Erythrocyte G6PD activity and GSH level as risk factors for vascular complications among type 2 diabetics in Osogbo, Nigeria

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Abstract

Background: Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the oxidation of glucose-6-phosphate (G6P) to produce nicotinamide adenine dinucleotide phosphate coenzyme (NADPH), a major defense against oxidative damage in erythrocytes. This study aims at evaluating enzymatic G6PD activity and erythrocyte glutathione (GSH) as a biomarker of vascular complications among type 2 diabetics.

Methods: Fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), erythrocyte G6PD (eG6PD) activity and erythrocyte GSH (eGSH) level were determined along with measured anthropometrics in 120 known type 2 diabetics (comprising 60 diabetics without vascular complications and 60 diabetics with vascular complications) and 50 age and sex matched apparently healthy non-diabetes individuals recruited for this study.

Results: Result revealed significant reduced eG6PD activity and eGSH levels in type 2 diabetics especially among those with vascular complications as compared to subjects without diabetes, with a slight correlation between eGSH level and eG6PD activity of diabetics with vascular complications.

Conclusion: The study, therefore, advocates measurement of G6PD activity and GSH level in type 2 diabetics to properly monitor the progress of the disease.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD EC 1.1.1.49) enzyme is a rate-limiting/housekeeping cytoplasmic enzyme of the pentose phosphate pathway (PPP) that catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconolactone and concomitant reduction of NADP⁺ to nicotinamide adenine dinucleotide (NADPH), which represents the only source of NADPH in erythrocytes [1]. G6PD is widely distributed in many species ranging from prokaryotes to eukaryotes with sequence identity resembling each other, in humans the G6PD encoding gene is located at the telomeric region of the long arm of the X chromosome (band Xq28) with 13 exons, 12 introns, and length of 18kb [2,3]. The polypeptide sequence of the dimer (sometimes tetramer) enzyme with about 515 amino acids contains conserved region of an octapeptide lysine residue (for enzymatic activity), heptapeptide dinucleotide (for binding activity) and a pentapeptide near the substrate binding site. Mutation of the G6PD gene results in protein variants with different levels of enzyme activity and a wide spectrum of biochemical and clinical phenotypes [4,5].

Traditionally, digested glucose from carbohydrate metabolism is transported into cells from plasma to undergo phosphorylation forming glucose-6-phosphate (G6P) through the hexokinase/glucokinase enzymes. The formed G6P may be used either in glycolysis to produce energy in the form of adenine triphosphate (ATP) and NADH, used to store energy in the form of glycogen, or used by the PPP (otherwise known as the hexose monophosphate shunt) to form ribose-5-phosphate, which is required for nucleic acid (ribose) synthesis, and nicotinamide adenine dinucleotide phosphate (NADPH) generated from NADP by G6PD enzyme. NADPH is the main cellular antioxidant (reductant) defense mechanism found in the body [2].

NADPH, a hydrogen carrier is required by many essential cellular systems like glutathione recycling, nitric oxide synthesis, cytochrome p450 system, and others for proper functioning and there is increasing evidence that G6PD activity is of major importance for NADPH production for defense against oxidative stress rather than for ribose production. Diabetes mellitus, the most common endocrine disease of carbohydrate metabolism is being considered a free radical disease owing to the increase in the production of free radical [6]. Diabetes mellitus causes an increase in metabolic flux of the polyol pathway with increase cellular demand for NADPH by the NADPH-dependent aldose reductase and subsequent deficiency generation of the endogenous
non-enzymatic antioxidant (glutathione) defense mechanisms. Therefore, this study aims at evaluating enzymatic G6PD activity and GSH level as risk factors for the development of vascular complications among type 2 diabetics.

2. Materials and methods

2.1. Subjects

This case-control hospital-based study was conducted at the State Specialist Hospital, Osogbo, Southwest Nigeria, using a simple non-probability sampling technique, the study protocol was in accordance with Helsinki declaration and approved by the Ethics Committee of the Osun State University. One hundred and twenty type 2 diabetics between 30 and 65 years old were recruited from the Hospital diabetes clinic. Type 2 diabetics were those previously diagnosed in the Hospital using WHO standard [7], and classified according to the presence or absence of diabetes vascular complications. Sixty of the type diabetics were with vascular complications (DM+VC), vascular complications were defined as any documented vascular disease (also diagnosed previously in the hospital) at least a year after the diagnosis of type 2 diabetes using standard medical protocols. Diabetes eye complications (retinopathy-31 subjects, cataract-8 subjects) were diagnosed after dilated pupil ophthalmoscopic examination in a darkened room [8], diabetes peripheral neuropathy (14 subjects) was diagnosed based on the presence of two or more signs of paresthesia, absent pinprick, light touch sensation, sense of position and absent tendon reflexes or muscular atrophy. Overt nephropathy (two subjects) was based on the criteria that a patient repeatedly had either a urinary albumin excretion rate of >200 µg/min or a positive urinalysis for protein using a reagent strip [9,10]. Type 2 diabetes ischemic heart disease (three subjects) was indicated based on the standard 12 lead electrocardiogram with the presence of Q wave or of left bundle branch block while, diabetic foot (two subjects) is established by any pathological features of the foot resulting directly from diabetes mellitus. The remaining 60 diabetics were those without any vascular complications (DM-VC) (Figure 1). Exclusion criteria include type 1 diabetics or secondary diabetics, type 2 diabetics using oral hypoglycemics other than biguanides, smokers, recent surgery and acute or chronic infection or undergoing isotope diagnostic or irradiation therapy. The control subjects were 50 apparently healthy subjects recruited among the staff of Osun State University but with similar age and sex distribution to those of the diabetes patients. They were subjected to complete medical examination to exclude the presence of any medical problems. Informed consent was obtained from all the participants before the commencement of the study while, a structured research questionnaire was administered on each subject to collect relevant demographic diabetes information. In addition, anthropometric data to calculate body mass index (BMI) and blood pressure were collected using standardized methods. The clinical characteristics of the diabetics and control subjects are as indicated in Table 1.

Table 1. Demographic and laboratory data of the study population.

| Parameters       | Controls | Type 2 diabetics | DM-VC | DM+VC |
|------------------|----------|------------------|-------|-------|
| Sex (♂ / ♀)      | 20/30    | 45/75            | 25/35 | 20/40 |
| Age (years)      | 54.16 ± 9.61 | 53.38 ± 9.38    | 53.97 ± 9.71 | 53.35 ± 8.41 |
| DD (years)       | NA       | 4.55 ± 2.91      | 4.43 ± 2.55 | 6.67 ± 2.84 |
| Family History with DM | 5 [10]  | 67 (55.83)       | 35 (58.33) | 32 (53.33) |
| BMI (kg/m²)      | 20.91 ± 0.85 | 25.71 ± 4.13    | 24.86 ± 3.43 | 26.56 ± 4.61 |
| Obese (%)        | NA       | 13 (10.83)       | 5 (8.33)    | 8 (13.33) |
| Overweight (%)   | NA       | 47 (39.17)       | 20 (33.33) | 27 (45) |
| Normal weight (%)| 50 (100) | 60 (50)          | 35 (58.34) | 25 (41.67) |
| SBP (mmHg)       | 117.00 ± 13.48 | 130.62 ± 8.51   | 130.65 ± 8.71 | 130.58 ± 8.38 |
| DBP (mmHg)       | 83.70 ± 4.77  | 88.93 ± 5.22     | 87.73 ± 5.16 | 90.13 ± 5.05 |
| Hypertensive (%) | NA       | 13(10.83)        | 4 (6.67)    | 9 (15) |
| G6PD deficiency (%) | 3 [6]  | 7 (5.83)         | 4 (6.67)    | 3 [5] |

Result expressed as mean, standard deviation and number (percentage)

DD = Diabetes duration
BMI = Body mass index
SBP = Systolic blood pressure
DBP = Diastolic blood pressure

Figure 1. Patient distribution in the diabetes group.
2.2. Blood samples and laboratory analysis

Fasting blood sample was obtained by venipuncture into fluoride oxalate (2 mL) and heparinized (3 mL) tubes for the biochemical estimation of fasting plasma glucose. FPG estimated using enzymatic glucose oxidase/hydrolysis method, glycosylated hemoglobin-HbA1c using ion exchange resin method. Methemoglobin reduction test as described by Amiweru and Olatunji [11] was used to screen all subjects for G6PD deficiency. Erythrocyte Glucose-6-Phosphate dehydrogenase (G6PD) activity was determined based on the enzyme ability to reduces NADP to NADPH, and the rate of reduction of NADP⁺ was measured at 340nm while erythrocyte glutathione was determined using modified method of Chakrabarty et al. [12] where glutathione in the protein-free red cell lysates was made to react with DTNB solution and the complex formed read at 412nm. Plasma total antioxidant capacity (pTAC), was also measured on the basis of the ability of plasma antioxidants plasma to reduce Fe²⁺-TPTZ to Fe³⁺-TPTZ [13].

2.3. Statistical analysis

Normal distribution was analyzed with Kolmogorov Smirnov test while Kruskal–Wallis test was used if parameters did not follow normal distribution. Differences between the groups were tested with t-test for independent variables or Mann-Whitney-U test for nonparametric variables. Pairwise comparisons between multiple groups were analyzed with one-way analysis of variance (ANOVA). Correlations were analyzed with Pearson’s correlation coefficient or Spearman correlation for nonparametric variables. Significance was assumed at p < 0.05. All statistical analyses were performed using Graph-Pad prism version 8 software.

3. Result

The study group consisted of 120 patients (mean age: 53.38 ± 9.38 years), DM-VC group were 60 individuals (mean age: 53.97 ± 9.71 years) and DM+VC group were also 60 subjects (mean age: 53.38 ± 8.41 years). The mean diabetes duration was 4.55 ± 2.91 years in all patients as compared to control subjects (4.69 ± 0.71 years). DM+VC group had significantly higher frequency of systemic hypertension and significantly higher levels of systolic and diastolic blood pressure and plasma glucose than the control subjects. The Mean FPG and HbA1c in type 2 diabetics were 6.92 ± 1.74mM and 7.43 ± 1.49%, respectively, as compared to control subjects (4.69 ± 0.71mM and 4.96 ± 0.69%) were significantly (p < 0.05) high, indicating poor glycemic control among diabetics (Table 1). All the G6PD-deficient subjects had undetectable levels of enzymatic red blood cell activity (total deficiency) and none of the subjects (either control or diabetics) with G6PD deficiency had previously been treated for G6PD enzyme deficiency in the past 2 years before their enrollment in this study.

The DM-VC and DM+VC had higher FPG and HbA1c levels of 5.38 ± 0.38mM, 6.16 ± 0.53% and 8.46 ± 0.45mM, 8.69 ± 0.68%, respectively, than the control group (4.69 ± 0.71mM, 4.96 ± 0.69%). However, no significant difference (p > 0.05) was recorded in the FPG of DM-VC group compared to the control individuals, but a significant (p < 0.05) two-fold rise in the FPG level of the DM+VC group was recorded compared to the normal individuals. Therefore, the DM+VC and DM-VC were considered to be under poor (PDC) and good (GDC) glycemic diabetes control, respectively, at the time of the experiment. Conversely, the level of HbA1c was significantly (p < 0.05) increased in DM-VC and DM+VC diabetic groups (Table 2).

The eG6PD enzymatic activity, eGSH and pTAC levels of type 2 diabetics (6.18 ± 1.85mU/gHb, 47.64 ± 18.34mg/gHb and 0.76 ± 0.24mM) respectively, were significantly (p < 0.05) higher than control (8.28 ± 0.57mU/gHb, 66.23 ± 3.55mg/gHb and 1.48 ± 0.33mM) group. eG6PD activity, eGSH and pTAC levels of DM+VC (GPG) group (4.52 ± 0.88 mU/gHb, 29.66 ± 3.42mg/gHb and 0.64 ± 0.22mM) were significantly (p < 0.05) decreased two-folds compared to the control group. The DM-VC (GGC) group also has a statistically (p < 0.05) similar but reduced eG6PD activity, eGSH and pTAC levels of DM+VC (PGC) group (4.52 ± 0.88 mU/gHb, 29.66 ± 3.42mg/gHb and 0.64 ± 0.22mM) respectively, as compared to control subjects (4.69 ± 0.71mM and 4.96 ± 0.69%).

Table 2. Biochemical levels of glycemic control, G6PD activity, GSH and TAC levels of the study population.

| Group          | FPG (mmol/L) | HbA1c (%) | G6PD activity (mU/gHb) | GSH (mg/gHb) | TAC (mM) |
|----------------|--------------|-----------|------------------------|--------------|----------|
| Control subjects | 4.69 ± 0.71a | 4.96 ± 0.69a | 8.39 ± 0.61a | 66.23 ± 3.55a | 1.48 ± 0.33a |
| Type 2 Diabetics | 6.92 ± 1.74a | 7.43 ± 1.49b | 6.18 ± 1.85b | 47.64 ± 18.34b | 0.76 ± 0.22b |
| DM -VC         | 5.37 ± 0.59c | 6.16 ± 0.53c | 7.88 ± 0.60c | 65.61 ± 3.15c | 0.89 ± 0.18c |
| DM +VC         | 8.46 ± 0.45d | 8.69 ± 0.96d | 4.52 ± 0.88d | 29.66 ± 3.24d | 0.64 ± 0.22d |

Result expressed as mean ± standard deviation

Values with different subscripts (a, b, c, d) down a column are significantly different from each other (p < 0.05).

DM-VC and DM+VC denote diabetic group without vascular and with vascular complications, respectively.
Figure 2. G6PD activity as a function of GSH level in plasma of DM-VC group.

4. Discussion

In the present investigation, the overall G6PD deficiency prevalence from our study was 5.88%, a value lower to previously reported deficiency among adults in our locality [14]. Diabetic patients from our study have 5.83% deficiency prevalence [of these, three (5.0%) were from diabetics with vascular complications and four (6.67%) were from diabetics without vascular complications] while control subjects have 6% deficiency prevalence, a lower prevalence value as compared to Engwa et al. [15], who did similar prevalence study of G6PD deficiency among type 2 diabetics.

This study also revealed that the mean enzymatic activity of G6PD is significantly lower in diabetics than nondiabetes subjects and also significantly lowered in diabetics with vascular complications than those without vascular complications. The reason for the decreased activity of the enzyme might be due to the reported hyperglycemia which causes activation of protein kinase-A and subsequent phosphorylation of G6PD enzyme thereby lowering the enzyme activity [1,6,16–19].

Likewise, our study revealed a reduced red cell glutathione level in type 2 diabetics as compared to control subjects. The decreased level is more pronounced among type 2 diabetics with vascular complications than those without vascular complications, supporting the hypothesis of Likidlilid et al. [20], who stated that the decreased red cell glutathione may be due to high rate of oxidative stress in diabetics caused by reduced pentose phosphate pathway activity or increased glutathione peroxidase activity as glutathione system prevents oxidative stress derangement in erythrocytes. This study however, in agreement with Bozzi et al [21], who showed a link between low G6PD enzymatic activities leading to an inefficient NADPH generating system and a decrease level of reduced glutathione which may become critical upon oxidative stress moreover, evidence from both in-vivo and in-vitro studies had shown that glutathione depletion enhances NADPH-dependent lipid peroxidation in diabetics.

5. Conclusion

The novelty of this study was the evaluation of G6PD activity and glutathione level in diabetics with and without vascular complications. Uncontrolled hyperglycemia in diabetes leads to vascular complications with concomitant decrease in G6PD enzyme activity and reduced glutathione level. Evaluation of G6PD activity and glutathione level can, therefore, predict diabetes injury due to inappropriate oxidation/anti-oxidation process in type 2 diabetics. We, therefore, advocate large-scale longitudinal study to validate the result of this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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