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Clinical and biological diagnosis of malaria and dengue fever among febrile patients in hospitals of Bangangte, Cameroon

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ABSTRACT

Malaria and dengue are vector borne diseases with shared endemic profiles and symptoms. This study aimed at investigating through the prevalence of malaria-dengue co-infection in febrile patients and to assess the level of knowledge about dengue fever among healthcare workers in Bangangté. A cross-sectional study was conducted from January to June 2021 in five healthcare facilities of Cameroon. Malaria diagnosis was performed by rapid tests and thick smears, and dengue virus detection was done using rapid test. A total of 60 healthcare workers (24 medical doctors, 36 nurses) and 200 patients with history of fever were enrolled. Overall findings revealed predominance of malaria (53%), followed by dengue (8.5%). Malaria-dengue co-infection was recorded in 6% of cases. Positivity rates were 53% with thick smears and 65% with rapid malaria tests. For dengue positivity rates were 5.50% for NS1 Ag, 6.50% for IgM, and 2% for IgG. Out of the 60 participating healthcare workers 55% had a low-knowledge level about dengue. The prevalence of dengue-malaria co-infection is low in Bangangté, but it's now known that the dengue virus is circulating in the area. Furthermore, the knowledge of healthcare workers on this arbovirus is poor, justifying the necessity for increasing surveillance for dengue and like diseases; and advocacy for the continue education for healthcare practitioners.

Keywords: Malaria, dengue, malaria-dengue co-infection, laboratory diagnosis, knowledge.

INTRODUCTION

Malaria and dengue are amongst the most common vector-borne diseases throughout the world and appear as major public health concerns in sub-Saharan Africa. Salam et al. reported that there are 390 million dengue cases each year, from which 96 million develop clinical symptoms which result in 20-25,000 deaths per year, mainly amongst children in developing countries (Bhatt et al.,

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2013). Before 1970, only 9 countries were known to experience severe dengue epidemics. In 2015, the number of notified cases increased to 3.2 million, right from 2.2 million in 2010 (Bhatt et al., 2013). The disease is nowadays endemic in more than 100 countries in Africa, America, Eastern Mediterranean, Southeast Asia, and Western Pacific. Southeast Asia and Western Pacific are the most affected. The number of cases in the American continent, Southeast Asia, and the Western Pacific exceeded 1.2 million in 2008 and 3.2 million in 2015 (Demanou et al., 2010; Tchuandom et al., 2019).

In Cameroon, most febrile cases are thought to be related to malaria or typhoid fever. Case management is routinely conducted in several healthcare facilities based on clinical symptoms rather than biological evidence with laboratory tests. Widespread in all tropical and subtropical areas of the world, dengue is the leading public health problem posed by arboviruses (Simo et al., 2019). These two pathologies share several similar signs and symptoms, including fever, headache, asthenia, and myalgias for their simple form, but the dual infection was first described only in 2005 (Bigna et al., 2018). However, their clinical picture may be more severe in case of coinfection than the one recorded in single infection. If malaria is endemic in Cameroon, dengue infections are barely reported while according to certain sources documented malaria and dengue coinfections are not known (Antonio-Nkondjio et al., 2019; Danwang et al., 2021).

Dengue virus (DENV) infection was first identified in Cameroon in 1987, but many cases may remain undiagnosed, misdiagnosed and/or confused with other tropical diseases. A previous cross-sectional study conducted in 7 urban and 3 semi-urban hospitals in Cameroon to determine the seroprevalence of dengue virus in 961 febrile children (≤15 years) revealed that DENV seroprevalence was 14.4%. Malaria cases accounted for 40.6% of the study population and typhoid fever, 2.3% while dengue-malaria coinfection was reported in 5.3% of cases (Tchuandom et al., 2019; Tchuandom et al., 2020). In order to contribute to a better management of the fever, the present investigation aimed at determining the prevalence of dengue in Bangangté as well as the rate of coinfection with malaria. Moreover, it aimed at assessing the level of knowledge of health workers on dengue. This will highlight clues for clinical differentiation between these two microbial conditions which, because they share closely related clinical characteristics represent real puzzles in clinical diagnosis by healthcare workers.

MATERIALS AND METHODS

Ethical considerations

The ethical clearance was obtained from the Institutional Ethical Committee of Université des Montagnes [N° 2021/061/UdM/PR/CIE] and all research authorizations from participating health facilities [Cliniques Universitaires des Montagnes, Bangangté District Hospital, Ad Lucem Hospital of Bangangté, Cradle of Angels Hospital and Petit Bangoua Hospital].

Study site and design

The study was conducted in five health facilities located in the city of Bangangté, headquarter of the Ndé Division, West Cameroon. Namely, these health facilities were: The Cliniques Universitaires des Montagnes, the Bangangté District Hospital, the Ad Lucem Hospital, Cradle of Angels Health Center and Petit Bangoua. Bangangté is located at 1340 m altitude on the national road number 4, 49 km southwards from Bafoussam, the regional headquarter and 249 km from Yaoundé *via* Obala. The urban area is drained by the Ngam River that flows into the Noun. With an annual temperature of 20.4°C and rainfall of about 1950 mm per year reflecting tropical climate, the general environment is conducive to dengue and malaria infections. This descriptive cross-sectional study was conducted over a period of 6 months, from January 11 to June 15, 2021.

Study population

The study population consisted of all patients admitted with fever hospitalized or not, as well as all the healthcare workers (doctor, nurse, care assistant) of the participating health facilities.

The inclusion criteria for patients were, more specifically, all patients hospitalized as outpatients or inpatients with sudden onset or patients experiencing a fever that began during the preceding seven days. Concerning the health staff, the inclusion criteria were for all health workers recruited in health structures for at least 6 months. Both categories had to complete informed consent forms in order to participate. Patients receiving antimalarial medication were excluded. Participants were consistently recruited. After signing the informed permission form, the participant was free to leave the study at any time, as specified, without penalty or effect on medical follow-up.

Sample collection

This was done by questions, physical examination and blood sample collection according to the inclusion criteria. Each healthcare professional would fill in a data survey sheet designed to assess of knowledge on dengue. For the prevalence of malaria-positive cases among participants, routine biological diagnostic tests (rapid tests and thick smears) were performed. The biological tests for dengue included NS1 antigen (NS1-Ag), and IgM/IgG antibodies (Qingdao Hightop Biotech).

Biological validation of tests results

For participants

The Dengue NS1/IgM/IgG Antigen test kit is a test for the identification of the four DENV serotypes in serum, plasma or whole blood.

After disinfection of the patient's finger with alcohol, whole blood was collected with a vacutainer needle. After collection, three drops (about 100µlenvironmentally) of the sample and four drops (about 120ul) of diluent were placed in the well with a disposable dropper.

The cassette was read after 15-20 min according to the manufacturer instructions. IgM was positive if both C and T1 lines appeared, and IgG was positive when both C and T2 lines appeared. In all cases, negative results were reported when a single stained line was observed in the control reading window (C) of the test device.

For the healthcare workers

Data for socio-demographic variables were related to the profession (doctor, nurse, care assistant) and experience based on the number of years of practice. For Variables related to knowledge about dengue, collected data were diagnosis, definition, symptoms, vector, mode of transmission, complications, biological diagnosis, prevention. The level of knowledge was qualified as good, average, insufficient and bad for more than 85%, between 65 and 84%, between 50 and 64% and less than 50%, respectively.

Statistical analysis

Data analysis was performed with the software SPSS (Statistical Package for Social Sciences) version 25.0. Categorical variables were expressed in frequency and percentages and compared by the Chi-square test. Factors influencing knowledge were investigated by logistic regression in univariate and multivariate analysis. Namely, qualitative variables for patients were age, sex, symptoms, clinical signs and biological pieces of information. The significance threshold used was 5% (p value<0.05) throughout the study.

RESULTS

Sociodemographic characteristics of participants

A total of 260 participants were enrolled. They included 200 patients and 60 healthcare workers. On the patients age, the largest number of participants were found between 1 and 40 years (75% of the study population). Between 40 and 80 years, the frequencies were relatively similar. Female patients represent 3/5 of the total with a sex-ratio of 0.69. The clinical and biological pieces of information recorded allowed identification

of cases of malaria and dengue (Table 1), as well as malaria-dengue coinfection with varied frequencies.

In total, 47% [n=94] of the patients were infected with malaria only, while 2.5% [n=5] were infected with dengue only. Malaria-dengue co-infection was found in 6% of cases [n=12]. However, in 32.5% of cases (n=65), patients were seronegative for malaria and dengue.

Data indicate higher positivity rate for rapid test than the thick smear, while NS1-Ag, and IgM positivity rates were three times higher than IgG's. Malaria and dengue related age distribution was summarized and displayed in Figure 1.

This data graphics further revealed that malaria was 20 times more frequent than dengue fever infection and almost 10 times more frequent than malaria-dengue coinfection.

Clinical characteristics of malaria and dengue

For each infection type, the clinical characteristics (symptoms and physical signs) were identified through bivariate statistical analysis and listed as displayed in Table 2. Major findings from this table indicate three different types of infections. Fever was the most common symptom. It also discloses that dengue/malaria coinfection was significant association with myalgias and headache, while tachycardia was significantly associated with malaria, and blurred vision with dengue.

Assessment of healthcare workers' knowledge on dengue

The 60 healthcare workers consisted of 24 medical doctors, 17 nurses and 19 healthcare assistants. The results of the questionnaire's overall data analysis showed that healthcare workers' understanding of dengue was generally low. Table 3 was created to compare the years of experience and healthcare professionals' understanding of dengue.

It appears that the number of years of practice does not affect the knowledge level. In order to have a trend on the frequency of dengue diagnosis in these health facilities, the answers collected from the healthcare workers were computerized. It appeared that almost all the healthcare workers (96.7%) have never thought about medical diagnosis of dengue.

Table 1: Prevalence of Malaria and Dengue among the study population.

Pathologies	Identification test	Frequency (%)	Frequency (%)
		Positive	Negative
Malaria	Thick smear	106 (53)	94 (47)
	RDT	130 (65)	70 (35)
Dengue	NS1-Ag	11 (5,5)	189 (94.5)
	IgM	13 (6.5)	187 (93.5)
	IgG	4 (2)	196 (98)

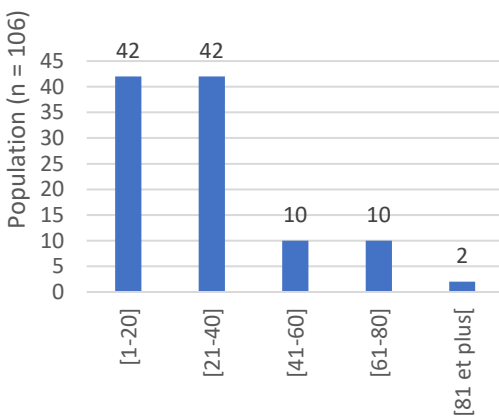
Legend: NS1-Ag: Dengue virus non-structural antigen 1; IgM: Immunoglobulin M; IgG: Immunoglobulin G; RDT: Rapid diagnosis test.

Table 2: Symptoms and physical indicators for malaria, dengue and the coinfection.

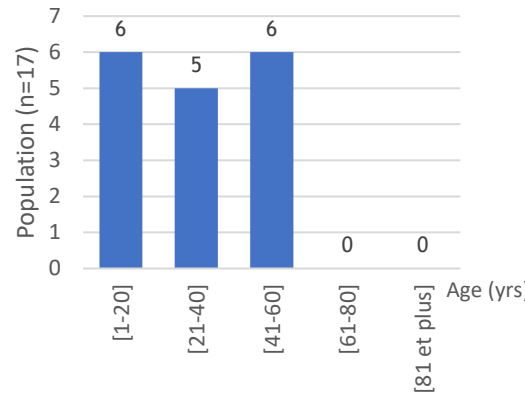
	Malaria (n=106)		Dengue (n=17)		Malaria-dengue coinfection (n=12)	
Clinical signs and symptoms	**Frequency (n) and percentage (%)**	**p-value**	**Frequency (n) and percentage (%)**	**p-value**	**Frequency (n) and percentage (%)**	**p-value**
Fever	92(86.6%)	0.185	17(100)	0.140	12(100)	0.221
Headache	81(76.4)	0.003	11(64.7)	0.833	9(75.0)	0.543
Asthenia	65(61.3)	0.921	14(82.4)	0.059	10(83.3)	0.102
Myalgia	13(12.3)	0.544	4(23.5)	0.084	4(33.3)	0.011
Abdominal pain	36(34.0)	0.194	4(23.5)	0.543	3(25.0)	0.697
Arthralgia	24(22.6)	0.516	4(23.5)	0.923	3(25.0)	0.967
Vomiting	44(41.5)	0.118	6(35.3)	0.914	5(41.7)	0.701
Nausea	8(7.5)	0.177	0	0.298	0	0.389
Ocular pain	1(0.9)	0.492	0	0.595	0	0.659
Blurred vision	1(0.9)	0.257	2(11.8)	0. 003	1(8.3)	0.106
Hepatomegaly	12(11.3)	0.129	1(5.9)	0.686	1(8.3)	0.983
Splenomegaly	6(5.7)	0.203	1(5.9)	0.679	1(8.3)	0.429
Tachycardia	17(16)	0.016	4(23.5)	0.084	3(25.0)	0.110
Bradycardia	0		0		0	
Adenopathy	4(3.8)	0.057	0		0(0.0)	0.610

Table 3: Healthcare workers' knowledge and working experience.

Experience (year)	**0-2years**		**3-5years**		**6-10 years**		**> 11years**	
Level of awareness	N	%	N	%	N	%	N	%
Good (> 85%)	8	13	2	3	2	3	2	3
Average (85-65%)	1	2	7	12	2	3	3	5
Insufficient (64-50%)	3	5	1	2	0	0	3	5
Bad (> 50%)	3	5	10	17	10	17	10	17
Total	8	13	20	33	14	23	18	30



Age distribution of malaria cases



Age distribution of dengue cases

Figure 1: Age distribution for Malaria and dengue fever cases.

DISCUSSION

Dengue virus and *Plasmodium* coinfection is a leading cause of hospitalization in sub-Saharan Africa (Galani et al., 2021). This coinfection worsens the symptoms of the disease and increases the risk of death. Furthermore, dengue virus is not often taken into consideration during the biological diagnosis of febrile cases. According to Salam et al. (2018) (Salam et al., 2018), this ignored issue represents a risk for patients and a real threat for the whole population in areas where malaria is endemic. It is in this context that we carried out the present study which focused on determining the prevalence of dengue-malaria coinfection in febrile patients as done by previous authors (Monamele & Demanou, 2018), and assessing the level of knowledge of healthcare personnel on dengue in Bangangté health facilities.

Previous investigators identified potential vectors for these viruses (Fontenille & Toto, 2001), and the others, the IgG and IgM antibody testing (Ndip et al., 2004). The first case of coinfection between dengue and malaria was observed by a team from the virology department of the Centre Pasteur du Cameroon (Monamele & Demanou, 2018). Subsequently, other authors reported the same coinfection in the Far North and the South of Cameroon (Tchuandom et al., 2020; Galani et al., 2021). Findings from the present investigation revealed 17 (8.50%) of dengue cases with the NS1-antigen and IgM-IgG antibodies. These results are firm indications supporting the silent circulation of DENV in the city of Bangangté, as described by Fokam et al. (2010), who reported 2.50% of DENV-1 in febrile patients by inhibition of hemagglutination and complement fixation (Fokam et al., 2010). They further confirm our suspicion that other etiologies of febrile fever than *Plasmodium* affect patients in Bangangté, in connection with daily rates of negative malaria tests in local laboratories. Moreover, a very high prevalence of malaria compared to dengue was observed. In addition, most of the dengue-positive patients were coinfected with *Plasmodium*. There is no palpable evidence of the presence of *Aedes aegypty* which is the

dengue virus vector in Bangangté. Accordingly, the cases of dengue identified are likely imported. In fact, previous studies have shown that such conditions like a prolonged duration of stay in an epidemic area, and non-compliance with personal protective measures are factors associated with increased risk of contracting the disease (Krippner & von Laer, 2002).

The bivariate analysis revealed that there is no association between malaria and age (P-value = 0.194); and sex (P-value = 0.111), unlike dengue infection which is associated with age (P-value = 0.031). Furthermore, no association was observed between dengue infection and sex (P-value = 0.295). Previous studies have shown that several factors could favor overt symptoms of dengue fever and malaria in contaminated hosts. These risk factors include comorbidities, particularly diabetes and hypertension. Age, gender, nationality, socioeconomic status, viral serotype, and access to health care were also shown not significantly associated with disease severity (Fontenille & Toto, 2001; Abuamalh et al., 2020).

Additional features in data analysis also indicated that although fever was the most common symptom for each infection type, it did not appear as a predictive indicator, neither for malaria (p = 0.185) nor for dengue (p = 0.140). Headache was observed as a predictive symptom of malaria, with a high a level of significance (p = 0.003). This significance could be explained by the fact that many malaria cases generally experience headaches. Headache is clinically related to the sequestration of parasitized red blood cells within the cerebral capillaries. This sequestration associates with excessive production of pro-inflammatory cytokines and the thrombosis of micro-vessels responsible for the pain. Blurred vision was also statistically significant for dengue (p= 0.003). Unlike headache for malaria, this currently resist explanation. Although visual blur is a symptom of dengue, it does not appear as a specific, like fever, myalgia, arthralgia and headache. In a comparative study conducted on 416 patients on the clinico-biological differential criteria malaria-dengue in the endemic area of Cayenne in Guyana by Epelboin et al. (2012) rather reported skin rashes (p<0.001) (Epelboin et al., 2012). This finding could be justified by the fact that in the that study, cases of dengue hemorrhagic fever were observed, contrasting with data from the present investigation in which only cases of classical dengue fever were recorded. Tachycardia was also significantly associated with malaria (p=0.016), unlike dengue (p=0.084).

Conclusion

The overlooked dengue infection in Bangangté health facilities as observed in the present survey could be associated with the poor level of knowledge of healthcare personnel about dengue (<50%) that was highlighted, and not the nber of years on duty. This may represent a major indicator for the cross-transmission of dengue in Bangangté. Overall findings revealed a challenge that should be met in other ensure more accurate diagnosis based on biological indicators with laboratory tests. New outbreaks of infectious diseases are consistent with this view about broadening the range of laboratory tests in areas where malaria is endemic. That will motivate the putting in place of epidemiological surveillance systems to control and limit the spread or eradicate dengue and other arboviral diseases which share the same symptoms upon infection and the same mode of transmission.

COMPETING INTERESTS:

The authors of the present manuscript declare no competing interests.

AUTHORS' CONTRIBUTIONS

All authors made a substantial contribution to the design of the work, the acquisition, analysis and interpretation of data. They also drafted the manuscript and revised it critically for important intellectual content, and approved the version to be published.

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