SCIENTIFIC ARTICLES

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

INFLUENCE DE PLASMODIUM FALCIPARUM PALUDISME FALCIFORME VASO-OCCLUSIVES crise en Yaoundé , Cameroun

Mbanya DNS, Tayou Tagny C, Lyli O and Kaptué LN
Faculty of Medicine and Biomedical Science, University of Yaoundé I, Cameroon;
Institut Supérieur des Sciences de la Santé, Université des Montagnes

CORRESPONDENCE
Dr Claude Tayou Tagny
PO BOX 5739 Yaoundé Cameroon
Email: tayouclaude@yahoo.fr

KEYWORDS
Plasmodium Falciparum, Malaria, Sickle Cell Disease

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 = 15 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 épaisses 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.

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 y avait 66,6% d'entre eux avec des films épaisses positives 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 épaisses 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.

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[1,2,3] 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.[4] Each slide was examined by two different technicians and the mean of their parasite counts recorded.

The mean parasite density (MPD) for each slide was calculated, based on a modification of the following WHO formula.[4]

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

Where L = number of leucocytes (estimated at 6000/µL)
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'

Group of patients	Age (years)	Frequency of positive thick film		Mean Parasite density(/ul)	
	Median [range]	N(%)	P value	Median [range]	P value
Patients in crisis state *n=60*	15[2-35]	40 (66.6)	0.003	40[20-1440]	0.13
Patients in steady state *n=40*	17[1-38]	14(35.0)		20[20-660]	

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.[5] 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.[6,7] Malaria caused by Plasmodium falciparum is the most severe, with potentially fatal complications.[8,9]

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.[10] In their study Kotila & al[11] 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[12] 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.[12] 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.[11] 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.

ACKNOWLEDGMENTS

We thank the Yaoundé University Teaching Hospital for the different lab analysis.

REFERENCES

1. Williams TN, Mwangi TW, Wambua S, Alexander ND, Kortok M, Snow RW, Marsh K. Sickle cell trait and the risk of Plasmodium falciparum malaria and other childhood diseases. J Infect Dis. 2005;192(1):178-86.
2. De Paz FJ, Romero A, Diez D, Botella C, Torrus D, Moscardó C. Malaria and sickle cell disease. Haematologica. 2006;91(12 Suppl):EIM03.
3. Crompton PD, Traore B, Kayentao K, Doumbo S, Ongoiba A, Diakite SA, Krause MA, Doumtabe D, Kone Y, Weiss G, Huang CY, Doumbia S, Guindo A, Fairhurst RM, Miller LH, Pierce SK, Doumbo OK. Sickle Cell Trait Is Associated with a Delayed Onset of Malaria: Implications for Time-to-Event Analysis in Clinical Studies of Malaria. J Infect Dis. 2008;198(9):1265-1275.
4. Payne D. Use and limitations of light microscopy for diagnosing malaria at primary health care level Bull World Health Organ. 1988;66(5):621-6.
5. Lewison G, Srivastava D. Malaria research, 1980-2004, and the burden of disease. Acta Trop. 2008 ;106(2):96-103.
6. Quakyi IA, Leke RG, Befidi-Mengue R, Tsafack M, Bomba-Nkolo D, Manga L, Tchinda V, Njeungue E, Kouontchou S, Fogako J, Nyonglema P, Harun LT, Djokam R, Sama G, Eno A, Megnekou R, Metenou S, Ndountse L, Same-Ekobo A, Alake G, Meli J, Ngu J, Tietche F, Lohoue J, Mvondo JL, Wansi E, Leke R, Folefack A, Bigoga J, Bomba-Nkolo C, Titanji V, Walker-Abbey A, Hickey MA, Johnson AH, Taylor DW. The epidemiology of Plasmodium falciparum malaria in two Cameroonian villages: Simbok and Etoa. Am J Trop Med Hyg. 2000;63(5-6):222-30
7. Zhou A, Megnekou R, Leke R, Fogako J, Metenou S, Trock B, Taylor DW, Leke RF.Prevalence of Plasmodium falciparum infection in pregnant Cameroonian women. Am J Trop Med Hyg. 2002;67(6):566-70
8. Ikome LE, Ndamukong KJ, Kimbi H. Prevalence and case-control study of cerebral malaria in Limbe of the South-West Cameroon. Afr J Health Sci. 2002;9(1-2):61-7
9. Chiabi A, Tchokoteu PF, Toupouri A, Mbeng TB, Wefuan J.The clinical spectrum of severe malaria in children in the east provincial hospital of Bertoua, Cameroon. Bull Soc Pathol Exot. 2004 ;97(4):239-43
10. Kotila TR. Management of acute painful crises in sickle cell disease. Clin Lab Haematol. 2005;27(4):221-3.
11. Achidi EA, Apinjoh TO, Mbunwe E, Besingi R, Yafi C, Wenjighe Awah N, Ajua A, Anchang JK.Febrile status, malarial parasitaemia and gastro-intestinal helminthiases in schoolchildren resident at different altitudes, in south-western Cameroon. Ann Trop Med Parasitol. 2008 Mar;102(2):103-18.
12. Binka FN, Morris SS, Ross DA. Patterns of malaria morbidity in children in northern Ghana. Trans Roys Soc trop Med Hyg 1994; 88: 381 – 385.