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Frequency of Allo-Immunization in Sickle Cell Disease: Case of the Patients at the Laquintinie Hospital in Douala-Cameroon

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Abstract: *Background:* Allo-immunization is an immune response to foreign antigens after exposure to genetically different cells or tissues. Allo-immunized patients with sickle cell disease (SCD) appear to experience worse survival compared to non allo-immunized patients. Therefore, to contribute to the management of patients living with SCD and improve the blood transfusion process, we aimed to determine the frequency of allo-immunization in SCD's patients. To realize this study, we carry out a cross sectional study in one Hospital of Douala-Cameroon. *Method:* Plasma was prepared from collected blood sample, electrophoresis was done and depending on the migration on the gel; the type of electrophoresis was determine. Blood group ABO/Rh was done. After electrophoresis, depending on the result obtained those that was not homozygote (SS) and heterozygote sickle cell anemia (AS) was discarded. The check for irregular agglutinins was done using the indirect Coombs test. Clinical and biological characteristics of the different participants were studied and analyzed using a Statview statistical software. *Result:* We obtained 104 sickle cell patient, out of these we had 55% of positive RAI, with an average transfusion of 7.35. Also 83 patients were homozygote sickle cell anemia for a mean age of 15.75 years old (ranging from 1 to 52 years). The result obtained shows that the number of blood transfusion for homozygote sickle cell patient increases with age and that homozygote sickle cell patient received more blood transfusion than heterozygote patient. It can also be said that allo-immunization in these patients originate from the multiple blood transfusion received that were not fully compatible and that it affects more children (from 1 – 15), they accounted for about 53.85% of the total population. Our result obtained implies that in the future, sickle cell patient will have difficulty in finding compatible blood for treatment and is a real problem since transfusion is the major standard of care for patient suffering from sickle cell anemia in Cameroon. The absence of treatment may lead to the death of the patient. To help reduced allo-immunization, extended compatibility test in all the red blood cell system and routinely screening for the presence of irregulars agglutinins may reduce the frequency of allo-immunization.

Keywords: Allo-immunization, Homozygote Sickle Cell Patient, RAI, Blood Transfusion

1. Introduction

Blood transfusion is a lifesaving therapy for patients with chronic anemias, including patients with sickle cell disease and myelodysplastic syndrome. It remains a cornerstone treatment that has considerably reduced a wide range of treatment modalities ranging from vaccination against capsulated organisms, use of antibiotics to treat infections, analgesics to treat painful episodes, fluid administration to correct and prevent dehydration, regular folic acid supplementation, prophylactic anti-malarial and blood

transfusion among others [1].

Although red cell transfusion of patients with SCD does not always required patients in steady state condition, transfusion remains the mainstay therapy for many of the complications of SCD [2–4]. However, it is associated with complications that include iron overload, infections, acute lung injury, anaphylaxis, and allo-immunization [3–5]. Allo-immunization is an immune response to foreign antigen following exposure to genetically different cells or tissues [6]. It depends on the number of blood transfusions received and the antigenic difference between the donor and the receiver. It is mostly caused by irregular agglutinins that are found on red blood cells [7]. It is a natural event in condition such as pregnancy, but it is usually an undesirable outcome of blood transfusion or transplant [4]. In the case of transfusion, blood group antigens can be immunogenic in individuals who lack the corresponding antigen on their red blood cells (RBCs). This mismatch can occur during transfusion of antigen positive blood into someone who is antigen-negative, or during pregnancy when the mother lacks a blood group antigen that is contained on the fetal RBCs. In the former case, this can result in immunization of the transfusion recipient and the production of alloantibody that may cause a hemolytic transfusion reaction [5]. This process also takes place during sickle cell anemia. Sickle cell disease is a group of disorders that affects hemoglobin [1]. People with this disease have atypical hemoglobin S, which can distort red blood cells into a sickle, or crescent shape. In case of severe sickle cell disease bone marrow transplant can be done. But the most common treatment for sickle cell disease is blood transfusion because it helps in increasing the oxygen carrying capacity and decreases the proportion of sickle cell disease, but this remains a treatment with side effect because it gives a different genetic polymorphism of antigens to the patient and increase the possibility of developing alloantibodies [1].

Globally, the prevalence of anti-erythrocyte allo-immunization is variable from one region to another and from one country to another. In Dehli (India) the frequency of allo-antibodies among polytransfused patients was 5.2% in 2016 [4] and the antibodies were predominantly from the Rhesus and Kell system. In sub-sahara Africa, the frequency of blood donors with erythrocyte allo-antibodies was less than 1% [7], a frequency obtained from a study which investigated blood donors from Cameroon, Ivory Coast and Benin. In a study carried out in Mali in October 2010, the authors concluded that the frequency of red blood allo-immunization is very high in polytransfused patient and the screening before transfusion increase the immunologic security of the patient [7]. The incidence of red blood cell allo-immunization in patients with sickle cell anemia is 18-46% [8].

In the willingness to improving the management of patients with sickle cells diseases in Cameroon, we aimed to determine the frequency of RBC allo-immunization. The goal this study was to show the impact of blood transfusions on polytransfused like sickle cell disease patient.

2. Methods

2.1. Study Design and Population

This was study was a cross-sectional study. Sickle Cell Disease Patient from Laquintinie Hospital in Douala was included in this study. A total of 104 SCD were recruited over a period of 2 months. The inclusion criteria was all transfusion dependent SCD patients as well as those that have had a history of at least two transfusions of ABO-and Rh D matched RBC during their lifespan. A questionnaire was used to collect demographic and medical data including patient's age, sex, age on first RBC transfusion, ABO and Rh blood group, hemoglobin concentration, number of blood units transfused, transfusion reactions, frequency and specificity of alloantibodies by direct interview of patients or their guardians and from medical files. Clinical files and transfusion records were analyzed in all SCD patients for the presence of allo-antibodies in SCD patients.

2.2. Laboratory Analysis

Data from the immunohaematology laboratory and characteristics of red blood cell concentrates distributed from the start of the setting in local patient care were collected. Systematic laboratory examinations in each transfused sickle cell patient are: electrophoresis of hemoglobin on cellulose acetate and in alkaline medium, detection of irregular agglutinins (RAI) and direct test of compatibility, performed as an indirect antiglobulin test (coombs test). Blood group ABO / RH D has been completed.

ABO blood typing was performed by the antigenic method of Beth Vincent and confirmed by the serum method of Simonin. The detection of irregular agglutinins was carried out by the method of indirect coombs with human antiglobulin and the antibodies obtained were observed at a microscope.

2.3. Statistical Analysis

The statistical analysis of all data was done using the Statview Statistical Package version 5 (SAS University Edition, SAS Institute Inc., Cary, USA). Data were expressed in frequencies and proportions; the chi^2 test was applied for the comparison of frequencies. The parameters were calculated for a confidence interval of 95%; the significance level was set at 0.05.

2.4. Ethical Considerations

This study was approved by the "Université des Montagnes" Ethics Committee ((AUTHORIZATION N ° 2020/166 / UdM / PR / CIE). A research authorization to collect data and analyze samples from eligible patients was issued by the Laquintinie Hospital in Douala (N ° 03927 / AR / MINSANTE / DHL / CM). Before starting our study, an information letter on the objectives and progress of the investigation was given to the sickle cell disease patient. All eligible participants had given their free and informed consent and assent with signature of the participants. The confidentiality of the research results was respected by using a unique identification code for each participant.

3. Results

3.1. Socio-demographic Characteristics and Frequency of Sickle Cell Patients

During the study period, the samples collected from n=104 participants indicated that majority (59%) were female and a minority for males (41%), with ages ranging from 1 to 52 and an average age of 15.75. The sex ratio obtained was 1.42 in favor of women, majority of the patient where from O positive blood group, and children accounted about 54 of our total population. And also the distribution of the frequency of blood transfusion received shows that the number of transfusions varies from 2 to 34 with an average of 8 and that they were more homozygote sickle cell disease than heterozygous sickle cell disease. The professions of student, primary and secondary was the most represented with a frequency of 30 and 56% (Table 1). The distribution of the study population was cosmopolitan in the city of Douala. The result obtained after electrophoresis shows that the amount of patient that were homozygote sickle cell disease was greater (80%) than those with heterozygote sickle cell disease (20%) (Figure 1).

Table 1. *Demographic distribution of the population.*

Sex with age range			
Age range	**Female (n)**	**Male (n)**	**Total**
]1 – 12]	21	19	40
]12 – 22]	20	17	37
]22 – 32]	13	5	18
]32 – 42]	5	1	6
]42– 52]	2	1	3
Total	61	43	104

Profession	**n**	**%**
Primary, secondary	56	53.85
Student	30	28.85
official	8	7.69
Unemployed	5	4.81
Informal sector	5	4.81
Total	104	100

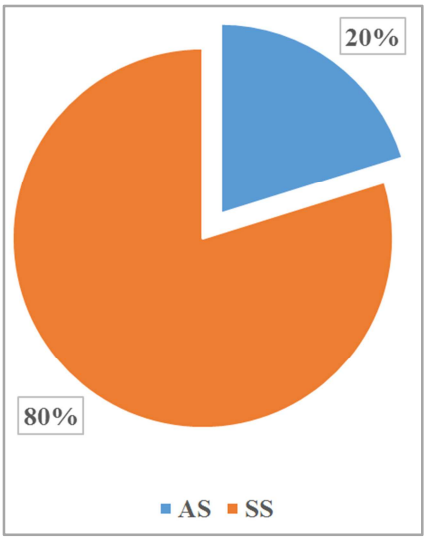

Figure 1. *Genotype of hemoglobin.*

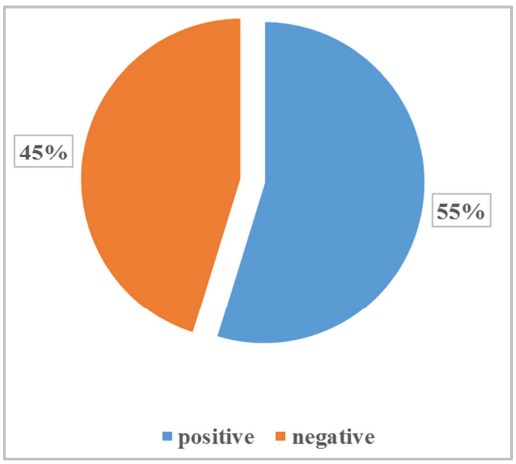

Figure 2. *Frequency of allo-immunisation.*

3.2. Distribution of the Frequency of Blood Transfusion Received Couple with Hemoglobin Genotype

The distribution of the study population by the frequency of blood transfusion received couple with the hemoglobin genotype shows that the patient that where homozygote sickle cell disease (SS) received more transfusion than heterozygote patient (AS) (p = 0,001) (table 3).

In our population study the O positive was predominant (40.38%) over the others blood group, and the AB blood group was comparatively low (9.62%). The mean of blood transfusion received was 7 per sickle cell patient (Table 2).

Table 2. *Distribution of the population according to blood group.*

GS RH		
Blood group/RH	**n**	**(%)**
A positive	23	22.12
AB positive	10	9.62
B positive	29	27.88
O positive	42	40.38
Total	104	100

Table 3. *Frequency number of transfusion received couple with hemoglobin genotype.*

Blood range	**n**	**%**	**n AS**	**% AS**	**n SS**	**% SS**	***p***
[2 - 9]	72	69.23	21	100	51	61.45	**0,003
[9 - 15]	21	20.19	0	0	21	25.3	**0,002
[15 - 22]	5	4.81	0	0	5	6.02	**0,004
[22 - 28]	3	2.88	0	0	3	3.61	**0,003
[28 - 34]	3	2.88	0	0	3	3.61	0,005
Total	104	100	21	100	83	100	**0,001

95% confidence interval. **Significant.

3.3. Distribution of the Frequency of Blood Transfusion Received Couple with the Result of Indirect Coombs

The mean of blood transfusion was 7 with an interval more represented between 2 and 7. 57 patients (55%) had positive indirect blood test. Sickle cell anemia patient with a positive indirect coombs test (55% positive for research for irregular agglutinin) had a higher blood transfusion frequency than those for negative agglutinin (p = 0,003) (table 4). We

noticed that patient that was positive to Research for Irregular Agglutinins tends to received more blood transfusion.

Table 4. *Frequency number of transfusion received couple with the result of RAI.*

Range	n	%	Negative RAI	Positive RAI	*p*
[2– 9]	72	69.23	46	26	0,006
[9 – 15]	21	20.19	1	20	**0,02
[14 - 22]	5	4.81	0	5	0,09
[22 - 28]	3	2.88	0	3	0,1
[28 - 34]	3	2.88	0	3	0,07
Total	104	100	47	57	**0,003

95% confidence interval. **Significant.

3.4. Frequency of Anti-erythrocyte Allo-immunization

Of the 104 sickle cell patients who received transfusions, 57 developed an anti-erythrocyte allo-immunization, either a frequency of 55%. This frequency was significantly higher in homozygous patients than heterozygotes in whom the frequency of blood transfusion is also higher ($p = 0.003$).

4. Discussion

We determined the frequency of allo-immunization in sickle cell patients aged 1 to 52 years. Our objectives were to describe the frequency of allo-immunization in relation to the genotype of hemoglobin and the impact of blood transfusions on sickle cell disease patient. Our study was cross-sectional; patient recruitment was done in the hematology service of the Laquintinie hospital in Douala. The frequency of allo-immunization (positive RAI) was 55%, after confirmation of agglutinations with a microscope by the indirect Coombs test. This frequency is far inferior to the one obtained in Morocco by Atouf in 2013 which was 90.4%, also in Ivory Coast carried by Sekongo in 2017 the obtained a frequency of 28.6%. This can be explained by the difference in the total population of study which was 2027 in Morocco by Atouf, 42 in Ivory Coast compare to us which was 104 [5, 16]. These results obtained implies that in the future, sickle cell patient will have difficulty in finding compatible blood for treatment and it is a real problem since transfusion is the major standard of care for patient suffering from sickle cell anemia in Cameroon. The results obtained suggest that erythrocyte allo-immunization constitutes a real public health problem and is the main cause of transfusion rejection; and in extreme cases involve the patient's vital prognosis [3].

The average age of the sickle cell disease patient was 15.75 years (ranging from 1 – 52 years). Children (1 – 15 years) accounted for 53.85% and 46.15% for adults. This can be due to the increasing of the sickle cell disease patient. The results are superimposed on those of Sekongo in 2017 (Ivory Coast) who observed an average age of 24.45 years (ranging from 4 – 68 years) and children accounted for 28.6%, adults 71.4% [5-10]. The difference in the result can be explained by total population of study, which was 42 in Ivory Coast [9]. The distribution of the study population was cosmopolitan in the city of Douala. Our population of study had more females (61) than males (43) and a sex ratio of 1.42. This can be due to the fact in Douala female are predominant over males. This observation differs from the studies carried in Morocco by Atouf in 2013 who reported a sex ratio of 1.06 in favor of women and in Ivory Coast carried by Sekongo in 2017 who had a sex ratio of 1 (both the number of males and females where equal) [5, 12, 13]. It can be explained that in our population study and in the population study in Morocco 2013 there were more females than males compare to the population study in Ivory Coast 2017 (which was 21 males and 21 females).

The distribution of antigens blood group in the ABO system is comparable with those of previous studies conducted in Africa [14, 15]. In fact, at the end of our study we obtained the O group (40.38%), A group (22.12%), B group (27.88%) and the AB group (9.62%) which are similar to the one obtained by Sekongo in 2017 the A group (26%), O group (50%), B group (17%) and AB group (7%) even Boateng in 2019 obtained a similar result, A group (21.4%), B group (19.1%), O group (54.7%) and AB group (4.8%) [5, 14, 16].

Our patients were predominantly sickle cell homozygous SS (80%). Our results are similar to those obtained in 2017 by Sekongo in Ivory Coast who obtained 76.2% of sickle cell anemia. This could be due to the gravity of the disease in this form with acute or chronic complications which justify the transfusions of much blood for treatment. The patient that where homozygote sickle cell disease (SS) received more transfusion than heterozygote patient (AS) (p=0.001). This strengthens our idea that patients that are homozygote sickle cell (SS) received more transfusions than heterozygote sickle cell patient AS, cause of allo-immunizations [16].

The frequency of allo-immunization (positive RAI) was 55%, after confirmation of agglutinations with a microscope after the indirect Coombs test. Sickle cell anemia patient with a positive research for irregular agglutinin had a higher blood transfusion frequency than those for negative agglutinin with (p= 0.003). These frequency is far inferior to the one obtained in Morocco by Atouf in 2013 which was 90.4%, also in Ivory Coast carried by Sekongo in 51 the obtained a frequency of 28.6%. This can be explained by the difference in the total population of study in Morocco by Atouf, 42 in Ivory Coast compare to us which was 104 [11, 16]. The frequency of positive RAI was observed in patients with transfusions greater or equal to 5, and the average transfusion was 7.35 ranging from 2 to 34 transfusions. This is a problem because transfusion is the only standard of care for sickle cell anemia in our country. Also we noticed that patients who were homozygote sickle cell disease (SS) with increasing age received more transfusion and almost all the patient that was SS and had received more than 5 transfusions was positive for the presence of irregular agglutinins.

5. Conclusion

The present work shows that 55% of the sickle cell

population presents allo-immunizations. The frequency of allo-immunization is significantly related to the total number of transfusions received. Sickle cell disease is a hemoglobinopathy which major standard of care is based on transfusion but this remains a treatment with side effects because it gives a different genetic polymorphism of antigens to the patient. Older age of patients, increase in number of blood units transfused; in order to prevent allo-immunizations, all transfusion must be phenotyped.

State of Current Knowledge on the Subject

1) Sickle cell anemia: sickle cell anemia is the most common hereditary genetic disease in the world;
2) Blood transfusions: its management requires blood transfusions;
3) Allo-immunization: non-compliance with good transfusion practices contributes to the development of allo-immunizations in recipients.

Contribution of Our Study to Knowledge

1) Good compliance with transfusion practices. This work highlights the need to respect good transfusion practices in order to prevent accidents linked to transfusion incompatibilities.
2) This work shows that it is essential before any blood transfusion to carry out an extended compatibility test in all the red blood cell system to prevent the appearance of allo-immunizations.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This study was presented during the Bachelor degree in November 2020 at the Université des Montagnes.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Celianthe Guegang Guegang conducted experimental design, data interpretation, and manuscript writing. Romaric De Manfouo Tuono conducted experimental design and interpretation of data and made a major contribution to writing the manuscript. Simon Ngamli Fewou contributed to data interpretation, statistical analysis, and manuscript writing. All authors read and approved the final manuscript.

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