Massive *Plasmodium falciparum* visceral sequestration: a cause of maternal death in Africa

P. Castillo[1], C. Menéndez[1,2], A. Mayor[1,2], C. Carrilho[3], M. R. Ismail[3], C. Lorenzoni[3], F. Machungo[4,5], N. Osman[4,5], L. Quintó[1], C. Romagosa[1,2], C. Dobaño[1,2], P. L. Alonso[1,2] and J. Ordi[1]

1) *Barcelona Centre for International Health Research (CRESIB), Department of Pathology, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain,* 2) *Centro de Investigaçao em Saude de Manhiça, (CISM),* 3) *Department of Pathology, Maputo Central Hospital, Maputo,* 4) *National Malaria Control Programme, Ministry of Health, Maputo, and* 5) *Department of Gynaecology, Maputo Central Hospital, Universidad Eduardo Mondlane, Maputo, Mozambique*

Abstract

Sequestration of *Plasmodium falciparum*-infected erythrocytes (PfIE) in the capillaries of the central nervous system (CNS) is the pathognomonic feature of cerebral malaria, a condition frequently leading to death. Sequestration of PfIE in the placental intervillous spaces is the characteristic feature of malaria in pregnancy and is associated with low birthweight and prematurity. Although both patterns of sequestration are thought to result from the expression of different parasite proteins involved in cytoadhesion to human receptors, scant information exists on whether both conditions can coexist and whether this can lead to death. We conducted a prospective autopsy study including all consecutive pregnancy-related deaths in a tertiary-level referral hospital in Maputo, Mozambique, between October 2002 and December 2006. Extensive sampling of all major viscera was performed. All cases showing parasites in any of the viscera were included in the analysis. From 317 complete autopsies PfIEs were identified in ten women (3.2%). All cases showed massive accumulation of PfIE in small capillaries of the CNS but also in most visceral capillaries (heart, lung, kidney, uterus). Placental tissue, available in four cases, showed a massive accumulation of maternal PfIE in the intervillous space. Coma (six women) and dyspnoea (five women) were the most frequent presenting clinical symptoms. In conclusion, massive visceral sequestration of PfIE with significant involvement of the CNS is an infrequent but definite direct cause of maternal death in endemic areas of Africa. The PfIE sequestered in cerebral capillaries and the placenta coexist in these fatal cases.

Keywords: Africa, cerebral malaria, maternal mortality, *Plasmodium falciparum*, severe malaria
Original Submission: 20 August 2012; **Revised Submission:** 4 October 2012; **Accepted:** 4 October 2012
Editor: E. Bottieau
Article published online: 13 October 2012
Clin Microbiol Infect 2013; **19:** 1035–1041
10.1111/1469-0691.12068

Corresponding author: J. Ordi, Department of Pathology, CRESIB—Hospital Clínic, Universitat de Barcelona, Villarroel 170, 08036-Barcelona, Spain
E-mail: jordi@clinic.ub.es

Introduction

Pregnant women are more susceptible to *Plasmodium falciparum* malaria than non-pregnant women or men [1,2]. The ability of *P. falciparum* infected erythrocytes (PfIE) to accumulate in the placental intervillous spaces, a condition described as placental malaria, is considered to contribute to this phenomenon [1]. In areas of stable transmission, where women are semi-immune and often asymptomatic during infection, malaria in pregnancy is associated with maternal anaemia, low birthweight and prematurity, but rarely with severe disease [1,3].

Cerebral malaria (CM) is a serious complication of *P. falciparum* infection, contributing to over three-quarters of the 655 000 malaria-related deaths per year worldwide [4]. Ninety percent of CM-associated deaths occur in children and, although recent estimations suggest that the malaria mortality burden in adults is larger that previously thought [5], the general consensus is that CM in adulthood almost exclusively affects non-immune individuals living in non-endemic areas or where endemicity is low [4].

Sequestration of PfIE in the micro-vasculature of the central nervous system (CNS) and the intervillous spaces of the placenta is considered to be a key feature in the pathogenesis of CM and placental malaria, respectively [6]. The capacity of *P. falciparum* to sequester is related to its ability to adhere to a variety of host endothelial receptors through parasite antigens expressed on the surface of erythrocytes, the best known of which is *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) [7]. Despite immense diversity in global *var* genomic repertoires, genes can be classified into different groups (A–E) [8]. Transcription of A and B groups has been associated with symptomatic and severe malaria [9,10], whereas C genes have been linked to asymptomatic infections and CM [9]. The single and relatively conserved member of group E, *var2csa*, is transcribed by placental isolates and provides high-affinity binding to chondroitin sulphate A (CSA) present in placental trophoblasts [11]. The extent to which different subsets of parasites may coexist and cause disease in a patient remains unknown.

In a previous study on the causes of maternal mortality conducted in Mozambique between 2002 and 2004 we identified a series of women who died of malaria [12,13]. The current study aimed to analyse the pattern of parasite sequestration in women showing parasites in the autopsy study.

Methods

Study design

All women dying in the Maputo Central Hospital between October 2002 and December 2006 and fulfilling the standard definition of the WHO for a maternal death were initially included in the study. Verbal informed consent was asked of the closest relatives. The study protocol was approved by the National Mozambican Ethics Committee and the Hospital Clinic of Barcelona Ethics Review Committee. A detailed description of the study design and the causes of maternal mortality has been published elsewhere [12,13]. Three hundred and seventeen complete autopsies were performed, representing 86.8% of the deaths occurring during the study period.

Study area

The Maputo urban area has low malaria transmission, although some of the peripheral suburbs are considered to have moderately stable transmission with seasonal variations and the surrounding rural areas have moderate and stable transmission. Malaria incidence is higher during the rainy season (October to May) than during the dry season (June to September) [14]. The reported human immunodeficiency virus (HIV) seroprevalence in pregnant women for 2004 was 20.7% for the Maputo area (Ministry of Health, Mozambique, 2004).

Pathological and laboratory methods

A complete dissection with macroscopic evaluation of each organ by a pathologist using a standardized macroscopic protocol was performed in all cases. At least the following samples were taken from each woman for histological study: CNS (three blocks including frontal and parietal lobes and cerebellum), right and left lung, liver, spleen, right and left kidney, heart and placenta when available. Tissue specimens were fixed in 10% buffered formalin and embedded in paraffin wax using standard procedures. A blood sample (100 μL) was obtained from the inferior vena cava and stored on filter paper. Four-micrometre sections were stained with haematoxylin & eosin. Detection of integrated HIV provirus was determined by qualitative DNA PCR using the standard Amplicor HIV-1 kit (Roche, Johannesburg, South Africa) on blood collected on filter paper.

Selection of cases

In all cases the presence of PfIE was investigated in CNS, liver, lung, spleen, kidney, heart, pancreas, uterus and placenta, by scanning all slides at low magnification (100×) with polarized light. In at least one of the slides of each organ, 50 high-power fields (1000×) were analysed to completely exclude the presence of PfIE. We selected as study group all cases showing unequivocally the presence of any PfIE in any of the organs of the autopsy. The medical records of all patients included in the study were reviewed to retrieve the clinical and epidemiological data.

Microscopy and quantification of sequestered parasites

Under immersion oil (1000×) 100 perpendicularly cross-sectioned capillaries were evaluated and the contents were counted by a single observer in a section of the following organs: CNS, lung, kidney, heart, pancreas and uterus. A capillary was defined as a blood vessel, circular or oval in profile, with a maximum to minimum diameter of <2:1 and with at most one visible endothelial cell nucleus in the wall. All parasites were counted. The number of vessels showing sequestration was quantified and calculated as a percentage of the total vessel count. The number of PfIE in each single capillary was recorded. The presence or absence of intravascular macrophages, and the number per cross-sectioned capillary were also registered, as well as the presence of microthrombi, haemorrhages and any other histological abnormalities. These analyses in placenta, liver and spleen were performed using a high-power field (1000×) as reference.