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Research Article

Glucose-6-Phosphate Dehydrogenase Levels and Oxidative Stress Markers Among Cancer Patients in Jos, Nigeria

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) is a sensitive cytosolic antioxidant enzyme that could be associated with carcinogenesis. Hence, its plasma levels are a good indicator to monitor cancer-induced cellular stress. This study aimed to determine the correlation between Glucose-6-phosphate dehydrogenase and oxidative stress markers among cancer patients in Jos, Nigeria. This case-control study involved 100 subjects (60 cancer patients and 40 healthy control subjects). Their blood samples were collected to measure the levels of G6PD and oxidative stress markers (malondialdehyde, total plasma peroxide, total antioxidant potential, and oxidative stress indices). Twenty-four (40.0%) of the cancer patients were G6PD deficient. Of this, 13 (54.2%) were females G6PD. Of the G6PD deficient cancer patients, 11(45.8%) were male, conversely, 16 (44.4%) of the cancer subjects who had normal G6PD were males. Of the cancer patients, 26.7%; 13.3%, 11.7 % and 10% had prostate, breast cancer, chronic lymphocytic leukemia (CLL), and hepatocellular carcinoma (HCC), respectively were the most frequent. There was no significant association between G6PD deficiency and cancer (X^2=0.025, p=0.804). Among G6PD deficiency cancer patients, the oxidative stress markers were significantly (p<0.05) higher compared to the control group. These findings showed that relatively more of the cancer patients had normal G6PD status even in increased cellular oxidative stress which could be due to host genetic factors. This suggests the need for further experiments on molecular characterization of mechanisms responsible for the findings.

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Keywords

Glucose-6-Phosphate Dehydrogenase, Oxidative Stress, Malondialdehyde, Plasma Peroxide, Cellular Oxidation

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is among the most important intracellular antioxidant enzyme in the metabolic pathway that supplies reducing energy to cells in response to oxidative insults by maintaining the levels of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) that maintains the level of glutathione in cells against oxidative damage [1]. G6PD catalyses the first reaction, oxidation of glucose-6-phosphate to 6- phosphogluconolactone accompanied by reduction of $NADP^+$ to NADPH, which is the rate-limiting and primary control step of the NADPH generating portion in the hexose monophosphate shunt. G6PD is a guardian of cellular redox potential during oxidative stress [1]. The co-enzyme NADPH is commonly used for reductive biosynthesis and maintenance of cellular redox potential and reductive biosynthesis of fatty acids, isoprenoids, and aromatic amino acids [2]. NADPH is also used to keep glutathione in its reduced form. The reduced glutathione (GSH) acts as a scavenger for dangerous oxidative metabolites in the cell and converts harmful hydrogen peroxide to water with the help of glutathione peroxidase (GSHPx) [3, 4]. the perturbed NADPH production increases the sensitivity to reactive oxygen species (ROS) and provokes apoptosis and necrosis thus highlighting the role of G6PD in defending against oxidative damage [5-7]. Many pathways are known to maintain cellular NADPH levels, the major NADPH-producing enzymes in the cell are glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) in the pentose phosphate pathway (PPP), malic enzyme (ME) in the pyruvate cycling pathway, and isocitrate dehydrogenase (IDH) in the tricarboxylic acid (TCA) cycle [8].

The activity of IDH1, ME1, and 6PGD remains unchanged during oxidative stress, while G6PD is the only NADPH-producing enzyme that is activated1. As erythrocytes lack the citric acid cycle, the Pentose phosphate shunt is the only source of NADPH. G6PD deficiency is a hereditary X- linked disorder and the most prevalent enzyme defect in humans and affects an estimated 400 million people worldwide, especially in populations historically exposed to endemic malaria [9]. The most common clinical manifestations are neonatal jaundice and acute haemolytic anaemia, which is caused by the impairment of the erythrocyte's ability to remove harmful oxidative stress triggered by exogenous agents such as drugs, infection, or fava bean ingestion [10]. Haemolytic anaemia caused by infection and subsequent medication is a clinically important concern in patients with G6PD deficiency. This issue has been a primary focus for many decades with efforts to understand the impact of *Plasmodium* infection (malaria) and antimalarial drugs [11].

Although haemolytic anaemia is the only known disease occurring in G6PD deficient subjects, G6PD deficiency has been demonstrated in other tissues as well. Ann-Joy *et al* [12] documented that oxidative stress due to G6PD deficiency is a factor in the development of nasopharyngeal carcinoma. If oxidative damage is one of the contributing factors to the pathogenesis of cancer, patients with a defective antioxidant system should be more prone to oxidative damage and hence, to accelerated cancer development. Haggar and Boushey [13] also reported that sex, black race, obesity, diabetes, acromegaly, sedentariness, excessive consumption of alcohol, processed/red meat, and smoking habits as factors raising the risk of developing cancer. Paradoxically, the position of G6PD in the metabolic pathway leading to nucleic acid synthesis supported the hypothesis that its deficiency interferes with normal cell function and replication and leads to a protective effect against the development of cancer [14]. Oxidative stress owed to G6PD deficiency as a factor in the development of nasopharyngeal carcinoma has been reported [12]. Paradoxically, G6PD deficiency interferes with normal cell function and replication leading to a protective effect against cancer development [14]. Researchers do not have clear proof or disprove the hypothesis that G6PD deficiency protects against cancer. There is a paucity of data on the prevalence of G6PD deficiency in cancer patients in Jos, however, the prevalence of G6PD deficiency in Jos University Teaching Hospital (JUTH) has been reported to be 20% [15]. Consequently, there are conflicting reports of G6PD deficiency-induced cancer development [12, 14]. This study aimed to determine the correlation between Glucose-6-phosphate dehydrogenase and oxidative stress markers among cancer patients in Jos, Nigeria.

2. Materials and Methods

2.1. Study Area and Population

This study was conducted at the Jos university teaching hospital (JUTH). This hospital is in the north central zone of Nigeria. The hospital provides specialist care for cancer pateints and also serves as referral center for neighboring

states of Bauchi, Benue, Taraba, Nasarawa, Kaduna and Plateau. The study population was comprised of sixty cancer patients attending Jos University Teaching Hospital and forty healthy individuals (controls). The control group were recruited from among students and staff at Jos University Teaching Hospital.

This study includes confirmed cancer patients of both sexes between the ages of 18-65 years attending Jos University Teaching Hospital and age-matched healthy individuals.

This study excluded individuals with diseases such as diabetes, cardiovascular disease and kidney disease.

2.2. Sample Collections

Five ml of blood was collected from every participant by venipuncture, 2ml dispensed into EDTA (ethylene diamine tetraacetic acid) specimen bottles for G6PD screening and the remaining 3ml dispensed into plain specimen bottles, allowed to clot and centrifuged at 3500 rpm for 5 minutes and separated for biochemical analysis.

2.3. Laboratory Analytical Methods

2.3.1. G6PD Screening

Glucose-6-phosphate dehydrogenase was determined using the methaemoglobin reduction method as previously described by Amiwero and Olatunji [16]. The choice for the methaemoglobin reduction method was because it has high specificity and sensitivity (93%) and cost-effectiveness in developing countries. This test is based on the following principle: Sodium nitrite oxidized haemoglobin (Hb) to methaemoglobin (Hi) when no methylene blue was added. Methylene blue, a redox dye stimulates the pentose phosphate pathway resulting in the enzymatic conversion of methaemoglobin back to haemoglobin in subjects with normal G6PD levels. In G6PD deficient subjects, blockage in the pentose phosphate pathway prevents this reduction. Thus, there is no enzymatic conversion of methemoglobin to haemoglobin.

2.3.2. MDA

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured by the method of Niehans and Samuelson [17]. The assay is based on the reaction of malondialdehyde with thiobarbituric acid, forming an MDA-TBA_2 adduct that absorbs strongly at 535nm.

2.3.3. Total Antioxidant Potential (TAP)

The total antioxidant potential was performed according to the method described by Benzie and Strain [18]. The method is based on the reduction of the ferric-tripyridyltriazine complex to form its ferrous form colour (blue Fe^{2+} TPTZ complex) in the presence of antioxidant which is read spectrophotometrically at 593nm.

2.3.4. Total Plasma Peroxide (TPP)

TPP determined using ferrous oxidation xylenol orange (FOX2) method as described by Benzie and Strain [18]. The determination of TPP was based on the principle that ferrous butylated hydroxytoluene-xylenol orange complex reacts with plasma hydrogen peroxide to form a colour complex measured spectrophotometrically at 560mm. H_2O_2 was used as standard.

2.3.5. Oxidative Stress Index (OSI)

OSI, an indicator of the degree of oxidative stress, is the ratio of the TPP to the TAP as described by Benzie and Strain [18].

2.4. Statistical Analyses

All data obtained from this study were cleaned and analyzed by Medcalc software version 22.023. categorical variables were presented in frequencies on bar charts. Continuous variables were presented as mean ± SD and the differences between groups were determined by One-way ANOVA. Dunnett's Multiple Posttest was used to determine the location of differences. Moreover, the univariate statistic was used to identify the association of G6PD deficiency and cancer. Probability (p) values < 0.05 were considered statistically significant at a confidence interval of 95%.

3. Results

Figure 1 presents the frequency of G6PD deficiency among the cancer patients and the healthy control groups. Figure 2 presents the frequency of G6PD status based on gender in cancer patients.

Of the 60 cancer patients, 36 (60%) had normal G6PD values while 24 (40%) were G6PD deficient. On the other hand, 25 (62.5%) of the healthy control subjects had normal G6PD values while 15 (27.5%) were G6PD deficient. There was no significant association between G6PD deficiency with the presence of cancer (p = 0.8018, OR=0.9, 95% CI: 0.3954 - 2.0486).

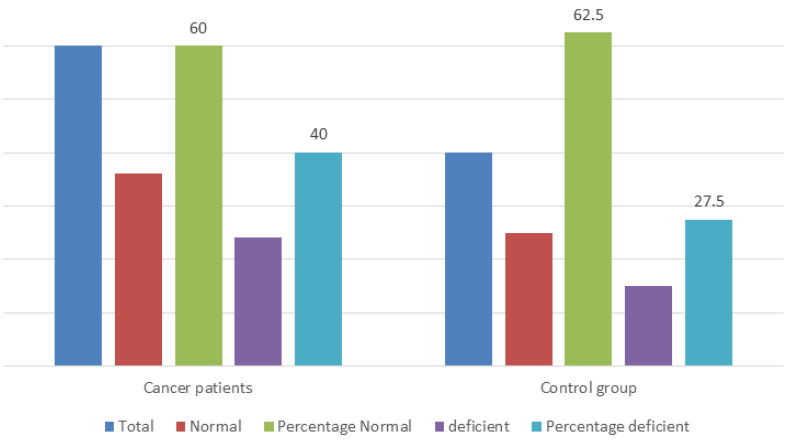

Figure 1. *Frequency of G6PD deficiency among the study groups.*

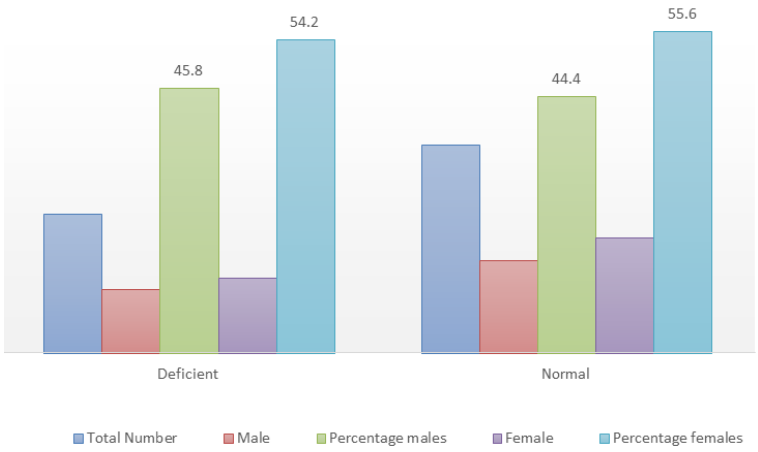

Figure 2. *G6PD status based on gender in cancer patients.*

It is observed that 11 (45.8%) of the 60 cancer subjects who were G6PD deficient were male while the remaining 13 (54.2%) females were G6PD deficient. Conversely, 16 (44.4%) of the cancer subjects who have normal G6PD were males while the remaining 20 (55.6%) who have normal G6PD were females.

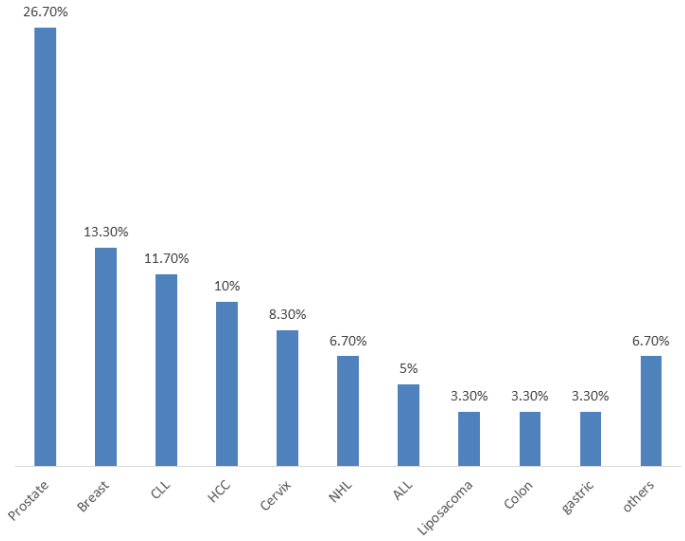

Figure 3. *Cancer type distribution among the test group.*

Of the cancer patients, 26.7%; 13.3%, 11.7 % and 10% had prostate, breast cancer, chronic lymphocytic leukaemia (CLL), and hepatocellular carcinoma (HCC), respectively (Figure 3). Moreover, 8.3%, 6.7% and 5% had cervical cancer, acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL), respectively. Furthermore, those with liposarcoma colon and gastric cancer made up 3.3% each (Figure 3).

Table 1 Shows oxidative stress markers and some antioxidant parameters among cancer patients and control subjects where malondialdehyde (MDA), total plasma peroxide (TPP) are statistically insignificant. Total antioxidant potential (TAP), OSI had significant differences.

Table 1. *Oxidative stress markers among cancer patients in JUTH.*

Parameters	Control	Cancer	P-value
MDA (nmol/ml)	0.93 ±0.03	0.98 ±0.03	0.267
TPP (µmol/L)	83.58 ±3.64	95.36 ±4.87	0.051
TAP (µmol/L)	239.44 ±14.04	189.97 ±10.65	0.006*
OSI	41.72 ±2.81	58.47 ±7.10	0.015*

The mean TPP level was significant higher (p<0.05) in test group B when compared to control group C. Also, TAP was significantly lower (p<0.05) in test group B when compared to the control group D. Furthermore, OSI was significantly lower (p<0.05) in test group B when compared to control group C and D. There was no significant difference (p>0.05) in MDA of the test groups when compared to the control group.

Table 2 shows the mean SD ±difference of MDA, TPP, TAP and OSI and Pos-hoc analyses to identify the location of the differences in the cancer patients.

Table 2. *Oxidative stress markers in G6PD deficient cancer patients.*

GROUP	N	MDA (nmol/ml)	TPP (µmol/L)	TAP (µmol /L)	OSI
A	24	0.977±0.0379	99.664±7.659	190.861±18.456	69.508±17.707
B	36	0.972±0.4797	92.779±6.351	189.438±13.255	51.843±4.057
C	15	0.938±0.0398	88.938±4.985	232.463±22.660	44.407±4.526
D	25	0.911±0.043	80.202±5.044	244.086±18.097	39.923±3.599
p value		p>0.05	p>0.05	p>0.05	p>0.05
POST HOC					
A vs B		p>0.05	p>0.05	p>0.05	p>0.05
A vs C		p>0.05	p>0.05	p>0.05	p>0.05
A vs D		p>0.05	p>0.05	p>0.05	p>0.05
B vs C		p>0.05	p<0.05*	p>0.05	p<0.05*
B vs D		P>0.05	P>0.05	P<0.05*	P<0.05*

Group A = Cancer patients with G6PD deficiency, Group B =Cancer patients with normal G6PD status, Group C = Control subjects with G6PD deficiency and Group D = Control subjects with normal G6PD status, p<0.05= significant.

Table 3 shows the mean SD ± difference of MDA, TPP, TAP, and OSI by gender of the cancer patients.

Table 3. Oxidative stress markers in cancer patients of different genders.

Parameter	Male (n=27)	Female (n=33)	*P*-value
MDA (nmol/ml)	0.984 ±0.0233	0.881 ±0.048	$p>0.05$
TPP (µmol/L)	86.643 ±4.736	81.067 ±5.382	$p>0.05$
TAP (µmol/L)	241.227 ±21.4397	7.972 ±18.850	$p>0.05$
OSI	43.556 ±4.689	40.212 ±3.415	$p>0.05$

$p<0.05$= significant

The MDA, TPP, TAP, OSI, vitamin C, vitamin E and total bilirubin levels were not significantly higher in males when compared to their female counterparts ($p>0.05$).

4. Discussion

Findings from this study revealed a 40% frequency of G-6-PD deficiency among cancer patients at the Jos University Teaching Hospital, Nigeria. The prevalence of Glucose 6-phosphate dehydrogenase is high among cancer patients in JUTH. Moreover, it was also observed that 37.5% of apparently healthy subjects were G6PD deficient which is similar to a previous report of 37.6% in Sokoto state of Nigeria [19]. Other studies in the malaria-endemic regions of the world also documented a high prevalence of G-6-PD deficiency [4, 10, 20]. Consequently, the 40% prevalence of G-6-PD deficiency may be unconnected with cancer development.

Among the study participants, G6PD deficiency was more prevalent in males. G6PD deficiency is an X-linked inherited recessive disorder common with men and females who are only affected when it's homozygous or if inactivation of their normal X chromosome occurs, thus females are carriers [21]. In this study, a relatively higher (54.2%) frequency of female cancer patients was G6PD deficient than (45.8%) of the male counterparts as observed. This suggest that inactivation of the X chromosome in G6PD deficient individuals may be involved in the pathogenesis of cancer [21, 22].

Furthermore, this study showed a significant decrease in the total antioxidant potential among cancer patients as compared to the control. This finding corroborated with that of Akiibinu *et al* [23] who reported a significantly low TAP level in prostate cancer but showed a significant increase in oxidative stress index in the patients as compared to the control group. Our findings showed the increase in OSI was attributed to the overall increased activity of the oxidative stress caused by disease in the body system because of DNA damage and oxidants generated which overwhelmed the antioxidant mechanism to effectively control oxidative stress.

Our result also indicated a statistically significant increase in TPP among cancer patients in JUTH who are G6PD normal compared to G6PD deficient controls and reduced TAP in cancer patients with normal G6PD activity and normal controls. OSI in G6PD normal cancer subjects in comparison with controls of both normal and deficient G6PD activity. These findings show that G6PD deficiency has a significant effect on oxidative stress markers, especially the OSI levels indicating the overall oxidative attack on the body system. Oxidative stress causes different diseases via major critical steps including membrane lipid peroxidation, protein oxidation, DNA damage, and disturbance in reducing equivalents of the cell [24-26].

Antioxidants are substances even at a low concentration that significantly inhibit oxidative processes while often being oxidized themselves. The primary function of antioxidants prevent cellular injury, DNA damage, lipid peroxidation, and cancer development. However, our result was different from another report by Haggar *et al* [13] that documented gender as a risk factor in developing cancer which suggests oxidative stress markers activity does not affect cancer individuals based on their gender.

5. Conclusion

Findings from this study showed a high G6PD deficiency among cancer patients and healthy individuals in Jos University Teaching Hospital. Moreover, it also indicated that TAP and OSI were significantly low when compared to the control. Hence, oxidative stress resulting from G6PD deficiency may contribute to the development of cancer, and cancer patients who need therapy that precipitates hemolytic crisis should be screened for G6PD deficiency before treatments (chemotherapy and radiation) that could induce an oxidative attack. These findings showed that host genetic factors could be significant factors for G6PD deficiency, hence suggests the need for further experiments on molecular characterization of mechanisms responsible for the findings.

Abbreviations

ANOVA	Analysis of Variance
CLL	Chronic Lymphocytic Leukemia
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetraacetic Acid

FOX2	Ferrous Oxidation Xylenol Orange
G6PD	Glucose-6-phosphate Dehydrogenase
GSH	Glutathione
GSHPx	Glutathione Peroxidase
Hb	Haemoglobin
HCC	Hepatocellular Carcinoma
H2O2	Hydrogen Peroxide
IDH	Isocitrate Dehydrogenase
JUTH	Jos University Teaching Hospital
ME	Malic Enzyme
MDA-TBA2	Malondialdehyde with Thiobarbituric Acid
Hi	Methaemoglobin
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
OSI	Oxidative Stress Index
PPP	Pentose Phosphate Pathway
ROS	Reactive Oxygen Species
SD	Standard Deviation
TBARS	Thiobarbituric Acid Reactive Substances
TAP	Total Antioxidant Potential
TPP	Total Plasma Peroxide
TCA	Tricarboxylic Acid

Author Contributions

The protocols and methodology used in this study have been reviewed and approved by the ethical research committee of the Jos University Teaching Hospital. All participants gave written informed consent before being recruited in this study.

Conflicts of Interest

The authors declared no conflict of interest.

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