ORIGINAL ARTICLE

Glutathione peroxidase activity in obese and nonobese diabetic patients and role of hyperglycemia in oxidative stress

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ABSTRACT

Background: Both hyperglycemia and obesity are known to cause oxidative stress, which leads to many complications associated with diabetes mellitus. A large number of diabetic patients are obese. Glutathione peroxidase (GPx) is an important indicator of level of oxidative stress.

Materials and Methods: In the present study, we assessed GPx levels in 20 healthy controls, obese, and nonobese diabetic patients (*n*=20 each) and analyzed the effect of insulin treatment for 24 and 48 weeks on GPx activity. GPx activity was measured using biochemical method. The GPx activity was also correlated with glycemic status of obese and nonobese diabetic patients [fasting plasma glucose (FPG) levels] after insulin therapy. Statplus software was used for statistical analysis.

Results: We found that there is suppression of GPx activity in diabetic patients as compared to healthy controls (70.9 ± 9.6 U/mg protein) and suppression is more in case of obese (23.4 ± 3.8 U/mg protein) than nonobese diabetics (41.5 ± 3.5 U/mg protein). Both obese (26.05 ± 4.03 U/mg protein) and nonobese (48.7 ± 4.8 U/mg protein) diabetics had increase in GPx activity after 24 weeks of insulin treatment. Further, insulin treatment led to improvement in oxidative stress after 48 weeks in both obese (28.4 ± 6.4) as well as nonobese diabetics (51.8 ± 5.4). The nonobese group showed extremely significant (P<0.001) increase in GPx activity after 24 and 48 weeks both, while obese group showed significant (P value<0.05) increase in GPx activity with insulin treatment only after 48 weeks. A negative correlation was found between postinsulin GPx levels and FPG of obese and nonobese diabetics. The correlation was more strong in case of nonobese than obese diabetics.

Conclusion: Higher levels of oxidative stress in obese diabetics even after control of hyperglycemia by insulin treatment reflect the importance of obesity in contributing to oxidative stress.

Key Words: Diabetes, obesity, oxidative stress

INTRODUCTION

Type 2 diabetes mellitus (T2DM) has reached global epidemic proportions. The disease affects over 150 million people worldwide, a number that is expected to double by 2025.[1] Diabetes is a prooxidant state characterized by increased reactive oxygen species (ROS).[2] Oxidative stress may be defined as an imbalance between production and degradation of ROS. One of the major hypotheses to explain the onset of diabetic complications is a DM-induced increase in oxidative stress.[3] Obesity further complicates T2DM by increasing insulin resistance and blood glucose concentrations.[4] American Diabetes Association and the National Institutes of Health have recommended that health care professionals advise obese diabetic patients to lose weight.[5,6] Though both hyperglycemia and obesity go hand in hand to cause oxidative stress, the contribution of obesity to this stress has not been quantified. Glutathione peroxidase (GPx),

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an enzyme whose main biological role is to protect the organism from oxidative damage by free radicals, is key indicator of oxidative stress. The red cell has been a central focus of research on GPx because it is thought to undergo a high endogenous rate of H_2O_2 production from hemoglobin autoxidation.[7] Optimum level of glutathione is required in body which in turn potentiates GPx activity to stay healthy. GPx activity is considered to represent the initial protective response required for adjusting the H_2O_2 concentration under normal physiological conditions as well as after oxidative insult.[8] The reports of GPx response in diabetics have been controversial.[9-12] In the present study, attempts were made 1) to know and compare the level of GPx activity in normal healthy, obese diabetic and nonobese diabetic patients, 2) to assess the contribution of obesity to oxidative stress in diabetic patients with respect to this antioxidant enzyme, GPx, and 3) to assess GPx activity after insulin therapy and correlate it with control of hyperglycemia (fasting plasma glucose levels).

MATERIALS AND METHODS

This was a prospective cohort study on 40 patients with T2DM, aged 45–65 years old. Diabetic patients were divided into two cohorts, obese diabetic and nonobese diabetic ($n=20$ each), obesity was defined as BMI > 30. The study also included 20 normal healthy volunteers as controls.

Patients, who had a history of any acute or chronic disease other than diabetes, either at present or in recent past, were excluded from the study, to minimize possible confounding of results due to oxidative stress by other diseases. All the patients to be included in study were informed about the objectives of this study and any potential benefits or losses. A standardized patient consent form, approved by Institutional Ethics Committee, was used for this purpose.

Measurement of fasting plasma glucose

Fasting plasma glucose (FPG) was measured by GOD-POD method, using kit procured from Span Diagnostics, India.

Measurement of GPx activity

Heparinized blood samples were collected before the start of insulin therapy and after 24 and 48 weeks of treatment. The activity of GPx in erythrocytes was measured as described elsewhere.[13,14] Briefly, the oxidized glutathione (GSSG) produced during GPx reaction was immediately reduced by nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione reductase. Therefore, the rate of NADPH consumption was regarded as the rate of GSSG formation during the GPx reaction. Cells were lysed in cell lysis buffer containing protease inhibitors (50 mM Tris/HCl, pH 7.4; 1 mM EDTA; 500 mM PMSF). The cell suspension was homogenized with a sonicator on ice and centrifuged at 10,000 rpm for 10 min. Protein concentrations of supernatants were determined by the method of Bradford with BSA as the standard and were subjected to GPx activity determination. The reaction mixture (1.0 ml) c ontaining 50 mM potassium phosphate (pH 7.0), 1 mM sodium azide, 2 mM GSH, 0.2 mM NADPH, 1 unit/ml glutathione reductase, 1.5 mM cumene hydroperoxide, and 20–100 ml of samples were incubated at 25°C for 5 min. The reaction was initiated by the addition of cumene hydroperoxide. The kinetic change was spectrophotometrically recorded at 340 nm (37°C) for 3 min. GPx activity was calculated after subtraction of the blank value, as µmol of NADPH oxidized/minute/mg protein (U/mg protein). GPx control specimens were prepared from pooled heparinized plasma aliquoted into 1.0 ml cryovials and were stored at 76°C. These quality control samples have been shown to be stable for 4 years.[15] The results were compared between the two cohorts and normal healthy individuals acting as controls.

Statistical analysis

The data were expressed as mean ± standard deviation. Paired *t* test was done to compare enzyme activity between two groups (obese and nonobese). Analysis of variance (one-way Anova) was used to compare enzyme activity in the same set of groups (either obese or nonobese) at two different times. $P < 0.05$ were considered significant, while $P < 0.001$ were extremely significant. Pearson's coefficient of correlation was done to assess association between enzyme activity and glycemic control after insulin therapy in obese and nonobese diabetics. Statplus software was used for statistical analysis.

RESULTS

GPx activity in normal healthy, diabetic nonobese, and diabetic obese patients

An appreciably high magnitude of GPx activity was recorded in normal healthy controls. The data obtained show GPx activity to the order of 70.9 ± 9.6 U/mg protein in healthy controls, while in obese diabetic patients devoid of treatment, it was 23.4 ± 3.8 U/mg protein. There was 3.03-fold suppression of GPx activity in obese diabetic patients as compared to

healthy control. Non-obese diabetic patients devoid of any insulin treatment showed a GPx activity of 41.5 ± 3.5 U/mg proteins which accounted to 1.71-fold suppression as compared to normal individuals. These results showed that GPx activity suppression was more in obese diabetics than nonobese diabetics. The suppression in the GPx activity in preinsulin samples of obese patients in comparison to nonobese diabetic patients was 1.77-fold more.

Effect of duration of insulin treatment on GPx activity in nonobese and obese diabetic patients

There was a consistent improvement in FPG and GPx activity of diabetic patients, after 24 and 48 weeks of insulin treatment [Table 1, Figure 1]. There was better glycemic improvement in nonobese than obese diabetics after 24 weeks.

After 24 weeks, GPx activity was observed to be 48.7 ± 4.8 U/mg protein in nonobese and 26.05 ± 4.03 in obese patients. Both nonobese and obese diabetics had increase in GPx activity (1.17- and 1.08-fold, respectively) at 24 weeks of insulin treatment. After 48 weeks, GPx activity was observed to be 51.8 ± 5.4 U/mg protein in nonobese and 28.4 ± 6.4 in obese patients. The improvement was 1.25-fold and 1.21-fold over 48 weeks in nonobese and obese diabetics respectively. The nonobese diabetics had more increase in GPx as compared to obese diabetics at 24 and 48 weeks ($P < 0.001$).

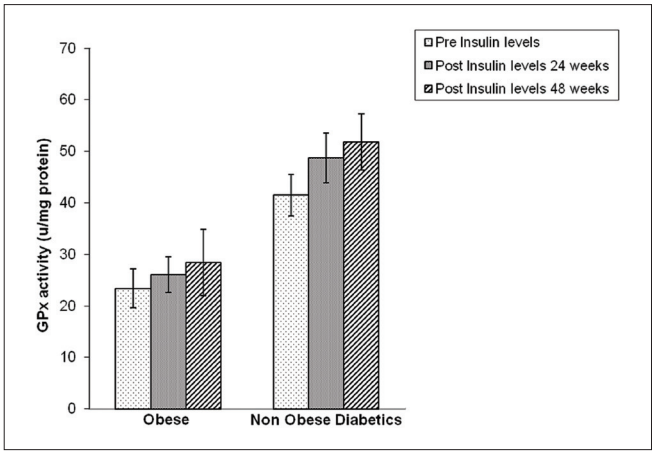

Figure 1: Preinsulin and postinsulin GPx activity status of obese and nonobese diabetics (*n*=20 each)

On further analysis of data for obese and nonobese groups, nonobese group showed extremely significant ($P < 0.001$) increase in GPx activity with insulin treatment after 24 and 48 weeks both, while obese group showed significant ($P < 0.05$) increase in GPx activity with insulin treatment only after 48 weeks.

GPx activity and FPG levels in nonobese and obese diabetics after insulin therapy

A negative correlation was found between postinsulin GPx activity and FPG levels of nonobese and obese diabetics after 24 and 48 weeks [Figure 2].

DISCUSSION

In this study, we investigated the effect of obesity on oxidative stress status in T2DM and found that there was decrease in GPx activity of diabetic patients as compared to healthy controls. Other studies have also recorded higher levels of GPx activity in healthy persons as compared to obese and diabetic subjects.[16-18] We have also studied the associations of GPx activity in obese and nonobese patients with T2DM and found decrease in GPx activity was significantly marked in obese diabetics than nonobese diabetics, indicating higher levels of oxidative stress in obese diabetics. Many clinical studies have recognized fair association between obesity leading to oxidative stress in T2DM.[19-21] Majority of diabetic patients are obese (55–60%). The more fat along with lower insulin levels in a diabetic patient leads to greater level of inflammation and oxidative stress in body as shown in our study. The possible sources of increased oxidative stress might include increased generation of free radicals or impaired host antioxidant defense system. Previous studies have shown that levels of free radicals are increased in diabetes and obesity.[20,22]

There was sustained improvement in GPx activity after 24 and 48 weeks of insulin treatment, indicating a reduction in oxidative stress. This improved GPx activity correlated with improvement in FPG levels. This correlation between GPx activity and glycemia (measured by FPG or HbA1C) has been validated by other studies also.[23] Though both obese and nonobese diabetics showed reduction of oxidative stress, the

Table 1: Glycemic and GPx activity status of obese and nonobese diabetics*

Diabetic patients	Glucose levels (preinsulin)	Glucose levels (24 weeks)	Glucose levels (48 weeks)	GPx activity (preinsulin)	GPx activity (24 weeks)	GPx activity (48 weeks)
Obese diabetic	283 ± 18	170 ± 12	164 ± 14	23.4 ± 3.8	26.05 ± 4.03	28.4 ± 6.4
Nonobese diabetic	252 ± 15	163 ± 10	159 ± 12	41.5 ± 3.5	48.7 ± 4.8	51.8 ± 5.4

*Fasting plasma glucose level (mg/dl) and GPx activity (U/mg proteins)

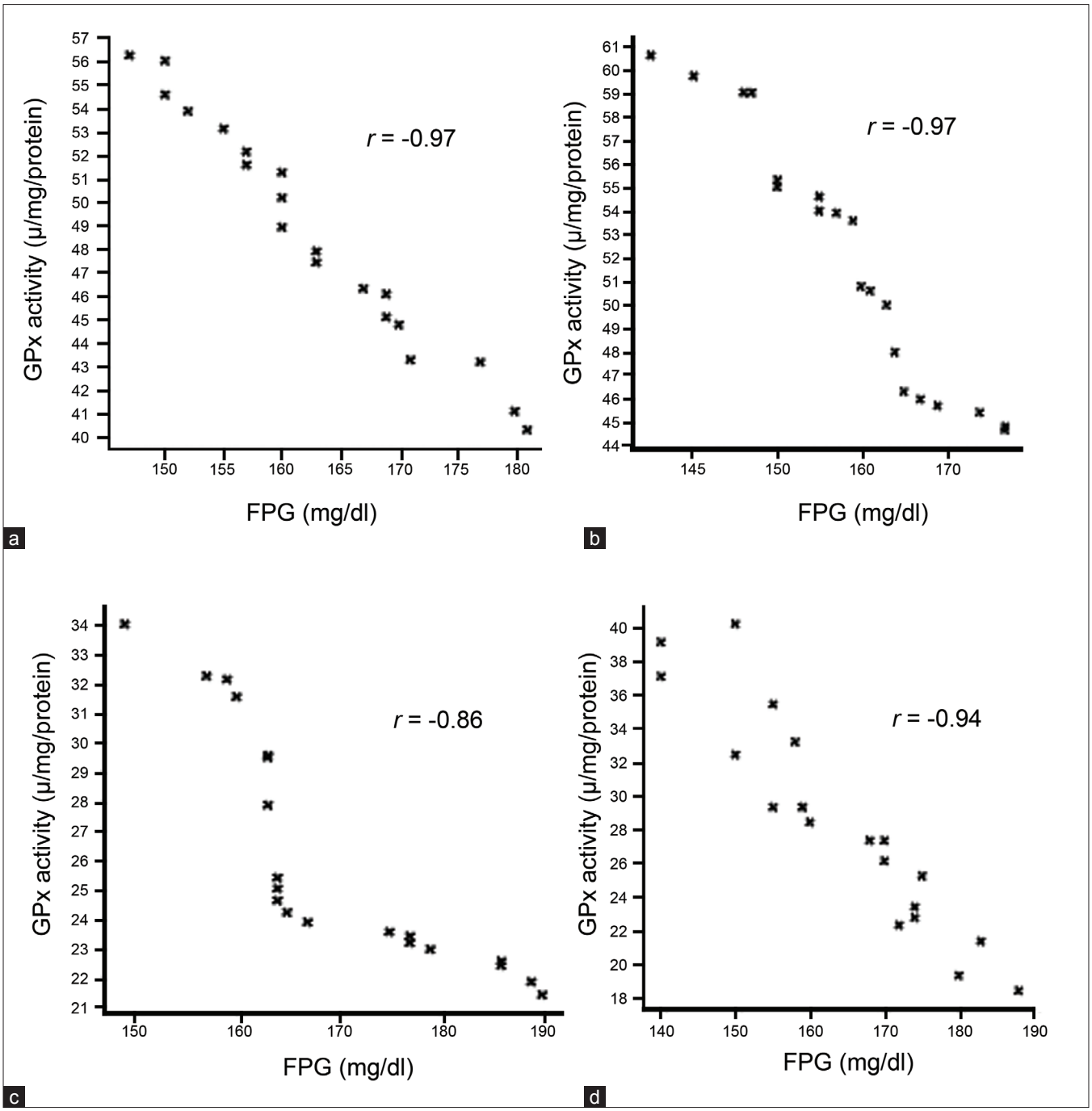

Figure 2: Correlation of GPx activity (U/mg/protein) and FPG (mg/dl) after insulin therapy in nonobese diabetics (24 weeks) (a), nonobese diabetics (48 weeks) (b), obese diabetics (24 weeks) (c), and obese diabetics (48 weeks) (d); *r*=correlation coefficient

reduction was more significant in nonobese diabetics than obese diabetics. This led us to conclude that there is an association of oxidative stress with hyperglycemia in diabetic patients and significant additional contribution by weight gain. These findings are in accordance with other studies which have shown increased oxidative stress in obese diabetics.[20,21] Although obese diabetic persons can be responsive to insulin, beneficial effects of weight loss on the metabolic parameters of many diabetic patients are well documented.[24-26]

Another noteworthy finding of this study was the correlation between postinsulin GPx activity and FPG levels among obese and nonobese diabetics. The GPx activity was significantly increased after control of hyperglycemia in diabetic patients after insulin therapy. A negative correlation was found between postinsulin GPx levels and FPG of obese ($r=-0.93$) and nonobese

diabetics (r=−0.98) at 48 weeks. Although at 24 weeks of post-insulin therapy GPx levels and FPG of obese (r=−0.86) and nonobese diabetics (r=−0.97) were negatively correlated but correlation was more strong in case of nonobese than obese diabetics. There are similar studies describing negative correlation of GPx activity with metabolic parameters (FPG and HbA1c) in various diabetic patients such as those classified on basis of presence or absence of vascular complications and the degree of metabolic control.[22,27] In this study, we could not assess HbA1c due to financial constraints, although we agree that it is a better marker of glycemic control.

Thus we found that there is an increase in oxidative stress in T2DM Mellitus than in normal persons, and stress is more marked in obese diabetics. Improvement in oxidative stress of both obese and nonobese diabetics after 24 and 48 weeks of insulin treatment indicates the role of hyperglycemia as promoter of oxidative stress. Further, higher oxidative stress in obese diabetics, both before as well as after control of hyperglycemia, indicates that obesity may act as an independent and significant contributor to this process, worsening the oxidative stress of patients and contributing to diabetic complications.

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