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High Risk of Transfusion-Transmitted Malaria (TTM) from Student Blood Donors Living in the Town of Douala, Cameroon

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Abstract

Objective: Despite its contribution in managing and saving human lives, blood transfusion nonetheless can represent one obvious hazard in the transmission of many infectious diseases, among which malaria. This study aimed at determining the risk of transfusion-transmitted malaria (TTM) from student donors.

Methods: A cross-sectional study was carried out in January 2015 in students living in the town of Douala, Cameroon. One hundred and seventy nine (179) students aged between 18 and 32 years were included in the study and their blood tested for the presence of malaria parasites using thick blood films. A questionnaire form was administered to each participant for documenting socio demographical, clinical and malaria-related data.

Results: The prevalence of malaria infection among donors was 27.54%. Overall prevalence of the asymptomatic malaria was 10.17% which accounted for 47.36% of all cases of malaria infection. Malaria prevalence was higher in males compared to their female counterparts (29.85%), in those aged 21-25 years old (32.55%) and who were not using insecticide-treated bed nets (26.31%). Mean parasite density was the highest in males, 21-25 years old and bed nets users with 139 ± 346 parasites/µl, 132 ± 341 parasites/µl and 156 ± 476 parasites/µl respectively. None of the factors tested were found to be associated with an increased risk of malaria infection (p-value>0.05).

Conclusion: This study has highlighted a potential high risk of TTM from student donors. In many endemic areas malaria diagnosis is overlooked thus increasing the risk of TTM and constraining its appraisal. This study fills the gaps a little in field of blood transfusion safety in our setting and we expect it will be helpful to adequately define policies in order to undermine the misperceptions about TTM such as screening malaria parasite and selection of potential donors in blood banks prior to the transfusion.

Keywords: Malaria; Transfusion-Transmitted malaria; risk; asymptomatic carriage; diagnosis; blood donation; Douala

Introduction

Blood transfusion is a rapid and effective public health intervention used for persons with multi-factorial life-threatening anemia. Although contributing in managing and saving human lives, blood transfusion nonetheless can induce immunological adverse reaction and represent one obvious hazard for transmission of many infectious diseases, among which malaria [1-3]. According to the latest estimates, 214 million cases of malaria occurred globally in 2015 and the disease led to 438 000 deaths. The burden is heaviest in the WHO African Region, where an estimated 88% and 90% of all malaria cases and deaths occur respectively, and in children aged less than 5 years, who account for 78% of all deaths [4].

Transfusion-transmitted malaria (TTM) is a real public health problem. It can impair the health of recipients living in endemic malaria areas and even be fatal despite their relative semi-immunity [3]. Children and pregnant women represent the bulk of the recipients of the blood transfusion and therefore are the first-line victims of TTM-related deleterious and lethal effects.

Malaria is endemic in Cameroon with a prevalence rate of 29%. It is the major cause of morbidity and mortality among the most vulnerable groups: children under five (18%), pregnant women (5%), people living with HIV/AIDS (5.5%), and the poor (40%), the last of which constitutes two thirds of the total population estimated at 19 million [5]. Like in other endemic countries of Sub Saharan Africa (SSA), the demand for blood transfusion is increasingly important. This increases the risk of TTM in recipients especially children and pregnant women. Unfortunately, the diagnosis of malaria in donors is not done routinely in most countries in SSA and this is often missed [1,3,6]. This paradox may be explain by the dearth of epidemiological data on TTM as well as the emphasis on the diagnosis of others infectious diseases (HIV/AIDS infection, hepatitis B, C, D and G) in blood donors without regard for malaria believed as less dangerous. There is a real lack of data about TTM in Cameroon, especially in the town of Douala, since blood transfusion has become a commercial transaction as outlined by Uneke et al. [1]. The implications in terms of malaria-related morbidity and mortality are important since the children, pregnant women and probably immunocompromised people like HIV/AIDS patients

constitute the bulk of the blood recipients and are also the groups the most at risk of malaria [2,3]. Thus, this pilot study aimed at determining the risk of transfusion-transmitted malaria (TTM) from student donors.

Methodology

Study design

This was a prospective and cross sectional study carried out in January 2015 for three days at the Faculty of Science of the University of Douala in the town Douala, Littoral Region Cameroon. Douala is located on latitude 3°48'N and longitude 10°08'E, near the Atlantic coast 1 m above sea level and receives over 3,500 mm of rainfall annually. It is the main business city in Cameroon. It is located within the Congo-Guinean phytogeographical zone characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November [7].

Study population

Participants apparently healthy, having a history of blood donation and who signed an informed consent form for their participation were included in the study. Conversely, participants who did not meet any of these criteria were excluded from the study. Thus, a convenient sample of 179 students was obtained in the study. The age ranged from 18 to 32 years. A questionnaire form was administered to each participant to document socio demographical, clinical and malaria-related data.

Prior to parasitological examination, the aim and objectives of the study were explained in a language students could better understand (French or English), and their questions were answered. Furthermore, an informed consent was obtained from each participant.

Parasitological examination

In order to establish parasitaemia, blood was collected by finger prick. The middle left finger was pricked (unless a wound on that finger) and blood was deposited on a slide to perform a thick blood film. Slides were air-dried and then transported to the laboratory where they were fixed and stained with Giemsa according to the methodology described by Cheesbrough (2004) [8]. Briefly, thick smears that were air-dried for 30 minutes, was stained with 10% Giemsa for 20 minutes. Thereafter, stained slides were allowed to air dry and stored not more than one day until microscopic examination.

Microscopy was used for identification and quantification of malaria parasites by a senior. Thick blood films were considered positive when asexual forms (trophozoites and schizonts) and or gametocytes were present in the blood film. Slides were declared negative after observing at least 100 high power fields without detecting any parasites. Malaria parasites were counted against 200 leukocytes and expressed as number of parasites per µl of blood (parasite density) with the assumption that there was an average white blood count of 8000 leukocytes/µl of blood [9]. Parasitaemia was classified as low (<500 parasite/µl of blood), moderate (501–5000 parasites/µl of blood) and high (>5000 parasites/µl of blood) as described by Allen and colleagues [10].

In order to ensure quality assurance of parasitological data, thick smears-based results were classified as valid (positive or negative slides) and invalid (not read slides) as outlined in literature [11].

Asymptomatic malaria was defined as the presence of malaria parasite with an axillary temperature of <37.5°C. Symptomatic malaria was defined as the presence of malaria parasite with an axillary temperature of ≥ 37.5°C [4].

Statistical analyses

All data were keyed in an Excel sheet, checked for consistency and statistical analyses performed with SPSS software version 16 for windows (SPSS Inc., Chicago, IL, USA). Data were presented in a table as proportion with 95% confidence interval (95%CI) or mean ± Standard Deviation (SD) for qualitative and quantitative variables respectively. Goodness-of-fit chi-square test was used for inferential statistics for analyzing qualitative variables and one-way ANOVA (analysis of variances) was used to compare mean value between two groups or more. Logistic regression was used to identify factors associated with malaria infection. Statistical significance was set at $P<0.05$.

Results

Baseline data

During the study period a total of 179 donors consisted of 91 females (50.84%) and 88 males (49.16%) were enrolled in the study and a female/male sex ratio of 1.03 was recorded. Age of donors was ranging between 18 and 32 years and the mean age was 23 ± 3 years. Regarding the age groups, the majority of participants (64.25%) were aged between 21-25 years old (Table 1). Most of the participants (83.79%) reported to use antimalarial preventive method of which Insecticide-Treated Nets (ITNs) were mainly used (79.33%; 95%CI=77.7-88.48). A large number of participants were using one preventive method only (44.69%; 95%CI=72.16-85.04). The other baseline participants' data are presented and summarized in Table 1.

Variables	Categories	Frequency (%)	P-value
Gender	Females	91 (50,84)	0.823
	Males	88 (49.16)	
Age (years)	<21	24 (13.4)	<0.0001
	21-25	115 (64.25)	
	>25	40 (22.35)	
History of malaria episode	No	22 (12.29)	<0.0001
	Yes	157 (87.71)	
Antimalarial drugs intake*	No	88 (50.00)	1
	Yes	88 (50.00)	
Reported use of preventive method	No	29 (16.21)	<0.0001
	Yes	150 (83.79)	
Number of preventive methods	None	31 (17.32)	<0.0001
	One	80 (44.69)	
	Two	56 (31.28)	
	Three	10 (5.59)	

	Four	2 (1.12)	
Bed nets*	No	31 (20.67)	<0.0001
	Yes	119 (79.33)	
Insecticide residual spraying*	No	110 (73.33)	<0.0001
	Yes	40 (26.67)	
Coils*	No	132 (88.00)	<0.0001
	Yes	18 (12.00)	
Cleaning up*	No	97 (64.67)	<0.0001
	Yes	53 (35.33)	

Data are presented as frequency (percentage); * = total frequency is less than 179 because of missing data; Goodness-of-fit chi-square was used to compare proportions; p-value<0.05 are considered statistically significant

Table 1: Baseline data of participants.

Prevalence of Transmitted-Transfusion Malaria and associated factors

Forty one (41) slides were not read. Actually, microscopy of blood films showed 38 cases (27.54%; 95% CI = 15.88 - 27.79) of malaria infection. Malaria infection was higher in males (29.85%), students aged 21-25 years old (32.55%) and in donors not sleeping under ITNs (33.33%). However, no statistical significant difference was found (Table 2). The mean parasite density was 116 ± 329 parasites/µl of blood with extremes values of 0 and 2160 parasites per µl of blood. Furthermore, mean parasite density was highest in these three categories with 139/µl, 132/µl and 156/µl respectively as presented in the Table 3.

Variables	Categories	Infected (%)	OR (95%CI)	P-value
Gender	Females	18 (25.35)	1	0.3034
	Males	20 (29.85)	1.984 (0.538 - 7.315)	
Age (years)	<21	2 (11.11)	1	0.9736
	21-25	28 (32.18)	1043191.977 (NA)	
	>25	8 (23.53)	512929.397 (NA)	0.975
History of malaria episode	No	6 (35.29)	1	0.3694
	Yes	31 (25.62)	0.483 (0.099 - 2.365)	
Antimalarial drugs intake	No	20 (28.57)	1	0.6188
	Yes	5 (33.33)	1.415 (0.360 - 5.557)	
Reported use of bed net	No	7 (33.33)	1	0.9181
	Yes	25 (26.04)	1.076 (0.264 - 4.386)	
Armpit temperature	<37.5°C	18 (25.35)	1	0.221
	≥ 37.5°C	20 (30.77)	2.207 (0.621 - 7.845)	

OR=Odds ratio; %95CI=confidence interval; Logistic regression was used to compute OR; NA=Not available; p-value<0.05 are considered statistically significant

Table 2: Factors associated with malaria prevalence.

Variables	Categories	Mean parasite density ± SD (/µl)	P-value
All donors		116 ± 329	
Gender	Females	95 ± 314	0.4362
	Males	139 ± 346	
Age (years)	<21	118 ± 480	0.7029
	21-25	132 ± 341	
	>25	75 ± 172	
Reported use of bed net	No	156 ± 476	0.4427
	Yes	99 ± 255	

Data are presented as mean ± standard deviation (sd) for qualitative and quantitative values respectively. One way ANOVA tests were used to compare proportions and mea values respectively; p-value<0.05 are considered statistically significant.

Table 3: Highest mean parasite density.

Asymptomatic malaria

Asymptomatic malaria was defined as a temperature below 37.5°C associated with positive malaria testing in the absence of clinical signs presuming malaria. Overall prevalence of asymptomatic malaria was 10.17%. When considering samples made up of infected persons, asymptomatic malaria accounted for 47.36% of all cases of malaria.

Discussion

In this study, the prevalence of malaria in participants was 27.54%. This result is in line with malaria prevalence related data in donors in Sub-Saharan Africa that range from 0.6% to 50% [12]. This result is higher than that obtained by Koanga et al. in a study carried out in the same town [6]. These authors found a malaria prevalence of 12.82% in student donors from the University of Douala, Cameroon. Although slightly different by its design, this study also depicts a significant rate of transfusion-transmitted malaria. The participants were donors and apparently healthy. As a result, the risk for Transfusion-Transmitted Malaria (TTM) may be high in this urban setting since it is an endemic area of malaria. Malaria diagnosis prior to any blood transfusion is overlooked. There is a lack of interest of its diagnosis in blood banks and blood donation has become a commercial transaction [13]. According to Uneke et al. [1], this trend has become a dominant feature in the Sub-Saharan regions. The implications are important since the children, pregnant women and probably immunocompromised people like HIV/AIDS patients constitute the bulk of the blood recipients and are also the groups the most at risk of malaria [2,3,12-15]. Furthermore, non-immune immigrants from outside malaria regions also run a real risk of contracting malaria from blood transfusion [16,17]. When compared to others foreign studies, result of the present study is higher than [14] but similar to Okocha et

al. (2005), Agboola et al. (2010). These authors have reported 13%, 30.2% and 28% respectively [14,18,19].

The males were more infected than females (29.85%) as well as the participants aged 21-25 years old (32.55%) although both gender and age group did not significantly affect the risk of malaria infection ($p>0.05$). These findings are in line with others authors [1,3,6].

Globally, the parasite density was low (116 ± 329 parasites/µl of blood) in the participants. Atchade and colleagues found the same trend in 2,515 voluntary blood donors [2]. This may explain the fact of an effective immunity in the participants. Furthermore, it appeared that a low proportion (8.37%) of participants has recently taken an antimalarial drug. Thus, some parasite density would have been higher than observed. The implication of this result in diagnosis practice would be the increased difficulty to track positive slides in the health facilities as there is a link between the parasite density level and chance to find at least one parasite [20]. As a result, potential donors harboring malaria parasites may be missed.

The overall prevalence of asymptomatic malaria was 10.17%. This reported prevalence is similar to that obtained by Erhabor et al. (2007) and Owusu-Ofori et al. (2010) [21,22], but lower than that found by Uneke et al. (2006) and Atchade et al. (2013) [2,3]. When considering infected persons, asymptomatic malaria accounted for 47.36% of all cases of malaria. This result is not surprising since in malaria endemic areas the asymptomatic carriage is common [12]. These ones are preferentially selected for blood donation in health facilities, according to WHO Guidelines, as they do not present symptoms. Again, Giemsa standard method was used for malaria diagnosis in the study. Many others have pointed out limitations in medical practice although it is still considered as gold standard and widely used in health facilities [15,22]. Indeed, it is mainly impugned because it is time-consuming, microscopist-dependent and inadequate for examining a large volume of samples [15]. Thus, some asymptomatic carriers may be missed because of negative result testing. Asymptomatic malaria is an epidemiological situation in malariology that undermines any attempt to evaluate the real incidence of TTM. The fact that they can be missed by diagnosis testing complicates the ability to differentiate between post-transfusional malaria (that is acquired from transfused blood) and naturally acquired malaria (that is from mosquito bite) [12,15]. The genotyping methods are to date the only ones to solve this problematic situation.

Many other methods based on immunology and molecular biology, mainly rapid diagnostic tests (RDTs) and polymerase chain reaction (PCR) respectively, have been developed to overcome the abovementioned limitations of microscopy. Importantly, these methods have nonetheless limitations which jeopardize their clinical value for diagnosing TTM in blood banks [15]. The former do not offer a better sensitivity than microscopy and their sensitivity decreases as parasitaemia falls below 100 parasites/µl. In addition, results falsely positive are commonly observed as the parasite antigens detected can remain for many days to weeks in the bloodstream. So, these methods are insufficiently sensitive to screen adequately all donated blood and as a consequence, some malaria-infected donors can be missed. On the other hand, methods based on molecular biology are expensive, labor-intensive and require a long analysis time [15], restricting thereby their usage in research. Besides, rejection of donated blood would be higher with PCR methods as they are too sensitive and therefore could considerably jeopardize the blood supply as previously outlined [15]. Owusu-Ofori and colleagues have even argued that none of these methods commonly used for malaria diagnosis are practical, affordable and suitability sensitive to blood banks in Africa [12]. In this context, the possibility of systematic prophylaxis of recipients with antimalarial drugs could constitute an interesting alternative, as it prevents any deferral of donor and the occurrence of TTM. Further studies, especially in our setting, are needed to accurately define the best strategy to deal with the prevention of TTM.

Donors sleeping under Insecticide-treated Nets (ITNs) have showed a lower risk of malaria infection (26.31% *vs* 33.33%). Thus, ITNs would mitigate the risk of infection in people using them efficiently. ITNs are an effective tool for malaria control [23,24], when properly used [25]. In addition, many authors have shown that ITNs had greatly impacted on exposure to Anopheles transmission by reducing it significantly [26,27]. The ITNs-related protective effect by the fact they reduce the risk of mosquito bite by constraining physically and chemically the vector-human contact.

The present study had some limitations. Firstly, the sample size was small enough. Thus, puissance of statistical tests could have been insufficient to identify some risk factors associated with malaria infection. Secondly, we used thick blood films for diagnosing malaria infection. Some asymptomatic carriers of malaria parasites would have been missed in the study. However, this method is routinely used in blood banks of healthcare facilities in Cameroon. Thus, our methodology reflects what is done in standard clinical practice.

Conclusion

This study aimed at determining malaria prevalence in student blood donors and possible risk of Transfusion-transmitted malaria (TTM). This has highlighted a high potential risk of TTM from participants. In many endemic areas, malaria diagnosis is overlooked thus increasing the risk of TTM and constraining its appraisal. Thus, it is crucial to define adequate policies for screening malaria parasite and selection of potential donors in blood banks prior to transfusion. This requests the developing of others strategies or new diagnostic tools since there is no screening tools for malaria practical, affordable, and suitably sensitive for use in blood banks in Africa. Furthermore, this study fills the gaps a little in field of blood transfusion safety in our setting and we expect it will be helpful to adequately define policies in order to undermine the misperceptions about TTM-related burden in medical practice and ultimately avoid any risk of TTM to recipients mainly children and pregnant women.

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