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Malondialdehyde, Superoxide Dismutase, Glutathione Peroxidase, Catalase and GSSH in Newly Diagnosed Type 2 Diabetic Subjects

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ABSTRACT

To analyze the malondialdehyde (MDA), superoxide dismutase (SOD), glutathione dismutase (GPX), catalase (CAT) and glutathione (GSSH) in newly diagnosed type 2 diabetic (T2DM) patients.Case control study Department of Medicine, Jinnah Post Graduate and Medical Centre, JPMC, Karachi from October 2013 to February 2014.50 normal adult controls and 70 newly diagnosed T2DM were included as per criteria. Blood samples were collected for analysis of blood glucose; HbA1c, MDA, SOD, GPX, CAT and GSSH. SPSS 21.0 was used for data analysis (p- ≤ 0.05).MDA was significantly elevated in diabetics 5.9 ± 1.9 compared to controls 4.7± 2.8 µmol/ml respectively (p=0.01) and anti oxidant enzymes- SOD, GPX, CAT and GSSH were reduced in diabetics compared to controls (p=0.0001) Glycated HbA1c and Blood glucose (R) showed positive association with MDA (r = 0.355 and p= 0.026).The serum MDA levels were observed significantly high in newly diagnosed type 2 diabetics with low anti oxidant enzymes. Antioxidants may be prescribed to newly diagnosed type 2 diabetics to halt the vascular complications.

Introduction

Prevalence of Diabetes mellitus (DM) is increasing throughout the World. Incidence of DM is on rise in Asian countries including Pakistan. By the year 2030, the Asian countries are forecasted will be the capital of diabetes mellitus.[1] Most common type of DM is the non-insulin dependent called type 2 DM (T2DM). It accounts for >90% of disease burden and is characterized by chronic hyperglycemia. Chronic hyperglycemia activates polyol pathway of glucose metabolism resulting in accumulation of polyols which cause osmotic cell injury. Chronic hyperglycemia alters protein functioning through non-enzymatic glycation.[2]

Oxidative stress is a hallmark of chronic hyperglycemia due to accelerated free radical formation and lipid peroxidation in both extracellular and intracellular compartments.[3] Several mechanisms of oxidative stress have been suggested such as; polyols, glucose oxidation and non enzymatic proteins glycation, all these generate the free radicals.[4]

Free radicals are chemically reactive. They react with cell biomolecules particularly the polyunsaturated fatty acids (PUFA) of cell membrane. PUFA oxidation induces lipid peroxidation and generates free radicals such as the peroxides, peroxy and short chain aldehyde radicals. One of the most reliable markers of lipid peroxidation is the malondialdehyde (MDA).[5] MDA increases the oxidative load in positive feedback fashion, resulting in aggravation of vascular complications of DM. Lipid peroxides interfere with vascular endothelium and β-cells of Islets of Langerhans, this in turn induces vascular injury and disturbs the glycemic control. Chronic hyperglycemia is also speeds up alternate biochemical pathways resulting in increased free radical generation. Chronic exposure of vascular endothelium to free radicals such as superoxide (O^{2-}) exerts deleterious effects on vascular integrity, physiology, *in-vivo* clot formation and intra endothelial lipid deposition.[6]

Nature has guarded against free radicals by anti oxidant enzyme systems such as SOD, GPX, CAT and GSSH. Various non-enzyme mechanisms are also working altogether.[6,7] The anti oxidant enzymes scavenge the free radicals e.g. SOD neutralizes superoxide (O^{2-}) through dismutation. In normal subjects, there exists a natural balance between oxidative and antioxidant systems which maintain healthiness. An imbalance between two systems, results in increased oxidative load to cell, and tissue. The end result of increased oxidative load in DM subjects is vascular injury resulting in micro-vascular and macro-vascular injury. Cell injury initiates a vicious cycle which multiplies the problem resulting in organ damage. Increased oxidative load and weakened anti oxidant systems have been reported.[8-10] Hence it is worth to analyze the oxidative stress and anti oxidative systems in DM subjects prevention of diabetic vascular complications. Diagnosed diabetics need to be evaluated for the oxidative stress by time to prevent organ damage; this will reduce morbidity and mortality. The present study analysed the superoxide dismutase (SOD), glutathione dismutase (GPX), catalase

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(CAT), reduced glutathione (GSSH) and malondialdehyde (MDA) in newly diagnosed T2DM subjects presenting at our tertiary care hospital.

Subjects and Methods

The present case control study included 120 subjects at the Department of Medicine, Department of Medicine, Jinnah Post Graduate and Medical Centre, JPMC, Karachi from October 2013 to February 2014. Of 120 subjects; 50 were normal adults taken as control group (Group A) and 70 newly diagnosed T2DM taken as cases (Group B). Non-probability purposive sampling technique was adopted for the selection of subjects. Inclusion and exclusion criteria were mandatory for the participants. Inclusion criteria were newly diagnosed T2DM cases of 20-50 years of age. Cases having chronic liver disease, renal disease, cardiac failure, ischemic cardiac disease, chronic lung disease and smokers were excluded. Cases taking diuretic drugs, vitamin and mineral formula supplements and HMG-co A reductase inhibitors were also excluded. Patients attending the Department of Medicine and Diabetic outpatient department were communicated about the purpose of study. They were informed in detail the advantages and disadvantages of study. The participants were open to withdraw from study protocol at any time without telling any reason, and this behavior will not affect their treatment. They were informed about blood sampling is needed for the study. Finally, a sample of 120 subjects was selected which were confident to comply with study protocol. Patient's biodata, history and vitals were noted by medical officer on a pre-structured proforma. This was followed by examination by consultant physician. Care was taken of inclusion and exclusion criteria were strictly obeyed. 10 ml of blood was taken into vacutainers by Venepuncture from ante cubital fossa. Blood samples were centrifuged at 4000 rpm for ten minutes to separate the sera which were stored at -20^0C for analysis. Blood glucose, glycated HbA1, blood urea nitrogen and serum creatinine were measured. Superoxide dismutase (SOD), glutathione dismutase (GPX), catalase (CAT), reduced glutathione (GSSH) and malondialdehyde (MDA) were detected on assay kits (Fortress Diagnostics and Cayman Chemical, USA). Biochemical tests were performed on Cobas e 411 analyzer (Roche Diagnosis GmbH, Mannheim, Germany). The study protocol was approved by Ethical review committee. Signing written informed consent proforma was mandatory for participants. Data was entered in *Statistical Package for Social Sciences (SPSS)* version 21.0. Continuous and categorical data was analyzed by student's t test and Chi square test respectively. Microsoft excel was also used for bar graphs. $P \leq 0.05$ was taken statistically significant.

Results

The present study included 50 normal control subjects (Group A) and 70 newly diagnosed type 2 DM (Group B) subjects. Age mean ± SD was noted as 41.0±8.5 and 45.2±7.97 years respectively. Male predominated with male to female ratio of 1.38 and 2.12 in group A and B respectively. Study subjects of 2 groups were age and sex matched ($p>0.05$). Blood pressure, blood urea nitrogen (BUN), serum creatinine and blood glucose showed statistically significant differences between cases and controls ($p<0.05$). Body weight, height, BMI, pulse, systolic and diastolic BP, blood glucose, glycated HbA1, Blood urea nitrogen (BUN) and serum creatinine are summarized in table 1. All of above variables showed statistically significant differences ($p<0.05$) between 2 groups except for the body weight ($p>0.05$). Anti oxidant enzymes- SOD, GPX, CAT and GSSH were reduced in diabetics compared to controls (p=0.0001) (table 1, Graph 1-4). MDA was significantly elevated in diabetics 5.9 ± 1.9 compared to controls 4.7± 2.8 µmol/ml respectively (p=0.01) (table 1). Bar graph 5 shows the MDA levels in diabetics and controls respectively. Pearson`s correlation is shown in table 2. Glycated HbA1c and Blood glucose (R) showed positive association with MDA with r-value of 0.355 and 0.026 respectively with significant p-value.

Table I. Characteristics and Laboratory findings of study subjects (120)

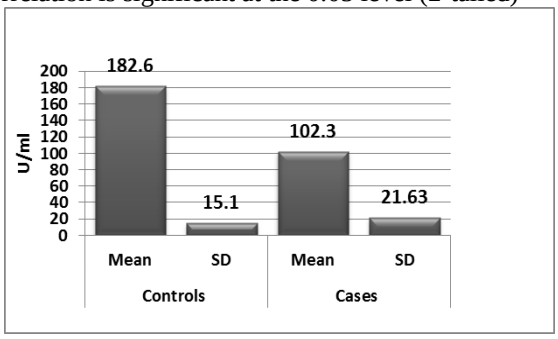
	Group A (Controls) (n=50)	Group B (T2DM) (n=70)	p-value
Age (years)	41.0±8.5	45.2±7.97	0.21
Male	29 (85%)	34 (68%)	0.39
Female	21 (42%)	16 (32%)	0.81
Weight (kg)	75.4±11.0	80.35±12.0.	0.051
Height (cm)	160.0±9.5	161±9.5	0.81
BMI (kg/m^2)	29.4±5.3	27.5±5.1	0.01
Pulse (bpm)	76±17	71±9.0	0.09
Systolic BP (mmHg)	132.5±8.7	133.2±14.4	0.019
Diastolic BP (mmHg)	69.0±4.9	75.4±11.5	0.001
Blood glucose (R) (mg/dl)	134.8±8.3	261.6±62.8	0.0001
Glycated HbA1 (%)	6.05±0.4	8.3±1.5	0.0001
BUN (mg/dl)	9.45±2.1	11.4±4.8	0.012
Serum creatinine(mg/dl)	0.93±0.18	1.07±0.24	0.021
Serum SOD (U/ml)	182.6±15.1	102.3±21.63	0.0001
Serum GPX (U/ml)	231.8±40.1	104.3±25.9	0.0001
Serum CAT (nM/ml)	848.1±101.8	449.2±82.7	0.0001
Serum GSSH (µM/ml)	4.95±0.72	3.60±0.39	0.001
Serum MDA (µmol/ml)	4.7±2.8	5.9±1.9	0.01

Table 2. Pearson`s correlation showing correlation co-efficient (r)

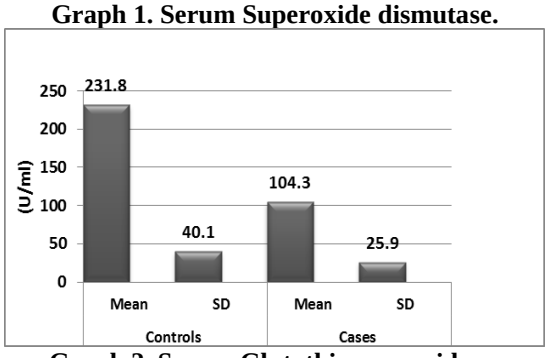
		Glycated HbA1 (%)	Blood Glucose (R) (mg/dl)
Serum MDA µmol/ml)	r-value	0.355**	0.206*
	P-value	0.0001	0.024

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

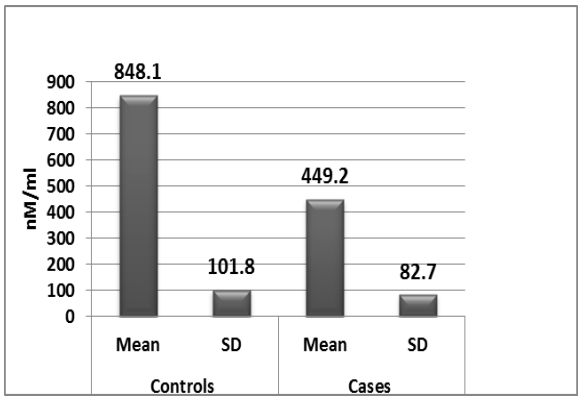
*. Correlation is significant at the 0.05 level (2-tailed)



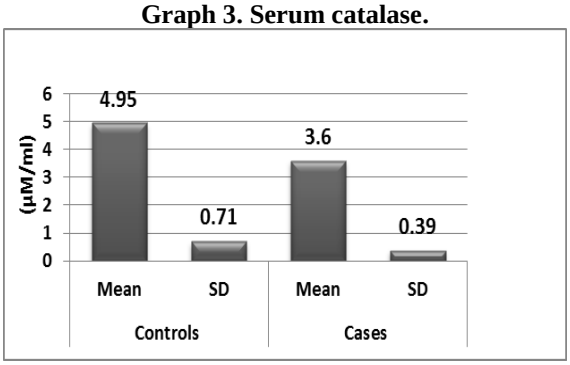
Graph 1. Serum Superoxide dismutase.



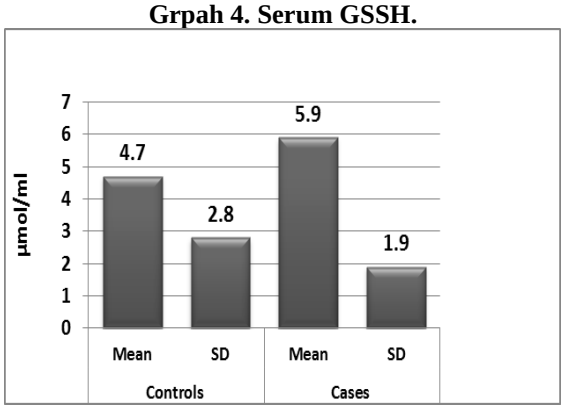
Graph 2. Serum Glutathion peroxidase.



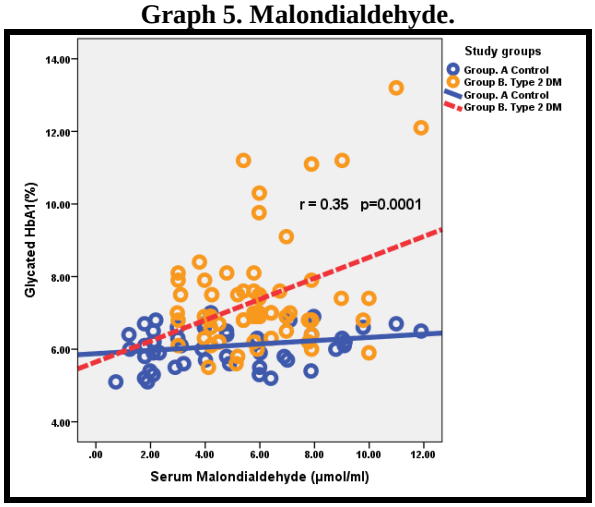
Graph 3. Serum catalase.



Grpah 4. Serum GSSH.



Graph 5. Malondialdehyde.



Graph 1. Scatter graph showing correlation of Malondialdehyde and glycated HbA1.

Discussion

Reactive oxygen species overwhelm the anti oxidant enzyme systems of body leading to oxidative damage in diabetics.[11,12] Endothelial dysfunction is the main hallmark of DM. The gluco-lipotoxicity complicates the vascular dysfunction and makes the person susceptible to oxidative stress. Chronic hyperglycemia multiplies the vascular complications many fold by free radical formation and lipid peroxidation.[13,14] Free radicals are biochemically reactive and breakdown the cell membrane phospholipids leading to malondialdehyde (MDA) formation MDA interacts with LDLc molecule making it prone to be deposited within the vascular endothelium and this initiates atherosclerosis MDA also facilitates non- enzymatic glycosylation of proteins.[15,16].

The present study is the first research reporting on the SOD, GPX, CAT, GSSH and MDA in newly diagnosed T2DM patients. The present study reports the anti oxidant enzymes and reduced glutathione were low in diabetics compared to controls (p=0.0001). Lipid peroxidation marker, the MDA was significantly raised in diabetics 5.9 ± 1.9 compared to controls 4.7± 2.8 µmol/ml respectively (p=0.01). Pearson`s correlation showed positive association between high blood glucose and HbA1c with MDA (r = 0.355, p= 0.026). The findings of present study are in keeping with previous studies.[17-20] This indicates the newly diagnosed T2DM subjects are already carrying a significant lipid peroxides load of which they are not aware. Also the treating physicians do not know the problem. The problem of vascular complications may be halted in beginning if attention is paid. Increased MDA in the presence of reduced SOD, GPX, CAT and GSSH are linked to insulin resistance and vascular endothelial injury leading to vascular complications.[19,20] Our findings are in keeping with above studies. Increased MDA levels in newly diagnosed T2DM are a comparable finding to previous studies.[20,21] Bhuttia et al has reported elevated MDA levels in Sikkimes diabetics along with dyslipidemia and raised HbA1c. They concluded that the MDA was positively correlated with both micro- and macro-vascular complications, these findings support our present study.[22] ROS are independently associated with the vascular endothelial dysfunction and complications and the imbalance of lipid peroxides and anti oxidants- the SOD, CAT and GPX activity are operating synergistically in the pathogenesis of diabetic vascular complications.[17-20] These findings are support the present study. Dave et al[23] has recently reported raised MDA and low serum bilirubin in type 2 diabetics. However, MDA was not associated with the diabetic retinopathy. The findings of MDA, SOD, GPX and CAT are consistent to previous studies.[22,24-26] In present study, newly diagnosed T2DM showed low anti oxidants; SOD, GPX, CAT and GSSH and raised malondialdehyde (MDA), this shows the oxidative stress begins earlier at the very onset of diabetes mellitus.

Conclusions

In conclusion, the serum malondialdehyde levels were observed significantly high in newly diagnosed type 2 diabetics with low anti oxidant enzymes; superoxide dismutase, glutathione dismutase, catalase and reduced glutathione. Antioxidant therapy may be prescribed to newly diagnosed type 2 diabetics to halt the vascular complications.

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