INFLUENCE OF PLASMODIUM FALCIPARUM MALARIA ON SICKLE CELL VASO-OCLUSION CRISIS in Yaoundé, Cameroon

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ABSTRACT
BACKGROUND
Sickle cell disease and malaria infections are common in Cameroon. Malaria infection is thought to influence the occurrence and severity of crisis in sickle cell patients.

OBJECTIVE
To investigate the relationship between malaria infection and vaso-occlusive crisis in sickle cell disease patients.

METHODS
In order to investigate the clinical severity of painful vaso-occlusive crisis in sickle cell anaemia patients suffering from malaria infection, 60 SS Homozygous patients aged 2 – 35 years (median = 15 years) with painful crisis and 40 SS Homozygous sickle cell patients in ‘steady state’ aged 1 – 38 years (median = 17 years) were recruited into the study. The clinical severity of the crisis was graded as 0.1 and 2 based on an arbitrary scale for increasing pain. For each participant thin and thick blood films were made from capillary blood, stained according to standard methods and examined for malaria parasites. Chi square and student t tests were used for statistical analysis.

RESUME
CONTEXTE
Infections de la drépanocytose et le paludisme sont fréquentes au Cameroun. Le paludisme est pensé pour influencer la fréquence et la gravité de la crise chez les patients drépanocytaires.

OBJECTIF
Pour étudier la relation entre l’infection du paludisme et de la crise vaso-occlusive chez les patients drépanocytaires.

MÉTHODES
Afin d’étudier la sévérité clinique de douloureuse crise vaso-occlusive chez les patients de drépanocytose souffrant de paludisme, 60 SS patients homozygotes âgés de 2 à 35 ans (âge médian = 35 ans) à la crise douloureuse et 40 SS patients drépanocytaires homozygotes dans “état stable” âgés de 1 à 38 ans (médiane = 17 ans) ont été recrutés dans l’étude. La gravité clinique de la crise a été évalué à 0,1 et 2 sur la base d’une échelle arbitraire pour augmenter la douleur. Pour chaque participant frottis sanguins minces et épais sont fabriqués à partir de sang capillaire, colorées selon des méthodes standard et examinés pour les parasites du paludisme. Carrés et étudiant tests t Chi ont été utilisés pour l’analyse statistique.
RESULTS
Of the 60 patients in crisis, pain in 46.7% was classified as Grade 1, in 46.7% as Grade 2 and in 6.6% as Grade 0. There were 66.6% of them with positive thick films for malaria parasites compared to 35.0% of the patients in ‘steady state’ (p=0.003). All parasites were shown on thin film to be Plasmodium falciparum. More than 50% of the patients in crisis with positive thick films were in Grade 2 of the clinical grading.

CONCLUSION
These findings show that falciparum malaria remains a major cause of morbidity and contributes significantly to the occurrence and severity of painful vaso-occlusive crisis in sickle cell disease patients.

INTRODUCTION
Sickle cell anaemia is a chronic non-communicable genetic disorder which results from the genetic mutation of the sixth amino acid of the beta chain of normal adult haemoglobin, from glutamic acid to valine. The resultant sickle haemoglobin (Haemoglobin S) tends to precipitate in the presence of low oxygen tension causing red cells to adopt the peculiar sickled form. This is responsible for the physiopathology of sickle cell anaemia within capillaries, and in the reticulo-endothelial system, evidenced by painful vaso-occlusive crisis, haemolysis and chronic anaemia. Several factors enhance this process including hypoxia, dehydration, hypothermia and infections. Although infections like malaria are said to be less frequent in sickle cell anaemia this infection has been frequently diagnosed in sickle cell sufferers (personal observations) and is believed to initiate and worsen sickle cell crisis. To investigate the relationship between sickle cell crisis and malaria infection, a group of homozygous sickle cell anaemia patients in painful crisis were screened for malaria parasites. For comparison, another group of homozygous sickle cell anaemia patients in ‘steady state’ were included as controls.

METHODS
This was a hospital-based cross-sectional study into which consenting homozygous sickle cell anaemic patients attending the Sickle Cell Centre in Yaoundé were recruited. For each patient a medical interview and a physical examination provided relevant information (age, sex, etc.). For those in painful crisis, the intensity of the pain was graded into:
- Grade 0 = Mild pain, with normal mobility
- Grade 1 = Moderate pain and much difficulty with mobility
- Grade 2 = Severe pain with immobility

A drop of capillary blood was obtained from a finger prick unto a microscope slide for a thin blood film. A second drop was obtained for a thick film. Thin films were air-dried and fixed for 1 minute in absolute methanol before colouring with May-Grunwald (3 minutes) and then Giemsa stains (15 minutes) after leaving for 1 minute in PBS. Thick films were dried for at least 2 hours and directly stained with 10% Giemsa in Phosphate-Buffered-saline for 10 minutes. Microscopic examination was carried out using the x100 objective of a light microscope. At least 100 fields were screened before a slide was considered negative. The parasite density was determined by counting the parasites in 100 fields and multiplying by 4 to obtain the number of parasites/µL. Each slide was examined by two different technicians and the mean of their parasite counts recorded.

RÉSULTATS
Sur les 60 patients en situation de crise, la douleur dans 46,7% a été classé comme une année, dans 46,7% de grade 2 et de 6,6% en année 0 Il avait 66,6% d’entre eux avec des films épaisse positifs pour les parasites du paludisme par rapport à 35,0% de la patients en “état stable” (p = 0,003). Tous les parasites ont été présentés sur le film mince pour être à Plasmodium falciparum. Plus de 50% des patients en crise films épaisse positives étaient de grade 2 de la classification clinique.

CONCLUSION
Ces résultats montrent que le paludisme à falciparum reste une cause majeure de morbidité et contribue de manière significative à la survenue et la gravité de la crise douloureuse vaso-occlusive chez les patients drépanocytaires.

The mean parasite density (MPD) for each slide was calculated, based on a modification of the following WHO formula:

\[
\text{MPD (parasite/µL)} = \frac{\text{L} \times \text{MP}}{100}
\]

Where \( \text{L} \) = number of leucocytes (estimated at 6000/µL)
\( \text{MP} \) = mean parasites/100 leucocytes

Chi square and student t tests were used for statistical analyses. P values <0.05 were considered significant.

RESULTS
There were 60 homozygous sickle cell sufferers in painful crisis and 40 in ‘steady state’. The patients in painful crisis were aged 2 - 35 years (median = 15 years) while those in ‘steady state’ were aged 1 - 38 years (median = 17 years).

Based on the intensity of pain, there were 4 of the 60 patients (6.6%) classified as Grade 0, 28 (46.7%) in Grade 1 and 28 (46.7%) in Grade 2. All the 40 patients in ‘steady state’ had no pain at all.

There was a significant difference in the number of positive thick films for malaria parasites in the group of patients in painful crisis (66.6%) compared to those in ‘steady state’ (35%) ; p = 0.003. All parasites were confirmed on thin film to be Plasmodium falciparum. The median MPD was 40/ul (range 20 – 1440 parasites/ul) in the patient group in crisis compared to 20/ul (range 20 - 660 parasites/ul) in the ‘steady state’ group; with a P value= 0.13 as shown in Table 1.

### Table 1: Comparison between Patients in crisis and patients in ‘steady state’

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>Age (years)</th>
<th>Frequency of positive thick film</th>
<th>Mean Parasite density/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median [range]</td>
<td>N(%)</td>
<td>P value</td>
</tr>
<tr>
<td>Patients in crisis state n=60</td>
<td>15[2-35]</td>
<td>40 (66.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>Patients in steady state n=40</td>
<td>17[1-38]</td>
<td>14(35.0)</td>
<td>20[20-660]</td>
</tr>
</tbody>
</table>
DISCUSSION
Malaria parasites are preferentially transmitted to man by the female anopheles mosquito. Because of ecological conditions and socio-economic factors, West and Central Africa are most affected. There are five species of malaria parasites that transmit malaria to man (Plasmodium falciparum; Plasmodium malariae; Plasmodium ovale; Plasmodium vivax and Plasmodium knowlesi). Plasmodium falciparum is most frequent in the tropical regions and is responsible for more than 80% of malaria infection in Cameroon. Malaria caused by Plasmodium falciparum is the most severe, with potentially fatal complications.

Sickle cell disease was shown in this study to contribute significantly to the occurrence and severity of painful vaso-occlusive crisis. Malaria is described as the initiating factor for vaso-occlusive crisis in many sickle cell sufferers. In their study Kotila & al diagnosed malaria in 34% of sickle cell sufferers with fever or pains. However, fever may result from other factors including bacterial infections, which are also frequent in sickle cell patients. Our study was conducted from September 2011 to August 2012 in the same site. This period covers the two major seasons (dry, raining) in Cameroon. Thus, prevalence of malaria, and average frequency of sickle cell crisis reported in this study took into account the customary climatic changes.

Microscopy was the method employed to detect malaria parasitaemia in this study. Although there are other methods such as rapid diagnostic tests, (RTD) based on antigen detection and molecular studies, microscopy gives other important information such as species, and stages of parasite development and density. Microscopy is labour-intensive, time-consuming, and somewhat subjective, but in skilled hands, it can be quite sensitive for parasitaemia of ≤50/µL (0.001%). Microscopy remains the most widely used tool to detect malaria parasitaemia in Africa, both at the primary and secondary health levels. The findings in this study suggest that malaria was a precipitating factor to bone pain in sickle cell sufferers. The clinical manifestations of malaria range from fever through rigors, to more severe systemic manifestations. The occurrence and greater severity of pain in the study group compared to the control group may be explained by the vaso-occlusion induced by the malaria infection. Based on the intensity of pain as graded in the present study, the majority were in Grade 1 and 2 (93.4%) while no sufferer in the ‘steady state’ (control group) had pain.

While parasitaemia remains an objective criterion for defining malaria severity, Binka & al defined dense parasitaemia as MPD ≥ 4000 parasites/µl. In this study, the median MPD was 40 (20 - 1440) parasites/µl in the test group, and 20 (20 - 660) parasites/µl in the control group. These findings would therefore not meet the criteria set by Binka & al. However, some patients tolerate high parasite densities clinically while others succumb even to low densities. This apparent discrepancy between symptoms and degree of parasitaemia may be explained by the differences in stages of development of parasite as suggested by Achidi & al. The severity of symptoms would be more associated with the number of parasites sequestrated rather than with the numbers in circulation. It is therefore not surprising that patients in our study group with relatively low parasitaemia presented with clinically severe crisis.

CONCLUSION
It may be concluded from the findings of this study that malaria contributes significantly, not only in initiating sickle cell vaso-occlusive crisis, but in aggravating it. Therefore malaria prevention measures are essential in the care of sickle cell patients particularly in malaria endemic regions.

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REFERENCES