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Abnormal findings on dipstick urinalysis of out-patients with malaria in Abakaliki, Nigeria

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ABSTRACT

Background & objectives: Malaria, one of the major health challenges of the tropics affecting about 500 million people, particularly the children and pregnant women have been associated with changes in urine compositions. The present study was undertaken to document the urinary abnormalities in malaria patients based on malaria species and the level of malaria parasitaemia.

Methods: Febrile patients (n = 365) with positive Giemsa-stained blood films for malaria recruited from Out-patient Department of Ebonyi State University Teaching Hospital, Abakaliki participated in the study. Patients were classified into two categories (+ and ++) based on parasite density. Apparently healthy individuals (n = 81), without malaria parasite on both thick and thin films of comparable age and gender acted as control group. Urine sample (10 ml) was collected from each participant and analysed using standard laboratory methods and techniques.

Results: Seventy-four (20.3%) of the patients had *Plasmodium falciparum* malaria. Although all the urine parameters were higher in the malarial patients in comparison to the control, only bilirubinuria and urobilinogenuria were statistically significant (p <0.05). Also, bilirubinuria, urobilinogenuria, haematuria and proteinuria were significantly (p <0.05) higher in *P. falciparum* infection than in infections with other malaria species, but only in *P. falciparum* infection, bilirubinuria and urobilinogenuria were significantly (p <0.05) higher at higher parasitaemia.

Conclusion: Even though positive blood film for malaria parasite remains the gold standard for the diagnosis of malaria, urinary abnormalities, such as bilirubinuria, urobilinogenuria, proteinuria and haematuria may aid in identifying patients with severe malaria parasitaemia, especially the falciparum malaria.

Key words Bilirubinuria; jaundice; falciparum malaria; urinalysis; urobilinogenuria

INTRODUCTION

Malaria remains one of the major health challenges of the tropics, affecting about 500 million people, particularly the children and pregnant women[1], and with greatest impact in Africa, India, Southeast Asia and Latin America. 'Imported malarias' have also been reported in some regions in Europe and North America due to increased immigration of natives of these malaria endemic regions[2]. Malaria, a protozoan disease caused by a unicellular parasite of the genus, *Plasmodium* is transmitted through the bite of an infected *Anopheles* mosquito[3]. Four species of human malaria parasites have been identified, namely *P. falciparum, P. vivax, P. malariae* and *P. ovale*[4]. However, in Africa, *P. falciparum* has been reported as the most widely spread species with high case fatality, accounting for about 1–3 million deaths each year worldwide[5].

Urine analysis, the first of all laboratory tests, still remains a most valuable and highly important means of diagnosis in clinical medicine[6]. For instance, studies have shown that positive urine tests for haematuria and/or proteinuria in mass screening settings were significant predictors of end-stage renal disease[7]. Malaria nephropathy presenting as nephrotic syndrome has been reported in patients having *P. malariae* infection from Nigeria, Uganda and Yemen[8]. It has also been reported that transitory acute glomerulonephritis occurs often in association with *P. falciparum* infection. Microscopic haematuria, mild proteinuria usually <1 g in 24 h has been encountered in as many as 25–50% of malarial patients. In Kassala town of Eastern Sudan, Karoum and Mohammed[9] reported albuminuria (71.2%), pyuria (53.8%), haematuria (45% and granular casts (71.4%) in patients with malaria infections. These observations led the authors to suggest that malaria may have significant effect on urine, especially, the presence of albuminuria and to a lesser extent haematuria. Among the population of malaria endemic areas, self diagnosis and treatment of malaria has partly been based on yellow colouration of urine. However, studies that documented urinary abnormalities in malaria infection are few and the extent to which malaria species and the degree of

parasitaemia affects urinary composition in patients with malaria infections remains largely unknown. The aim of the present study, therefore, is to document the urinary abnormalities in malaria patients based on malaria species and the level of malaria parasitaemia.

MATERIAL & METHODS

This study was conducted at the Outpatient Department of Ebonyi State University Teaching Hospital, Abakaliki. Malaria transmission in the area is perennial but usually at peak towards the end of the rainy season. Ethics and Research Committee of Ebonyi State University Teaching Hospital approved the protocol of the study. The procedures, effects and benefits of the study were explained to the participants after which their oral consents were obtained. For recruitment of patients, every consecutive febrile patient was rapidly screened by a new generation immunochromatographic assay technique (ICT). The assay was done with commercial kit (ACON Laboratories Inc., San Diego, USA) in accordance with manufacturer's instructions. For confirmation of malaria infection/species and counting of malaria parasites, each blood sample was analysed for malaria parasite infection by performing the microscopy of Giemsa-stained thick and thin blood films. The Plus System was used for the determination of parasite density in accordance with WHO standard[10]. In all, 402 patients were recruited for the study.

Additionally, medical and sociodemographic information of patients was obtained by a questionnaire. A total of 37 patients were excluded from the study based on medical history. They comprised of 21 with symptoms suggestive of urinary tract infection, 8 with history of renal disease, 5 with history of liver disease (viral hepatitis), and 3 HIV-seropositive patients.

In all, 365 patients (170 males and 195 females) with malaria were eventually included in the study. Patients were classified into two categories: + and ++ based on parasite density and 81 apparently healthy individuals (without malaria parasite on both thick and thin films) of comparable age and gender, selected from staff and students of Ebonyi State University Teaching Hospital acted as control group.

Clean-cached urine sample (10 ml) was collected from each patient. Standard laboratory methods and techniques were used to analyse urine samples. Briefly, aliquot of 5 ml of the unspunned sample was tested with dipstick (Medi-Test Combi 9, MACHERY-NAGEL GmbH, Duren) strictly according to the procedure specified by the manufacturer. Leucocyte esterase was recorded positive in the presence of any shade of colour change after 2 min. Bilirubin, urobilinogen, protein, and blood were considered positive if there was any colour change during one minute. Packed-cell volume (PCV) was determined as previously described[11]. All malarial patients were treated appropriately by the attending physician.

Statistical analysis

The data generated were analysed by SPSS® for Windows® ver 16.0 (SPSS Inc., Chicago, IL, USA). Differences between the groups were compared using Student's *t*-test and $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows that comparable numbers of male and female were selected for the subjects and controls and 74 (20.3%) of the malarial patients were infected with *P. falciparum*. Although the malarial subjects and controls were of comparable age, the former had significantly ($p < 0.05$) lower packed cell volume and haemoglobin concentration in comparison to the latter (Table 2).

From Fig. 1, although all the urine parameters were higher in the malarial patients in comparison to the controls, only bilirubin and urobilinogen were statistically significant ($p < 0.05$). Figure 2 shows the urine abnormalities in relation to species of malaria parasite. Urine bilirubin, urobilinogen, blood and protein were significantly ($p < 0.05$) higher in *P. falciparum* infection in comparison to infection with other malaria species. However, urine

Table 1. Sex distribution of malarial subjects and controls

Subjects	Total	Male	Female
P. falciparum	74	36 (48.6)	38 (51.4)
Other species	291	129 (44.3)	162 (55.7)
Control	81	39 (48.1)	42 (51.9)

Figures in parentheses are percentages

Table 2. Comparison of age and packed-cell volume between malarial subjects and control*

Parameters	Control (n = 81)	Malarial subjects (n = 365)	*p*-values
Age (yr)	32.7 ± 36.1	27.8 ± 20.5	0.093
PCV (%)	40.5 ± 5.07	36.0 ± 5.99	0**
HbC (g/dl)	13.5 ± 1.23	11.9 ± 1.19	0.001**

*Values are presented as mean ± standard deviation; **$p < 0.05$; PCV: Packed-cell volume; HbC: Haemoglobin concentration.

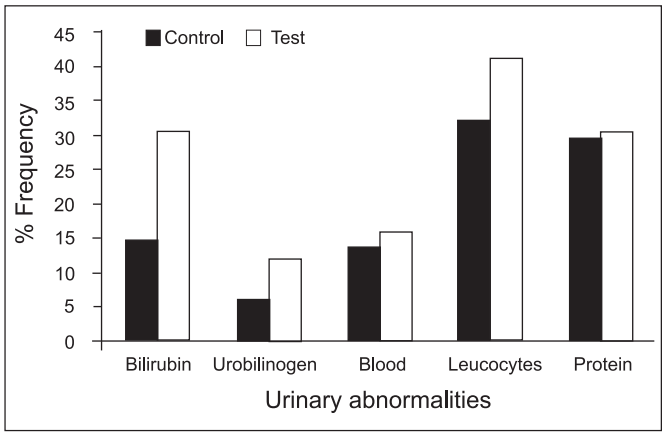

Fig. 1: Comparison of urine abnormalities between control and malarial subjects

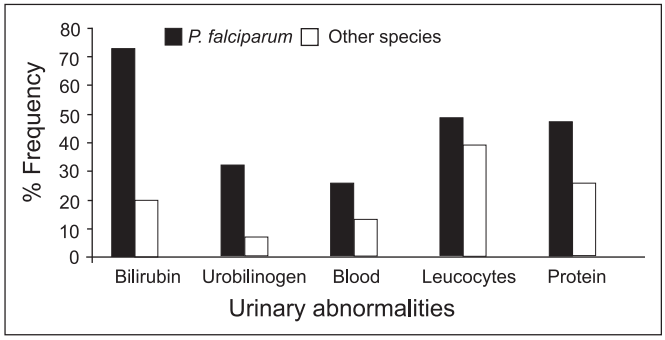

Fig. 2: Comparison of urine abnormalities between patients infected with *P. falciparum* and other species of malaria

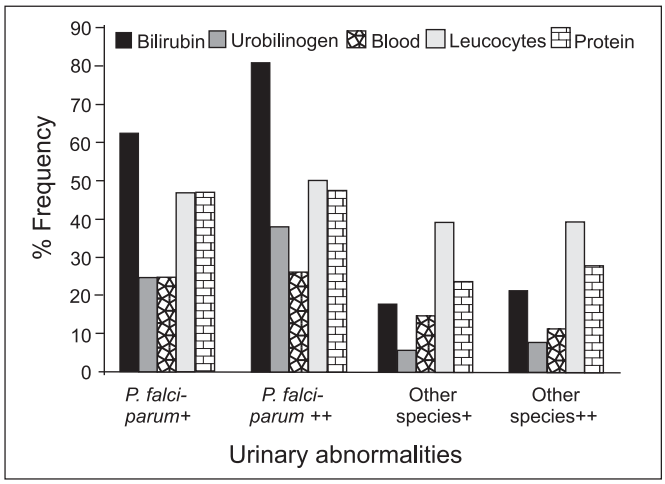

Fig. 3: Urine abnormalities in relation to malaria parasitaemia

leucocytes were comparable (p >0.05) among the two groups.

Although regardless of malaria species, urinary abnormalities seem to increase with malaria parasitaemia (Fig. 3) but only in *P. falciparum* infection urinary bilirubin and urobilinogen were significantly higher at higher malaria parasitaemia.

DISCUSSION

This study has documented significantly higher urinary bilirubin and urobilinogen in malarial patients in comparison to healthy controls. Moreover, patients infected with *P. falciparum* excreted significantly higher bilirubin, urobilinogen, blood and protein than do patients infected with other species of malaria, with abnormalities increasing with malaria parasitaemia, especially in *P. falciparum* infection. Also of note is the prevalence of *P. falciparum* (20.3%) and the significantly lower packed cell volume and haemoglobin concentration observed in malarial subjects in comparison to the controls. According to Ahsan *et al*[12], jaundice is the leading clinical presentation of complicated falciparum malaria.

In the present study, significantly high incidence of urinary bilirubin and urobilinogen in malarial subjects in comparison to controls suggests either hepatic involvement or haemolysis. Moreover, the significantly higher incidence of urinary bilirubin, urobilinogen, blood, and protein in patients infected with *P. falciparum* in comparison to patients infected with other species of malaria indicates that falciparum malaria may be one of the causes of hepatic disorder and severe jaundice in malarial patients in this part of the world. Study has shown that in patients presenting with fever and mainly conjugated hyperbilirubinaemia, there should be a high index of suspicion for falciparum malaria even in the face of negative blood films[12].

Malarial involvement of liver is now a known entity with its specific histopathological changes and cases with altered liver function test[13,14]. Although in the present study, determination of plasma bilirubin and liver enzymes were not part of the original design, the appearance of bilirubin in urine shows that it is of the conjugated type and by extrapolation, there was conjugated hyperbilirubinaemia in malarial subjects in comparison to the controls, which was significantly higher in *P. falciparum* infection than in other forms of malaria infection. Jaundice in malaria infection is caused by many factors including intravascular haemolysis of parasitized red blood cells, haemolysis of non-parasitized red blood cells (innocent bystanders), possible micro-angiopathic haemolysis associated with disseminated intravascular coagulation (DIC), hepatic dysfunction, associated haemoglobinopathies (not uncommon in malaria-prone areas), drug-induced haemolysis (including quinine, etc.), G-6-PD deficiency, etc.

The other causes of jaundice in malaria could be coexistent viral hepatitis[15]. However, none of our patients had viral hepatitis and the increased bilirubinuria and uro-

bilinogenuria observed in the present study cannot be attributed to viral co-infection. It has been shown that intravascular haemolysis of parasitized and non-parasitized red blood cells is considered as an important factor in the causation of mild to moderate jaundice with the bilirubin predominantly of the unconjugated type[16]. However, predominantly conjugated hyperbilirubinaemia has been reported in *P. falciparum* malaria[13,17]. Apart from intravascular haemolysis and DIC, hepatocellular jaundice secondary to histopathological changes of liver in malaria have been reported. Coincidentally, we observed significantly lower PCV and HbC in malaria subjects in comparison to the controls, reaffirming anaemia as an accompaniment of malaria infection[18]. Anaemia in our malarial patients may partly be attributed to increased destruction of red blood cells, thus, buttressing the fact that the observed bilirubinuria and urobilinogenuria may be associated with intravascular haemolysis. Although demonstration of bilirubinuria and urobilinogenuria in malaria patients may be a big relief for clinicians working in malaria endemic resource-limited countries as they may be of diagnostic and prognostic value as surrogate markers of bilirubinaemia, the clinical utility of rapid testing for bilirubin and urobilinogen in urine need to be confirmed in future studies.

Significantly higher incidence of haematuria and proteinuria in *P. falciparum* infection in comparison to infection with other species of malaria parasites observed in the present study is in agreement with the findings of other researchers[3,9]. This also suggests renal impairment in malaria infection, which is more in falciparum infection. Haematuria and proteinuria in malaria patients have been associated with immune complex nephritis[19]. Although renal diseases such as nephritis are common in developing countries, where survey screening has shown a high prevalence for proteinuria with or without haematuria[20,21] the simultaneous occurrence of haematuria and proteinuria in our subjects has important clinical implications. However, we are constrained to relate our findings to the likelihood of renal impairment in our malaria patients due to lack of data on renal function tests. Nevertheless, renal impairments, such as glomerulonephritis and nephrotic syndrome have been reported in malaria patients, which have been attributed to increased capillary permeability as a result of systemic inflammatory response[1,22] or mild impairment in electrolyte and fluid balance[23].

CONCLUSION

We, therefore, conclude that even though urinalysis is not an alternative diagnostic tool for malaria infection, urinary abnormalities, such as bilirubinuria, urobilinogenuria, proteinuria and haematuria may help in identifying patients with severe malaria parasitaemia, especially the falciparum malaria. This preliminary findings need to be confirmed in a well designed study as it may be of value in the management of malaria patients in resource constrained settings of developing countries.

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