Prevalence of Hepatitis B Surface Antigen and Hepatitis C Antibodies in Sickle Cell Disease Children under Sixteen in Two University Hospitals of Lome

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Authors’ contributions

This work was carried out in collaboration among all authors. Author SD was the project owner. Author AC was data collector for Campus. Author KT was data collector for Sylvanus Olympio. Author MK was helped in writing protocol. Author VK was lab technician. Author TA was lab supervisor. Author KMG was patients’ doctor at Sylvanus Olympio. Author ADG was head of Sylvanus Olympio’s paediatric unit. Author AYS was head of Campus’ haematology unit. Author AYD was project supervisor. Author PB was financial support. All authors read and approved the final manuscript.

ABSTRACT

Introduction: Sickle cell disease causes chronic anemia with the need for transfusions. The risk in children to get transfusions transmitted infections is high.

Aims: Determine the prevalence of HBsAg and HCV antibodies in sickle cell disease children under sixteen in Lome (Togo).

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Study Design: It is a cross-sectional study.

Place and Duration of Study: Sample: Haematology Unit of Campus University Hospital and Paediatric Unit of Sylvanus Olympio University Hospital of Lomé collected between February to May 2016. Sample processing: Campus Hospital Laboratory.

Methodology: We collected blood in sickle cell disease patients in Campus and Sylvanus Olympio university hospitals at Lomé and informations about sickle cell type, transfusion, and hepatitis B vaccination. Sera were tested with Cobas e411 Roche® in the determining of hepatitis B surface antigen (HbSAg) and hepatitis C antibodies (HCVAb). Epi Info was used for statistical analysis. Significant associations were found when \( P < 0.05 \).

Results: Total of 172 patients from Campus Hospital and 79 from Sylvanus Olympio were included. Sex ratio and SS phenotype were 0.93, 69.8% and 1.32, 64.5% respectively. HBsAg was detected in 1.7% from Campus and 7.6% in Sylvanus Olympio. One patient from Campus carried HCVAb. Significant association between hepatitis B and sex (\( P = 0.02 \)) and hepatitis B and vaccination were found (\( P = 0.0003 \)). Males were more infected and patients who were unvaccinated carried HBsAg.

Conclusion: Vaccination against viral hepatitis and best blood donation screening are necessary to avoid these viral diseases in sickle cell disease children.

Keywords: Children; HBs antigen; HCV antibodies; sickle cell disease; Togo.

1. INTRODUCTION

Sickle cell disease, one of haemoglobinopathies, is the most common monogenic disorders worldwide. It due to haemoglobin abnormality. It is very widespread in sub-Saharan Africa, India, Saudi Arabia and Mediterranean’s countries [1].

Haemoglobin the principal constituent of red cells has two chains \( \alpha \) and \( \beta \). In sickle cell disease, the Sixth amino-acid; glutamic acid is replaced by valine in \( \alpha \) chain. The obtained haemoglobin named Haemoglobin S, agglomerates in the red blood cells. They are crescent shaped and do not live as long as normal red blood cells. Patients present then with chronic anaemia.

In treating the anaemia, patients are habitually transfused to maintain their haemoglobin level at 10 to 11 g/dl using haemoglobin AA blood [2]. Blood transfusions are associated with a risk of transmission of Hepatitis virus A, B, C, D and E, Human Immunodeficiency Virus (HIV), Cytomegalovirus (CMV), Epstein- Baar Virus (EBV), parvovirus B19, Human Herpes Virus type 6, Human T lymphotropic [3].

Beside that, sickle cell disease patients are very vulnerable to infections. More than 90% of children with sickle cell disease die before diagnosis can be made and those who survive, die from infections in Kenya such as bacteraemia [4].

Transfusion blood is made secure by isolating the donor’s blood that has positive markers of these infections. In the past, only syphilis was serologically screened in blood donors. But as the transfusion transmitted infections became a public concern most of bloods for transfusions are screened before giving blood to patients. Then many studies related seroprevalence in blood donors. In Madagascar, it was 0.47% for HIV, 3.21% for HBV, 0.98% for HCV and 1.18% for syphilis. In Morocco, prevalence for HCV was decreasing, 0.91% in 2000 to 0.5% in 2010. In Nigeria in 2012, the seroprevalence of HIV, HBV, HCV and syphilis in blood donors was respectively 2.9%, 1.6%, 0.2% and 0.2% [5]. In Togo, in 2012, seroprevalence of HCV was 15.2% of 158 donors, 5.7% for HIV and 9.5% for HBV [6].

Sickle cell disease was responsible of 7.2% of children’s death in Gabon at 1992. The first cause of death is due to anaemia in children under 5 years of age [7]. Sickle cell disease children need transfusions earlier in their first life and have more risk to get transfusions transmitted infections. The problem lies in the absence of screening for HCV in blood donors in Togo. In 2005, the prevalence of HCV, HBV was 6.5% and 20.2% respectively in sickle cell disease patients [8].

One of the complications of sickle cell disease is acute liver failure. In patients who have been co-infected by hepatitis virus, the prognosis is very poor [9]. In a concern of the high prevalence which was described in 2005 and the risk in children, we decided to screen Hepatitis B and C in sickle cell patients who are aged less than 16 years.
2. PATIENTS AND METHODS

This is a cross-sectional study carried out from 8 February 2016 to 6 May 2016 (3 months) at two university hospitals: Campus and Sylvanus Olympio at Lomé in Togo. The patients were brought to the Clinical Haematology department of Campus and to Paediatric unit of Sylvanus Olympio. Blood-samples had been taken and brought to be processed at the laboratory of Campus in the Immmuno-serology unit.

2.1 Patients

2.1.1 Inclusions

In the study, we included all sickle cell disease patients aged 16 or less, who are regularly shown or new admitted to the two hospitals.

2.1.2 Exclusions

Patients whose sickle-cell phenotype is not yet known, who had other haemoglobinopahies and who refused to do this study are excluded.

2.2 Medical Informations

Before sample-collection, parents’ consent was obtained and they answered a survey about name, surname, date of birth; sickle-cell phenotype, vaccination against hepatitis B and about transfusions.

2.3 Laboratory Analysis

Blood samples were collected in EDTA tubes. We took 2 ml of blood from infants (0-1 year) and 5 ml from the older-children (1-16 years). Centrifugation was done and HBs Antigen (HBsAg) for hepatitis B and HCV antibodies (HCVAb) for hepatitis C were screened. That has been done by Electrochemiluminescency method with Cobas e411 ® Roche Diagnostics. It is an immunological test for the qualitative detection of HBsAg and HCV-Ab with both sensitivity of 100%, and a specificity of 99.88%, 99.94% respectively.

2.4 Data Analysis

Each data was saved on Excel® and Epi-Info ©software. Statistical association between hepatitis B, with specific variables (age, transfusion, vaccination, type of sickle cell disease) was determined. For the statistical tests, since the hospitals are located in the same city, the analysis was made by combining the results of the two hospitals. Chi-square test was done, significant association was found when \( P < 0.05 \).

3. RESULTS

3.1 Demographical Statements

During the study, a total of 251 blood samples were collected; 172 in Campus and 79 at Sylvanus Olympio.

Campus Hospital

At this Hospital, 83 males and 89 females were included and the sex ratio (M/F) was 0.93 (Table 1). The mean age was 12 and the lowest was 1 and the oldest was 16. All patients had sickle cell disease but for some it was associated with another haemoglobinopathy. Their repartition was 69.8% SS (n=120), 28% SC (n=48), 1.1% SF (n=2) and 1.1% of SAFA2 (n=2). 53.5% (n=92) of patients have been transfused at least once. All are not hospitalized and have been vaccinated.

Sylvanus Olympio

Consent was obtained in 79 patients. We had 45 males and 34 females, sex ratio (M/F) was 1.32. Patients’ age was 1 to 16 and the mean age was 4.5. Phenotype SS was most at 64.5% (n=51). The remaining are SC (19%; n=15), SF (12.7%; n=10) and Sβthal (3.8%; n=3). 43% (n=34) was hospitalized in Sylvanus Olympio. 55.7% of patients have been transfused. Only 14% (n=11) are vaccinated.

3.2 HBsAg and HCV Antibodies [Tables 1,2]

During the study, a total of 3.6% patients were HBsAg positive (n=6 of Campus hospital and n=3 of Sylvanus Olympio). Sickle cell phenotype in positive patients are SS (n=5), SF (n=2), SC (n=1) and Sβthal (n=1). All the patients with HBsAg are male and aged 3-14. Association was found between sex and Hepatitis B (p=0.03) and between vaccination and hepatitis B (p=0.0004). For the screening of antibodies against HCV, only one positive patient (0.39%) was found. Serological screening did not show HBV-HCV coinfestion.
Table 1. Percentage of positive antigen HBs (AgHBs) and HCV antibodies (HCV Ab)

|                      | Sylvanus Olympio (N=79) | Campus (N=172) |
|----------------------|-------------------------|----------------|
|                      | Number | Percentage | Number | Percentage |
| AgHBs                | 6      | 7.6%       | 3      | 1.7%       |
| HCV Ab               | 0      | -          | 1      | 0.58%      |

N: Number of all patients

Table 2. Comparison between the different variables and the presence of HBsAg

| Variables                  | Number | AgHBs positif (Percentage) | P      |
|---------------------------|--------|---------------------------|--------|
| Age (years)               |        |                           |        |
| ≤ 3                       | 40     | 2 (5%)                    | 0.6    |
| 3 - 16                    | 211    | 7 (3.31%)                 |        |
| Sex                       |        |                           |        |
| Masculin                  | 129    | 9 (7%)                    | 0.03   |
| Feminin                   | 122    |                           |        |
| Vaccination               |        |                           |        |
| Yes                       | 183    | 2 (1.63%)                 | 0.0004 |
| No                        | 68     | 7 (10.29%)                |        |
| Transfusion               |        |                           |        |
| Yes                       | 137    | 4 (2.91%)                 | 0.54   |
| No                        | 115    | 5 (4.34%)                 |        |
| Sickle Cell Phenotype     |        |                           |        |
| Homozygote                | 171    | 5 (3%)                    | 0.4    |
| Heterozygote              | 80     | 4 (5%)                    |        |

4. DISCUSSION

The two hospitals are reference centers for sickle cell diseases patients; All phenotypes were represented in both of two hospitals; in paediatric unit, we had SF phenotype for 12.7% and in Campus, we have the persistence of HbF for 1.1%.

Transfusion was related being the origin of some infections with sickle cell disease patients. Mba et al. reported in 2017, the prevalence of HIV, HCV and syphilis in blood donors in Gabon which were 3.4%, 1.2% and 3.2% respectively [10]. Although screening was done to blood before giving them to patients, it still was a risk. In fact, donors who are in the incubation phase when the markers are undetectable by the techniques used pass through screening. Generally the diagnostic method used by national transfusion centers in limited resources countries are serological. In Libreville, Bangui, Lomé, [6,10,11]. Authors related that the risk is increased with multiples transfusions. Sack and al. showed a statistically significant difference for the presence of HCVAb in sickle cell patients having received more than 10 transfusions compared to those having received less than 10 transfusions [12]. In Nigeria, a study showed that the mean number of transfusion was higher among patients who were sero-positive for both HbsAg (5.0 +/- 6.6) and HCVAb (4.6 +/- 6.7) when compared to patients who were negative for both viruses (2.7 +/- 3.0 and 2.9 +/- 3.2) for HBsAg and HCVAb respectively [13]. Gody and al found that more than three transfusions are significantly associated with hepatitis B contamination in 2014 [11]. These risks being high, it therefore becomes necessary to have to develop a more effective and relevant selection process to blood donation in African francophone countries [13]. In our study, we didn’t have a relation between transfusions and hepatitis B (P= 0.54). Maybe it due to the young age of our patients. Indeed, with age and lot of crisis they will receive more transfusions.

Sickle cell disease patients were vulnerable to infections as bacteraemia, meningititis, pneumopathy and osteomyelitis [14]. That is why vaccinations were highly recommended to them. Mok and al. in their study, suggested that low dose intradermal hepatitis B vaccination made antibodies titre stable up 10 UI/l in patients with β thalassaemia and sickle cell disease [15]. During our study, we found a significant difference in patients carrying HBsAg who were unvaccinated compared to those who were vaccinated (p=0.0004). Vaccination against hepatitis B early in the life, is the best prophylaxis as recommended by WHO [16]. In fact, in 2005 Segbena, et al. found at the CHU campus a prevalence of 24.5% in children aged 0-15 years. We can notice that from 2005 to 2016, there was a decrease in the prevalence (p = 0.0005). This decrease could be explained by a vaccination campaign against hepatitis B in sickle cell disease children from 2006 in our country.

Viral hepatitis has been long recognized as the most common infection transmitted by blood. Incidence of HBV and HCV are high in sickle cell disease patients and responsible of comorbidities [17,18]. At Bangui, 14.3% of sickle cell children were infected by HBV and 6.48% at Yaoundé; also in that place, 16.67% were positive for HCV antibodies [11,12]. Compare to our study, those
percentages were higher. Indeed, we found for HBV prevalence, 7.6% at Sylvanus Olympio and 1.7% at Campus. The low percentage was probably due to the age of our patients and to the fact that they were mostly vaccinated. It was supposed that for patients less than ten years, their contamination as the fact that we didn’t find a relation between transfusions and hepatitis was probably due to family contact. We didn’t do in our study a family screening of viral hepatitis. During our study males were more infected than females; an association was found between hepatitis B and sex (P= 0.03). That association was found in a survey in northern Uganda where females were more infected than males [19]. A previous study in Lomé reported that men were more infected than women [20].

In our study, only one person was HCVAb positive at Campus (0.53%). That percentage was lower than others authors who found a relation between transfusions and positive HCV antibodies [15,21]. A former study in our country showed two (2) positives while screening HCV antibodies in 57 children less than fifteen in 2005 [8]. Although few prevalence, that is the way to make a plea to screen, hepatitis C in blood donations.

5. CONCLUSION
This study allowed us to realize that the carriage of HBsAg in sickle cell disease young population is higher than that of HCV antibodies. However, the best alternative to avoid a high prevalence of these diseases in this vulnerable population is the better immunization coverage associated with screening for viral markers of hepatitis B and C on all blood donations.

DISCLAIMER
The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT
Before sample-collection, parents’ consent was obtained and they answered a survey about name, surname, date of birth; sickle-cell phenotype, vaccination against hepatitis B and about transfusions.

ETHICAL APPROVAL
This study had an agreement on national bioethics committee. All results were given to doctors for patients’ treatment.

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COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES
1. OMS. OMS] Drépanocytose et autres hémoglobinopathies [Internet]. WHO. [cité 10 août 2017]. Available: http://www.who.int/mediacentre/factsheets/fs308/fr/
2. Adewoyin AS, Obieche JC. Hypertransfusion therapy in sickle cell disease in Nigeria. Adv Hematol. 2014;2014:923593.
3. Yap PL. Transfusion transmitted viral infections-recent developments in blood donor screening. Postgrad Med J. Nov 1990;66(781):906-9.
4. Williams TN, Uyoga S, Macharia A, Ndila C, McAuley CF, Opi DH, et al. Bacteraemia in Kenyan children with sickle-cell anaemia: A retrospective cohort and case–control study. Lancet. 2009;374(9698):1364-70.
5. Santi L, Montanari G, Berardi S, Patti C, Frigerio M, Sama C, et al. Liver Cirrhosis in a patient with sickle cell Trait (Hb Sβ+ Thalassemia) without Other Known Causes of Hepatic Disease. Case Rep Gastroenterol. 2009;3(3):275-9.
6. Dagnra AY, Prince-David M, Agbenu S, Ouro-Akpo T, Hounkpati F. Prevalence and risk of transmission of HCV after screening for HIV and HBV in blood donors. Med Mal Infect. 2002;315-9. French
7. Koko J, Dufilott D, M’Ba-Meyo J, Gahouma D, Kani F. Mortality of sickle cell children in a pediatric ward in Central Africa. Archives of Pediatrics. 5th Ed. 1998:965–9. French
8. Segbena A-Y, Prince-David M, Samdapawinde Kagoné T, Dagnra AY. Human immunodeficiency virus and viral hepatitis C and B virus in sickle cell patients at CHU Campus in Lomé (Togo). Clinical and Biological Transfusion. 12th Ed. 2005:423-6. French
9. Ballas SK, Kesen MR, Goldberg MF, Lutty GA, Dampier C, Osunkwo I, et al. Beyond the definitions of the phenotypic complications of Sickle Cell Disease: An Update on Management. Scientific World Journal [Internet] : 2012. [cited 11 feb 2016]. Available:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3415156/
10. Jean Marie Eko MBA, Mireille Jophrette Ntsame NDONG, Cyrille Biseye. Socio-demographic characteristics associated with the risk of transmission of HIV, HCV and Treponema pallidum by first-blood donors in Libreville (Gabon): Three-year dynamics from 2009 to 2015. Int J Biol Chem Sci. 11th Ed. 2017;350-9. French
11. Gody JC, Essomo Megnier-Mbo CM, Chelo D, Guindo A, Gabato W, Bureau JJ, et al. HIV and HBV in a cohort of sickle cell children transfused at the Bangui Pediatric Complex. Health Sci Dis. 2014;15 (2):1-5. French.
12. Sack FN, Noah DN, Zouhaïratou H, Mbanya D. Carrying of the HBs antigen and anti-HCV antibodies in homozygous sickle cell anemia at the Yaoundé Central Hospital.. Pan Afr Med J [Internet]; 2013. [cité 11 juillet 2017]:14. Available:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3612872/French
13. Fasola FA, Odaibo GN, Aken’Ova YA, Olaleye OD. Hepatitis B and C viral markers in patients with sickle cell disease in Ibadan, Nigeria. Afr J Med Med Sci. 2003;32(3):293-5.
14. Tayou CT, Kouao MD, Touré H, Gargour J, Fazul AS, Ouattara S, et al. Transfusion safety in francophone African countries: An analysis of strategies for the medical selection of blood donors. Transfusion. 2012;52(1):134-43.
15. Bégué P, Castello-Herbreteau B. Serious infections in sickle cell children: Clinical aspects and prevention. Arch Pediatrics. 2001:8:732-41. French.
16. Mok Q, Underhill G, Wonke B, Aldouri M, Kelsey M, Jefferies D. Intradermal hepatitis B vaccine in thalassaemia and sickle cell disease. Arch Dis Child. Avr. 1989;64(4): 535–40.
17. World Health Organisation. WHO | Weekly Epidemiological Record, [Internet]. WHO. 2017;92(27):369–392. [cited 3rd oct 2017]. Available:http://www.who.int/wer/2017/wer 9227/en/
18. Nouraie M, Nekhai S, Gordeuk VR. Sickle cell disease is associated with decreased HIV but higher HBV and HCV comorbidities in US hospital discharge records: a cross-sectional study. Sex Transm Infect. 2012;88(7):528–33.
19. Ochola E, Ocama P, Orach CG, Nankinga ZK, Kalyango JN, McFarland W, et al. High burden of hepatitis B infection in Northern Uganda: Results of a population-based survey. BMC Public Health. 7 août. 2013; 13:727.
20. Kolou M, Katawa G, Salou M, Gozo-Akakpo KS, Dossim S, Kwarteng A, et al. High Prevalence of Hepatitis B Virus Infection in the Age Range of 20-39 Years Old Individuals in Lome. Open Virol J. 2017;11:1-7.
21. Hassan M, Hasan S, Giday S, Alamgir L, Banks A, Frederick W, et al. Hepatitis C virus in sickle cell disease. J Natl Med Assoc. 2003;95(10):939-42.