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ORIGINAL ARTICLE

ANTIOXIDANT STATUS AND LIPID PROFILE IN RELATION TO GLYCOSYLATED HEMOGLOBIN LEVELS IN TYPE 2 DIABETES MELLITUS PATIENTS

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ABSTRACT: OBJECTIVE: Type 2 Diabetes Mellitus (Type 2 DM) is a metabolic disorder related to either insulin deficiency or resistance. Glycosylated haemoglobin (HbA1c) is used to monitor long term regulation of blood glucose. Studies relating HbA1c with antioxidant status and lipid profile are very few. Present study was carried out to determine the relation between HbA1c and antioxidant markers along with lipid profile in these patients. **METHODOLOGY:** Blood samples were collected from healthy controls (n=55) and type 2 DM patients (n=52) and their fasting lipid profile and HbA1c levels were determined using auto analyser. Antioxidant status markers such as serum thiols, ceruloplasmin and GST were measured spectrophotometrically. **RESULT:** There was significant increase in fasting plasma glucose (FPG) ($p<0.001$), HbA1c ($p<0.001$), LDL-C ($p<0.001$) and ceruloplasmin ($p<0.001$), and there was significant decrease in serum total antioxidant status ($p<0.001$), total thiols ($p<0.001$), GST ($p<0.001$), and HDL-C ($p<0.001$) in type 2 DM patients compared to healthy controls. HbA1c correlated positively with FPG ($r=0.749$, $p<0.001$), LDL-C ($r=0.513$, $p<0.001$) and negatively with HDL-C ($r=-0.715$, $p<0.001$), total thiols ($r=-0.704$, $p<0.001$) and GST ($r=-0.426$, $p<0.001$). **CONCLUSION:** Findings in our study support the fact that increased glycation of biomolecules and enhanced generation of free radicals lead to deficient antioxidant status coupled with altered lipid profile may possibly increase the susceptibility to atherogenesis.
KEYWORDS: Type 2 diabetes mellitus, Glycated haemoglobin, Antioxidant status, Total thiols.

INTRODUCTION: Type 2 diabetes mellitus (DM) is a heterogeneous group of syndromes characterized by an elevation of fasting plasma glucose (FPG).[1] Type 2 DM is routinely monitored by determining blood glucose for short period or by glycated haemoglobin (HbA1c) for long term monitoring.[2] A number of studies have revealed that the level of HgbA1c is directly proportional to FPG concentrations.[3] The reaction between glucose and beta chain of haemoglobin (Hb) is a slow, irreversible, non-enzymatic, is continuous over the life span of the red blood cells (RBC's) and is proportional to the blood glucose concentration to which the red cell is exposed.[4]

Type 2 DM has been traditionally accepted as a risk factor for the development of coronary heart diseases (CHD). It has been established that hyperglycemia will lead to alteration in lipid profile, increase in low density lipoprotein cholesterol (LDL-C) and decrease in high density lipoprotein cholesterol (HDL-C).[5] Oxidized LDL-C has been proved to be main triggering event in the atherogenesis process leading to microvascular obstruction and its related complications.[6] Uncontrolled diabetes leading to prolonged hyperglycaemia has been studied to be triggering event in glycation of biomolecules and generation of glycated end products. Recently, hyperglycaemia has been shown to be directly related to the generation of free radicals and there by leading to bimolecular damage.[7,8]

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Presence of antioxidants will protect biomolecules from oxidative damage, and there are different types of antioxidants protecting biomolecules within and out of cell. One of the major antioxidants within and out of the cell is the thiol system of antioxidant mechanism. Total thiols are present either in free form as reduced glutathione (GSH) or bound to proteins, mainly to albumin.[9,10]

Previous studies have shown relation between oxidative damage to biomolecules leading to atherogenesis process and long standing hyperglycaemia in type 2 DM. It has been shown that atherogenic changes mostly occur during the postprandial stage when blood glucose levels are at higher range. Hyperlipidaemia as a consequence long standing hyperglycemia, is directly related to to atherogenesis process. The interlinking of these pathways is required to be studied in different clinical settings.[11]

The current study has been undertaken to know the relation between HbA1c, fasting plasma glucose, lipid profile and major antioxidant markers type 2 DM patients in comparison to healthy controls.

MATERIALS AND METHODS: SUBJECTS: The study was carried out on 52 type 2 DM and 55 healthy controls. We have included all diagnosed type 2 DM patients having HbA1c >6% and FPG >126 mg%.

Type 2 DM patients having diagnosed complications like ischemic heart disease, diabetic nephropathy, diabetic retinopathy and other microvascular complications were excluded from the study group. Type 2 DM patients were either on insulin therapy or oral hypoglycaemic agents or both. The mean duration of type 2 DM was 5 years, and mean age of patients was 55 years, none of the patients were known to suffer from any acute illness or chronic inflammatory condition at the time of study. The healthy controls were not on any medication and were free from any acute or chronic illness. Informed consent was obtained from all subjects involved in the study. This study was approved by institution ethical committee for human research.

Samples and Reagents: Blood samples were drawn under aseptic conditions into both plain vacutainers and vacutainers containing EDTA with sodium fluoride, and were centrifuged at 5000 rpm for 15 minutes. Whole blood was used for HbA1c and plasma was used for FPG determination.

The serum so obtained was immediately assayed for total antioxidant activity, total thiols, ceruloplasmin, glutathione-S-transferase (GST), total antioxidant activity and lipid profile. Special chemicals like 5' 5' dithio- bis-(2-nitrobenzoic acid) (DTNB), 1-chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), p- phenylenediamine (PPD), were obtained from Sigma chemical co. (St Louis, MO, USA). All other reagents used were of analytical grade.

METHODS: Serum total total thiols were measured by a spectrophotometric method using DTNB.[12] 900µl of 0.2M Na2HPO4 containing 2mM Na2 EDTA, 100µl of serum and 20µl of 10mM DTNB in 0.2M Na2HPO4 were taken in an Eppendorf tube and warmed to 37 degrees centigrade. The solution was mixed with a vortex mixer and transferred to a cuvette and the absorbance was measured at the end of 5 min at 412nm. Simultaneously sample and reagent blanks were also prepared and their values were ascertained at 412nm. The absorbance of the sample and the reagent blank were subtracted from the serum absorbance values. The calibration curve was produced using glutathione dissolved in phosphate buffer saline (PBS). The total thiol concentration in the serum was determined from the standard curve using molar exctinction coefficient 1600 $M^{-1}.Cm^{-1}$.

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Serum Ceruloplasmin was determined using diamine oxidase method Sheinberg et al.,[13] In this method, the action of ceruloplasmin on P-phenyl diamine (PPD) is used to measure the amount of ceruloplasmin present in the serum. Violet colour was read at 546nm using control tube as blank. Briefly, 3ml of PPD was added to both the blank and the test followed by the addition of 600µl sodium azide only to the blank. 60µl of serum was added to both the blank and the test andboth tubes were incubated at 37degrees centigrade for 15 minutes. This was followed by the addition of 600µl of sodium azide only for the test and the O.D. was read at 546nm. Serum ceruloplasmin was calculated in mg/dl using formula T-B×237, we have expressed ceruloplasmin values in SI units i.e., in µ moles/L using conversion factor mg/dl×10.

Serum GST activity was measured spectrophotometric method by et al.,[14] Briefly, 850µl of phosphate buffer of pH 6.5, 50µl of CDNB, 50µl of GSH were added and incubated at 37 degrees centigrade for 10 minutes. This was followed by the addition of 50µl of serum. The OD was read at 340nm at 1 minute interval for 5 minutes. Serum GST activity was calculated from mean absorbance difference using extinction coefficient $9600M^{-1}.Cm^{-1}$

Serum total antioxidant activity (AOA) was measured by Koracevic et al's method described elsewhere.[15] HbA1c in whole blood was determined by immunoturbidimetric method in Cobas Integra 400. FPG and serum lipid profile levels were determined using the automated analyzer Hitachi 912.

Statistical Analysis: Statistical analysis was performed using the Statistical Package for Social Sciences. (SPSS-14, Chicago, USA). Independent sample t test and Mann Whitney rank sum test were used to compare mean values. Pearson's correlation was applied to correlate between the parameters. The results were expressed as mean±SD and a $p < 0.001$ was considered statistically significant.

RESULTS: There was a significant increase in the FPG ($p<0.001$) HbA1c ($p<0.001$), LDL-C ($p<0.001$) and ceruloplasmin ($p<0.001$), and there was significant decrease in serum total antioxidant activity ($p<0.001$), total thiols ($p<0.001$), GST ($p<0.001$), and HDL-C ($p<0.001$) in type 2 DM patients compared to healthy controls. (Table/figure 1&2) HbA1c correlated positively with FPG ($r = 0.749$, $p<0.001$), LDL-C ($r=0.513$, $p<0.001$) and negatively with HDL-C ($r=-0.715$, $p<0.001$), total thiols ($r=-0.704$, $p<0.001$) (Table/figure 3) and GST ($r=-0.426$, $p<0.001$).

DISCUSSION: Oxidative stress has been implicated in the aetiology and progression of type 2 DM. This could be attributed to increased generation of free radicals and decreased total antioxidant levels in these patients.[16] Studies have shown that HbA1c and FPG both have shown positive correlation with lipid hydroperoxides (LPO),[17] and it has been reported that there is positive correlation between the degree of hyperglycemia and oxidative stress,[18] These reports indicate that there is a direct relation between degree of hyperglycemia and oxidative damage to biomolecules. In our study we have also found significant increase in HbA1c and FPG in type 2 DM patients. Our results have shown significant positive correlation between HbA1c and LDL-C and negative correlation with total thiols and HDL-C. This possibly indicates increased free radical generation with increase in glycation of biomolecules including albumin and lipoproteins. This possibly may damage functional capacity of these biomolecules.

Oxidative damage to lipoproteins like LDL may initiate the atherogenesis process, where oxidized LDL can be engulfed by scavenger macrophages and initiate the lipid accumulation in vessel wall leading to atherogenesis.[19] We have also observed significant decrease in HDL-C and increase in LDL-C in our patient group, and negative correlation HDL-C with HbA1c. This may indicate decreased anti-atherogenic potential of HDL-C in these patients in prolonged hyperglycaemic states as indicated by rise in HbA1c. It has been reported that advanced glycation end products (AGE'S) are elevated in type 2 DM patients with coronary and carotid angiopathy. AGE's have shown possible role in complications of uncontrolled type 2 DM.[20]

We have also observed significant decrease in total antioxidant activity, total thiols and GST activity in type 2 DM patients indicating depletion of antioxidant pool in this population. This may possibly indicate increased presence of free radicals generated due to various causes including prolonged hyperglycemia. Recently it has been reported that there is a significant decrease in both serum and urinary total thiols and authors have also reported that such thiols correlated negatively with FPG.[21] We have also observed that HbA1c was correlating negatively with total thiols and GST. This finding supports our previous statement, where prolonged hyperglycemia depletes the antioxidant pool by generating of free radicals.

In conclusion, findings in our study support the fact that increased glycation of biomolecules and enhanced generation of free radicals lead to deficient antioxidant status. Decreased antioxidants coupled with altered lipid profile may possibly increase the susceptibility to atherogenesis in type 2 DM patients.

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Table/Fig 1: Independent sample t test for all the determined biochemical parameters in both healthy controls and type 2 DM cases (Values expressed as mean ± Standard error of mean, both minimum and maximum value observed).

	Healthy controls (n =55)	**Type 2 DM patients (n=52)**
FPG (mg/dl)	83.00±1.34 Max:99.40 Min:64.10	157.57±7.72* Max:300.00 Min:97.00
HbA1c (mg%)	5.79±0.08 Max:6.80 Min:4.69	7.69± 0.19* Max:10.90 Min:5.70

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TC (mg/dl)	154.52±3.70 Max:198.00 Min:110.00	159.61±5.34 Max:248.00 Min:98.00
TG (mg/dl)	189.20±2.83 Max:237.00 Min:156.00	167.17±11.11 Max:448.00 Min:41.00
HDL (mg/dl)	106.32±1.18 Max:130.00 Min:90.00	37.13±2.16* Max:91.00 Min:10.00
LDL (mg/dl)	46.56±1.18 Max:65.00 Min:35.00	89.04±4.22* Max:163.00 Min:37.40
Total thiols (µmoles/L)	327.79±7.56 Max:412.00 Min:233.00	29.17±1.24* Max:53.75 Min:14.38
Ceruloplasmin (mg/dl)	30.40±0.82 Max:39.00 Min: 20.00	56.55±2.90* Max:120.87 Min:14.22
GST (IU/L)	1.21±0.07 Max:2.01 Min:0.26	0.68±0.03* Max:1.25 Min:0.31
AOA (mmol/L)	0.85±0.01 Max:0.96 Min:0.63	1.74±0.07* Max:2.97 Min:1.00

Table 1

*p<0.001 compared to healthy controls.

Table/Fig 2: Mann Whitney rank sum test for all the determined biochemical parameters in both healthy controls and type 2 DM cases.

	Mean rank		**Sum of ranks**		**Z**	**Asymp. Sig. (2-tailed)**
	Case	**Control**	**Case**	**Control**		
FPG(mg/dl)	81.40	28.09	4233.0	1545.0	-8.882	0.0001
HbA1c (mg %)	77.13	32.13	4011.0	1767.0	-7.501	0.0001
TC(mg/dl)	54.69	53.35	2844.0	2934.0	-0.224	0.8220
TG(mg/dl)	45.89	61.66	2386.5	3391.5	-2.628	0.0090
HDL(mg/dl)	26.56	79.95	1381.0	4397.0	-8.899	0.0001
LDL(mg/dl)	77.97	31.34	4054.5	1723.5	-7.773	0.0001
Totalthiols(µmoles/L)	26.50	80.00	1378.0	4400.0	-8.915	0.0001
Ceruloplasmin(..)	78.60	30.75	4087.0	1691.0	-7.976	0.0001
GST(IU/L)	38.77	68.40	2016.0	3762.0	-4.949	0.0001
AOA(mmol/L)	81.50	28.00	4238.0	1540.0	-8.923	0.0001

Table 2

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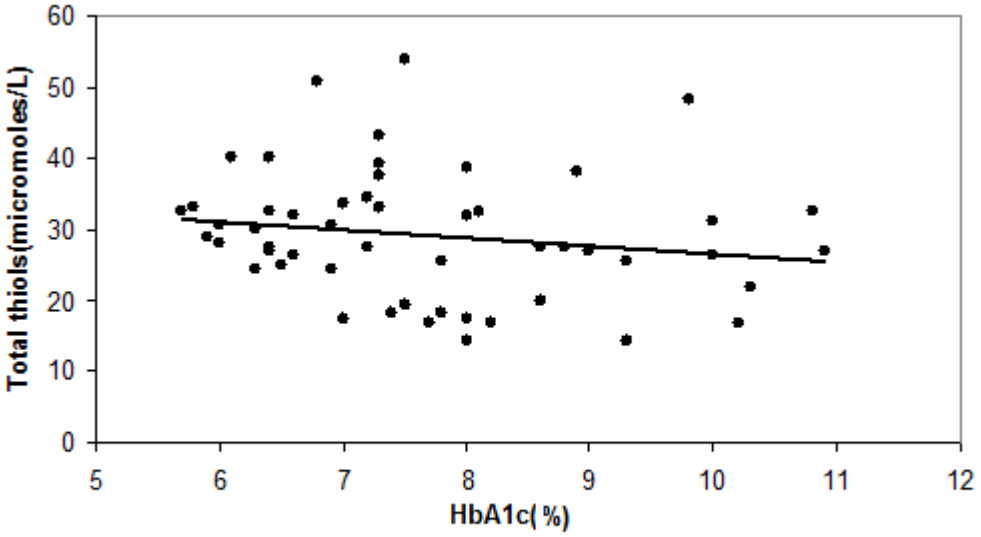

Table/Figure 3: Correlation between total thiols and HbA1c in type 2 DM cases

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