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ORIGINAL ARTICLE

The Prevalence of α-Thalassemia and Its Relation to *Plasmodium falciparum* Infection in Patients Presenting to Clinics in Two Distinct Ecological Zones in Ghana

George Ghartey-Kwansah[1], Johnson N. Boampong[1], Benjamin Aboagye[1], Richmond Afoakwah[1], Elvis O. Ameyaw[1], and Neils B. Quashie[2,3]

[1]*Department of Biomedical and Forensic Sciences, University of Cape Coast, Cape Coast, Ghana,*
[2]*Centre for Tropical Clinical Pharmacology and Therapeutics, School of Medicine and Dentistry, University of Ghana, Accra, Ghana, and*
[3]*Epidemiology Department, Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana*

Abstract

Thalassemia and sickle cell disease constitute the most monogenic hemoglobin (Hb) disorders worldwide. Clinical symptoms of α^+-thalassemia (α^+-thal) are related to inadequate Hb production and accumulation of β- and/or γ-globin subunits. The association of thalassemia with malaria remains contentious, though from its distribution it appears to have offered some protection against the disease. Data on the prevalence of thalassemia in Ghana and its link with malaria is scanty and restricted. It was an objective of this cross-sectional study to determine the prevalence of thalassemia in areas representing two of Ghana's distinct ecological zones. The relationship between thalassemia and *Plasmodium falciparium* (*P. falciparum*) infection was also ascertained. Overall, 277 patients presenting to health facilities in the study areas were recruited to participate. Tests were carried out to determine the presence of α^+-thal, sickle cell and malaria parasites in the blood samples of participants. The outcome of this study showed an α^+-thal frequency of 19.9% for heterozygotes ($-\alpha/\alpha\alpha$) and 6.8% for homozygotes ($-\alpha/-\alpha$). *Plasmodium falciparum* was detected in 17.7% of the overall study population and 14.9% in those with α^+-thal. No association was observed between those with α^+-thal and the study sites ($p > 0.05$). A test of the Hardy-Weinberg law yielded no significant difference ($p < 0.001$). Findings from this study suggest a modest distribution of α^+-thal in Ghana with no bias to the ecological zones. Although the prevalence and parasite density were relatively low in those with the disorder, no association was found between them.

Keywords

Coastal zone, forest zone, heterozygote, homozygote, *Plasmodium falciparum*, thalassemia

History

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Introduction

Thalassemia is not a rare genetic disease but one of the most common human genetic abnormalities ever known (1). It is a group of genetic blood disorders [α- and β-thalassemia (α- and β-thal)] characterized by anemia due to enhance red blood cell (RBC) destruction (2).

Initially described in the tropical and subtropical regions, thalassemia is now common all around the world because of migration (3). Globally, the disorder affects 5.0 to 10.0% of the population in the Mediterranean, 20.0 to 30.0% in West Africa, and about 68.0% in the South Pacific (4). α-Thalassemia appears to occur in areas with historically endemic malaria. As to whether the thalassemic condition confers protection against malaria remains contentious. Some researchers have reported a connection between the condition and severity of malaria (5,6), while others could not find any (7).

Address correspondence to Dr. Neils B. Quashie, P.O. Box GP 4236, Accra, Ghana. Tel: +223-302-500381. Fax: +233-302-50302-02182. E-mail: nquashie@noguchi.mimcom.org; neilsquashie@hotmail.com

Information on α^+-thal in Ghana is scanty and restricted to a small area and its association with malaria remains inconclusive. Most of the previous studies of the disorder in Ghana have been carried out in the forest zones of the country, disregarding the other ecological zones. Therefore, this study seeks to determine the prevalence of thalassemia in parts of Ghana representing the two major ecological zones in the country and ascertain its link with *Plasmodium falciparum* (*P. falciparum*) infection.

Materials and methods

The study was conducted in health facilities located in the central and western regions of Ghana. The central region (CR) is located within latitudes 5°1'N and 6°18'N and longitudes 0°22'W and 2°10'W and covers an area of 9826 km^2, which is about 6.6% of the land area of Ghana. It lies in both the forest and coastal zones of the country and has an estimated population of 2,201,863. The prevalence of malaria parasitemia reported for this region is 37.1% (8). Although ethnicity is diverse in this area there are three major ethnic groups dominating. The western region (WR) covers an area

of approximately 23,921 km^2 and lies between latitude 5.5000° N and longitude 2.5000° W. The region has about 75.0% of its vegetation within the high forest zone of Ghana, and is also the wettest part of Ghana. The prevalence of malaria parasitemia reported for the region is 39.0% (8). The region is dominated by five major ethnic groups. For the purpose of this study, two health facilities each located in the forest or coastal zones of the WR and CR regions were chosen. In the coastal area, the study was conducted at the Elimina Health Centre, Elimina (CR) and Essikado Government Hospital, Sekondi-Takoradi (WR), and in the forest zone it was carried out at the Twifo Praso District Hospital, Twifo Praso (CR) and Tarkwa Government Hospital, Tarkwa (WR) (Figure 1).

All patients presenting at the out-patient departments of the health facilities in the study areas at the time of recruitment, and who reside permanently in the area, were recruited to participate in the study without bias. The only exceptions were those brought to the emergency departments of the health facilities in critical condition or residing outside the selected communities. Detailed information on the study was made available to adult participants and parents or guardians of children, and they were encouraged to ask questions about any aspect of the study that was unclear to them. Potential participants were recruited only after giving their informed consent. Based on an estimated prevalence of 26.0–33.0% (9) with 95% confidence interval (CI) (10), a total of 277 patients were considered enough and recruited to participate in the study. Demographic characteristics (gender and age) were obtained for each participant.

Two mL of venous blood were then drawn from each participant into EDTA tubes for hematological indices and other tests. Thick and thin Giemsa stained blood films for detection of malaria parasites were prepared on the day of recruitment for each participant. Parasites, when present, were counted against 200 leukocytes, and the parasite density expressed as parasites per 1 µL blood.

Genomic DNA was extracted from whole blood using a DNA extraction kit (Qiagen GmbH, Hilden, Germany) (11). The extracted DNA was typed for the presence of thalassemia using previously described protocols (12,13). All participants were initially screened for the presence of sickle cell disease using the standard sodium metabisulphate oxygen reduction test. When positive for the test, variant forms of Hb were determined using the cellulose acetate electrophoresis method described by Marengo-Rowe (14) with slight modification.

Approval for this study was obtained from the Ghana Health Service Ethics Review Committee. Written, signed/thumb printed and dated informed consent was obtained from adult participants, and in the case of children, from parents/guardians before being enrolled in the study.

Figure 1. Map of the CR and WR of Ghana, showing the study areas (red triangle). Insert: map of Ghana.

Statistical analysis

Data analysis was performed by the Statistical Package for the Social Sciences (SPSS), version 16 for Windows (SPSS Inc., Chicago, IL, USA) and MINITAB version 15 (Minitab Inc., State College, PA, USA; www.minitab.com). We used the χ^2 test, to assess whether the data followed the Hardy-Weinberg equilibrium and to determine the association between the presence of α^+-thal and malaria. A p value less than 0.05 was considered statistically significant.

Results

The ages of the 277 patients who participated in the study ranged from 1 to 84 years (mean age of 28.3 years; 73 males and 204 females) (Table 1). An overall α^+-thal prevalence of 74/277 (26.7%) was determined, 19 (6.8%) of whom were homozygous. When those with α^+-thal trait were stratified according to study areas, no significant difference was observed between the cases and study area: 29.7, 20.3, 24.3 and 25.7% were observed for Elimina Health Centre, Elimina, Twifo Praso Government Hospital, Twifo Praso, Essikado Government Hospital, Sekondi-Takoradi, and Tarkwa Municipal Hospital, Tarkwa, respectively (Figure 2). Using the χ^2 test, no association between the sex of the participant and α-thal was observed ($p > 0.05$).

Only 3.0% of the samples analyzed were observed to be carriers of the sickle cell trait, with none of them being homozygous. These were observed mainly for samples from Essikado and Twifo Praso. There was no coinheritance of the sickle cell trait and α^+-thal. No malaria parasites were found in those with sickle cell trait. When the Hardy-Weinberg equilibrium was tested using the χ^2 test of Goodness of Fit, a significant difference ($p < 0.001$) was observed. This indicates that the data does not conform to the Hardy-Weinberg law.

Forty-nine out of the 277 participants had *P. falciparum* in their blood samples, resulting in an overall parasite rate of 17.69%. The mean parasite density (MPD) was categorized as low (1–999 parasites/μL blood), moderate (1000–9999 parasites/μL blood) and high (≥10,000 parasites/μL blood) as previously defined (15). In general, low and moderate *P. falciparum* parasite densities were found to be associated with homozygous α^+-thal (Figure 3). Moderate MPD was higher in those with the heterozygous trait compared to those with the homozygous trait or normal individuals. High MPD was observed to be lower in heterozygous α^+-thal than in the normal subjects. In those with the homozygous trait, there were only two individuals with low or moderate conditions and none had high MPD. Only 39.5 and 44.7% of those with a normal genotype had low or moderate MPD, respectively, while 22.2 and 66.7% of those with the heterozygous trait had low or moderate MPD, respectively.

Association of α^+-thal with malaria parasite density was not statistically significant ($p = 0.643$) (Table 2). Similarly, no association was observed between the disorder and either the study sites or the two ecological zones (coastal or forest) (Table 3).

Table 1. Demographic data of the study participants.

	Genotype			
Gender	αα/αα	−α/αα	−α/−α	Total
Males	51	13	9	73
Females	152	42	10	204
Mean age	30.2	27.4	26.8	

αα/αα: normal α-globin genotype; −α/αα: heterozygous state; −α/−α: homozygous state.

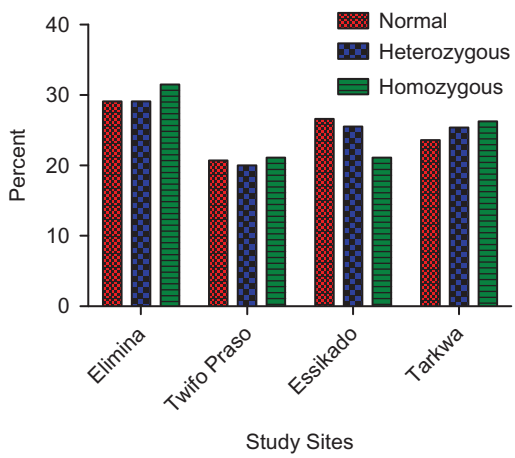

Figure 2. Distribution of α-thal at the study sites in the CR and WR of Ghana.

Discussion

α-Thalassemia is the most frequent hemoglobinopathy of humans with high prevalence observed in malaria endemic areas of the world. The distribution of this inheritable autosomal recessive blood disorder seems to suggest that it has been selected to protect against malaria infection. However, outcomes from various studies including the current study, do not totally support this assertion. In this study, an overall prevalence of the disorder in two distinct ecological zones in Ghana was determined as 19.9% for heterozygotes (−α/αα) and 6.8% for homozygotes (−α/−α). This observation is consistent with reports from earlier studies by Mockenhaupt *et al.* (9) and Opoku-Okrah *et al.* (16), conducted in the Ashanti region of Ghana, showing a

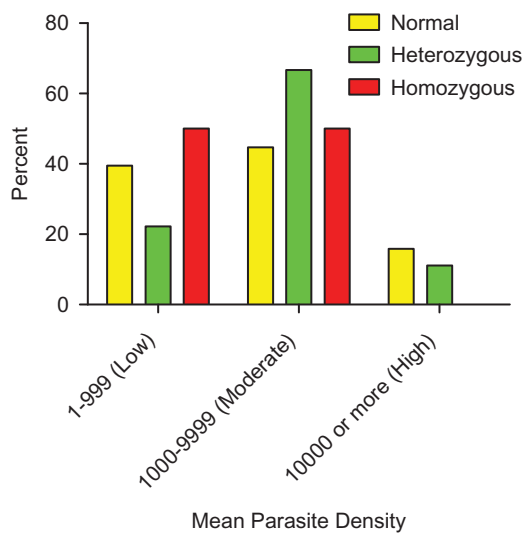

Figure 3. Parasite densities in the normal, heterozygous and homozygous groups.

prevalence rate between 20.0 and 30.0%. However, it contrasts findings from similar studies carried out in the same region and also in Kenya and Tanzania that showed a higher prevalence of over 35.0% (15,17,18). Our report is quite similar to the observed gene frequency in various studies conducted in different Nigerian populations indicating a range of 0.19–0.39 (19–22).

Distribution of α^+-thal in the two ecological study areas, coastal and forest, was found to be similar. When the disorder was categorized according to the four study sites, a relatively uniform observation was again made, which was confirmed statistically by lack of significant association ($p > 0.05$). This observation supports the report of uniform distribution of α^+-thal in Ghana by Mockenhaupt *et al.* (23) in a previous study. However, it differs from reports of heterogeneity in α^+-thal allele in studies elsewhere (24,25). Although the communities in the study areas are of different tribes, there is a lot of intermarriage and easy tribal migration. These factors probably contributed significantly to the uniformity of the disorder seen in these areas. Interestingly, these observations contrast reports from other countries such as Iran, where mutations in the thalassemia gene were found mostly in the coastal areas, probably as a consequence of limited intertribal marriages in the area (26). In Nepal, where strict practice of the caste system limits intertribal marriages and promotes consanguineous marriage, distribution of the condition also appears to lack uniformity (27).

Observations made in this study suggest that gender plays no role in the determination of α^+-thal as a similar prevalence rate was observed in males and females in all the study areas (Table 1). This finding is in line with global observation, as most reports showed that males and females are equally affected.

Thalassemia complications that may not be applicable in our situation have been described (28). In a previous study, conducted in Ghana by Franklin *et al.* (15), it was reported that α^+-thal was a key contributor to microcytic hypochromic anemia in cases that were suspected to be iron deficient. It is equally important to add that iron deficiency is reported to equally affect α^+-thal individuals at the same rate as normal subjects (29). It is suggested that anemic patients who do not respond to iron therapy should be screened for α-thal after ruling out β-thal and managed accordingly.

This study recorded a malaria parasite prevalence of 15.0% for the coastal and 12.9% for the forest areas. This outcome is similar to observations made in a previous study that indicated a parasite prevalence rate of 13.2% (15). However, it is significantly lower than the country's malaria prevalence of 27.5% and the reported 39.0 and 37.1%, respectively, for the WR and CR of Ghana where the study was conducted (8). The relatively low prevalence level observed in this study probably reflects the decrease in incidence of the disease recently reported in the country. The decrease could be due to the intervention measures introduced by the National Malaria Control Program (NMCP) (30). These measures included insecticide spraying, sleeping under insecticide-treated mosquito nets, administration of intermittent treatment with anti-malaria drugs and prompt and effective case management. All the same, it is worth mentioning that with the NMCP goal to eliminate the disease from Ghana by 2020, the current overall prevalence level of 17.7% in the current study is still high, too far from the target, and should be of concern to all stake holders.

Analysis carried out in this study showed no significant difference ($p = 0.539$) in the prevalence of malaria parasites between α^+-thalassemic (14.9%) and normal individuals (18.7%). Although there was reduced presence of malaria parasites in the former group, no association was observed between the two ($p = 0.64$). This observation does agree with other reports (7,31,32) yet contradicts the studies by Franklin *et al.* (15) and Pattanapanyasat *et al.* (33), conducted in Ghana and elsewhere, indicating that α^+-thal protects against malaria *via* a reduction in the parasite density (and growth) and that

Table 2. Prevalence of the malaria parasite and association with genotype.

	Genotype					
Malaria status	αα/αα	−α/αα	−α/−α	Total	χ^2	*p* value
Number of subjects	203	55	19	277		
Malaria parasites present	38	9	2	49		
No malaria parasites seen	165	46	17	228		
Prevalence of malaria	18.72%	16.36%	10.53%	17.69%	0.884	0.643

αα/αα: normal α-globin genotype; −α/αα: heterozygous state; −α/−α: homozygous state.

Table 3. Association between α^+-thalassemia and study area.

	Genotype				
Study Sites in Ghana/Ecological Zone	αα/αα	−α/αα	−α/−α	χ^2	*p* value
Elimina Health Centre, Elimina	59	16	6	0.361	0.999
Twifo Praso Government Hospital, Twifo Praso	42	11	4		
Essikado Government Hospital, Sekondi-Takoradi	54	14	4		
Tarkwa Hospital Municipal, Tarka	48	14	5		
Coastal zone	113	30	10	0.078	0.962
Forest zone	90	25	9		

αα/αα: normal α-globin genotype; −α/αα: heterozygous state; −α/−α: homozygous state.

the homozygous α^+-thal was more protective than the heterozygous state.

A study conducted in Ghana by Ohene-Frempong *et al.* (34) showed that the genotype characterized by Hb SS (β_S/β_S) and Hb SC (β_S/β_S) are dominant in the sickle cell disease population in the country. They reported a frequency rate of 30.0%, and also indicated that 2.0% of Ghanaian newborns have the disease. In the light of their report, the 3.0% carrier rate observed in this study is significantly low and unexpected. We find it difficult to find an explanation for this low level of prevalence. Though ethnicity could play a role, the lack of national data on sickle cell disease prevalence for the study communities limits our discussion. The sickling test used for the initial screening in this study has been described as very reliable (35). However, it has been said that if the sodium metabilsulphite used has deteriorated or if the cover slip is not properly sealed a false-negative result may be produced (35), a situation that to the best of our knowledge and observation did not happen in our case. The presence of glucose-6-phosphate dehydrogenase (G6PD) in the study participants was not investigated.

Conclusions

We concluded that α^+-thal is uniformly distributed in the coastal and forest zones of Ghana and had no association with the study sites. The presence and density of *P. falciparum* is relatively low in those with α^+-thal but an association between the disorder and the infection could not be established. Distribution of α^+-thal, as determined in this study, did not follow Hardy-Weinberg law.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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