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Preliminary Study on Occult Hepatitis B in Sickle Cell Anemia

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Abstract

Introduction: The transmission of occult hepatitis B Virus (HBV) is a significant public health issue among transfused sickle cell disease (SCD) patients. **Object**: The aim of this study was to determine the prevalence of occult hepatitis B in SCD patients and to establish the association between HBsAg and anti HBc, according to the number of blood transfusions received. **Patients and Methods**: This epidemiological, descriptive and analytical study was conducted in three sickle cell care centers in Kinshasa from January to June 2018, involving 384 transfused SCD patients. **Results**: The study revealed that the age group 10 - 20 years was the most represented with a predominance of males (male-female sex ratio of 1.07). 65 patients of the study population tested positive for anti-HBc but negative for HbsAg and had occult hepatitis, representing 19.0 % of the total **Conclusion**: Occult hepatitis B is a particular form of HBV infection that is increasingly attracting the attention of clinicians in the SCD patients.

Subject Areas

Hematology, Infectious Diseases, Public Health

Keywords

Occult Hepatitis B, Sickle Cell Disease, Transfusion, Elisa, Determine

1. Introduction

Sickle cell anemia, a major global genetic disease, particularly impacts Africa, where it affects around 300,000 births annually and contributes significantly to childhood mortality [1] [2].

Like most countries in sub-Saharan Africa, the Democratic Republic of Congo (DRC) is one of the countries severely affected by sickle cell disease, with a prevalence of heterozygous and homozygous forms, estimated respectively at 25% and 2% [3] [4].

This high rate of death is the consequence of several complications, some linked to the disease itself, but others due to comorbidities and therapy. Blood usage is frequent during transfusions as well as transfusion exchanges. The latter are also of vital interest in certain acute and chronic situations [5].

Of all the complications of infectious origin, those due to the hepatitis B virus are the most frequent post-transfusion infections, with the risk of development of occult hepatitis. Thirty years ago, several studies demonstrated that HBsAg-negative and Anti-HBc-positive blood donors were capable of transmitting HBV [6] [7].

The Center for Mixed Medicine and SS anemia SS Mixed (CMMASS), a pivotal site in our study, consults 250 to 300 sickle cell patients monthly and administers approximately 250 transfusions per month [8]. This increases the risk of HBV transmission and the development of occult hepatitis B through blood transfusions. Research indicates that donors positive for Anti-HBc antibodies have a positivity rate ranging from 0% to 15% [9]. Additionally, an international Survey in 2008 estimated the prevalence of occult HBV infection among blood donors at 8.55% per million donations [9]. The safety of blood for HBV, including occult B infection, has improved significantly, but the possibility of transmitting occult B infection remains [10].

The aim of this study was to determine the prevalence of occult hepatitis B in SCD patients and to establish the association between HBsAg and anti HBc, according to the number of blood transfusion received

2. Patients and Methods

- **Setting, type and period of study**

This was an epidemiological, descriptive and analytical study, which concerned the period from January to June 2018. It took place in the City Province of Kinshasa, more precisely in three health facilities specialized in the care sickle cell disease in particular: the Center for Mixed Medicine and SS anemia (CMMASS), the Kingasani Hospital Center (CHK) and the Lisungi Health Center located in Binza Ozone.

- **Population study**

Homozygous sickle cell patients who consulted the 3 health facilities mentioned above.

- **Inclusion criteria**

Any homozygous sickle cell patient (having a document attesting to his status),

having benefited from at least one blood transfusion, who presented himself during the study period, and who gave his informed consent for the adult patient or whose parents have given informed consent for the minor patient.

➢ **Non-inclusion criteria**

Any homozygous sickle cell patient who has not received a transfusion, or whose informed consent has not been obtained, was not included in this study.

➢ **Sample size**

The sample size was calculated using the following formula:

$$n = \frac{z^2 \times p \times (1-p)}{d^2}$$

- n: required sample size;
- z: the 95% confidence coefficient (typical value of 1.96);
- p: was estimated at 50% by default, given that the prevalence of occult hepatitis among sickle cell patients in our environment is not known;
- d: represented the degree of precision of 5% (typical value 0.05%).

$$n = \frac{(1.96)^2 \times 0.5 \times (1-0.5)}{(0.05)^2} \geq 384$$

The minimum number of sickle cell patients required to carry out the study was 384.

➢ **Collection of data**

The collection of sociodemographic, epidemiological, and clinical information was carried out by completing pre-established information sheets at the Specialized Centers for the Management of Sickle Cell Disease selected.

➢ **Laboratory analyses**

Five milliliters of blood were drawn from each patient under complete aseptic conditions into heparinized tubes. The samples obtained were directly placed in an insulated container with cold accumulators and sent day to the Cremer laboratory (Faculty of medicine at the University of Kinshasa).

- First step: Involved the centrifugation of the samples using the Hettich EBA 20 centrifuge at 2600 revolutions per minute for 6 minutes.
- Second step: The results of the analyzes were recorded directly in a register.
- Third step: The search for two serological markers of HBV by Elisa and Determine.

➢ **Statistical analyzes**

Statistical analysis in this study involved processing quantitative variables (such as age and number of blood transfusions) using measures like mean ± standard deviation, mode, minimum, maximum, and quantiles. Qualitative variables (HBsAg status, anti-HBc Ab status, occult hepatitis B status, sex, age groups, and number of blood transfusions) were summarized with counts (n) and percentages (%). Frequencies were reported with 95% confidence intervals (95% CI).

Comparisons between quantitative variables were conducted using the Student

t-test, while the Chi-square test was employed to test equality between proportions. Associations among qualitative variables, including the frequency of occult hepatitis B in sickle cell patients and other study parameters, were assessed using the Chi-square test and Fisher's exact test.

Statistical significance was determined at the 0.05 level ($p < 0.05$). Results were visually represented through graphs and tables. Data analysis was performed using IBM SPSS Statistics 20 software for Windows.

➢ **Ethical considerations**

Ethical considerations, including those relating to the confidentiality of results, were scrupulously observed. Indeed, informed consent was obtained from all adult patients, or from parents for any minor patients included in this study.

A consent form was signed by each patient or by their legal representative for minor patients. The subjects included in the study were not exposed to any particular risks linked to certain manipulations.

3. Results

3.1. Demographic Epidemiological Characteristics of the Study Population

In total, 384 sickle cell patients were collected in the present study.

The average age of this study population was 16.46 ± 10.29 years. Age 10 was the most common. The age group of 10 - 20 years was the most represented (42.2%).

More than half (51.8%) of the study population was male with a sex ratio of 1 man: 1 woman. There was no significant difference ($p = 0.475$) between the gender proportions in the study population.

Among the 384 sickle cell patients recorded in the present study, 342 (89.06%) sickle cell patients seronegative for HBsAg (study subpopulation) were identified positive for anti-HBc Ab in isolation.

The average age of this study subpopulation was 16.30 ± 10.55 years, with extremes ranging from 1 year to 53 years. The mode was 10 years and the median age was 14 years.

More than half (71.6%, n = 145/342) of the study population was aged 20 or less. The male gender was represented at 52.0% (n = 178/342) for a sex ratio of 1 man: 1 woman.

Table 1 shows the distribution of demographic characteristics (age and gender) in the study population.

Table 1. Distribution of demographic characteristics.

Variable	Effectif (N)	Percentage (%)	p
Age range (Year old)			
1 - 9	104	30.4	0.0001
10 - 20	141	41.2	

Continued

21 - 30	54	15.8	
31 - 40	34	9.9	
41 - 53	9	2.6	
Gender			
Male	178	52.0	0.482
Female	164	48.0	

3.2. Biological Characteristics of the Study Population

The vast majority (90.9%; n = 311/342) of the study population was transfused at most 10 times. Of a total of 342 sickle cell patients seronegative for HBsAg, 19.0% (65/342) were seropositive for Anti-HBcAb. There was a significant difference ($p < 0.0001$) between the % of patients with occult hepatitis B and that of patients without occult hepatitis B.

Table 2 describes the transfusion and biological parameters (number of transfusions and occult hepatitis B in the study population).

Table 2. Transfusion and biological parameters.

Variable	Effectif (N)	Percentage (%)	p
Number of Blood Transfusion			
1 - 5	179	52.3	0.0001
6 - 10	132	38.6	
11 - 26	31	9.1	
Occult Hepatitis B			
Negative	277	81.0	0.0001
Positive	65	19.0	

3.3. Distributions of the Frequency of Occult Hepatitis B

There were no significant links between age ($p = 0.137$), sex ($p = 0.819$), number of blood transfusions ($p = 0.960$) and the occurrence of occult hepatitis B.

Table 3 summarizes the distribution of occult hepatitis B according to age, sex and number of blood transfusions.

Table 3. Distribution of occult hepatitis B according to age, sex and number of blood transfusions.

	Status	Age (years)						Sex			Number of Blood Transfusion			
		1 - 9	10 - 20	21 - 30	31 - 40	41 - 53	p	Male	Female	p	1 - 5	6 - 10	11 - 26	p
Occult Hepatitis B N (%)	Négatif	92 (26.9)	112 (32.7)	42 (12.3)	25 (7.3)	6 (1.8)	0.137	145 (42.4)	132 (38.6)		146 (42.7)	106 (31.0)	25 (7.3)	0.960
	Positif	12 (3.5)	29 (8.5)	12 (3.5)	9 (2.6)	3 (0.9)		33 (9.6)	32 (9.4)		33 (9.6)	26 (7.6)	6 (1.8)	

4. Discussion

The present study noted a rate of 19.0% of occult hepatitis among sickle cell patients negative for HBsAg. This rate is 2 to 3 times lower than those already reported in other studies in sub-Saharan Africa (ASA) [11] [12]. However, rates lower found in the present study have also been reported in North Africa, Cameroon and Burkina Faso [13]. The high prevalence found in the present study is correlated with the geographical distribution of the seroprevalence of viral hepatitis B markers, which is characterized by a distribution in an area of high endemicity (sub-Saharan), an area of medium endemicity (Mediterranean) and an area of low endemicity (European). Furthermore, it can also be justified by the large sample size in the present study and also by low coverage of the hepatitis B vaccine and by the method used.

Indeed, the technique used to search for markers of occult hepatitis B has a sensitivity of around 100%, likely to lead to false positives. The presence of anti-HBc antibodies may indicate active immunization by vaccination [14] [15].

As reported in most studies around the world, the present study did not note a significant difference between the genders. Indeed, there is no evidence of any gender predominance in sickle cell disease, or in the transmission of hepatitis B, or even occult hepatitis B [16] [17].

More than half of the population in the present study was under the age of 20. However, the age group of 10 to 20 years was the most represented. This over-representation of adolescents can be explained by the improvement in the management of sickle cell disease [18]. Actually, with the administration of hydroxyurea, the establishment of transfusion exchanges, gene therapy, bone marrow transplantation, we can today observe an increase in this life expectancy with children reaching and far beyond adulthood in Western countries [19]. This increase in life expectancy, which by the way, was estimated at 40 years for the period 2005-2008 in France, has largely contributed to the change in the causes of mortality, which have moved from vaso-occlusive events and infections, chronic complications and organ failure [19] [20].

In addition, a study carried out by the National Health Monitoring Institute (InVS) on the entire population observed that the life expectancy of sickle cell patients has doubled in 20 years, with a median age at death which is increased from 18 years in 1981-1985 to 36 years (34 years for men, 38 years for women) in 2001-2005 [21].

The vast majority of the study population was transfused at least 10 times.

Indeed, blood transfusion is the main therapy used in sickle cell patients, especially in emergency situations in countries with limited resources. It helps improve tissue oxygenation and limit crises (vaso-occlusive crisis, hemolytic crisis, splenic sequestration crisis, thoracic crisis) [5]. Unlike the present study which found no link between age, sex, number of transfusions, and the occurrence of occult hepatitis. A study carried out in Egypt showed a significant link between occult hepatitis B and age over 6 years, as did the number of transfusions [22]. Indeed, the

risk of contracting an infection transmissible through blood transfusion increases with the number of blood transfusions.

In principle, the absence of links between the number of blood transfusions and the occurrence of occult hepatitis B could be explained by effective transfusion safety (selection of blood donors, biological qualification of labile blood products) applied in transfusion structures. Blood and the various sickle cell monitoring centers.

In our study, the frequency of occult hepatitis was higher in the age group of 10 to 20 years, and in sickle cell patients having been transfused at most 10 times, a situation which could be explained by susceptibility to transfusion blood to which sickle cell patients are subject, which susceptibility increases with time. In addition, several African, European and American studies have shown a significant link between hepatitis B (occult Hepatitis B) and the number of blood transfusions [23] [24].

The present study presented some limitations and strengths. It was limited by its transversal approach. Indeed, a cohort study would have made it possible to determine the different predictors of occult hepatitis B in the study population. The detection of viral DNA was not sought in this study. In addition, the limited number of centers included in the present study could be the basis of certain biases.

Nevertheless, the present study has the merit of being the first to estimate the prevalence of occult hepatitis B in our environment. It demonstrated the extent of occult hepatitis B in the transfused sickle cell population. The results of the present study will have implications in research and routine practice.

5. Conclusion

Occult hepatitis B is a particular form of HBV infection that is increasingly attracting the attention of clinicians. Appropriate and effective transfusion safety measures must be ensured to minimize the transmission. There is a great proportion of occult hepatitis B cases among sickle cell patients who have received up to 10 transfusions. HBV vaccination remains an essential means to prevent the potential risk of infection related to iterative transfusions.

Conflicts of Interest

The authors declare no conflicts of interest.

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