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Red Cell Catalase Activity in Diabetics

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Abstract: The relationship between red cell catalase activity and glucose concentrations was examined. Red cell catalase activity, whole blood glucose concentration, red cell lysate glucose concentration and packed cell volume (PCV) were estimated in 40 diabetic patients and a control group of 30 healthy subjects. The diabetic group had a significantly lower catalase ($p<0.01$) activity than the control. As would be expected, the blood glucose concentration of the diabetic group was significantly higher ($p<0.01$) than that of the control group. Same was observed for the red cell lysate glucose concentration. There was no significant difference in the packed cell volume of both groups ($p<0.01$). Red cell catalase correlated negatively and significantly with red cell lysate glucose ($r = -0.29$, $p<0.05$); in the diabetic subjects. There was no significant correlation of red cell catalase with either the red cell lysate glucose concentration ($r = 0.088$; $p>0.05$) or with the blood glucose concentration ($r = -0.130$; $p>0.05$) in the control group. The findings above suggest that raised red cell glucose may exhaust red cell catalase. Low level of red cell catalase in diabetics may be a risk factor for the complications of diabetes mellitus.

Key words: Diabetes, catalase, fasting blood glucose

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia. Clinical expression of diabetes is dependent on both genetic and acquired factors (Expert committee on the diagnosis and classification of diabetes mellitus, 1998). It is now recognized that diabetes is an epidemic disease in most countries that are undergoing socioeconomic transitions. Diabetes mellitus, a syndrome characterized by chronic hyperglycemia is associated with most common complications such as atherosclerosis, nerve damage, renal failure, male impotence and infection (Bennett, 1994). World wide an estimated 150 million people are affected by diabetes, and this number is likely to reach 300 million by the year 2025 if successful strategies are not implemented for it's prevention and control (King *et al*., 1998).

In recent studies, some evidence suggest that oxidative stress may play some important role in the etiology of diabetes and diabetic complications (Shinn, 1998). Oxidative stress arises from an imbalance between reactive oxygen species production and antioxidant levels. The adverse effects of free radicals are recognized in several disorders and it may possibly be involved in β-cell destruction; and in the pathogenesis of diabetes mellitus (Oberley, 1988). Aerobic metabolism is always accompanied by the production of reactive oxygen species. Consequently all aerobic organisms possess some sort of antioxidant defense system with enzymatic and non-enzymatic constituents which are capable of preventing excess radical production, neutralizing free radicals and repairing the damage caused by them (Sies, 1993). Damage to the antioxidant system has been proven to play a role in various disorders such as arthritis, bursitis and gout.

Antioxidant enzymes primarily account for intracellular defense. The three major antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase. Hydrogen peroxide (H_2O_2) is a by-product of normal cellular respiration and is also formed from superoxide anion, which is also a free radical. H_2O_2 has been reported to damage pancreatic b-cells and inhibit insulin signaling (Murata *et al*., 1998). The enzyme catalase has a predominant role in controlling the concentration of H_2O_2. Low catalase activity may contribute to a variety of disorders such as type 2 diabetes (Gaetani *et al*., 1996).

In diabetic patients, an altered balance between reactive oxygen species production and antioxidant levels has been reported, though there is still lack of data regarding the actual status of antioxidant enzymes in diabetic patients (Nourooz-Zadeh *et al*., 1997).

This study investigated the activity of the antioxidant enzyme catalase in the red cell of diabetics, the relationship between catalase activity and fasting blood glucose and if red cell catalase measurement can be used for monitoring diabetic control.

Materials and Methods

Subject selection and sample collection: Forty patients (21 males and 19 females) of Nigerian origin with confirmed type 2 diabetes mellitus, attending the

University of Calabar Teaching Hospital Diabetic Clinic were used as test subjects. All the subjects were 30 years and above. Thirty age-matched non-diabetic apparently healthy subjects were used as controls. Venous blood samples (3 mL) were obtained from all patients and controls after overnight fast. 20 µL of the sample was collected into a plain serum bottle containing 10 mL of cold distilled water and used for determination of catalase activity in erythrocytes. 2 mL of the sample collected into a fluoride oxalate bottle was used for determination of fasting blood sugar. Blood was also collected into heparinized capillary tubes and used to determine the packed cell volume of the subjects.

Estimation of red cell catalase activity: Catalase was determined by a titrimetric method.

Procedure: To 0.5 mL of 0.24 substrate solution was added 0.5 mL H_2SO_4 and mixed well. This mixture was titrated with standard permanganate till a faint pink color is obtained. The titre was read off the pipette.
The 20 µL of blood was pipetted into a plain bottle containing 10 mL of cold distilled water. This was shaken vigorously to form a haemolysate. Into a Universal container, 0.5 mL of 0.24% substrate solution and 0.1 mL of the heamolysate were added. These were allowed to react for 5 min and the reaction was stopped with 0.5 mL H_2SO_4 and mixed well. The solution was then titrated with 0.005M, $KMnO_4$ till a permanent light pink color was obtained. The titre value was read off the pipette.
Catalase was expressed as µmol of H_2O_2 degraded by enzyme /ml/min. 0.24% w/v H_2O_2 contains w/v 0.24g/100 mL of the substrate. To convert this to Mol/l

$$\frac{0.24\times10}{34} = 0.07\text{Mol/l}$$

0.07 Mol/l to mmol/l = 0.07×1000
=70 mmol/l
that is 1000 mL contains 70 mmol
therefore 0.5 mL substrate contains

$$\frac{70\times0.05}{1000} = 0.035\ \text{mmol/l} \equiv 35\ \mu\text{mol}$$

Mean volume of 0.005 M $KMnO_4$ required for titration of 35 µmol of H_2O_2 after 3 titrations = 2.55mL
I.,e., 2.55 of 0.005 M $KMnO_4$ = 100% substrate
100% substrate = $[S]_0$ = 0.5mL substrate solution
If the volume of $KMnO_4$ that titrates undecomposed H_2O_2 after 5 min is x, then concentration of (undecomposed) H_2O_2 is

$$\frac{x}{2.55} \times 35\mu\text{mol} = \frac{35x}{2.55} = 13.7\ \mu\text{mol}$$

therefore $[S]_5$ = 13.7xµmol
Concentration of decomposed H_2O_2 (amount of substrate decomposed) in 5 min per 0.1 mL of red cell lysate = $[S]_0 - [S]_5$
Concentration of decomposed H_2O_2 (amount of substrate decomposed) in 5 min per 1 mL of red cell lysate = 10 ($[S]_0 - [S]_5$)
Therefore amount of substrate decomposed in 1 min per milliliter of red cell lysate

$$= \frac{10\ ([S]_0 - [S]_5)}{5}$$

Estimation of glucose in the whole blood and red cell lysate: Glucose was analyzed using glucose oxidase kits from Randox, United Kingdom.

Whole blood glucose: The first stage was the preparation stage (deproteinization). Here 0.1 mL of whole blood was added to 0.4 mL of 5% Na_2WO_4, 0.4 mL of 2/3N H_2SO_4 and 0.1 mL of distilled water. This was then centrifuged at 3000 rpm for five min and the supernatant, which was protein free, was obtained. 100 µL of the supernatant was mixed with 1000 µL of the glucose reagent. Also 100 µL of the glucose standard was mixed with 1000 µl of the glucose oxidase reagent. Both the test and the standard mixtures were incubated at 37°C for 10 min. After 10 min of incubation, the absorbances of the standard and that of the tests were read on a colorimeter at 546 nm against that of a reagent blank within 60 min.

Red cell lysate glucose: An aliquot of the blood sample was washed with normal saline. Here 1/8 of the centrifuge tube was filled with the sample and made up to ¾ of the centrifuge tube at 3000 rpm for five min and the supernatant removed, leaving only the cells. This washing was done twice for each sample. A 1 in 4 dilution of the washed cells was made with distilled water (0.1 mL of washed red cells to 0.2 mL of distilled water). The essence of which was to lyse the red cells. 0.1 mL of the lysed red cells was added to 0.4 mL of 5% Na_2WO_4, 0.4 mL of 2/3 H_2SO_4 and 0.1 mL of distilled water. This was centrifuged at 3000 rpm for 5 min. Into tubes labeled test and standard, 100 µL of the supernatant and 100 µL of the glucose standard were pipetted and 1000 µL of the glucose oxidase reagent added. The test and standard mixtures were incubated at 37°C for 10 min. After 10 min of incubation, the absorbances of the standard and that of the tests were read on a colorimeter at 546 nm against that of the reagent blank within 60 min. The reagent blank for the whole blood, red cell lysate and plasma glucose concentration estimation was prepared by incubating 1000 µL of the glucose oxidase reagent for 10 min at 37 °C.

Table 1: Comparison of some biochemical parameters of diabetics with non-diabetics

Parameters	Diabetics	Non-diabetics	Calculated t-value	Critical t-value	p-value	Remark
Catalase activity (imol/ml/min)	27.9±16.5	57.9±9.1	9.1	2.68	<0.01	S
Whole blood glucose (mmol/l)	7.1±3.8	4.2±0.7	4.1	2.68	<0.01	S
Red cell lysate glucose (mmol/l)	2.7±1.6	1.7±0.6	3.2	2.68	<0.01	S
PCV	39.0±7.2	41.7±0.6	2.0	2.68	>0.01	NS
n	40	30				

Mean±S.D, NS-Not significant, S-Significant

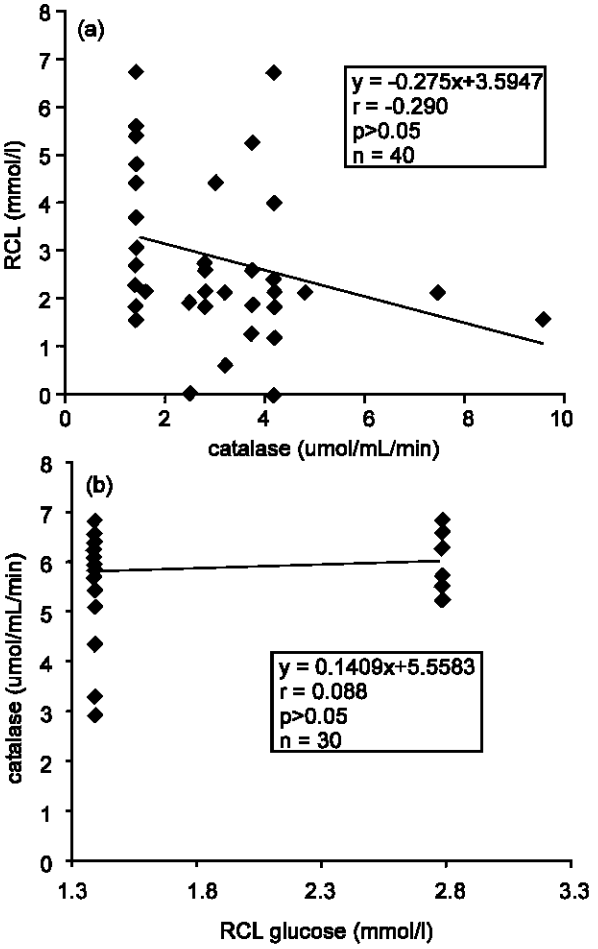

Fig. 1: Correlation plot of red cell catalase against red cell lysate glucose in (a) diabetics (b) non-diabetics

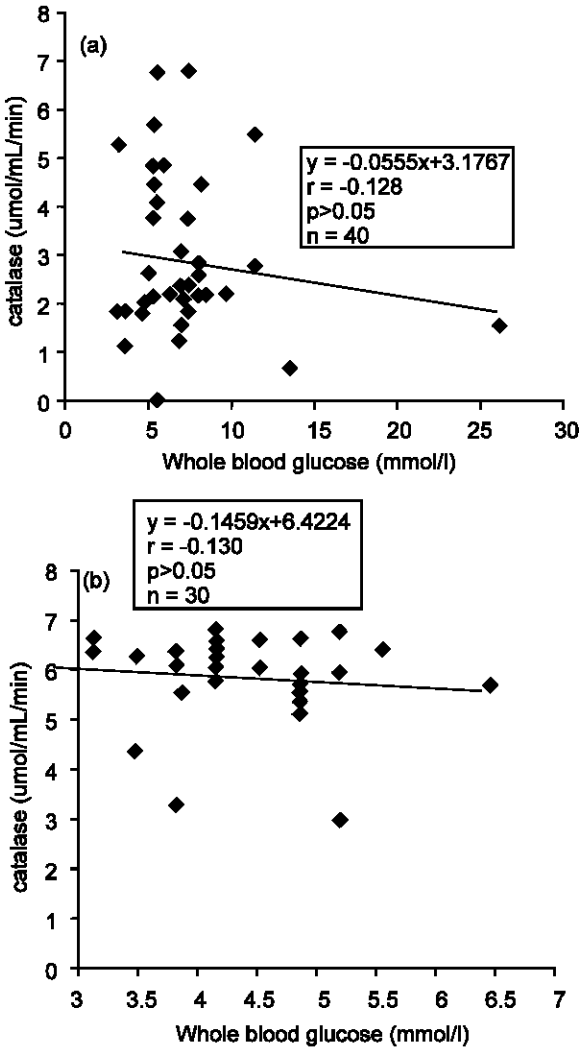

Fig. 2: Correlation plot of red cell catalase against whole blood glucose in (a) diabetics (b) non-diabetics

Calculation: The various concentrations of glucose in whole blood and red cell lysates were then calculated using the formulae

$$\frac{\text{Abs of test}}{\text{Abs of standard}} \times 100 = \text{conc. of glucose (mmol/l)}$$

This test is not influenced by uric acid, ascorbic acid, gluthathione, anticoagulant and creatinine in physiological concentrations.

Reference values: -Serum or plasma (fasting) 4.2-6.4 mmol/l Or 75-115 mg/dl.

Results

The mean value of red cell catalase activity in diabetics was 27.9±16.5 µmol/ml/min, which was found to be significantly lower ($p<0.01$) than that of the control subjects (57.9±9.1 µmol/ml/min). The glucose concentration of whole blood and red cell lysate of the diabetics were significantly higher ($p<0.01$) than those of

the controls. There was no significant difference in the mean packed cell volume of the diabetic and non-diabetic subjects (Table 1). Red cell catalase correlated negatively with red cell glucose in diabetics (Fig. 1). Correlation with whole blood glucose was not significant (Fig. 2). There was no correlation between catalase and red cell glucose in controls. There was also no correlation between red cell catalase and whole blood glucose.

Discussion

In this study, the mean red cell catalase activity was significantly lower in diabetics than in controls as shown by the results in Table 1. Some other studies have reported no alteration in the activity of the red cell catalase in diabetics (Dohi *et al*., 1998; Asayama *et al*., 1989). Others reported decreased catalase activity (Tagami *et al*., 1992) in aortic endothelial cells while Langenstroer and Piper (1992) reported an increase in these cells. These other investigations used animal models with induced diabetes, the duration of diabetes was not a factor in those other studies.

The activity of erythrocyte superoxide dismutase (SOD) has been shown to be decreased in diabetes (Matkovics *et al*., 1982) and Kawamura *et al*. (1992) showed that red blood cell Cu/Zn superoxide dismutase (SOD) is glaciated both *in vitro* and *in vivo*, leading to its inactivation. SOD glaciation causes increased hydrogen peroxide production. The percentage of glaciated SOD has been found to be higher in diabetics than in non-diabetics (Adachi *et al*., 1991) and thus an increased level of hydrogen peroxide production is inevitable. The resulting H_2O_2 inhibits SOD activity.

It is also known that in diabetic patients, autoxidation of glucose results in the formation of hydrogen peroxide (H_2O_2) which also enhances inhibition of SOD. Therefore the accumulation of hydrogen peroxide is believed to be one of the explanations for decreased SOD activity. Our study shows a decreased catalase activity in the red cells of diabetics in whom hydrogen peroxide is likely to accumulate. The decrease may therefore be a consequence of SOD glaciation or glaciation of catalase as reported for SOD. Exhaustion of the red cell catalase could also result from the accumulation of H_2O_2 on which the enzyme activity would be expended. In other tissues, the accumulation of H_2O_2 might lead to increased activity of catalase as the pressure of H_2O_2 might induce more catalase synthesis. However the red blood cell has no genetic apparatus for such synthesis, therefore the consequence might be the exhaustion of the enzyme in this cell. This speculation fits well into the results obtained in this study. The reduction in catalase activity is shown in this study to be inversely related to intracellular glucose concentration of the red cells. Unfortunately, glaciated hemoglobin was not measured, but it is expected that the level of glaciation will increase with the concentration of the intracellular glucose. Consequently, it is also expected that glaciation would have an inverse correlation with catalase activity. The packed cell volumes (PCV) of the diabetics and non-diabetics in this study were identical, therefore the results obtained are unlikely to be affected by differences in packed cell volume.

A recent study has suggested that the antioxidant defense mechanisms are overwhelmed in diabetic patients (Akintonwa, 2004) and this is believed to be responsible for the various complications of diabetes including diabetic wounds. The findings in this study seem to fit into this picture.

We conclude that in diabetes red cell catalase activity is decreased, and is inversely related to glucose concentrations in the red cell. Low catalase activity may be a risk factor for diabetes mellitus and its complications. Our findings suggest a role for antioxidants in the management of diabetes mellitus.

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