Indian Journal of Clinical Biochemistry, 2007 / 22 (1) 91-94

COMPARISON BETWEEN ERYTHROCYTE HEMOGLOBIN AND SPECTRIN GLYCOSYLATION AND ROLE OF OXIDATIVE STRESS IN TYPE-2 DIABETES MELLITUS

Y. S. Mahindrakar, A. N. Suryakar, R. D. Ankush, R. V. Katkam and K. M. Kumbhar.

Dept. of Biochemistry, Dr. V. M. Govt. Medical College, Solapur (Maharashtra)

ABSTRACT

New findings on organization of blood cell cytoskeleton represent an exciting aspect of modern cell biology and hematology, which is an interesting investigation to study diabetes. The present study was undertaken in 150 subjects. Out of these, 30 subjects were controls (Group I) and 30 were type-2 diabetics without any complication (Group II), while remaining 90 subjects were type-2 diabetics with complication (Group III). We determined erythrocyte spectrin and hemoglobin glycosylation and also estimated plasma lipid peroxide, nitric oxide and erythrocyte glutathione peroxidase activity to assess the status of oxidative stress.

There was a significant increase in spectrin ($P < 0.001$) and hemoglobin ($P < 0.001$) glycosylation in Group II and III as compared to Group I and spectrin glycosylation was nearly three times more as compared to hemoglobin, whereas plasma levels of lipid peroxide ($P < 0.001$) as well as nitric oxide ($P < 0.001$) were found to be significantly increased and GPx activity ($P < 0.001$) was significantly decreased in Group II and III as compared to Group I. However, it was also observed that spectrin ($P > 0.05$) and hemoglobin ($P > 0.05$) glycosylation was not significantly different in Group II and III. In contrast, there was significant rise in lipid peroxide ($P < 0.001$), nitric oxide ($P < 0.001$) and fall in GPx activity ($P < 0.001$) in Group III when compared to Group II. Increased erythrocyte protein glycosylation and oxidative stress is clearly evident from our study. However, to understand the exact interplay between these two mechanisms, further studies are required.

KEY WORDS

Type-2 diabetes, Spectrin, Glycosylation, Oxidative stress

INTRODUCTION

Chronic elevation of plasma glucose causes many complications in diabetes mellitus. People with type-2 diabetes mellitus develop characteristic microvascular complications such as retinopathy, nephropathy and neuropathy. There is also an increased risk of macrovascular complications such as cardiovasculopathy, cerebrovasculopathy and peripheral vasculopathy (1). A variety of hematological abnormalities are seen in diabetes. These include increased erythrocyte aggregation, decreased deformability of erythrocytes, increased platelet aggregation, and adhesion predisposed to sluggish circulation, endothelial damage and focal capillary occlusion (2).

Address for Correspondence:

Dr. A. N. Suryakar,
Prof. and Head, Dept. of Biochemistry,
Dr. V. M. Govt. Medical College,
Solapur. (Maharashtra)

De novo oxidative damage, a result of increased protein glycosylation could participate in the mechanism, whereby diabetic erythrocytes may acquire membrane abnormalities (3). Spectrin is a very important protein of erythrocyte membrane and a target for glycosylation and further oxidation, which might be responsible for increased number of poorly deformable erythrocytes found among diabetic erythrocytes (4, 5). Enhanced glycosylation by elevated glucose concentration may induce the formation of oxygen derived free radicals through protein glycosylation, which releases early and late glycosylation end products, contributing to enhancement of oxidative stress (6). Both protein glycosylation and protein oxidation are biochemical alterations occurring in diabetes (7). Under physiological conditions, autooxidation of glucose leads to hydrogen peroxide, reactive oxygen species and reactive ketoaldehydes, which modify the cellular proteins leading to their fragmentation by free radical mechanism. This protein fragmentation is inhibited by antioxidants confirming that tissue damage associated with diabetes has an oxidative origin (8). Evidence has accumulated indicating that the generation of reactive oxygen species (oxidative stress) may

play an important role in the etiology of diabetic complications. This hypothesis is supported by evidence that many biochemical pathways strictly associated with hyperglycemia (glucose autooxidation, polyol pathway, prostanoid synthesis, protein glycosylation) can increase production of free radicals. A rational extension of this proposed role of oxidative stress is the suggestion that the different susceptibility of diabetic patients to microvascular and macrovascular complications may be related to endogenous antioxidant status (9). Diabetes causes dyslipidemia and increases susceptibility to lipid peroxidation (10). Nitric oxide, a free radical produces diverse cellular responses, both beneficial and detrimental (11). To counteract the harmful effects of free radicals, antioxidant defense mechanism operates to detoxify or scavenge these free radicals (12). Glutathione peroxidase (GPx) is one of the important enzymatic antioxidants.

Therefore, the present study was carried out to understand the interplay among oxidants such as lipid peroxide and nitric oxide and the antioxidant GPx, along with the glycosylation status of erythrocyte spectrin and hemoglobin in type-2 diabetes mellitus patients with and without complications.

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry, Dr. V.M. Govt. Medical College, Solapur during the period, August 2003 to March 2005 in collaboration with the Department of Medicine, Shri Chhatrapati Shivaji Maharaj General Hospital, Solapur. The study was approved by institutional ethical committee and informed consents were obtained from all the participants of the study. The present study was conducted on 150 subjects and they were divided into three different groups.

Group I : 30 healthy controls.
Group II : 30 type-2 diabetic patients without any complication.
Group III : 90 type-2 diabetic patients with complications, which further consist of 30 retinopathy, 30 nephropathy and 30 cardiovasculopathy patients.

The selected subjects were not on supplementations such as antioxidants, minerals, or any kind of medications for the related complications, which could affect the study parameters. Diagnosis of type-2 diabetes mellitus was done according to the criteria proposed by the American Diabetes Association (13). Further, retinopathy was confirmed by fundal examination by observing presence of microaneurysm, soft exudates and intra retinal hemorrhages. Nephropathy was considered to be present if there was proteinuria (1gm/L). Cardiovasculopathy was diagnosed by ECG findings and myocardial infarction.

Total 10 ml fasting blood was drawn in heparinised vacutainers. Fasting plasma glucose level was measured by using commercial kits. Estimation of glycosylated hemoglobin was carried out by modified method of Fluckiger and Winterhalter (14). Spectrin was isolated by the method of Coetzer and Zail (15). Concentration of hemoglobin was measured by using commercial kits and concentration of erythrocytic spectrin was measured by Lowry method (16). Further, equal concentrations of spectrin and hemoglobin were adjusted and glycosylation status was determined by using fructose as external standard (17). Erythrocytic glutathione perxidase was determined by using the kits from Randox Lab U.K. Levels of plasma lipid peroxide was estimated, as malondialdehyde, by Kei Satoh's method (18), and plasma nitric oxide was determined by the method of Cortas and Wakid (19). Two tailed student 't' test was used for statistical analysis.

RESULTS

Table 1 : Plasma glucose, glycosylated hemoglobin, spectrin glycosylation and hemoglobin glycosylation in different groups

Parameter	Group I	Group II	Group III
Fasting plasma glucose mg/dl	94.8 ± 8.7	183.6 ± 14*	185.9 ± 10.0* #
Glycosylated Hemoglobin %	3.46 ± 0.3	9.99 ± 0.6*	10.3 ± 0.4* #
Spectrin Glycosylation nmole of fructose/mg Spectrin	8.59 ± 0.7	29.3 ± 1.3*	29.21 ± 1.1* #
Hemoglobin Glycosylation nmole of fructose/mg Hemoglobin	4.25 ± 0.7	9.88 ± 1.1*	10.14 ± 1.1* #

Values expressed as mean ± SD * P < 0.001, as compared with Group I and # P > 0.05, as compared to Group II.

DISCUSSION

Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from a defect in insulin secretion, insulin action or both. Increase in reactive oxygen species in diabetes mellitus is due to autooxidation of glucose, protein glycosylation and active polyol pathway (20). The membrane protein glycosylation enhancement occurring in diabetes could be one of the reasons for lowered erythrocyte membrane fluidity in diabetes (21).

Table 2 : Plasma lipid peroxide, nitric oxide and erythrocyte glutathione peroxidase in different groups.

Parameter	Group I	Group II	Group III
Lipid Peroxide (MDA) nmole/ml	1.80 ± 0.4	4.31 ± 0.16*	5.09 ± 0.2* #
Nitric Oxide (Nitrite) µmole/L	49.14 ± 5.3	61.36 ± 0.8*	66.15 ± 1.6* #
Erythrocyte Glutathione Peroxidase U/gm Hb	63.59 ± 3.8	56.51 ± 4.3*	46.07 ± 2.7* #

Values expressed as mean ± SD * P < 0.001, as compared with Group I and # P < 0.001, as compared with Group II.

In the present study, it was observed that glycosylation of both spectrin and hemoglobin was increased in type-2 diabetes patients as compared to controls. Glycosylation status of both these proteins was similar in patients with complications and patients without complication. Further, in type-2 diabetes, the spectrin glycosylation was nearly three times more than that of hemoglobin glycosylation. This may be due to 7% of total amino acids as lysine in spectrin, which is believed to be the easily glycosylated (5).

In the present study, lipid peroxidation was significantly increased in type-2 diabetics as compared to controls. Further, these values were significantly higher in patients with complications when compared with the patients without complication although they had similar protein glycosylation status. These findings suggest that, type-2 diabetic patients differ in their susceptibility to oxidative stress. Increased levels of lipid peroxide may cause oxidative injury to blood cells, cross-linking in membrane proteins and lipids (8).

Augmented production of nitric oxide was observed in type-2 diabetics as compared to controls. Further, these values were significantly higher in patients with complications as compared to patients without complications. Abnormal nitric oxide metabolism is related to advanced diabetic complications in type-2 diabetes mellitus, which may be the consequence of increase in inducible nitric oxide synthase gene expression (22).

Erythrocyte GPx activity was found to be decreased in type-2 diabetic patients as compared to controls. Further, significant fall in GPx activity was observed in patients with complications as compared to patients without complication. GPx can remove hydrogen peroxide and other peroxides with reducing power of glutathione (23). GPx is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress (24). Increase in lipid peroxidation observed in present study and glycosylation of the enzyme may result in decreased activity of GPx.

The fundings of present study show that free radicals are increasingly produced in type-2 diabetes mellitus patients and may play a significant role in development of complications. Though free radical production is believed to be the consequence of protein glycosylation and glucose autooxidation, the organism's susceptibility to oxidative stress and lipid peroxidation of cellular structure may play an important role in development of diabetic complications. The alterations in glycosylation status of erythrocyte membrane spectrin might affect integrity, life span, deformability and viscoelasticity of type-2 diabetic erythrocytes. However, to understand the causative factors and underlying biochemical mechanisms in elegant detail need further elucidations.

REFERENCES

1. Yeolekar ME, Borade PS. Classification of diabetes and its implications. J Gen Med 2002; 14: 7-9.
2. Raman R, Biswas J. Occular complications of diabetes mellitus. J Gen Med 2002; 14: 21-7.
3. Scwartz RS, Madsen JW, Raybicky AC, Nagel RL. Oxidation of spectrin and deformability defects in diabetic erythrocytes. Diabetes 1991; 40: 701-8.
4. Lux SE. Spectrin Actin membrane skeleton of normal and abnormal red blood cells. Semin. in Hematol 1979; 16: 21-51.
5. McMillan DE, Brooks SM. Erythrocyte spectrin glycosylation in diabetes. Diabetes 1982; 31: 64-9.
6. Konukoglu D, Kemerli GD, Sabunca T, Hatemi HH. Protein carbonyl content in erythrocyte membrane in type-2 diabetic patients. Horm Metab Res 2002; 34: 367- 70.
7. Resm H, Peketin C, Guner G. Erythrocyte membrane cytoskeletal protein glycation and oxidation in short term diabetic rabbits. Clin Exp Med 2001; 1: 187-93.
8. Parthibhan A, Vijaylingam S, Shanmughsundaram K, Mohan R. Oxidative stress and the development of diabetic complications, antioxidant and lipid peroxidation in erythrocytes and cell membrane. Bio Int 1995; 19: 987-92.
9. Aggarwal KK. Diabetes and oxidative stress. Asian J Diabetol 2001; 3: 12.
10. Pasaoglu H, Suncak B, Bukan N. Lipid peroxidation and resistance to oxidation in patients with type-2 diabetes mellitus. Tohoku J Exp Med 2004; 203: 211-8.
11. Moncada S, Higgs A. The L-Arginine-Nitric oxide pathway. New Eng J Med 1993; 329: 2002-12.

12. Irshad M, Chaudhari S. Oxidant antioxidant system; Role and significance in human body. Ind J Exp Bio 2002; 40: 1233-9.

13. Sekerglu M, Sahin H, Dulger H, Algun E. The effect of dietary treatment on erythrocyte lipid peroxidation, Superoxide dismutase, Glutathione Peroxidase and serum lipid peroxidation in patients with type-2 Diabetes Mellitus. Clin Biochem 2000; 33: 669-74.

14. Flukiger R, Winterhalter KH. Invitro synthesis of HbA_{1C}. Fed. Eur Biochem Soc Lett 1976; 71: 356-60.

15. Coetzer T, Zail S. Spectrin tetramer-dimmer in hereditary elliptocytosis. Blood 1982; 59: 900-5.

16. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193: 265-75.

17. Parker KM, England JD, Costa JD, Hess RL, Goldstein DE. Improved colorimetric assay for glycosylated hemoglobin. Clin Chem 1981; 27: 666-72.

18. Satoh K. Serum lipid peroxide in cerebrovasular disorders determined by new colorimetric method. Clin Chim Acta 1978; 90: 37-43.

19. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem 1990; 36:1440-3.

20. Gurler B, Vural H, Yilmaz N, Oguz H, Satici A, Aksoy N. The role of oxidative stress in diabetic retinopathy. Eye 2000; 14: 730-5.

21. Lux SE, John KM, Ukena TE. Diminished spectrin extraction from ATP depleted human erythrocytes. Evidences relating spectrin to changes in erythrocyte shape and deformability. J Clin Invest 1978; 61: 815-27.

22. Moris BJ, Markus A, Gleen CL. Association of a functional inducible nitric oxide synthase promoter varient with complications in type-2 diabetes. J Mol Med 2002; 80: 96-104.

23. Young IS, Woodside JV. Antioxidant in health and disease. J Clin Path 2001; 34: 176-86.

24. Condell RA, Tappel AL. Evidence for suitability of glutathione peroxidase as a protective enzyme: Studies of oxidative damage restoration and proteolysis. Arch Biochem Biophys 1983; 223: 407-16.