Engwa et al., J Diabetes Metab 2018, 9:8
DOI: 10.4172/2155-6156.1000800

Journal of Diabetes and Metabolism

Research Article Open Access

Relationship between Type 2 Diabetes and Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency and Their Effect on Oxidative Stress

Godwill Azeh Engwa[1,6*], Friday Nweke Nwalo[2], Gregory Eze Chibuzor[1], Endaline Chiamaka Ejiagha[1], Micheal Chinweuba Abonyi[3], Theophilus Ejiofor Ugwu[3], Kenneth Ikechukwu Obiudu[1], Kingsley Nwonu Agbafor[4], Opeolu Oyejide Ojo[5] and Benjamin Ewa Ubi[6]

[1]Department of Chemical Sciences, Godfrey Okoye University, Thinkers Corner, Enugu P.M.B 01014, Nigeria

[2]Department of Biotechnology, Federal University, Ndufu-Alike Ikwo (FUNAI), Abakaliki P.M.B. 1010, Nigeria

[3]Department of Internal Medicine, College of Medicine, Enugu State University Teaching Hospital (ESUTH), Enugu, Nigeria

[4]Department of Biochemistry, Ebonyi State University, P.M.B 53 Abakaliki, Nigeria

[5]Diabetes Research Group, Department of Biology, Chemistry and Forensic Science, School of Science, Faculty of Science, University of Wolverhampton,Wulfruna Street, WV1 1LY Wolverhampton, UK

[6]Department of Biotechnology, Faculty of Science, Ebonyi State University, Abakaliki P.M.B 53, Nigeria

[*]**Corresponding author:** Engwa Azeh Godwill, Department of Chemical Sciences, Godfrey Okoye University, P.M.B 01014, Thinkers Corner, Enugu, Nigeria, Tel: +2348068473306; E-mail: engwagodwill@gmail.com; gengwa@gouni.edu.ng

Received date: July 28, 2018; **Accepted date:** August 14, 2018; **Published date:** August 20, 2018

Abstract

Objective: Though the relationship between glucose-6-phosphate dehydrogenase (G6PD) deficiency and type 2 diabetes (T2D) has been reported, their interaction to influence oxidative stress is not fully understood. This study was conducted to investigate the relationship between T2D and G6PD deficiency and assessed their effect on oxidative stress.

Methodology: A total of 73 T2D and 75 non-diabetic (ND) out-patients at Enugu State University Teaching Hospital (ESUTH) in Enugu, Nigeria were recruited. They were screened for G6PD deficiency, oxidative stress markers; lipid peroxidation, protein peroxidation and glycated haemoglobin (HbA1c) and antioxidant enzymes; superoxide dismutase (SOD) and catalase activities were assayed.

Results: Oxidative stress was influenced by T2D as malondialdehyde (MDA), protein carbonyl and HbA1c levels were significantly higher ($p<0.05$) in T2D patients compared to ND patients. Significantly ($p<0.05$) decreased SOD and catalase activity were also observed in T2D patients. G6PD deficiency and T2D showed a significant relationship ($p<0.05$) in male patients ($\chi^2=5.070$; $p=0.024$). Moreover, there was no significant ($p>0.05$) interaction between G6PD deficiency and T2D to influence oxidative stress in patients.

Conclusion: The interaction between G6PD deficiency and T2D did not influence oxidative stress though there was a possible relationship between G6PD deficiency and T2D in male patients.

Keywords: G6PD deficiency; Type 2 diabetes; Oxidative stress; Antioxidants; Glycated haemoglobin

Introduction

The global prevalence of diabetes is constantly increasingly. The burden of the disease has risen from 108 million (4.7%) in 1980 to 422 million (8.5%) in 2012. More than 90% of all cases of the disease are of type 2 diabetes (T2D) [1]. Though this disease was previously known to be highly prevalent in developed countries, recent data indicate rising prevalence rates in middle and low-income countries. For instance, the current rate for Nigeria is 1.9%, translating to over 3.85 million people living with the disease. Nigeria currently has the highest prevalence rate in Africa [2].

T2D is a disease characterised by elevated blood glucose levels as a result of the inability of insulin to recognise its receptor and promote the metabolism of glucose [3]. In a state of hyperglycaemia, glucose is known to generate free radicals through several mechanisms [4]. Glucose can be autooxidised in the presence of transition metals to an enediol radical anion which is further converted into reactive ketoaldehydes and to superoxide anion radicals [5]. Glucose also promotes lipid peroxidation of low density lipoprotein (LDL) via a superoxide-dependent pathway to generate free radicals [6,7]. Glucose can promote free radical formation by interacting with proteins to form amadori product [8] and then advanced glycation end-products (AGEs), including glycated haemoglobin [9,10]. These processes make glucose a major source of free radicals in diabetes. Excessively high levels of these free radicals lead to a state of oxidative stress [11] causing damage to nucleic acids [12], cellular proteins [13], lipid membranes [14] and eventually cell death. Cell damage of this nature forms the basis for some of the secondary complications of diabetes mellitus [15].

In response to oxidative stress, the antioxidant defence system, constituting both the enzymatic and non-enzymatic antioxidants, is activated to scavenge free radicals [16]. Superoxide dismutase (SOD) emerges to dismutate superoxide anion radicals to hydrogen peroxide (H_2O_2) [17]. H_2O_2, if not removed can be converted in the presence of a transition metal to extremely reactive hydroxyl radicals [18]. Thus,

catalase and glutathione peroxidase further degrades H_2O_2 [19]. For glutathione peroxidase to reduce H_2O_2 to H_2O and O_2, it requires reduced glutathione (GSH) [20]. In the process, GSH is converted to oxidized glutathione (GSSG) [20] and then regenerated by glutathione reductase which utilizes nicotinamide adenine dinucleotide phosphate (NADPH) as the reducing agent [21] NADPH is generated by glucose-6-phosphate dehydrogenase (G6PD), a key enzyme in the pentose phosphate pathway [22].

G6PD is coded by an X-linked housekeeping gene in humans, located at the telomeric region of the X-chromosome long arm (band Xq28) [22]. G6PD deficiency is a common enzyme defect that occurs as a result of mutation in the G6PD gene. It affects over 400 million people worldwide with highest frequencies (5-30%) recorded in Africa, Asia, the Mediterranean area, and the Middle East with male presenting high prevalence than female [23-25]. The X-linkage of this gene has important implications as hemizygous males for this gene could either be normal or G6PD deficient while females who have two copies of the gene G6PD gene, could either be normal, deficient (homozygous), or intermediate (heterozygous) [23-25]. G6PD deficient individuals are vulnerable to oxidative stress [25]. As such, there has been considerable thought that G6PD deficiency may be associated with oxidative stress in T2D due to insufficient or limited production of NADPH which regenerates GSH, a physiologic antioxidant to scavenge glucose-generated free radicals [26]. Reports of studies investigating the relationship between G6PD deficiency and diabetes have been inconsistent with contrasting findings [27-29]. Moreover, majority of these studies only investigated the relationship between G6PD deficiency and diabetes as well as their independent influence on oxidative stress [27-29] without assessing the interaction between G6PD deficiency and T2D on oxidative stress. Hence, this study addressed this gap by assessing the relationship between G6PD deficiency and T2D and assessed the effect of this interaction on oxidative stress.

Materials and Methods

Participants' recruitment and study design

This study was conducted at Enugu State University Teaching Hospital (ESUTH) in Enugu Nigeria. The study design was reviewed and approved by the Ethical Committee of ESUTH Enugu, Nigeria with approval number: ESUTHP/C-MAC/RA/034/174. Written informed consent was obtained from all participants before the commencement of the study. Outpatients above 30 years of age without any critical health condition or complications of diabetes and are not on hospital admission were recruited for the study. Breastfeeding and/or pregnant women, HIV positive patients as well as patients without oxidative stress related illnesses such as malaria, atherosclerosis, hypertension, and cardiovascular diseases were excluded from the study. Outpatients visiting the hospital were screened for T2D by measuring their fasting blood glucose and were considered diabetic according to IDF criteria [30] with at least one-year history of the disease. Following the screening, a total of 148 patients were recruited which includes 73 T2D patients and 75 non-diabetic (ND) patients. To assess the effect of T2D on oxidative stress, lipid peroxidation, protein oxidation and glycated haemoglobin (HbA1c) as oxidative stress markers as well as SOD and catalase were assayed. To assess the relationship between G6PD deficiency and T2D, and their effect on oxidative stress, the patients were further screened for G6PD deficiency to differentiate G6PD deficient from non-deficient patients. Also, the relationship between G6PD deficiency and T2D was assessed independently for males and females. The study design is summarized in Figure 1.

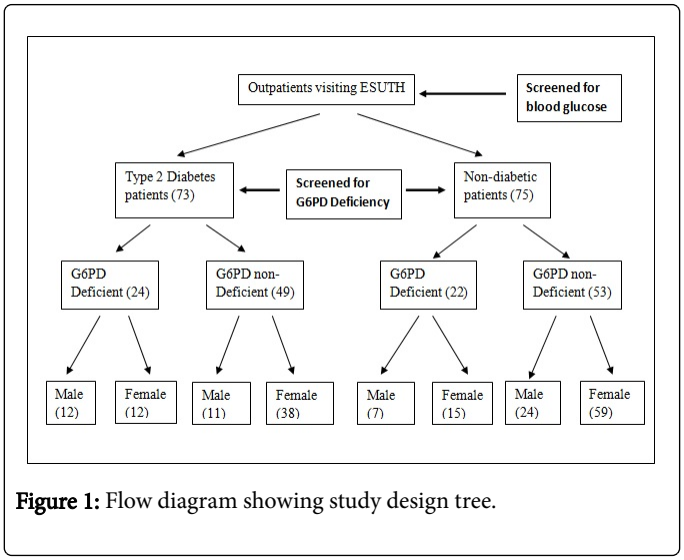

Figure 1: Flow diagram showing study design tree.

Data collection

Following recruitment, participants were administered a research questionnaire which sought relevant demographic information including age, sex, ethnicity, location and disease history. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automatic sphygmomanometer. In addition, anthropometric data including body weight, height and waist circumference (WC) of participants were collected. Weight and height of participants were used for the estimation of Body Mass index (BMI) as weight (Kg)/ height^2 (m^2).

Laboratory analysis

Following an overnight fast, blood sample (2 ml) was collected from participants into EDTA tubes. Whole blood was used for the assessment of all biochemical parameters in this study. Fasting blood glucose (FBG) was measured by finger-prick method using an Accucheck glucometer (Roche Holding AG, Basel, Swizerland) while the HbA1c level was assayed using the A1CNow kit (PTS diagnostics, Indianapolis, USA).

Measurement of catalase activity

Catalase activity was measured as described by Mahmoud [31] with slight modifications. Briefly, homogenized blood sample (100 µl) was mixed with phosphate buffer (1 ml) and hydrogen peroxide (1 ml) as substrate for catalase. For quality control, incubations were also performed using water (100 µl) instead of blood sample and/or without hydrogen peroxide.

All the tubes were well mixed and incubated at 37°C for 3 min after which 2 ml of 5% potassium dichromate in glacial acetic acid solution was added. The tubes were then heated at 100°C for 10 min, cooled with tap water and centrifuged at 2500 rpm for 5 minutes to remove precipitated protein. The absorbance of samples was recorded at 570 nm against the blank incubation carried out with water instead of blood sample. The catalase activity (kU/L) was calculated according to

the formula: $\frac{2.303}{t} \times \left[Log\left(\frac{S^0}{S-M}\right)\right] \times \frac{V_t}{V_s}$ with t=incubation time; S °=Absorbance of control incubation containing catalase standard, S=Absorbance of unknown sample, M=Absorbance of incubation containing blood sample without hydrogen peroxide (correction factor), Vt=Total volume of reagents in test tube, Vs=Volume of blood sample.

Superoxide dismutase activity

Superoxide dismutase activity was determined as described by Marklund and Marklund [32] with slight modifications. Briefly, homogenized blood sample (50 µl) was added to 450 µl of 8 mM pyrogallol and 500 µl of 20 mM phosphate buffer (pH 6.6). The absorbance was read at 420 nm for 3 minutes against a blank preparation containing distilled water instead of sample in pyrogallol and phosphate buffer. SOD activity was expressed as U/mg. One unit of SOD activity was defined as that amount of SOD required to cause 50% inhibition of pyrogallol autoxidation per 3 ml of assay mixture.

Measurement of lipid peroxidation

Lipid peroxidation was assessed in blood samples by a modified method of Niehaus and Samuelsson [33] which measures the concentration of thiobarbituric acid reactive substances (TBARS) using a colourimetric assay. Whole blood (100 µl) was mixed with 2 ml of a solution containing 0.37% thiobarbituric acid, 15% trichloroacetic acid and 0.25 mol/l hydrogen chloride reagent (1:1:1 volume ratio). The resultant mixture was incubated at 100°C in a water bath for 30 minutes after which it was then cooled. The clear supernatant was collected and the absorbance was taken at 535 nm against the blank to determine the quantity of malondialdehyde (MDA) formed. Concentration of MDA was calculated using the equation: $C = \frac{A}{E} \times L$ where, A=the absorbance of the sample, E=the extinction coefficient (1.56 × 105 M-1cm-1) and L=length of the light path (1 cm).

Assessment of protein oxidation

Protein carbonyl content of blood samples was quantified, as a marker of oxidative stress, following the method of Mesquita et al. [34]. Blood samples (400 µl) was added to equal volume of 10 mM DNPH (prepared in 0.5 M H3PO4) solution and incubated for 10 minutes at room temperature. This was followed by the addition of NaOH (6M, 200 µl) and further incubated at room temperature for 10 minutes. The absorbance was measured at 450 nm against the blank which contained equal volume of distilled water instead of blood samples. The concentration of carbonyl content was calculated using the equation: $C = \frac{A}{E} \times L$ where, A=absorbance of the sample, E=molar extinction coefficient for DNPH (22,000 M-1cm-1) and L=length of the light path (1 cm).

Assessment of G6PD deficiency

This was assayed by the methaemoglobin reduction test described by Brewer et al. [35].. Briefly, a mixture of whole blood (500 µl) and glucose (0.28M, 50 µl) was transferred into a set of three tubes labeled "Test", "Deficient-Control" and "Normal-Control". In the tube labeled "Test" was added 50 µl of 0.18M sodium nitrite and 50µl of 0.4mM methylene blue solutions. Sodium nitrite (50 µl) and equal volume of saline solutions were added to the tube labeled "Deficient-Control" while 100 µl of saline solution only was added to the tube labeled "Normal-Control". All tubes were well mixed, corked with cotton wool and incubated at 37°C for 3 hours. After incubation, 100 µL of the incubated mixture was transferred into new set of tubes labeled as before followed by the addition of 5 ml of 0.02M (pH 6.6) phosphate buffer for colour development. A dark-brown or grey colour like the "Deficient control" was indicative of G6PD Deficient while a red colour like the "Normal-Control" was considered as G6PD Non-Deficient (Normal). G6PD deficient hemizygous males and homozygous females presented a dark-brown colouration just like the "Deficient control" while G6PD deficient heterozygous females present an intermediate expression; a colouration between dark-brown and grey.

Statistical Analysis

Data was analyzed using Statistical Package for Social Science (SPSS) version 16. Quantitative values were expressed as Mean ± Standard Error of the Mean (SEM) or Standard Deviation (SD) while frequencies were presented as proportions and percentages. Pearson chi-square (χ^2) test was used to compare the proportional differences of G6PD deficiency in T2D and ND patients as well as in male and female. Mean differences of demographic, clinical data, oxidative stress and antioxidant indices between T2D and ND patients were compared using parametric independent sample t-test. Interaction between T2D and G6PD deficiency for oxidative stress and antioxidant indices was assessed by Two Way ANOVA. Pearson correlation test was used to assess the association between continuous variables. A confidence interval of 95% was taken and a p-value less than 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of patients

Among the 148 study participants, 73 (49.3%) were T2D patients while 75 (50.7%) were non-diabetic (ND) patients. Among the T2D patients, 23 (15.3%) were male and 50 (34.0%) were female while among the ND patients, 31 (20.8%) were male and 44 (29.9%) were female. There was no significant sex difference between T2D and ND patients (χ^2=1.541, p=0.214). All the patients were between 30 and 92 years. Age, BMI, WC, and FBG were significantly higher (p<0.05) in T2D patients compared to ND patients. The demographic and clinical characteristics of the patients are summarised in Table 1.

	T2D patients (n=73)		ND patients (n=75)		p-value
	Male (n=23)	Female (n=50)	Male (n=31)	Female (n=44)	
Age (years)	60.17 ± 9.90	55.90 ± 9.77	52.85 ± 19.46	48.83 ± 14.914	0.011
Height (m)	1.66 ± 0.08	1.56 ± 0.064	1.69 ± 0.11	1.57 ± 0.09	0.22

Weight (kg)	83.89 ± 19.34	76.21 ± 27.14	76.15 ± 19.78	70.61 ± 15.63	0.154
BMI (Kg/m2)	30.59 ± 6.41	31.36 ± 11.46	26.91 ± 7.29	28.55 ± 6.06	0.086
WC (cm)	100.61 ± 10.88	101.21 ± 12.69	94.95 ± 20.57	91.87 ± 12.65	0.013
SBP (mmHg)	132.72 ± 18.39	133.21 ± 22.03	136.10 ± 20.46	132.83 ± 25.66	0.745
DSP (mmHg)	80.44 ± 11.53	78.67 ± 11.38	83.80 ± 13.69	82.09 ± 18.64	0.239
FBG (mg/dL)	173.67 ± 109.50	175.79 ± 94.03	67.60 ± 39.77	67.65 ± 38.46	<0.0001

Table 1: Demographic and clinical characteristics of participants. Results are presented as Mean ± SD; SD: Standard deviation; SBP=Systolic blood pressure; DSP=Diastolic blood pressure; FBG=Fasting blood glucose; BMI=Body mass index; WC=Waist circumference.

Effect of T2D on antioxidant and oxidative stress markers

Data on activities of antioxidant enzymes and markers of oxidative stress in T2D and ND participants are presented in Figure 2. Catalase activity was significantly reduced by 19% (p<0.01) in T2D patients compared with their ND counterparts (Figure 2A). Similarly, SOD activity was significantly reduced by 20% (p<0.05) in T2D participants (Figure 2A). However, 1.4-fold significant increase (p<0.001) in blood MDA concentration was observed in T2D participants compared to their ND counterparts (Figure 2B). A similar upward trend was observed for protein carbonyl in T2D participants (1.4-fold, p<0.001, Figure 2B). Expectedly, average HbA1c values in the T2D population was 1.9-fold significantly higher (p<0.01) compared to ND participants (Figure 2C).

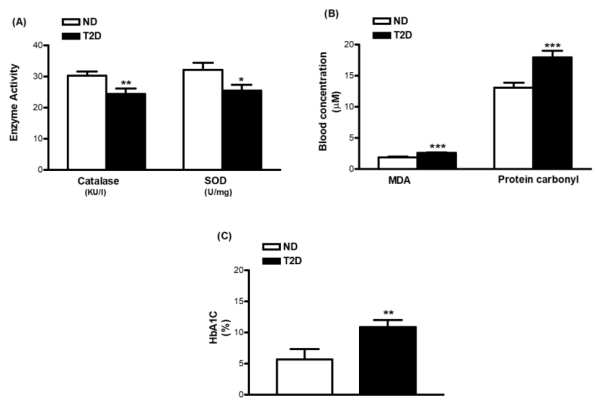

Figure 2: Oxidative stress indices among T2D and ND patients. Values are expressed as mean ± SEM with n=75 for control patients (ND) and n=73 for T2D patients (T2D). ***p<0.001, **p<0.01, *p<0.05 compared with control patients for each parameter.

Effects of G6PD deficiency on antioxidant and oxidative stress markers

The influence of G6PD deficiency on activities of antioxidant enzymes and markers of oxidative stress in T2D and ND participants is presented in Figure 3. Catalase activity significantly reduced by 17% (p<0.05, Figure 3A) in G6PD deficient participants compared with their G6PD non-deficient counterparts. Similarly, a 16% reduction in SOD activities was observed in G6PD deficient participants but this drop was not statistically significant (Figure 3A). However, no significant difference (p>0.05) was observed in the blood concentrations of MDA and protein carbonyl in both G6PD deficient and non-deficient participants (Figure 3B). Similar, HbA1c values were observed not to be significantly different (p>0.05) in G6PD deficient and non-deficient participant (Figure 3C).

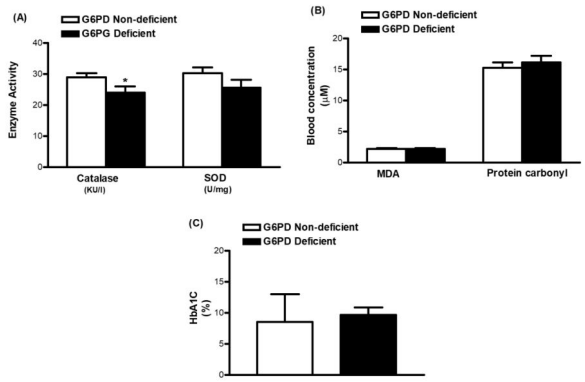

Figure 3: Oxidative stress indices among G6PD deficient and G6PD non-deficient patients. Values are expressed as mean ± SEM with n=102 for G6PD non-deficient patients and n=46 for G6PD deficient patients (T2D). *p<0.05 compared with G6PD non-deficient patients for each parameter.

Relationship between G6PD deficiency and type 2 diabetes

In total, 46 of 148 patients were G6PD deficient with an overall prevalence of 31.1%. Among these patients, 24 (16.4%) were T2D while 22 (14.9%) were ND patients. Chi square analysis carried out to assess the relationship between T2D and G6PD deficiency showed no significant relationship between G6PD deficiency and T2D (χ^2=0.217; p=0.641) as shown in Table 2. Since G6PD is a sex-linked disorder, the relationship between G6PD and T2D was assessed independently for each sex. Chi square analysis as shown in Table 2 showed significant relationship between G6PD deficiency and T2D in males (χ^2=5.070; p=0.024). Among the females, 27 were deficient of which 10 were homozygous while 17 were heterozygous (intermediate). However, no significant association (χ^2=1.164; p=0.281) was observed between G6PD deficiency and T2D in females (Table 2).

		Diabetes status			Computed χ^2 value	Standard χ^2 value at p=0.05	p-value
		T2D	ND	Total			
G6PD status	Deficient	24 (16.2%)	22 (14.9%)	46 (31.1%)	0.217	3.841	0.641
	Non-deficient	49 (33.1%)	53 (35.8%)	102 (68.9%)			
	Total	73 (49.3%)	75 (50.7%)	148 (100%)			
Sex	**G6PD Status**	**Diabetes status**			**Computed χ^2 value**	**Standard χ^2 value at p=0.05**	**p-value**
		T2D	ND	Total			
Male	Deficient	12 (22.2%	7 (13.0%)	19 (35.2%)	5.07	3.841	0.024
	Non-deficient	11 (20.4%)	24 (44.4%)	35 (64.8%)			
	Total	23 (42.6%)	31 (57.4%)	54 (100%)			
Female	Deficient	12(12.8%)	15 (16.0%)	27 (28.7%)	1.164	3.841	0.281
	Non-deficient	38 (40.4%)	29 (30.9%)	67 (71.3%)			
	Total	50 (53.2%)	44 (46.8%)	94 (100%)			

Table 2: Relationship between G6PD deficiency and T2D (A) and in male and female (B).

Interaction between T2D and G6PD deficiency on antioxidant and oxidative stress markers

A two-way analysis of variance computed to further investigate the interaction between G6PD deficiency and T2D on antioxidant and oxidative stress indices revealed no significant difference in the data set at $p<0.05$ as shown Table 3.

Parameters (n=148)	T2D (n=73)		ND (n=75)		2 WAY ANOVA (p-value)
	Deficient (n=24)	Non-Deficient (n=49)	Deficient (n=22)	Non-Deficient (n=53)	
Catalase (KU/L)	20.36 ± 2.81	26.84 ± 1.99	29.92 ± 3.03	31.94 ± 1.89	0.371
SOD (U/mg)	22.37 ± 3.72	26.96 ± 2.63	29.07 ± 3.88	33.35 ± 2.50	0.962
MDA (µM)	2.46 ± 0.18	2.63 ± 0.13	1.94 ± 0.19	1.82 ± 0.12	0.369
Protein carbonyl (µM)	16.81 ± 1.77	18.59 ± 1.25	14.96 ± 1.91	11.94 ± 1.19	0.126
HbA1c (%)	8.8 ± 1.65	7.3 ± 2.96	13.0 ± 2.3	4.25 ± 4.56	0.347

Table 3: Interaction between T2D and G6PD deficiency on antioxidant and oxidative stress markers.

Correlation between antioxidant, oxidative stress indices, age and FBG

Analysis of the correlation between antioxidant enzymes, markers of oxidative stress, age and FBG levels is summarised in Table 4. Results of the analysis indicated that age significantly ($p<0.05$) correlated positively with protein oxidation and negatively with catalase while FBG significantly ($p<0.05$) correlated positively with MDA and protein oxidation. No statistically significant correlation was observed between age or FBG with other antioxidants and markers of oxidative stress.

(n=148)	Catalase (KU/L)		SOD (Ug/mg)		MDA (µM)		Protein carbonyl (µM)	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
Age (years)	-0.162	0.05	0.011	0.893	0.126	0.136	0.217	0.01
FBG (mg/dl)	-0.114	0.182	-0.039	0.649	0.218	0.01	0.241	0.004

Table 4: Correlation between age, FBG, antioxidant and oxidative stress indices. *r*=Pearson correlation.

Discussion

T2D is a disease characterized by high levels of glucose in blood (hyperglycaemia) and it's usually prevalent in people above 40 years of age. Hyperglycaemia is known to induce the formation of free radical [4], promoting oxidative stress and damage to cellular macromolecules such as lipids, proteins and nucleic acids [12]. Lipid peroxidation by free radicals produces highly reactive aldehydes such as MDA which damages cell membranes [15]. This provides the basis for the consideration of serum MDA level as a biomarker for free radical-

mediated lipid damage and oxidative stress [36]. The significantly (p<0.001) high levels of MDA in T2D participants observed in this study confirms previous reports of elevated levels of thiobarbituric acid-reactive substances (TBARS) in the serum as well as in red blood cells of diabetes patients [37].

Endogenous proteins are targets for free radical attacks via the oxidation of methionine, cysteine, and/or tyrosine residues; forming carbonyls as the oxidation product. Therefore, there is a direct link between the generation of free radicals (such as observed in T2D) and increased serum levels of protein carbonyls [38]. In agreement with previous studies, this study revealed significantly high levels (p<0.001) of protein carbonyl content in T2D compared with ND participants [38].

Measurement of blood levels of HbA1c has becomes a classical method for assessing the extent of oxidative damage resulting from hyperglycaemia and glycaemic control in T2D. It is clearly understood that, due to prolonged hyperglycaemia, autoxidation of glucose occurs and the resulting oxidized glucose (free radicals) condenses non-enzymatically with protein in blood, particularly haemoglobin [39]. As such, elevated levels of blood glucose in T2D could increase the level of HbA1c. As expected, findings in this study showed significantly higher level (p<0.05) of HbA1c in T2D patients compared to the control. The significantly high levels of protein oxidation, lipid peroxidation as well as HbA1c in T2D participants suggest T2D to promote oxidative stress. More so, this study showed FBG to positively correlate with protein oxidation as well as lipid peroxidation suggesting possible influence of T2D on oxidative stress as previously reported [40].

Increase in oxidative stress (lipid peroxidation, protein oxidation and HbA1c) due to glucose autoxidation may possibly affect or inhibit the activities of enzymatic and non-enzymatic antioxidants as previous reported [40-42]. In congruence with this assertion, our study showed significantly (p<0.05) reduced activities of SOD and catalase activity in T2D patients. This observation is consistent with previous reports of reduced SOD [43,44] and catalase [45] activities as well as reduced total antioxidants level [46] in T2D patients. This may be due to the debilitating effects of high levels of free radicals damaging antioxidant producing cells. Reduced erythrocyte antioxidant enzyme activities have been reported in T2D condition [47]. Likewise, reduced catalase activities in patients with diseases associated with oxidative stress, such as atherosclerosis and schizophrenia, has been reported [48]. The increase in protein and lipid oxidation damage as well as reduced activities of antioxidant enzymes further attributes T2D to promote oxidative stress [49,50].

With the depletion of enzymatic antioxidants, the body relies on non-enzymatic antioxidant sources or other physiological molecules to supplement the antioxidant defence system against oxidative stress. One of such physiological molecule is NADPH. NADPH plays a critical role in the regeneration of GSH, a non-enzymatic antioxidant that decomposes free radicals to non-reactive species in the body [26]. NADPH is produced by G6PD in the pentose phosphate pathway during the metabolism of Glucose-6-phosphate [51]. Due to its immensely important role in free radical scavenging, it is logical to think that G6PD deficiency may promote oxidative stress and be associated with T2D. This study showed similar levels of oxidative stress markers in G6PD deficient and non-deficient participants suggesting that G6PD deficiency did not influence oxidative stress as previously reported by Obasa et al. [52] which reported no association between oxidative stress and G6PD deficiency in babies. More so, when the relationship between G6PD deficiency and T2D was assessed, this finding revealed no significant relationship between the two parameters as previously reported [27-29]. However, further comparison of the relationship between G6PD deficiency and T2D independently in male and female showed a significant association in male patients. This suggests that G6PD deficiency may be associated with T2D in male patients. On the other hand, there was no association between G6PD deficiency and T2D in female patients. Similar finding has been reported previously showing association between G6PD deficiency and diabetes among men but not in women [53].

Available research evidence with respect to the relationship between diabetes and G6PD deficiency indicates divergent opinions. For instance, our results agree with the findings of Heymann et al. [29] which showed G6PD deficiency as a potential risk factor for diabetes but conflicts with the reports of Adinortey et al. [54]. Even though extensive statistical analysis conducted in this study to investigate the interaction between G6PD deficiency and T2D on oxidative stress showed no significant relationship, there was possible association between G6PD deficiency and T2D in male patients. It is therefore important that research efforts are dedicated to resolving this divergent view of the role of G6PD in the development of oxidative stress in T2D.

Conclusion

This study showed possible association between G6PD deficiency and T2D in male patients. These findings also indicate an association between oxidative stress and T2D as significantly increased levels of lipid peroxidation, protein oxidation and HbA1c was observed in T2D participants coupled with reduced activities of antioxidant enzymes in the same cohort. However, oxidative stress was not associated with G6PD deficiency neither did G6PD deficiency interact with T2D to influence oxidative stress. Further investigations may be necessary to better explain the contribution of G6PD deficiency to the development of oxidative stress in T2D as there are conflicting reports on the association between these parameters.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgment

The authors are thankful to the students of medical laboratory science and medical students of ESUTH Enugu for their assistance in blood and data collection. This study was partially supported by the competitive research grant of Godfrey Okoye University, Enugu Nigeria.

Authors' Contributions

GAE, BEU and FNN contributed to the conception of the study. GAE, GEC, ECE, TEU and MCA were involved in the design and collection of data. GAE, GEC, ECE and KIO performed the laboratory and statistical analysis. GAE, GEC, TEU, KNA, BEU, FNN and OOO interpreted the data, drafted and revised the manuscript. All authors read and approved the final manuscript.

References

1. World Health Organization (2016) Global report on diabetes.

2. International Diabetes Federation (2016) International Working Group on the Diabetic Foot.
3. Rosenbloom AL, Joe JR, Young RS, Winter WE (1999) Emerging epidemic of type 2 diabetes in youth. Diabetes Care 22: 345-354.
4. Winterbourn CC (2008) Reconciling the chemistry and biology of reactive oxygen species. Nat Chem Biol 4: 278-286.
5. Baynes JW, Thorpe SR (1999) Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes 48: 1-9.
6. Tsai EC, Hirsch IB, Brunzell JD, Chait A (1994) Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. Diabetes 43: 1010-1014.
7. Kawamura M, Heinecke JW, Chait A (1994) Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide dependent pathway. Eur J Clin Invest 94: 771-778.
8. Hamada Y, Araki N, Koh N, Nakamura J, Horiuchi S, et al. (1996) Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. Biochem Biophys Res Commun 228: 539-543.
9. Hori O, Yan SD, Ogawa S, Kuwabara K, Matsumoto M, et al. (1996) The receptor for advanced glycation end-products has a central role in mediating the effects of advanced glycation end-products on the development of vascular disease in diabetes mellitus. Nephrol Dial Transplant 11: 13-16.
10. Mullarkey CJ, Edelstein D, Brownlee M (1990) Free radical generation by early glycation products: A mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 173: 932-939.
11. Rains JL, Jain SK (2011) Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 50: 567-75.
12. Dinc Y, Akc T, Alademir Z, Ilkova H (2002) Assessment of DNA base oxidation and glutathione level in patients with type 2 diabetes. Mutat Res 505: 75-81.
13. Kalousová M, Skrha J, Zima T (2002) Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. Physiol Res 51: 597-604.
14. Halliwell B, Chirico S (1993) Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 57: 715S-724S.
15. Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. Circ Res 107: 1058-1070.
16. Young IS, Woodside JV (2001) Antioxidant in health and diseases. J Clin Pathol 54: 176-186.
17. Johnson F, Giulivi C (2005) Superoxide dismutases and their impact upon human health. Mol Aspects Med 26: 340-352.
18. Halliwell B, Clement MV, Long LH (2000) Hydrogen peroxide in the human body. FEBS Lett 486: 10-13.
19. Margis R, Dunand C, Teixeira FK, Margis-Pinheiro M (2008) Glutathione peroxidase family-an evolutionary overview. FEBS Lett 275: 3959-3970.
20. Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 121: 1191-212.
21. Kunwar A, Priyadarsini KI (2011) Free radicals, oxidative stress and importance of antioxidants in human health. J Med Appl Sci 1: 53-60.
22. Cappellini MD, Fiorelli G (2008) Glucose-6-phosphate dehydrogenase deficiency. Lancet 371: 64-74.
23. Manganelli G, Masullo U, Passarelli S, Filosa S (2013) Glucose-6-phosphate dehydrogenase deficiency: disadvantages and possible benefits. Cardiovasc Hematol Disord Drug Targets 13: 73-82.
24. Luzzatto L, Nannelli C, Notaro R (2016) Glucose-6-Phosphate Dehydrogenase Deficiency. Hematol Oncol Clin North Am 30: 373-393.
25. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E (2009) The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Mol Dis 42: 267-278.
26. Gaskin RS, Estwick D, Pedi R (2001) G6PD deficiency: its role in the high prevalence of diabetes mellitus. Ethn Dis 11: 749-54.
27. Pinna A, Contini EL, Carru C, Solinas G (2013) Glucose-6-Phosphate Dehydrogenase Deficiency and Diabetes Mellitus with Severe Retinal Complications in a Sardinian Population, Italy. Int J Med Sci 10: 1907-1913.
28. Carette C, Dubois-Laforgue D, Gautier JF, Timsit J (2011) Diabetes mellitus and glucose-6-phosphate dehydrogenase deficiency: from one crisis to another. Diabetes Metab 37: 79-82.
29. Heymann AD, Cohen Y, Chodick G (2012) Glucose-6-Phosphate Dehydrogenase Deficiency and Type 2 Diabetes. Diabetes Care 35:1-9.
30. WHO-IDF (2014) Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation.
31. Mahmoud HH (2016) New Method for Assessment of Serum Catalase Activity. Indian J Sci Technol 9:1-4.
32. Marklund S, Marklund G (1974) Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. Eur J Biochem 47: 469-474.
33. Niehaus WG, Samuelsson B (1968) Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 6: 126-30.
34. Mesquita CS, Raquel O, Fátima B, Dulce G, João VR, et al. (2014) Simplified 2,4 dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Anal Biochem 458: 69-71.
35. Brewer GJ, Tarlov AR, Alving AS (1960) Methaemoglobin Reduction Test: A New, Simple, in Vitro Test for Identifying Primaquine-Sensitivity. Bull World Health Organ 22: 633-40.
36. Shodehinde SA, Oboh G (2013) Antioxidant properties of aqueous extracts of unripe Musa paradisiaca on sodium nitroprusside induced lipid peroxidation in rat pancreas in vitro. Asian Pac J Trop Biomed 3: 449-457.
37. Singh M, Shin S (2009) Changes in erythrocyte aggregation and deformability in diabetes mellitus: a brief review. Indian J Exp Biol 47: 7-15.
38. Pandey KB, Mishra N, Rizvi SI (2010) Protein oxidation biomarkers in plasma of type 2 diabetic patients. Clin Biochem 43: 508-511.
39. Wolff SP, Jiang ZY, Hunt JV (1991) Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med 10: 339-352.
40. Saddala RR, Thopireddy L, Ganapathi N, Kesireddy SR (2013) Regulation of cardiac oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with aqueous extract of Pimpinella tirupatiensis tuberous root. Exp Toxicol Pathol 65:15-19.
41. Sheweita SA, Mashaly S, Newairy AA, Abdou HM, Eweda SM (2016) Changes in Oxidative Stress and Antioxidant Enzyme Activities in Streptozotocin-Induced Diabetes Mellitus in Rats: Role of Alhagi maurorum Extracts. Oxidative Med Cellular Longevity 2016: 1-8.
42. Giugliano D, Ceriello A, Paolisso G (1996) Oxidative stress and diabetic vascular complications. Diabetes Care 19: 257-267.
43. He K, Li X, Chen X, Ye X, Huang J, et al. (2011) Evaluation of antidiabetic potential of selected traditional Chinese medicines in STZ-induced diabetic mice. J Ethnopharmacol 137: 1135-1142.
44. Kim CH (2013) Expression of extracellular superoxide dismutase protein in diabetes. Arch Plast Surg 40: 517-521.
45. Kedziora-Kornatowska KZ, Luciak M, Blaszczyk J, Pawlak W (1998) Effect of aminoguanidine on erythrocyte lipid peroxidation and activities of antioxidant enzymes in experimental diabetes. Clin Chem Lab Med 36: 771-775.
46. Ganjifrockwala FA, Joseph JT, George G (2017) Decreased total antioxidant levels and increased oxidative stress in South African type 2 diabetes mellitus patients. J Endocrinol Metab Diab South Africa 1: 1-5.
47. Varashree BS, Bhat PG (2011) Correlation of lipid peroxidation with glycated haemoglobin levels in diabetes mellitus. Online J Health Applied Sci 10: 1-4.
48. Goth L, Vitai M (1996) Hypocatalasemia in hospital patients. Clin Chem 42: 341-342.

49. Ceriello A (2000) Oxidative stress and glycemic regulation. Metabolism 49: 27-29.
50. Wolff SP, Jiang ZY, Hunt JV (1991) Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med 10: 339-352.
51. Beutler E (1994) G6PD deficiency. Blood 84: 3613-3616.
52. Saeed TK, Hamamy HA, Alwan AA (1985) Association of glucose-6-phosphate dehydrogenase deficiency with diabetes mellitus. Diabet Med 2: 110-112.
53. Obasa TO, Mokuolu OA, Ojuawo A (2011) Glucose 6 phosphate dehydrogenase levels in babies delivered at the University of Ilorin teaching hospital. Nig J Pediatr 38: 165-169.
54. Adinortey MB, Owusu RK, Galyuon KA, Ekloh W, Owusu I, et al. (2011) G-6-PD deficiency - a potential risk factor for development of diabetes mellitus. J Med Med Sci 2: 1017-1021.