



Erythrocyte Glutathione-S-Transferase Activity in Diabetics and its Association with HBA1c

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Article ID: WMC002004

Article Type: Original Articles

Submitted on: 01-Jul-2011, 05:26:29 AM GMT **Published on:** 02-Jul-2011, 01:55:02 PM GMT

Article URL: http://www.webmedcentral.com/article_view/2004

Subject Categories: CLINICAL BIOCHEMISTRY

Keywords: Glutathione-S-transferase, Glycated Hemoglobin (HbA1c), Glutathione, Antioxidant Enzymes, Free Radicals, Oxidative Stress

How to cite the article: Velladath S U, Das A, Kumar R, K N. Erythrocyte Glutathione-S-Transferase Activity in Diabetics and its Association with HBA1c. WebmedCentral CLINICAL BIOCHEMISTRY 2011;2(7):WMC002004

Source(s) of Funding:

This project is funded by Manipal University.

Competing Interests:

None

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Abstract

The generation of reactive oxygen species is increased in both types of diabetes and is closely associated with oxidative stress. Erythrocytes are equipped with a highly effective anti-oxidant defence system. Oxidative denaturation of hemoglobin leads to the release of heme into the RBC membrane and the released heme is capable of oxidizing membrane proteins via a thiyl radical intermediate. GST can bind free heme that is released during Hb oxidation presumably reducing damage to RBC membrane. We hypothesized that as oxidative stress and hyperglycemia are major etiologic and pathologic factors of diabetes mellitus, then the antioxidant enzyme, Glutathione – S – Transferase (GST) in red blood cells, are likely to have a role in the glycation of Hb in diabetic patients. Hence we designed this study to evaluate the activity of red cell Glutathione-S-Transferase and correlate its association with HbA_{1c}. This is a cross – sectional study, conducted in subjects attending Kasturba Hospital, Manipal. All patients who were advised to do a blood test for glycated haemoglobin were included in this study. Hemolysed blood samples were excluded. Erythrocyte GST activity was determined using the method of Habig et al and glycated Hb (HbA_{1c}) was estimated using the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood by Roche Cobas Integra 400 auto analyzer. 68 patients who were confirmed to have type 2 diabetes mellitus were included in diabetic group whereas rest 32 patients without diabetes were included in non-diabetic group. The data were analysed using Mann Whitney U test and Pearson correlation coefficient test by SPSS (v. 14.0) software. From this study we could found out that there is no significant difference in erythrocyte GST activity between diabetics and non-diabetics (p value = 0.08). However a positive correlation between erythrocyte GST activity and HbA_{1c} concentration was observed in diabetic patients ($r = 0.239$, $p = 0.089$). The chronicity of the disease along with treatment modalities might have played a significant role in the outcome of our study as the subjects included in diabetic group were selected without considering their glycemic control status.

Introduction

It is well known that oxidative stress plays an important role in the pathophysiology of diabetes mellitus by impairing various cellular functions. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance leading to diabetic complications [1].

The easy accessibility, finite life span and relative simplicity of erythrocytes make them an attractive model to study the oxidative stress status of the body in diabetes mellitus. Erythrocytes, which are equipped with a highly effective anti oxidant defence system, can scavenge free radicals by the action of their antioxidant enzymes [2,3].

In erythrocytes, glutathione detoxifies free radicals through GST conjugation. GST also possesses peroxidase activity and can directly attack the peroxides. Interaction of free radicals with hemoglobin can denature Hb. Oxidative denaturation of hemoglobin leads to the release of heme into the RBC membrane and the released heme is capable of oxidizing membrane proteins via a thiyl radical intermediate. GST can bind free heme that is released during Hb oxidation presumably reducing damage to RBC membrane [4-6].

Considering all these factors we hypothesized that if oxidative stress and hyperglycemia are major etiologic and pathologic factors contributing towards diabetes mellitus and its complications, then Glutathione – S – Transferase in red blood cells, are likely to have a role in the glycation of Hb in diabetic patients. Hence we designed this study to evaluate the activity of red cell Glutathione-S-Transferase and correlate its association with Glycated Hemoglobin (HbA_{1c}).

Methods

This study was designed as a cross – sectional study, conducted in subjects attending Kasturba Hospital, Manipal. All patients who were advised to do a blood test for HbA_{1c} were included in this study. Hemolysed blood samples were excluded.

Specimen Collection: Na₂EDTA-Fluoride anticoagulated venous blood was used for both GST activity and HbA1c estimations. The blood sample was centrifuged at 3000 rpm for 20 min to separate the packed red cells. The separated red cells were washed 3 times with normal saline.

Erythrocyte GST activity

Erythrocyte GST activity was determined using the method of Habig et al (1974). This assay is based on the principle that GST catalyzes the conjugation of L-glutathione with CDNB through the thiol group of the glutathione to form GS-DNB Conjugate, which has maximum absorbance at 340 nm. The rate of increase in the absorption is directly proportional to the GST activity in the sample[7].

The hemolysate was diluted 1 in 5 with phosphate buffer and used for the assay. To the reaction tube, 0.1 ml of 30 mM GSH, 0.1 ml of CDNB and 2.7 ml of 100 mM KPO₄ buffer (pH 6.5) were added. The reaction was initiated by adding 0.1ml of hemolysate, mixed thoroughly, and absorbance was recorded at 340 nm every minute for 5min period. The average change in absorbance $\Delta A(\text{avg})$ per minute was calculated.

Estimation of Glycated hemoglobin (HbA1c)

The concentration of HbA1c was estimated using the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood by Roche Cobas Integra 400 auto analyzer. In this method the polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody polyhapten complex which can be determined turbidimetrically. Here the hemoglobin concentration has been determined in a second channel. Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically.

Statistical analysis

Erythrocyte GST activity in diabetics and non-diabetics were compared with Mann Whitney U test. The Pearson correlation coefficient test was applied to know the association of erythrocyte GST with HbA1c. A p value of ≤ 0.05 was considered statistically significant. The analysis was performed using SPSS (v. 14.0) software.

Results

After the eligibility criteria, a total of 100 subjects were included in this study. The subjects were divided into "diabetic" and "non-diabetic" based on clinical diagnosis. 68 patients who were confirmed to have type 2 diabetes mellitus were included in diabetic

group whereas rest 32 patients without diabetes were included in non-diabetic group [Table 1.].

Erythrocyte GST activity in diabetic patients

The erythrocyte GST activity in the diabetic patients was assessed by comparing with that in non-diabetics. Data were expressed as median and interquartile ranges. The GST activity in the diabetic group was 83.3(41.6-145.8)IU/L and that of non-diabetic group was 83.4 (47-153.6)IU/L. The Mann Whitney U test revealed that there is no statistically significant difference in the distribution of erythrocyte GST between diabetic patients and non-diabetic patients (p value = 0.08).

Association between erythrocyte GST and HbA1c

The association of erythrocyte GST and HbA1c was studied considering the possible effect of glycemic control on erythrocyte GST. The Pearson's correlation study yielded a mild positive correlation ($r = 0.239$, $p = 0.089$) between erythrocyte GST and HbA1c among the diabetic patients [Fig. 1.] and no association in non-diabetic patients [Fig. 2.].

Discussion

Increased hyperglycaemia induced oxidative stress is known to be a component of molecular and cellular tissue damage, associated with complications of diabetes. Impaired antioxidant status is known to be an indicator of oxidative stress..

Glutathione metabolism and GST distribution in the tissues may play an important role in the etiology, pathology and prevention of diabetes. Low levels of GSH in diabetics are found to potentiate the effects of the increased reactive oxygen species. Changes in GSH dependent enzyme activities, such as glutathione peroxidase, γ -glutamyl transpeptidase, and glutathione S-transferase(GST) are also noted in diabetes.

Evidences from few studies suggest that erythrocyte GST tend to decrease in diabetic patients. But there are studies which show increased GST activity in diabetics independent of their glycemic status. According to our study, there is no significant difference in erythrocyte GST activity between diabetics and non-diabetics. Decreased red blood cell GSH as well as increased serum total GST levels may be due to a compensatory mechanism of the antioxidants to combat the oxidative stress in diabetic conditions with or without complications. The regulation of GST is subject to a complex set of endogenous and exogenous parameters. These include developmental, gender, and tissue-specific factors, as well as a large number of xenobiotic-inducing agents.

Conclusion

The association of erythrocyte GST with HbA_{1c} was tested since HbA_{1c} is an established marker of glycemic control. The hypothesis underlying this study was that there will be a correlation between erythrocyte GST and HbA_{1c}. Our study shows no significant difference in erythrocyte GST activity among diabetic and non diabetic patients. However a positive correlation between erythrocyte GST activity and HbA_{1c} concentration was observed in diabetic patients. The chronicity of the disease along with treatment modalities might have played a significant role in the outcome of our study as the subjects included in diabetic group were enrolled without considering their glycemic control status.

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Acknowledgements

We are thankful to **Dr. B Rajashekhar**, DEAN, MCOAHS-Manipal, MU and **Dr.(Sr.) Licy CD**, Prof. and Head Dept. of MLT, MCOAHS-Manipal, MU for their support

Illustrations

Illustration 1

Table. 1. The Biochemical parameters in diabetics and non-diabetics

	Diabetic(n=68)	Non-diabetic(n=32)
Age*	58(43-65)	52(39-63)
Males(n)	44	22
Females(n)	24	10
Blood sugar(mg/dL)*	189(131.4-229)	101(87-106)
HbA ₁ C (% of Hb)*	10.3(7.2-12.1)	4.7(3.9-6.2)
GST(IU/L)*	83.3(41.6-145.8)	83.4 (47-153.6)

*Median (interquartile range)

Illustration 2

Fig. 2. The association of erythrocyte GST and HbA1c in Diabetics

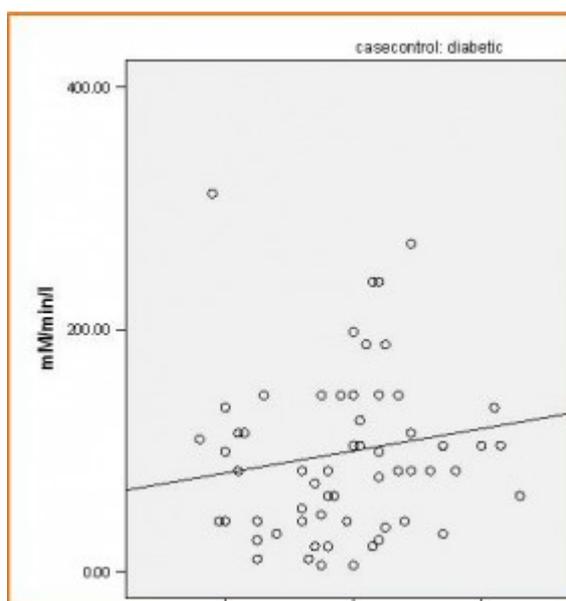
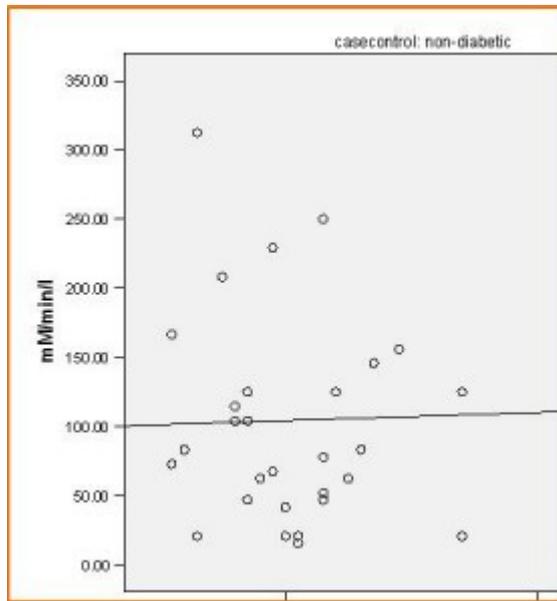


Illustration 3

Fig. 2. The association of erythrocyte GST and HbA1c in Non-diabetics



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