Dipstick urinalysis findings in children with *Plasmodium falciparum* in the South Tongu District: A case-control study

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ABSTRACT

Background: Malaria ranks among the major health and developmental challenges facing some of the poorest countries in tropical and sub-tropical regions across the globe. We determined urinary abnormalities and its relationship with parasite density in children ≤12 years with *Plasmodium falciparum* infection. **Materials and Methods:** From December 2013 to March 2014, we randomly recruited 116 participants comprising 58 malaria patients (cases) and 58 healthy controls from the Comboni Mission and the Sogakope District Hospitals both in the South Tongu district. Blood was collected for the estimation of hemoglobin and total white blood cells; thick and thin blood films were used for the determination of malaria parasite density. Urine was collected for the measurement of the various biochemical components using the automated urine analyzer. A pretested questionnaire was used to obtain demographic and clinical data. **Results:** Urine protein ($P < 0.001$), blood ($P < 0.001$), bilirubin ($P < 0.001$), urobilinogen ($P < 0.001$), and ketones ($P = 0.001$) were significantly higher in individuals with *P. falciparum* infection than in healthy controls. Proteinuria ($P = 0.247$; $r = 0.155$), hematuria ($P = 0.142$; $r = 0.195$), bilirubinuria ($P = 0.001$; $r = 0.438$), urobilinogenuria ($P = 0.876$; $r = 0.021$), and ketonuria ($P = 0.136$; $r = 0.198$) were positively correlated with malaria parasite density; however, only bilirubinuria was significantly higher at higher parasitemia. **Conclusion:** Malaria has a significant effect on the chemical composition of urine with bilirubin positively correlated with parasite density. Dipstick urinalysis can be used together with light microscopy in resource-limited malaria-endemic areas to accurately diagnose falciparum malaria infection.

Key words: Children, dipstick, malaria, *Plasmodium falciparum*, urinalysis

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INTRODUCTION

Malaria ranks among the major health and developmental challenges facing some of the poorest countries in the tropical and sub-tropical region across the globe.[1] An estimated 3.3 billion people were at risk of malaria in 2010, although of all geographical regions, population living in sub-Saharan Africa have the highest risk of acquiring malaria; in 2010, 81% of the cases and 91% of the deaths are estimated to have occurred in the world health organization

(WHO) African Region with children under 5 years of age and pregnant women being most severely affected.[2]

In Ghana, malaria is one of the leading causes of mortality in children under 5 years.[3] Similarly, studies have indicated that about 3.5 million people suffer from malaria every year, and approximately 20,000 children die from malaria

annually with 25% of the deaths occurring in children under the age of 5 years.[4]

Urine analysis remains the most valuable and highly important means of diagnosis in clinical medicine, as it helps to detect many diseases even before symptoms appear.[1]

Liver dysfunction and renal impairment have been reported in severe malaria infections,[5,6] and these observations led to the suggestion that malaria infection may have a significant effect on urine composition.[1] Studies conducted in Nigeria and Sudan examined urinary abnormalities among the general population with malaria infection and reported parasite density using the semi-quantitative (plus) system instead of the standard quantitative system.[1,7] However, studies on urinary abnormalities in *Plasmodium falciparum* infection, and the extent to which the degree of malaria parasitemia affects urinary composition in Ghanaian children remains scarce. Considering the endemicity of malaria in Ghana, and the associated mortality and morbidity, particularly in children and pregnant women, accurate diagnosis and proper management are very necessary. It is imperative, therefore, to determine urine abnormalities and its relationship with parasite density in children with *P. falciparum* infection in a resource-limited malaria-endemic setting.

MATERIALS AND METHODS

This randomized study was conducted at Comboni Mission and Sogakope District Hospitals, in the South Tongu District of the Volta region from December 2013 to March 2014. A total of 116 participants (age- and sex-matched) comprising 58 children aged ≤12 years with confirmed falciparum malaria (cases) and 58 healthy children (controls) were recruited. Children with other variants of malaria and those with ailments such as renal disease, liver disease, or urinary tract infection were excluded. Medical and sociodemographic data were obtained with the aid of a structured pretested questionnaire. The Institutional Review Board of University of Cape Coast and the Ethical Committee of the Hospitals concerned approved the study. Written informed consent was obtained from the participants before recruitment into the study.

Venous blood (3 ml) was obtained for the determination of hemoglobin (HGB), white blood cell count (WBC), and malaria parasite density. Smears for malaria parasite were stained with Giemsa and examined using standard protocols.[12] HGB concentration and total WBC count were estimated using the Mindray auto-hematology analyzer BC-3000 Plus (Shenzhen, China). Malaria infection was grouped into mild/moderate (parasite density <7000/μL) and severe (parasite density >7000/μL).[5]

Dipstick urinalysis strips (Accu-Tell Ref ABT-UM-A33) was used to detect the following parameters in urine provided by the participants; protein, glucose, bilirubin, urobilinogen, ketones, blood, and leukocytes. The outcomes were measured with the Mindray urine analyzer UA-66 (Shenzhen, China).

IBM SPSS Statistics for Windows, Version 21.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Continuous variable estimates were expressed as mean ± standard error mean (SEM), and Student's *t*-test used to determine the variation amongst healthy individuals and malaria patients while Mann–Whitney U-test was used for the analysis of categorical variables. Spearman's rho correlation coefficient was used to show correlation of the parameters measured. Statistical significance level was set at $P < 0.05$.

RESULTS

Table 1 summarizes the mean age, HGB concentration, and parasite density of the study participants. The participants included 58 malarial patients (29 males and 29 females) and 58 controls (29 males and 29 females), indicating that comparable numbers of malaria cases and controls were selected for this study. The independent sample *t*-test showed no significant difference between mean ages of cases and controls. Although the malaria cases and controls were of comparable age, the former had significantly ($P < 0.001$) lower HGB concentration than the latter. There was a high mean parasite density in the cases, signifying severe infection of the participants with malaria.

Table 2 categorizes malaria infection among the cases into two, those with mild infection and those with severe infection. The majority of the cases (86.2%) were severely infected.

Table 3 shows the percentage distribution of the urinary parameters of the study participants. Although the concentration of all the urine parameters were elevated in the cases in comparison to the controls, only protein, blood, bilirubin, urobilinogen, and ketones were statistically significant.

Table 1: Demographic, hemoglobin concentration, and parasite density study participants

Parameter	Cases (*n* = 58)	Controls (*n* = 58)	*P*
Age (years)	6.3±0.5	6.4±0.5	0.986
HGB (g/dL)	9.5±0.25	12.0±0.14	<0.001
Parasite density/μL	110,912.21±18,146.60	—	—

P > 0.05 – Not significant; *P* < 0.05 – Significant; Values are reported as mean ± SE of mean for "*n*" number of children. HGB – Hemoglobin; SE – Standard error

Table 2: Parasite density of participants with malaria

Category	*n* = 58	Parasite density/μL
Mild/moderate infection (<7000/μL)	8 (13.8)	22,882.75±799.29
Severe infection (>7000/μL)	50 (86.2)	128,292.92±19,991.01

Parasite density <7000/μL – Mild/moderate infection; Parasite density >7000/μL – Severe infection

Figure 1 is a graphical (visual) presentation of the comparison of the significant dipstick urinalysis findings between cases and controls.

The dipstick urinalysis findings in relation to parasite density of the participants is summarized in Table 4. The Spearman's rho correlation test showed positive correlation between proteinuria, hematuria, bilirubinuria, urobilinogenuria, and ketonuria with malaria parasite density. However, only bilirubinuria was significantly ($P = 0.001$) higher at higher parasite density. Glycosuria and leukocyturia were negatively correlated with malaria density.

DISCUSSION

Accurate diagnosis of malaria is integral to the appropriate and effective treatment of affected individuals and in the reduction of malaria-related morbidity and mortality.[13-15]

This study related dipstick urinalysis findings to malaria parasite density, with the aim of using dipstick urinalysis in conjunction with other diagnostic tests to accurately diagnose malaria in resource-limited malaria-endemic settings. Our findings indicated a significantly higher urine protein, blood, bilirubin, urobilinogen, and ketones in individuals with *P. falciparum* infection in comparison to healthy controls. Although proteinuria, hematuria, bilirubinuria, urobilinogenuria, and ketonuria were positively correlated with malaria parasitemia, only bilirubinuria was significantly ($P = 0.001$) higher at higher parasitemia.

The presence of significant bilirubinuria and urobilinogenuria in malaria patients is similar to observations made in other West African countries[1] and is suggestive of either hepatic involvement or hemolysis. However, this is attributable to malaria since bilirubinuria and urobilinogenuria were positively correlated with

malaria parasite density, with bilirubinuria increasing with increasing parasite density. Consequently, a study has shown that in patients presenting with fever and mainly conjugated hyperbilirubinemia, there should be a high index of suspicion for falciparum malaria even in the face of negative blood films.[16] Malaria is known to have an effect on the liver with cases of altered liver function tests being reported.[17] Although the determination of plasma bilirubin was not part of this study, the appearance of significantly higher bilirubinuria in the cases in comparison to the controls suggests the presence of conjugated bilirubin and by extrapolation, there was conjugated hyperbilirubinemia.

Jaundice is a common manifestation of severe falciparum malaria that may be caused by many factors including intravascular hemolysis of parasitized and nonparasitized red blood cells and hepatic dysfunction.[18-20] The co-existence of malaria with viral hepatitis could be another
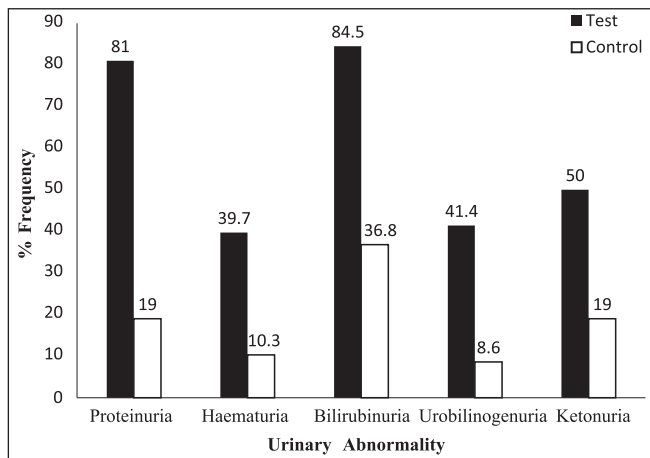
Table 3: Percentage distribution of dipstick urinary findings among study participants

Urine parameter	Cases (*n* = 58)	Controls (*n* = 58)	*P*
Protein			
Absent	11 (19.0)	47 (81.0)	<0.001
Present	47 (81.0)	11 (19.0)	
Blood			
Absent	35 (60.3)	52 (89.7)	<0.001
Present	23 (39.7)	06 (10.3)	
Bilirubin			
Absent	09 (15.5)	37 (63.8)	<0.001
Present	49 (84.5)	21 (36.8)	
Urobilinogen			
Normal	34 (58.6)	53 (91.4)	<0.001
Increased	24 (41.4)	05 (8.6)	
Glucose			
Absent	55 (94.8)	58 (100.0)	0.081
Present	03 (5.2)	—	
Leukocytes			
Absent	41 (70.7)	48 (82.8)	0.133
Present	18 (29.3)	10 (17.2)	
Ketones			
Absent	29 (50.0)	47 (81.0)	0.001
Present	29 (50.0)	11 (19.0)	

Absent – Negative; Present – Trace or 1+ or 2+ or 3+ or 4+ on the dipstick urinalysis

Table 4: Spearman's rho correlation coefficient for the urinary abnormalities in falciparum malaria-infected children

Urine parameter	Correlation coefficient (*r*)	*P*
Protein	0.155	0.247
Blood	0.195	0.142
Bilirubin	0.438**	0.001
Urobilinogen	0.021	0.876
Glucose	−0.030	0.822
Leukocytes	−0.048	0.722
Ketones	0.198	0.136

**Correlation is significant at the 0.01 level (two-tailed)

Figure 1: Differences between the significant urinary abnormalities of the cases and controls

cause of jaundice.[19,21] However, the selection procedure excluded people with viral hepatitis and thus the increased bilirubinuria and urobilinogenuria observed in the present study cannot be attributed to co-infection with viral hepatitis.

The significantly lower HGB concentration observed in cases in comparison to the controls reaffirms anemia as a complication of severe malaria infection.[22] Similar results was obtained from a study conducted in Nigeria.[1] The anemia may partly be attributed to increased destruction of red blood cells in high parasitemia hence reducing HGB levels leading to anemia. This buttresses the fact that the observed urobilinogenuria may be a consequence of intravascular hemolysis.

As indicated in this study, there was significant proteinuria and hematuria in cases compared to controls. These findings are in agreement with earlier studies.[7,23,24] Proteinuria is an early manifestation of renal disease.[5,25] In healthy kidneys, proteins are completely filtered from the bloodstream and subsequently reabsorbed, allowing no protein or only untraceable amounts of protein into the urine. Persistent presence of considerable amounts of protein in the urine is a useful indicator of a form of kidney disease.[5] Studies have shown that positive urine tests for hematuria and/or proteinuria in mass screening settings were significant predictors of end-stage renal disease.[26] Therefore, the presence of significant levels of proteinuria and hematuria in cases in comparison to controls is suggestive of renal impairment. However, these findings are not conclusive of renal impairment due to the lack of data on renal function tests in this study. Nevertheless, renal impairments such as glomerulonephritis and nephrotic syndrome have been reported in malaria patients.[17]

This study also observed significant ketonuria in the cases which might have resulted from some form of caloric deprivation (starvation) or an overnight fast due to loss of appetite.[25]

In contrast to the findings of this study, research conducted in Nigeria observed significantly higher urobilinogenuria at higher parasitemia in *P. falciparum* infection.[1] The difference between these studies might be as a result of the methods used in conducting the urinalysis. This study assessed the dipstick urinalysis using the automated urine analyzer as compared to the previous study where dipstick color changes were assessed visually.

The small sample size used and the short duration of the study, however, remain the limitations of this study. In view of these, it is recommended that further studies should be conducted using a large sample size with enough time duration where dipstick urinalysis findings could be correlated with renal function and liver function markers in children with falciparum malaria infection. This would help in knowing the diagnostic value of dipstick urinalysis in detecting renal impairment and liver dysfunction in falciparum malaria in resource-limited malaria-endemic areas.

CONCLUSION

It is evident that severe malaria infection has a significant effect on urine, which may lead to urine abnormalities, such as proteinuria, hematuria, bilirubinuria, and urobilinogenuria. Urinalysis could be used in conjunction with microscopy in health facilities, especially in resource-limited malaria-endemic settings for accurate diagnosis and prognosis of severe malaria infection, particularly falciparum malaria.

Conflicts of interest
There are no conflicts of interest.

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