Comparison Of The Lipid Composition Of The Membrane Of Sickle Cell (SS) To That Of Normal Red Blood Cells (AA)

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SUMMARY

Comparing the lipid composition of the membrane of sickle cell (SS) to that of normal red blood cells (AA) is the subject of this study. We handled the blood samples collected from January 2012 to June 2012 in the LARBA Laboratory (laboratory for research in applied biology). Spectrophotometry, electrophoresis, chromatography are the methods used to enhance our results. At the end of the work, out of 663 cases studied, we obtained 76.63% of persons with AA hemoglobin and 23.37% of persons with SS hemoglobin. A alkaline Ph (Ph=8.6) as well as acid Ph (Ph=6.2), hemoglobin A has a higher migration rate than hemoglobin S. Optical density in AA is higher for sugar, cholesterol, triglycerides, phospholipids, and total protein compared to SS. People with AA hemoglobin have a higher lipid composition than people with SS hemoglobin. In order to improve the rigidity and membrane strength of abnormal red blood cells; without, however, interfering with the different roles played by the plasma membrane of these red blood cells, the genetic program of membrane lipid biosynthesis should be determined.

Keywords: hemoglobin, sickle cell disease, membrane, lipid composition

INTRODUCTION

Sickle cell disease or sickle cell anemia, also known as sicklanemia or Herrick's disease, is an inherited blood disorder due to the presence of pathological hemoglobin S (Hb S) in the red blood cells. It is an inherited genetic disease affecting the red blood cells, in which a child can only be sick if both parents are transmitters, i.e. asymptomatic carriers of the sickle cell disease gene [1; 2] It is an autosomal, recessive hereditary disease that affects both boys and girls and only occurs when one is a carrier of two disease genes. This disease is genetic and therefore not contagious. Of all the abnormal hemoglobins, hemoglobin S is by far the most common and the most serious in terms of public health. Indeed, sickle cell disease affects more than fifty million children in Black Africa on both sides of the equator. The situation in Benin is a perfect reflection of the sickle cell disease problem that occurs in a very large part of West Africa where the economic resources of the populations are generally limited. One person in four carries hemoglobin S and 4% of the Beninese population is affected by hemoglobinopathy SS and double heterozygosity SC [2]. Until the last thirty years hemoglobinopathies were responsible for a high infant mortality rate (50% of deaths at 1 year of age and 90% before 5 years of age) [2; 3]. These data are therefore of interest to pediatricians, hematologists, biochemists and epidemiologists. Technician biologists are interested in the numerous screening and follow-up tests to be carried out in affected subjects [4]. 4] It is therefore becoming imperative, especially for Africans, to search for the basis of this disease and the means to reverse it, hence the importance of this work on the plasma membrane of red blood cells. Our work aims to determine above all the membrane and plasma lipid composition of sickle cell (SS) to that of normal red blood cells (AA).

PATIENTS AND METHODS

From January 2012 to June 2012, a comparative study was carried out with patients from the LARBA Laboratory (Laboratory of Applied Biology Research) on the red blood cell membrane. The comparative study of the lipidic composition of the sickle cell membrane compared to normal ones was carried out on 663 samples. Sickle cell patients and apparently normal patients were all subjected to hemoglobin electrophoresis beforehand. Sickle cell patients and normal subjects who were transfused within two months prior to study entry were excluded, as well as those with a history of other conditions such as thalassemia; diabetes; spherocytosis; elliptocytosis and hereditary persistence of fetal hemoglobin (PHHbF). Children under 6 years of age are excluded from our study framework because of the persistence of fetal hemoglobin. Informed patient consent is obtained prior to blood sampling. Spectrophotometry, chromatography and densitometry are other procedures that have been combined with electrophoresis to achieve this comparison.

Statistical analysis

Values are averages ± sd. Statistical analysis of the data is performed using STATISTICA (version 4.1; Stat-Soft, Paris, France). The data were evaluated by analysis of variance. Duncan's multiple test was used to compare patients with AA and SS hemoglobin. Differences were considered significant when $P<0.05$.

RESULTS

The distribution of the sample according to the type of hemoglobin electrophoresis is shown in Figure 1. There are 76.63% AA hemoglobin and 23.37% SS hemoglobin; a difference of 53.26%.

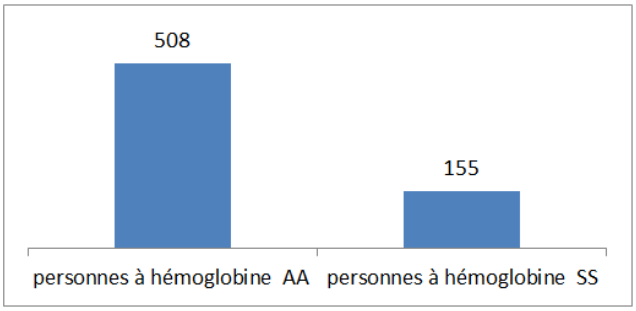


Figure 1: **Distribution of the sample according to the type of hemoglobin electrophoresis**

Figure 2 shows a photograph of the migration of hemoglobin HbA and hemoglobin HbS.

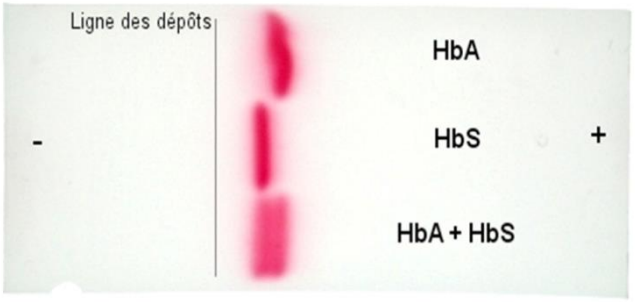
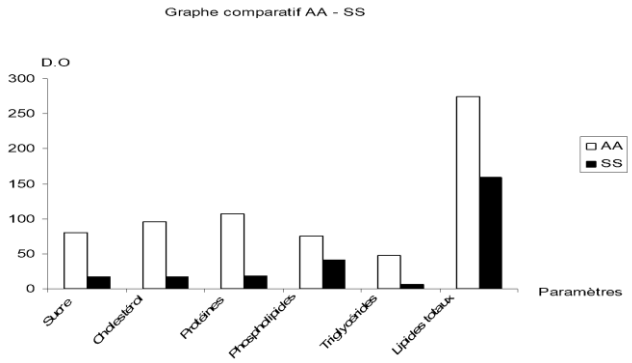


Figure 2: Electrophoresis of hemoglobins A and S

The graph 1 shows the spectrophotometric determination of certain biological constants contained in the red blood cell membrane.



These are sugars, cholesterol, triglycerides, total lipids, phospholipids, and total proteins.

Discussion

This study, based on the comparison of the lipid composition of the sickle cell membrane to that of normal red blood cells, was performed on 663 cases of which 76.63% were AA hemoglobin patients and 23.37% were SS hemoglobin patients; a difference of 53.26%. This explains the 4% of the Beninese population affected by SS hemoglobinopathy [2]. There is therefore a small proportion of the SS type [5].

	Normal Nouveau né	Normal adulte	Hétérozygote	Drépanocytose homozygote	Double hétérozygote
Hémoglobine	A F > 80% A2	A A2 < 3,50%	A S = 35 à 45 % A2 < 3,50%	S > 90% F = 2 à 20 % A2 < 3,50%	S C

Figure 3 : Schéma d'une électrophorèse

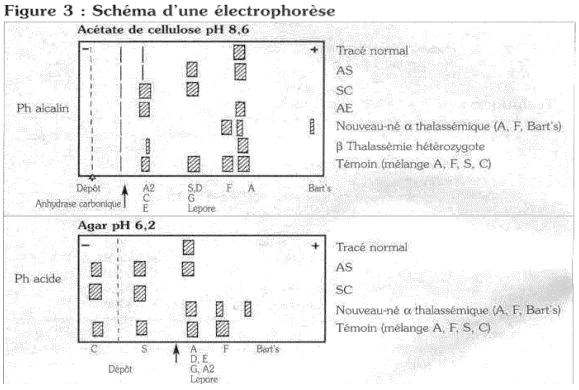


Table 1: Percentage of different haemoglobins by subjects

The difference in hemoglobin load leads to a different mobility in electrophoresis. This difference in charge is visualized by alkaline pH electrophoresis on cellulose acetate where hemoglobin S migrates behind hemoglobin A [6;7]. The disadvantage here is that other abnormal hemoglobins such as hemoglobin D can migrate to the same location as hemoglobin S; this difficulty is circumvented by the use of the isoelectric focusing method, a method used in the neonate [8;9]. Hemoglobin S, which is less charged than hemoglobin A due to the substitution of glutamic acid by valine at position 6 of the beta globin chain, migrates less quickly and can thus be distinguished on the cellulose acetate strip [3; 10]. The higher the protein concentration in a gel portion, the stronger the coloration. The concentrations of the different categories of proteins can therefore be assessed by optical measurement of the staining density on the strip (Figure 4).

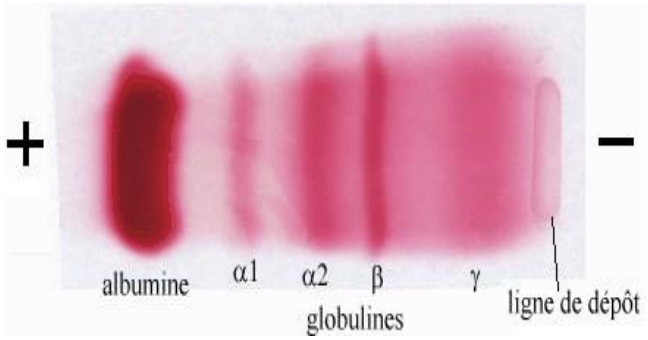


Figure 4: Schematic representation of serum protein migration

In newborns, the fetal hemoglobin level is higher than 80%; in adults the A2 level is lower than 3.50% and in sickle cell patients the HbS is higher than 90%, HbF between 2 and 20% and A2 lower than 3.50%. We then retain that in the normal individual (AA), we have more than 97% HbA.

The results obtained in electrophoresis confirm those obtained in spectrophotometry. On the electrophoresis curves of the serum lipids of the subjects, the very high peak shows that the sample is indeed plasma; and this peak represents the fibrinogen which is only contained in the plasma. The peak representing the total lipids shows that the amount is high in each type of hemoglobin and confirms the results of the spectrophotometry. In AA subjects, no pre lipoproteins or chylomicrons were present. In SS subjects, there was a clear band of chylomicrons.

During these studies, we measured certain biochemical constants, namely sugars, cholesterol, triglycerides, phospholipids, and total proteins.

The comparative graph of the different values shows that for people with AA hemoglobin; all the biochemical constants dosed, namely: sugars, cholesterol, total protein, triglycerides, are high compared to those of SS individuals. With protein electrophoresis, the same comparison is observed: People with hemoglobin AA have a high level of total proteins compared to SS individuals. Lipid electrophoresis confirms the results obtained with the spectrophotometer. The comparison remains the same when switching from AA to SS. The thin layer chromatography used in this study was not able to measure specific lipids such as phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine. It just allowed their revelation in the different samples. We can therefore say that these specific lipids are present in the plasma membrane of red blood cells, whatever the type of hemoglobin. In order to have more precision, especially to quantify these specific lipids of the plasma membrane of red blood cells, it is therefore necessary to use high performance gas chromatography (HPLC) [11]. It can therefore be said that the anomaly also affects the biochemical constants measured.

It should also be noted that the presence of HbS hemoglobin in homozygous individuals (HbS//HbS) is a source of early mortality in them. It is therefore necessary to raise awareness of the dangers of marriage between partners who may give birth to a homozygous child [12].

Conclusion

The plasma membrane constitutes the palisade of red blood cells [13; 14]. This palisade owes its rigidity to its lipidic composition. The different assays performed during our work show differences in lipid composition according to the type of hemoglobin. Lipid quantities decrease from AA to SS; and membrane rigidity also decreases. Thus showing that there is a difference in lipid composition not only in the membranes of AA to SS red blood cells but also in the serum.

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