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Immunoglobulin levels in malaria infected Nigerians with and without abnormal haemoglobin

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Summary

Comparative studies were made between malarial parasitaemia in Nigerians with and without abnormal haemoglobins. The three main classes of immunoglobulins (i.e. IgG, A and M) were assayed in these groups of patients and the mean values were compared. Those with abnormal haemoglobins S or C (HbS or HbC) were compared with those with normal control haemoglobin A (HbA). HbSS malarial patients have the highest mean values of the 3 classes of immunoglobulins. This is followed by HbAS patients while patients with normal Hb have lowest mean values for IgG and IgM. The significance of the results is discussed.

Resume

Les etudes comaparatif etaient effectue entre parasitemie paludique chez les Nigerians avec ou sans l'haemoglobines anormal. Les trois classe essentiel des anti corps (Ca d IgG, A, et M) etaient titre dans ces groupes de malades et les valeurs moyenne etaient compare. Ceux avec l'hemoglobine anormal S ou C (HbS ou HbC) etaient compare avec la controle, l'hemoglobuie normal A (HbA). Les malades paludique HbSS avaient les valeurs moyenne plus superieur de la trois classe des anticorps. Les malades HbAS suivre alors que les malades avec l'hemoglobine normal avaient les valeurs plus inferieur pour IgG et IgM. La portee de ces resuitats e st discute.

Introduction

Malaria and sickle cell disease are two major causes of morbidity and mortality in tropical homogenous black African populations; nevertheless certain unestablished factors do act to reduce the frequency and sometimes the severity of malaria in individuals with haemoglobinopathies[1,2]. Since the advent of continuous culture of malaria parasites, there have been reports that *in vitro*, the growth of *Plasmodium* is retarded in cells containing abnormal haemoglobin[3,4,5,6,7,8]. There have also been clinical and epidemiological reports that individuals with abnormal haemoglobin do enjoy some protection against infection by *Plasmodia* [9].

To date not much is known about the possible relationship between the genes responsible for abnormal haemoglobin and immune response to malaria by individuals carrying such genes. For example sickle cell disease is known to be associated with low resistance to infection[7]. A good understanding of the immune response to malaria of individuals carrying sickle cell gene may provide clues for chemotherapeutic and/or immunological strategies for treating such individuals in Nigeria as they constitute a resonable proportion of the population. Furthermore, malaria vaccines are now being developed; to establish the safety, immunogenicity and efficacy of the malaria vaccines, there should be a clinical trial which should be followed by an epidemiological evaluation among the entire population including individuals with haemoglobinopathies. The results obtained in such studies may serve as a useful baseline data for the evaluation of the efficacy of the malaria vaccine in malaria endemic area.

We have therefore designed comparative studies to assess the immune responses to malaria of individuals with and without abnormal haemoglobin in Ibadan, Nigeria, a malaria endemic area.

Materials and methods

A total of 125 patients who reported to the hospital with complaints such as headache, body pain, nausea and lack of appetite, suggestive of malaria were admitted into the programme after clinical

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examination. These patients comprising of 36 homozygons HbSS seen at Haematology clinic of the Ring Road State Hospital, Ibadan; 32 HbAS, 48 HbAA and 9 HbAC patients, all seen at Jericho Nursing Home, Ibadan. The age and sex of each were recorded and the axillary temperature noted. Venepuncture at antecubital fossa was done and 10 ml of blood was taken and distributed as follows: 2ml was put into sequestrene bottle and the remaining put in the plain clean container for the preparation of sera used for the estimation of immunoglobulins. Thin and thick blood films were made from sequestrene sample and stained by Leishman and Geimsa as described by Charterjee[10]. The blood films were examined using X 100 objective lens of the microscope.

Immunoglobulins G, M and A were estimated by single radial immunodiffusion technique of Mancini[11]. Only malaria positive samples were assayed.

Determination of Hb type: The haemoglobin types were determined by cellulose acetate electrophoresis using Tris- EDTA Aborate buffer (pH 8.9) [12].

Statistical analysis: Correlation between haemoglobin genotype and proportion of parasite incidence was determined by Z-score test while comparison of the mean values of the immunoglobulins, testing the null hypothesis that there is no difference between mean values observed in individuals with HbAA and patients with haemoglobinopathies was tested by Student t- test.

Results

Parasitological examination

The effect of Hb type of *P. falciparum* incidence is shown in Table 1, of the 48 HbAA patients that reported with symptoms suggestive of malaria 36 (61.4%) were positive for parasitaemia. On the other hand, 13 (21.0%) and 10 (16.1%) of 32 HbAS and 36 HbSS patients respectively were found to be positive for *P. falciparum*. All the 9 HbAC patients showed positive parasitaemia.

The proportion of the HbAA patients that are malarial parasite negative in their blood is significantly lower than the proportion of HbSS. ($Z = 2.409$, $P < 0.05$) (see Table 2). In the same way the proportion of HbAA patients that are malaria positive is higher than that of HbAS patients ($Z = 1.348$, $P = 0.05$). No significant difference is observed between HbAA and HbAC patients.

Immunoglobulin levels

Table 3 summarises the effect of malaria infection on immunoglobulin levels in Nigerians with different haemoglobin electrophoretic types. On the average, HbSS patients have the highest mean values of the 3 classes of immunoglobulin assayed. This is followed by HbAS while patients with normal haemoglobin have lowest means for IgG & IgM.

Table 1: The effect of haemoglobin electrophoretic type on malaria parasite incidence in individuals in Ibadan, Nigeria

Group	AA	AS	AC	SS
Parasitaemia	30/48 (61.4%)	13/32 (21.0%)	9/9 (14.5%)	10/36 (16.1%)
Without Parasitaemia	18/48 (28.6%)	19/32 (30.2%)	09 (0.0%)	26/36 (41.3%)
Age mean (years)	25.6 S.D = 16.2	22.1 S.D = 12.8	18.1 S.D = 5.7	17.7 S.D = 5.9 yrs

Figures in parenthesis indicate percentage of total samples examined for all the groups. The only specie of parasite seen is *P. falciparum*
S.D = Standard deviation

Table 2: Comparison between HbAA and abnormal haemoglobins and parasitaemia

Group	Parasitaemia	Negative	Total
Hb type AA SS	Positive 30 (62.5%) 10 (27.8%) Z = 2.078 P < 0.05	Negative 18 (37.5%) 26 (72.2%) Z = 2.409 P < 0.05	Total 48 36
AA AS	30 (62.5%) 13 (40.6%) Z = 1.348 P = 0.05	18 (37.5%) 19 (59.4%) Z = 0.983 N/S	48 32
AA AC	30 (62.5%) 9 (100%) Z = 4.242 N/S	18 (37.5%) 0 (0.0%)	48 9

N/S = Not significant

Table 3: Effect of malaria infection on immunoglobin levels in individuals with different haemoglobin electrophoretic types

Parameters	AA	AS	Group SS	AC	AA/SS	Pairs compared AA/AS	AA/AC
IgG	X = 861 S.D = 405	X = 994 S.D = 489	X = 1406 S.D = 516	X = 832 S.D = 384	t = 3.124 P = 0.005	t = 0.88 P = 0.40	t = 144 P = 0.40
IgM	X = 138 S.D = 102	X = 190 S.D = 136	X = 1441 S.D = 821	X = 160 S.D = 108	t = 8.765 P < 0.001	t = 1.372 P = 0.1	t = 0.551 P > 0.1
IgA	X = 133 S.D = 40	X = 128 S.D = 41	X = 197 S.D = 55	X = 172 S.D = 34	t = 3.714 P = 0.001	t = .431 P > 0.5	t = 2.479 P < 0.025

X = Mean values (mg/d)
S.D = Standard deviation

Discussion

Although there is now general agreement that sickle cell trait confers considerable protection against malaria, the actual mechanism by which it does so is still undetermined. Mackay and Vivarelli[13] suggested that the presence of malaria parasites might cause cells containing HbS to sickle and then be removed by phagocytosis. Miller *et al* [14] pointed out that parasitized cells tend to adhere to the walls of the blood vessels and that cells adhering to the walls of the capillaries and veins might become sickled and so interfere with the multiplication of the parasite. This may not be true entirely; infact adherence of the parasite to the walls could help in its development and may be the reason why schizonts are not commonly found in peripheral blood. Allison[15] had postulated that *Plasmodium* might have a high rate of oxygen uptake which might induce sickling of the cells of heterozygotes. This may not be correct because since the advent of continuous culture of malaria parasite it is infact known that Plasmodium is microaerophilic.

Furthermore, Allison[15] had come out to suggest that *P. falciparum* might be unable to break down and utilize sickle cell haemoglobin as effectively as normal adult haemoglobin (HbA). The understanding of the biology of the parasite[16] shows that the food

vacuole of *Plasmodium* is acidic and it is because of this acidic nature that chloroquine attacks the parasite. That malaria parasite cannot utilize HbS as effectively as HbA might be due to the acidic nature of its food vacuole in which HbS is not soluble. The present studies have confirmed earlier report[17] that the incidence of malaria parasitaemia is lower in carriers of sickle cell haemoglobin than individuals homozygous for HbA (Table 1). The proportion of malaria/positive patients in HbAA group were found to be higher than those of SS ($Z = 2.409$, $P < 0.05$) (Table 2); so also a higher proportion of HbAA patients were found to be positive for *P. falciparum* in their blood film than HbAS patients as shown in Table 2 ($Z = 1.348$, $P < 0.05$). There is no difference in parasitaemia in HbAA and HbAC patients. All the 9 HbAC patients who reported with complaints suggestive of malaria showed positive parasitaemia. However, because of the sample size (9 HbAC) it will be difficult to conclude that HbC will support the growth or development of *Plasmodium* or not; even though Dei-cas *et al.* [5] had reported that the presence of HbC makes the parasite to show signs of functional impairement.

Immunoglobulins: Cornille - Brogger *et al.* [17] and Molineaux *et al.* [18] had reported while comparing sickle cell trait individuals with normal HbAA individuals in malaria hyperendemic area, that after the first one year of life, the sickle cell trait have on the average a lower IgM and IgG levels and that sickling is sufficient to protect against malaria. The present study reports immunoglobulin levels in malaria infected patients. HbSS malaria patients have the highest mean values of the 3 classes of immunoglobulin assayed (Table 3). This is followed by HbSS patients that were found to have significantly higher mean values of IgG and IgM.

When compared statistically only malaria HbSS patients were found to have significantly higher mean values of IgG, IgM and IgA ($t = 3.124$, $P < 0.005$, $t = 8.765$, $P < 0.001$ and $t = 3.714$, $P = 0.001$ respectively). There were no significant differences between mean values of IgM and IgG when malaria HbAS and HbAC patients were compared with malaria HbAA patients. However, there was significant difference between mean values of IgA between HbAA and HbAC patients.

From the present findings it appears that humoral immune response of HbSS individuals to malaria appears to be higher than individuals with normal HbAA. However, it should not be concluded that the HbSS individuals show higher immune response to malaria than HbAA since HbSS patients are known to be prone to other infections. But since both groups were under the attack of *Plasmodium falciparum* as at the time their blood samples were taken, the findings still suggest that HbSS malaria patients may synthesize immunoglobulins at a higher rate than HbAA patients with malaria infections.

To actually confirm this, the estimation of specific malaria antibodies in all the groups is going on in our laboratory. This will ascertain whether the higher immunoglobulin levels in patients with sickle cell is actually in response to malarial antigen stimulation. A clearer understanding of the immune response to malaria by individuals with and without abnormal haemoglobins in malaria endemic area may provide further clues to the possible genetic link between abnormal haemoglobin and protection against malarial infection.

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References

1. Allison AC. Protection afforded by sickle cell trait against subtertian malaria infection. Brit. Med. J. 1954; 1: 290- 294.
2. Fleming AF, Stoney J, Monineauax L, Iroko EA, Attai EDE. Prevalence of haemoglobins relationship between sickle cell trait, malaria and survival. Ann. Trop. Med. Parasitol. 1979; 73 (2): 169-172.
3. Friedman MJ. Erythrocyte mechanism of sickle cell resistance to malaria. Proc. Nat. Acad. Sci. U.S.A. 1978; 75: 1994.
4. Pasvol G. Foetal haemoglobin and malaria. Lancet 1 1972; 1269-1272.
5. Dei-Cas E, Slomimamy C, Charet P, Prensier G, Ajana F, Ramage C, Veines A. *In vitro* growth and chloroquine sensitivity of *Plasmodium falciparum* (FCR - 3 strain) in red blood cells containing HbC. Parisitol. Res. 1987; 73 (4): 306-312.
6. Yuthavong Y, Butheg P, Buryoratueji A,

Fucharem S, Khusmith S. Inhibitory effect of beta-zero thalasaemia/haemoglobin E erythrocytes in *Plasmodium falciparum* growth *in-vitro*. Am. J. Clin. Pathol. 1987; 88 (3): 409-415.

7. Luzzato L, Nwachukwu - Jarret ES, Redy S. Increased sickling of parasitized eruthrocytes as mechanism of resistance against malaria in sickle cell trait. Lancet 1970; 1: 319.
8. Yuthavaong Y, Butheg P, Buryoratueji A, Fucharem S, Khusmith S. Impaired parasite growth and increased susceptibility to phagocytosis of *Plasmodium falciparum* infected alpha thalasaemia or haemoglobin constant spring red blood cells. Am. J. Clin. Pathol. 1987; 89 (4): 521-525.
9. Marsh KL, Otoo RJ, Hayes DC, Carson, Greenwood BM. Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relations to protection against infection. Trans. Roy. Soc. Trop. Med. Hyg. 1989; 83: 293-303.
10. Charterjee KD. In Parasitology - Helminthology and Protozoology, 11th Ed. Calcuta 1976; pp. 72-92.
11. Mancini G. A single radial diffusion method for immunological quantitation of proteins. Colloquine Protides. Biol. Fluids 1965; 11: 370-373.
12. Dacie JV, Lewis SM. In Practial Haematology 5th Ed. Churchill Livingstone. 1975; pp. 241-246.
13. Mackey JP, Vivarelli P. Sickle cell anaemia. Brit. Med. J. 1954; 1: 276.
14. Miller MH, Neel JV, Livingstone FB. Distribution of parasites in cells of sickle cell trait carriers infected with *P. falciparum*. Trans. Roy. Soc. Trop. Med. Hyg. 1956; 50: 294.
15. Allison AC. Genetic factors in resistance to malaria. Ann. N.Y. Acad. Sci. 1961; 91 (3): 710-729.
16. Oduola AMJ. Private Communication 1990.
17. Cornille - Brogger P, Fleming AF, Kagan I, Matsunshima T, Moloneaux L. Immunological response to malaria in normals and subject with sickle cell trait. Ann. Trop. Med. Hyg. 1979; 73 (2): 174-183.
18. Molineaux L, Fleming AF, Cornille - Brogger P, Kagan I, Storey J. Malaria, immunoglobulins and anti-malaria B antibodies in sickle cell disease. Ann. Trop. Med. Parasitol. 1979; 73 (4): 301-310.

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