ORIGINAL ARTICLE

Analysis Of Serum Malondialdehyde And Glutathione Peroxidase Levels In Type 2 Diabetes And Its Correlation With HbA₁c

Shilpashree YD¹, Devaki RN²

ABSTRACT

Introduction: Diabetes mellitus has rapidly gained the status of a potential epidemic in India. It is associated with high incidence of mortality and morbidity. The pathogenesis of diabetes and its complication remain elusive; however oxidative stress is the most common factor that has been suggested. The present study was undertaken to evaluate the oxidative stress and antioxidant status and to correlate these with glycaemic control in type 2 diabetes.

Materials and Methods: This cross sectional study was carried out in the Department of Biochemistry, JSS Medical College, Mysore. Thirty patients with type 2 diabetes mellitus and 30 unrelated age and sex matched controls were included in the study. Glycated haemoglobin was estimated in whole blood. Serum malondialdehyde and glutathione peroxidase levels were estimated by thiobarbituric acid method and pagelia & valentine method respectively. SPSS version 16 for windows was employed for statistical analysis.

Results: Mean serum malondialdehyde levels were significantly greater in type 2 diabetics. There was a statistically significant negative correlation between serum malondialdehyde and glutathione peroxidase in type 2 diabetics ($r=-0.176$). Highly significant negative correlation was found between glutathione peroxidase and HbA₁c in diabetic controls. ($r=-0.168$).

Conclusion: There exists an inverse relationship between oxidative stress and antioxidants in type 2 diabetics and is a result of poor glycaemic control.

Keywords: antioxidant enzymes, diabetes, glycaemic control, lipid peroxidation, oxidative stress, glutathione peroxidase.

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INTRODUCTION

Free radical generation and dissipated antioxidant system are increasingly being singled out in current research works as causative factors in the pathogenesis of diabetic mellitus and its complications.¹ Reactive oxygen species (ROS) produced due to increased blood sugar levels cause damage to the cells ultimately resulting in the clinical manifestation of diabetes mellitus. High ROS generation leads to impaired defence system causing increased damage due to oxidative stress.² Malondialdehyde(MDA) [(CH₂(CHO))₂] is a naturally obtained reactive species used as a biomarker to measure the levels of oxidative stress.³ It is produced due to lipid peroxidation of polyunsaturated fatty acids present on the cell membrane by reactive oxygen species. Glutathione peroxidase (GPx) is a potent antioxidant enzyme which requires the element selenium for its activity.⁴ GPx removes H₂O₂ by using it to oxidize reduced glutathione (GSH) into oxidized glutathione.

$$2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O}$$

GPx works in conjunction with superoxide...
dismutase (SOD) in removing H$_2$O$_2$ from human cells. Although SOD works in conjunction with catalase, GPx is more important because the latter is located in the same subcellular compartment as SOD.\textsuperscript{5} Long-term control of blood glucose is measured by HbA$_{1c}$ levels. Glycation is the process of non-enzymatic addition of a carbohydrate residue to amino group of proteins. Slow, non-enzymatic reaction between glucose and amino groups forms post-translational glycated proteins. One percent reduction in HbA$_{1c}$ levels will decrease long term complications of diabetes up to an extent of 30\%. Measurement of HbA$_{1c}$ levels is more beneficial in monitoring blood glucose levels as it circumvents the need of fasting and frequent blood draws; is not affected by recent food intake or transient fluctuations in blood sugar levels.\textsuperscript{6} Very few studies were available among the residents of Mysore regarding status of oxidative stress and antioxidant enzyme GPx in type 2 DM. The present study was undertaken to evaluate the serum levels of oxidative stress marker MDA and antioxidant glutathione peroxidase and their correlation with glycaemic control.

MATERIALS AND METHODS

This cross-sectional study was conducted, in the Department of Biochemistry, JSS Medical College, Mysore during the period between February 2012 to January 2013. The study was conducted after obtaining the approval of institutional ethical committee. After explaining the details of the study a written informed consent was taken from all the participants. Thirty participants in the age group 40-80 years were randomly selected from type 2 diabetics who visited the outpatient Department of Medicine of JSS Hospital, Mysore. Participants with acute or chronic infections, fever, anaemia, malignancy, acute and chronic nephritis, cirrhosis, congestive heart failure were excluded from the study. None of the participants were on antioxidant supplementation. Thirty unrelated age and sex matched apparently healthy individuals were included as control participants.

Collection of sample

Fasting, un-haemolysed venous blood (5ml) was drawn from all the participants using universal precautions. Two ml of blood sample collected in EDTA vacutainers was used for estimation of HbA$_{1c}$ and GPx in whole blood. Three ml of the fasting blood sample was collected in plain vacutainers and serum was carefully separated and stored at -20°C until biochemical analyses and was used to estimate blood glucose and MDA.

Biochemical analysis

Fasting blood glucose was estimated by GOD-PAP method using RANDOX KIT-GL 3815 in the RandoxImola auto analyser.\textsuperscript{7} Assessment of oxidative stress was done by quantifying the thiobarbituric acid reactivity as MDA in spectrophotometer.\textsuperscript{8,9} GPx was estimated by Pagelia and Valentine method using RANSEL KIT in the Randox Imola auto analyzer.\textsuperscript{10} HbA$_{1c}$ was estimated by using RX SERIES HA 3830 KIT in the RandoxImola auto analyzer.\textsuperscript{11}

STATISTICAL ANALYSIS

SPSS for windows version-16 (2007) was employed for statistical analysis. Comparison between cases and controls was calculated using analysis of variance (ANOVA), independent sample’s t test and Pearson correlation coefficient test.

RESULTS

The mean values of FBS, MDA, GPx and HbA$_{1c}$ in cases and healthy controls are shown in table 1. The serum MDA level was significantly elevated (p<0.001) and the GPx level was significantly (p<0.001) decreased in type 2 diabetics compared to controls. Increase in HbA$_{1c}$ level was also significant (p<0.001) in type 2 diabetics compared to healthy controls. Figure 1 shows the correlation between MDA and GPx in cases. There was a significant negative correlation between plasma MDA and GPx in cases (r= -0.191). There was a positive correlation (Figure 2) between plasma MDA and HbA$_{1c}$ (r = 0.482) Correlation is significant at the 0.01 levels (2-tailed) indicating that as HbA$_{1c}$ increases, MDA also increases. Correlation study revealed inverse relationship (Figure 3) between GPx and
HbA1c (r= -0.664).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Type 2 Diabetics</th>
<th>Healthy Controls</th>
</tr>
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<tbody>
<tr>
<td>FBS (mg/dl)</td>
<td>124.86±22.09</td>
<td>93.26±8.90</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.7±1.02</td>
<td>5.13±0.54</td>
</tr>
<tr>
<td>GPx (u/l)</td>
<td>4935.4±1449.31</td>
<td>7604.27±1968.01</td>
</tr>
<tr>
<td>MDA(nmol/ml)</td>
<td>5.05±2.64</td>
<td>1.81±0.61</td>
</tr>
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</table>

Table-1: Biochemical parameters in the study participants

DISCUSSION

In the present study, the serum levels of MDA and GPx were measured and their relationship vis-à-vis HbA1c was studied. The values were compared between healthy controls and type 2 diabetics. The study proves the imbalance of oxidative stress and antioxidant status in type 2 diabetes as revealed by an increase in the levels of MDA and decrease in GPx levels. A positive correlation between MDA and HbA1c and between MDA and blood glucose were also observed. The present study concurs with previous studies in the same field of research. The elevation of MDA levels is a result of a hyperglycaemic state that induces the overproduction of oxygen free radicals in diabetes. Activation of polyol and hexosamine pathways, protein kinase C and advanced glycation end products production in a hyperglycaemic state leads to oxidative stress, which in association with dysfunctional mitochondria and endoplasmic reticula promote increased free radical production. The free radicals produced promote cellular damage and contribute to the manifestation of diabetes and its complications. GPx is an antioxidant enzyme found in the mitochondria, cytoplasm and nucleus. Hydrogen peroxide is reduced to water by GPx utilizing the hydrogen of reduced glutathione forming Glutathione disulfide (GSSG). Reduced glutathione is resynthesized from GSSG by glutathione reductase, using the cofactor NADPH generated by glucose 6-phosphate dehydrogenase. The correlations of MDA and GPx with HbA1c show a positive and negative correlations respectively suggesting that good glycaemic control is essential for proper balance of oxidative and antioxidant status in diabetes. The diabetes induced alterations in the levels of GPx can be altered with supplementation of antioxidants.

CONCLUSION

To conclude, increase in oxidative stress which is measured as high MDA levels and decrease in antioxidant status measured as low GPx levels along with their association with glycaemic control and decreased antioxidant status could lead to the of complications associated with diabetes. Therefore, it appears reasonable to suggest supplementation of antioxidants to treat elevated oxidative stress and lipid peroxidation that may predispose diabetic patients to complications associated with diabetes. Future research focussing on the therapeutic role of antioxidant supplementation may be rewarding in diabetes.
REFERENCES


