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Decreased Blood Activity of Glucose-6-Phosphate Dehydrogenase Associates with Increased Risk for Diabetes Mellitus

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency predisposes affected individuals highly susceptible to oxidative stress, which is one of the risk factors for diabetes. To evaluate the relationship between blood level of G6PD activity and diabetes in Taiwan, blood G6PD activity was analyzed among 237 patients with diabetes and 656 healthy subjects. A significant difference in the distribution of G6PD activities as grouped by an increment of 100 U/10^{12} red blood cells (RBCs) was observed between diabetic patients and healthy subjects. The odds ratio for diabetes was 1.46 (95% confidence interval = 1.11–1.92) for every decrement of 100 U/10^{12} RBC G6PD activities in these subjects. The data indicate that low G6PD activity is another risk factor for diabetes.

Key Words: Oxidative stress; antioxidants; glucose-6-phosphate dehydrogenase; G6PD deficiency; diabetes.

Introduction

Enhanced oxidative damage may play an important role in a variety of many common degenerative disorders such as cancer, diabetes, cataracts, and cardiovascular diseases *(1–5)*. Radical formation in biologic systems mostly involves the reduction of molecular oxygen leading to the formation of highly reactive oxygen species, such as superoxide anion, hydroxyl radical, hydrogen peroxide, hypochlorous acid, and nitric oxide. These radicals can react and damage many biomolecules in a number of ways.

Diabetes mellitus is a common disease with multiple etiologic factors. Oxidative damage has been suggested as one of the factors to play a major role in the pathogenesis of this disease *(1,2)*. Oxidative stress may act as a common pathway for diabetes and its later complication *(1)*. Increased release of free radicals and decreased antioxidants may be responsible for an increased lipid peroxidation in poorly controlled diabetic subjects *(6)*, as well as contribute to the onset of diabetic complication *(2,7)*.

Glucose-6-phosphate dehydrogenase (G6PD)–deficient individuals are under high oxidative stress *(8–10)* because the major biochemical function of G6PD is to generate NADPH, the essential reducing equivalent protecting cells against oxidative attack. For example, G6PD-deficient erythrocytes are more susceptible to free radical–induced oxidative damage leading to a dramatic decrease in their deformability than normal erythrocytes *(10)*. Such increased oxidative stress has led to a decrease in antioxidant capability including plasma level of vitamins C and E in G6PD-deficient individuals *(11)*.

It seems clear that G6PD deficiency predisposes affected individuals highly susceptible to oxidative stress and oxidative stress, is a risk factor for diabetes. However, the relationship between diabetes and G6PD activity in blood is not very clear. Several studies showed a positive association between G6PD deficiency and diabetes mellitus, suggesting that G6PD-deficient individuals should be more susceptible to become diabetic *(12–14)*. In addition, individuals with G6PD deficiency had an abnormal glucose tolerance test that was attributed to a failure to secrete adequate insulin in response to the glucose load *(15)*. By contrast, there was no statistically significant difference in the prevalence of G6PD deficiency between male subjects with non-insulin-dependent diabetes mellitus (NIDDM) (7.63%) and the normal male population (7.29%) *(16)*.

There is a high prevalence of G6PD deficiency in malaria-frequent regions, including Africa, southern China, Southeast Asia, and southern Europe. Since G6PD deficiency affects more than 200 million people worldwide and the relationship between G6PD deficiency and diabetes is not crystal clear *(12–16)*, a major objective of the current study was to prove our hypothesis that G6PD deficiency is one of the underlying causes of diabetes. To test this hypothesis, blood G6PD activity was determined in patients with diabetes in Taiwan, a subtropical island. In addition, the defect in the G6PD gene was determined in diabetic patients with low G6PD activity. Such an approach should help to elucidate the relationship between G6PD activity and diabetes because it has been suggested that diverse point mutations

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Table 1
Distribution of G6PD Activities in Diabetic and Healthy Subjects

G6PD activity ($U/10^{12}$ RBCs)[a]	Diabetic subjects			Control subjects		
	Total (%) (n = 237)	Male (%) (n = 136)	Female (%) (n = 101)	Total (%) (n = 656)	Male (%) (n = 522)	Female (%) (n = 134)
≤100	13 (5.5)	10 (7.4)	3 (3.0)	32 (4.9)	32 (6.1)	0 (0)
101–200	14 (5.9)	11 (8.1)	3 (3.0)	39 (5.9)	34 (6.5)	5 (3.7)
201–300	160 (67.5)	87 (64.0)	73 (72.3)	372 (56.7)	290 (55.6)	82 (61.2)
301–400	44 (18.6)	26 (19.1)	18 (17.8)	180 (27.4)	143 (27.4)	37 (27.6)
≥401	6 (2.5)	2 (1.5)	4 (4.0)	33 (5.0)	23 (4.4)	10 (7.5)
Range		5.2–508.9			0–560.9	
Mean (SD)[b]		254.7 (77.4)			270.9 (83.7)	

[a] χ^2 test for diabetic vs control subjects, $p < 0.05$.
[b] Student's t-test for diabetic vs control subjects, $p < 0.01$.

Table 2
Distribution of Gene Mutations in Diabetic and Healthy Subjects

G6PD gene mutation (cDNA number)	Diabetic subjects (%) (n = 10)	Control subjects (%) (n = 27)
95 A to G	0 (0)	2 (7.4)
392 G to T	0 (0)	0 (0)
493 A to G	0 (0)	1 (3.7)
1024 C to T	0 (0)	3 (11.1)
1360 C to T	0 (0)	1 (3.7)
1376 G to T	5 (50.0)	14 (51.9)
1388 G to A	5 (50.0)	6 (22.2)

might cause phenotypic heterogeneity in G6PD deficiency *(17–22)*. Moreover, a questionnaire-based study on daily activities in patients with diabetes was conducted to further evaluate the association between G6PD activity and diabetes.

Results

The prevalences of G6PD deficiency (using <100 $U/10^{12}$ red blood cells [RBCs] as a cutoff point) among males and females with diabetes were compared with those of healthy control subjects (Table 1). In comparing male samples, the prevalence in diabetes was not statistically different from that in control subjects. The prevalence of G6PD deficiency for females was higher for diabetes (3.0%) than for control subjects (0%) but was not statistically significant ($p = 0.069$). Moreover, the mean G6PD activity among diabetic patients (254.7 $U/10^{12}$ RBCs) was significantly ($p = 0.009$) lower than that of healthy subjects (270.9 $U/10^{12}$ RBCs). In addition, a slight but significant statistical difference in the distribution of G6PD activities as grouped by a decrement of 100 $U/10^{12}$ RBCs was observed between diabetic patients and healthy subjects using the χ^2 test ($p = 0.022$), as shown in Table 1.

Among individuals with low G6PD activity (<100 $U/10^{12}$ RBCs), the nucleotide substitution at cDNA 1376 G to T accounted for 50% of G6PD-deficient subjects in the diabetes group (Table 2). This frequency was similar to that found in the healthy population with low G6PD activity. However, the other predominant form of nucleotide substitution of G6PD mutation found in the diabetic group was cDNA 1388 G to A (50.0%), and such a frequency was not different from that found in the healthy population (22.2%) ($p = 0.481$).

A total of 893 individuals (237 diabetic patients and 656 healthy control subjects) completed the questionnaire (100% response rate). There were significant differences in age, gender, and education between diabetic and healthy individuals ($p = 0.000$) (Table 3). The intake contents of vitamins (A, C, E) with antioxidant capability for both diabetic and control subjects are given in Table 4. The intake levels (≥2 d/wk) of vitamins A, C, and E were significantly higher for control subjects (5.4%) than for diabetic patients (0.5%) ($p = 0.000$). In addition, patients with diabetes consumed less (<2 d/wk) ($p = 0.049$) vegetables and fruits and exercised less frequently ($p = 0.001$) than healthy control subjects.

In the logistic models that estimated the associations between G6PD levels and diabetes, important factors such as age, gender, education, intake of vitamins (A, C, E) and vegetables/fruits, drinking behavior, and exercise were adjusted. Such a model demonstrates a strong association between G6PD activity and increased risk for diabetes. Table 5 shows that for every decrement of 100 $U/10^{12}$ RBC G6PD activities, an increased risk of 1.46 (odds ratio [OR]) (95% confidence interval [CI] = 1.11, 1.92) was observed ($p = 0.006$).

Discussion

Diabetes is a widespread disease with multiple etiologic factors. In the current study, decreased G6PD activity was identified as another risk factor for the pathogenesis of diabetes in Taiwan. A multiple logistic regression clearly indicated

Table 3
Characteristics of Study Participants

Host characteristic	Frequency (%) Diabetic subjects (n =237)	Control subjects (n =656)
Age (yr)[a]		
<50	0	0.6
50–54	21.1	32.0
55–59	21.1	29.2
60–64	18.1	21.2
65–69	19.8	11.2
≥70	19.8	5.8
Gender[a]		
Male	57.4	79.7
Female	42.6	20.3
Education[a]	15.35	32.55
≥High school	15.3	32.5
<High school	84.7	67.5
Marital status		
Single	0.5	1.7
Married	88.7	88.1
Other	10.8	10.2
Nationality of mother		
Chinese	4.0	5.1
Taiwanese	76.2	69.4
Hakka	18.3	24.6
Aborigines	1.5	1.0
Nationality of father		
Chinese	4.5	4.7
Taiwanese	76.7	69.4
Hakka	17.3	24.9
Aborigines	1.5	1.0

[a] χ^2 test for diabetic vs control subjects, $p < 0.01$.

Table 4
Percentage Distribution of Supplement Use and Lifestyle of Study Participants

Supplement/lifestyle	Frequency (%)[a] Diabetic subjects	Control subjects
Intake of vitamins (A, C, E)[b]		
Always	0.5	5.4
Seldom	3.0	13.8
None	96.5	80.8
Intake of vegetables and fruits[c]		
Always	89.2	93.6
Seldom	9.4	6.4
None	1.5	0
Smoking status		
Yes	14.8	18.2
Quit	6.4	7.4
No	78.8	74.4
Drinking behavior[b]		
Yes	10.8	22.6
Quit	4.4	3.0
No	84.7	74.4
Exercise[b]		
Always	0	0
Seldom	61.2	76.0
None	38.8	24.0

[a] Frequency = "always" if the answer was >2 d/wk, "seldom" if the answer was <2 d/wk, and "none" if the answer was 0 d/wk.
[b] χ^2 test for diabetic vs control subjects, $p < 0.01$.
[c] χ^2 test for diabetic vs control subjects, $p < 0.05$.

that low levels of G6PD activity were associated significantly with diabetes (OR = 1.46; $p = 0.006$). In this multiple logistic regression, independent variables including age, gender, education, intake of vitamins (A, C, E) and vegetables/fruits, drinking behavior, and exercise were all taken into consideration.

The prevalence of G6PD deficiency was not significantly different between male and female patients with diabetes and healthy control subjects. However, the observed differences in G6PD deficiency between females with diabetes (3%) and healthy females (0%) might be in part attributed to the regulation of endocrine or other sex-linked functions. The interrelationship between G6PD deficiency and diabetes among females deserves further attention.

Our study adds support to the notion that G6PD deficiency does predispose affected individuals to certain degenerative diseases such as diabetes *(12–14)*. However, this notion is not without challenge. In contrast to our findings, a recent study from Taipei demonstrated that there is no association between G6PD deficiency and diabetes *(23)*. Such a discrep-

Table 5
Adjusted ORs and 95% CIs for Associations Between Diabetes and Personal Characteristics/Lifestyle

Risk factor	Diabetes OR	95% CI of OR
G6PD activity (per 100 U/10^{12} RBC decrement)	1.46[c]	1.11–1.92
Age (per 5-yr increment)	1.49[c]	1.27–1.73
Gender (female/male)	0.83	0.53–1.31
Education (≥high school/<high school)	0.55[d]	0.32–0.94
Intake of vitamins (A, C, E)[a]	0.28[c]	0.14–0.56
Intake of vegetables and fruits[a]	0.65	0.31–1.35
Drinking behavior[b]	0.67[d]	0.50–0.91
Exercise[a]	0.64[d]	0.41–0.99

[a] Baseline = none.
[b] Baseline = no drinking.
[c] $p < 0.01$.
[d] $p < 0.05$.

ancy could be attributed to (1) difference in study population because many people living in Taipei originated from mainland China whereas most of those living in the Tao-Yuan area were native Taiwanese; and (2) difference in lifestyle such as the eating of betel nuts, which increase oxidative stress *(24–26)*, because many people in the Tao-Yuan area eat betel nuts, whereas very few people in Taipei city have such a habit.

Another important factor that could account for the discrepancy between the Taipei study *(23)* and our current findings is the difference in dietary habit such as the supplementation of antioxidants. It is more common for people living in Taipei to take antioxidant supplementation than those living in the Tao-Yuan area. In our study, both blood G6PD activity and intake of antioxidant were found to be risk factors for diabetes. It has been reported that people with lower intake of antioxidants have a higher risk for diabetes *(6)*. Taken together with our previous finding that G6PD-deficient individuals have lower antioxidant capability *(11)*, these findings support our hypothesis that G6PD deficiency alone or with an additional factor(s) will render affected individuals highly susceptible to developing certain degenerative disorders such as diabetes.

Clinical and biochemical analyses have identified nearly 400 putative variants of G6PD *(20)*. However, minimal data about the associations between G6PD gene variants and degenerative diseases have been available. In our study, we observed that the main form of G6PD gene mutation in patients with diabetes was cDNA 1376 G to T (50.0%). This is consistent with the high frequency of this mutation found in the general population of Taiwan *(27)*. By contrast, our study also showed that the percentage distribution of G6PD gene mutation, cDNA 1388 G to A, in the diabetic group (50.0%) was higher than that in healthy subjects (22.2%). This might be related to DM-related gene transmitted from the ancestor of G6PD-deficient subjects.

In Taiwan, a subtropical region with a high prevalence of G6PD deficiency (3% among all males), it would be important to find out more about the relationship between G6PD deficiency and degenerative disorders such as diabetes. Such an approach would help to provide ideal medical care policy such as supplementation with antioxidants for individuals with G6PD deficiency.

Materials and Methods

Study Population

Subjects were recruited from the medical outpatient department of Chang Gung Memorial Hospital, including patients with a history of NIDDM for more than 1 yr after being diagnosed at the hospital. The study was designed to have one hospital-based control group, which consisted of individuals with no history of diabetes from the physical checkup department at the Chang Gung Memorial Hospital. Individuals with cataracts, hypertension, and other cardiovascular diseases were excluded from the study. All individuals were ages 45–75 yr and provided informed consent to complete a questionnaire.

Questionnaires

From September 1999 to May 2000, the self-administered questionnaires were completed by 237 diabetic subjects and 656 healthy subjects. A total of 893 subjects completed questionnaires in which they indicated personal characteristics (i.e., age, gender, education, marital status, sub-ethnic origin of father/mother), intake of vitamins (including vitamin A, C, E) and vegetables/fruits, smoking status, drinking behavior, and exercise during the year preceding the survey. We defined the frequency categories as follows: frequency = always if the answer was >2 d/wk, frequency = seldom if the answer was <2 d/wk, and frequency = none if the answer was 0 d/wk.

Blood Sample Collection

After obtaining informed consent at the questionnaire interview, blood specimens were collected from diabetic individuals and control subjects of Chinese origin residing in Taiwan. A sample of venous blood was taken from every patient to test for G6PD activity level *(19)*. All blood samples were stored at 4ºC for not longer than 5 d until measurement of G6PD activity. In patients with G6PD deficiency, DNA analysis was performed to identify the nature of molecular defect *(21,28)*.

G6PD Activity Assay

G6PD activity in fresh RBCs was quantitatively measured using kit no. 345-B (Sigma, St. Louis, MO) as previously described *(19)*. The chemicals in the reagent kit include oxidized $NADP^+$, maleimide (to inhibit 6-phosphogluconate dehydrogenase), and lysing buffer. The activity of G6PD can be quantitated spectrophotometrically by colorimeter at an absorbance of 340 nm.

Genomic DNA and Sequencing Analysis

Genomic DNA was extracted from lymphocyte nuclei by the method of Kunkel et al *(28)*. Molecular defect of G6PD deficiency was determined using the amplification-created restriction site method first described by Chang et al. *(27)*.

Statistical Analyses

All data were analyzed with the Statistical Analysis System statistical package (version 6.12; SAS, Cary, NC). The significance level was 0.05. Student's *t*-test and χ^2 test were used to make comparisons between diabetic and healthy individuals with respect to G6PD distribution, personal characteristics, and daily activities. Multiple logistic regression models were constructed to estimate the strength of associations between diabetes and G6PD deficiency. When ORs were used to estimate associations between G6PD activity and diabetes, a 100 U/10^{12} RBCs decreased for G6PD activity

level was used. Additionally, age, gender, education, intake of vitamins (A, C, E) and vegetables/fruits, drinking behavior, and exercise were adjusted in the logistic regressions.

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