Oxidative DNA damage and Lipid Peroxidation as Markers of Oxidative Stress among Type 2 Diabetic Patients

Original Article

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Summary:

Background:
Type 2 diabetes mellitus is a chronic disease that is accompanied by the production of free radicals which will ultimately increases the level of oxidative stress

Aim:
To show whether there is a relation between level of lipid peroxidation and that of oxidative DNA damage as represented by the level of 8OHGuanosine in type 2 diabetes.

Subjects and Methods:
Multiple biochemical parameters were obtained from 60 patients with type 2 diabetes (25 males and 35 females) on oral hypoglycemic agents with disease duration ranging from (1 – 15) years, and 40 matching healthy normal control subjects.

Biochemical parameters included in the study involved the measurements of HbA1c, serum lipid profiles (Total Cholesterol, Triglycerides, HDL, LDL and VLDL), serum lipid peroxides (Malodialdehyde, Oxidized HDL and Oxidized Non-HDL) and serum 8OHdGuanosine as a measurements of the level of oxidative DNA damage.

Results:
The study showed a significant positive relationship between the level of lipid peroxidation and the level of 8OHdGuanosine level in type 2 diabetic patients in a pattern proportion to that of the glycemic control, Dyslipidemia and oxidative stress.

Keywords: type 2 diabetes, lipid peroxides, oxidative DNA damage, HbA1c

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Introduction:

Type 2 diabetes (T2DM) is a devastating and debilitating disease defined by high concentration of glucose in the blood (Hyperglycemia). Chronic hyperglycemia has been shown to cause several microvascular and neuronal complications that often results in blindness, kidney and heart failure, stroke, neuropathies and limb amputation[1]. It account for 90% to 95% of all new diagnosed cases of diabetes. The worldwide point prevalence of which is estimated to be 8.6% in subjects more than 20 years and 20.1% in subjects more than 65 years of age[2]. The direct and indirect medical costs associated with diabetes were estimated at $132 billion in 2002[3].

Diabetes is associated with a high risk of cardiovascular disease (CVD). The management of diabetic dyslipidemia, a well-recognized and modifiable risk factor, is a key element in the multifactorial approach to prevent CVD in individuals with type 2 diabetes[4]. On the other hand oxidative stress, caused by imbalance between the production of reactive forms of oxygen and their elimination, leads to oxidative damage of biomolecules[5]. Reactive oxygen species play an important role in various diseases including diabetes hypertension and atherosclerosis[6]. The consequences of oxidative stress are multiple and invariably ominous. They include lipid peroxidation, resulting in the destruction of membrane lipids and oxidative DNA damage, collectively leading to the loss of cell viability, either via necrotic or apoptotic pathways[7].

Oxidation of DNA may lead to mutation (and hence to carcinogenesis); the most common altered base, 8- oxo-guanine, can pair with A rather than C, and so if it is present during replication, C into A transversions may result[5]. Oxidative DNA damage can be measured by 8-Hydroxy deoxy-Guanosine (8OHdG). The accumulation of this oxidative biomarker in serum provides evidence of increased oxidative damage in patients with diabetes mellitus[8]. Also it is used to estimates the level of oxidative DNA damage in various disease states and in the aging process, and to investigate the protective effects of antioxidants *in vitro*[9,10].

Subject and Methods:

Sixty patients with type 2 diabetes mellitus (twenty five male and thirty five female) were included in this study, aging ranges between (35 – 60) years, with a disease duration ranging between (1 – 15) years. They were on oral hypoglycemic agents with no history of hepatic, renal or absorptive disorders. At the same time forty subjects with a matching age and sex groups were selected as a normal control subjects.

Ten milliliter of venous blood sample was drawn by plastic disposable syringe, two milliliters were put in an EDTA tubes for the measurements of HbA1c, and the rest was used for measurements of serum lipid profiles (Total Cholesterol, Triglycerides, HDL, LDL and VLDL), serum lipid peroxides (Malodialdehyde, Oxidized HDL and Oxidized Non-HDL), and serum oxidative DNA damage (8 OHdGuanosine level).

Serum 8OH dGuanosine was measured by an ELISA Kit[7]. Serum lipid peroxides were measured using the Thiobarbituric acid method. Serum Oxidized HDL was measured after precipitation of all lipoproteins, except HDL by phosphotungestic acid- $MgCl_2$ reagent. The supernatant was used for estimation of oxidized HDL by the same method used for the measurements of total MDA and serum lipid profiles were measured using enzymatic method.

Results:

Table (1) illustrates the distribution of 60 diabetic patients according to the level of HbA1c level, depending on American Diabetic Association HbA1c Quidelines[11].

By referring to *Table (2)*, one can conclude a significant elevation in the level of 8OHdGuanosine in all diabetic groups when compared with control subjects. The

three groups show a significant difference when compared with each other.

In *Table (3)* a significant elevation in the level of Malodialdehyde in all diabetic groups can be seen when compared with controls, there is also significant variations between diabetic groups when compared with each other.

There are significant differences in the level of oxidized HDL and oxidized Non-HDL in all diabetic groups when compared with control subjects as illustrated in *table (3),*. Also this table shows a significant difference in diabetic groups when compared with each other.

A significant elevation in all lipid parameters for all diabetic groups can be seen except for the serum HDL which was found to be reduced in diabetic groups when compared with control subjects *Table (4)*.

There is a significant positive correlation between Serum 8OHdGuanosine concentration and HbA_{1c} level in controls ($r= 0.67$, $p<0.05$) and diabetic groups ($r= 0.58$, $p<0.05$) as demonstrated in Figures *(1) and (2)* respectively.

On the other hand *figure (3)*shows a non-significant correlation between serum 8OHdGuanosine and serum Total Cholesterol in Control subjects, while *Figure (4)* shows a significant positive correlation between serum 8OH-dG level and serum Total Cholesterol in Diabetic group ($r= 0.16$, & $r=0.39$ respectively), at P value equal or less than 0.05.

Mean while a non-significant correlation between serum 8OH-dG level and serum MDA is noticed in Control group. While a significant positive correlation between them is noticed among Diabetic Patients as it is illustrated in *Figures (5) & (6)*, ($r= 0.03$ & 0.38 respectively), at p = or <0.05.

Table (1) Distribution of type 2 diabetic patients according to the level of HbA1c

Diabetic Groups	No. of cases	Percent %
T2DM HbA_{1c} <7%	15	25 %
T2DM HbA_{1c} 7-8%	19	31.6 %
T2DM HbA_{1c} >8%	26	43.4%
TOTAL T2DM	*60*	*100%*

Table (2): 8OHdGuanosine level (Mean ± SD) in Diabetic and Control groups

Groups	HbA_{1c} <7%	HbA_{1c} 7-8%	HbA_{1c} >8%	ANOVA	Controls
8OHdG ng/ml	53.8 ± 5.1	95 ± 14	106 ± 6.5	~	8.9 ± 0.7
t-test p-value*	0.001	H.S	H.S	H.S	~

- Student t-test was done between each diabetic and control groups
- P value is considered significant at 0.05 or less.
- H.S (Highly Significant) when P value = or $<10^{-3}$.

Table (3): Lipid peroxidation and its fractions (mean±SD) in different diabetic groups and control group

Groups	S.MDA Umol/L	t-test P-value*	Ox.HDL %	t-test P-value*	Ox.non-HDL %	t-test P-value*
T2DM HbA_{1c} <7%	0.78 ± 0.22	H.S	65 ± 10	H.S	35 ± 10	H.S
T2DM HbA_{1c} 7-8%	0.82 ± 0.24	H.S	62.4 ± 9.8	H.S	37.6 ± 9.8	H.S
T2DM HbA_{1c} >8%	0.86 ± 0.27	H.S	60 ± 11.9	H.S	40 ± 11.9	H.S
ANOVA P-value	~	H.S	~	H.S	~	H.S
Controls	0.49 ± 0.07	~	72.3 ± 15.8	~	27.6 ± 15.8	~

- Student t-test was done between each diabetic group and control.
- P value is considered significant at 0.05 or less.
- H.S (Highly Significant) when P value = or $<10^{-3}$.

Table (4): Serum Lipid Profile (mean±SD) in Diabetic and Control Groups expressed in mg/dl

Groups	HbA1c <7% n=15	HbA1c 7-8% n=19	HbA1c >8% n=26	ANOVA	Controls n=40
T.Ch	191.5 ± 35	196.2 ± 26	226.1 ± 48	~	171.1 ±23
t-test p-value*	0.05	H.S	H.S	H.S	~
TG	134.2 ±50	137.2 ± 37	215 ± 10	~	94.1 ± 27
t-test p-value*	0.01	0.01	H.S	H.S	~
HDL-C	30 ± 7	29 ± 8	27 ± 5.6	~	42 ± 5.3
t-test p-value*	H.S	H.S	H.S	H.S	~

VLDL-C	26.8 ± 10	26 ± 14	43.5 ± 22	~	18.7 ± 5
t-test p-value*	0.01	0.02	H.S	H.S	~
LDL-C	102 ± 19	155 ± 67	156 ±59	~	111 ± 22
t-test p-value*	0.1	0.01	H.S	H.S	~

- Student t-test was done between each diabetic group and control
- Significant p value at 0.05 or less.
- H.S (Highly Significant) when P value = or $<10^{-3}$.

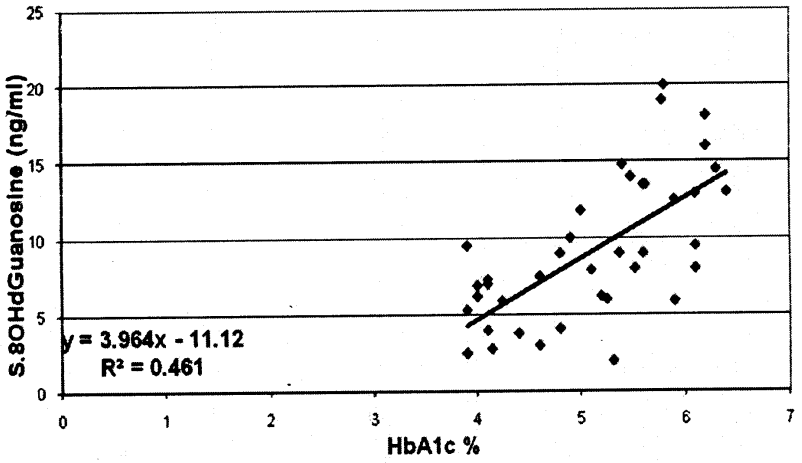

Figure (1): Correlation between Serum 8OH-dGuanosine and HbA_{1c} % in Controls, (r value = 0.67, P value < 0.05)

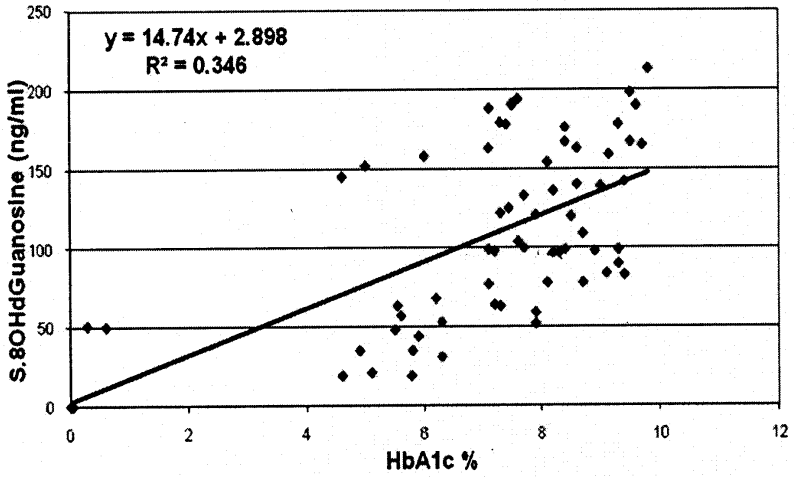

Figure (2): Correlation between Serum 8OH-dGuanosine level and HbA_{1c} % in type 2 diabetes mellitus. (r value = 0.58, P value < 0.05)

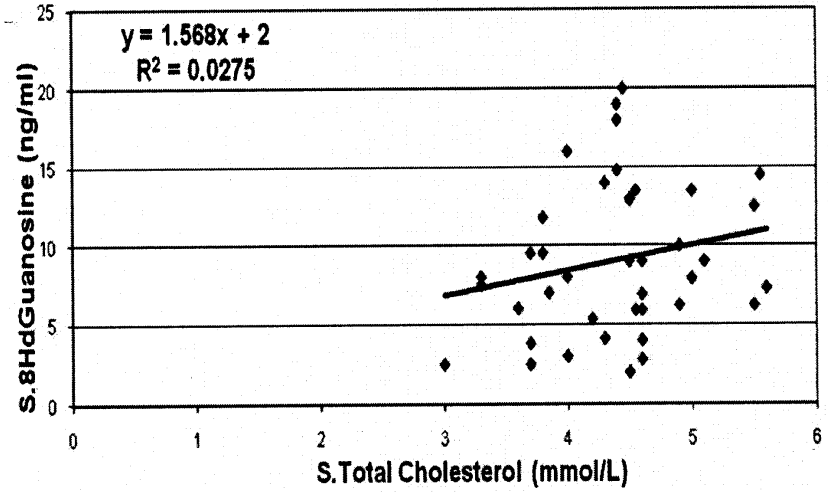

Figure (3): Correlation between serum 8OHdGuanosine level and serum Total cholesterol in Control subjects, (r value = 0.16, P value < 0.05)

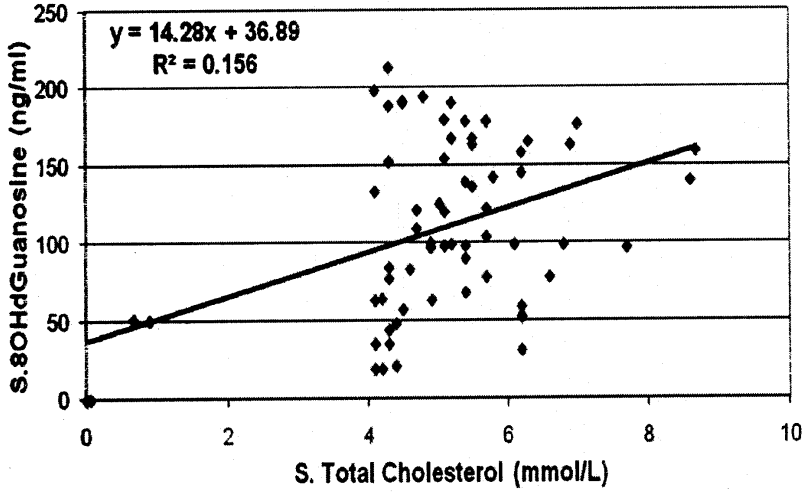

Figure (4): Correlation between serum 8OHdGuanosine level and serum Total cholesterol in Diabetic subjects. (r value =0.39, P = or < 0.05)

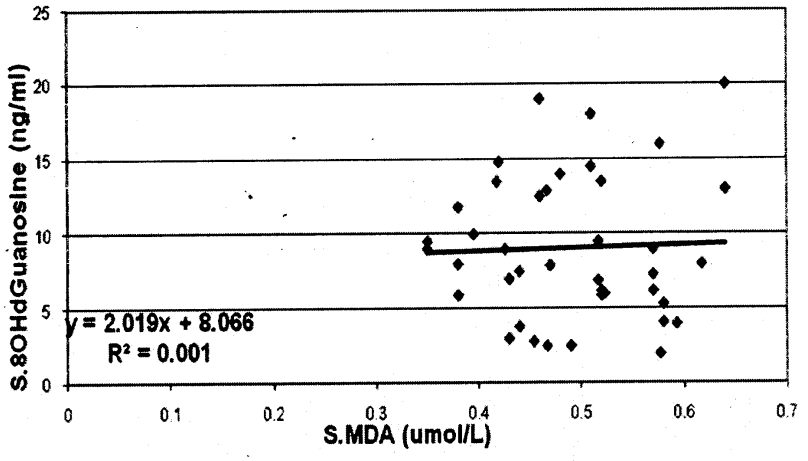

Figure (5): Correlation between serum 8OHdGuanosine level and serum Malondialdehyde in Normal Control subjects. (r value = 0.03 at P = or < 0.05)

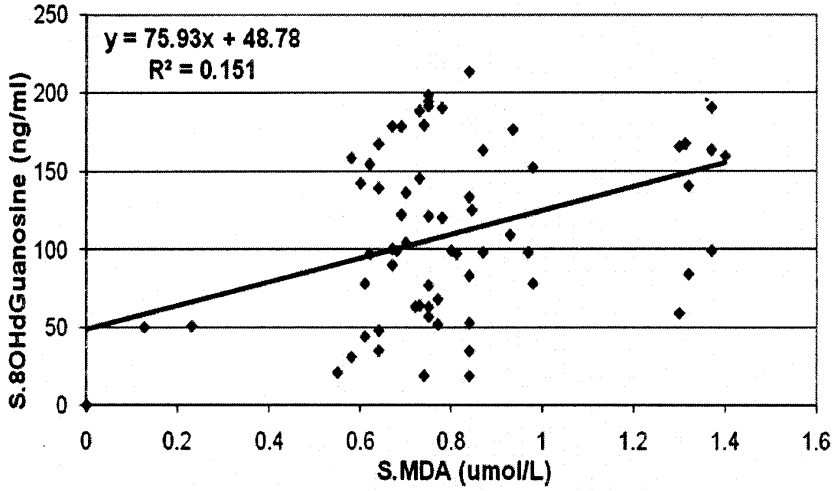

Figure (6): Correlation between serum 8OHdGuanosine level and serum Malondialdehyde in Type 2 Diabetic Patients. (r value = 0.38 at P value <0.05)

Discussion:

Excellent diabetic control implies the maintenance of near normal blood glucose concentration, as reflected by HbA1c <7%. The major cause of death, however, in patients with type 2 diabetes is cardiovascular disease and glucose control may have little impact upon diabetes mortality. In contrast control of other features of metabolic syndrome, such as hypertension, dyslipidemia & hypercoagulability appears to be more important in this regards[12].

Insulin resistance is of great importance not only in carbohydrate metabolism, but also with respect to lipid metabolism. It has long been established that there is a strong relationship between insulin resistance, compensatory hyperinsulinemia and hypertriglyceridemia[13].

The importance of perturbations in lipid metabolism is stressed by the fact that postprandial lipid intolerance in type 2 diabetes appears to be a very early hallmark of the disease, since it was demonstrated in normoglycemic relatives of type 2 diabetes patients who are at high risk of future diabetes[14].

Table *(4)* illustrate a significant elevation in the level of Serum Lipid profiles in all the diabetic groups and as there is more disturbances in the glycemic level there will be more pronounced lipid disturbances, except for the serum HDL-C which found to be reduced significantly when compared with control group.

The management of diabetic dyslipidemia, a well-recognized and modifiable risk factor, is the key element in the multifactorial approach to prevent CVD in individuals with type 2 diabetes[4].

The rise in serum MDA indicates an increased rate of lipid peroxidation which is mostly attributed to hyperglycemia[15]. Oxidative stress, caused by imbalance between the production of reactive forms of oxygen and their elimination, leads to oxidative damage of biomolecules. Reactive oxygen species play an important role in various diseases including diabetes hypertension and atherosclerosis. However, in spite of its deleterious damage, it may have beneficial effects involve physiological roles in cellular responses to anoxia[5,16].

Table (3) illustrates significant differences in total lipid peroxides and oxidized lipid subfractions in the enti

diabetic group compared with the normal controlled subjects. The rise in serum MDA indicates an increased rate of lipid peroxidation which is mostly attributed to hyperglycemia, these results are in accordance with the results obtained from else where[17,18].

This study also showed a significant elevation in serum 8OHdG concentration in all the diabetic groups as illustrated in *Table (2)* and as there is more disturbances in the glycemic control there will be more elevation in the level of 8OHdG as illustrated in *figures (1) & (2)*. This clearly indicates the relation between development of oxidative stress and the progression of type 2 diabetes mellitus. These results are similar with the results of others[19,20].

Figures (3) & (4) illustrate a positive correlation between serum 8OHdG and serum Total Cholesterol. In addition serum 8OHdG level appears to be significantly correlated with serum MDA in all the diabetic and normal control subjects. See *figures (5) & (6)*.

Oxidative stress, including lipid peroxidation, can negatively influence the antioxidant system and consequently cause greater DNA damage. Evaluation of DNA damage has been used as a biological marker in the detection, monitoring, and prognosis of chronic degenerative diseases[21]. Differences in the extent of DNA damage in the normal population have been reported to not only depend on aging but also depend on eating and smoking habits[20].

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