Glucose -6-Phosphate Dehydrogenase (G-6-PD) Status and Kidney Functions Evaluation in Patients with Acute Kidney Injury in Two Tertiary Hospitals, Sokoto

ABSTRACT

Background: Glucose-6-phosphate dehydrogenase deficiency has been implicated in the pathogenesis of a number of common diseases, including acute kidney injury. G6PD deficient individuals are prone to developing acute kidney injury if exposed to certain medication, food or infection. There is high prevalence of G6PD deficiency in Sokoto.

Aim: To determine the G6PD status and evaluate kidney function in patients with acute kidney injury (AKI).

Methodology: This was a case control study involving 206 participants comprising of 106 patients with acute kidney injury and 100 apparently healthy control subjects. The AKI patients were grouped into two; group A: AKI patients with G6PD deficiency, and group B: AKI patients with normal G6PD status. Their blood samples were collected and G6PD status determined by methaemoglobin reduction method, full blood count using Sysmex auto analyser, electrolytes by Ion Selective Electrode, cystatin C by ELISA technique, urea, creatinine and bilirubin using Diacetyl monoxime, Jaffe and Diazo methods respectively.

RESULTS: G6PD deficiency was established in patients with acute kidney injury. The values of Cystatin C, microalbumin, creatinine, urea were significantly higher in AKI patients compared with control, their values were higher in AKI patients with G6PD deficiency. There was no significant difference in bilirubin, and electrolytes values between the patients and control with the exception of bicarbonate which was significantly lower in the patients than the control. Haematocrit was lower while white cell count was higher in the patients than in the control.

CONCLUSION: AKI patients with G6PD deficiency had impaired kidney function profiles which was more pronounced than in AKI with normal G6PD status. Since it is recognized that certain medications can precipitate AKI, and because of high G6PD prevalence, there is need for G6PD screening before commencement of drug to patient whose G6PD is unknown.

Key words: G6PD, acute kidney injury, medication,

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is an intracellular enzyme that guards cell from oxidative stress by catalyzing the first reaction, the oxidation of glucose-6-phosphate to 6-phosphogluconolactone, and concomitantly reduces $NADP^+$ to NADPH, in the Pentose Phosphate Pathway (PPP). Due to lack of mitochondria in red blood cells, pentose phosphate pathway is the only source of producing NADPH [1]. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) functions purposely in reductive biosynthesis and maintenance of cellular redox potential; it is an essential cofactor in many reductive biosynthetic reactions [2]. oMaintenance of glutathione in its reduced form is also a role of NADPH, reduced glutathione (GSH) acts as a scavenger for dangerous oxidative metabolites in the cell, and it converts harmful hydrogen peroxide to water with the help of glutathione peroxidase [3]. If NADPH production is disturbed, sensitivity to reactive oxygen species (ROS) is high and could stimulate apoptosis and necrosis thus highlighting the role of G-6-PD in defending against oxidative damage [4]. G6PD deficiency is a hereditary X- linked disorder and the most common enzymopathy in humans and affects an estimated 400 million people in the world, especially in regions endemic to malaria [5]. Viral, bacterial and malarial infections are the most common triggers, but many drugs, foods, toxins and oxidative stress can also precipitate haemolysis [1]. The most clinically serious public health burden of G6PD deficiency is neonatal jaundice as a result of hyperbilirubinaemia, and puts infants at risk of kernicterus within the first few days of life [6]. G6PD deficiency has been linked to the development of diseases such as hypertension, diabetes, cancer and cardiovascular disease [7]. Studies from various parts of the world document G6PD deficiency prevalence rates as Spain, France and Singapore 1.57, 2.1 and 1.62% respectively were low, while that of Saudi Arabia, Nigeria and American Blacks 18.4, 40 and 14% respectively were high [8]. In earlier studies, the prevalence rates of G6PD deficiency in apparently healthy individuals were Ile-Ife 26.7% [9] Sokoto 37.6% [10] and 30% amongst neonates in Usmanu Danfodiyo University Teaching Hospital, Sokoto [11]. Acute kidney injury (AKI) have been described as a sudden decline in kidney excretory function characterized by elevated blood concentration of creatinine and nitrogenous waste products, with a decrease in urine output and impaired kidney functions to regulate fluid and electrolyte homeostasis (Makusidi *et al* [12] and Anigilaje *et al* [13] . AKI remains a major cause of morbidity and mortality, and can be community acquired, as a result of injury or infection before hospital admission, or can be hospital acquired, arising as a complication of hospital admission [14]. Different mortality rates of AKI have been reported across Nigeria, Makusidi *et al* [12] reported 26.4% in Sokoto , Okunola *et al* [15] 28.8% from Osogbo and Arogundade *et al* [16] 43.2% in Ile-Ife. In G6PD deficient individuals, massive intravascular hemolysis may lead to acute renal failure and acute tubular necrosis might complicate the severe hemolytic episode [17]. Glucose-6-Phosphate dehydrogenase deficiency has been documented to cause jaundice, hemolysis, and AKI secondary to pigment nephropathy after receiving anti-malarial [18]. Acute kidney injury due to intravascular haemolysis have been reported in patients whose hitherto their G6PD status remained unknown, consumed fava beans or unsafe drugs, but were later confirmed to be G6PD deficient during laboratory investigations [19, 20], hence this study was designed to investigate G6PD status and kidney function profile in patients with acute kidney injury in Usmanu Danfodiyo University Teaching Hospital and Specialist Hospital, Sokoto.

PATIENTS AND METHODS

This was a case control study, with a total of 206 subjects, age and gender matched, comprising of 106 patients with acute kidney injury and 100 apparently healthy control subjects. The AKI patients were grouped into two; group A: AKI patients with G6PD deficiency, and group B: AKI patients with normal G6PD status. The remaining 100 apparently healthy control subjects were also classified into two, group C: apparently healthy control with G6PD deficiency, and group D: apparently healthy control with normal G6PD status. The sample size was determined using the formula $n= z^2pq/d^2$, n= the desired sample when the population is greater than 10,000; Z= the desired normal deviate, usually set at 1.96, which corresponds to the 95% confidence level; P = using 6.6% as prevalence rate of AKI [21]; q =1-p; d=degree of accuracy desired, usually set at 0.05. Patients diagnosed of AKI by the consultant nephrologist were recruited for the study and apparently healthy individuals recruited among the staff and students of Usmanu Danfodiyo University Teaching Hospital, Sokoto as control. Patients with smoking habits, alcoholics or have history of chronic kidney disease or any other diseases were excluded from the study. Written informed consent was obtained from all the participants in this study (patients and control). Ethical approval for this study was obtained from the Ethics and Research Committees of Specialist Hospital, Sokoto, with reference number SHS/SUB133/VOL.1

Sample collection and processing

From each subject, 10 ml of blood was collected using a sterile disposable syringe and needle through clean venipuncture. Five ml of blood was transferred into ethylene diamine tetraacetic acid (EDTA) bottle and mixed properly, for G6PD screening and full blood count. The remaining 5 ml was transferred into plain tubes and allowed to clot at room temperature after which it was centrifuged at 3000 rpm for 5 minutes and the sera removed and placed in another plain tube for the analysis of cystatin C, microalbumin, electrolytes (Na^+, K^+, HCO^-_3, Cl^-), urea and creatinine.

Analytical procedures

Glucose-6-phosphate dehydrogenase was determined in EDTA blood sample by Methaemoglobin Reduction Method [22], full blood count (PCV, Haemoglobin, total white blood cell and differential counts) was determined by automated blood analyser (Sysmex KN2IN, Japan, 2007), bilirubin using Diazo method, electrolytes by Ion Selective Electrodes (K- Lite 8 – Series, China, 2018) and cystatin C by ELISA technique. Urea, creatinine, microalbumin, cystatin were measured using techniques of Mohammed and Boyde [23], Spierto *et al* [24], and Elving *et al* [25] respectively.

Data analysis

The data obtained were analyzed using statistical package for social science (SPSS) for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). The data were represented as Mean ± Standard deviation (SD). A *p*-value of less than 0.01 ($p < 0.01$) was considered significant. The results of the groups were compared by using one-way analysis of variance (ANOVA)

RESULTS

Table 1 shows the G6PD status of the AKI subjects and control group in the study population.

The results of the kidney function biomarkers of the study population are shown in table 2. The mean ± SEM of bicarbonate, urea, creatinine, microalbumin and cystatin C were significantly higher in groups A and B as compared with the control groups ($P<0.01$). There was no significant difference in the values of sodium, potassium and chloride in groups A and B as compared to the control groups. There was no significant difference in the values of total and conjugated bilirubin in groups A and B as compared to the control groups (table 3).

PCV was significantly lower in groups A and B as compared with the control group ($p<0.05$) while WBC was significantly higher. There were significant differences in the values of differential leucocyte count between the groups (table 4).

Table 1: G6PD status distribution of the study subjects

G6PD status	AKI group n=106	Frequency (%)	Control group n=100	Frequency (%)
Deficiency				
Male	43	81%	43	86%
Female	10	19%	7	14%
Normal				
Male	45	85%	35	70%
Female	8	15%	15	30%

G6PD= Glucose-6-phoshate dehydrogenase, AKI= Acute kidney injury

Table 2: Kidney Function Profile in G-6-PD deficient Patients with Acute Kidney Injury

	Patients' groups		Control groups		
	A	**B**	**C**	**D**	**P-value**
Parameters					
Na^+ mmol/l	132.21±1.89	133.53±1.52	133.96±0.57	131.94±0.66	p>0.01
K^+ mmol/l	4.10±0.16	4.21±0.14	3.98±0.08	3.95±0.08	p>0.01
Cl^- mmol/l	96.30±1.46	97.51±1.30	98.52±0.53	98.28±0.48	p>0.01
HCO^-_3 mmol/l	18.47±0.23[a,c,e]	19.04±0.32[a,e]	24.24±0.27	24.84±0.33	p<0.01
Urea mmol/l	17.86±0.96[b,d,f]	14.40±1.05[b,f]	4.87±0.18	4.90±0.18	p<0.01
Creat mg/dl	6.16±0.77[b,d,f]	4.92±0.68[b,f]	0.94±0.04	0.91±0.04	p<0.01
Microalb mg/dl	1.04 ± 0.08[b,d,f]	0.42 ± 0.04[b,f]	0.27 ± 0.01	0.29 ± 0.02	p<0.01
Cyst C mg/l	2.51±0.01[b,d,f]	2. 40±0.03[b,f]	0.82±0.13	0.80±0.12	p<0.01

Values are expressed as mean ± SEM; A= AKI with G6PD deficiency, B= AKI with normal G6PD, C= apparently healthy subjects with G6PD deficiency, D= control group with normal G6PD, creat = creatinine, [a]p <0.01= significantly lower compared with group D, [b]p <0.01= significantly higher compared with group D, [c]p <0.01= significantly lower compared with group B, [d]p <0.01= significantly higher compared with group B, [e]p<0.01=significantly lower compared with group C, [f]p <0.01= significantly higher compared with group C.

Table 3: Bilirubin Levels in G-6-PD deficient Patients with Acute Kidney Injury

	Patients' groups		Control groups		
Parameters	**A**	**B**	**C**	**D**	**P-value**
Total bilirubin mg/Dl	0.84±0.46	0.87±0.38	0.80±0.11	0.81±0.09	P>0.01
Conjugated mg/dL	0.36±0.90	0.37±0.89	0.35±0.14	0.36±0.10	P>0.01

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Table 4: Haematological Parameters in G-6-PD deficient Patients with Acute Kidney Injury

	Patients' groups		Control groups		
Parameters	**A**	**B**	**C**	**D**	**P-value**
PCV %	23.36 ± 0.91[a,c,e]	26.40 ± 0.73[a,e]	38.86 ± 0.66	41.86 ± 0.54	P<0.01
WBC x 10^9/l	7.65±2.39[b,f]	7.32±2.28[b,f]	5.57±1.34	5.55±1.30	P<0.01
Neutrophil %	62.45±4.21[b,f]	59.76±3.96[f]	55.39±1.76	57.69±1.51	P<0.01
Lymphocyte %	36.20±3.20[f]	37.23±3.76	42.61±2.13	41.90±2.41	P<0.01
Eosinophil %	1.35±0.90[a,c,e]	2.01±0.89[a]	2.00±0.10[a]	3.01±0.13	P<0.01
Monocyte %	0.00	2.00±0.87	0.00	1.00±0.13	P>0.01
Basophil %	0.00	0.00	0.00	0.00	P>0.01

Values are expressed as mean ± SEM; A= AKI with G6PD deficiency, B= AKI with normal G6PD, C= apparently healthy subjects with G6PD deficiency, D= control group with normal G6PD, [a]p <0.01= significantly lower compared with control (D), [b]p <0.01= significantly higher compared with control (D), [c]p <0.01= significantly lower compared with group B, [d]p <0.01= significantly higher compared with group B, [e]p<0.01=significantly lower compared with group C, [f]p <0.01= significantly higher compared with group C.

DISCUSSION

Glucose-6-Phosphate Dehydrogenase deficiency has been recognized as one of the most common inherited disorders. G6PD is responsible for producing reduced glutathione by catalyzing the first step of the pentose phosphate pathway which is the only antioxidant defense mechanism in erythrocytes. Erythrocytes deficient in G6PD exhaust available reduced glutathione rapidly and become more susceptible to oxidative damage as they expose to oxidative substances. Consequently, ongoing oxidative stress ends up with hemolysis. G6PD deficient subjects generally are asymptomatic unless they are exposed to contraindicated drugs, infections, and certain food [26]. From this study, there was no significant difference in the serum electrolytes levels of the patients and control groups, with the exception of bicarbonate which was significantly lower in the patients' groups than in the control groups, bicarbonate was also significantly lower in G6PD deficient AKI patients than in AKI patients with normal G6PD status. Severe metabolic acidosis have been reported in G6PD deficient acute kidney injury patients [19, 27], which may be due to excessive loss of bicarbonate from the kidney. Although we did not observe hyperkalaemia in our study, it was documented by Khandelia *et al* [27] in their study. Urea, creatinine, microalbumin and cystatin C values in patients' group were significantly higher than the control groups, their values were also significantly higher in G6PD deficient AKI patients than in AKI patients with normal G6PD status; higher values of these biomarkers are indices of impaired kidney function. Increased urea, creatinine, microalbumin and cystatin C values in G6PD deficient AKI patients than in AKI patients with normal G6PD status might have been due to G6PD deficiency. Our observations were similar to the findings of Khandelia *et al* [27], Ruan *et al* [20], and Hakeem *et al* [17]. G6PD deficient patients have been reported to develope acute kidney failure secondary to acute tubular necrosis and tubulointerstitial nephritis due to haemoglobinuria as a complication from severe hemolytic episodes [28, 29]. Although the cause of kidney injury secondary to hemoglobinuria have not been well understood, some studies attribute various factors such as exposure to ferrihemate which is nephrotoxic, converted from hemoglobin when pH is less than 6.5, obstruction of renal tubules by lysed erythrocytes which is nephrotoxic, intravascular coagulation leading to release of thromboplastin factors, impaired renal blood flow, and reduced glomerular filtration rate, or combination of these factors [18, 30]. There was no significant difference in the bilirubin (total and conjugated) values between the patient and the control groups, an indication that the conjugating ability of the liver was intact. From this study, the PCV values of patients group was significantly lower than the control group, it was also significantly lower in G6PD deficient AKI patients than in AKI patients with normal G6PD status. Anaemia is a common finding in patients with AKI, and is primarily due to the shortened red cell survival (from haemolysis associated with the G6PD deficiency), and also because of decreased levels of erythropoietin usually associated with AKI as a result of decrease kidney function. Intravascular haemolysis often occurs in G6PD deficient patients and AKI is a recognized complication in diseases typified by recurrent heamolysis. The WBC was significantly higher in the patients group than the control group; this increase might due to infection. Although in this study, we could not establish acute kidney injury precipitant, studies have linked exposure to contraindicated drugs, certain foods or infection in G6PD deficient individual to the development of acute kidney injury.

CONCLUSION

In conclusion, G6PD deficiency was established in patients with acute kidney injury and had impaired kidney function profiles, and more pronounced in G6PD deficient AKI patients than in AKI patients with normal G6PD status. Due to high prevalence of G6PD in Sokoto, certain medications can expose numerous patients with unknown G6PD deficiency to severe haemolytic crisis, hence G6PD screening is recommended before commencement of medications that could lead to acute kidney injury secondary to intravascular haemolysis in G6PD deficiency.

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