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Full Length Research Article

Relationships between *P. Falciparum* Density, Haptoglobin, Transferrin and Packed Cell Volume in Apparently Healthy Pregnant Women.

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Abstract

The present study investigates possible evidence of anaemia in apparently healthy pregnant women with *P. falciparum* parasiteamia. Hence, 82 apparently healthy pregnant women aged 20-39 years reporting for routine antenatal care were recruited for the study. They were screened for *P. falciparum* parasiteamia, those with positive *P.falciparum* became 'Asymptomatic group' (n=60), while those with negative *P.falciparum* became 'aparasitaemia group, (n=22). Further analysis made on the blood samples collected from both groups includes serum estimations of haptoglobin and transferrin and packed cell volume (PCV). The result showed no significant difference in packed cell volume, serum transferrin and haptoglobin concentrations between both groups (P>0.1 for each case). Different levels of associations were observed between *P.falciparum* density and packed cell volume (r = -0.3, P<0.01); haptoglobin concentration (r = -0.319, P<0.05) but no such association was observed with transferrin concentration This study shows that continued increase in *P. falciparum* density might affect haptoglobin metabolism and may result in anaemia.

Keywords:
Malaria, PCV, transferrin, haptoglobin, pregnancy.

INTRODUCTION

In Nigeria, a malaria endemic area it was recently reported that pregnant women could remain asymptomatic despite *P. falciparum* infection[1]. It is also clear that malaria parasites density differs in instances of asymptomatic and clinical malaria[2-5] and the degree of parasitaemia may influence the pathologic and biochemical presentations of individuals presenting with either of these conditions. Reports have shown that in clinical cases of malaria, aneamia is a prominent factor[6, 7], which is possibly caused by destruction of malaria infected red blood cells by the reticulo-endothelial system and haemolysis of such infected cells[8,9]. Both conditions could result in aneamia if severe. Hence, the present study was designed to assess influence of asymptomatic *P. falciparum* parasiteamia on certain biochemical parameters that could show evidence of aneamia in apparently healthy pregnant women.

MATERIALS AND METHODS

Subjects: Eighty-two apparently healthy pregnant women aged 20-39 years reporting for routine antenatal care at University Teaching Hospital (UCH) Ibadan were recruited for this study. They were selected after data on their health status obtained from questionnaire containing relevant information responded to by them indicated healthy status before they were screened for *P. falciparum* parasitaemia. Those with positive *P.falciparum* became 'Asymptomatic group' (n=60), while those with negative *P.falciparum* became 'aparasiteamic group, (n=22). Further analysis made on the blood samples collected from both groups includes serum estimations of haptoglobin and transferrin and packed cell volume (PCV). *The*

study design was approved by the UI/UCH, Board of ethical committee PIMRAT Ibadan and the subjects gave inform consents.

***P. falciparum* malaria parasite screening**: The *P. falciparum* malaria parasite was detected by microscopic examination of Giemsa stained thin and thick blood films. The parasitaemia was expressed as number of malaria parasites per microliter of blood as described by Rooth et al[10].

Analysis of serum levels of transferrin and haptoglobin: The serum levels of transferrin and haptoglobin were estimated by the Single Radial Immuno-diffusion method as modified by Salimonu et.al[11]. The procedure is briefly described. Equal volumes of the minimal anti-transferrin or anti-haptoglobin (Randox Laboratories Limited, United Kingdom) titers that gave clear precipitin rings and 3% molten noble agar was mixed and poured on respective slide plates. By means of a circular metal punch wells were made on the semi-solidified agar-antibody plates into which 5µl of test samples and standards were applied and kept in a humid Chamber at room temperature for 24 hours. The diameters of the precipitin rings formed on the wells for the respective plates were measured in two directions at right angles to the nearest 0.1mm using immunodiffusion precision Viewer (Div. Travenol Laboratories. Inc. Costa Mesa, CAL. USA). The diameters of the different concentrations of the haptoglobin standard or pooled human sera were plotted on semi-log paper against haptoglobin concentration or percent pooled sera. Hence the corresponding concentrations of haptoglobin and transferrin in each sample were extrapolated from the respective standard curves based on individual precipitin diameters. The result of the serum transferrin concentration was reported as percent of adult human sera while haptoglobin was expressed in mg/dl.

Preparation of pooled adult human sera: Blood samples collected from ten adult subjects (7 males, 3 females) were separated and serum components of each sample collected and added together in equal volumes and mixed homogenously. These subjects were apparently healthy blood donors. The pooled sera were stored in aliquots frozen. The concentration of transferrin in the pooled sera was assumed 100 percent.

Selection of non-pregnant adults for the determination of percent transferrin concentrations.

Determination of packed cell volume: The packed cell volume (PCV) was determined by micro-heamatocrit centrifugation of EDTA-whole blood collected into a capillary tube. The volume of the packed red cells was measured in a micro-haematocrit reader and expressed as relative mass of packed red cells present in a sample of whole blood (%).

Statistical analysis: This was performed using Stac-pac Gold package. The mean (±SD) was determined for the variables in both groups while Pearson correlation was used to evaluate the levels of associations between the variables. Levels of significance was considered at p-value <0.05.

RESULTS

The mean malaria parasites density per µl of blood in asymptomatic pregnant women with *P. falciparum* parasitaemia was 262±190 (range101-770 parasites per microliter of blood). However, the mean (±SD) serum concentration of transferrin (% human sera) was 126±36 in asymptomatic group and 115±26 in aparasitaemic group (p>0.1). While the mean (±SD) serum haptoglobin concentration (mg/dl) was 25.1±18.3 in asymptomatic group and 23.1±17.4 in aparasitaemic group (p>0.1). Similarly, the packed cell volume (%) was not significantly different in asymptomatic and aparasitaemic groups of pregnant women (P>0.1). See table 1.

However, significant associations were observed between the malaria parasites density; and packed cell volume (r = -0.3, P<0.01); and haptoglobin (r = -0.319, P<0.05) but not with transferrin (r = -0.244, P>0.05). Furthermore, a significant negative association was observed between serum haptoglobin and transferrin concentration (r = -0.449, P<0.01) and between serum transferrin concentration and packed cell volume (r = -0.469, P<0.01) in asymptomatic pregnant women with malaria infection but not in uninfected pregnant women (r = -0.271, P>0.1; r = -0.111, P>0.1) respectively.

However, there was lack of association between the serum haptoglobin concentration and packed cell volume in asymptomatic (r = -0.025, p>0.1) and uninfected (r = -0.191, p>0.1) pregnant women respectively.

Table 1.
Mean (±SD) P.falciparum density, serum transferrin, haptoglobin and packed cell volume in asymptomatic and aparasitaemia pregnant women.

Variables	MPD	PCV	Transferrin	haptoglobin
Asymptomatic (n=60)	262.0 ±19.0	33.6 ± 4.1	126.0 ± 36.0	25.1 ± 18.3
Aparasitaemia (n=22)	-	33.3 ± 4.3	115.0 ± 26.0	23.1 ± 17.4

DISCUSSION

The presentation of asymptomatic malaria parasiteamia is different from that of severe/clinical malaria parasiteamia in pregnancy. This is probably due to differences in malaria parasites density in blood of infected individuals[1].

The packed cell volume in the present study did not indicate anaemia in asymptomatic pregnant women with malaria infection. Similar report of lack of anaemia has been made on non-pregnant adults with asymptomatic malaria parasitaemia[3]. Packed cell volumes less than 33% has been reported as suggestive of anaemia[11], while packed cell volume less than 25% has been associated with severe malaria infection in children[6].

In the present study, the strong negative association between malaria parasites density and packed cell volume in these subjects suggest the likelihood that anaemia may occur with further degree of parasitaemia. In an earlier report we observed asymptomatic malaria parasites threshold in pregnant women[1] and the parasites density in the present study is within this range. Thus suggesting that parasites densities exceeding this range may result in progressive drop in packed cell volume indicating the presence of anaemia. Elsewhere, in Southern Cameroon[7] and South-Western Nigeria[6] young children presenting with severe malaria parasitaemia have been shown to present with packed cell volume as low as 25% indicating severe anaemia. This was attributed to the malaria parasites densities observed in these young children.

Haptoglobin is known as transport protein for free haemoglobin in blood. The present study observed that serum concentration of haptoglobin was not affected by asymptomatic malaria parasiteamia in pregnant women. However, the wide range of values encountered in both groups of subjects studied might have confounded any significance difference in mean values. Thus suggesting that factors modulating the metabolism of haptoglobin may be multiple. The haptoglobin value further suggest that the degree of parasitaemia in the pregnant women with asymptomatic malaria did not possible induce haemolysis of red blood cells as is often the case in cases of severe malaria infection that results in anaemia.

Study elsewhere has shown that the range for different acute phase proteins were wider in African women compared with Western adults[13]. The authors also observed no differences in mean haptoglobin levels amongst pregnant, lactating and non-pregnant females. However, the significant negative association observed in the present study, between malaria parasites density and haptoglobin concentrations suggest that malaria parasitaemia may be one of the modulating factors affecting metabolism of haptoglobin possibly through haemolysis of red blood cells. This is often intensified under higher degree of parasitaemia.

The percent human transferrin in asymptomatic pregnant women with malaria infection is higher than was observed in the uninfected counterparts although no significant difference was observed. Although studies have reported that concentrations of serum iron and transferrin are low in the presence of acute phase reaction[12, 14], the present study did not observe findings consistent with the above reported cases. The lack of massive evidence for haemolysis in asymptomatic pregnant women with malaria infection, unlike was reported in severe/clinical cases of malaria[8,9,15], was reflected in the lack of significant association between malaria parasites density and serum transferrin concentration in the present study. This may explain the differences in observation in the present study and those of Karunawera et al[8] and kurtzhals et al[9]. This shows that *P. falciparum* density may not directly affect the metabolism of transferrin.

Serum transferrin concentration might have influenced the packed cell volume and serum haptoglobin concentration in asymptomatic pregnant women with malaria infection. This was reflected in the level of association observed between serum transferrin and these biochemical parameters. These levels of association were lacking in the aparasitaemia pregnant women studied. This finding suggests that in malaria endemic areas, asymptomatic individuals present with serum transferrin concentration that is inversely related to serum haptoglobin concentration and packed cell volume.

It is therefore possible that there exist relationships between malaria parasite density and serum concentrations of haptoglobin, transferrin and packed cell volume. However, biochemical evidence of changes may depend on degree of parasitaemia.

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