LIPID PEROXIDATION IN ERYTHROCYTES OF PATIENTS WITH TYPE 2 DIABETES MELLITUS

P. Ashok Kumar and G. Rajagopal

Department of Biochemistry, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar-608 002.

ABSTRACT

Lipid peroxidation by measurement of Thiobarbituric Acid Reactive Substances (TBARS) and antioxidant status by determining the activities of the enzymes, Catalase (Cat), Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) and the level of Reduced Glutathione (GSH) in the erythrocytes of patients with type 2 Diabetes Mellitus were investigated. It was observed that the level of TBARS in the erythrocytes was increased by 50% showing a significant generation of free radicals in the erythrocytes of these patients. The activities of both Cat and SOD were enhanced while that of GPx was not altered. The level of GSH was also not changed.

KEY WORDS

Glutathione, Type 2 Diabetes, Catalase, Peroxidase, Superoxide Dismutase

INTRODUCTION

Glutathione cycle operates in the erythrocytes for the disposal of hydrogen peroxide generated in the cell supplementing the function of catalase. Reduced glutathione (GSH) and hydrogen peroxide are the twin substrates for glutathione peroxidase (GPx). GSH is formed from its oxidised form, GSSG by the enzyme, Glutathione reductase, which requires enzyme cofactor, NADPH. Glucose oxidation through the pentose phosphate pathway operates in the erythrocytes precisely to supply this NADPH(1). Normally, insulin promotes the operation of this pathway. In Diabetes mellitus, due to inadequate function of insulin, the efficiency of this pathway is hampered and as a result decreased quantities of NADPH will be formed. The present investigation is therefore aimed at further understanding of glutathione metabolism in the erythrocytes of diabetic patients by determining GSH levels along with associated glutathione peroxidase activity and Catalase activity in the erythrocytes and assessment of related disturbance of lipid peroxidation by measuring Thiobarbituric acid

reactive substances (TBARS) and the enzyme superoxide dismutase.

MATERIALS AND METHODS

Twenty patients in the age group of 50-65 years attending Rajah Muthiah Medical College Hospital were selected for this study. All these patients were recently diagnosed cases of Type 2 Diabetes Mellitus based on the abnormal GTT and clinical findings. All these patients were from the surrounding villages and they belonged to the non- obese type of Type 2 Diabetes Mellitus. Almost all of them were agricultural laborers. None of these patients were chronic smokers or alcoholics and did not suffer from any systemic diseases like hypertension or any diabetic complication. 20 controls in the same age group were selected from among the staff.

The haemolysate of RBCs was prepared as described by Lakshmi and Rajagopal(2).

After removal of plasma and buffy coat, the packed Red Blood Cells (RBCs) obtained were washed twice with physiological saline and then lysed with hypotonic phosphate buffer pH 7.4. The haemolysate was separated by centrifuging at 10000 rpm for 15 mins at 2°C

The following estimations were carried out in the haemolysate:

Author for Correspondence :

P. ASHOK KUMAR
Department of Biochemistry,
Rajah Muthiah Medical College,
Annamalai University,
Annamalai Nagar-608 002.

• Haemoglobin Estimation – Drabkin and Austin(3)

Haemoglobin (Hb) was estimated in haemolysate by the cyanmethaemoglobin method of Drabkin and Austin (1932). The haemoglobin reacts with ferricyanide and forms methaemoglobin which gets converted to cyanmethaemoglobin by the cyanide.

• Reduced Glutathione – Beutler *et al.*(4)

Reduced glutathione (GSH) was determined according to the method of Beutler and Kelley (1963). The method is based on the development of yellow colour due to reaction of 5, 5-Dithio (bis) nitrobenzoic acid (DTNB) with compounds containing sulphhydryl groups.

• Glutathione Peroxidase – Rotruck *et al.*(5)

The activity of glutathione peroxidase was assayed by the method of Rotruck *et al.* (1973) with modifications. A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of reduced glutathione. After a specified period of enzyme action; the remaining reduced glutathione content was measured by the method of Beutler and Kelley (1963).

• Catalase- Sinha *et al.*(6)

Catalase activity was assayed by the method of Sinha (1972). Dichromate reacts with acetic acid when heated in the presence of hydrogen peroxide to form chromic acetate which is measured at 620 nm. The enzyme preparation was allowed to split H_2O_2 and the reaction was stopped at different time intervals by the addition of dichromate/acetic acid reagent. The remaining H_2O_2 was measured as chromic acetate at 620 nm.

• Plasma Glucose – By GOD – POD method (7)

Plasma glucose level was estimated using Boehringer Mannheim Kit by enzymatic glucose oxidase peroxidase (GOD-POD) method. The plasma glucose is expressed as mg/dl.

• ThioBarbituric Acid Reactive Substances– Ohkawa *et al.* (8)

Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS). TBARS in haemolysate was assayed by the method of Ohkawa (1979). This method is based on the formation of red pigment by condensation of lipid peroxidation breakdown products like MDA with thiobarbituric acid (TBA).

• Superoxide Dismutase- Kakkar *et al.* (9)

The activity of Superoxide Dismutase (SOD) was assayed by the method of Kakkar *et al.* (1984) based on the formation of NADH-Phenazine methosulfate-nitro blue tetrazolium formazan.

The results were analyzed statistically by students "t" test.

RESULTS AND DISCUSSION

The results of this investigation are presented in Table I and II. Lipid peroxidation is increased in the erythrocytes of diabetic patients as shown by the increase in the level of TBARS by 50%. The activity of superoxide dismutase is enhanced by 9.4% in the erythrocytes of the patients. These results show higher oxidative stress and generation of free radicals in the RBCs of these patients. Earlier, Parthiban(10) has reported oxidative stress associated with development of diabetic complications; Oberley(11) has observed the role of free radicals in diabetes.

The level of GSH was not significantly enhanced in diabetic patients. It is well known that NADPH is necessary for reducing GSSG to GSH by glutathione reductase in the red cells. However the present study shows that GSH level though expected to be decreased, is not significantly altered. The activity of glutathione peroxidase is also not significantly changed. This may be explained from the associated observation that the activity of Catalase is enhanced by 157% in the red cells of the diabetics, taking care of the disposal of hydrogen peroxide in the cell particularly when glutathione peroxidase is helpless for want of adequate quantities of its second substrate, GSH. Therefore GSH gets underutilised and remains almost unaltered.

This investigation is of interest from the point of view that in diabetes mellitus even if GSH formation is hampered due to diminished efficiency of the HMP shunt pathway of glucose oxidation, the second enzyme in the red cell rises to the occasion and disposes off the hydrogen peroxide formed in the cell so that 'internal environment' in the erythrocyte is taken care of to the maximum possible extent. But in spite of this mechanism, lipid peroxidation and free radical generation prevails in the RBCs of diabetics.

ACKNOWLEDGEMENT

We thank **Dr.T.S. Xavier**, Dean, Faculty of Medicine for allowing us to utilize the facilities in the Division of Biochemistry and RMMC Hospital for this study.

TABLE I

Levels of TBARS and GSH in erythrocytes in Type 2 Diabetes

Parameters	Control	Type 2 Diabetes	P Value
TBARS (n.mol/gm of Hb)	4.5 ± 0.45*	6.75 ± 0.58	<0.001
GSH (mmol/gm of Hb)	14.17 ± 0.64*	14.74 ± 0.86	NS
PLASMA GLUCOSE (mg/dl)	89.15 ± 2.15	164.35 ± 2.72	<0.001

Mean ± S.E.

(n=20)

TABLE II

Activity of Cat, GPx and SOD in the erythrocytes in Type 2 Diabetes Mellitus.

Parameters	Control	Type 2 Diabetes	P Value
SOD (U/mg of Hb)	2.98 ± 0.32*	3.26 ± 0.39	<0.001
GPx(U/mg of Hb)	25.15 ± 0.97	26.52 ± 1.15	NS
CATALASE (μmol H ₂ O ₂ decomposed/min/gm of Hb)	2441.75 ± 10.91	6282 ± 17.52	<0.001
PLASMA GLUCOSE (mg/dl)	89.15 ± 2.15	164.35 ± 2.72	<0.001

* Mean ± S.E.

(n=20)

REFERENCES

1. Murray, K, Daryl K. Garner, Peter A Mayer, Victor N Rodwell in Harper's Biochemistry 25th edn Lange 2000.
2. Lakshmi, S and Rajagopal, G(1998) Reduced Glutathione level and Catalase activity in erythrocytes in patients with Diabetes Mellitus, Biomedicine. 18(1) 37-39.
3. Drabkin, DL, and Austin, JM, (1932) Spectrometric studies and spectrometric constants for common haemoglobin derivatives in human, dog and rabbit blood. J.Biol.Chem.
4. Buetler and Kelly, (1963) The effect of sodium on RBC Glutathione . J.Experientia 19 : 96.
5. Rotruck, JT, Pope, L Ganther, HE, Swanson, AB. (1973) Selenium biochemical role as a component of Glutathione Peroxidase. Science. 179 :588.
6. Sinha, K.A. (1972) Colorimetric assay of catalase. J. Biochem. 47 : 389.
7. Trinder, P, (1969). Annals clin. Chem ; 6 :24
8. Ohkawa, H Ohisi, N and Yagi, K. (1979) Assay of lipid peroxides in animal tissues by thiobarbituric reaction. Annal. Biochem. 95 :351
9. Kakkar, P, Das, B and Viswanathan, PN, (1984) Indian J. of Biochemistry and Biophysics 21:130
10. Parthiban, A, Vijayalingam, S, Shanmugasundaram, KR, Mohan, S, (1995) Oxidative stress and development of diabetic complications –antioxidants and lipid peroxidation in erythrocytes and cell membrane. Cell-Biol-Int. Dec: 19(12):987-910
11. Oberley, LW, (1988) Free radicals and diabetes. Free radical. Biol. ed.,5: 113.