

**ASSESSMENT OF THE ACTIVITY OF GLUCOSE-6-PHOSPHATE
DEHYDROGENASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS IN
EKPOMA, SOUTH-SOUTH NIGERIA*****¹Festus OO., *²Dada FL., ²Iweka FK., ³Eyaufe AO., ³Osagie RN.,
¹Akiyang EE.**

Department of ¹Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria; ²Chemical Pathology, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria. ³Medical Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

*Corresponding Author: olkof2004@gmail.com

ABSTRACT

Glucose-6-phosphate dehydrogenase (G-6-PD) is an enzyme in the pentose phosphate pathway (PPP) which reduces NADP to NADPH while oxidizing glucose-6-phosphate. In turn, NADPH then provides reducing equivalents needed for the conversion of oxidized glutathione to reduced glutathione, which protects against oxidant injury. The activity of G-6-PD was determined in type 2 diabetes mellitus patients and control subjects using enzymatic colourimetric method. A total of one hundred (100) subjects consisting of sixty (60) diabetes mellitus patients (test) and forty (40) apparently healthy subjects (control) were involved in the study. The mean \pm standard deviation of G-6-PD activity in type 2 diabetic patients was $2.53 \pm 1.34 \mu\text{g Hb}$ while the control was $14.44 \pm 3.27 \mu\text{g Hb}$. The results showed that G-6-PD activity in type 2 diabetic patients was significantly lower ($p < 0.05$) compared to the control subjects. This finding therefore suggests that there is a decrease in G-6-PD activity in type 2 diabetic patients. For that reason, monitoring of G-6-PD activity may be an important tool in preventing diabetic injury due to inappropriate antioxidation process.

Keywords: Glucose-6-phosphate dehydrogenase, glutathione, diabetes mellitus, oxidant, oxidative stress

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorders characterized by high levels of sugar (glucose) in the blood (David and Gardner, 2011). It is said to occur when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces and hence, an increased concentration of glucose in the blood known as hyperglycaemia (Warrell *et al.*, 2005; David and Gardner, 2011). Although an estimated 16 million people in the United States are known to be affected, worldwide, more than 140 million people suffer from diabetes, making it one of the most common non communicable diseases (Zimmet *et al.*, 2005). According to the American Diabetes Association (2003), DM is said to be associated with increased thirst, dehydration, weight loss, blurred vision, fatigue and occasionally comma. By implication, DM is a leading cause of end-stage renal disease, adult onset blindness, and non traumatic lower extremity amputations in many parts of the world.

The World Health Organization (1999) has classified three main types of DM. The Type 1 DM; which was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes" (Lambert and Bingley, 2002) results from the body's failure to produce insulin and presently requires the person to inject insulin or wear an insulin pump. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated autoimmune attack (Rother, 2007). The Type 2 DM; which was previously referred to as non insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes" is said to results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency or relatively reduced insulin secretion (David and Gardner, 2011). The third form; gestational diabetes, occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of type 2 DM (Cooke and Plotnick, 2008). Other forms of diabetes mellitus include

congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes (Cooke and Plotnick, 2008; Lawrence *et al.*, 2008).

Glucose-6-phosphate dehydrogenase (G-6-PD) is an enzyme in the pentose phosphate pathway, a metabolic pathway that supplies energy to a number of cells (notably erythrocyte) and maintained the level of the coenzyme – Nicotinamide adenine dinucleotide phosphate (NADPH) (Takizawa *et al.*, 1986). It catalyses the entry of glucose-6-phosphate into the pentose phosphate shunt and in the case of the red cells, this alternate anaerobic pathway for glucose metabolism is the only source of reduced NADPH, which is required for methaemoglobin reductase activity and the maintenance of the level of reduced glutathione. NADPH and reduced glutathione in turn maintain an effective redox potential, protecting cell membrane sulfhydryl group, enzymes and haemoglobin against oxidative stress (Takizawa *et al.*, 1986).

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is a recessive X-link trait, placing males at highest risk for symptomatic diseases and this often manifested in several distinct clinical patterns (Gomez-Gallego, 2000). Inherited as an X-linked disorder, G-6-PD deficiency affects 400 million people worldwide (Gaskin *et al.*, 2001). Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in the alternative pathway causes the build-up of glucose and thus there is an increase of advanced glycation end products (AGE), it also causes a reduction of NADPH which is necessary for the formation of nitric oxide (NO) (Gaskin *et al.*, 2001). The disease is highly polymorphic with more than 300 reported variants. It confers protection against malaria, which accounts for its high gene frequency (Cappellini and Fiorelli, 2008). Most common is haemolysis after exposure to oxidative stress which could be due to ingestion of certain drugs or food (eg fava beans) or exposure to oxidant free-radicals generated by leucocytes during infection (Gomez-Gallego, 2000).

Previous study by Gaskin *et al.*, (2001) has related the high prevalence of type 2 diabetes mellitus and hypertension in blacks in the West with G-6-PD deficiency. Hence, this study assesses the activity of glucose-6-phosphate dehydrogenase in patients with type 2 diabetes mellitus in Ekpoma, south-south Nigeria.

MATERIALS AND METHODS

Study duration: This four (4) months study was carried out between September 2011 and January 2012 at Irrua Specialist Teaching Hospital (ISTH), Irrua.

Study population: ISTH located in Irrua the Local Government Head Quarter of Esan Central Local Government Area (ECLGA) of Edo State. It is the teaching Hospital of the Ambrose Alli University and serves as a referral institute for urban and rural population.

Subjects: Subjects enrolled for the study comprised a total of one hundred (100) subjects, made up of sixty (60) type 2 diabetes mellitus patients (test) and forty (40) apparently healthy individuals (control) groups.

Ethical consideration: Ethical approval was sought and given by the research and Ethic Committee of ISTH, Irrua while informed consent was obtained from willing participants.

Sample collection: After an informed consent was obtained from subjects, four milliliters of blood samples were collected by venipuncture technique from subjects (both test and control). 2 ml of the sample collected was dispensed into EDTA and fluoride oxalate bottles each, properly swirled to avoid clotting.

Assay: The glucose concentrations of the samples were determined using glucose oxidase-peroxidase (GOPD) method and the haemoglobin concentration were estimated colorimetrically using Drabkin's solution. G-6-PD activity was estimated using enzymatic colourimetric method described by Lohr and Waller, (1974).

Statistical analysis: The data obtained were analysed statistically, the Mean±standard deviation values were calculated in each case. The Students t- test statistical method was employed for comparison using SPSS software package version 16.0. A P-value ($p \leq 0.05$) was considered statistically significant at 95% confidence level.

RESULTS

Table 1 shows the mean glucose concentration of diabetes mellitus (277.33 ± 122.44 mg/dl) and that of its counterpart apparently healthy subjects (67.36 ± 6.61 mg/dl). The mean G-6-PD activity of patients with type 2 diabetes mellitus was lower when compared with the non diabetic control group.

Specifically, the mean G-6-PD activity of patient with type 2 diabetes mellitus was shown to be $2.53 \pm 1.34 \mu\text{g Hb}$ while that of the control subjects (non diabetes group) was $14.44 \pm 3.27 \mu\text{g Hb}$. This

difference in mean G-6-PD activity between type 2 diabetes mellitus group and non-diabetes group (control) was significantly different ($p < 0.05$).

Table 1: Glucose concentration and G-6-PD activity of patients with type 2 diabetes mellitus (test) and apparently healthy subjects (control) groups

Parameters	Control (n=60)	Test (n=60)	t-value	p-value
Glucose (mg/dl)	67.36±6.61	277.33±122.44*	13.22	< 0.05
G-6-PD activity ($\mu\text{g Hb}$)	14.44±3.27	2.53±1.34*	21.89	< 0.05

Results are mean \pm standard deviation; values in a row having different superscript is statistically different ($p \leq 0.05$) from control; G-6-PD = Glucose - 6 - phosphate dehydrogenase

DISCUSSION

Diabetes mellitus is a common and complicated metabolic disorder/disease. Studies indicate that blood glucose and its oxidant derivatives have a key role in the pathogenesis of diabetes mellitus (Haffner et al., 1998). Specifically, the activity of enzyme “glucose-6-phosphate dehydrogenase” (G-6-PD), an antioxidant system, has been reported to be important in preventing the complications of diabetes mellitus (Hamiton et al., 2004).

In the present investigation, the G-6-PD activity in type 2 diabetic patients was significantly lower ($p < 0.05$) compared to the non-diabetic subjects. This finding is in agreement with the reports of Wan et al., (2002) and Gwo-Hwa et al., (2002) who reported significantly lower activity of G-6-PD in type-2 diabetic individuals. Hence, unsuitable control of blood glucose may decrease G-6-PD activity and increase diabetes mellitus complications. This can be attributed to the fact that, G-6-PD deficiency predisposes affected individuals highly susceptible to oxidative stress, which is one of the risk factors for diabetes.

Gupta et al (1997), in a similar survey showed that erythrocytic G-6-PD activity was lower in diabetic rats compared to healthy rats. It was reported that the enzyme activity gradually rose to normal limit after insulin administration (Gupta et al., 1997). The finding of this study was however at variance with the work of Joshi et al. (2001) where they observed a slight increase in the activity of G-6-PD in patients with type-2 diabetes mellitus.

Several surveys have also concluded that hyperglycaemia resulting from resistance to insulin, leads to increase of cAMP. This may activate protein

kinase A, which causes inhibition and phosphorylation of G-6-PD activity and decreases NADPH as reported by Zhang et al., (2002). However, significant increase in G-6-PD mutations in the ketosis prone diabetes (KPD) population has been implicated, suggesting that G-6-PD deficiency alone does not predispose individuals to ketosis-prone diabetes (Mauvais et al., 2004). Therefore, the significant reduction of G-6-PD activity in type 2 diabetic patients may be due to the long episode of hyperglycaemia which was absent in the control subjects.

Diabetic hyperglycaemia may lead to serious complications and decrease G-6-PD activity through glycation process and oxidative stress. This itself aggravates diabetic injury due to inappropriate antioxidation process. Therefore, patients with type 2 diabetes mellitus who are G-6-PD deficient should avoid exposure to foods and drugs that can trigger haemolysis as this may result in haemolytic episodes related to G-6-PD deficiency occurring intermittently.

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AUTHORS' CONTRIBUTIONS

Festus OO., supervised this study with the assistance of Dada FL., Iweka FK., Eyaufe AO., Osagie RN. and Akiyang EE. All authors were involved in the preparation of this article.