Evaluating Blood Glucose-6-Phosphate Dehydrogenase Activity with Oxidative Stress: A Study in Uncomplicated Type 2 Diabetes Mellitus Patients

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ABSTRACT

Background: Diabetes mellitus (DM) is chronic hyperglycemia condition affecting multiple organs due to metabolic disorder. Insulin secretion, function, or both are affected for which one of the factors attributed is due to increased free radical activity. Nicotinamide adenine dinucleotide phosphate (NADPH) produced in HMP shunt pathway is regulated by the rate-limiting glucose-6-phosphate dehydrogenase (G6PD). When there is an imbalance between the production of reactive oxygen species and the antioxidant system that detoxifies, then it is called oxidative stress. This pathway is regulated by the reductant concentration of NADPH.

Aims and objectives: The current study was taken up to evaluate and correlate oxidative stress and insulin resistance with G6PD activity in type 2 DM (T2DM) patients.

Materials and methods: A total of 100 (76 males 24 females) T2DM patients with equal age- and sex-matched healthy controls were selected for the study. Glucose-6-phosphate dehydrogenase was measured by chemical method in semiauto analyzer. Total oxidative stress measured as ferrous oxidation in xylenol orange and total antioxidant capacity estimated as ferric-reducing ability of serum by spectrophotometer. Glucose was measured by glucose oxidase-peroxidase method in an autoanalyzer. SPSS Version 20 software was used for statistical analysis.

Results and observations: Increased serum G6PD levels were found in DM patients which significantly correlates with the increase of oxidative stress and high glucose levels (p value < 0.01).

Conclusion: Estimation of blood G6PD activity may be used as a test to know the extent of oxidative status in DM patients for its implications in further clinical complications.

Keywords: Fasting plasma glucose, Ferrous oxidation in xylenol orange, Ferric-reducing ability of serum, Glucose-6-phosphate dehydrogenase, Nicotinamide adenine dinucleotide phosphate.

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INTRODUCTION

According to World Health Organization (WHO),¹ as of October 2013, 347 million people have diabetes mellitus (DM). The WHO projects that the seventh leading cause of death worldwide will be DM by the year 2030. The state of Odisha comprises 44 million people, and according to estimates, around 1.5 million people in Odisha are suffering from DM against the country’s 62 million.² Many diseases are associated with oxidative stress, and type 2 DM (T2DM) is one of them. The etiopathogenesis is due to increase in oxidative stress along with inadequate antioxidant defense systems as revealed by many studies. A significant role is being played by free radicals in the progression of this multifactorial disease. The imbalance between generation of oxidants and antioxidants results in oxidative stress. Glucose auto-oxidation, protein glycation, formation of advanced glycation end products, etc. are the different intricate mechanisms resulting in origin of free radicals. These are equally damaging toward macromolecules such as DNA, molecules of extracellular matrix, lipoproteins, etc.³⁻⁵

These free radicals induce cellular damage and β-cell dysfunction causing decrease in insulin secretion and signaling.⁶ Generation and detoxification of reactive oxygen species (ROS) maintains the redox balance in normal physiological state. Any alteration in this tightly controlled system leads to cell damage and death. Redox balance is affected and regulated by many diseases including diabetes.⁷⁻¹¹

The chief source of nicotinamide adenine dinucleotide phosphate (NADPH) is the HMP shunt pathway, regulated by the antioxidant enzyme glucose-6-phosphate dehydrogenase (G6PD). NADPH is required for synthesis of nitric oxide, glutathione recycling within the cells, cytochrome P450, and others.¹²,¹³ Activity of G6PD is affected by various signal transduction mechanisms such as transcription, posttranslational modifications, sorting, and transport that require interaction with other proteins.¹⁴,¹⁵ Pronounced changes in environment, food availability, and lifestyle have resulted in escalating the rates of diabetes.

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Chronic hyperglycemia and its oxidant derivatives play a major role in pathogenesis of DM which is demonstrated by several research studies. Activity of enzyme G6PD as an antioxidant system is important in preventing and postponing complications of DM.

Aims and Objectives

- The current study was designed to evaluate G6PD activity in uncomplicated T2DM.
- Oxidant activity and antioxidant activity were correlated with levels of G6PD.

Materials and Methods

This is a hospital-based, case–control prospective study. It includes 100 diagnosed cases of T2DM and equally matched healthy controls during the period of December 2014 to December 2015. Patients with autoimmune disorders, smokers, hypertension, alcoholics, and tobacco users were excluded in this study. Ethical Committee approval was obtained to conduct the study. Written consents were obtained from the participants (patient and control groups).

A morning sample of venous blood (5 mL) was collected after overnight fast in a day by sterile disposable syringe under aseptic condition. Serum was used for analysis of fasting insulin (FI), ferrous oxidation in xylene orange (FOX-2). Plasma was used for the analysis of ferric-reducing ability of serum (FRAP) and glucose. All the tests were done within 8 hours of collection and separation of serum. Serum total oxidant level was measured by FOX-2 assay and measured spectrophotometrically at 560-nm wavelength. The results were expressed in μmol H2O2/L. The total antioxidant capacity was measured by FRAP and measured spectrophotometrically at a wavelength of 593 nm. The results were expressed in μmol/liter equivalent of FeSO4 solution. Insulin was assessed by commercial kit supplied by US Diagnostics Roche. It was estimated in Roche Cobase411 autoanalyzer by electrochemiluminescence method and glucose by glucose oxidase and peroxidase (GOD–POD) method by fully automated Toshiba TBA 120 FR procuring kit from AGAPPE diagnostics. G6PD was estimated in vitro by kinetic method in blood at 340 nm by procuring kit from Far Diagnostics.

Statistical analysis of the data was done using independent Students t test. Correlations were calculated by Karl Pearson’s coefficient.

Results

In our study, we have seen that DM is more common in the middle-aged group, with the mean age of patients being 52 years, more prevalent in males, and 79% of cases belonging to the age group of 40–60 years (Table 1 and Fig. 1).

The physical attributes such as age, body mass index (BMI), waist circumference, and blood pressure (BP: systolic and diastolic) were elevated but not statistically significant among the study groups (p value > 0.05) (Table 2).

The FOX-2, FI, and fasting plasma glucose (FPG) levels significantly raised in DM, showing increased oxidative stress in diabetic patients. Ferric-reducing ability of serum was significantly less in patients showing decreased antioxidant levels.

G6PD is an intracellular enzyme and barely shows any serum activity. But in our study, we found that serum activity is increased in diabetic patients (1.92 ± 0.55) when compared to healthy individuals (0.09 ± 0.06) as shown in Table 3 and Figure 2.

The correlation between activity of G6PD and oxidative stress in diabetic individuals is shown in Figure 3. Thus, the level of G6PD in the plasma increases with the increase in oxidative stress.

On correlating G6PD with FPG, it was observed that in diabetic patients G6PD is positively and significantly correlated with FPG which shows that G6PD activity increases with hyperglycemia in diabetic cases Table 4 and Figure 4.

Discussion

Diabetes mellitus has become a global disaster placing considerable burden on healthcare services. Increased lifespan, population explosion, unplanned urbanization, low education level, increased external debt, and reduction in national healthcare expenditure are the reasons behind the “epidemiological transition” of this
The study carried out by Patel et al. provides direct evidence of the antioxidant activity in T2DM patients indicating oxidative stress. Due to chemical diversity, the collective action of all the natural antioxidants when assessed is potentially more useful toward an individual’s health. The availability of NADPH and feedback inhibition by ATP decides the activity of HMP shunt pathway, as 10% of glucose is utilized by this pathway under normal physiological conditions. Oxidative stress causes increased flux of glucose through this pathway. As the ratio between NADPH–NADP decreases, G6PD activity increases to provide more NADPH. When cells are exposed to various external stimuli, the level of NADPH, the cells principal reductant, decreases. Activity of G6PD is triggered following diminished the level of NADPH. Hence, G6PD activity may increase in serum due to reduced availability of substrate glucose-6-phosphate in DM patients.

Superoxide dismutase, catalase, thioredoxin, and the glutathione system are some important antioxidant systems in our body. But until now, the antioxidant property of G6PD has not been properly evaluated. The functioning of entire antioxidant system depends on NADPH12,13 Research done by Zhang et al.27 as well as others have indicated that chronic hyperglycemia impairs the activity of G6PD by decreasing NADPH production in vascular endothelial cells. They carried on their research work further and for the first time found that by increasing G6PD activity, there is a remarkable improvement that by increasing G6PD activity, there is a remarkable improvement in the level of redox enzymes and redox status. Henceforth, the change in redox status leads to decrease in cell death and enhanced cell growth in endothelial cells. A signaling molecule gets modified which influences the activities of other antioxidant enzymes due to overexpression of G6PD. This modified redox status affects the activity of ROS-scavenging antioxidant enzymes in the form of posttranslational modification.

**CONCLUSION**

The present study showed an increase in G6PD activity in DM patients which was positively correlated with plasma hydroperoxide level measured as FOX-2. The plasma antioxidant levels measured by FRAP was found to be reduced in DM patients. Despite the fact that these enzymatic changes are comparatively very small, they are physiologically significant. Further studies...
in the larger group of patients is required for identification of the important role of G6PD activity. This knowledge will give us a clear understanding about the oxidative stress–attributed complications in T2DM.

REFERENCES