Available online at www.elixirpublishers.com (Elixir International Journal)

Physiology and Anatomy

Elixir Physio. & Anatomy 94 (2016) 40266-40269

ISSN: 2229-712X

Superoxide dismutase, Glutathione peroxidase, Malondialdehyde, Lipids and Glycated HbA1 in newly diagnosed type 2 diabetes mellitus patients.

Dr Naresh Kumar[1], Dr Ahmed Abdul Rahman Abbasi[1], Dr Jagdesh Kumar[1], Dr Arsalan Ahmed Uqaili[2] and Dr Joti Bai[3]

[1]Worked as Medical Officer in Lohana Medical Center, Karachi.
[1]Working as Medical Officer in Isra university Hyderabad
[2]Working as Lecturer in Department of Physiology, Isra University Hyderabad.
[3]House intern Liaquat University Hospital, Jamshoro.

ARTICLE INFO

Article history:
Received: 5 March 2016;
Received in revised form:
4 May 2016;
Accepted: 9 May 2016;

Keywords
Superoxide dismutase,
Glutathione peroxidase
Malondialdehyde Ascorbic Acid,
Uric acid,
ZincGlycated HbA1.

ABSTRACT

Objective: To determine superoxide dismutase (SOD), glutathione dismutase (GPX) malondialdehyde (MDA), uric acid, zinc, ascorbic acid, albumin, blood lipids and glycated hemoglobin A1 (HbA1c) in newly diagnosed type 2 diabetic patients. Study Design: Case control study Place & Duration: Department of Medicine -Liaquat University of Medical and Health Sciences Hospital from December 2014- August 2015. Subjects & Methods: 95 newly diagnosed type 2 diabetics and 55 controls selected through non-probability purposive sampling according to inclusion and exclusion criteria. 8-12 hour fasting was ensured for blood samples. BUN, serum creatinine, albumin, bilirubin, uric acid, blood glucose, HbA1c, blood lipids, triglycerides, cholesterol, glucose, MDA, SOD, GPX, zinc and Ascorbic acid were determined by standard methods. Data was entered in *Statistix 8.1*. (USA) Continuous and categorical data was analyzed by student's t test and Chi square test respectively. Microsoft excel was used for graphing. Data was analyzed at 95% confidence interval (p- $\leq$ 0.05). Results: Blood pressure, obesity, blood glucose, blood urea nitrogen (BUN) and serum creatinine showed statistically significant differences ($p<0.05$). VLDL, triglycerides, LDLc were elevated in diabetics, while HDLc was reduced. MDA, SOD, GPX, zinc, ascorbic acid, uric acid and bilirubin showed significant differences ($p<0.05$). MDA in diabetics and controls was noted as 5.17±0.81 vs. 2.15±0.62 µmol/ml respectively ($p=0.0001$). SOD, GPX, AA, Zn++, UA, serum albumin and serum bilirubin were decreased in diabetics.
Conclusion: The superoxide dismutase (SOD), glutathione peroxidase (GPX), uric acid, bilirubin and zinc were reduced and malondialdehyde (MDA), altered blood lipids and glycated HbA1 were increased in diabetics in present study.

Introduction

Prevalence and incidence of diabetes mellitus (DM) is fastest growing in the world and many Asian countries like Pakistan are forecasted to be the "diabetes capital" by the year 2030.[1] Type 2 DM (T2DM) is commonest type of diabetes as it accounts for >90% of disease burden. Chronic hyperglycemia of DM damages tissues by non-enzymatic glycation of proteins and by accumulation of polyols.[2] DM is characterized by increased oxidative stress caused by elevated glucose levels in extracellular and intracellular compartments.[3] Oxidative stress is caused by several mechanisms, such as oxidation of glucose and non-enzymatic glycation of proteins like collagen, which in turn generates excessive free radicals.[4] Free radicals react with biomolecules, in particular the polyunsaturated fatty acids (PUFA) of cell membranes which are the major targets. PUFA produce lipid peroxides through oxidative destruction.

Lipid peroxidation generates the peroxides, peroxy and short chain aldehyde radicals. Malondialdehyde (MDA) is a reliable marker of lipid peroxidation.[5] MDA augments the oxidative destruction and in turn aggravates the diabetes complications. Vascular endothelium and β-cells of pancreas are susceptible to damage by lipid peroxides. Other hyperglycemia associated biochemical pathways are also associated with increased production of free radicals. Vascular endothelium, if exposed chronically to glucose, produces superoxide free radicals (O^{2-}).[6] SOD and GPX enzymes, vitamins – the ascorbic acid, tocopherols, retinols, and the bilirubin and uric acid, all play role against free radicals. They scavenge the free radicals and prevent tissue damage. A balance between antioxidant mechanisms against oxidant load is vital for cell, tissue and organ protection. These mechanisms also reduce the tissue susceptibility against complications which might erupt. Increased lipid peroxidation is associated with a decline of anti-oxidant enzymes, resulting in cell damage, which in turn gives rise to a positive vicious cycle. Previous studies reported the reduced anti-oxidant mechanisms and exaggerated oxidative stress in DM.[6,7] Inbuilt physiological anti-oxidant enzymes and non-enzymatic systems act synergistically to protect against tissue injury and disease onset. Non enzyme anti-oxidant mechanisms include the zinc (Zn++), ascorbic acid (AA), uric acid (UA), albumin and bilirubin which are normally circulating in the blood plasma.

Tele: +92-3332755701
E-mail address: arsalanuqaili@yahoo.com

They scavenge free radicals, protect against oxidative injury and comprise body`s primary extracellular defense.[6-7] As the incidence and prevalence of DM is increasing in Pakistan, there is a dire need to evaluate into various anti-oxidant systems and injurious lipid peroxidation markers for better patient management in order to reduce the mortality and morbidity. The present study was designed to assess the SOD, GPX, uric acid, ascorbic acid, zinc, malondialdehyde, blood lipids and glycated hemoglobin A in newly diagnosed type 2 diabetic subjects.

Subjects and Methods

The study subjects of present case control study were selected from the Department of Medicine, Liaquat University Hospital Hyderabad/Jamshoro from December 2014- August 2015. 95 newly diagnosed type 2 diabetics (ND-T2DM) and 55 healthy subjects (age and gender matched) were selected as controls. Study subjects were selected through non-probability purposive sampling according to inclusion and exclusion criteria. Newly diagnosed T2DM subjects having age between 20 and 50 years were included. Subjects with concomitant cardiac failure, chronic lung disease, chronic renal disease, liver disease, taking drugs – lipid lowering, vitamins, minerals, diuretics, Metformin and smokers were excluded. Subjects were facilitated to comply with protocol. 8-12 hour fasting was ensured for blood samples. BUN, serum creatinine, albumin, bilirubin, uric acid, blood glucose, HbA1c and blood lipids were analyzed (Cobas e 411 analyzer- Roche Diagnosis GmbH, Mannheim, Germany). Triglycerides and cholesterol were determined by enzymatic colorimetric (CHOD-PAP & GPO-PAP) methods. Precipitant method was used for HDL-Cholesterol. Friedewald's formula (LDL-C = TC - HDL-C – (TG/5)) was used for LDL-Cholesterol.[8] "*Glucose oxidase*" method was employed for glucose determination. MDA, SOD and GPX were detected by assay kit (Cayman Chemical, USA).[9] Zinc and Ascorbic acid were determined by Centronic GmbH-Germany Kit [10] and by the method as mentioned.[11] Data was entered in *Statistix 8.1*. (USA) Continuous and categorical data was analyzed by student's t test and Chi square test respectively. Microsoft excel was used for graphing. Data was analyzed at 95% confidence interval (p- $\leq$ 0.05).

Results

The demographic characteristics are shown in table I. The study subjects were age and gender matched (p>0.05). BMI, blood pressure, obesity, blood glucose, blood urea nitrogen (BUN) and serum creatinine showed statistically significant differences between cases and controls (p<0.05). VLDL, triglycerides, LDLc were elevated in diabetics, while HDLc was reduced in cases. MDA, SOD, GPX, zinc, ascorbic acid, uric acid and bilirubin showed significant differences (p<0.05). MDA in diabetics and controls was noted as 5.17±0.81 vs. 2.15±0.62 µmol/ml respectively (p=0.0001). SOD, GPX, AA, Zn++, UA, serum albumin and serum bilirubin were decreased in diabetics. MDA, SOD and GPX are shown in graphs 1, 2 and 3.

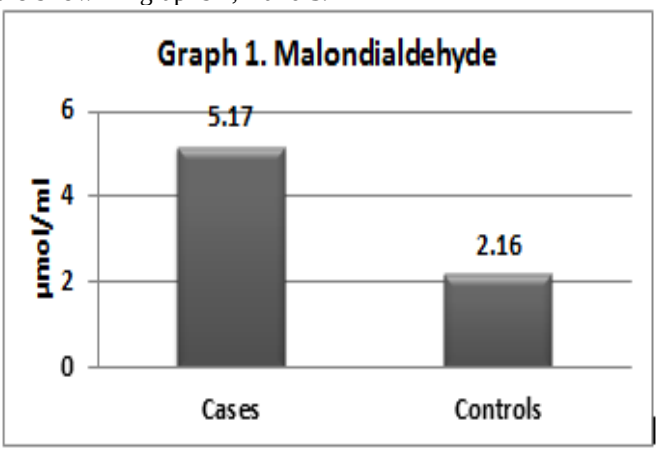
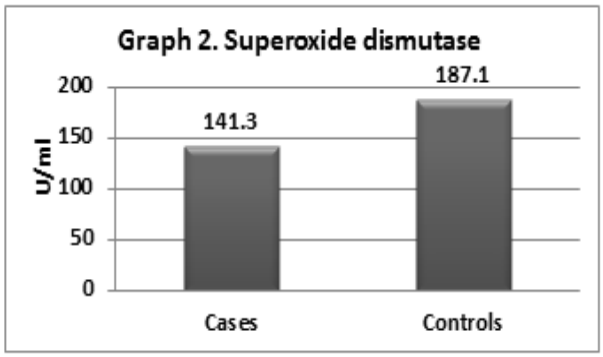
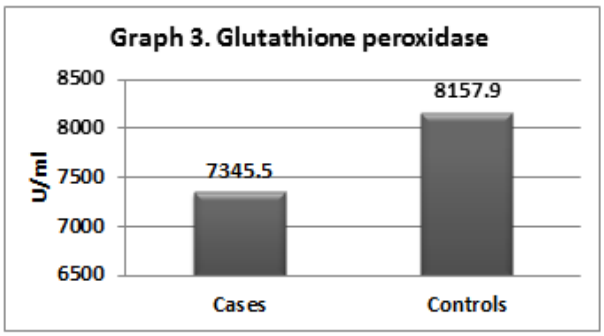


Table 1. Demographic characteristics and Laboratory findings of study subjects.

	Cases (n=95)	Controls (n=55)	p-value
Age (years)	49.7±8.3	49.5±6.1	0.53
Male	76 (80%)	41 (74.5%)	0.19
Female	19 (20%)	14 (25.4%)	0.06
Weight (kg)	56.9±19.7	55.7±16.73	0.07
Height (cm)	163.3±4.5	161.2±5.6	0.81
BMI (kg/m^2)	28.5±7.1	27.3±4.3	0.01
Pulse (bpm)	83±15	76±17	0.09
Blood Pressure- systole (mmHg)	157.5±17.5	136±12.3	0.023
Blood Pressure- diastole (mmHg)	97±11.8	72±11.9	0.041
Hypertension	71 (74.7%)	11 (20%)	0.0001
Obesity	45 (47.3%)	23 (41.8%)	0.00
Blood glucose (F) (mg/dl)	139±37.5	91±9.8	0.0001
Blood glucose (R) (mg/dl)	243±51.0	134±31.1	0.0001
Glycated HbA1 (%)	11.3±5.3	5.6±1.9	0.0001
BUN (mg/dl)	13±4.6	9.3±2.5	0.02
Serum creatinine(mg/dl)	1.3±0.61	0.8±0.12	0.07
Triglycerides (mg/dl)	251±11.8	134.2±45.1	0.001
Cholesterol-Total (mg/dl)	231.1±13.9	118.3±20.7	0.0001
HDLc (mg/dl)	33.4±6.7	39.9±5.9	0.01
LDLc (mg/dl)	139.3±12.3	97.2±10.4	0.001
VLDL (mg/dl)	40.9 ± 10.2	27.3 ± 9.1	0.01
Ascorbic acid (mg/dl)	0.67±0.32	1.10±0.11	0.001
Zinc (µg/dl)	47.9± 1.12	59.4±10.1	0.005
Albumin (g/dl)	4.01±0.31	4.98±0.73	0.004
Bilirubin (mg/dl)	0.41±0.12	0.67±0.36	0.003
Uric acid (mg/dl)	2.04±0.67	3.59±1.7	0.001
Superoxide dismutase (U/ml)	141.3±24.92	187.1±33.7	0.03
Glutathione peroxidase (U/ml)	7345.5±134.0	8157.9±119.0	0.000
Malondialdehyde (µmol/ml)	5.17±0.81	2.15±0.12	0.002





Discussion

It is for the first time, the present study reports a case control study on the superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), uric acid, bilirubin and zinc, blood lipids, glycated HbA1 in T2DM. Our findings are comparable to previous studies cited.[12-15] However, the MDA levels of present are much elevated and SOD and GPX revealed more reductions in diabetics. This shows increased lipid peroxidation and altered anti-oxidant enzymes in diabetics, both of which are linked to insulin resistance, endothelial dysfunctions and diabetic microvascular complications,[12,15] hence our findings are in support to previous studies. Altered SOD, GPX and malondialdehyde were found in chronic hyperglycemia. MDA, a marker of lipid peroxidation, was raised in T2DM and is comparable to a previous studies.[21, 15]

A reduced SOD and GPX activity increases the susceptibility of body tissues to reactive oxygen species (ROS) induced lipid peroxidation and free radical formation, which are closely associated with diabetic microvascular complications. ROS are independent risk factors for the endothelial dysfunction and vascular complications.[11] It been has reported that imbalance between lipid peroxidation and reduced SOD and GPX activity are operating synergistically and simultaneously in diabetics.[12,15] Our findings are consistent with the above cited studies. Non-enzymatic anti-oxidants- the uric acid, zinc, bilirubin, serum albumin, serum α-tocopherol, ascorbic acid, serum β-carotene, serum retinol and retinal are capable of scavenging the ROS and reducing the oxidative stress.[12]

The present study revealed reduced uric acid, bilirubin and zinc levels in diabetics; hence it indicates increased oxidative stress. Zinc is a weak anti-oxidant, [12] our findings are comparable. Zn++ functions in various ways; such as by increased insulin secretion and as an enzyme, thereby normalizing the blood glucose levels.[13] Our findings of low Zn++ in diabetics is consistent with previous studies.[14,15] Previous studies had reported low Zn++ due its urinary loss in diabetics.[12,15] Increased ROS in diabetics occurs due to auto oxidation of glucose, advanced glycation end products and lipid peroxide generation.[16] Above mechanisms are delayed by Zn++, hence supplementation reduces ROS generation. This shows that hypozincemia may accelerate the tissue damage by ROS in susceptible diabetics. Our findings of low zinc and Ascorbic Acid (AA) are in accordance with the previous studies.[17, 18]

Other studies had reported low AA due to increased reactive species, [19, 20] which are comparable to present study. Other studies have reported a competitive inhibition of ascorbic acid by glucose at the level of membrane transporter, which may be corrected by mega doses of AA.[17, 18] Mega doses of AA as high as 2 grams each day improved glycemic parameters in diabetics.[21,22]

Uric acid (UA) also functions as anti-oxidant in body fluids and is capable of scavenging free radicals- singlet oxygen, doublet oxygen (O^{2-}) and OH^{-} radicals. Concentration of UA is 10 times more than that of AA in body fluids.[23-24] UA was reduced in diabetics in present study. Serum bilirubin and albumin were found to be low in diabetics, which is consistent with the previous studies.[25-26]

The present study reports reduced anti-oxidants; both enzymes and non-enzymes – the superoxide dismutase (SOD), glutathione peroxidase (GPX), uric acid, bilirubin and zinc. Elevated malondialdehyde (MDA), altered blood lipids and increased glycated HbA1 were found in T2DM in present study.

Conclusions

The study found reduced levels of Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Uric Acid, Bilirubin and zinc. While, Malondialdehyde (MDA) and Glycated Hemoglobin (HbA1c) levels were found to be increased and blood lipid levels, altered. A poor glycemic control was observed in diabetics, as indicated by HbA1c. Practicing physicians should do their best to control blood glucose and lipid levels and to maintain the balance between oxidant and antioxidant mechanisms in order to minimize the vascular complications of Diabetes.

References

1. Parmar D, Vidja K, Ghugare B. Impact of duration of diabetes and age: on lipid profile and glycaemic control in type 2 diabetic patients. Int J Res Med 2013;2(1):69-72

2. Meshram R, Ghate J, Ghugare B, Koundinya S, Parmar D. Lipid Peroxidation And Erythrocyte Enzymatic Antioxidants In Type II Diabetes Without Complications. Natl J Integr Res Med 2015; 6(5): 17-21

3. Giugliano D, Ceriello A. Oxidative stress and diabetic vascular complications. Diabetes Care 1996; 30(1):257-267.

4. Alti T, Keven K, Avei A, Kutlav S, Turkeapar N, Varli M, et al. Oxidative stress and antioxidant status in elderly diabetes mellitus and glucose intolerance patients. Arch Gerontol Geriatr 2004; 39(3):269-75.

5. Cheesman KH, Slater TF. An introduction to free radical biochemistry. Br Med Bulletin 1993; 49(3); 481-493.

6. Maritim AC. Diabetes, oxidative stress and antioxidants. J Biochem Mol Toxicol 2003; 17:24-38.

7. Mahboob M. Lipid peroxidation and antioxidant enzyme activity in different organs of mice exposed to low level of mercury. J Environ Sci Health B 2001; 36:687-97.

8. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment

of high blood cholesterol in adults (adult treatment panel III). *J Am Med Assoc* 2001; 285(19): 2486– 97.
9. Gavino VC, Miller JS, Ikharebha SO, Milo GE, Cornwall DG. Effects of polyunsaturated fatty acids and antioxidants on lipid peroxidation in tissue cultures. J Lipid Res 1981; 22:763-9.
10. Johnsen, Eliasson R. Evaluation of a commercially available kit for the colorimetric determination of zinc. International Journal of Andrology 1987; 10 (2); 435-40.
11. Ayekyaw. A simple colorimetric method for ascorbic acid determination in blood plasma. Clinica chemica Acta1978; 86: 153-7.
12. West IC. Radicals and oxidative stress in diabetes. Diabet Med 2000; 17: 171-80.
13. Ford Earl S, Mokdad Ali H, Giles Wayne H, Brown David W. The Metabolic Syndrome and Antioxidant Concentrations. Diabetes 2003; 52:2346-52.
14. Marjani A. Plasma lipid peroxidation zinc and erythrocyte Cu- Zn SOD enzyme activity in patients with type 2 DM in Gorgan city. The Internet J of Endocrinology 2005; 2(1): 1540-2606
15. Evliaoglu O. Serum levels of Cu, Zn, and Mg in type 1 and type 2 diabetic patients. Turkish National Biochemical Congress 2002; 285-6.
16. Zargar A H. Copper, zinc and magnesium level in NIDDM. The Asian J of Diabetology 2000; 2 (3): 43-99.
17. Lai MH. Antioxidant effects and insulin resistance improvement of chromium combined with vitamin C and e supplementation for type 2 diabetes mellitus. J Clin Biochem Nutr 2008; 43(3):191-8.
18. Dulal HP, Lamsal M, Sharma SK, Baral N, Majhi S. Status of iron, oxidant and antioxidants in chronic type 2 Diabetes mellitus patients. *Nepal Med Coll J 2013; 15(3): 208-211*.
19. Ray G, Husain S A. Oxidants, antioxidants and carcinogensis. Indian J of Exp. Biol. Non 2002; 40:1213-32.
20. Hisalkar PJ, Patne AB, Fawade MM. Assessment of plasma antioxidant levels in type 2 diabetes patients. Int J Biol Med Res. 2012; 3(2): 1796-1800.
21. Dakhale GN, Chaudhari HV, Shrivastava M. Supplementation of Vitamin C Reduces Blood Glucose and Improves Glycosylated Hemoglobin in Type 2 Diabetes Mellitus: A Randomized, Double-Blind Study. Adv Pharmacol Sci 2011; 2011: 1-5.
22. Ford S E. The metabolic syndrome and antioxidant concentrations. Diabetes 2003; 52: 2346-52.
23. Akbas EM, Timuroglu A, Ozcicek A, Ozcicek F, Demirtias L, Gungor A, et al. Association of uric acid, atherogenic index of plasma and albuminuria in diabetes mellitus. Int J Clin Exp Med 2014;7(12):5737-5743
24. Ceriello A. New insights on oxidative stress and diabetic complications may lead to a causal antioxidant therapy. Diabetes care 2003; 26:1589-96.
25. Ziegler D. Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneropathy and cardiovascular autonomic neuropathy. Diabetes Care 2004; 27:2178-83.
26. Minetti M. Bilirubin is an effective antioxidant of peroxynitrite mediated protein oxidation in human blood plasma. Archives of Biochemistry and Biophysics1998; 352(2); 165-74.