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Cardiovascular changes in patients with non-severe *Plasmodium vivax* malaria☆

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ABSTRACT

Background: Cardiovascular system involvement in patients with *Plasmodium vivax* malaria has been poorly addressed. The aim of this study was to evaluate cardiac structures and function, and serum markers of cardiovascular injury in patients with the non-severe form of vivax malaria in Manaus, Amazonas State, Brazil.
Methods and results: We prospectively evaluated 26 patients with vivax malaria in an outpatient referral hospital and compared results with a control group of 25 gender- and age-matched healthy individuals. Patients underwent clinical evaluation, laboratory tests, and transthoracic echocardiography at first evaluation (day zero, D0) and seven days (D7) after malaria diagnosis. At D0 echocardiography showed higher left ventricular (LV) systolic diameter (28.8 ± 2.82 vs 30.9 ± 4.03 mm; $p = 0.037$) and LV diastolic volume (82.4 ± 12.3 vs 93.8 ± 25.9 ml; $p = 0.05$), and lower LV ejection fraction (Teicholz method: 73.2 ± 6.59 vs 68.4 ± 4.87%; $p = 0.004$) in patients compared to controls. Right ventricle (RV) fractional area change (54.7 ± 5.11 vs 50.5 ± 6.71%; $p = 0.014$) was lower, and RV myocardial performance index (0.21 ± 0.07 vs 0.33 ± 0.19; $p = 0.007$), and pulmonary vascular resistance (1.13 ± 0.25 vs 1.32 ± 0.26 Woods unit; $p = 0.012$) were higher in patients than controls. Patients presented higher serum levels of unconjugated bilirubin (0.24 ± 0.15 vs 1.30 ± 0.89 mg/dL; $p < 0.001$), soluble vascular cell adhesion molecule–1 (sVCAM-1; 453 ± 143 vs 1983 ± 880 ng/mL; $p < 0.001$), N-terminal prohormone brain natriuretic peptide (0.59 ± 0.86 vs 1.08 ± 0.81 pg/mL; $p = 0.045$), and troponin T (861 ± 338 vs 1037 ± 264 pg/mL; $p = 0.045$), and lower levels of plasma nitrite (13.42 ± 8.15 vs 8.98 ± 3.97 μM; $p = 0.016$) than controls. Most alterations had reversed by D7.
Conclusion: Patients with non-severe *Plasmodium vivax* malaria present subclinical reversible cardiovascular changes.

1. Introduction

Malaria, a common parasitic disease affecting humans, is one of the most important public health issues in developing countries [1]. In 2013, there were 104 countries with endemic malaria, with approximately 198 million people affected and an estimated 584 thousand deaths [1].

Malaria pathophysiology has been extensively studied. However, since the first reports by Laveran [2] in 1884 describing myocardial and coronary changes in patients dying from malaria, few studies have carefully evaluated the cardiovascular system in malaria. These clinical and experimental studies have suggested that acute infection is accompanied by parasite sequestration and obstruction in microvascular coronary and myocardial injury caused by parasite released proteins as well as inflammatory cytokines and anemia [3–8]. More recently, falciparum malaria patients were shown to present endothelial

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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dysfunction with impaired vascular nitric oxide bioavailability and increased pulmonary artery pressure [4,9].

All studies on cardiovascular involvement in malaria have been performed on *Plasmodium falciparum* malaria, which is related to the most severe form of the disease affecting several organs and systems [10]. Of the various Plasmodium species, *Plasmodium vivax* was previously considered to cause a benign non-fatal infection. However, in the last decade several reports have linked *P. vivax* to systemic complications involving the central nervous system, renal and respiratory failure, abnormal bleeding, anemia, and jaundice [11–16]. To the best of our knowledge, there are no studies analyzing the cardiovascular system during *P. vivax* malaria. In this study, we evaluated cardiac structures and function by Doppler-echocardiogram and plasmatic markers of cardiovascular injury in patients with the non-severe form of *P. vivax* malaria in Manaus, Amazonas State, Brazil.

2. Materials and methods

2.1. Study subjects

In a case–control study, we prospectively evaluated outpatients with *P. vivax* malaria attending the Dr. Heitor Vieira Dourado Tropical Medicine Foundation (FMT-HVD), in Manaus, Brazil, between December 2012 and March 2013. The FMT-HVD is a tertiary care center for infectious diseases, where patients can either seek attention directly or be referred for specialized care in neighboring municipalities. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by FMT-HVD Research Ethics Committee. All individuals signed the informed consent.

Patients aged 18–60 years were eligible to participate if they had no known illness and were diagnosed with *P. vivax* malaria to be treated out of hospital (*P. vivax* group, n = 26). Age and gender-matched individuals testing negative for malaria comprised the Control group (n = 25). The controls had similar economic conditions and lived in the same neighborhood as the patients. Exclusion criteria included electrocardiographic changes suggesting regional abnormalities, heart valve disease, and previously diagnosed severe diseases such as stage C heart failure, renal or liver insufficiency, cancer, pregnancy, malaria other than *P. vivax*, or severe malaria needing in-hospital treatment according to the World Health Organization [1]. All patients had positive thick blood smear and real-time qPCR assay for *P. vivax* malaria. Controls tested negative in both tests. Patients were treated with a combination of chloroquine and primaquine or a combination of artesunate and amodiaquine.

All individuals were subjected to the following: medical history evaluation, physical examination, 12-lead resting electrocardiogram, transthoracic Doppler-echocardiogram, and Laboratory investigation. In patients, clinical and laboratory evaluation was performed before treatment (day zero, D0) and seven days after starting treatment (day seven, D7). Medical history and physical examination were performed to assess general health and to clinically exclude diseases or conditions described in the exclusion criteria. Blood pressure was measured by the auscultatory technique with a conventional mercury sphygmomanometer.

2.2. Echocardiographic evaluation

A standard echocardiography system (General Electric Medical Systems, Vivid 3) was used to measure cardiac structures as previously described and following American Society of Echocardiography recommendations [17–20]. All echocardiograms were performed by the same examiner (JMBBF). With individuals positioned in left lateral decubitus position and monitored with an electrocardiographic lead, the following echocardiographic cuts were performed: short parasternal axis to measure ventricles, aorta and left atrium; apical 2, 4 and 5 chambers to evaluate ventricular cavities and systolic and diastolic function. The average of three measurements was calculated for each variable. The following left ventricular (LV) structures were measured by two-dimensional guided M-mode images: diastolic and systolic diameters (LVDD and LVSD, respectively), and diastolic and systolic volume (LVDV and LVSV, respectively). LV systolic function was evaluated by measuring ejection fraction according to the Teicholz index, endocardial fractional shortening, and myocardial performance index (Tei index) [21]. Right ventricle (RV) was structurally evaluated by measuring diastolic and systolic areas. RV systolic function was evaluated by fractional area change (FAC), and Tei index. Pulmonary vascular resistance (PVR) was estimated by Doppler echocardiography [22] according to the formula: PVR = tricuspid regurgitation peak velocity/right ventricular outflow tract velocity time integral) X 10 + 0.16. Pulmonary artery systolic pressure (PASP) was estimated by Doppler echocardiography using the modified Bernoulli equation [22]: PASP = 4 X (tricuspid regurgitation peak velocity) [2] + right atrial pressure. Right atrial pressure was estimated from inferior vena cava diameter [22].

2.3. Laboratorial analysis

Venous blood samples were obtained after a 12–15 h overnight fast in EDTA-coated tubes. Plasma was frozen at −80 °C in tubes containing 5 μL/mL antioxidant butyl hydroxytoluene (BHT, 20 μM), proteases inhibitor (aprotinin, 2 mg/mL), phenylmethylsulphonyl fluoride (PMSF, 1 mM), and benzamidine (2 mM). Nitrite plasma concentration was quantified by colorimetry using a commercially available nitric oxide assay kit (Cayman, Chemical Company, AnnArbor, Michigan, USA). Concentrations of N-terminal prohormone brain natriuretic peptide (NT-proBNP) and troponin T were measured by ELISA using commercially available kits (USCN Life Science Inc., Houston, Texas, USA). Soluble vascular cell adhesion molecule (sVCAM)-1 concentration was analyzed by immunoassay using a commercially kit (R&D Systems, Inc., Minneapolis, Minnesota, USA). All kits used in this study are available for laboratory research use only, not for human diagnostics.

Subjects were tested for malaria by thick blood smear. Parasite density was calculated by the arithmetic mean of two concordant readings; the white blood cell count was obtained from total blood count analysis as previously described [23]. In case of discordance (species-specific, or in the density quantification whenever a discrepancy was higher than 10%), a third reading was performed by a senior investigator (WMM). Real-time qPCR was performed as previously described [24] to confirm *P. vivax* malaria.

2.4. Statistical analysis

Variables are presented as mean and standard deviation or median and minimum and maximum values. Comparisons between periods were performed by Student's *t* test for dependent data and comparisons between groups were performed by unpaired Student's *t* test. Categorical parameters were compared by Fisher's exact test. The association between variables was assessed with Pearson's correlation coefficient. The level of significance was 5%. Statistical analyses were performed using IBM SPSS Statistics software Version 21.

3. Results

Baseline characteristics for controls and patients at day zero (D0) are presented in Table 1. Heart rate, although within the normal range, was higher in *P. vivax* group than Controls.

The *P. vivax* group had a mean peripheral parasitemia of 2844 ± 3286 parasites/mm^3, ranging from 87 to 11,806 parasites/mm^3. Laboratory data are shown in Table 2. *P. vivax* D0 had increased plasma concentrations of unconjugated bilirubin, troponin T, NT-proBNP, and sVCAM-1 and decreased platelet count and nitrite levels compared to Controls. At D7, unconjugated bilirubin, troponin T, NT-proBNP, and sVCAM-1 were lower, and platelet count and nitrite levels higher than D0. Sixty