Effect of Combined G6PD Deficiency and Diabetes on Protein Oxidation and Lipid Peroxidation.

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Abstract

Oxidative Stress, an imbalance in the pro oxidant / antioxidant homeostasis occurs by a large number of physiological and nonphysiological processes and in several human diseases including diabetes.

The use of protein carbonyl protein (PC) and malanodialdehyde (MDA) as biomarkers of oxidative stress are the most widely measured markers for protein and lipid oxidation. These are measured by spectrophotometric assay. the aim of the study is to measure the effect of G6PD – deficiency on the oxidative markers and the antioxidant GSH in diabetic and non diabetic individuals because of the high incidence of G6PD – deficiency in Jordan and many parts of the world

The results show that diabetes and/or G6PD deficiency are positively connected to protein and lipid oxidation. The effect is additive in protein oxidation when both disorders are present. The antioxidant, GSH level, is not affected by diabetes but reduced to 50% by G6PD deficiency.

Background

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary disorder in humans, found mainly in people of Mediterranean, Southeast Asian and African descent and affecting over 400 million people (1).G6PD deficiency is an X-linked disorder caused mostly by diverse point mutations. Although few G6PD variants cause chronic hemolysis, the most common clinical manifestation of its deficiency is an oxidative stress-induced hemolytic anemia, caused by certain drugs and chemicals, infections or ingestion of fava beans (Vicia faba) (1).

More than 450 million people worldwide have diabetes mellitus (DM), the vast majority having type 2 DM. This condition is now defined as a group of metabolic disorders characterized by hyperglycemia resulting from defects in action and / or secretion of insulin. Hyperglycemia is one of the most important causes of oxidative stress and the production of oxidants in diabetes. Increased oxidative stress has been suggested to contribute to the pathogenesis and development of diabetic complications (2).

Reactive oxygen species (ROS), including free radicals, are highly reactive molecules generated by the redox reactions that occur as part of normal aerobic cell metabolism and exposure to certain environmental factors. The imbalance between production and scavenging of free radicals due to an increase in the oxidative flux or a decrease in the antioxidant ability is responsible for cellular and tissue damage in several chronic and acute diseases, including cardiovascular disease, DM, cancer, rheumatoid arthritis, Alzheimer disease, other neurodegenerative disorders, stroke, sepsis and others. The resulting oxidative stress damages cellular components such as proteins, lipids, and nucleic acids (3). For instance, ROS attack against proteins and lipids modifies amino acid residues generating protein carbonyl (PC) and induces lipid peroxidation generating malondialdehyde (MDA). Both PC and MDA are considered biomarkers for protein and lipid damage, respectively (3, 4). Additionally, determining the fate of low molecular antioxidants like reduced glutathione (GSH), is also an important an indicator of oxidative damage (4).
In Jordan, the incidence of G6PD deficiency among normal male adults is about 5 to 10% (5). Six different missense mutations were found with a frequency of G6PD Mediterranean about 55% (5). G6PD deficiency is a public health issue in Jordan because of the hemolytic crisis after ingesting a popular seasonal food fava beans (5). In addition, the prevalence of DM (17.1%) and impaired fasting glycemia (7.8%) is high in Jordan and is increasing (6). Since the role of G6PD deficiency in the pathogenesis of DM is not truly clear, it is of high concern to study the combined effect of G6PD deficiency in diabetic subjects in a population that has a high incidence of both. Therefore, the present study aimed to investigate G6PD deficiency’s effect on the levels of oxidative markers and antioxidants in individuals with diabetes and non-diabetics.

**Methods**

Blood samples were collected from diabetic and non-diabetic patients with sufficient or deficient G6PD level at the University of Jordan Hospital and the National Center for Diabetes, after obtaining informed consent. The study protocol was approved by the Ethics Committee and the Deanship of Academic Research of the University of Jordan.

Fluorescent spot screening for G6PD deficiency was carried on the blood samples of all individuals (7). Quantitative G6PD assays were performed spectrophotometrically for all G6PD-deficient and normal subjects (7). G6PD levels in G6PD-deficient subjects of both, diabetics and non-diabetic, were severely deficient with a mean equal to 5–10% of the normal level.

MDA as a marker for lipid peroxidation, was measured in plasma colorometrically by its reaction with thiobarbituric acid by a modification of the method of Nichans and Samualson (8). PC as a marker of protein oxidation, was measured in the plasma by a 2,4 dinitrophenyl hydrazine (DPNH) spectrophotometric assay (9). GSH as an antioxidant marker, was measured in whole blood by the spectrophotometric assay method which involves oxidation of GSH by the sulfhydryl reagent 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm (7).

**Results**

We observed a 5.5 to 6-fold increase in PC levels in patients with diabetes and in G6PD-deficient subjects more than in non-diabetic subjects with sufficient G6PD subjects \((p < 0.001)\). This fold increase was doubled in diabetic patients with G6PD deficiency and was significantly higher than in G6PD-deficient and diabetics with sufficient G6PD \((p < 0.001)\) (Table 1). Furthermore, the MDA level was significantly increased by 28–41% in the G6PD-deficient, diabetics with sufficient G6PD, and diabetics with G6PD deficiency more than in non-diabetic and G6PD-sufficient subjects. On the other hand, GSH was significantly reduced to half in G6PD-deficient subjects and in diabetics with G6PD-deficiency. GSH level in people with diabetes was equivalent to non-diabetic with sufficient G6PD (Table 1).
Table 1
Level of MDA, PC, and GSH in G6PD-deficient, diabetics with deficient G6PD, diabetics with sufficient G6PD and non-diabetics with sufficient G6PD

| Subjects                           | n   | MDA (nmol/ml) | PC (nmol/mg protein) | GSH (µmol/g Hb) |
|------------------------------------|-----|---------------|----------------------|-----------------|
| G6PD-deficient                    | 14  | 9.33 ± 1.40*  | 3.50 ± 0.95*         | 3.19 ± 1.88*    |
| Diabetics with deficient G6PD     | 12  | 10.05 ± 0.73* | 6.53 ± 0.71*         | 2.72 ± 0.35*    |
| Diabetics with sufficient G6PD    | 68  | 9.13 ± 1.88*  | 3.92 ± 0.95*         | 5.85 ± 0.98     |
| Non-Diabetics with sufficient G6PD| 30  | 7.11 ± 1.07   | 0.64 ± 0.15          | 6.00 ± 0.40     |

Values are in mean ± SD *p < 0.001 when compared to non-diabetic with sufficient G6PD subjects

* p < 0.001 when compared to G6PD-deficient and diabetics with sufficient G6PD subjects

MDA, malondialdehyde; PC, protein carbonyl; GSH, reduced glutathione; G6PD, glucose-6-phosphate dehydrogenase

Discussion

The balance between ROS and antioxidants determines the oxidative status of cells. Although GSH level is not reduced in diabetic subjects since it is likely to be maintained by the NADPH supplied by the normal G6PD, hyperglycemia can promote ROS accumulation through activation of multiple metabolic pathways: 1) increased flux of glucose through the polyol pathway, 2) increased formation of advanced glycation end products (AGEs), 3) activation of protein kinase C (PKC), and 4) increased hexosamine pathway flux (2). These activated biochemical pathways generate excess ROS leading to oxidative stress, and protein and lipid damage, as shown by the nearly 6-fold increase and 28% increase in PC and MDA levels respectively.

The pentose phosphate pathway is the only source of NAPDH in the erythrocyte. Therefore, G6PD-deficient erythrocytes are unable to maintain GSH to protect sulfhydryl groups against oxidative damage. Theoretically, G6PD deficiency should be a disadvantage in patients with diabetes since NADPH is essential to restore the antioxidant GSH. Furthermore, NADPH is a necessary co-factor for the synthesis of NO, a potent vasodilator with anti atherogenic effects. On the other hand, G6PD deficiency can benefit people with diabetes because decreased NADPH supply may damp aldose reductase. The latter is the first step in the polyol pathway which limiting the excess polyols in patients with diabetes and therefore lowers vascular damage, and also accounts for decreased cholesterol synthesis and a favorable lipid profile (3, 10, 11).

ROS contribute to pathogenesis of several hereditary disorders of erythrocytes, including sickle cell anemia (SCA), thalassemia, and G6PD deficiency (4, 10). Increased oxidative stress in G6PD deficient
erythrocyte is documented because of the limited supply of the reducing power NADPH (4). Factors responsible for the oxidative stress in thalassemia and SCA are Hb-instability and excess iron. In thalassemia excess α- or β-chains are oxidized to metHb and superoxide ion which is converted to hydroxyl free radical in presence of released free iron. In SCA, met-HbS is produced at higher rate and less stable than metHbA producing ROS (4). In thalassemia, the increase in carbonyl groups was 7-fold (4) as high as in G6PD deficiency (Table 1). Therefore, the coinheritance of G6PD deficiency and DM is expected to aggravate each other as it was observed that patients having both disorders have a poorer prognosis (10, 11). Our data support these clinical findings, as shown by the additive damage in protein oxidation and the decrease in GSH level in DM with G6PD deficiency (Table 1). Some studies also showed that the clinical impact of G6PD deficiency in patients with SCA could also worsen hemolysis in SCA patients, while other studies showed no influence (12). However, it is imperative to evaluate G6PD status in the patients with DM or SCA to avoid oxidative medications such as the hypoglycemic drug glibenclamide and the anti-malaria drug primaquine, which might predispose persons to severe hemolysis. It will also be interesting to test if the increase in the PC level correlates with the severity of diabetes, especially for G6PD-deficient subjects and to monitor the efficacy of antioxidants supplementation in controlling the PC level and finally the complications.

Conclusion

The results show that diabetes and/or G6PD deficiency are connected with protein oxidation and lipid peroxidation. The effect is additive when both disorders are present together in case of protein oxidation. The antioxidant, GSH level is not affected by diabetes alone but reduced to 50% by G6PD deficiency

Declarations

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Authors’ Contributions

All authors contributed, reviewed and approved the final version.

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