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RESEARCH ARTICLE

PREVALENCE OF G6PD DEFICIENCY IN PATIENTS WITH CHRONIC KIDNEY DISEASE OF UNKNOWN ORIGIN IN NORTH CENTRAL REGION OF SRI LANKA: CASE CONTROL STUDY

Jayasekara JMKB[1], Dissanayake[2] DM, Gunaratne MDN[3], Sivakanesan R[4], Dissanayake DMTS

[1]Department of Pathology, Faculty of Medicine, University of Peradeniya, Sri Lanka
[2] Department of Pathology, Faculty of Medicine, University of Peradeniya, Sri Lanka
[3]Department of Mathematics, Faculty of Engineering, University of Moratuwa, Sri Lanka
[4] Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Sri Lanka
[5]SACTRC, Faculty of Medicine, University of Peradeniya, Sri Lanka

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ABSTRACT

The study was initiated with the objective to identify the role of G6PD deficiency in chronic kidney disease patients with unknown etiology (CKD-U) in North Central Region (NCR) of Sri Lanka. 104 recently diagnosed, biopsy proven CKD-U patients (cases) and 208 age and sex matched controls were selected from same areas.

Demographic information were collected and G6PD activities were determined in both cases and controls. The severity of G6PD deficiency was interpreted. Collected information were analyzed by using two-way ANOVA experimental design model and mean separation. The mean G6PD activity of the case group was significantly lower ($p < 0.001$) than the control group. Twenty percent (20%) of the patients had G6PD deficiency whereas only 2% in control group. Smoking, histories of malaria, alcohol consumption were significantly contributed for the disease. Prevalence of G6PD deficiency was high among CKD-U patients and which may play a major role in the pathogenesis of disease.

INTRODUCTION

Glucose-6-phosphate Dehydrogenase (G6PD) deficiency is the commonest enzymopathy in the world (Sarkar *et al.,* 1993). The enzyme G6PD catalyzes the entry step of G6P into the Pentose Phosphate Shunt that is the only source for NADPH, which is required to maintain an effective redox potential protecting red cell membrane against oxidative stress and injury. The highest prevalence rates (with gene frequencies from 5-25%) of glucose-6-phosphatase dehydrogenase (G6PD) deficiency are found in tropical Africa, the Middle East, tropical and subtropical Asia, some areas of the Mediterranean, and Papua New Guinea(Luzzatto *et al.,*1969). The severity of glucose-6-phosphatase dehydrogenase (G6PD) deficiency varies significantly among racial groups because of different variants of the enzyme. Severe deficiency variants primarily occur in the Mediterranean population (Gray, 1973; Beutler, 1990). However the prevalence of G6PD deficiency in Sri Lankan population is still unidentified.

Individuals with G6PD deficiency can present with a spectrum of disorders including acute massive haemolysis due to drugs, haemolysis complicating illness such as viral hepatitis, Favisam, herb and chemical induced haemolysis, neonatal jaundice and congenital non spherocytic haemolytic anaemia. Deaths due to acute renal failure are well documented in patients with massive haemolysis due to tubular necrosis, especially in those with underlying diseases of the liver such as hepatitis (Vives-Corrons *et al.,* 1982).In G6PD deficient individuals, their tissue G6PD enzyme levels are also lower than normal in leukocytes, platelets, liver, kidneys and adrenals. However there are alternate pathways in nucleated cells for the generation of NADPH, most of these subjects do not suffer from any other cellular dysfunction or disease. In some variants of G6PD deficiency with absence of leukocyte G6PD, there may be abnormal leucocytes function and such subjects present with proneness to infection, similar to chronic granulomatous disease (Gray, 1973).

Another study reported that patients with viral hepatitis and thyrotoxic periodic paralysis (TPP) have a disproportionally high incidence of G6PD deficiency. Severe jaundice in G6PD subjects with viral hepatitis results in an increased admission rate into hospital. Although TPP patients are usually males and there have been reports of familial tendency. It would seem that the genetic predisposition to TPP is linked to the G6PD deficient gene in the Southern Chinese (McFadzean and Yeung, 1969). The distribution of G6PD deficiency is similar to thalassaemia and is thought to be due to the selective advantage of these phenotypes against endemic malaria infection in the past. Luzzatto *et al* 1969; have shown that G6PD-deficient cells are protective against malaria and both homozygous female and hemizygous males should also be

* Corresponding author**:** Tel: +94776971907, +94812387572
E-mail address: kbjayasekara@gmail.com

protected and has a survival advantage against malaria endemics.

Twenty years ago health researchers in Sri Lanka have observed high incidence of new form of chronic kidney disease of unknown etiology (CKD-U) has emerged in high malaria endemic areas in past. CKD-U was identified as interstitial nephritis which suggested a toxic etiology (Wanigasuriya *et al.,* 2007) and common etiologies such as diabetes and hypertension were not contributed. Though the population at risk is scattered in the North Central Region (NCR), large number of patients have been detected in Medawachchiya, Padaviya and Girandurukotte, Medirigiriya and Nikawewa areas which are high endemic areas for malaria from 1900s. Moreover the epidemics occurred in 1906, 1911, 1914, 1919, 1928, 1934/35, 1936-1946, 1968/1969 and 1987 and large number of deaths due to malaria were also reported. Thus remained population after epidemics was more susceptible for G6PD deficiency because G6PD deficiency patients had more survival advantage than others during the malaria endemics in this region (Report of anti malaria campaign-2007).

The survey carried out by Abeyrathna et al in 1976 reported that, 20.7% had G6PD deficiency in ancient villages in North Central Region of the country where CKD-U is prevalent now. Over 3% were noticed among resettled areas in NCR and over 5% were noticed among school children.

However the etiology of the CKD-U in North Central Region of Sri Lanka still remains a mystery (Herath *et al.,* 2005). According to the studies carried out by scientist in Sri Lanka, high groundwater fluoride content, bleaching of heavy metals such as cadmium from chemical fertilizers into water sources, usage of aluminum vessels to store drinking water were postulated as risk factors for CKD-U and some studies clearly indicate that the disease is affecting mainly the male farming community in some parts of the North Central Region(NCR) of Sri Lanka and familial occurrence also significantly contributed for the disease (Chandrajith *et al.,* 2010).
Previous studies have shown that a significant number of CKD patients have family members with the same disease and no further investigations were carried out to find whether it is due a genetic factor or due to the exposure to the same etiological factor. One such possibility is Glucose-6-phosphate Dehydrogenase (G6PD) deficiency which is the commonest enzymopathy in the world and also identified high in CKD-U high prevalence areas. The literature showed that G6PD deficiency is so far related only to acute renal failure and no association with chronic renal failure was found (Sakhuja *et al.,* 1999.). Hence excisting study was initiated with the objective to identify the role of G6PD deficiency in CKD patients with unknown etiology in the areas of NCR.

MATERIALS AND METHODS

The ethical clearance for the study was taken from the Ethical review committee, Faculty of Medicine, University of Peradeniya and informed consent was taken from each individual who participated in the study. List of biopsy proven CKD-U patients (600 patients) were traced from renal clinics in NCR (Medawachchiya, Padaviya and Girandurukotte clinics) for the study and one hundred and four (104) recently diagnosed patients were selected randomly by using random number table. Two hundred and eight (208) age and sex matched healthy individuals were selected as controls from the adjecent house of each CKD-U patient. CKD due to hypertension, diabetes or any other identifiable cause was excluded. Patients who had blood transfusions during last two months were excluded. The diagnostic criteria used for CKD-U includes absence of diabetes mellitus, hypertension, urinary tract infections or other renal diseases in the history, presence of proteinuria on two occasions ,decreased GFR , presence of radiological and pathological evidence for interstitial nephritis (Biopsy proven). Healthy individuals(absent of any chronic disorder) were selected after two consecutive urine samples showed absence of proteinuria by using urine protein turbidimetric assay (urine protein < 15 mg/dl, detection limit 4-200mg/dl, sensitivity 4mg/dl).

The demographic data (Age, sex, occupation) and history of haematuria, malaria, snake bite, use of Indigenous medicine (Ayrurvedha treatment with duration, type etc) were collected from both cases and controls by using interviewer administered questionnaire. Family history of CKD-U from both cases and controls were also collected with pedigrees for three or more generations. Three (3) ml of blood was collected into EDTA tubes for G6PD assay and assays were done on both cases and control groups. The G6PD enzyme activity was determined by measurement of the absorbance change at 340 nm due to the reduction of NADP in peripheral blood samples. Full blood counts of all blood samples were carried out by using Mindray fully automated (5500-series) Heamatology analyzer. G6PD activity was expressed as mU per 10^9 Red blood cells and laboratory mean G6PD activity was established by using 40 healthy individuals (20 males and 20 females) from CKD-U low prevalence areas. The severity of G6PD deficiency was interpreted according to the laboratory mean. Comparison of G6PD levels and other collected information between case and control group were analyzed by using two-way ANOVA experimental design model and mean separation with LSD (Least Significant Different) method.

RESULTS

One hundred and four (104) patients selected randomly included 80 male and 24 female. The age ranged from 9 to 64 years and mean age was 44 $\pm$10 years. One hundred and sixty (160) male and 48 female healthy individuals included as control group with mean age of 43$\pm$9 years. Mean G6PD activity (LSD) of both cases and controls were analyzed and compared. The mean G6PD activity of the case group (185.78 mU/10^9 RBC) was significantly lower ($p < 0.001$) than the control group (215.71 mU/10^9 RBC). The following differences were noticed in both male and females in cases and controls respectively however no significant difference was noticed between two female groups and between male and female in control group (Table 1).

Table 1 Difference of G6PD levels among male and female in both case and control groups

case / control	Sex	Mean G6PD activity(LSD) mU/10^9 RBC	P value
Case	Male(80)	175.31 [a]	[a]& [b] , p<0.01
	Female(24)	196.25 [b]	[a] &[c] , p< 0.01
Control	Male(160)	222.38 [c]	[b] & [c], p< 0.01
	Female(48)	209.03 [bc]	[a] & [bc], p< 0.01

a, b, c,d,- different G6PD activities

Normal G6PD activity for the laboratory was established as 225±16 mU/10^9 RBC by analyzing 40 healthy individuals selected from CKD-U non affected area (20 males and 20 females) and G6PD activities of patients and controls of the study were compared using the above laboratory mean. Prevalence of G6PD deficiency among CKD-U patients and controls were estimated separately and G6PD activity less than 10% of the laboratory mean who considered as severe deficiency, 10-40% as moderate, 40-60% as mild and over 60% as normal activity (Ruwende, 1998 ; Gregg, 2000). (Figure1).

(Wanigasuriya *et al.,* 2007). Further smoking, histories of malaria, alcohol consumption were significantly contributed for the disease compared with the controls which increase the susceptibility to renal disease. Whereas history of snake bite was not significantly contributed. The literature showed that G6PD deficiency only related to the acute kidney failure no associations were found with chronic kidney disease. However elevated urinary albumin was detected in G6PD deficiency mouse model that showed G6PD deficiency alone can promote persistence albuminuria.(Fornoni, 2008; Suliman,2008) which is the early indicator of chronic kidney disease.

Table 2 Odds ratios of other risk factors

Risk factor (variable)	Level	Case n (%)		Control n(%)		Odds ratio	P value	95 % CI Lower limit-upper limit
Smoking	Yes	56	54%	76	37%	2.02	0.0038	1.25-3.26
	No	48	46%	132	63%	1.00		
Alcohol consumption	Yes	54	52%	70	34%	2.13	0.0020	1.31-3.44
	No	50	48%	138	66%	1.00		
History of Malaria	Yes	70	67%	108	52%	1.91	0.0101	1.16-3.11
	No	34	33%	100	48%	1.00		
History of Snake bites	Yes	26	25%	40	19%	1.40	0.2407	0.80-2.45
	No	78	75%	168	81%	1.00		
Family history of CKD-U	yes	38	36%	38	18%	2.57	0.0005	1.51-4.38
	No	66	64%	170	82%	1.00		

Four (4%) percent of CKD-U patients had severe G6PD deficiency and 6% and 10% moderate and mild deficiency respectively (total 20%). However the control group showed only 2% of both moderate and mild deficiency. The demographic data (Age, sex, occupation) and history of haematuria, malaria, snake bite, usage of Indigenous medicine (Aryurvedha treatment with duration, type etc) were collected from both cases and controls and analyzed. The following table showed comparison of above parameters. Table 2-Odds ratios of other risk factors Thirty six percent (36%) of the patient group and only 18% of controls had family history of CKD-U (table 2) however pedigree analysis showed no evidence of clear Mendelian inheritance in both groups.

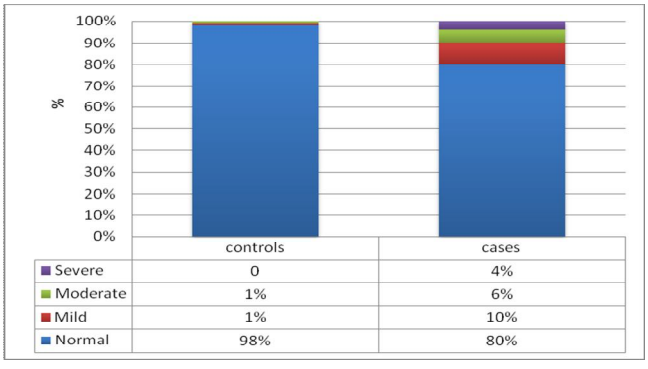

Figure 1 prevalence of G6PD deficiency among cases and controls

DISCUSSION

The results of the current study showed that prevalence of G6PD deficiency was high among CKD-U patients than healthy individuals and G6PD activity was significantly low in patients group. Male patients showed lowest G6PD activity (table 2) adversely males were mostly affected than females with CKD-U in high prevalence areas with the ratio of 2.4 to 1

The familial occurrence of the disease is also evidenced in the pedigree analysis with no indication of clear Mendelian inheritance could be due to exposure of the siblings to the etiological agent rather than direct genetic/inherited background for the disease and G6PD mutations needs to be identify in future in both CKD-U patients and healthy individuals to confirm the findings of the current study.

Finally we can conclude that G6PD deficiency may play a considerable role in the pathogenesis of chronic kidney disease due to unknown origin.

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