

Association of haptoglobin phenotypes with malaria infection in Kano, Northern Nigeria

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ABSTRACT

Background: It has been documented that haptoglobin phenotypes have been linked to malaria susceptibility but this assertion still remains inconclusive. The aim of this study was to determine the association of haptoglobin phenotypes to malaria infection in Kano, Northern Nigeria. **Materials and methods:** A total of 261 children (211 malaria-infected and 50 non-malaria infected children), aged 1-12 years, were recruited for a study between January and December, 2018. Blood sample was collected from each participant into tripotassium EDTA tube and plain container for detection of malaria of parasites from prepared blood films and haptoglobin phenotyping, respectively using standard techniques. **Results:** Haptoglobin 1-1 phenotype had highest prevalence for malaria (46.4%) among the malaria-infected children compared to haptoglobin 2-1 phenotype (33.6%) and haptoglobin 2-2 phenotype (19.9%). The species detected were *Plasmodium falciparum* (98.6%), *Plasmodium malariae* (0.9%) and *Plasmodium ovale* (0.5%). There was no relationship between haptoglobin phenotypes and plasmodium species (P=0.6210). **Conclusion:** *Plasmodium falciparum* is the predominant *Plasmodium species* in Kano. Haptoglobin 1-1 phenotype had the highest prevalence among the malaria-infected children but there were no significant differences in the prevalence rates of haptoglobin phenotypes in malaria-infected children compared to non-malaria infected children. However, haptoglobin phenotypes and *Plasmodium species* showed no relationship.

Keywords: Association, haptoglobin phenotype, malaria

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INTRODUCTION

Malaria is a vector-borne infectious disease caused by a protozoan parasite of the *genus Plasmodium*, which has four species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*) that can infect humans. However, *Plasmodium knowlesi* has recently been observed to naturally infect humans as a zoonosis in Southeast Asia (1).

Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. In Nigeria, malaria accounts for 60% of outpatients' visits and 30% of hospitalizations among children under five years of age while the prevalence of malaria in children under five years was observed to be close to 50% in the Southwest, North central and Northwest regions but 27.6% in the Southeast region (2).

Haptoglobin (Hp) is an acute-phase plasma protein that binds rapidly and irreversibly with free haemoglobin (Hb) after the occurrence of malaria-induced haemolysis (3).

Haptoglobin is the free body's main tool for removing circulating free plasma haemoglobin, which is a potent pro-oxidant that is capable of entering and damaging renal glomeruli, and thus leading to the generation of reactive oxygen species in the absence of haptoglobin (4-6).

The haptoglobin gene is located on chromosome 16q 22.1 and it consists of two different loci, called haptoglobin alpha (Hp - α) and haptoglobin beta (Hp - β), which code for the α -chain and β -chain, respectively of the haptoglobin protein [6]. In humans, haptoglobin is polymorphic with two co-dominant alleles, Hp¹ and Hp², resulting in three phenotypes; Hp¹⁻¹, Hp¹⁻² and Hp²⁻² (6,7).

Hp¹⁻¹ is biologically the most effective in binding free haemoglobin and suppressing inflammatory responses associated with free haemoglobin while Hp²⁻¹ is moderately active but Hp²⁻² is biologically the least active (8).

The different haptoglobin phenotypes influence the progression of various infections and inflammatory diseases, including malaria due to their phenotype-dependent binding affinity to haemoglobin (Hp¹⁻¹ > Hp²⁻¹ > Hp²⁻²) and the CD163 receptor on monocytes and macrophages (Hp²⁻² > Hp²⁻¹ > Hp¹⁻¹) (6). According to Hunt et al. (9), haptoglobin acts in at least two ways: (i) it is inimical to malaria parasite replication and (ii) it is needed for an effective anti-parasite immune response.

Divergent views have been expressed by the previous authors on the relationship between haptoglobin phenotypes and malaria. Hp¹⁻¹ phenotype has been reported to be susceptible to falciparum malaria by some authors (10,11) while other researchers reported no significant association between haptoglobin phenotypes (polymorphisms) and severe malaria (12,13).

However, Bienzle et al. (13) observed that the children with Hp²⁻² may be at greater risk to develop severe malaria compared to Hp¹⁻¹ individuals but Quaye et al. (10) demonstrated that Hp²⁻² phenotype is associated with reduced susceptibility to malaria.

Conflicting reports have been documented by previous researchers on the relationship between haptoglobin polymorphisms and malaria infection in some African countries while in Nigeria, scanty information is available. Therefore, the aim of this study was to determine the association of haptoglobin phenotypes to malaria infection in Kano, Northern Nigeria.

MATERIALS AND METHODS

A total of 261 children (211 malaria-infected and 50 non-malaria infected children), aged 1-12 years, were recruited between January and December, 2018 for a study on the association of haptoglobin phenotypes to malaria infection in Kano, Northern Nigeria after the ethical approval from the Research Ethics Committee of Aminu Kano Teaching Hospital, Kano, through a letter dated 7th August, 2017 and reference numbers NHREC/21/08/2008/AKTH/EC/2033 and AKTH/MAC/SUB/12A/P-3/VI/2133, and informed written consent obtained from parents and guardians.

Five milliliters of venous blood was collected by venipuncture aseptically from every participant. Out of this, 3ml of blood was put into tripotassium EDTA tube while the remaining 2ml was put in a plain container. The blood sample in EDTA container was used for the detection of malaria parasites and plasmodium species using thick and thin blood films stained by Giemsa stain (14) while the serum from the centrifuged blood in the plain container was used for haptoglobin phenotyping using discontinuous SDS-PAGE as described by Elagib *et al.* (11).

Statistical analysis

The data obtained from this study were analyzed using statistical package for social sciences (SPSS Software, version 20; SPSS Inc Chicago, USA 2012). The data were expressed as percentages and the comparison of results was done using Chi-square test while $P < 0.05$ was considered to be significant.

RESULTS

Table 1 shows the distribution of haptoglobin phenotypes in malaria-infected and non-malaria infected children. Among the 211 malaria-infected children, 98 (46.4%) were associated with Hp¹⁻¹, 71 (33.6%) were linked to Hp²⁻¹ and 42 (19.9%) were associated with Hp²⁻² while out of 50 non-malaria infected children (controls), 20 (39.4%) were associated with Hp¹⁻¹, 23 (46.4%) were linked to Hp²⁻¹ and 7 (14.2%) were associated with Hp²⁻². However, there were no statistically significant differences in the prevalence rates of Hp¹⁻¹, Hp²⁻¹ and Hp²⁻² in malaria-infected children compared to non-malaria infected children ($P = 0.2424$).

Table 2 reveals the distribution of plasmodium species in malaria-infected children. Out of 211 malaria-infected children, 208 (98.6%) of the cases were caused by *Plasmodium falciparum*, 2 (0.9%) were caused by *Plasmodium malariae* and 1 (0.5%) was caused by *Plasmodium ovale*.

Table 3 displays the relationship between haptoglobin phenotypes and Plasmodium species. Out of 98 children with Hp¹⁻¹ phenotype, 96 (97.96%) were infected with *P. falciparum*, 1 (1.02%) with *P. malariae* and 1 (1.02%) with *P. ovale* while 71 children with Hp²⁻¹ phenotype were infected with *P. falciparum* but none was infected with *P. malariae* (0%) and *P. ovale* (0%). However, out of 42 children with Hp²⁻² phenotype, 41 (97.62%) were infected with *P. falciparum*, 1 (2.38%) was infected with *P. malariae* and none was infected with *P. ovale* (0%).

There was no significant relationship between haptoglobin phenotypes and plasmodium species ($P=0.6210$).

Table 1: Distribution of haptoglobin phenotypes in malaria and non-malaria infected children.

Haptoglobin phenotype	Malaria-infected children n (%)	Non-malaria infected Children n (%)	P-value
1 – 1	98 (46.4)	20 (39.4)	0.2424
2 – 1	17 (33.6)	23 (46.4)	
2 – 2	42 (19.9)	7 (14.2)	

Table 2: Distribution of Plasmodium species among malaria infected children in Kano.

Plasmodium Species	Frequency (n)	Percent (%)
Falciparum	208	98.6
Malaria	2	0.9
Ovale	1	0.5

Table 3: Relationship between haptoglobin phenotypes and Plasmodium species

	Malaria-infected pateints with Hp 1-1 n (%)	Malaria-infected patients with Hp 2-1 n (%)	Malaria-infected pateints with Hp 2-2 n (%)	P-value
Number	98 (46.4)	71 (33.6)	42 (19.9)	
<i>Plasmodium falciparum</i>	96 (97.96)	71 (100)	41 (97.62)	0.6210
<i>Plasmodium malaria</i>	1 (1.02)	0 (0)	1 (2.38)	
<i>Plasmodium ovale</i>	1(1.02)	0 (0)	0 (0)	

DISCUSSION

Conflicting reports have been documented by previous researchers on the relationship between haptoglobin polymorphism and malaria infection (10-13).

Our study has shown that the frequencies for Hp¹⁻¹, Hp²⁻¹ and Hp²⁻² were 98 (46.4%), 71 (33.6%) and 42 (19.9%) in malaria-infected

children compared to 20 (39.4%), 23 (46.4%) and 7 (14.2%), respectively in non-malaria infected children, however, the different frequencies in Hp¹⁻¹, Hp²⁻¹ and Hp²⁻² between malaria and non-malaria infected children were not statistically significant. These findings are at variance with the conflicting reports of Quaye et al. (10) that had frequencies of 63.9%, 29.2% and 6.9%

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for p¹⁻¹, Hp²⁻¹ and Hp²⁻², respectively in Sudanese patients with cerebral malaria and Atkinson et al. (3) that observed ratio 1:2:1 for Hp¹⁻¹, Hp¹⁻² and Hp²⁻², respectively in Tanzanian population. However, differences in the frequencies of haptoglobin genotypes in various ethnic groups and regions have been associated with genetic, ethnic and environmental factors (3,15,16).

Hp¹⁻¹ phenotype had the highest prevalence in malaria-infected children in this study. This is in line with the reports on Sudanese patients with complicated and uncomplicated falciparum malaria (11), Ghanaian patients with severe malaria (10) and Malian Fulanis infected with *Plasmodium falciparum* (15) but in contrary to the reports from Kenya that associated the highest prevalence with malaria infection to Hp²⁻¹ phenotype (3) and that of Dogon in Mali to Hp²⁻² phenotype (15).

These divergent views expressed by various researchers could justify ethnicity and environmental factor as determinants for the variation in the frequencies of haptoglobin phenotypes in malaria-infected patients.

The study has further supported the earlier reports on patients with Hp²⁻² being associated with low level of malaria infection (3,10,11,13,15). This has been linked to protective effect of Hp²⁻² phenotype against malaria by earlier authors (3,10).

The study further revealed that among the malaria infected patients, *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax* had frequencies of 98.6%, 0.9%, 0.5% and 0%, respectively. These findings are in agreement with earlier studies in different parts of Nigeria (17-20). However, the low prevalence of *Plasmodium vivax* has been associated with the absence of the erythrocytic Duffy antigen in many Africans because Duffy antigen has been postulated

to be necessary for the entry of the *P. vivax* merozoite into an erythrocyte (21) while low frequency of *P. malariae* as evident in the previous studies (17-20) could be linked to low sensitivity of microscopy as approximately 50% of *P. malariae* positive samples detected by PCR were undetected by microscopy (22) coupled with the asymptomatic nature of non-falciparum infections associated with the reduction in seeking for medical treatment (23).

In our study, it was observed that there was no relationship between haptoglobin phenotypes and *Plasmodium* species. This observation is in line with previous studies which showed no clear association between malaria infection in general and haptoglobin genotype (12,13,16).

In conclusion, the study has shown that *Plasmodium falciparum* is predominantly the cause of malaria infection in Kano, Northern Nigeria, however, the patients with haptoglobin¹⁻¹ phenotype had the highest prevalence among the malaria-infected children while the haptoglobin²⁻² phenotype had the least. There were no significant differences in the prevalence rates of haptoglobin phenotypes in malaria and non-malaria infected children. The haptoglobin phenotypes and *Plasmodium species* in malaria infected patients showed no significant relationship.

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Conflict of interest: None

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