Original Article

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Correlation of Erythrocyte Glutathione Peroxidase activity with high levels of HbA1c

H. Nandita Mallya[1], Varashree B S[1], Shruti N Bhatkalkar[1], Revathi P Shenoy[1]

Abstract

Hyperglycemia induces increased generation of reactive oxygen species and decreased action of antioxidant enzymes which leads to oxidative stress. Antioxidant enzymes prevent the action of reactive oxygen species and protect the cells from oxidative damage. Glutathione peroxidase is one of the antioxidant enzyme. The aim of the study was to evaluate the association between antioxidant enzyme glutathione peroxidase activity in erythrocytes with elevated HbA1c levels.

Material and Method: Total of 92 whole blood samples were taken based on their HbA1c levels and were divided control (n=52) and hyperglycaemic group (n=40). Glutathione peroxidase activity in erythrocyte was measured. Statistical difference between groups was analyzed using independent t test. Pearson's correlation analysis was performed to determine the relationships between variables.

Results: There was no significant difference in glutathione peroxidase activity between the groups. There was statistically significant ($p < 0.001$) increase observed for HbA1c and blood glucose in their mean±SD values from control to hyperglycaemic group. There was no significant correlation observed between glutathione peroxidase and HbA1c as well as blood glucose. The results indicate that elevated levels of HbA1c does not alter the activity of erythrocyte glutathione peroxidase activity.

Keywords: HbA1c, Glutathione peroxidase, hyperglycemia, erythrocyte, glucose, antioxidant, oxidative stress

Introduction

Oxidative stress is caused due to increased production of reactive oxygen species and decreased action of antioxidant system. It is said to play a major role in pathogenesis of various disorders such as atherosclerosis, chronic renal failure, hypertension, cancer and diabetes mellitus[1] . In condition such as prolonged hyperglycemia, there is increased genenration of reactive oxygen species through the mitochondrial electron transport chain[2] and glucose autoxidation[3] which then leads to increased oxidative stress[2-4]. Oxidative stress induced by hyperglycemia has been found to be a major link between diabetes and diabetic related complications [5].

Another cause for oxidative stress in hyperglycaemic conditions is due to decreased activity of antioxidant system [6,7]. Antioxidant system is involved in getting rid of the free radicals that are produced, so as to maintain the redox homeostasis. This antioxidant system will detoxify reactive oxygen species by blocking their production, or by sequestering the transition metals in the presence of which free radicals are formed. There are two sources of antioxidant system. Out of these, endogenous system constitutes of antioxidant enymes of the body. Antioxidant enzymes act as primary defence against reactive oxygen species and they include superoxide dismutase, catalase and glutathione peroxidase. This study is focused specifically on activity of glutathione peroxidase. It is a selenium containing enzyme that has peroxidase activity. Its role in the body is to protect cells or tissues from oxidative damage. It does so by reducing harmful reactive oxygen species such as lipoperoxides and organic hydroperoxides using glutathione as reducing agent. Reduced glutathione is then oxidized by Glutathione reductase to complete the cycle. Deficiency of this enzyme can cause oxidative stress.

Together with oxidative stress, hyperglycemia also leads to increase in the levels of glycated hemoglobin (HbA1c) [8]. It is formed by non-enzymatic glycation of haemoglobin upon exposure to glucose. This formation of the glycated hemoglobin occurs normally in the presence of normal blood glucose levels. However, as blood glucose levels increases HbA1c levels increase as well[9]. HbA1c measurement provides a three month average blood glucose concentration. This test is most useful in monitoring the treatment of patients with diabetes[10] It is also used as a marker for chronic hyperglycemia[11] This study was designed to evaluate the association between antioxidant enzyme glutathione peroxidase activity in erythrocytes with elevated HbA1c levels as there was only little information available regarding the association between the two parameters. The expectation is that there will be a significant decrease in the activity of erythrocyte glutathione peroxidase when the HbA1c levels are extremely high. This is because of the oxidative stress caused due to production of extremely high levels of HbA1c in hyperglycemia which may alter the activity of the antioxidant enzyme

[1]Department of Biochemistry, Kasturba Medical College, Manipal, Manipal Academy Of Higher Education, Manipal, Karnataka, India-576104.

Address of Correspondence
Dr. Varashree B S,
Associate professor, Department of Biochemistry, Kasturba Medical College, Manipal, Manipal Academy Of Higher Education, Manipal, Karnataka, India-576104.
Email address: varashree.bs@manipal.edu.

Table 1: Comparison of blood glucose, HbA1c and glutathione peroxidase among control and hyperglycaemic group (n=92)

Parameters	Control	Hyperglycemic	P
n	52	40	
Blood Glucose	98.33± 15.8	261.6± 87.7	<0.001*
HbA1c	5.31± .253	10.97± 1.22	<0.001*
Glutathione Peroxidase	0.317± 0.101	0.342 ± 0.141	0.3 NS

Table 2: Correlation of Glutathione peroxidase with HbA1c and blood glucose in control and Hyperglycemic group.

		r	p
Control	HbA1c	0.213	0.129 NS
	Blood Glucose	0.161	0.274 NS
Hyperglycemic	HbA1c	0.08	0.623 NS
	Blood Glucose	0.18	0.294 NS

glutathione peroxidase in erythrocytes.

Material and Method

Study protocol was approved by the Institutional Ethics Committee, Kasturba Medical College, Manipal, India. A total of 92 whole blood samples that were referred for HbA1c test and blood glucose test were collected from the Clinical Laboratory of Biochemistry, KMC, Manipal after proper anonymization. The samples were collected based on their HbA1c levels and age (ranging from 35-65 years old) of the patient. HbA1c and blood glucose values for the samples were obtained from the laboratory. Whole blood was centrifuged at 2000rpm to separate the serum. Erythrocyte pellet was used for the estimation of Glutathione peroxidase. Estimation was done by first extracting the enzyme from the erythrocyte pellet by adding 5% meta-Phosphoric acid to it and centrifuging at 3000x g for 10mins at 4°C. Enzyme activity was estimated in the supernatant using an assay medium containing 100mM phosphate buffer, 0.1mM EDTA, 2mM Sodium azide, 2.5U/L Glutathione reductase, 10mM GSH, 2.5mM NADPH and reaction was started by adding 1.5mM Hydrogen peroxide. Enzyme activity was calculated using the formula ΔA/6.21 M-1 Cm-1. [12,13]

Statistical Analysis

The results were expressed as mean ± standard deviation. The statistical difference between groups was analyzed using independent t test. Pearson's correlation analysis was performed to determine the relationships between variables. The results were considered significant at $P < 0.05$. The statistical calculations were done using Statistical Package for the Social Sciences version 16.0.

Results

Samples were divided into two groups based on their HbA1c levels. Samples with HbA1c levels ranging from 4.5% - 5.7% were placed in Control group (n=52) and from 9.6% to 14% were placed in Hyperglycemic group (n=40). Mean±SD values of blood glucose, HbA1c and glutathione peroxidase among control and hyperglycaemic group is put in Table 1. There was no significant difference between the two groups for glutathione peroxidase whereas a significant difference (p value <0.001) was observed for blood glucose and HbA1c between the two groups.

There was no correlation found between glutathione peroxidase neither with HbA1c nor with blood glucose. (Table 2)

Discussion

Uncontrolled hyperglycemia has been suggested to produce abnormally high levels of reactive oxygen species. The increased levels of free radicals formed in hyperglycemia together with products of non-enzymatic glycation, glucose oxidation and lipid peroxidation causes damage to enzymes, cell functioning and insulin resistance due to oxidative stress. [14] Oxidative stress is a common feature of uncontrolled hyperglycemia in humans as well as animals[15-21]. Antioxidant enzyme such as glutathione peroxidase encounters with the reactive oxygen species and helps to repair the damage caused by them. Different studies have reported variations in activity of antioxidant enzymes in different tissues like liver, kidney, muscle, erythrocytes etc., in normal and diabetic condition and an increase in the glutathione peroxidase activity was observed in erythrocytes[22]. Whereas there are studies imply there was significant decrease in the activity of erythrocyte glutathione peroxidase when compared between diabetic and non- diabetic groups. [23,24] At the same time there are studies that found no significance difference in activity of glutathione peroxidase in eryhtrocytes between the two groups[25,26]. From Table 1 it is clear that the present study showed no significant decrease in erythrocyte glutathione peroxidase activity when compared between control and hyperglycemic group. However there was a significant increase ($p < 0.001$) observed in mean±SD values of HbA1c as well as blood glucose when compared between control and hyperglycemic group. Table 2 illustrates the correlation coefficient of glutathione peroxidase activity with HbA1c and blood glucose. There was no significant correlation observed between the enzyme and other two parameters. There is discrepancy among the results from previous studies and the present study. There could be various reasons for this which is yet to be cleared. Although, a recent study has shown that erythrocyte glutathione peroxidase activity begins to improve as insulin treatment is started [27].This could be one of the reasons for variations in the results .

Conclusion

There was no significant difference found between control and hyperglycemic groups regarding erythrocyte glutathione peroxidase activity. Also, there was no significant correlation observed between erythrocyte glutathione peroxidase activity and HbA1c as well as blood glucose.

References

1. Mates JM, Perez-Gomez C, NunezDeCastro I. Antioxidant enzymes and human diseases. Clin Biochem 1999; 32:595–603.

2. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, .et. al., Normalizing mitochondrial superoxide production

blocks three pathways of hyperglycaemic damage. Nature 2000; 404:787–790

3. Wolff SP, Dean RT: Glucose autoxidation and protein modification: the potential role of 'autoxidative glycosylation' in diabetes. Biochem J 1987; 245:243–250

4. Yoshifumi Saisho. Glycemic Variability and Oxidative Stress: A Link between Diabetes and Cardiovascular Disease? Int J Mol Sci. 2014; 10: 18381–18406.

5. G. Negi, A. Kumar, R. P. Joshi, P. K. Ruby, and S. S. Sharma, "Oxidative stress and diabetic neuropathy: current status of antioxidants," Institute of Integrative Omics and Applied Biotechnology Journal, 2011; 2: 71–78

6. Rains, J. L.; Jain, S. K. Oxidative stress, insulin signaling and diabetes, Free Radic Biol Med. 2011; 50: 567–575

7. Mercuri F, Quagliaro L, Ceriello A. Oxidative stress evaluation in diabetes. Diabetes TechnolTher. 2000;2:589-600

8. Shariq I. Sherwani, Haseeb A. Khan, Aishah Ekhzaimy, Afshan Masood, and Meena K. Sakharkar. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights. 2016; 11: 95–104.

9. EJ Ikekpeazu, EE Neboh, FE Ejezie, MD Ibegbu, and IE Ike. Oxidative Stress and Glycaemic Control in Type 2 Diabetic Patients in Enugu, South-East Nigeria. Ann Med Health Sci Res. 2011;1: 123–128.

10. Laura S. Greci, Mala Kailasam, Samir Malkani, David L. Katz, Ilja Hulinsky, Ramin Ahmadi, .et.al., Utility of HbA1c Levels for Diabetes Case Finding in Hospitalized Patients With Hyperglycemia. Diabetes Care 2003;26: 1064-1068.

11. Ekpebegh, CO, B Longo-Mbenza, and E Blanco-Blanco. "Glycosylated Haemoglobin Is Markedly Elevated In New And Known Diabetes Patients With Hyperglycaemic Ketoacidosis". African Health Sciences 14.3 (2014): 526. Web.

12. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Res. 1984;44:5086–91.

13. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun. 1976;71:952–8

14. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia 2001; 44:129–146.

15. Low PA, Nickander KK. Oxygen free radical effects in sciatic nerve in experimental diabetes. Diabetes 1991; 40:873–877.

16. Jennings PE, Jones AF, Florkowski CM, Lunec J, Barnett AH. Increased diene conjugates in diabetic subjects with microangiopathy. Diabet Med 1987; 4:452– 456.

17. Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersole de SC, Monnier VM, Witztum JL, Kurokawa K. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic

nephropathy. J Clin Invest 1997; 100:2995–3004.

18. Nishikawa T, Edelstein D, Brownlee M. The missing link: a single unifying mechanism for diabetic complications. Kidney Inter, Suppl 2000; 77:S26–S30.

19. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia 2001; 44:129–146.

20. Mohan IK, Das UN. Oxidant stress, antioxidants and nitric oxide in non-insulin dependent diabetes mellitus. Med Sci Res 1997; 25:55–57.

21. Tuzun S, Girgin FK, Sozmen EY, Mentes G, Ersoz B. Antioxidant status in experimental type 2 diabetes mellitus: effects of glibenclamide and glipizide on various rat tissues. Exp Toxicol Pathol 1999; 51:436–441.

22. Sailaja Devi MM, Suresh Y, Das. Preservation of the antioxidantstatus in chemically-induced diabetes mellitus by melatonin. J Pineal Res 2000; 29:108-15.

23. Goodarzi MT, Varmaziar L, Navidi AA, Parivar K. Study of oxidative stress in type 2 diabetic patients and its relationship with glycated hemoglobin. Saudi Med J. 2008 ;29:503-6.

24. G. S. Dave and k. Kalia hyperglycemia induced oxidative stress in type-1 and type-2 diabetic patients with and without nephropathy. Cell Mol Biol. 2007;53:68-78.

25. Mohsen Firoozrai, Mitra Nourbakhsh , Maryam Razzaghy-Azar. Erythrocyte susceptibility to oxidative stress and antioxidant status in patients with type 1 diabetes: Diabetes Research and Clinical Practice 2007;77: 427–432

26. Ozden, Meltem et al. "Erythrocyte Glutathione Peroxidase Activity, Plasma Malondialdehyde And Erythrocyte Glutathione Levels In Hemodialysis And CAPD Patients". Clinical Biochemistry 2002;35.4: 269-273.

27. Singhai, Monil, Rajeev Goyal, and AbulFaiz Faizy. "Glutathione Peroxidase Activity In Obese And Nonobese Diabetic Patients And Role Of Hyperglycemia In Oxidative Stress". Journal of Mid-life Health 2011;2: 72.

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