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Research article

Performance assessment of a widely used rapid diagnostic test CareStart™ compared to microscopy for the detection of *Plasmodium* in asymptomatic patients in the Western region of Cameroon

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ABSTRACT

While malaria remains a serious public health concern, its rapid or prompt diagnosis in remote areas is important in the fight against the disease. The study aimed to evaluate the performance of widely used Rapid Diagnostic Test (RDT) kits for routinely detection of *Plasmodium* asymptomatic patients. A total of 400 asymptomatic participants of both sexes aged between 1-89 years from Menoua Division (Santchou and Dschang) were tested for malaria infection using both microscopy and CareStart™ RDT. The prevalence of malaria was higher when using the standard gold tool (Microscopy) (26.0%) compared to RDT (21.8%) with a significant difference (P < 0.05). However, a strong agreement was observed between both tests (kappa = 0.883; P < 0.0001). RDT CareStart™ sensitivity and specificity were 83.65% and 100% respectively while the positive predictive value and negative predictive value were 100% and 95.57% respectively. RDT sensitivity increased with parasite density while false negative (40.4%; n = 17) were observed only when parasite density was low (<500 parasites per microliter of blood). RDT kits appear to be good tools in areas where malaria diagnosis through microscopy is not feasible. However, the low sensibility observed when parasite density is low could be a concern.

1. Introduction

Cameroon is a country where malaria is an important public health threat with the whole country being exposed to the high risk of transmission [1,2]. Although significant progress to eradicate the disease has been made in the recent years, it remains prevalent with about 3.7 million suspected cases per year [1,3]. In 2019, the report of National Malaria Control Program (NMCP) highlighted 2,133,523 cases of disease responsible of 25,9% of medical consultations in health centers, 49% of hospitalizations and about 2000 deaths registered [1]. In addition to vector control, global malaria control programs advocated cases diagnostic and treatment as one of important strategies for its achievements [4]. Before starting the treatment, a good diagnosis had to be done to limit the expansion of drug resistance and to enable the identification of malaria-free patients [5].

In Cameroon as in other countries, microscopy is still considered as a standard technique in malaria diagnosis in hospitals [6,7]. However, a number of challenges have been observed in the use of microscopy as routine diagnostic tools in health care centers including: lack of qualified human resources, lack of stable electrical power supply, the quality of blood slides, low parasite densities, and altered parasite morphology caused by chemoprophylaxis or empiric therapy [8,9,10,11]. To face these challenges, rapid diagnostic tests (RDT) have been widely used in areas with limited diagnosis resources. CareStart™ RDT test targets antigens histidine-rich protein-2 (PfHPR2) for *Plasmodium falciparum* [12]. RDT has the advantages to be easier to carry and use, and required less experienced/qualified personnel. Also, it gives results from the whole blood within 20 min using a very little amount of blood [13,14,15]. However, some limitations to the technique include the persistence of

PfHRP2 antigens and false-positive reactions due to other infectious agents or immunological factors [16].

Despite the numerous advantages that RDT offers, microscopy still remains the reference standard method in malaria endemic regions [17, 18,19]. Therefore, this study aimed to evaluate the performance of widely used RDT kits available for routine detection of *Plasmodium* in asymptomatic patients.

2. Materials and methods

2.1. Study site

The study was conducted in two localities (Santchou and Dschang) of the Menoua Division in the western region of Cameroon. Menoua Division is an outskirt of the Cameroon Western Highlands Plateau. It is made up of 06 sub-Divisions (Dschang, Fokoue, Fongo-Tongo, Nkong-Ni, Penka-Michel, and Santchou).

The chief-town of the Menoua Division is Dschang, situated between 9°50′ and 10°20′of East longitude, 5°10′and 7°0′ of North latitude; and its altitude lies between 1,300m and 1,400m. Its vegetation is of wooded savannah type and its climate is of Guinean-Soudano type with two seasons (rainy and dry seasons) [20]. The mean annual rainfall is 1,872mm with a relative humidity comprised between 64,3% and 97%. The average annual temperature is 20 °C with February being the hottest month. According to the report of the third population census of Cameroon, Dschang has an estimated population of 120,207 inhabitants [21].

Santchou is located between 5°16′N and 9°58′E at 786m of altitude with a surface area of 95,05km^2. The annual average temperature in Santchou is 22.5 °C. Its annual average precipitation is 1,364.4mm with a relative humidity of 92%. Santchou has a very complex vegetation and its climate is equatorial of the Guinean type and has four unequal seasons, namely: The long rainy season which runs from mid-August – October-The small rainy season (from March to June); The great dry season (mid-October to March); The short dry season (June – mid-August). . It constitutes part of the Mbo plain; it is limited to the north by the town of Dschang, the capital city of the Menoua Division, to the North-West by the city of Mendji in the South-West Region, to the South by the cities of Kekem and Melong In the littoral Region, and to the East by the city of Fokoué. During the 2005 population census, the community counted 37, 479 habitants representing a density of 394 hab/km^2 with 9 428 inhabitants [21]. Rice, cassava, and Ginger cultivation representing the economic activity of the area was responsible for the rapid expansion of the population in the beginning of the 1980s.

2.2. Study design

Prior to this study, authorizations for investigations were obtained from the administration of each locality (senior divisional officer, Director of the Santchou/Dschang District Hospital and chiefs of the community). Also, an ethical clearance was obtained from Cameroon National Ethics committee of human research (ref N°2019/04/CE/ CNERSH/SP). After having explained to the population the purpose of the study and procedure of sampling, only people who signed the informed consent form were enrolled in the study. For children less than 18 years, consents were obtained from their parents. The population included in this study were asymptomatic, of both sexes and of different ages living in Santchou or Dschang.

This study was a cross sectional study during which demographic characteristics and malaria status (using RDT and microscopy) of each participant were collected from door to door. The sample size was calculated using Lorentz formula.

2.3. Laboratory methods

Venous blood collected from participants was used for the detection of malaria parasites by microscopy. Using clean slides, thick blood films were prepared, stained, and examined under the microscope according to WHO [22,23] as described elsewhere [17,24]. For the correctness of the observation, each slide was examined or observed by two expert scientists from VBID-URBEA. A crosscheck was done by independent scientists on randomly selected slides and slides where results were different from the first two scientists. All observed randomly selected slides by independent scientists confirmed the first results while three (03) from five slides with opposite results were positive. During examination, the presence and density (parasitemia) of *Plasmodium* species were determined. Malaria parasite density was calculated as follows: number of parasites x8000/number of count leucocytes. Using the same venous blood, malaria rapid diagnostic test kit was performed on the field (in situ) for malaria parasite detection (malaria infection) using CareStart™ Malaria PAN (pLDH) Ag RDT (ACCESSBIO, USA (sensitivity [98.50% and specificity 97% according to manufacturer, Year of Manufacture: 2018)) according to the manufacturer guidelines. Results were read after 20–25 min. When results were negative, only a control line appeared, while for positive results, a control line, T and/or PAN appeared. An absence of a control line implied that the test was invalid.

2.4. Statistical analysis

Data were entered in Microsoft Excel spreadsheet. After checking errors and missing values, data were transferred to IBM- Statistical Package for Social Sciences (IBM -SPSS) version 22.0 for statistical analyses. Pearson's Chi-Square test was used to evaluate differences in proportions. Sensitivity (a/(a+c), specificity (d/(b + d)), positive (a/ (a+b))and negative (d/c + d) predictive values were calculated using two-by-two cross tables where "a" is the number of positive tests in both TDR and Microscopy, "b" is the number of positive TDR with negative microscopy, "c" number of negative TDR with positive microscopy and "d" the number of both negative TDR and microscopy. Determination of agreement between CareStart™ and microscopy was done using Cohen's kappa test. Kappa value was interpreted according to Florkowsky [25] with a value of <0.59, 0.6–0.79, 0.8–0.9 and >0.9 considered as weak, moderate, strong and almost perfect respectively. The likelihood ratio for positive ((LR+) (calculated as LR+ = sensibility/(1 – specificity)) and negative (LR-) (calculated as LR- = (1-sensibility)/(Specificity)) tests were assessed. Results were considered as good when LR+ was >10, and LR-<0.1. The diagnostic accuracy was explored from receiver-operating characteristic (ROC) curve. Using the area under the curve (AUC) for diagnostic accuracy; <0.5, 0.5–06, 06–0.7, 0.7–0.8, 0.8–0.9 and 0.9–1.0 were considered equivalent to test not useful, bad, sufficient, good, very good and excellent respectively [26,27,28].

3. Results

3.1. Characteristics of the study population, malaria prevalence and parasite density

Overall, 400 participants were included in this study and their blood were tested by microscopy and TDR for malaria parasites detection. The age of participant varied from 1 to 89 years (mean age ±SD: 21.62 ± 17.7)) with a sex ratio of 1:2 in favour of females (62.3% and 37.8% respectively for females and males). In general, the prevalence of malaria infection in the study asymptomatic population was high (n = 104; 26%) and age-dependant. Younger children less than 05 years were less infected compared to other age groups except for elders (>60 years) with no statistical difference ($\chi^2 = 1.95; P = 0.74$). Prevalence of malaria was not associated with gender ($\chi^2 = 1.95; P = 0.74$) but varied significantly with locality ($\chi^2 = 20.79; P < 0.001$) (Table 1). From the 26 % (n = 104) of confirmed malaria positive cases by microscopy, parasites density ranged from 16 to 4001 parasites per microliter of blood with a mean density of 886.82 ± 840.39 parasites/microliter of blood.

Table 1. Malaria prevalence according to population characteristics.

		Tested	Positive	Prevalence (%)	*P value*
Gender	Males	151	43	28.48	0.54
	Females	249	61	24.50	
Age (year)	0–5	67	14	20.90	
	6–15	109	34	31.19	0.74
	16–30	134	35	26.12	
	31–60	72	18	25.00	
	>60	18	3	16.67	
Locality	Dschang	200	32	16.00	0.000
	Santchou	200	72	36.00	

3.2. Comparison of RDT CareStart® and microscopy

A high prevalence was observed with microscopy (n = 104; 26%) compared to TDR (n = 87; 21.8 %) with a significant difference (χ^2 = 1.92; P = 0.05). The sensitivity and specificity of RDT were 83.65% and 100% respectively. The positive predictive value was 100% while the negative predictive value was 94.57%. The LR+ was impossible to compute due to 100% specificity while LR-was 0.0017 meaning that asymptomatic patients have less than 1% chance to be diagnosed positive with TDR when they are negative. The diagnostic accuracy for malaria rapid diagnostic test CareStart™ was excellent (0.96). A strong agreement for the accuracy of malaria diagnosis between CareStart™ and microscopy was recorded (Kappa = 0.88; P < 0.001) (Table 2).

It was observed that the sensitivity of RDT varied with parasite density (χ^2 = 340.5; P < 0.001). About 40.48% (17/42) of samples with low parasitemia (<500 parasites per microliter of blood) were undetectable with CareSart™ malaria Rapid Diagnostic Test while samples with parasites load greater than 500 parasites per microliter of blood was perfectly detectable with RDT (Table 3).

4. Discussion

As recommended by WHO, microscopy is the gold standard technique for malaria detection in health facilities. With the absence of qualified technicians and prompt parasites detection, RDT is also recommended as a point of care tools [29]. This study aimed to evaluate the performance of widely used CareStat™ RDT on detection of *Plasmodium* in asymptomatic participants in Menoua Division. The prevalence of asymptomatic malaria was high in study sites locality (especially in Santchou) and this calls for concern because it could serve as a reservoir of malaria parasites that could help increasing malaria transmission from infected to uninfected individuals. The high prevalence recorded may be due to the fact that the study area is a meso-endemic malaria area harbouring the

main malaria vectors in the country, *Anopheles gambiae* s.l [30,31]. Several studies indicated high malaria prevalence on patients visiting hospitals in the study area [31,32]but this prevalence is low compared to 31.8% (38.26% and 25.49% in Santchou and Dschang respectively) found in 2011 by Tchuinkam *et al* [31]. In Mezam Division situated in the North West region of the country, malaria prevalence was found higher than the one reported in this study [17], while in Douala, prevalence of malaria was low [24]. Prevalence of malaria was age and gender-dependent with younger children, elders and females being the less infected, maybe due to their wide used of mosquito nets and their indoors behaviour at night compared to other groups [33,34,35]. The sensitivity of RDT was lower while the specificity was higher than those compared in the manufacturer declaration. Sensitivity was also lower compared to those observed in previous studies who recorded higher sensitivity and specificity using the same RDT [27,36,37,38,39]. Also, Metoh *et al* [17] observed high sensitivity and specificity with another histidine-rich protein 2 RDT test. As mentioned by Metoh *et al* [17] 2020, this lower sensitivity than what is recommended (95%) could impair control intervention since fraction of the malaria infected populations will be left untreated in the case where only RDT is used as a diagnostic tool. The low sensitivity of this RDT may be due to genetic mutation of parasite histidine-rich protein 2 [12]. This deserves further investigation. Importantly, high specificity of RDT was observed in this study. The high specificity may be due to the dominance of *P. falciparum* in the study area. High specificity of RDT was also observed in previous studies in Nigeria [40,41] and Cameroon [17] and elsewhere [27] using another RDT.

According to Emmanuel *et al* [42], low performance of RDT in field conditions may be due to several factors including; environmental conditions (such as high temperatures during transportation and storage), quality issues, disease-related factors (e.g. parasite species and density etc.), and also host factors (such as the treatment history). In Menoua, temperature is low and could not affect the quality of the RDT test kits. During this study, treatment history of patients/populations enrolled was not monitored while malaria parasite density was observed. It was found that the sensitivity of used RDT increased with parasitemia. With CareStart™ RDT, 17 false-negative results were obtained and all the samples had low parasitemia. The low parasitemia was found elsewhere as a limit for the use of alternative tools for malaria diagnostic [24,42]. In Tanzania, high sensitivity was obtained compared to others [36,37] due to the high parasite density in the tested population [27].

Table 2. Performance of CareStart™ RDT with microscopy as standard method.

	Microscopy	
	Positive	Negative
CareStart™		
Positive	87	0
Negative	17	296
Measures of diagnosis performanace	**Value**	
Sensitivity	83.65 [67.0–101]	
Specificity	100	
Positive predictive value	100	
Negative predictive value	94.57 [84.1–105.98]	
Diagnostic accuracy	0.96 [0.86–1.01]	
Likehood ratio for positive test	-	
likehood ratio for negative test	0.0017	
Cohen's kappa, P value	0.883; P < 0.0001	

Table 3. Relationship between parasite density and performance of Carestart™ Rapid Diagnostic Test.

Parasites/µL	Tested	Negative	Positve	%
0	296	296	0	0.0
[1–500]	42	17	25	59.5
[501–1000]	31	0	31	100.0
[1001–5000]	31	0	31	100.0
Total	400	313	87	21.8

5. Conclusion

RDTs are important diagnostic tools and useful in areas where microscopy is not available for malaria detection. Based on the low sensitivity of CareStart™, it could not be used to replace microscopy but should be prioritized as point of care tools for prompt malaria detection. The level of parasite in blood (parasitemia) should be considered when using this tools. On one hand, in an endemic area, this tool would definitely be a key device in malaria treatment. On the other hand, in a hypo endemic area, it could be a concern.

Declarations

Author contribution statement

Roland Bamou: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Zidedine Nematchoua-Weyou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Timoléon Tchuinkam: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Michel Lontsi-Demano: Laura Gilberine Ningahi, Melanie Adele Tchoumbou, Blaise Armand Defo-Talom and Marie Paul Audrey: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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