8-OHdG performs dynamic equilibrium between DNA oxidative damage and DNA repair , its measurement is useful to evaluate DNA damage in whole body [5, 6].

The 8-OHdG is produced from guanosine residues by the oxidation of DNA with intracellular reactive oxygen species (ROS) [7]. The level of 8-OHdG is increased in patients with diabetes, and it has been reported that 8-OHdG is a biomarker of the patients with early stage diabetic complications [7].

The biochemical markers of oxidative stress associated with chronic hyperglycemia are prevalent in developmental pediatrics age group [8]. Numerous authors attempts to employ markers of oxidative stress as additional clinical manifestations to evaluate the metabolic status in diabetes or to predict the risk of developing late complications have been undertaken [9–12].

We are aiming in this work to measure serum MDA level and urinary 8hydroxydeoxyguanosine (8-OHdG) in type 1 DM Egyptian children and to correlate their values with severity of type 1 DM.

MATERIAL AND METHODS

A case control study was conducted on 182 subjects, the patients group consisted of 132 children with T1DM referred to Pediatric Clinic of National Research Centre. Diagnosis of T1D was established according to the criteria of the American Diabetes Association [13]. Fifty healthy children of same age group and sex distribution with neither symptoms nor family history of T1D or any other autoimmune disorders served as a control group. All patients required insulin for glycemic control at the time of diagnosis. Conventional regimen includes the administration of three injections of insulin, mainly a combination of regular short-acting and intermediate-acting insulin (usually before breakfast, lunch and dinner time, respectively), coupled with self-monitoring of blood glucose (SMBG) and adjustments of insulin dosage in response to the individual's glycemic control.

Full history taking and thorough clinical examination including anthropometric measurements (height and weight) were done by the instruments followed the International Biological Program (IBP) [14]. BMI was calculated as weight divided by the height squared (Kg /m^2). Relevant laboratory investigations were performed for all patients and controls including glycosylated hemoglobin (HbA1C) levels and lipid profile (cholesterol, triglyceride , HDL and LDL) .

Informed consents were obtained from the parents of our subjects according to guidelines of ethical committee of National Research Centre, Egypt.

Laboratory Methods

Laboratory tests:

Fasting (12 - 14 hours) venous blood samples were withdrawn from all subjects on plain blood-collection tubes. Part of each sample will be mixed with EDTA for glycated Hb (HbA1c) detection, and serum was separated from the remaining blood in the plain tube after clotting by centrifugation at 3000 xg for 5 minutes for malondialdehyde (MDA) and lipid profile detection. Separated serum was stored at - 20ºC until

analysis. Random urine samples were collected & stored at - 20 ºC until used for detection of Urinary 8-hydroxydeoxyguanosine (8-OHdG).

Estimation of lipid profile:

Serum levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) were measured by standardized enzymatic procedures, using kits supplied by Roche Diagnostics (Mannheim, Germany) on the Olympus AU 400 automated clinical chemistry analyzer. Low density lipoprotein cholesterol (LDL-C) was calculated according to formula of Friedewald et al as follows:

LDL – C = Total cholesterol – Triglycerides/5 + HDL-C [15].

Quantitative determination of human MDA in serum.

PRINCIPLE MDA ELISA kit applies the quantitative sandwich enzyme immunoassay technique. The microtiter plate had been pre-coated with a monoclonal antibody specific for MDA. Calibrators or samples were then added to the microtiter plate wells and MDA if present would bind to the antibody pre-coated wells. In order to quantitatively determine the amount of MDA present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for MDA was added to each well to sandwich the MDA immobilized on the plate. The microtiter plate was incubated, and then the wells were thoroughly washed to remove all unbound components. Next, a substrate solution was added to each well. The enzyme (HRP) and substrate were allowed to react over a short incubation period. Only those wells that contain MDA and enzyme-conjugated antibody exhibited a change in color. The enzyme-substrate reaction was terminated by addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. A calibration curve was plotted relating the intensity of the color (O.D.) to the concentration of calibrators. The MDA concentration in each sample was interpolated from this calibration curve.

Assay Range: 0.75- 100 nmol/ml.
Sensitivity: 0.515 nmol/ml.
Intra assay precision: CV< 10%.
Inter assay precision: CV< 12%.

Human urinary 8-Hydroxy-deoxyguanosine (8-OHdG) ELISA

Urinary 8-OHdG was determined by competitive enzyme-linked immunosorbent assay (ELISA) (Glory Science co., Ltd, USA , , www.glorybioscience.com, Cat.No #:90357, Tel: 001-830-734-0090 .) The procedure was performed according to the manufacturer's instruction.

The kit is for the quantitative level of 8-OHdG in the sample. Purified anti Human 8-OhdG antibody was adopted to coat microtiter plate and make a solid-phase antibody, then 8-OHdG in urine samples was added to wells, then 8-OHdG antibody was Combined with labeled HRP to form antibody-antigen -enzyme-antibody complex. After washing completely, TMB substrate solution was added, TMB substrate became blue color at HRP enzyme-catalyzed. The reaction was terminated by the addition of a stop solution and the color change was measured at a wavelength of 450 nm. The concentration of 8-OHdG in the samples was then determined by comparing the O.D. of the samples to the standard curve. Detection range: 10-300 nmol/ml.

Statistical analysis

Statistical Package for Social Science (SPSS) program version 15 (Chicago, IL, USA) was used for the analysis of data. All numerical variables were expressed as mean± SD. For multiple independent variables, ANOVA test was performed and least significant difference (LSD) method for multiple comparisons. Spearman's correlation was used for detection of the relation between two variables. P-value was considered significant if it was less than 0.05.

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RESULTS

Our study included 132 patients with type 1 diabetes (52males and 80 females) and 50 healthy children (25 males and 25females). All the patients with diabetes were on intensive insulin therapy regimen. Table 1 shows the comparison anthropometric and clinical data of T1DM cases and control including: (mean and ±SD) of age, BMI, disease duration, insulin dose ,systolic , diastolic blood pressure and glycated hemoglobin (HbA1c) and lipid profile .

Table 1 Descriptive data of T1DM cases and controls

Variable	Controls (50)	Diabetics (132)	P Value
Age (year)	11.65±3.54	12.98±3.35	1.40
DurationT1DM(years)	NA	4.68±3.24	
BMI kg/m2	21.42±6.95	22.13±4.39	.412
WC/HT	.45±.17	.46±.12	.714
WC/HIP	.86±.07	.83±.056	.041*
Percentage body fat	23.86±10.67	24.14±6.42	.827
SBP mmHg	99.86±11.43	105.09±11.20	.017*
DBP mmHg	64.86±9.0	66.51±7.74	.289
Height for age Z score	-.24 ±1.14	-.27±1.36	.900
Weight for age Z score	.50 ±1.82	.67±1.33	.526
Insulin dose IU/kg/day	NA	1.05±.37	
HbA1C%	5.59 ±.69	7.54±1.67	.000**
Cholesterol (mg/dl)	172.24±44.76	192.33±52.34	0.019*
TG (mg/dl)	89.40±35.02	111.14±50.99	0.003**
HDL(mg/dl)	63.72±14.02	65.18±14.24	0.578
LDL(mg/dl)	87.79±42.75	104.75±48.91	0.044*

BMI, body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; WC/HT, waist circumference /height; WC/HIP, waist circumference /hip circumference, TG, triglyceride, HDL, high density lipoprotein, LDL , low density lipoprotein, NA, not available . ** Highly significant,* significant

Distribution of serum MDA in diabetic patients and controls reveals a high statistically significant difference between patients and controls regarding serum MDA (P value = .000) Fig. (1).

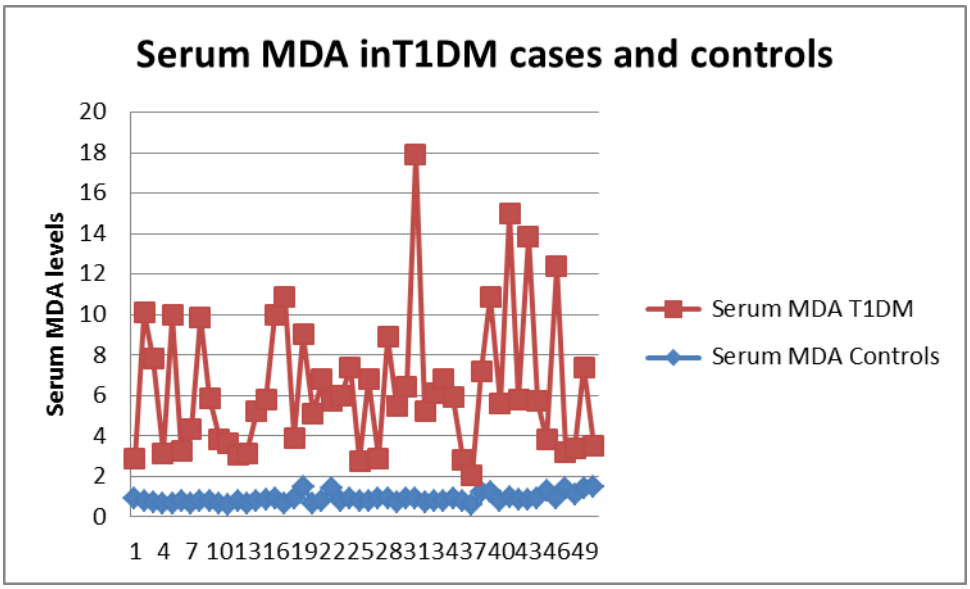

Fig (1) Distribution of serum MDA in diabetic patients and controls.

Also Urinary 8 hydroxy -deoxyguanosine shows a high statistically significant difference between diabetic patients and control group (P value = .000). Fig (2) reveals distribution of Urinary 8 hydroxy -deoxyguanosine in diabetic patients and controls.

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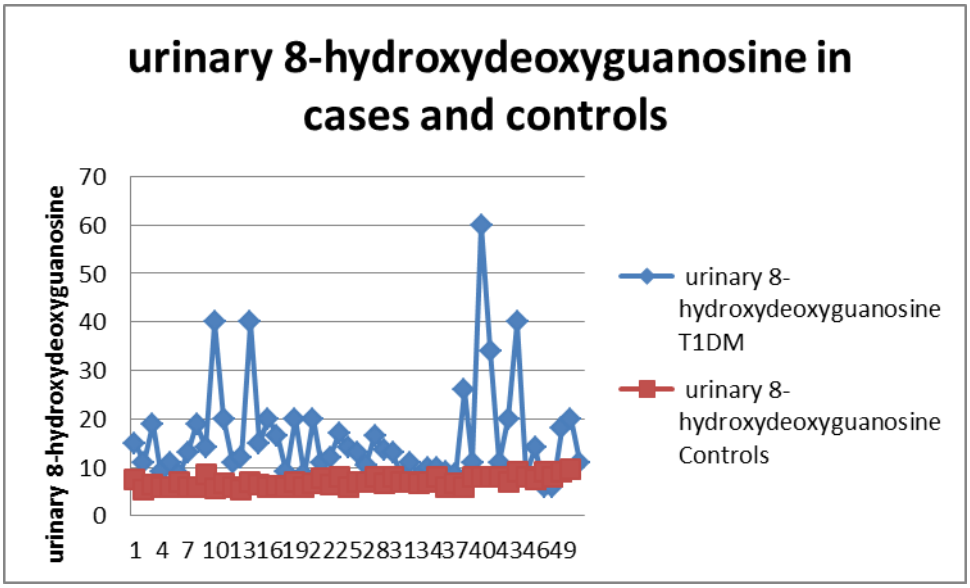

Fig (2) Distribution of Urinary 8 hydroxy -deoxyguanosine in diabetic patients and controls

In our studied subjects , we divides them according to BMI into normal weight ≤ 25 and overweight/ obese ≥ 25 . Table (2) shows a high statistically significance difference between groups regarding MDA, 8 hydroxy -deoxyguanosine and HbA1C

Regarding MDA results in between groups, there were statistically significant difference between DM normal wt , normal wt. controls and obese controls (P =.000 , .000 respectively). In addition there were statistically significant difference between obese DM, DM normal wt ,normal wt. controls and obese controls (P = .000 , .000 &000 respectively).

8 hydroxy -deoxyguanosine measurement results revealed statistically significant difference between DM normal wt , normal wt. controls and obese controls (P =.000 &.025 respectively). With statistically significant difference between obese DM , normal wt. controls & obese controls (P=003 , .000 &000 respectively).Furthermore obese controls showed statistically significant difference with DM normal wt. and obese DM (P .025 &.003).

HbA1C levels revealed statistically significant difference between DM normal with normal wt. controls (P=.000). In addition levels in obese DM found statistically significant difference with normal wt. controls and obese controls (P=.000 & .009 respectively) Table(2).

Table (2) Comparison MDA , 8 hydroxy -deoxyguanosine & HbA1C results in between subgroups.

		N	Mean	Std. Deviation	Minimum	Maximum	
MDA nmol/L	Normal WT T1DM	80	5.25	3.45	1.50	17.00	.000
	Obese T1DM	52	6.72	3.93	1.50	15.00	
	Normal WT controls	35	.80	.18	.56	1.50	
	Obese controls	15	1.51	.23	.80	3.50	
8 hydroxy -deoxyguanosine ng/ L	Normal WT T1DM	80	14.7 4	8.98	6.00	60.00	.000
	Obese T1DM	52	16.52	16.06	7.00	100.00	
	Normal WT controls	35	6.67	.79	5.30	8.50	
	Obese controls	15	8.07	.93	6.00	9.50	
HbA1C	Normal WT T1DM	80	7.41	1.61	4.00	12.20	.000
	Obese T1DM	52	7.81	1.76	4.60	11.00	
	Normal WT controls	35	5.47	.71	4.00	6.50	

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	Obese controls	15	5.95	.50	5.20	6.50	

The difference between groups were sig except between normal wt T1DM &obese T1DM groups in all items by LSD.

Urinary level of 8-OHdG is significantly positively correlated with MDA (r 0.365, P=.001) , in addition 8-OHdG shows a negative correlation with age (r=-.202 , P = .048) in diabetic group table (3).

Table (3) Spearman's rho Correlations in T1DM

		MDA mmol/L	8 hydroxy - deoxyguanosine ng/ L	HBA1C	DURATION (years)	BMI	AGE (years)
MDAmmol/L	Correlation Coefficient	1.000	.365(**)	-.204	-.002	.051	-.085
	Sig. (2-tailed)	.	.001	.077	.987	.620	.411
8 hydroxy - deoxyguanosine (8-OHdG) ng/ L	Correlation Coefficient	.365(**)	1.000	-.134	.005	-.141	-.202(*)
	Sig. (2-tailed)	.001	.	.247	.960	.170	.048

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

In controls correlations are present between MDA and 8-OHdG , furthermore correlations between BMI with both MDA and 8-OHdG (table 4).

Table (4) Spearman's rho Correlations in controls

		MDA mmol/L	8 hydroxy - deoxyguanosine ng/ L	HBA1C	BMI	AGE (years)
MDAmmol/L	Correlation Coefficient	1.000	.616(**)	.024	.598(**)	.095
	Sig. (2-tailed)	.	.000	.910	.000	.511
8 hydroxy - deoxyguanosine (8-OHdG)ng/ L	Correlation Coefficient	.616(**)	1.000	.077	.679(**)	.033
	Sig. (2-tailed)	.000	.	.722	.000	.821

** Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Several studies on the formation of reactive oxygen species (ROS) and oxidative stress have demonstrated that the hyperglycaemia-induced process of peroxide generation in the mitochondrial chain of electron transport plays a key role in the activation of pathways responsible for the development of diabetic progression and complications [16].

In present study we focused on specific marker of oxidative damage to DNA that is called urinary 8-OHdG . Also glycated hemoglobin and MDA levels as markers for glycemic control, serum protein glycation.

Urinary levels of 8-OHdG were significantly higher in diabetic children than controls, this results in agreement with Goodarzi et al., 2010 [17] and Mitsutaka Ono et al.,2014 [18].The 8-OHdG is produced from guanosine residues by the oxidation of DNA with intracellular reactive oxygen species (ROS) [19]. The level of 8-OHdG is increased in patients with diabetes, and it has been reported that 8-OHdG is a biomarker of the patients with early stage diabetic complications [19].

Previous studies revealed the association between oxidative DNA damage and diabetic complications, so that urinary 8-OHdG levels were significantly higher in diabetic patients with nephropathy and retinopathy [20].

Results of our study also revealed that our diabetic children have a statistically significant higher level of MDA than controls. This result in concordance with Goodarzi et al., 2010[17].

Same as Dave et al., 2015 [21] showed that serum MDA levels were found to be higher in diabetics as compared to controls (P = 0.00). In addition malondialdehyde and protein carbonyl group levels were gradually higher in diabetic children and adolescents than in control subjects ($P < 0.0001$)[22] . Same results showed increased MDA in diabetic children when compared with healthy children)[23] .

Also a significantly high level of malondialdehyde was observed in type 2 diabetic subjects with insulin in addition to hypoglycaemic drugs (HGDI) when compared to subjects with hypoglycaemic drugs alone (HGD). In addition studies by Griesmacher et al. and Ruiz et al. [24, 25] revealed same results.

Our results related to high statistically significance difference between normal weight and obese controls regarding MDA, 8 hydroxy -deoxyguanosine and HbA1C is due obesity. Studies related to this point , revealed an increase in oxidative stress associated with obesity, examined by various methods – measurements of, malondialdehyde formation (MDA), oxidized LDL, and other parameters . A preivous review showed studies of obesity and type 2 diabetes are associated with an increase in oxidative stress [26]. In addition Sfar et al 2013 observed that in childhood period, there was increase in obesity related oxidative stress. Moreover the complications of an increased BMI, obesity itself might be considered as a risk factor of free radical production with an increased antioxidant response [27]. Our obese and nonobese type 1 diabetic children showed increase in oxidative stress with no significant variation. This could be explained by the possibility that type1 diabetes has stronger oxidative stress than obesity.

Lipid profile levels in our study shows a statically significant increase in cholesterol, triglyceride, & low density lipoprotein in diabetic children than controls. Henry 2001 [28] revealed that common pattern observed in diabetic patients when compared to normal subjects is a low level of HDL and moderately high levels of Triglyceride, LDL, and cholesterol. Typical diabetic dyslipidemia pattern is mainly associated with insulin resistance and poor glycemic control [29].Our diabetic cases showed a high statistically significant increase in glycated hemoglobin than controls , which indicted poor diabetic control .

CONCLUSIONS

In conclusion, the gained results support that oxidative stress in Type 1 diabetic children can lead to damage to DNA, may start early in disease course. Therefore, urinary 8-OHdG and MDA can be a beneficial marker of oxidative stress assessment and glycemic control should be intensified to prevent diabetic complications. Using antioxidant medication could help in delaying diabetic complications.

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