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Humoral immunity and haemoglobin F (HbF) status in steady state adult Nigerian sickle cell disease patients with asymptomatic malaria

Aim: Nigeria, where malaria is prevalent, has the largest sickle cell gene pool in the world. To this end, there is a need for increased understanding of the pathophysiology of HbSS patients in a malaria endemic zone to reduce mortality. Haematology Department, University College Hospital, Ibadan, Nigeria.

Materials and methods: The levels of HbF, IgG, IgA, IgM, transferin (TRF), C-reactive protein (CRP), and haptoglobin (HPT) were determined in 14 Nigerians having HbAA genotype with malaria parasitaemia (HbAA +MP), 17 Nigerians having haemoglobin AA genotype without malaria parasitaemia (HbAA –MP), 15 Nigerians having haemoglobin SS genotype without malaria parasitaemia (HbSS –MP), and 14 Nigerians having HbSS with malaria parasitaemia (HbSS +MP).

Results: The levels of Ig classes were statistically similar in all the groups. CRP was significantly increased in HbSS –MP compared with HbAA +MP or HbAA –MP. The levels of TRF and HPT were significantly reduced in HbSS –MP or HbSS +MP compared with HbAA –MP. Also TRF and HPT were significantly reduced in HbSS +MP compared with HbSS –MP.

Conclusion: These observations may be related to RBC haemolysis especially in HbSS +MP.

Key words: Sickle cell disease, malaria parasitaemia, immunoglobulins, inflammation, haemolysis

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Introduction

Sickle cell disorder is the most important genetic haematological disease that affects the people of black African descent. About 2% of all babies born to Nigerian parents have sickle cell anaemia (1). The distribution of indigenous sickle cell disorder coincides with the distribution of falciparum malaria. Possession of sickle cell trait confers a natural protection against death from malaria in children while sickle cell anaemia (HbS) easily develops severe hyper-haemolytic anaemia and are least fitted for survival in malaria environment (2). Increased mortality of HbS patients due to malaria was supported by Molineaux et al. (3) who reported improved survival of Nigerian children following introduction of anti-malaria measures.

Levels of HbF molecules interfere with polymerization of HbS molecules thus inhibiting sickling (4), and HbF has been reported to correlate with severity of disease in HbSS (4). A study concluded that HbF prevents the growth of *P. falciparum*. The mechanism was reported to involve resistance to alteration by malaria haemoglobinase and super stability of HbF (5).

Sickle nature of RBC in sickle Cell Disease (SCD) patients was reported to be responsible for the increased susceptibility of SCD subjects to malaria. Also defective immune responses in SCD could also be conjectured. Abnormal immune responses in SCD patients are reduced neutrophil count and activity (6), reduced

C4 (7), decreased C3 (8), increased C1 inhibition, and reduced C3 activator (9). These defective immune factors are among important naturally acquired immunity to erythrocytic stages of malaria parasites (6).

For successful management of SCD patients in malaria endemic areas, there is a need for more research to provide additional information for increased susceptibility of sickle cell disease patients to malaria parasites.

Materials and methods

Patient recruitment

Sixty subjects were recruited for the study. They were 29 consecutive SCD patients (15 males and 14 females) in steady state (without crisis within the previous 3 months of recruitment) from the Department of Haematology, University College Hospital, Ibadan, Nigeria and 31 HbAA controls (17 males and 14 females) selected among the blood donors and staff of the University College Hospital, Ibadan, Nigeria. They were aged between 26 and 55 years.

Ethical clearance was obtained from the Institutional Ethical Review Committee and informed consents were obtained from the subjects before sample collection.

A 10 ml blood sample was collected by venipuncture from each participant; 6 ml of which was put in bottles containing E.D.T.A (ethylene diamine tetra-acetic acid) for the estimation of HbF (10) and haemoglobin electrophoresis, and the detection of malaria parasites using standard methods (11) while the remaining 4 ml was put in plain bottles from which serum was separated for the determination of Ig classes and acute phase proteins using the immunodiffusion method (9).

Statistical methods

All statistical analyses were performed using SPSS 15.0. Results were expressed as mean ± SD. The difference between mean and standard deviation was compared using Student's t test. Pearson's correlation coefficient was used to establish a relation between immunological parameters and HbF. Values of $P < 0.05$ were considered statistically significant.

Results

As shown in Table 1, the level of HbF was significantly increased in HbSS –MP or HbSS +MP compared with HbAA –MP or HbSS +MP. The levels of immunoglobulins classes (IgG, IgA, and IgM) were not statistically different when the groups were compared.

The level of CRP was significantly raised in HbSS –MP compared with HbAA –MP or HbAA +MP. The levels of TRF and HPT were significantly reduced in HbSS –MP or HbSS +MP compared with HbAA –MP. The levels of CRF and HPT were significantly reduced in HbSS –MP compared with HbAA +MP but were significantly raised in HbSS –MP compared with HbSS +MP.

As shown in Table 2, only CRP in HbSS +MP showed positive correlation with immunological parameters in SCD patients while in Table 3 none of the immunological parameters showed correlation with HbF in HbAA Nigerian subjects.

Discussion

SCD is clinically one of the most important haemoglobinopathies. Over the years many advances have been made in understanding sickle cell crisis and several principles about their complications were established (12). The most popular principle is that the bizarre shaped sickle cells adhere to endothelium to induce the vaso-occlusive process that occurs in almost all the vascular beds leading to ischaemic injury with organ dysfunction and early death (13). In contrast, it was reported that sickle red blood cells are least adhesive and that location of vascular occlusion is in the microvascular postcapillary venules (14). It was hypothesized that vaso-occlussive process is probably a continuum (15), where some adhesive cells are continually present in the post capillary venules but when the number increases, and other ancillary factors are favourable; vaso-occlusion will begin (15). Few of such ancillary factors are proposed by the present study.

Certain acute phase proteins have been linked with the severity of anaemia and sickle cell crisis. Based on the hypothesis that vaso-occlusive crisis in SCD is gradual and continuous, the authors of this study

Table 1. The levels (mean ± standard deviation of HbF, Ig classes, and acute phase proteins (mg/dL) in HbSS Nigerians with or without malaria parasitaemia compared with controls.

	N	HBF	CRP	TRF	HPT	IgG	IgA	IgM
HbAA -MP	14	0.86 ± 0.44	6.5 ± 5.09	1.68 ± 0.29	1.41 ± 0.53	1564 ± 435.30	285 ± 202	102 ± 65
HbAA+MP	17	1.13 ± 0.37	5.94 ± 4.72	1.51 ± 0.45	1.48 ± 0.67	1607 ± 345.30	360 ± 216	500 ± 165
HbSS -MP	15	4.65 ± 5.0	30.00 ± 37.58	1.15 ± 0.30	0.65 ± 0.65	1539 ± 265.43	318 ± 88	605 ± 175
HbSS+MP	14	4.54 ± 2.54	27.00 ± 35.71	1.10 ± 0.20	0.35 ± 0.38	1611 ± 266.7	306 ± 167	133 ± 113
F-, P value		8.31,0.00	3.40,0.02	10.54,0.00	14.18,0.00	0.06,0.98	0.05,0.60	0.62,0.61
t-,P value [a]		1.48,0.08	0.36,0.72	1.24,0.23	0.31,0.76	0.30,0.76	0.99,0.33	0.90,0.30
t-,P value [b]		2.83,0.01	2.31,0.03	4.88,0.00	3.47,0.00	0.22, 0.83	0.58,0.56	1.07,0.29
t-,P value [c]		5.36,0.00	2.00,0.50	6.18,0.00	6.05,0.00	0.34,0.74	0.31,0.76	0.88,0.39
t-,P value [d]		2.91,0.01	2.62,0.01	2.63,0.01	3.58.0.00	0.12,0.91	0.70,0.49	0.17,0.86
t-,P value [e]		5.51,0.00	2.29,0.30	3.14,0.00	5.58,0.00	0.04,0.97	0.76,0.45	0.83,0.41
t-,P value [f]		0.07,0.95	0.21,0.84	0.51,0.61	1.47,0.51	0.17,0.86	0.25,0.81	1.01,0.32

[a] = HbAA-MP compared with HBAA +MP
[b] = HbAA -MP compared with HbSS -MP
[c] = HbAA -MP compared with HbSS +MP
[d] = HbAA +MP compared with HbSS -MP
[e] = HbAA +MP compared with HbSS +MP
[f] = HbSS -MP compared with HbSS +MP

Table 2. Correlation of HbF in Nigerians having HbSS genotype with immunological factors.

		HbF	
		r	P
HbSS +MP	CRP	+0.61	0.02
	TRF	+0.11	0.71
	HPT	+0.27	0.36
	IgG	+0.42	0.14
	IgA	-0.09	0.76
	IgM	-0.12	0.70
HbSS, -MP	CRP	-0.02	0.95
	TRF	-0.12	0.66
	HPT	-0.19	0.50
	IgG	+0.10	0.73
	IgA	+0.04	0.88
	IgM	+0.16	0.56

Table 3. Correlation of HbF in Nigerians having HbAA genotype with immunological parameters.

		HbF	
		r	P
HbAA +MP	CRP	-0.34	0.18
	TRF	-0.06	0.83
	HPT	-0.56	0.21
	IgG	+0.03	0.91
	IgA	+0.18	0.48
	IgM	-0.31	0.23
HbAA, -MP	CRP	+0.01	0.96
	TRF	-0.15	0.62
	HPT	-0.18	0.52
	IgG	+0.22	0.45
	IgA	-0.31	0.29
	IgM	+0.01	0.99

determined the levels of certain acute phase proteins, immunoglobulin classes, and HbF in steady state SCD patients. It is also recognized that malaria parasite initiates vaso-occlusive crisis while at the same time triggering haemolysis of RBCs that already have shortened life span (16).

The level of CRP was significantly raised while TRF and HPT were significantly reduced in HbSS-MP compared with HbAA-MP. Also the levels of Ig classes were not significantly different in HbSS-MP compared with HbAA-MP. The implication of these results is that our HbSS subjects were in a state of low grade inflammation and were not having clinical infections. More so, these responses are likely a consequence of the frequent infarcted tissue damage that characterized SCD. The reduced level of TRF or raised level of CRP observed in HbSS group might be consistent with the known role of these proteins as acute phase reactants. HPT is known to complex with intravascularly liberated haemoglobin that is removed from circulation by reticuloendothelial system thus reduced level of HPT in SCD is expected where intravascular and extravascular haemolysis are constant features (12).

TRF is significantly reduced in SCD patients. This may be due to increase loss in urine or faeces, malnutrition as a result of metabolic damage, and most likely due to acute phase response to the chronic tissue damage that occurs in SCD patients. The resting metabolic rate is 20% higher in HbSS compared with HbAA in Jamaican cohort (17). Factors contributing to this increased rate were reported to include greater cardiac work secondary to anaemia, sub-clinical chronic inflammatory, and metabolic demands of a greatly expanded bone marrow. For reasons yet unclear these increased demands are not met by increased caloric intake (18). These may explain low levels of TRF in HbSS since TRF is one of the biochemical indicators of nutritional status. Reduced TRF level in SCD disease may therefore partly explain the increased susceptibility of these patients to pyogenic bacteria; as low circulating transferrin will lead to excess free iron in the blood. Iron is vital for growth and proliferation of *Plasmodium* especially in the developing merozoites to trophozoites in the RBC (19, 20). This has formed the basis why iron chelator was proposed as antimalarial chemotherapy. There are several opinions for the sources of iron available for the growth of malaria parasite. It was reported that TRF is the source of iron for *Plasmodium* (19), while others reported that *Plasmodium* uses a labile intracellular pool of iron for its metabolism (20).

CRP is a non-specific acute reactant that is raised in many acute or chronic states. SCD patients are susceptible to infectious agents, which are frequently associated with raised CRP. Therefore, the use of CRP is limited in clinical setting. The finding in this study of significant positive association between CRP and HbF in HbSS with falciparum malaria may suggest the use of serum concentration of CRP as an indicator of complications in SCD patients.

HbF molecules interfere with polymerization of HbS molecules thereby inhibiting sickling; high HbF levels protect the HbSS patients from many of the early life threatening complications of the disorder. HbF is also protective against malaria thus high HbF level is expected in HbSS patients (particularly HbSS +MP) compared with HbAA as shown in our study.

There is accumulating evidence for the role of IgG antibodies in protection against malaria infection. Kinyanjui et al. (21) described long term antibody response to malaria in Kenyan children. Antibody response generated a peak after 7 days and lasted for approximately 6 weeks. An IgM response was reported to be minimal and a reflection of secondary malaria antibody response. Both IgG and IgM were not significantly raised in HbAA +MP compared with HbAA -MP thus indicating asymptomatic nature of malaria in our subjects.

This study concludes that reduced TRF and HPT in SCD patients may be a consequence of low grade haemolysis and that reduced TRF and HPT contribute to their susceptibility to malaria and certain infections by making iron available for proliferation.

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