SL Clinical Medicine: Research

SCIENTIFIC LITERATURE

Research Article

Detection of Sickle Cell Trait and Dengue Virus Serological Markers in Blood Bags: A Challenge in the Management of Persons Affected by Sickle Cell Disease

Salomon Bonsi Tchuandom[1,2], Abel Lissom[3,4], Thibau Flaurant Tchouangueu[5], Cecile Flavienne Elomo[6], Jean Claude Djontu[7] and Constantin Tchakounte[8]

[1]Blood Bank, Central hospital of Yaoundé, Cameroon

[2]Public School of Medical Laboratory Technicians, Cameroon

[3]Department of Biological Science, University of Bamenda, Cameroon

[4]Fondation Congolaise pour la Recherche Médicale, Rep of Congo

[5]Department of Microbiology, Haematology and Immunology, University of Dschang, Cameroon

[6]Blood Bank, Jamot hospital of Yaoundé, Cameroon

[7]The Biotechnology Center, University of Yaoundé I, Cameroon

[8]Department of Biochemistry, University of Dschang, Cameroon

ARTICLE INFO

Received Date: August 29, 2022
Accepted Date: September 14, 2022
Published Date: September 15, 2022

KEYWORDS

Sickle cell; Dengue virus; Transfusion

Citation for this article: Salomon Bonsi Tchuandom, Abel Lissom, Thibau Flaurant Tchouangueu, Cecile Flavienne Elomo, Jean Claude Djontu and Constantin Tchakounte. Detection of Sickle Cell Trait and Dengue Virus Serological Markers in Blood Bags: A Challenge in the Management of Persons Affected by Sickle Cell Disease. SL Clinical Medicine: Research. 2022; 5(1):126

Corresponding author:
Abel Lissom,
Department of Biological Science,
University of Bamenda, Cameroon,
Email: lissomabel@yahoo.fr

ABSTRACT

Background: Blood transfusions still remains the cornerstone in the management of sickle cell disease in African countries, hence the management of blood bags remains a public health concern. We aimed to determine the prevalence of the sickle cell trait and dengue virus serological markers in blood bags intended for transfusion to sickle cell patients.

Methods: A cross-sectional survey was conducted at the Yaoundé Jamot hospital using 963 blood bags intended for transfusion to sickle cell patients. Blood samples were screened for the sickle cell trait by microscopy and using the Capillarys Haemoglobin kit (Sebia®). Serological markers of dengue virus were determined using Tell me fast® Combo NS1-IgM/IgG Rapid test and confirmed by an in-house DENV-IgG/IgM-ELISA.

Results: From the 963 selected blood bags, 7.6% had AS haemoglobin genotype. Though the family donors were the predominant (69.5%) study population, they were significantly ($p< 0.0001$) less positive (4.0%) to AS haemoglobin genotype as compared with voluntary donors (17.7%) and blood bag replacement donors (9.5%). Out of 24.7% positive cases with Dengue virus markers, 15.2%, 6.7% and 2.9% were positive with IgM, IgG and dual IgM/IgG, respectively. The rate of detecting blood bags with both Dengue virus markers and sickle cell trait was of 2.3% in this study.

Conclusion: These findings highlight a likely risk of transfusing blood with both sickle cell trait and dengue virus markers to hospitalized sickle cell patients, necessitating a review of the blood management system that cares for the sickle cell patients.

KEY MESSAGES

- What is already known on this topic? Children with sickle cell disease are often hospitalized for a long period and blood transfusion is an important option in their management.

- What this study adds? The findings of this study highlight a likely risk of transfusing blood with both sickle cell traits and dengue virus markers to hospitalized sickle cell disease patients.
- How this study might affect research, practice or policy: this calls for reviewing the blood bank management system for the care of sickle cell patients in Cameroon and Africa.

INTRODUCTION

Sickle cell disease is an ubiquitous hemoglobinopathy that predominantly affects subjects in sub-Saharan Africa [1,2]. It is characterized by the presence of abnormal Haemoglobin S (HbS) in the red blood cells, which alters the structure of the erythrocytes, resulting in reduced oxygen levels, blocked veins and organ damage. Affected individuals are homozygous carriers that have inherited an abnormal haemoglobin beta gene from each parent [1,2]. Individuals with sickle cell trait are heterozygous carriers of one normal allele and one abnormal allele encoding HbS. In Sub-Saharan Africa, and more particularly in Cameroon, for the past three decades only 12.5% couples underwent premarital screening [3,4]. The increase in the prevalence of sickle cell disease remains the major consequence [3]. Recent data show that sickle cell disease affects about 6000 newborn annually in Cameroon and 2-3% of the general population [5-7]. Blood transfusions can serve as symptomatic treatment for sickle cell disease [8,9]. In addition to the serious socio-economic issues in the affected families, the management of sickle cell disease in African countries faces safety issues in health care delivery [9,10] such as management of blood bags transfusion with sickle cell traits [11] or serological markers of dengue virus (DENV) [12].

Studies on the screening of sickle cell traits in blood donors in Cameroon are scarce although approximately 22.5% of the general Cameroonian population is a sickle cell trait carrier [2,13]. The majority of sickle cell trait carriers are asymptomatic and could present themselves to donate blood in transfusion centres. Transfusion of blood with the sickle cell trait will result in a decrease in oxygen supply in the recipient and consequently increased acidosis. Hypoxia and acidosis in sickle cell blood recipients maintain and aggravate thrombosis and haemolysis and promote the production of inflammatory mediators, increasing the risk of vaso-occlusive crises [2,14]. Increased levels of inflammation mediators may be more observed in individuals with DENV-specific antibodies prior to a new bite by a mosquito infested with a different serotype than the one responsible for the production of previous antibodies [12,15] and would be responsible for the risk of dengue severity in sickle cell children. This could deteriorate the clinical condition or induce vaso-occlusive crisis [1,2,7]. Our previous work showed that 24.8% of blood donors eligible for transfusion carry serological markers of DENV [12]. DENV infection is generally a mild and asymptomatic arboviruses disease in 80% of cases [16]. Children with sickle cell disease are often hospitalized for a long period and blood transfusion is an important option in their management [10]. Transfusion of blood carrying sickle cell traits and/or serological markers for DENV could exacerbate the clinical state of children with sickle cell disease [2,10,12] and their exclusion from blood transfusions present a real challenge in the management of patients with sickle cell disease. Currently, little is known about the sero-epidemiological situation of sickle cell traits and serological markers for dengue virus in blood units relevant for the management of sickle cell disease. The aim of the present study was to determine the prevalence of sickle cell traits and DENV serological markers in blood bags prior to blood transfusion in order to improve the management of sickle cell patients.

MATERIAL AND METHODS

Study design

This cross-sectional survey was carried out from January 2020 to February 2021 at the Yaoundé Jamot Hospital. The random blood sample collection included blood bags from eligible blood donors aged between 18-57 years who donated blood at the blood bank of the Yaoundé Jamot Hospital for sickle cell recipients after cross-match tests. Blood bags that were not compatible with sickle cell recipients were excluded from the study. After signing a consent form, sociodemographic and clinical data (sex, age, quarter, school level etc.) were recorded from participants. Blood samples were collected in EDTA tubes. The sickle cell traits were screened using microscopic analysis, followed by alkaline agarose gel electrophoresis using Capillarys Hemoglobin (e) kit (Sebia®) for the confirmation of the positive results. Serological markers of DENV in plasma samples were determined using Tell me fast®

Combo NS1-IgM/IgG Rapid test and confirmed by an in-house ELISA test for dengue specific IgM/IgG antibodies. All data were analysed with respect to diagnostic results of sickle cell traits, DENV biomarkers and the sociodemographic parameters of the participants.

Ethics approval and consent to participate

Ethical clearance for this study was obtained from the Centre Regional Ethics Committee for Human Health Research (N°3352/AP/MINSANTE/DRSPC/CRERSH). All participants provided a written informed consent. Data were processed using specific identifiers for privacy and confidentiality purposes. Individual diagnostic results of dengue and sickle cell trait or disease generated during the course of this study were provided free of charge to the respective participants.

Sample collection and processing

Samples were collected at the blood bank. A volume of 5 ml of blood was collected in EDTA tubes from the blood bags of eligible blood donors. A proportion of whole blood was used for sickle cell trait test. The remaining blood sample was centrifuged at 4000 rpm for 5 min, the plasma was harvested and aliquoted in single-use volumes. One aliquot of plasma of each sample was directly used for DENV biomarker Rapid Diagnostic Test (RDT), and the other aliquots were stored at -20°C for the confirmation of anti-DENV antibodies by ELISA.

Screening of DENV serological markers

Each plasma sample was analysed for the detection of NS1 antigen, DENV-specific IgG and IgM antibodies using a Tell me fast® Combo Dengue NS1-IgG/IgM Rapid Test (Biocan Diagnostics Inc. Canada). The interpretation of the RDT results was done in conformity with the manufacturer's instructions. IgM and IgG antibodies against DENV were confirmed using an in-house indirect ELISA assay as we have previously described [17].

Screening sickle cell trait (AS)

Sickling test (Sodium metabisulphite method): Equal volumes of EDTA anticoagulated blood and 2 % sodium metabisulphite ($Na_2S_2O_5$) were mixed on a cleaned labelled slide, covered with a cover slip and incubated at room temperature for 30 min. For the detection of the crescent-shaped sickle cells, the slides were analyzed by microscopy using the 40X objective for sickle cells detection [11]. The blood samples of individuals with known sickle cell trait and without the sickle cell trait were used as positive and negative controls respectively, throughout the test. Agarose gel electrophoresis was performed on samples with sickled red blood cells.

Hemoglobin electrophoresis (agarose gel method at alkaline pH): Haemoglobin genotyping was done according to the recommendation of the hydragel 7 hemoglobin kit (Sebia®). Briefly, the red blood cells were beforehand washed 3 times by adding 10 volumes of physiological solution (NaCl 0.9 g / dl) to 1 volume of the pellet, and by centrifuging the mix at 4000 rpm for 5 min. The agarose gel (ready to use) was soaked in the buffer solution, and the filter paper was used to adsorb the buffer from the hemolysate deposition area, while preventing the agarose gel from drying out. The washed blood (40 µl) was diluted in 100 µl of hemolysis solution (pH 6.1), then incubated for 5 min at room temperature. A volume of 10 µL hemolysate (samples and controls) were introduced into the slots of the deposition area and incubated for 5 minutes at room temperature. The liquid not absorbed by the agarose gel was removed using the filter paper. The gel was placed in the electrophoresis tank filled with buffer (pH 9.2 ± 0.5 ready to use). After running the gel at 340 V (50 mA) for 15 min, it was stained with amidoblack for 5 min. The gel was blotted and allowed to air dry, after which it was labelled and the results were read (Capillarys Hemoglobin -Sebia®) and compared to controls. A combination of hemolysate from a sickle cell trait (AS) and HbF trait samples (AFS) served as a control.

Statistical analysis

Data were processed and statistically analyzed using Graphpad Prism software (version 8). Sickle cell trait and serological markers of DENV rates were calculated by considering sickle cell trait (AS) and the presence of IgM and /or IgG antibodies respectively. Chi2 and Fisher Exact tests were used to determine the associations between the different independent variables. Statistical significance was confirmed when $p < 0.05$.

RESULTS

Socio-demographic profile of the study population

In total, 963 donors (875 men and 88 women) were recruited in this study (Table 1). The age varied from 18 to 57 years, with similar age means (±SD) of 29.93 ± 7.83 years and 29.85 ± 7.60 years for men and women respectively. Family donors were the most represented (69.5%) study population.

While a very minor proportion (0.52%) had primary education, up to 57.11% of participants undertook higher education.

Table 1: Demographic characteristics of donors.

	men (n=875)	women (n=88)	Total (n=963)
Age mean (± SD)[a]	29.93 ± 7.83	29.85 ± 7.60	
Age ranges % (n)			
18-25	32.69 (286)	34.09 (30)	32.81 (316)
26-33	35.51 (337)	43.18 (38)	38.94 (375)
34-41	19.09 (167)	18.18 (16)	19.00 (183)
42-49	8.00 (70)	4.55 (4)	7.68 (74)
50-57	1.71 (15)	0 (0)	1.56 (15)
Types of Donors % (n)			
Voluntary	21.94 (192)	31.86 (28)	22.85 (220)
Family	69.83 (611)	65.91 (58)	69.47 (669)
Replacement	8.23 (72)	2.27 (2)	7.68 (74)
Types of activity % (n)			
Hawker	30.06 (263)	26.14 (23)	29.70 (286)
Non-motile	69.94 (612)	73.86 (65)	70.30 (677)
Educational Level % (n)			
Primary	0.57 (5)	0 (0)	0.52 (5)
Secondary	44.49 (389)	21.59 (19)	42.37 (408)
High school	54.97 (481)	78.41 (69)	57.11 (550)

[a]:the ages were expressed in mean ± Standard Deviation (SD); For each characteristic the proportions (%) were compared between men and women; n: number of donors

The detection of sickle cell traits in blood bag is associated with the type of donor

The samples from the blood bag of donors were screened for the sickle cell traits and analyzed with regard to the gender and the type of donor. A participant was declared positive to a sickle cell trait (AS) when the migration of his sample was at the same line with both Hb-A and Hb-S control (Figure1). As shown in Table 2, blood bags from 7.6% (73/963) donors were detected with sickle cell trait. The proportion of men donors with sickle cell trait was apparently greater (8.1%) than their women donors counterpart (2.3%), although the difference was not significant (p= 0.0545). With respect to the type of donor, sickle cell traits were mostly represented in voluntary donors (17.7%), followed by blood bags replacement donors (9.5%), and then the family donors (4.0%). There was a significant association between the detection of sickle cell traits and the type of blood donor (p<0.0001), with a higher risk of detecting the sickle cell traits in the volunteer blood donors (OR: 5.1 (3.1 – 8.6); p< 0.0001) and those who came for blood replacement (OR: 2.5 (1.04 - 5.9); p= 0.0400).

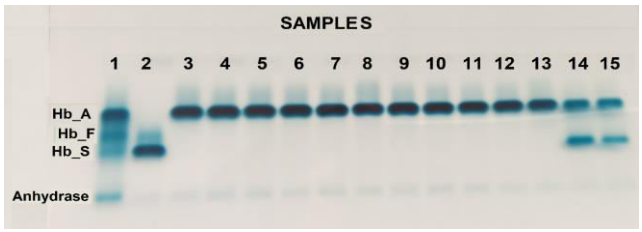

Figure 1: Agarose haemoglobin electrophoresis from samples with or without sickling cell trait in transfusion blood bags.

Genotype AA was found in 11 samples (lane 3 to 13), and genotype AS in 2 samples (lane 14 and 15). Controls for genotype AFS (lane 1) and genotype SS (lane 2) were include.

Table 2: Distribution of sickling status in transfused blood bags according to gender and types of donors.

	Sickling status (Haemoglobin genotype)		P-value	Univariate logistic regression	
	Negative (AA)	Positive (AS)		OR (95% CI)	p-value
Gender % (n)					
Women (n= 88)	97.7 (86)	2.3 (2)	0.0545	Ref.	-
Men (n= 875)	91.9 (804)	8.1 (71)		3.8 (0.9 – 15.8)	0.066
Types of Donors % (n)					
Voluntary (n= 220)	82.3 (181)	17.7 (39)	<0.0001	5.1 (3.1 – 8.6)	< 0.0001
Family (n= 669)	96.0 (642)	4.0 (27)		Ref.	-
Replacement (n= 74)	90.5 (67)	9.5 (7)		2.9 (0.4 – 22.2)	0.0400

The distribution of the genotype proportions (%) was compared with respect to gender and the type of donors.

Detection of dual DENV serological markers and sickle cells trait in blood bags of donors

Three serological DENV markers (NS1, IgM and IgG) were investigated, and the results illustrated in Table 3 shows their distribution according to hemoglobin genotypes. Out of the whole study population, 24.7% (238) participants were diagnosed positive for DENV markers. Among the blood bags positive to the serological marker of DENV, 15.16% (146/963), 6.65% (64/963) and 2.91% (28/963) were IgM,

IgG and dual IgM/IgG positive, respectively. None of the blood bags were positive for NS1 antigen. The proportion of blood donors with both DENV biomarkers and sickle cell trait was 2.3% compared to 5.3% with sickle cell trait only.

Table 3: Distribution of dual sickling status and dengue serological markers among transfused blood bags.

	Sickling status (Haemoglobin genotype)		Statistical test (p-value)	Univariate logistic regression	
	Negative (AA)	Positive (AS)		OR (95% CI)	p-value
Dengue screening results % (n)					
Positive	22.4 (216)	2.3 (22)	0.2614	1.3 (0.8 – 2.3)	0.265
Negative	70.0 (674)	5.3 (51)		Ref.	-
Dengue Biomarkers detected % (n)					
IgM	55.5 (132)	5.9 (14)	0.5185	2.9 (0.4 – 22.2)	0.3193
IgG	24.0 (57)	2.9 (7)		3.3 (0.4 – 28.3)	0.273
IgM + IgG	11.3 (27)	0.4 (1)		Ref.	-

n: number of donors. (%): percentage.

DISCUSSION

The aim of this study was to determine the prevalence of sickle cell trait and serological markers of DENV in blood bags of eligible donors at the Yaoundé Jamot Hospital. This, the survey was conducted in the blood bags intended for sickle cell patients after various compatibility tests. Out of 963 blood bags collected in this study, 90.86% came from men donors compared to 9.14% from women donors. These results show that, though men population are demographically similar to women population [18], they are generally predominant blood donors in the health centres [11,12]. This observation may be due to certain criteria that disqualify women during to the blood donation such as pregnancy, the menstruation, low weight, as well as the socio-cultural behaviours [19]. The ages of donors ranged from 18 to 57 with averages of 29.93 ± 7.83 and 29.85 ± 7.60 years for men and women respectively; suggesting that, younger individuals might be the predominant candidates for blood donation in Cameroon, as previously reported in a study carried out in Ghana [11]. Similarly to some studies conducted in Douala and Yaoundé [12,20], results from this work showed that family donors (69.47%) was also the predominant population.

The hemoglobin genotype screening revealed an overall prevalence of sickle cell trait in blood donors of 7.6%. This finding suggests the probability of having sickle cell traits in blood intended for sickle cell patients. These results are lower compared to those found in Ghana (11.3%) [11], probably because of the fact that the blood bags included in this study were those intended only for sickle cell patients. The disparity of sickle cell trait prevalence observed in men (8.1%) compared to women (2.3%) might be linked mainly to the low number of women included in the study. Moreover, no relationship has been established elsewhere between distribution of this hereditary disease and the gender [21]. There was a significant association between the type of donor and sickle cell trait cases ($p<0.0001$). The sickle cell traits were highly detected among voluntary donors, followed by blood bag replacement donors, and the family donors. One of the main advantages during blood donation in Cameroon is the free routine diagnostic of infectious diseases such as HIV/AIDS, Hepatitis virus infections, syphilis and sometimes malaria [9,22]. Hemoglobin genotyping is not among routine screenings for blood donation, but generally done in particular conditions such prenuptial events, pregnancy, or in cases with a family history of sickle cell trait or disease. The knowledge of hemoglobin genotype by each family member may have resulted in a very selective blood donation within the family itself, in order to reduce the risk of disease complication in the patient.

In contrast to our previous finding reporting 20.32% of positive cases with DENV antibodies in blood donors [12], this study showed an increased seroprevalence (24.71%) of DENV antibodies in blood bags. This elevation of the positive cases to DENV serological markers could be explained by an increased exposure to the bites of infected Aedes spp mosquitoes due to lock down related to COVID-19 situation. It is well reported that Aedes spp mosquitoes have a diurnal bite activity increased with human sedentary lifestyle, resulting in an increased susceptibility to DENV infection [23-25]. The transfusion of blood bags to sickle cell patients with anti-DENV antibodies or DENV may lead to a severe course of dengue disease [1].

Both the sickle cell trait and anti-DENV antibodies were detected in 2.3% of blood bags intended to be transfused in sickle cell patients in this study. This study demonstrated for the

first time, the simultaneous presence of sickle cell trait and anti-DENV antibodies in blood bags intended for sickle cell patients. The fact of having these two clinical factors in donor's blood bags is an alarm bell in the management of the blood banks and the care of sickle cell patients, especially children who represent the most vulnerable population. Moreover, the presence of Aedes mosquito larvae in the hospital could be responsible for nosocomial dengue, therefore drastically damage health of sickle cell patients [26,27]. The fact that the post-transfusion effects of blood bags positive to sickle cell trait or anti-DENV antibodies were not followed up in sickle cell patients, in the context of nosocomial dengue, is however the limitation of this study.

CONCLUSION

This study showed that there is an increased risk of detecting both sickle cell trait and serological markers of DENV in blood bags intended to the transfusion in the sickle cell patients who are in their critical phase of the disease in Cameroon. This findings should sound an alarm bell and prompt review of the blood bank management strategies as well as the management of sickle cell patients, mainly children who represent the most vulnerable population.

COMPETING INTERESTS

The authors declare that they have no competing interests

FUNDING STATEMENT

This study received a personnel founding by the authors. No author financial support was received by a funding agency.

ACKNOWLEDGEMENTS

We are grateful to the blood donors for their participation in this study and the administration of the Jamot Hospital for their strong and fruitful collaboration.

REFERENCES

1. Wilder-Smith A, Leong WY. (2019). Risk of severe dengue is higher in patients with sickle cell disease: a scoping review. J Travel Med. 26.
2. Ochocinski D, Dalal M, Black LV, Carr S, Lew J, et al. (2020). Life-Threatening Infectious Complications in Sickle Cell Disease: A Concise Narrative Review. Front Pediatr. 8: 38.
3. Ngwengi NY, Fon PN, Mbanya D. (2020). Distribution of haemoglobin genotypes, knowledge, attitude and practices towards sickle cell disease among unmarried youths in the Buea Health District, Cameroon. Pan Afr Med J. 37: 109.
4. Clark S, Koski A, Smith-Greenaway E. (2017). Recent Trends in Premarital Fertility across Sub-Saharan Africa. Stud Fam Plann. 48: 3-22.
5. Ngo Um SS, Seungue J, Alima AY, Mbono R, Mbassi H, et al. (2019). A cross sectional study of growth of children with sickle cell disease, aged 2 to 5 years in Yaounde, Cameroon. Pan Afr Med J. 34: 85.
6. Alima Yanda AN, Nansseu JR, Mbassi Awa HD, Tatah SA, Seungue J, et al. (2017). Burden and spectrum of bacterial infections among sickle cell disease children living in Cameroon. BMC Infect Dis. 17: 211.
7. Andong AM, Ngouadjeu EDT, Bekolo CE, et al. Verla SV, Nebongo D, (2017). Chronic complications and quality of life of patients living with sickle cell disease and receiving care in three hospitals in Cameroon: a cross-sectional study. BMC Hematol. 17: 7.
8. Ngo Sack F, Eboumbou C. (2013). Prevalence of HIV Seropositivity Among Sickle Cell Disease Patients at the Yaoundé Central Hospital. Health Sciences and Disease. 14.
9. Samje M, Fondoh VN, Nguefack-Tsague G, Kamalieuk KJL, Mbanya D, et al. (2021). Trends in serological markers of transfusion transmissible infections in blood donations at the Bamenda Hospital-based Blood Service, Cameroon. Transfus Clin Biol. 28: 228-233.
10. Wiencek JR, Booth GS. (2017). Sickle Cell Trait in the Blood Supply. J Appl Lab Med. 1: 773-775.
11. Antwi-Baffour S, Asare RO, Adjei JK, Kyeremeh R, Nana D. (2015). Prevalence of hemoglobin S trait among blood donors: a cross-sectional study. BMC Res Notes. 8: 583.
12. Tchuandom SB, Lissom A, Ateba GHM, Tchouangueu TF, Tchakounte C, et al. (2020). Dengue virus serological markers among potential blood donors: an evidence of asymptomatic dengue virus transmission in Cameroon. Pan Afr Med J. 36: 185.
13. Ama Moor VJ, Pieme CA, Chetcha Chemegne B, Manonji H, Nono NJinkio BL, et al. (2016). Oxidative profile of sickle cell patients in a Cameroonian urban hospital. BMC Clin Pathol. 16: 15.

14. Guarda CC, Silveira-Mattos PSM, Yahouedehou S, Santiago RP, Aleluia MM , et al. (2019). Hydroxyurea alters circulating monocyte subsets and dampens its inflammatory potential in sickle cell anemia patients. Sci Rep. 9: 14829.
15. Tchuandom SB, Tchadji JC, Tchouangueu TF, Biloa MZ, Atabinkeng EP, et al. (2019). A cross-sectional study of acute dengue infection in paediatric clinics in Cameroon. BMC Public Health. 19: 958.
16. WHO. (2022). Dengue and severe dengue. Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO. World Health Organization.
17. Tchuandom SB, Tchouangueu TF, Antonio-Nkondjio C, Lissom A, Ngono Djang JO, et al. (2018). Seroprevalence of dengue virus among children presenting with febrile illness in some public health facilities in Cameroon. Pan Afr Med J. 31: 177.
18. Cameroon population. (2021). live.
19. Asamoah-Akuoko L, Hassall OW, Bates I, Ullum H. (2017). Blood donors' perceptions, motivators and deterrents in Sub-Saharan Africa - a scoping review of evidence. Br J Haematol. 177: 864-877.
20. Cécile OE, Dongho EN, Texier G, Nda Mefoo J-P, Etapelong GS, et al. (2017). Residual risk of transfusion-transmitted malaria infection in a malaria endemic sub-Saharan African setting. Translational Medicine Communications. 2.
21. Ceglie G, Di Mauro M, Tarissi De Jacobis I, de Gennaro F, Quaranta M , et al. (2019). Gender-Related Differences in Sickle Cell Disease in a Pediatric Cohort: A Single-Center Retrospective Study. Front Mol Biosci. 6: 140.
22. Ankouane F, Noah Noah D, Atangana MM, Simo RK, Guekam PR, et al. (2016). [Seroprevalence of hepatitis B and C viruses, HIV-1/2 and syphilis among blood donors in the Yaounde Central Hospital in the centre region of Cameroon]. Transfus Clin Biol. 23: 72-77.
23. Adams B, Kapan DD. (2009). Man bites mosquito: understanding the contribution of human movement to vector-borne disease dynamics. PLoS One. 4: e6763.
24. Duong V, Lambrechts L, Paul RE, Ly S, Lay RS, et al. (2015). Asymptomatic humans transmit dengue virus to mosquitoes. Proc Natl Acad Sci USA. 112: 14688-14693.
25. Fustec B, Phanitchat T, Aromseree S, Pientong C, Thaewnongiew K, et al. (2021). Serological biomarker for assessing human exposure to Aedes mosquito bites during a randomized vector control intervention trial in northeastern Thailand. PLoS Negl Trop Dis. 15: e0009440.
26. Wiwanitkit V. (2016). Aedes Mosquito Larva in the Hospital: A Note From Thailand. Infect Control Hosp Epidemiol. 37: 1519.
27. Ehelepola NDB, Wijesinghe W. (2018). An Analysis of a Dengue Outbreak at a Large Hospital and Epidemiological Evidence for Nosocomial Dengue. J Trop Med. 2018: 9579086.