Original Article

The Effect of Alpha (+) -Thalassaemia on *P. falciparum* Malaria Parasitaemia in Children Attending Komfo Anokye Teaching Hospital

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Malaria reportedly accounts for 10% of Africa's disease burden and about 90% of the global morbidity and mortality affecting mostly children under 5 years old. Previous studies have ex-pressed varied opinion on the protection from severe *Plasmodium falciparum* malaria by α+-thalassaemia. A random cross-sectional sampling of 456 children at the Komfo Anokye Teach-ing Hospital (KATH), Kumasi, Ghana were tested for malaria parasite, complete blood count (CBC), serum ferritin, and α-globin genotype. Alpha (+)-thalassaemic children recorded a sig-nificantly lower (28,705/µL) mean parasite density (MPD) compared to non-α-thalassaemic children (35,483/µL) ($p<0.0001$). The homozygotes, α+-thalassaemia, recorded a significantly lower (691/µL) MPD compared to 26,350/µL for the heterozygotes and 35,483/µL for the non-α-thalassaemic children ($p = 0.0001$). Alpha (+)-thalassaemia was hypothesized to protect against malaria via a reduction in the parasite density. The homozygous α+-thalassaemias were more protective than the heterozygous. Microcytic hypochromic anaemia was found in 141 (59%) of the subjects of which 71 were α+-thalassaemia. Alpha (+)-thalassaemia was shown to be a possibly key contributor to microcytic hypochromic anaemia amongst cases that were suspected of iron deficiency. Suspected iron deficiency cases should therefore be screened for α-thalassaemia to avoid the unnecessary administration of iron supplement.

Key words*: *alpha+thalassaemia, malaria, RBC, microcytosis, Ghana

Introduction

The α+-thalassaemia is the most common single gene disorders in the world affecting 5 to 10% in the Mediterranean, 20 to 30% in West Africa, and approximately 68% in the South Pacific [1]. In Ghana, there have been reports of the high prevalence of α+-thalassaemia, especially the heterozygous affecting between 26-33% of the population [2,3]. The α+ thalassaemia is prevalent in areas where malaria is endemic thus providing a compelling example of natural selection. Malaria reportedly accounts for 10% of Africa's disease burden and the fact that about 90% of the global morbidity and mortality of malaria is suffered by Africa especially children below five years [4]. Though α+-thalassaemia is reported to confer protection against the fatal consequences of malaria, especially the type caused by *Plasmodium falciparum*, there is however, a degree of controversy as to whether it is the heterozygous α+-thalassaemia trait, the homozygous α+-thalassaemia trait or both α-globin genotype that offer protection, or indeed whether the protection applies to all forms of malaria. According to a study in Papua New Guinea (PNG), the risk of severe malaria was reportedly reduced by 60% and 34% in ho-

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mozygous and heterozygous α+-thalassaemic children, respectively [5]. Some studies have however, expressed varied opinion on the protection from severe *Plasmodium falciparum* malaria by α+-thalassaemia. Whereas a Ghanaian study found no protection for the homozygous status [3], a Kenyan study found protection for both homozygous and heterozygous conditions [6]. As is the case in sickle cell trait, the mechanism of the proposed protection might most likely be linked to a reduction in the parasite density [7,8].

Similarly, α+-thalassaemia is suggested to have a causative effect on anaemia, specifically microcytic hypochromic anaemia; mean cell volume (MCV) < 80 fL; mean corpuscular haemoglobin (MCH) < 27 pg). It had been indicated that about 50% of individuals analyzed had α+-thalassaemia as the cause of the microcytosis and hypochromia [9]. Similarly, Rahim found a considerable 20% prevalence of α+-thalassaemia in microcytic hypochromic anaemic patients [10]. These were also supported by findings that microcytic hypochromic red cell might be responsible for conferring protection from severe *P. falciparum* malaria in α+-thalassaemia [11].

We tested the hypothesis that heterozygous, homozygous or both α+-thalassemia, have effect on malaria parasitaemia, and that α+-thalassemia contributes to microcytic hypochromic anaemia.

Materials and Methods

Study site, subject population, and sample collection

The study was conducted at the Komfo Anokye Teaching Hospital (KATH), and the Kumasi Centre for Collaborative Research (KCCR), both located in Kumasi, with a projected population of about 1.2 million [12]. The ethnicity of Kumasi is predominantly Akans, who make up 77.7% of the population, while other smaller groups, including, but not limited to, the Mole-Dagbon (9.1%), Ewe (2.9%), and Grusi (2.9%), account for the rest [13]. A total of 456 children, aged 1 week to 10 years, were recruited between the months of May and December 2008. Five ml of blood was obtained by venepuncture after consent by parents or guardians. The Committee on Human Research Publication and Ethics (CHRPE), Kumasi gave approval for the study

Malaria parasite test

Thick and thin blood films were prepared, stained with Giemsa, and read for malaria parasites following standard, quality-controlled procedures. Parasitaemia was expressed as the number of asexual forms of the malaria parasites per microlitre. It was graded as low (1-999/µl), moderate (1000-9999/µl) and high (≥ 10000/µl) [14,15].

Complete blood count (CBC)

Haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and red cell count (RBC), were determined using the Cell-Dyn® 3700 (Abbott Diagnostics, USA). Microcytosis (MCV ≤ 80fL) and hypochromia (MCH ≤ 27pg) and RBC count ($<4.2x10^6$ /uL)) were used to exclude microcytic hypochromic anaemia.

Serum ferritin test

All samples that showed microcytosis were further evaluated for plasma ferritin [16] to exclude iron deficiency, leaving probable alpha thalassaemia as causes of microcytosis The test was conducted using a microparticle enzyme immunoassay (MEIA), (Abbott laboratories, USA.) technology, which uses a solution of suspended, submicron, sized latex particles to measure ferritin. Using this method plasma ferritin shows 79% specificity and 79% sensitivity for IDA and a cutoff of <273 µg/µl best predicted iron deficiency anaemia (IDA) [17]. The results were compared with the age-adjusted values described previously elsewhere [18].

Polymerase chain reaction (PCR)

The molecular characterization of the alpha plus $\alpha^{3.7}$kb heterozygous and homozygous were determined by DNA sequence analysis of each deletion breakpoint using the single-tube multiplex-PCR assay [19] capable of detecting any combination of the 6 common single and double gene deletion. Since each of the 6 deletions either partially or completely removes the α_2 gene, its positive amplification was used to indicate heterozygosity when a deletion allele is present. Amplification of the large 2.5Kb segment of the LISI gene 3' UTR was included as control for the amplification. The extraction of the DNA complied with the protocol described in the QIAamp DNA mini kit handbook [20].

Following the extraction of the DNA, each DNA

sample was amplified using a thermal cycler (Eppendorf, Germany). Along with the test sample, each PCR run included three positive control DNAs (mutant DNA (64 ng/µl), wild type DNA (71ng/µl), and heterozygote DNA (105ng/µl)) and a negative control of aqua ad injectabilia. The total reaction volume was 50 µl in adherence to the protocol of Chong [19]. Each 50µl reaction tube contained primers as shown in Table 1.

After the DNA, amplification the product was electrophoresed using 1.5% agarose gel. The images were then printed out on a black and white Polaroid film. The individual bands were then compared with the positive controls, and the different genotypes determined. The extent of deletion was confirmed by comparing the bands with the 100bp DNA ladder. Along the 100 bp DNA ladder, the 1800 bp point served as the wild type, the 2000 bp, the deletion type, while the heterozygote form comprised two strands, one of the 1800 bp, while the others were of the 2000 bp. The results were thus reported as wild type or normal (αα/αα), mutant or deletion type (-α/-α), and heterozygote (-α/αα).

Statistical analysis

Categorical data were analyzed using the χ^2 test for trend, and numerical data were compared using one way analysis of variance (ANOVA) or unpaired *t*-test and were determined using logistic regression from GraphPad prism version 5.00 for windows (GraphPad software, San Diego California USA). In all statistical test, a value of $p<0.05$ was considered significant.

Results

Age group, sex distribution and incidence of P. falciparum

Out of the total of 456 children (mean age 4.3 years) who were tested for malaria parasites, 60 (13.17%) were positive, with ages 1 week to 5 years having an incidence rate of 7.47% and a mean parasite density (MPD) of 8609.0 μL^{-1} compared to 5.70% incidence rate in the 6 to 10 year group with MPD of 51,740.0 μL^{-1} ($p<0.0001$) (Table 2).

Females had a higher (15.51%) infection rate compared to the males (11.52%), ($p=0.2706$). However, females recorded a significantly higher (50,180.0 μL^{-1}) MPD compared to males (9,513.0 μL^{-1}) ($p<0.0001$) (Table 2).

A total of 84 (35.59%) of the children were positive for α+-thalassaemia, (28.81% heterozygotes and 6.78% homozygotes) while 152(64.41%) were non-α-thalassaemic (Figure 1)

The incidence rates of malaria in children with α+-thalassaemic (16.67%)compared to non-α+-thalassaemic (26.32%) were not significant ($p = 0.2036$), (Table 3) although α+-thalassaemic children had a significantly lower (28,705/µL) MPD compared to non-α+-thalassaemic children (35,483/µL) ($p < 0.0001$). Interestingly, the homozygotes recorded a significantly lower (691/µL) MPD compared to 26,350/µL for the heterozygotes and 35,483/µL for the non-α-thalassaemic children ($p = 0.0001$). Severe malaria amongst α+-thalassaemic children (1.19%) and non-α+-thalassaemic children (1.97%) showed no significant difference ($p = 0.6605$).

Microcytic hypochromic anaemia (MCV ≤ 80fL, MCH ≤ 27pg and RBC count <4.2) was evident in 59%

Table 1 A collection of the primers used

Name	5'-3' Sequence	GenBank ID: Nucleotide
α_2/3.7-F	CCCCTCGCCAAGTCCACCC	HUMHBA4:5676-5694
3.7/20.5-R	AAAGCACTCTAGGGTCCAGCG	HUMHBA4:11514-11494
α_2-R	AGACCAGGAAGGGCCGGTG	HUMHBA4:74757457

Table 2 Age group, sex distribution and malaria incidence in children within the Kumasi metropolis

Age (yrs)	Total (456)		Male (269)		Female (187)	
	%(Infected)	MPD (/µL)	%(Infected)	MPD (/µL)	%(Infected)	MPD (/µL)
≤ 5	7.47(34)	8,609	6.69(18)	13,220	8.55(16)	15,900
6 to 10	5.70(26)	51,740	4.83(13)	30,380	6.95(13)	73,100
Total	13.17(60)	30,626	11.52(31)	9,513	15.51(29)	50,180

MPD = mean parasite density

Table 3 Incidence of malaria in α+-thalassaemic children within the Kumasi metropolis

		-α/αα & -α/-α (84)	-α/αα (68)	-α/-α (16)	αα/αα (152)
Malaria	Prevalence	16.67(14)	16.18(11)	18.75(3)	26.32(40)
	MPD	28,705.0	26,350.0	691.3	35,483.0
Severe malaria		1.19(1)	1.47(1)	0(0)	1.97(3)

MPD =mean parasite density;

Severe malaria = Hb ≤ 8.0g/dl with mean parasite density of >10,000 μL^{-1}

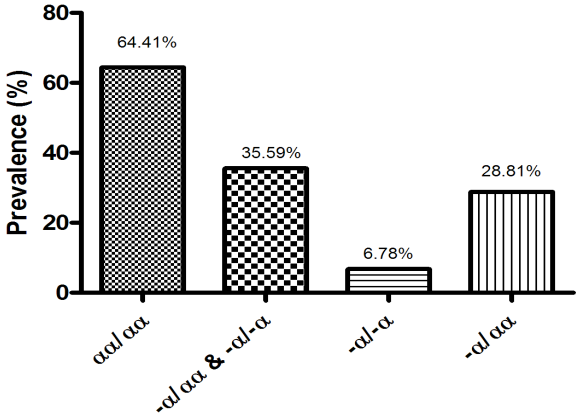

Fig.1 *Prevalence of α-thalassaemia genotypes in children within the Kumasi Metropolis.*

of the subjects. These subjects were further tested for serum ferritin (cut-off level of 273µg/µl), of which 120(85.0%) had normal/high levels, while 21(15.0%) had low levels. Out of the 120 with normal/high serum ferritin levels, 63(52.5%) were α+-thalassaemia, while 57(47.5%) had the normal genotype (αα/αα). Of the 21 with low serum ferritin levels, 8 (38.1%) were also α+-thalassaemia, while 13(61.9%) were negative for α+-thalassaemia (Table 4).

Mean RBC, MCV and MCH counts were higher in α+-thalassaemic children compared to non-α+-thalassaemic (Figure 2A, 2C, 2D) (p<0.0001, p<0.0005, p<0.0019) however, this was not the same for mean Hb (p = 0.1635) (Figure 2B).

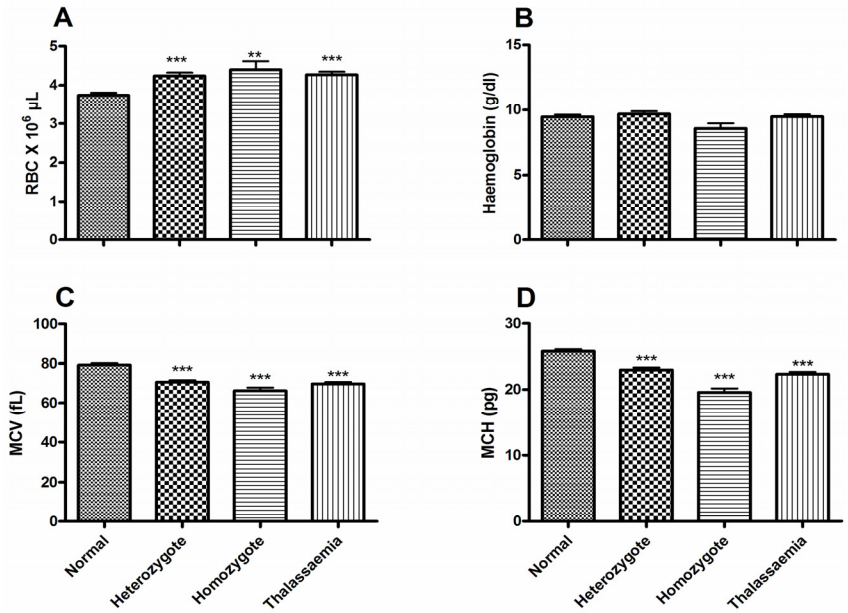

Fig.2 *Haematological parameters of α+-thalassaemic against non α-thalassaemic children.*
A: Mean RBC value in normal and alpha thalassaemic children; B: Mean Hb value in normal and alpha thalassaemic children; C: Mean MCV value in normal and alpha thalassaemic children; D: Mean MCH value in normal and alpha thalassaemic children.

Table 4 Characteristics of serum ferritin level by non-microcytic hypochromic, microcytic hypochromic (IDA) and α + thalassaemia

Non-Microcytic Hypochromic (MCV≥80 fl; (MCH≥27pg)		Microcytic Hypochromic (MCV<80 fl); MCH <27pg)			
Prevalence	186 (41%)	270 (59%)			
		Microcytic Hypochromic/Microcytic Hypochromic (IDA)/ (α + thal)			
Serum Ferritin (n=141) <273ng/μL		21 (15%)		120 (85%)	
α + thalassaemia genotype		α+ thal	αα/αα	α+ thal	αα/αα
		8 (38.1%)	13 (61%)	63 (52.5%)	57(47.5%)

Discussion

This study recorded malaria incidence rate of 13.16% similar to the 13.65% and 16,1% earlier reported [21,22] but differs from incidence rates of 66.90% and 77.80% previously reported [3,23]. The lower rate found in this study might be due to adherence to malaria-control protocols such as insecticide spraying, sleeping under insecticide-treated mosquito nets, administration of intermittent treatment with anti-malaria drugs, or strict adherence to laid down sanitary ordinances, or difference in rainfall patterns [24]. However, the rate of 13.16% suggests that malaria currently presents a leading cause of morbidity and mortality among urban African populations [25] with the projected rapid growth rate of 3.5% and a United Nation Population Prospects (UNPP) projection of 50% of African populations living in urban areas by 2025 [26]. Children between1 week to 5 years recorded a 7.47% incidence rate of malaria (MPD (8609/μL) compared to the 5.70% for 6 to 10 years (MPD 51,740/μL) ($p < 0.0001$) (Table 2). Age-group 1 week to 5 years appear more susceptible to malaria infection which may be due to their lower immunity against malaria infection [27]. Females compared to males, are apparently more susceptible to malaria parasitaemia (15.51% and 11.52% incidence rate respectively and MPD of 50,180/μL and 9,513 /μL ($p < 0.0001$) (Table 2) which might be due to differences in background immunity between males and females [28]. However, other findings [29] have reported contrasting finding, in which boys were rather more likely to be parasitaemic than girls, even though no reasons were given.

The prevalence of the $\alpha^{3.7}$ kb deletional type of α^{+}-thalassaemia was 35.59% (28.81% heterozygotes and 6.78% homozygotes,) (Figure 1) and compares with previous findings [1,2] but considerably lower than the 45% rate reported in Nigeria [23], or the 53.7% reported on the coast of Kenya [30] and 90% in the PNG [5].

This study recorded no significant differences ($p = 0.2036$) in the incidence rates of malaria between α+-thalassaemic children (16.67%) and non-α+-thalassaemic children (26.32%), although α+-thalassaemic children recorded a significantly lower (28,705/μL) MPD compared to non-α+-thalassaemic children (35,483/μL) ($p < 0.0001$) (Table 3). Interestingly, the homozygotes recorded a significantly lower MPD (691/μL compared to both the heterozygotes (26,350/μL) and non-α+-thalassaemic children (35,483/μL) ($p < 0.0001$) (Table 3).

These results suggest that α+-thalassaemia offer some degree of protection against malaria in children, as previously reported [3,4,5,30], and that the early exposure of very young thalassaemic children to both *P. falciparum* and *P. vivax* appears to provide the basis for better protection in later life [31].

As is the case in sickle cell trait, the mechanism of protection might most likely be linked to a reduction in the parasite density [7,8]. However, current studies have not been able to provide plausible mechanisms for the protection [32]. This is in spite of earlier suggestions [33,34] that the significantly higher level of haptoglobin, an acute phase protein in homozygous individuals, has been found to be toxic to *P. falciparum in vitro* and may be responsible for conferring protection from severe malaria. Later confirmation [35] suggested that the higher level of haptoglobin (Hp), in homozygous α+-thalassaemic individuals confers protection by removing free haemoglobin released during haemolysis. However, a recent study conducted in PNG however, suggested that an increased number of abnormally small erythrocytes associated with homozygous α^{+}-thalassaemia might be responsible for the protection against severe malarial anaemia [10].

Results from the current study, however, seem to lend credence to a previous report [7] that the mechanism might be due to a reduction in the degree of parasitaemia. This finding is supported by the observation that

the imbalance in globin chain production, which is a major characteristic of α+-thalassaemia, produces membrane oxidation by hemichromes and other molecules that produce reactive oxygen species – superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) that supposedly injure and kill malaria parasite [36-38].

Interestingly, this protective effect was most evident in homozygotes that recorded a significantly lower MPD compared to both the heterozygotes and non-α+-thalassaemic children as reported previously [4,5]. This finding however, conflicts with an earlier study conducted in Ghana [3], which reported that the observed protective effect of α+-thalassaemia was attributable only to the heterozygous. The present finding could be supported by the suggestion that the small red blood cells of α+-thalassaemia genotypes are less hospitable to parasite growth and multiplication [39]. This implies that the mechanism by which α+-thalassaemia appears to confer protection from malaria might be due to a reduction in the parasite burden. However, the fact that there is no significant difference (p = 0.2036) between the incidence rate of malaria in α+-thalassaemic and non-α+-thalassaemic children means that both groups were susceptible to the malaria infection, a point supported by previous findings [23,40]. It has also been indicated that, both normal and α+-thalassaemic RBCs were susceptible to *P. falciparum* invasion, however, the parasite multiplication rates were significantly reduced in the thalassaemic RBC population [7].

This study observed no significant differences (p = 0.6605) in the incidence rates of severe malaria amongst α+-thalassaemic (1.19%) and non α^+- thalassaemic (1.97%) children (Table 3).

Microcytic hypochromic anaemia was found in 141 (59%) of the subjects of whom 71 were α+-thalassaemia making alpha (+)-thalassaemia a key contributor to microcytic hypochromic anaemia amongst cases that were suspected to be iron deficiency and further suggests that suspected iron deficiency cases should be screened for α-thalassaemia so as to avoid the unnecessary administration of iron supplement [8,9]. In populations like Ghana with a high prevalence of thalassaemia trait (33%), and malaria (13%), serum transferrin receptor (sTfR) level may not be useful in diagnosing iron deficiency unless the patient's thalassaemia status is known [41]. It has however, been suggested that serum ferritin cut-off level of 273μg/μl is able to distinguish between microcytosis due to IDA and α+-thalassaemia [17]. This study also found a significant increase (p < 0.0001) in the mean RBC counts (Figure 2A), decrease in the mean MCV (p = 0.0005) (Figure 2C) and mean MCH values (p = 0.0019) (Figure 2D) for α+-thalassaemic children compared to non-α+-thalassaemic children. The increase in the small erythrocytes as recorded in this study might be responsible in conferring protection from severe parasitaemia by a reduction in parasite density.

Conclusion

The 13.16% incidence rate of malaria recorded in his study appears to be relatively low, suggesting some degree of compliance to malaria-control protocols; but could be regarded as a serious public health conundrum in an urban settlements like Kumasi.

Alpha thalassaemia protects against malaria by a reduction in the MPD and that the homozygous α+-thalassaemia were more protected. Alpha (+)-thalassaemia appears to be a key contributor to microcytic hypochromic anaemia, accounting for 52.5% of children that were microcytic hypochromic, suggesting that these individuals are screened for thalassaemia before the administration of iron supplement.

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References

1. Yaish HM (2005). Thalassemia. http://www.emedicine.com/ped/topic2232
2. Mockenhaupt FP, Rong B, Till H, Thompson, WN A, Bienzle U. Short report: increased susceptibility to *Plasmodium Malariae* in pregnant α-+-thalassemic women. Am J Trop Med Hyg 2001; 64(1-2): 6-8.
3. Mockenhaupt, FP, Ehrhardt S, Gellert S, Otchwemah RN, Dietz E, Anemana SD, Bienzle U. α^+-thalassemia Protects African Children from Severe Malaria. Blood 2004; 104 (7): 2003-2006.
4. Mockenhaupt FP, Ehrhardt S, Burkhardt J, Bosomtwe SY, Laryea S, Anemana SD, Otchwemah RN, Cramer JP, Dietz E, Gellert S, Bienzle, U. Manifestation and Outcome of severe malaria in children in northern

Ghana. Am J Trop Med Hyg 2004; 71: 1-3.
5. Allen SJ, O'Donnell A, Alexander NDE, Alpers MP, Peto TEA, Clegg JB, Weatherall DJ. α^+-thalassemia protects children against disease caused by other infections as well as malaria. Proc Natl Acad Sci U S A. 1997; 94(26): 14736-14741.
6. Williams TN, Wambua S, Uyoga S, Macharia A, Mwacharo J K, Newton CRJC, Maitland K. Both heterozygous and homozygous α^+-thalassemias protect against severe and fatal plasmodium malaria on the coast of Kenya. Blood 2005; 106 (1): 368–371.
7. Pattanapanyasat K, Yongvanitchit K, Tongtawa P, Tachavanich K, Wanachiwanawin W, Fucharoen S, Walsh DS. Impairment of *Plasmodium falciparum* Growth in Thalassemic Red Blood Cells: Further Evidence by Using Biotin Labelling and Flow Cytometry. Blood 1999; 93(9): 3116-3119.
8. Williams TN. Human red cell polymorphorisms and malaria. Curr Opin Microbiol 2006; 9: 388-394.
9. Borges E, Wenning, MRSC, Kimma EM, Gervasio SA, Costa FF, Sonati MF. High Prevalence of α-thalassemia among individuals with Microcytosis and Hypochromia without anemia. Brazilian J Med Biol Res 2001; 34(6); 759-762.
10. Rahim F. Genotyping of thalassemia in microcytic hypochromic anaemia patients from SouthWest Region of Iran. Pakistan J Med Sci 2008; 24(1): 23-28.
11. Fowkes FJ, Allen SJ, Alpers MP, Weatherall DJ, Day KP. Increased microerythrocyte count in Homozygous α^+-thalassemia contributes to protection against severe malaria anaemia. PLoS Med 2008; 5(3): 494-501
12. Ghana Demographic Health Survey (2003). Calverton, Maryland: Ghana Statistical Service (GSS), Noguchi Memorial Institute for Medical Research, and ORC Macro (2004)
13. Ghana Statistical Service 2000 Population and Housing Census: Analysis of District Data and Implication for Planning. 2005; Pp. 45-46
14. Agomo PU, Okonkwo CA, Asianya OO, Okoh II, Nebe OJ. Comparative Evaluation of Immuno-Chromatographic Test (ICT) and Parasight ®-F for the Rapid Diagnosis of *Falciparum* Malaria in Nigeria. African J Clin and Exp Microbiol 2001; 2: 44-46.
15. Meeusen EN, Bischof RJ, Lee CS. Comparative T-Cell responses during pregnancy in large animals and humans. Am J Reprod Immunol 2001; 46: 169-170.
16. Wonke B, Modell M, Marlow T, Khan M, Modell B. Microcytosis, iron deficiency and thalassaemia in a multi-ethnic community: a pilot study. Scand J Clin Lab Invest 2007; 67 (1) 87-96
17. Phiri K, Brabin B, Bates I, Molyneux M, Boele Van Hensbroak M. (2009). New cut-off values for ferritin and soluble transferrin receptor for the assessment of iron deficiency in children in a high infection pressure area. J Clin Pathol 2009; 000:1–4. doi:10.1136/jcp.2009.066498
18. Jacobs DS, Demott WR, Oxley DK. Laboratory Test Hand. 5th ed. Lexi-Comp Inc. 2001; p. 203
19. Chong SS, Boehm CD, Higgs DR, Cutting GR. Single-tube multiplex-PCR screen for communal deletional determinants of α-thalassemia. Blood 2000; 95: 360-362.
20. QIAamp® DNA Mini Kit Handbook. (2003). QIAGEN
21. Asante R. A study of the Haemoglobin status of children between the ages of four and twelve years in relation to malaria, anaemia and intestinal worms within the Kumasi Metropolis 2003; (Unpublished data)
22. Sabatinelli G, Bosman A, Lamizana L, Rossi P. Prevalence of malaria in Ouagadougou and the surrounding rural environment during the period of maximal transmission. Parasitologia 1986; 28: 17 – 31.
23. Mockenhaupt FP, Falusi AG, May J, Ademowo OG, Olumese PE, Meyer CG, Bienzle U. The contribution of α^+-thalassemia to anaemia in a Nigerian Population Exposed to Intense Malaria transmission. Trop Med Int Health 1999; 4(4): 302-307.
24. WHO. Malaria. (www.WHO.int/topics/malaria/en). 2007; Fact sheet N°94.
25. Donnelly MJ, McCall PJ, Lengeler C, Bates I, D'Alessandro U, Barnish G, Konnadsen F, Klinkenberg E, Townson H, Trape JF, Hastings IM, Mutero C. Malaria and Urbanization in Sub-Saharan Africa. Malar J 2005; 4: 1-5.
26. UNPP World Urbanization Prospects: The 2003 Revision. Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat. 2005; 1: 66-6747/R3.
27. Centers for Disease Control and Prevention. Traveller's Health. National Center for Health Sciences 2005; 1-6
28. Al-Taiar A, Jaffar S, Assabri A, Habori M, Azay A, Al-Mahdi N, Ameen K, Greenwood B M, Whittey CJM. Severe malaria in children in Yemen: two-site observational study. BMJ 2006;333: 1-3.
29. Klinkenberg E, McCall PJ, Wilson MD, Akoto AO, Amerasinghe FP, Bates I, Verhoeff FH, Barnish G, Donnelly MJ. Urban malaria and anemia in Children: a cross-sectional survey in two cities of Ghana. Trop Med Int Health 2006; 11(5): 578-588.
30. Wambua S, Mwangi TW, Kortok M, Uyoga SM, Macharia AW, Mwacharo JK, Weatherall DJ, Snow RW, Marsh K, Williams TN. The effect of α^+- thalassemia on the incidence of malaria and other diseases in children living on the Coast of Kenya. PLoS Med 2006; 3(5): e 158.
31. Weatherall DJ, Clegg JB. Thalassemia and malaria: new insights into an old problem. Proc Assoc Am Physicians 1999; 111(4): 278-282.
32. Pasvol G. Does Malaria Protect Against Malaria? Plos Med 2006; 3(5): e235
33. Imrie H, Carter M, Hadjuk S, Day KP. Killing of *Plasmodium falciparum* by human serum haptoglobin. Mol Biochem Parasitol 2004; 133: 93-98.
34. Imrie H, Fowkes JI, Michon P, Tavul L, Hume J. CC, Piper KP, Reeder JC, Day KP. Haptoglobin Genotype and α^+-Thalassemia in a Malaria-endemic area. Am J Trop Med Hyg 2006; 74(6): 965 – 971.
35. Heather I., Freya JI, Piper PK. Haptoglobin levels are

associated with haptoglobin genotype and alpha+- thalassaemia in a malaria- endemic area. Am. J. Trop. Med. Hyg 2006;74 (6): 965-971.
36. Grinberg LN, Rachmilewitz EA, Kitrossky N, Chevion M. Hydroxyl radial generation in beta-thalassemic red blood cells. Free Radic Biol Med 1995; 18: 611-615.
37. Sorensen S, Rubin E, Polster H, Mohandas N Schrier S. The role of membrane skeletal-associated alpha-globin in the pathophysiology of beta-thalassemia. Blood 1990; 75:1333-1336.
38. Clark IA, Chaudhri G, Cowden WB. Some roles of free radicals in malaria. Free Radic Biol Med 1980; 6: 315-321.
39. Ifediba TC, Stern A, Ibrahim A, Rieder RF. *Plasmodium falciparum* in vitro: diminished growth in Hemoglobin H disease erythrocytes. Blood 1985;65:452-455
40. Oppenheimer SJ, Hill AVS, Gibson FD, MacFarlane SB, Moody JB, Pringle J. The intera ction of alpha thalassemia with malaria. Trans R Soc Trop Med Hyg 1987; 81: 322-326.
41. Ong KH, Tan HL, Tam LP, Hawkins RCW, Kuperan P. Accuracy of serum trans ferrin receptor levels in the diagnosis of iron deficiency among hospital patients in a popula tion with a high prevalence of thalassaemia trait. Int J Lab Hematol 2008; 30: 487– 493.