Comparison between Microscopy and PfHRP2 based RDT for diagnosing Tropical Malaria in Febrile Patients in Mesoendemic Malaria Transmission Area of Eural Burundi, among Internally Displaced Populations after Tribal Conflicts

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Original Article

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Abstract:

Background: Malaria is the leading cause of mortality and morbidity in post-conflict country of Burundi. Because of the lack of qualified

healthcare workers and laborants, frequent cuts of power and insufficient funds to provide regular microscopic examination in rural parts of Burundi, where around 91% of population live, malaria rapid diagnostic tests (RDTs) are the most important tools in diagnostic process of tropical malaria. Cheap P. falciparum Histidine Rich Protein 2 (PfHRP2) based RDTs are broadly used. The aim of the study was to compare sensitivity and specificity of these tests comparing to microscopy as the golden standard, and to assess whether they are or not an appropriate tool for diagnosing malaria in remote settings in Burundi.

Methods: A cross-sectional study was conducted in health centre Gasura in rural part of northern Burundi, in meso-endemic malaria transmission area. 190 patients (13 – 80 years old) presenting with fever or history of fever in past 3 days were examined with ParaHIT f Ver. 1.0 Rapid Test for P. falciparum Malaria Dipstick (PfHRP2 detection based RDT) from finger prick, and the blood slide was prepared to be microscopically examined by expert laborant. Results were analysed and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined.

Results: Out of 190 examined patients, 134 patients were confirmed to have P. falciparum infection by microscopic examination (70,5% prevalence). Out of those 134 patients, 92 patients had positive RDT. 56 patients were negative for P. falciparum using microscopy, out of whose 45 patients had also negative RDT. Overall sensitivity and specificity of RDT comparing to microscopic examination, as golden standard, were 68,7% and 80,4%, respectively, while PPV and NPV were 89,3% and 51,7%, respectively. Sensitivity significantly increased with increasing parasite density, 51,1% sensitivity for 100 – 500 parasites/µl, 71% sensitivity for 501 – 1 000 parasites/µl, and 82,4% sensitivity for >1 000 parasites/µl.

Conclusion: Rapid diagnostic tests (RDTs) remain to be an important tool in diagnosis of tropical malaria in remote settings, because of its relatively easy performance, quick result and low price, but because of relatively low sensitivity and specificity, microscopic examination should be preferred over RDT in group of patients over 13 years old, where available. In case of treatment failure, diagnosis based only on RDT should be revised.

Conflict of interest:

The authors whose names are listed in the title of the article certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, or other equity interest), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Background

Burundi is the landlocked country localized in the South-East Africa. It is one of the smallest countries of this continent with high density of population, with the area of 27,834 km^2 and 10,395,931 inhabitants (2014 estimates). Only around 9% of the population live in urban areas, the rest remain in rural parts of the country. Burundi is characteristic by its varying altitude (the mean altitude is of 1,707 m). Since its independence in 1962, the population of Burundi has suffered from several civil wars, amongst which the most recent was held from 1993 until 2005 (Buru, 2015). According to the data of the World Bank, since the end of the civil war in 2005, Burundi annually ranked as the poorest country globally according to GDP until 2013. In 2014 it ranked the second poorest country with GDP 267,10 USD per capita (GDP, 2015). According to the Global Hunger Index 2014, this country suffers from extremely alarming food and hunger situation, and with the ratio of 35,6 it is the most starving country globally. The severity is even more marked in micronutrient deficiency, due to unbalanced diet and starving (GMI, 2015). As the consequence of the long lasting civil war, the quality and coverage of health facilities are low, as well as the education of healthcare workers (nurses and laborants particularly) (Kimuli, 2016; Bujdova, 2015, Polonova, 2015, 2016). Based on the varying altitude of the rural parts, Burundi consists of varying endemicity of malaria transmission, in general specified as hyperendemic areas (altitude <1,400 meters), meso- to hypoendemic areas (1,400 - 1,750 meters of altitude) and areas of no malaria transmission (altitude >1,750 meters) (Amasi, 2011). Population of Burundi is rather uneducated, particularly in health issues and only a proportion of the population is aware of the basic facts about malaria transmission, diagnosis, and treatment (Tagliaferri, 2012).

Malaria belongs to the major public health issues in Burundi. According to national data from Ministry of Public Health of Burundi, in 2013 confirmed uncomplicated malaria stood for 39,5% of all pathologies in health centres (4 168 908 cases), what makes it the most common reason of attending the healthcare facility in Burundi. It was followed by upper respiratory tract infection in 20,3%, and non-specified febrile diseases (suspicion of malaria with negative diagnostic test) in 12,4% of all pathologies. Confirmed severe malaria stood for 0,9% of all pathologies in health centres. In 2013 in health centres, 4 089 355 blood slide examinations were performed, with 59% positivity and 2 827 930 rapid diagnostic tests performed, with 63,5% positivity. The mortality for confirmed malaria in health centres stands for 30,4% of overall health centre mortality. In hospital level, malaria stands for 25,6% of overall hospitalizations, with mortality 36,3% of overall hospital mortality (Ministerie, 2014). According to these statistics, malaria is the leading cause of morbidity and mortality in Burundi.

Vast majority of the population seeking for healthcare attends health centres, which are most commonly without the presence of high qualified personnel (doctors or certified nurses), and sometimes even without the possibility of microscopic examination. Therefore, performance of rapid diagnostic tests remains the most important tool for diagnosis of malaria. These tests are distributed to health centres free of charge, as a part of national malaria transmission elimination program, but they mostly are one of the cheapest tests available on market, based on detection of presence of P. falciparum histidine-rich protein 2. The outcomes of several previous studies based on evaluation of sensitivity and specificity of PfHRP2 based RDTs in comparison with other diagnostic tools came up with different results, some

of those more and another less satisfactory. These studies were conducted in Tanzania (Kweka, et al. 2011; Buhalata, et al. 2011; Uganda (McMorrow, et al. 2010), Ethiopia (Hailu, Kebede, 2014), Nigeria (Ajumobi, 2015; Elechi, 2015), but none of them in Burundi. Therefore, the aim of this study is to determine sensitivity and specificity of widely used RDT based on the detection of presence of P. falciparum histidine-rich protein 2 comparing to microscopic examination of thick and thin blood smears (as golden standard) in febrile patients, over 13 years old, presenting in health-centre in rural Burundi.

Patients and Methods

A cross-sectional study was conducted in health centre Gasura, part of medical district Vumbi, province of Kirundo in northern Burundi, from June 2015 to July 2015. Area of the study lies in altitude 1,450 meters and belongs to mesoendemic malaria transmission areas. Health centre Gasura is the healthcare facility for the total population of 34,586 inhabitants.

190 patients aged 13 - 80 years attending Health centre Gasura with elevated body temperature (>37,5°C) or history of fever in past 3 days and with no history of malaria or taking antimalarial treatment in last 14 days, were examined for tropical malaria by both, PfHRP2 based RDT and microscopic examination (thick and thin blood film). The blood slides were prepared and evaluated by the expert laborant in laboratory of Health centre Gasura, without knowing the result of RDT.

Thick and thin smears from finger prick were put on the same slide, left to dry, fixed with methanol and stained with 10% Giemsa during 30 minutes. Then thick smears were examined under the microscope using 1000x magnification for the presence of parasites and confirmed to be P. falciparum monoinfection on thin smear. Parasite density was determined by counting the parasites against white blood cells and expressed as number of parasites per microliter (µl) of blood. Blood slide was considered negative after examining 100 plasmodium-free microscopic fields.

The blood from the same finger prick was used for rapid diagnosis of P. falciparum malaria using ParaHIT f Ver. 1.0. Rapid Test for P. falciparum Malaria Dipstick, manufactured in March 2014 with expiration in March 2016. Tests were stored according to manufacturer's recommendation at 25°C, protected from sunlight and humidity. The tests were all performed according to the instruction of manufacturer. After the disinfection of fingertip by alcohol wipe and air drying, sterile lancet was used to pierce the skin and capillary blood was collected to a plastic pipette and transported to a dipstick, which was then put to a tube containing 4 drops of a reaction buffer. Results were read after 25 minutes. Dipsticks showing two lines (test and control) of any intensity were considered positive, those showing only control line were considered negative. No invalid test was detected.

Data analysis and results

After collecting the data, sensitivity, specificity, positive predictive value (PDV) and negative predictive value (NPV) were determined. Sensitivity was determined as the proportion of number of patients with true positive RDT results to number of patients with positive microscopic result, while specificity was calculated as the proportion of number of patients with true negative RDT results to number of patients with negative microscopic result. PPV was calculated as the proportion of number of patients with true positive RDT results to number of all patients with positive RDT result and NPV was determined as the proportion of number of patients with true negative RDT result to number of all patients with negative RDT result.

Out of total 190 patients examined, 134 patients tested positive for P. falciparum infection by blood smear microscopic examination, which stands for prevalence of 70,5%. Out of those 134 patients, 92 patients had positive RDT. 56 patients tested negative for P. falciparum using microscopy, out of whose 45 patients had also negative RDT. Overall sensitivity and specificity of RDT comparing to microscopic examination, as golden standard, were 68,7% and 80,4% respectively, while PPV and NPV were 89,3% and 51,7% respectively.

When sensitivity was attributed to parasite density, it showed following results: for density 100 - 500 parasites/µl, sensitivity was 51,1%, increased sensitivity of 71% was proven in group of patients with density 501 - 1 000 parasites/µl and in group of patients with density >1 000 parasites/µl sensitivity was as high as 82,4%. No patient with density <100 parasites/µl was detected.

Discussion

Correct diagnosis of malaria and early treatment are crucial for eliminating mortality rate and transmission in endemic areas, therefore reliable diagnostic tests are highly required to be accessible in referral hospitals as well as in remote settings. Because many healthcare facilities in rural Burundi suffer from lack of qualified staff, frequent cuts of electricity or insufficient funds to provide microscopic examination, RDTs mostly based on detection of PfHRP2 are provided from the government as a part of national program for malaria transmission elimination. Annual report for 2013 of Ministry of Health of Burundi showed, that overall prevalence of malaria in Burundi in microscopically examined patients is 59%. In this study, the prevalence was 70,5%. This disproportion is probably due to altitude of Health centre Gasura, where the study was conducted, that lies in 1 450 meters in mesoendemic area (comparing to average altitude of Burundi, that is 1 707 meters, in hypoendemic area). Overall sensitivity of 68,7% and specificity of 80,4% are important limitations for correct tropical malaria diagnosis based exclusively on PfHRP2 RDTs, but in remote settings with lack of qualified laborants or no electricity access, they are still of important value. Although sensitivity was significantly increased with increasing parasite density (sensitivity of 51,1% for 100 - 500 parasites/µl, 71% for 501 - 1 000 parasites/µl and 82,4% for >1 000 parasites/µl) with $p < 0,01$, it does not achieve the values given in manufacturer's information, where sensitivity for >100 parasites/µl is claimed to be 99,4% and specificity 99,7% (9-16).

Conclusions

This study shows, that rapid diagnostic tests based on detection of PfHRP2 are of limited value in diagnostic process of tropical malaria in patients over 13 years old and therefore, microscopic examination should be recommended, where available. In remote settings without possibility of microscopy, they remain to be an important diagnostic tool, because of its relatively easy performance, quick result and low price. Although, because sensitivity and specificity of Pf HRP2 RDT was relatively low, diagnosis based only on Pf HRP2 RDT should be revised in case of treatment failure.

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