Gender Influence on Asymptomatic Malaria Diagnosis

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Short Report

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Abstract

The characterisation and identification of malaria reservoirs are major challenges for the efficacy of control and elimination programs. Malaria asymptomatic infections are highly prevalent in different malaria settings worldwide and constitute a great obstacle for disease control and elimination. The monitoring of asymptomatic malaria infection requires active case detection in many cases challenging the limit of diagnostic technology detection. We evaluated diagnostic methods and analyzed the gender dimension implication for asymptomatic malaria diagnosis on a high-endemic malaria region during the dry-season.

Introduction

Malaria is declining worldwide since the beginning of this century due to massive investment and remarkable expansion in the range and effectiveness of products and strategies available for its elimination [1]. As more countries start entering pre-elimination phase, strategies for detecting and target all hotspots of infection, whether geographic or demographic, become extremely important to treat all infections and interrupt malaria transmission [2]. Asymptomatic malaria infections remain a challenge for malaria control programs as it significantly influences transmission dynamics [3]. Therefore, understand malaria infections and its detection among asymptomatic groups is of crucial importance in the near future for malaria control worldwide.

Accurate detection of asymptomatic malaria infections provide better realistic estimations of malaria burden and improve malaria control interventions. Due to the inherent lack of clinical manifestations, common sub-patent level of parasites, different malaria parasite species, different malaria parasite cellular stages, and other multivariable factors, the parasitological detection of asymptomatic malaria infections is still a challenge.

The commonly observed low parasitaemia infections among asymptomatic cases constitute a silent reservoir for malaria transmission. In this context, present molecular polymerase chain reaction (PCR) based methods are important tools since it can achieve 100-fold greater sensitivity as compared to microscopy [4]. Inhere we compared the use of microscopy and PCR to detect asymptomatic malaria infections on a highly endemic African community.

Methods

We performed the golden standard microscopic malaria diagnosis in 57 malaria asymptomatic adults (average age 52, range 16–80 years, Fig. 1A) from Dundo, Angola during the dry season. Blood sampling and storage: Microscopy for malaria screening was performed between 3 and 10 of August 2019 at S. Francisco de Assis Health Centre, Camatundo, Dundo on Lunda Norte province of Angola on a volunteer health community check-up. Microscopic malaria diagnosis was performed applying WHO protocols, in brief: blood samples collected through finger-prick on glass slides for Giemsa-staining. The presence or

absence of parasites was conducted in an optical Microscopy (Leica) through Giemsa-stained blood smears observation under oil immersion (x100 magnification). Glass slides were then stored in individual sealable plastic bags at room temperature.

DNA extraction: The parasite DNA from all samples was extracted using the ZR DNA-Card Extraction Kit (Zimo Research). The extraction was performed according to the manufacturer's recommendations with one modification: the blood samples were removed from glass slides with the aid of a sterile swab impregnated with the lysis solution. Next, the end of the swab with the sample was cut and placed in a sterile tube with ZR DNA-Card Extraction Kit (Zimo Research) kit's lysis solution. The DNA samples were stored at -20 °C.

A previously reported PCR method for *Plasmodium* spp. typing was applied, using primers set 5'- TGG TAG CAC AAA TCC TTT AGG G -3' and 5'- TGG TAA TTG ACA TCC AAT CC -3' respectively, specific for cytochrome B gene. The amplifications were carried out in a final volume of 25 µL, containing 5 µL of extracted DNA, 0.25 µM of each primer, and 15 µL Supreme NZYTaqlI 2x Green Master mix (NZYTech). PCR was performed using a T100™ Thermal Cycler (Bio-Rad) under the following conditions: 95°C for 5 min, followed by 35 cycles of 94°C for 30s, 60°C for 30s, 72°C for 1 min and with extension step of 72°C for 5 min. The PCR product was confirmed by electrophoresis gel (1% agarose gel stained with GreenSafePremium (NZYTech)) and visualized using an UV transilluminator (Gel-Doc™ EZ Imager (BioRad)) and quickly cut to minimize the UV exposure. 100 ng/µL of PCR products and 10 µmol/L of each primer (described above) were sent for sequencing (STABvida).

The 430bp-fragment obtained by PCR was extracted and purified from the gel slice using GRS PCR & Gel Band Purification Kit (GRiSP) and insert in the pJET plasmid using CloneJET PCR Cloning Kit (Thermo Scientific™). The plasmid was isolated from the cultured bacteria using the NZYMiniprep (NZYTech) according to the manufacturer's instructions. 100 ng of plasmid DNA and 10 µmol/L of each primer (pJET1.2-forward and pJET1.2-reverse) were sequencing (STABvida) and analyzed with Geneious software.

Results

Within the patients included, eighteen patients showed high body temperature (> 37.5°C) which was not associated with malaria infections detected or gender (Fig. 1B). Overall, 35% (20/57) patients were diagnosed with malaria using at least one diagnostic method. The use of PCR significantly increased the number of malaria infections detect from 16% (9 infections by microscopy) to 35% (20 infections by PCR) (p-value = 0.03) Fig. 1C. All the 9 infections detected by microscopy were also detected by PCR confirming. The detection of additional 11 sub-microscopic infections by PCR increased malaria positivity in 55%.

Strikingly, the observed difference between microscopy and PCR diagnosis was determined by gender. Among males, PCR diagnosis had a residual increase to 8 infections from initial 6 infections detected by

microscopy. However, among females, a 4-fold increase was observed (7–29%, p-value = 0.02) Fig. 1D. No other variables were significantly different comparing gender groups.

To evaluate the interference of possibility *Plasmodium spp.* infections, we sequenced all positive PCR infections. Three out of the 20 positive infections showed suspected mixed infections of *P. falciparum* and *P. malariae*, being that the remaining malaria infections confirmed the presence of pure *P. falciparum* infections (85%, 90% CI 64%-94%). The PCR products with mixed *Plasmodium spp.* were cloned to isolate DNA chains to confirm the presence of *P. falciparum* and *P. malariae* (Genebank accession number: MW094305). No gender association with *Plasmodium spp.* mixed infections since only two *P. malariae* were found on the male group and one on the female.

Discussion

On this prospective study, a prevalence of 35% asymptomatic malaria cases is detectable among asymptomatic infections on this high-endemic region during dry-season. We were able to confirm the superior capacity of molecular tools to diagnose submicroscopic malaria infections, which accounted to a 55% increase for the total malaria cases detected. Interestingly, applying PCR diagnosis, we found that submicroscopic infections were more prevalent among females as compared with males. Several factors have been reported to influence asymptomatic cases of malaria such as age, gender, ethnicity, seasonality and/or geography. In particular, submicroscopic infections in women are commonly observed during pregnancy [5]. On our study, none of the women reported to be pregnant. Factors modulating clinical malaria are highly variable and interconnected making it challenging to find singular determinants causative for the gender association detected on all malaria settings [6]. Independently of the cause for the presence of higher number of submicroscopic infections observed on women, we showed that the methodological sensitivity for accurate detection of such infection bias the results output depending on the gender.

The detection of a gender influence of submicroscopic infections highlights the need to consider such variables and technologies when performing asymptomatic malaria studies. These observations are considerable for the improvement of malaria diagnosis effectiveness for future malaria' control programs and interventions towards malaria elimination.

Declarations

Ethical Approval and Consent to participate and Consent for publication

The international volunteer mission Porta Nova, School of Medicine, performed this activity University of Minho, Portugal under the permission of local authorities and following ethical principles for medical research stated in the 1964 Declaration of Helsinki. Individuals with no signs or complains of malaria infection were asked to sign an informed consent to be included on the study and results published.

Availability of data and materials

Data and materials are available

Competing interests

Authors declare no conflict of interest

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Authors' contributions

MG, AC, DB, AB, GS were responsible to perform field work. SM and PEF designed the study and performed laboratory work.

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Authors' information

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Figures

Figure 1

Comparison of asymptomatic malaria diagnosis by microscopy and PCR. A) Average age mean of the groups analyzed. B) Percentage of individual with body temperature above 37.5 °C among the groups analyzed. C) Percentage (%) of malaria infections detected by microscopy and by PCR). D) Difference of malaria diagnosis stratified by gender comparing microscopy and PCR. Asterix marks a Fisher's exact test with a p-value below 0.05.