Niger J Paed 2012; 39 (2):51- 55

ORIGINAL

Nwaneri DU
Ibadin MO

Haemoglobin genotype of children with severe malaria seen at the University of Benin Teaching Hospital, Benin city, Nigeria

DOI:http://dx.doi.org/10.4314/njp.v39i2.2

Received:19th April 2011
Accepted: 23rd October 2011

Nwaneri DU (✉)
Ibadin MO
Department of Child Health, University of Benin Teaching Hospital, PMB 1111, Benin City, Edo State, Nigeria.
E-mail: damiannwaneri@yahoo.com
Tel: +2348056321577, +23480139172309

Abstract: *Introduction:* Types of haemoglobin (Hb) genotype have been found to be crucial to the rate of red blood cell parasite invasion, multiplication, and destruction as well as outcome of malaria disease. In a bid to provide more information on the relationship between Hb genotype and level of protection conferred by genotype against severe forms of malaria, this study was undertaken. This is done through evaluation of forms of Hb genotype in children with severe malaria seen in University of Benin Teaching Hospital (UBTH), Benin City.

Patients and methods: This cross-sectional study was carried out on children (6 - 60 months old) admitted for severe malaria using standard World Health Organization (WHO) guidelines. Diagnosis of malaria was by microscopic demonstration of parasitaemia or serology (in those with negative parasitaemia). Hb genotype was done using the Hb electrophoresis.

Results: Ninety-six well nourished children; (56(58.3%) males and 40 (41.7%) females) mean age (± SD) 29.22 ± 16.02 months were recruited for the study. Sixty-eight (73.4%) of the 92 subjects had Hb genotype AA while 24(26.1%) Had abnormal Hb genotype. Prevalence of severe malaria in children with abnormal Hb was 20/24 (83.3%) as against 58/68(85.3%) observed in those with HbAA. Significantly fewer incidence of heavy malaria parasitaemia (3+ and 4+) was observed in children with abnormal Hb genotype. Heavy parasite density was the most important features of severe malaria in children with HbAA (p= 0.013) as against altered sensorium, prostration, and haemoglobinuria in children with abnormal Hb genotype (p = 0.003, 0.041, and 0.023 respectively). Children with HbAA were also about 3 times more likely to die from severe malaria (p = 0.567, O.R = 2.96) when compared with their counterparts with abnormal Hb.

Conclusion: Study supports a higher prevalence of severe malaria in children with HbAA when compared with those with abnormal Hb genotype. Altered sensorium, prostration and haemoglobinuria were the significant presenting features of severe malaria in children with abnormal Hb genotype in this study.

Key words: children, genotype, haemoglobin, mortality, severe malaria

Introduction

More than 300-500 million people suffer from malaria annually; of which two million deaths occurs.[1-4] About 90.0% of all malaria related deaths in the world today occur in Africa, and these deaths occur in children under the age of five years.[2-3] This is because majority of the infections in Africa are caused by Plasmodium falciparium, the most dangerous of the four Plasmodium species [2-3]. Among the unprotected children in rural areas of Southern Nigeria, the infection rate rises rapidly from 0. 2.0% during the first three months of life to about 50.0% by the age of one year and persists at a high level during childhood.[5.6] In Benin City, Nigeria 69.0% of Under-5s who had fever without specific localizing signs of infection were found to have malaria.[6]

A major phase in malaria parasite life cycle is red blood cell invasion and multiplication,[7] consequently leading to red blood cells destruction. However host immunity and types of haemoglobin (Hb) genotype have been found to be crucial to the rate of parasite invasion, multiplication, and destruction as well as outcome of the disease[7].

The relationship between Hb genotype and level of protection conferred against severe forms of malaria remains unclear. It was reported by Okam[8] in 2002 that children with heterozygous sickle cell traits have lower parasite rates and less fatal infections as compared to children with HbAA. These children with heterozygous Hb when infected with Plasmodium falciparum are more likely to survive the acute illness due to the presence of human leukocyte antigen (HLA) which has been suggested to play crucial role in the defense of host against malaria infection and reduce susceptibility to severe form of malaria[10]. The HLA-Bw53-restricted cytotoxic T lymphocytes are reported to recognize a conserved epitope of Plasmodium falciparum liver-stage antigen type 1 which is a crucial stage malaria disease progression10. hence children with heterozygous Hb are protected against severe malaria[7-10]. However, Konotey-Ahulu[11,12]. and Jones[13] in their separate studies observed that children with sickle cell disease are not immuned to cerebral malaria. These findings were corroborated by those observed by Luzzatto in some of his studies[14,15]. These authors concluded that these groups of individuals die quicker from cerebral malaria and would therefore need chemoprophylaxis against malaria.[11-15]

In a bid to provide more information on the relationship between Hb genotype and level of protection against malaria, this study was undertaken; involving the evaluation of types of Hb genotype in children (6 - 60 months old) with severe malaria seen in Children Emergency Room (CHER) of the University of Benin Teaching Hospital (UBTH), Benin City.
The study also entailed the determination of the prevalence of severe malaria in children with abnormal Hb genotype, comparison of the prevalence, presentation and outcome of severe malaria between children with abnormal Hb and those with HbAA genotypes.

Patients and Methods

The cross-sectional study was carried out in Children's Emergency Room (CHER) of UBTH, Benin City, Nigeria between January and April 2009. CHER is a 15 bedded unit of the paediatrics department of the hospital. There were at least three nurses on duty per shift. A consultant paediatrician (with sub-specialty interest on emergency paediatrics is incharge of all medical affairs in CHER. There were also two senior registrars and at least one registrar and a paediatrics casualty officer who work on shift basis. The nursing staff and the medical doctors are equipped to take care of common paediatrics emergencies using the principle of patient triage. The unit serves as a transition unit for patients admitted in the main paediatrics ward and has an average patient load of 150 per month.

Subjects were children aged 6 to 60 months admitted for severe malaria based on standard World Health Organization (WHO) guidelines[16]. They were recruited consecutively into the study. Ethics and Research Committee of UBTH, Benin City gave approval for the study. Written informed consent was also obtained from each parent or care-giver of the subjects recruited in the study. Researcher administered validated questionnaire was used to obtained socio-demographic data of each subject, anthropometry, symptoms before presentation, drug treatment and outcome. Outcome was defined as discharged home or dead.

Specimen collection/laboratory procedure

Diagnosis of malaria was supported by demonstration of parasitaemia by microscopy on venous blood or serology (in those with negative parasitaemia who had overwhelming features of severe malaria including response to anti-malarial drugs only). Thick smear was used to determined the parasite density and was described by WHO criteria for malaria parasite density[16,17]. Thin film was made to identify the species of the malaria parasite.[17]

Hb genotype was done by electrophoresis using cellulose acetate paper (Shandon Scientific Co Ltd) as described by Khon.[18] Two millimeter of venous blood washed in a solution of 0.9% normal saline by adding 9.0 mls of normal saline to 1ml of blood. The solution thus formed was centrifuged at 5000 rpm for 5minutes. The supernatant was discarded living the cells. About 3mls of water was added to 1ml of the cell to effect lysis of the red cells. 0.1ml of the haemolysate was then applied to the cellulose acetate strip at pH 8.4 and the strip was placed across the bars of electrophoretic chamber with a positive and negative electrode. The rate of migration on this electrophoresis machine was used to classify the different genotypes.
All laboratory results were recorded in a pro-forma.

Data Analysis

Data obtained were entered into a Microsoft Excel 2007 and analyzed using Statistical Package for Social Sciences (SPSS) 13.0 software. Quantitative variables were summarized using means and standard deviations. The significance of association between proportions was tested using chi-square or Fisher's exact tests (where appropriate) while student t-test was used for comparison of means. Binary logistic regression was done to obtain the independent predictors of severe malaria in the subjects using the Hb status (abnormal Hb genotype versus HbAA genotype) as dependent variables. Abnormal genotype is defined as all individual with heterozygous Hb genotype (example HbAS, HbSS, HbAC, etc).[18] The level of significance of each test was set at $p < 0.05$.

Results

Ninety- six well nourished children; 56(58.3%) males

and 40(41.7%) females were recruited into the study. The mean age was 29.22 ± 16.02 months (range 6- 60 months). Age and gender distribution of subjects is presented in Table 1.

Table 1: Age and gender distribution of the subjects

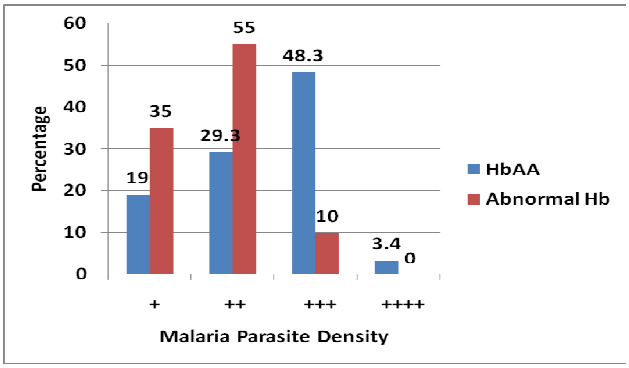
Age (Years)	Male (%)	Female (%)	Total (%)
6-12	7(12.5)	0(0.0)	7(7.6)
13-24	26(46.4)	13(36.1)	39(42.4)
25-36	14(25.0)	9(25.0)	23(25.0)
37-48	3(5.4)	5(13.9)	8(8.7)
49-60	6(10.7)	9(25.0)	15(16.3)
Total	56(100.0)	36(100.0)	92(100.0)

Four (4.2%) of the 96 subjects were excluded from the study because their genotype was unequivocal due to history of recent blood transfusion, thus the total number of subjects whose genotypes were obtained was 92. This comprised 56(60.9%) males and 36(39.1%) females. Sixty-eight (73.9%) of the 92 subjects had genotype AA while 24(26.1%) had abnormal Hb [Abnormal Hb included HbAS 16/24(66.7%); HbSS 7/24(29.2%) and HbAC 1/24(4.1%)]. Mean age of children with abnormal Hb (32.10 ±17.13 months) was comparable to the 30.64 ± 25.21 months obtained in those with HbAA genotype. The anthropometric measurements between the two groups were also comparable.

Prevalence of severe malaria in subjects with abnormal Hb was 20/24(83.3%) as against 58/68(85.3%) observed in children with HbAA (Fisher's exact; p = 0.75, OR = 0.86).
Plasmodium falciparum was the sole specie of malaria parasite identified.

Figure1 shows that subjects with abnormal Hb had significantly lower proportion of malaria parasite densities of 3+ and 4+ (p=0.013) as compared with those with HbAA genotype. Malaria parasite density independently was a significant feature of severe malaria in children with HbAA using Hb status of subjects as dependent variable (β = -0.286, t = -2.30, R2 = 0.215, p = 0.024).

Figure 1: Types of Hb genotype in relation to malaria parasite density in subjects.



$\chi 2$ = 10.75, df = 3, p = 0.013

Binary logistic regression model using Hb genotype status (abnormal Hb versus HbAA) as the dependent variable showed that symptoms such as altered sensorium, prostration, and haemoglobinuria were the significant presenting features of severe malaria in children with abnormal Hb when compared with HbAA children (p = 0.003, 0.041, and 0.023 respectively). Of note was that pallor and level of anaemia (pack cell volume at presentation) were not significant presenting features of severe malaria in neither patients with abnormal Hb nor those with HbAA (Table 2).

Table 2: Binary logistic regression models of severe malaria presentation in children with Hb genotype (abnormal Hb versus HbAA) as dependent variable.

	Abnormal Hb	HbAA				
Symptoms	n=20(%)	n=58(%)	β	t	O.R	p-value
Convulsion	16(80.0)	43(74.1)	-0.06	-0.50	1.40	0.617
Altered sensorium	15(75.0)	28(48.2)	-0.65	-3.10	3.20	0.003
Coma	5(25.0)	23(39.7)	0.15	1.07	0.50	0.287
Pallor	16(80.0)	42(72.4)	0.08	0.74	1.50	0.463
Prostration	13(65.0)	31(53.4)	0.47	2.09	1.60	0.041
Haemoglobinuria	8(40.0)	2(3.4)	-0.38	-2.33	18.70	0.023
Jaundice	8(40.0)	3(5.2)	-0.10	-0.60	12.20	0.552
Vomiting	8(10.0)	8(13.8)	-0.12	-0.96	4.20	0.339
Diarrhoea	4(20.0)	12(20.7)	-0.12	-0.98	0.96	0.332

β = measure of how strongly each variable influences the dependent variables, O.R = odds ratio, p-value,
Standard Error = 0.000 in all the indepenent variables.

Seven (29.2%) of the subjects with abnormal Hb genotype were on malaria chemoprophylaxis. All the subjects (abnormal Hb or HbAA) were treated with intra-venous quinine.

Table 3 shows the outcome of severe malaria in children with abnormal Hb and those with HbAA. The odds ratio shows that children with HbAA are 3 times more likely to die from severe malaria (p = 0.567, O.R = 2.96) when compared with their counterpart with abnormal Hb. However none of the symptoms were significant predictors of outcome of severe malaria in neither children with normal Hb genotype nor those with abnormal Hb genotype (Table 4). The model fitting information (R2 = 0.13, $\chi 2$ = 10.23, df = 10, p = 0.42).

Table 3: Outcome of severe malaria in children with abnormal Hb and HbAA

Outcome	Abnormal Hb(%)	HbAA (%)	Total (%)
Discharged	20 (100.0)	54(93.1)	74(94.9)
Died	0(0.0)	4(6.9)	4(5.1)
Total	20(100.0)	58(100.0)	78(100.0)

Fisher's Exact; p = 0.567, O.R = 2.96

Table 4: Multiple logistic regression models of predictors of outcome of severe malaria (using symptoms at presentation as independent variables) in children with normal Hb and those with abnormal Hb genotypes

Symptoms at presentation	β	SE	χ^2	Exp (β)	p-value
Convulsion	-17.1	0.00	0.00	3.91	0.99
Altered sensorium	-8.95	7846.02	0.00	0.00	0.99
Coma	-7.10	7844.52	0.00	0.00	1.00
Pallor	-17.81	0.00	0.00	1.84	1.00
Prostration	-16.51	5430.91	0.00	1.48	1.00
Haemoglobinuria	-1.99	1.52	0.00	0.14	1.00
Jaundice	18.25	1.30	0.00	8.45	1.00
Vomiting	16.54	5520.91	0.00	1.53	1.00
Diarrhea	-1.25	1.38	0.83	0.29	0.36
Constant	12.61	7847.11	0.00		1.00

SE = Standard Error, β = measure of how strongly each predictor variable influences the outcome variables, Exp (β) = Exponential (β)

Discussion

The study revealed a slightly lower prevalence (83.3%) of severe malaria in children with abnormal Hb when compared with the prevalence of 85.3% observed in children with HbAA. This does not support the assertion of selective protective effect against severe malaria conferred on individuals with abnormal Hb (HbAS, HbSS and HbSC). This finding is in contrast to the documentation by Aidoo et al[9] in 2002 which noted that children with genes for HbAS and HbSS had significantly fewer episodes of severe malaria when compared with children with HbAA genotype. Aidoo et al[9] views had been corroborated by Pasvol et al[7] and Okam.8The high degree of polymorphism in human leukocyte antigen genes had been suggested to account for the natural selection against susceptibility to a variety of infectious pathogens including malaria.[10] This phenomenon is also said to play a crucial role in the defense of individuals with abnormal Hb against severe malaria parasitaemia.[10-11] It may also however be argued that children with HbSS are on malaria chemoprophylaxis and as such, are less likely to have high parasite burden and frequent episodes of malaria including the severe forms.[10] In this present study nearly one-third of subjects were on malaria chemoprophylaxis, yet they presented with severe malaria hence debunking the protective effect of antimalarial chemoprophylaxis against severe malaria.

This study also showed that children with abnormal Hb have significantly lower incidence of heavy malaria parasitaemia when compared with their counterpart with HbAA. This finding is in consonance with that of Aidoo et al[9] in 2002 who observed, that children with abnormal Hb significantly had reduced risks of heavy malaria parasite densities (>10,000 parasites/uL) that is, they usually have lower parasites densities when compared with children with HbAA. The higher parasite density found in children with HbAA as observed in this present study was one of the major features of severe malaria in children with HbAA (R2 = 0.215, p = 0.024) and could offer some explanations for the slightly higher prevalence of severe malaria in children with HbAA as against those with abnormal Hb genotype.

Altered sensorium, prostration and haemoglobinuria were the significant presenting features of severe malaria in children with abnormal Hb in this study. These symptoms are easily recognizable by care-givers at home and hence may warrant their early presentation in health institutions, with attendant improved outcome.19 To buttress this fact is the better outcome of severe malaria in children with abnormal Hb in this study. Whereas all children with severe malaria and who had abnormal Hb genotype survived and were discharged home, 7.0% of those with HbAA died from severe malaria when compared with children with abnormal Hb (O.R= 2.96). HbAS specifically is associated with protection against mortality in children (2 - 16 months) which is the period they are at most risk of severe falciparium malaria. Some authors observed that HbAS is associated with protection against severe anaemia in the presence of any level of malaria parasitaemia, hence these children have lower risk of severe anaemic episodes.[7-9] Therefore individuals (especially children) infected with Plasmodium falciparum are more likely to survive the acute malaria illness if they have the HbAS genotype.[7,8,10,11]

Conclusion

This study conforms high prevalence of severe malaria and high parasite density in children with HbAA as against those with abnormal Hb. Presence of altered sensorium, prostration and haemoglobinuria are significant presenting features of severe malaria in children with abnormal Hb genotype and may be relied upon in effecting early presentation to competent health facility.

Authors contributions

Ibadin MO, Nwaneri DU Conception and design, Drafting the manuscript, Analysis and interpretation of data
Nwaneri DU Acquisition of data
Ibadin MO Revising the manuscript, for intellectual content
Ibadin MO, Nwaneri DU Final approval of the completed manuscript
Conflict of interest: None
Funding: None

Acknowledgment

The authors wish to thank Dr (Mrs) Isreal-Aina Yetunde and Dr Adigweme Ikechukwu for their assistance in data collection. Special thanks to Laboratory Scientists in the Department of Microbiology and Haematology UBTH, Benin City for sample analysis (malaria parasite and Hb electrophoresis).

References

1. WHO World malaria report 2008. http://malaria.who.int/wmr2008/malaria2008.pdf
2. Snow R, Craig H, Newton C, Steketer R. The public health burden of plasmodium falciparum malaria, deriving the numbers, working paper no:11, Fogarty international centre, National institute of health, 2003; 1-75.
3. WHO guidelines on prevention of the reintroduction of malaria/who regional office for the eastern Mediterranean. Publication series no: 34, ISSN 1020-0428.
4. Malaria Centers for Disease Control and Prevention, 1600 Clifton Rd. Atlanta, GA 30333, U.S.A. June 27, 2001.
5. Bruce-Chwatt LJ. Malaria in African infants and children in Southern Nigeria. Ann Trop Med Parasitol 1952; 46, 173-175.
6. Akpede GO, Sykes, RM. Relative contribution of bacteremia and malaria to acute fever without localizing signs of infections in under-5 children. J Trop Pediatr 1992; 38:295-298.
7. Pasvol G, Weatherall DJ. The red cell and the malaria parasite. Brit J Haematol 1980; 46: 165-170.
8. Okam M. Sickle cell and thalassaemic disorders. Available at http://sickle.bwh.harvard.edu/index.html. Accessed on 19/02/2011.
9. Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, Ter-Kuile FO, Kariuki S, et al. Protective effects of the sickle cell genes against malaria morbidity and mortality. Lancet 2002; 359: 1311-1312.
10. Hananantachai H, Patarapotikul J, Ohashi J, Naka I, Looaresuwan S, Tokunaga K. polymorphisms of the HLA-B and HLA-DRB1 genes in Thai malaria patients. Jpn J Infect Dis 2005; 58: 25-28.
11. Konotey-Ahulu FID. A non-sense mutation and protection from severe malaria. Lancet 2001; 358: 927-928.
12. Konotey-Ahulu FID. Malaria and sickle cell: 'Protection or no protection?'- Confusion reigns. Available at http://ucc.edu.gh/konotey/scell_protection. Accessed on 06/06/2010.
13. Jones KDJ. Malarial chemoprophylaxis. BMJ 2008; 337: a1875.
14. Luzzatto L. Genetics of red cells and susceptibility to malaria. Blood 1979; 54: 961-976.
15. Luzzatto L. Malaria. In: Recent advances in haematology 1985; 4: 109-126.
16. World Health Organisation: Action programme on severe and complicated malaria. Trans R Soc Trop Med Hyg 2000; 94: 190.
17. Monica Cheesebrough. Examination of blood for malaria parasite in district laboratory Practice in tropical countries. Part 1, second edition. Cambridge University press 2005.
18. Khon J. Separation of haemoglobins on cellulose acetate. J Clin Path 1969; 22: 109-111.
19. Tripathy R, Parida S, Mishra DP, Tripathy D, Das MC, Maguire JH, et al. Clinical manifestations and predictors of severe malaria in Indian children. Paediatr 2007; 120: e454-e460.