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Impact of Plasmodial Parasitaemia on the Quality of Erythrocyte Concentrates Distributed at the Blood Transfusion Center of the Regional Hospital of Bafoussam-Cameroon

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Abstract

Background: Blood transfusion is a potential route for transmission of Plasmodium, which lives mainly in erythrocytes and can survive low temperatures. Therefore, this study was undertaken in order to determine the plasmodial parasitaemia in labile blood products for the evaluation of the quality of erythrocyte concentrates (EC) distributed at the Blood Transfusion Center of the Bafoussam Regional Hospital.

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Material and methods: This was an analytical cross-sectional study carried out between March 15 and May 31, 2021 on 101 EC. The data collection was done on one hand by using a questionnaire and the technical sheet for the evaluation of the quality of the EC compared to the Cameroonian reference and on the other hand, by the analysis of the blood taken from the donors. This analysis was made through the examinations of RDT malaria, Thick blood smear, Thin blood smear, measurement of the hemoglobin level thanks to the HemoCue and hemogram using the Urit 3000 automaton. Data analysis was done using Epi info and SPSS software.

Results: The prevalence of malaria among donors of the Bafoussam Regional Hospital Blood Bank was 4.41%. By considering the three parameters simultaneously, a compliance rate of 34.65% was obtained. A positive, although non-significant, correlation was established between plasmodial parasitaemia and EC quality.

Conclusion: The importance of quality control of ECs is essential, sofar as the parasitaemia of plasmodial is not negligible with one bag of erythrocyte concentrate out of three non-compliant.

Key words: Bafoussam blood transfusion center; erythrocyte concentrate; plasmodial parasitaemia; quality.

1. Introduction

Blood transfusion consists of administering blood or one of its components from one or more healthy subjects called donors to a sick subject called the recipient [1, 2]. It is reputed to be a life-saving activity, sofar as it saves lives and improves health, but like any other therapy it presents risks [3]. Transfusional malaria is a real public health problem, due to repeated blood transfusions, little or not controlled and where the donors are mostly potential carriers of haematozoa, which can be inoculated by blood transfusion [2, 4, 5]. In Cameroon, malaria remains the major endemic and the leading cause of morbidity and mortality in the most vulnerable groups, namely children under five and pregnant women. Health statistics reveal that it is responsible for 35 to 40% of all deaths in health facilities, including 50% of morbidity in children under 5 [6]. Variations in climatic conditions, such as temperature, rainfall patterns and humidity, have a significant effect on the transmission of the disease, in a country like Cameroon [7]. The transfusion risk of malaria seems to be essentially linked to administration of whole blood or erythrocyte concentrate (EC), since Plasmodium, in fact, lives mainly in erythrocytes [8–11]. The erythrocyte concentrate is used to ensure the transport of oxygen and correct anemia [12]. To this, it must meet the quality standards required by the guide to blood transfusion characteristics for Cameroon set up two years ago [13]. The transfusion risk mainly involves an asymptomatic carrier donor of a haemoparasite which may be in the incubation phase like Plasmodium. [9, 14–16]. Recent data show an increase in the frequency of parasitic transfusion transmissions [17]. Quality control within a blood transfusion establishment implements means and methods of control to rule on the conformity of a product or a process. The Quality Control Manager's report should be based on the results as accurately as possible. The monitoring of blood products by quality control attests to their compliance with regulatory requirements and the quality objectives of the National Center for Blood Transfusion (CNTS) [18].Therefore, the act of transfusion must be beneficial to recipients of Labile Blood Products (LBP) by providing them with products with a higher level of quality and safety [3]. It is therefore important to question the quality of the erythrocyte concentrates produced at the Blood Transfusion Center of the Bafoussam Regional Hospital; hence the objective of this work, which

was to assess the impact of plasmodial parasitaemia in labile blood products and more particularly in erythrocyte concentrates.

2. Material and method

Type of study and recruitment sites

This was an analytical cross-sectional study that took place from March 09, 2021 to May 31, 2021 at the Blood Transfusion Center of the Bafoussam Regional Hospital (HRB).

Study population and sample size

The study population consisted of blood donors from the HRB blood bank as well as CE bags prepared there. Sampling was random, consecutive and non-exhaustive. Regarding recruitment, it was done consecutively, and the size was defined for convenience. To this, out of 297 donors registered during the study, we only retained 204, but 101 bags were retained.

Course of the study

All eligible donors following medical selection and having consented to participate in the study were chosen. Everyone was informed of the objectives and activities of the study through informed consent. After obtaining their informed consents, the questionnaire was submitted to them before the blood sample was taken. A dry tube was used to collect approximately 3 mL of post- blood donation tests through the blood bag tubing for Malaria RDT, Thick blood smear, and thin Blood Smear testing. Subsequently, the blood bags, which were of the double or triple type, were sent for the preparation of the EC. These bags were centrifuged using program 2 (5000 rpm for 15 min). Then, the extraction of the plasma to the corresponding satellite pocket was carried out using a manual press, which made it possible to obtain the EC. 3ml of EC was collected in a dry tube after been well homogenized manually in order to carry out the hemogram. The bags were then sealed using an electric sealer before being weighed using a previously tared scale.

Ethical considerations

For this study, we received a research certificate from the Dean of the Faculty of Medicine and Pharmaceutical Sciences of the University of Dschang; the administrative authorization of the Director of the Bafoussam Regional Hospital Blood Bank. The management and computerized processing of the data were carried out in strict confidentiality and anonymity kept in complete safety without primary analysis.

3. Data analysis

Statistical analyses were performed using chi-square, Fisher and Pearson tests using Epi Info 7.2.2.6 and SPSS 26 software. The Excel 2016 spreadsheet was used to enter the data and represent the results obtained. These data were expressed in the form of numbers and frequencies.

4. Results

During the study period, out of the 297 registered donation candidates, 204 were asked for this study because of the concordance with the medical selection criteria from the information sheet and the non-responsiveness to the pre-donation tests. They all responded favorably to participate in the study, which made it possible to have 204 bags of whole blood, corresponding to the first sampling. Subsequently, of the 204 bags of whole blood, 108 bags (either double or single) followed the fractionation process in order to obtain various labile blood products including packed red blood cells (RBC), which constituted the second sampling. Of the 108 RGCs prepared, 101 validated by biological qualification were evaluated in this study.

During this work, all the RDT-malaria were negative. On the other hand, 9 donors were positive after thick blood smear, for a plasmodial prevalence in the recruited population of 4.41%. This prevalence corresponds to the rate of infected labile blood products. The species that was identified during this study is Plasmodium falciparum with a parasite load of between 150 and 400 parasites/µL of blood.

The association between age, sex, type of donor, use of MILDA and asymptomatic carriage of Plasmodium sp. among donors is presented in Table 1. This table shows that there was no association between age, gender and type of donor ($p>0.05$). On the other hand, the use of MILDA was a protective factor against plasmodial infection ($p<0.05$).

Table 1: Risk factors associated with plasmodial infection in blood donors at the Bafoussam Regional Hospital.

Variables	**Modality**	**Infected donor** n (%)	**P**	**OR**	**IC95%**
Age	**[**18-35[	6(66.67)	0.7	1.33	0.32-5.54
	≥35	3(33.33)			
Sex	Male	8(88.89)	1	1.63	0.19-13.55
	Female	1(11.11)			
Type of donor	Volunteer	1(11.11)			
	Family	7(77.78)	0.37	ND	ND
	Renumbered	1(11.11)			
Use of MILDA	No	8(88.89)	<0.001	18,89	2.31-154.53
	Yes	1(11.11)			

P : P-value ; **OR :** Odd ratio **; IC95% :** Confidence interval at 95% ; **ND** : Not determined; n : total number

Pre-donation donors had an average hemoglobin level of 14.3 g/dl; the average volume of red blood cell concentrates was 257.98 mL; the average hematocrit was 55.16% and the average amount of hemoglobin was 44.45g.

The EC compliance rate according to Cameroonian standards is presented in Table2. It was 91.09% for the volumes, 38.61% for the hematocrit and 63.37% for the amount of hemoglobin. The compliance rate of packed red blood cells for all three parameters was 34.65%.

Table 2: Conformity of red blood cell concentrates according to Cameroonian standards.

Variables (unit)	Conformity	Total number (percentage)
Volume (mL)	Improper (< 180)	1 (0.99)
	Improper(>330)	8 (7.92)
	Complies with Cameroonian standards(180-330)	92 (91.09)
Hematocrit (%)	Improper(<60)	59 (58.41)
	Improper(>80)	3 (2.98)
	Complies with Cameroonian standards(60-80)	39 (38.61)
Hemoglobin quantity(g)	Improper (<40)	37 (36.63)
	Complies with Cameroonian standards (≥40)	64 (63.37)

The correlation between plasmodial parasitaemia and the quality of erythrocyte concentrates is presented in Table 3. This shows a positive and non-significant correlation between plasmodial parasitaemia and the quality of packed red blood cells (r^2=0.02 and P=0.2). It is noted that all parasitized pockets were non-compliant.

Table 3: Correlation between plasmodial parasitaemia and the quality of erythrocyte concentrates.

Variables	**Compliance of red blood cell concentrates**	
Parasitemia	Improper: n (%)	**Compliant :n (%)**
Unparasitized pockets	63 (64.29)	35 (35.71)
Parasitized pockets	3 (100)	0 (0)
Total	66(65.35)	35 (34.65)

P= 0,2 et **r^2= 0,02**

5. Discussion

This study established a plasmodial prevalence of 4.41%. This prevalence is significantly four times higher than that (1.5%) found in a study conducted on asymptomatic Plasmodium carriage among blood donors in the southern region of Madagascar in 2019[18]. Furthermore, Kwenti and his colleagues (2016) [19] in a similar study conducted in Yaoundé in Cameroon, found a prevalence of 8.1%; on the other hand, NDO and his colleagues (2016)[2] found an infection rate of 11.78% among donors at the Douala-Cameroon General Hospital blood bank. These differences in prevalence in the three cities (Bafoussam, Yaoundé and Douala) could be due to different climatic and demographic conditions, urbanization and waste management. Furthermore, Murphy and his colleagues (2018) [20] in Uganda had established a prevalence of 15.4% among blood donors, while Bassandja and his colleagues (2013) [16] had in turn established a prevalence of 28.3% among blood donors in Kisanganie in the DRC. These rates could be explained by the fact that, according to the WHO, these two

countries are respectively the third and second countries in the world who are the most affected by malaria. Other authors have reported much higher prevalences, notably Okocha and his colleagues (2005) [10] as well as Epidi *and his colleagues* (2008) [21], in Nigeria, with respective prevalences of 55% and 51.5%. The levels of infection vary on one hand according to the level of endemicity of malaria in each country, and on the other hand because of the rainy season, where the larval forms are numerous, resulting in high prevalence of malaria [22]. Moreover, in the present study, this prevalence was relatively low, probably because the study was conducted at the end of the dry season, the climate of which does not favour the survival of the malaria vector. Candidates for donation with a positive RDT were automatically eliminated in pre-donation. In addition, the operation of massive and free distribution of mosquito nets by the Cameroonian government since 2010 would have contributed to reducing the prevalence of asymptomatic carriers of Plasmodium in this study. The results of all these studies sufficiently indicate that the plasmodial risk for African blood transfusion exists, which implies the taking of effective preventive measures.

The parasite species identified during this work was Plasmodium falciparum, responsible for the most serious forms of malaria. Adusei and his colleagues (2017) [23] had identified this same species in Ghana. Muphy and his colleagues (2018) also identified this species as the main donor species. Similarly, Bassandja and his colleagues (2013) [24] had noted this species in the DRC, and confirmed that it was the most dangerous species, and also the most widespread in sub-Saharan Africa. This result in the face of these various studies could be justified by the fact that the WHO assigns Plasmodium falciparum the responsibility for 99.7% of the estimated cases of malaria in the African regions.

The results obtained during this work did not make it possible to establish a link between age, sex, type of donation and carriage of Plasmodium sp. in donors. The same was true for Kwenti and his colleagues (2019) [19], as well as for Bassandja and his colleagues (2013) [24]. Furthermore, blood donors who declared that they did not use the MILDA presented a higher risk of asymptomatic Plasmodium carriage than the others. These results agree with those of Lengeler and his colleagues (2018) [25] who had demonstrated that despite the increase in insecticide resistance in malaria vector populations across the world, evidence of the effectiveness of MILDA in reducing disease and malaria-related deaths remained solid.

The compliance rate of red blood cell concentrates for the volumes recorded in this study was 91.09%. Which is far from the result of Mbanya and his colleagues (2007)[26] who found a compliance rate for CE volumes of 57% during a similar study in a hospital in Yaoundé, Cameroon. The result obtained during this work could be due to the non-complete elimination of the plasma during the preparation of the EC. On the other hand, Eiman (2014) [27] and his team had found a compliance rate for CE volumes of 100%, this could be due to the fact that they had used apheresis as a method of collecting blood bags. unlike the manual method that was used in this study. Indeed, apheresis techniques are much more sophisticated sofar as they allow the collection of one or more blood components by extracorporeal blood circulation using a cell separator, the components not collected are reinjected into the donor or patient [28].

Hematocrit compliance was relatively low in this study. In Cameroon, Mbanya and his colleagues (2007)[26] had obtained 95% conforming units with respect to the hematocrit. Moreover, in Egypt Eiman and his

colleagues (2014)[27] had in turn reported 70% compliant units with respect to the hematocrit. These differences would be due to the reference values considered by the different teams. Indeed, they have established compliance rates on the basis of the European standard; although the Cameroonian reference system had been put on drawing inspiration from this one, the two nevertheless present significant differences. The non-compliance of RBCCs for hematocrit in this study could also be due to insufficient plasma extraction during their preparation. It would be necessary to review the process of preparing these units in order to extract an adequate volume of plasma without impacting either the final volume of the RBCC or the recommended hematocrit.

The hemoglobin quantity compliance rate recorded in this study was 63.37%. This is similar to that of Mbanya and his team in Cameroon in 2007 [26] who also found that 66% of RBCC bags were compliant for their hemoglobin content. It has been reported that low donor hemoglobin is often the cause of low hemoglobin in the prepared RBCC [29]. But the hemoglobin level of blood donors before the collection would not explain this amount of hemoglobin since all the donors had a hemoglobin level greater than or equal to 12 g/dl. Some authors such as Nevalainen and Lloyd [30] as well as Engelfreit and his colleagues [31] reported that hematocrit, white blood cell count and hemoglobin level of African subjects were different with age from European averages [32] , hence the recommendation made by Mbanya and his colleagues (2007) [26] for standardization of red blood cell concentrates adapted to physiological specificities and local realities. Moreover, the result obtained during this study is far (83.2%) from that reported by Nebie and his colleagues (2014) in Burkina Faso. The percentage of compliant pockets (34.65%) concerning the three parameters (volume, quantity of hemoglobin and hematocrit) was relatively low. Hezouwe and his colleagues (2019) [33] had found in Togo a compliance rate close to that established in this study (42.16%), this could be explained by the use of an analysis methodology similar to that implemented In this study. On the other hand, Yao and his colleagues (2014) in Côte d'Ivoire had established a largely satisfactory compliance rate (93.85%), their result was the result of a three-year experience of setting up a product quality control laboratory. labile blood [34]. Saloni and his colleagues (2016) in India had also argued that periodic quality control of blood products is essential to verify the adequacy and safety of these products and is part of good transfusion practice [35]. The correlation between the presence of parasites and the overall quality of packed red blood cells was positive and not significant. This result would probably be due to the modification of the amount of hemoglobin by the parasite. Indeed, erythrocytes infected with the malaria parasite develop alterations in their membrane, thus modifying their hemoglobin content [36, 37]. On the other hand, the penetration of merozoites into the red blood cells and their development induce in them typical modifications of their size, their tinctorial affinity and their shape. By developing inside red blood cells, the parasite finds itself in an unhospitable environment. These anucleated cells, devoid of intracellular organelles, do not have endocytosis machinery. It is then that the survival of Plasmodium requires the establishment of membrane compartments in order, on one hand, to import nutrients from the external environment and on the other hand, to export numerous proteins to the surface. of the red blood cell, thus modifying its physiological state [38].

6. Conclusion

From this study, it appears that the prevalence of malaria in donors was 4.41%, with a parasite load of between

150 and 400 parasites / µl of blood, the dominant species was Plasmodium falciparum; the overall EC compliance rate was 34.65%, with volume compliance of 91.09%, hematocrit of 38.61% and hemoglobin amount of 63.37% and that parasitaemia plasmodiale would have reduced the quality of red cell concentrates, especially with regard to the quantity of hemoglobin, which was revealed through an average rate (34.65%) of erythrocyte concentrates compared to the limit quality level which is 70% . Therefore, only one EC bag distributed out of three can be considered to be of high quality. It is therefore urgent to set up a more elaborate system for the quality assurance of labile blood products, and erythrocyte concentrates in particular, in order to reduce to the maximum the residual risk of malaria, but above all to obtain red blood cell concentrates of the required quality.

7. Conflict of interest

Authors declare no conflict of interest.

8. Source of funding

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