

Erythrocyte reduced glutathione level in type -2 diabetic patients with and without nephropathy

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Abstract

Background Diabetic nephropathy is the most common cause of end stage renal disease. Oxidative stress is being considered as a common pathogenic factor in diabetes mellitus and its complication.

Aim To assess the marker of oxidative stress in patients of type-2 Diabetic nephropathy.

Material and methods Total 90 subjects were included in this study, of these 30 were controls, 30 type-2 diabetic patients without any complication and 30 type-2 diabetic patients with nephropathy. The parameters assessed were plasma fasting glucose, glycated HbA_{1c}, Erythrocyte reduced glutathione level (GSH) and Plasma Malondialdehyde (MDA).

Result It was observed that, in diabetic nephropathy, there was significant increase in MDA (P<0.001) values and significant decrease in GSH (P<0.001), We found significant negative correlation between glycated HbA_{1c} v/s GSH while positive correlation was observed between glycated HbA_{1c} v/s MDA and significant negative correlation between GSH and MDA.

Conclusion Our study indicates that, the intensity of oxidative stress in nephropathy patients is greater than the patients without any complications.

Keywords: Diabetic nephropathy, oxidative stress, MDA, GSH.

INTRODUCTION

Nephropathy is a common complication of Type -2 DM and is frequent cause of death .

The diabetic kidney is characterised by increased perfusion , which generates increased glomerular filtration and intraglomerular pressure. Pathological changes result in initial microalbuminuria which progresses to more extensive proteinuria, loss of tubular filtration and ultimately renal failure.

Accumulating research suggests that oxidative stress is a significant contributor to the pathogenesis of diabetic nephropathy. The normal kidney generates a substantial amount of oxidative stress because of its high metabolic activity that is balanced by an extensive . antioxidant system. However in pathologic states such as hyperglycemia, nitroso-oxidant balance shifts towards pro-oxidant state that accelerates tissue oxidative stress.

Many biological pathways, such as glucose autooxidation, polyol pathway, prostanoid synthesis and protein glycation are triggered in hyperglycaemia state leading to increased production of free radicals. ¹

The ability of cell to resist damage caused by oxidative stress is determined by the capacity of an array of antioxidant defense system among which reduced glutathione is the most ubiquitous and abundantly available within human cell. GSH is tripeptide synthesized from glutamate, cysteine and glycine. Decrease in the reduced GSH level and impaired in GSH metabolism have been reported in erythrocyte of diabetics. Decrease in the reduced GSH level occurs both due to the competition between aldose reductase and glutathione reductase for NADPH, a cofactor and increased oxidative stress.²

In point of view, present study was undertaken to investigate whether there is any difference in the biomarkers of oxidative stress in type -2 DM and to explore their putative role in pathogenesis of diabetic nephropathy.

MATERIAL AND METHOD

In present study, outdoor as well as indoor patients admitted in Chhatrapati Shivaji Maharaj Govt Hospital Solapur were included. The diagnosis of diabetes mellitus was done on the basis of detailed clinical history, clinical examination and biochemical investigations, as per the ADA criteria. We have selected well diagnosed diabetic nephropathy patients on the basis of history, clinical examination, urinary albumin excretion by spot albumin creatinine ratio $\geq 30\mu\text{g}/\text{mg}$ creatinine and decrease in glomerular filtration rate.

Male and female patients diagnosed as type -2 DM with and without nephropathy in age group of 40 – 60 years were considered as cases. Control group included healthy volunteers who were consistent with patients according to age, sex and body mass index.

In order to achieve the laid down objectives, total 90 subjects were enrolled for present study and distributed into 3 group, 30 age and sex matched healthy controls as group1, 30 type2 diabetic patients without any complication as groupII and 30 type2 diabetic patients with nephropathy as groupIII. Subjects who had acute complications such as severe infections , major operations, trauma , GI disorders, severe cardiovascular / respiratory diseases, pregnant and breast feeding women. Subjects on any concomitant medication such as antioxidant vitamins , minerals, herbal treatment which may interact with glycemic status and oxidative stress parameters. Cigarette smokers and alcoholics were excluded from study.

Collection of sample

Blood samples were collected from Dept of medicine. After 12 hour fast , 2 ml venous blood was collected in fluoride bulb, centrifuged at 3000 r.p.m., plasma was separated and used for estimation of plasma glucose.

5ml venous blood was collected in heparin bulb, centrifuged, Haemolysate was used to measure glycated HbA_{1c}, and Erythrocyte GSH. Fasting plasma glucose (FPG) was measured by GOD- POD. Glycated haemoglobin (HbA_{1c}) by Resin binding method.

BIOCHEMICAL MEASUREMENTS

For erythrocyte enzymes, heparinized whole blood was kept in ice .Erythrocytes were separated and centrifuged at 4 °C for 20 min at 3500 rpm. Erythrocytes were rewashed for measurement of enzymes (\leq 24 hours). Hemoglobin concentration of the samples was measured by Drabkin's method to express erythrocyte GSH activity values in units

per gram hemoglobin.

BLOOD GLUCOSE

Fasting blood glucose was estimated by GOD- POD method³.

GLYCOSYLATED HEMOGLOBIN (HBA_{1c})

Glycosylated hemoglobin was measured using the Resin Binding method⁴

OXIDATIVE PARAMETERS

Malondialdehyde (MDA)

Plasma MDA concentration was determined by using the method described by S.K.Jain⁵ based on TBA reactivity.

ANTIOXIDANT PARAMETERS**Erythrocyte reduced glutathione (GSH)**

Erythrocytic GSH by Beutler's method.⁶ In this method, 5,5'-dithiobis (2-Nitrobenzoic Acid) (DTNB, Ellman's Reagent) reacts with GSH to form the chromophore, 5-Thionitrobenzoic Acid (TNB) and GS-TNB. Absorbance of this chromophore was measured at 412 nm.

STATISTICAL ANALYSIS

Results were expressed as mean \pm SD. Unpaired Student 't' test was applied to compare the results between the controls, Group 1 and Group 2. P value $<$ 0.05 was considered statistically significant

RESULT**Table no.1** Comparison of parameters between Group 1 & Group II

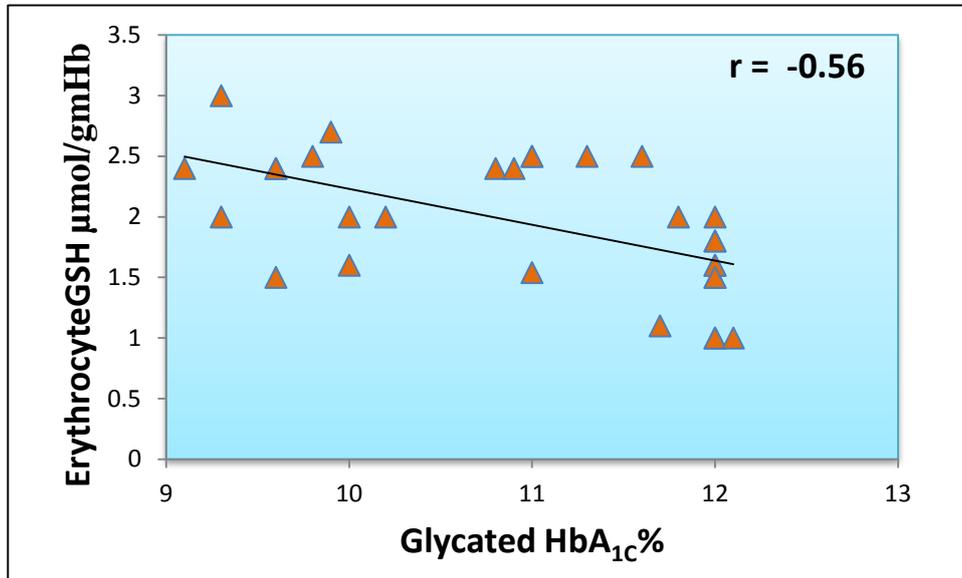
Parameters	Group I	Group II
FPG(mg/dl)	88.23 \pm 13.58	140.57 \pm 27.38 ^a
HbA _{1C}	4.95 \pm 0.36	7.4 \pm 0.79 ^a
Plasma MDA(nmol/ml)	1.84 \pm 0.65	4.13 \pm 1.07 ^a
Erythrocyte GSH (μ mol/g Hb)	4.46 \pm 1.01	2.82 \pm 0.88 ^a

Table no. 2 – Comparison of parameters between Group II & Group III.

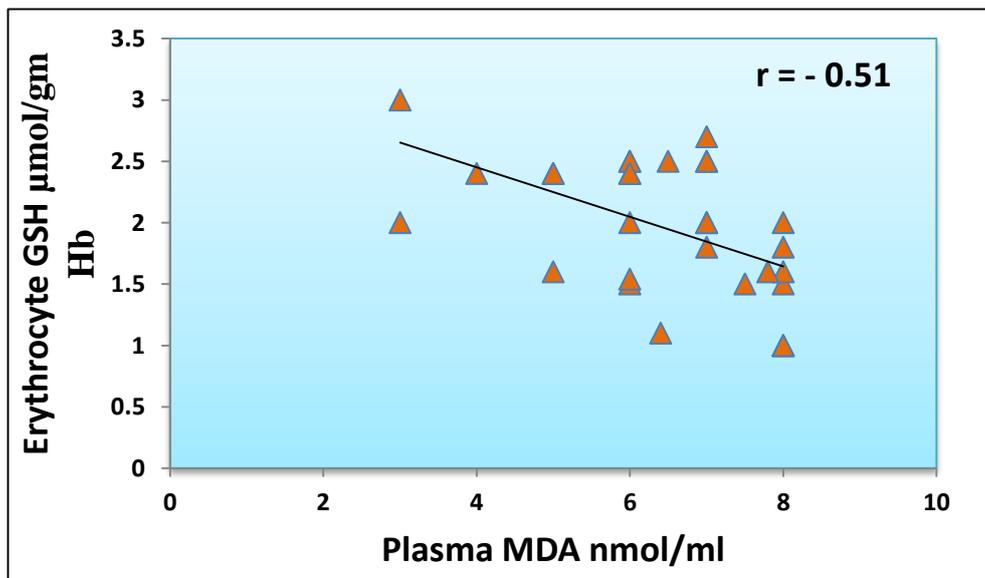
Parameters	Group II	Group III
FPG(mg/dl)	142.57 \pm 27.38	224.63 \pm 23.28 ^a
HbA _{1C}	7.37 \pm 0.79	10.92 \pm 1.04 ^a
Plasma MDA(nmol/ml)	4.13 \pm 1.07	6.34 \pm 1.47 ^a
Erythrocyte GSH(μ mol/g Hb)	2.82 \pm 0.88	1.96 \pm 0.56 ^a

a = p $<$ 0.001, b = p $<$ 0.05

Scatter diagram 1: Negative correlation between glycated HbA_{1C} and erythrocyte GSH in diabetes nephropathy patients.



Scatter diagram no.2: Negative correlation between plasma MDA & erythrocyte GSH in Type-2 DM with nephropathy patients.



DISCUSSION

In the present study glycemic status parameters such as fasting plasma glucose ($P < 0.001$) and glycated Hb ($p < 0.001$) levels were significantly increased in cases as compared to controls. Both the levels were significantly increased ($p < 0.001$) in the patients with group 2 than group 1. Results of the present study are consistent with previous studies⁷. Increase in HbA_{1c} is one of the causes for increased production of free radical by direct Aamadori reaction. Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to nonenzymatic glycation, autooxidation of glycation products, but also changes in the tissue content and activity of antioxidant defense systems .

In our study, we found significantly higher serum MDA levels in Type 2 diabetic patients. Serum MDA level was significantly higher in patients of Type 2 diabetes with nephropathy as compared to patients without nephropathy. This finding is in agreement to previous studies^{7,8,9,10,11,12,13}. This significant increase in MDA levels could be due to increased production of free radicals in diabetes mellitus patients. MDA is an end product of lipid peroxidation, could be responsible for the intermolecular cross linking of collagen through MDA leads to its stabilization and further glycation. This starts a vicious cycle as glycated collagen initiates further lipid peroxidation releasing more MDA. Our study shows that significant decrease concentration of GSH ($p < 0.001$) was found in cases as compared to control group, decline was of higher in group 2 patients as compared to group 1 patients , which was statistically significant ($p < 0.001$) . Result are in accordance with previous studies.^{9,10,11,12,13,14,15,16} Glutathione acts as a direct scavenger as well as co-substrate for Glutathione peroxidase. It is a major intracellular redox tampon system. Depletion of GSH impairs the activity of antioxidant enzymes resulting in oxidative damage induced diabetic nephropathy. There is a negative correlation between GSH and HbA_{1c} (scatter diagram no.1) in diabetic nephropathy patients, which confirms the link between hyperglycemia and GSH depletion. Indeed, in diabetic nephropathy, excess of glucose is preferentially used in polyol pathway , that consumes NADPH necessary for GSH regeneration by the GSH-Red enzyme and indirectly decrease the GSH level. As GSH is an important antioxidant molecule, its depletion leads to the increase of oxidative stress that demonstrated persistent hyperglycaemia and poor metabolic control significantly increased the risk for the development of nephropathy, GSH shows negative correlation with MDA (scatter diagram no.2) The main function of reduced GSH is to suppress lipid peroxidation, which occurs in plasma membranes and alters structure and permeability of membrane. Glutathione also functions to scavenges oxidants or xenobiotics by covalently binding them and transporting the resulting metabolites through an adenosine 5' triphosphate- dependent transport system. Inactivation of glutathione synthesis and thiol transport in diabetic patients increases the sensitivity of the cells to

oxidative stresses and these changes may lead to development of complications in type-2 diabetes mellitus.

GSH systems may have the ability to manage oxidative stress with adaptational changes in enzymes regulating GSH metabolism.

Thus, results of the present study indicate that, oxidative stress appears early in type-2 diabetic patients. Moreover, oxidative stress and compromised antioxidant status exacerbates type-2 diabetic nephropathy. Therefore, the measurements of these parameters seem to be the best biomarkers for the assessment of oxidative stress in diabetic patients and may be important for the prediction and prevention of diabetic complications. Further, lifestyle modification, intensive glycemic control and improvement of antioxidant status may be renoprotective in type-2 diabetes mellitus and warrants further elucidations

CONCLUSION

GSH were significantly altered in Type-2 DM with nephropathy and without nephropathy when compared to that in normal individuals, suggesting the role of uncontrolled hyperglycemia as a cause and consequence of oxidative stress. Hence determination of GSH in erythrocytes can contribute to know the extent of oxidative stress in diabetes and help in effective control and prevention of the onset and progression of complications like diabetic nephropathy.

REFERENCES

- 1 Pawan krishan & Vishal Arvind Chakkarwar Diabetic nephropathy: Aggressive involvement of oxidative stress J pharmacy Edu Res 2011; 2(1): 35-41.
- 2 Ismail Hakki Kalkan et al The relationship between the level of glutathione impairment of glucose metabolism and complication of diabetes mellitus. Pakistan Journal of Medical Sciences 2013 ; 29 (4) 938 – 942
- 3 Trinder P. Estimation of blood sugar level by GOD-POD method. Ann. Clin. Biochem 1969; 6: 24.
- 4 Trivelli LA, Ranney PH and Lai HT. Estimation of glycosylated hemoglobin by resin binding method. New Eng J Med. 1971; 284
- 5 Jain S.K. Hyperglycemia can cause membrane lipid peroxidation and osmotic fragility in human red blood cell. J. Biol Chem 1989; 264; 21340-45
- 6 Beutler E, Duron O, Kefly BM, Improved method for the determination of blood glutathione. Jlab Clin Med 1963; 61:882-8.

- 7 Manjulata Kumavat et al Antioxidant enzyme and lipid peroxidation in type 2 diabetes mellitus patients with or without nephropathy North American Journal of medical sciences 2013 ;5:213-219
- 8 D.A. Slatter, C.H. Bolton, A. J. Bailey The importance of lipid-derived Malondialdehyde in diabetes mellitus Diabetologia (2000) 43: 550-557.
- 9 S A.Mousa Oxidative stress in Diabetes Mellitus Romanian journal of biophysics 2008 ;18; 225-236.
- 10 Sundaran RK , Bhaskar A, Vijayalinam S, Vishwanathan M, Mohan R, Ahanmugsunderam. Antioxidant status and lipid peroxidation in type 2DM with and without complications.Clinical science 1996;90:255-260.
- 11 Kornelia Z,Kedziora, Kornotowaska, Merck Luciak, Jan Blaszezy k, Wojeieeh Pawlak. Lipid peroxidation and activites of antioxidant enzymes in erythrocyte of patients with noninsulin dependent diabetes with or without nephropathy.Nephrol Dial Transplant 1998 ; 13 : 2829-2832.
- 12 Ifeoma Nneka Nweke, Obioma C ,Ohaeri and Christian Chinyee Ezeala. Effect of vitamin on Malondialdehyde and glutathione levels in type 2 nigarians . The internet journal of nutrition and wellness 2009; 7(2)
- 13 Shakir Khan et al Oxidative stress in Patients with Type-2 Diabetes Mellitus, Journal of Rawalpindi medical college 2014; 18(1): 29-31
- 14 Dr. T. Angeline, Dr. AJAsirvatham , Dr W. Isabel Alteration in serum Zinc and Erythrocyte Reduced Glutathione Status in Newly Diagnosed Type 2 Diabetes Mellitus. International journal of scientific result 2013 ;2;430-432
- 15 S.I.Rizvi Intracellular reduced glutathione content in normal and type-2 diabetic erythrocytes , effect of insulin and epicanthin Journal of physiology and pharmacology 2001 52 (3) 483 -488
- 16 K. Gawlik et al Markers of antioxidant defense in patients with type-2 diabetes Oxidative Medicine and cellular longevity 2016; 1; 1-6