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ELLIPTOCYTOSIS AT UNIVERSITY HOSPITAL SOURO SANOU (CHUSS)

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Résumé: -

Elliptocytose au CHU Souro Sanou.
IL s'est agi d'une elliptocytose (anomalie de membrane du Globule rouge) dans une famille de la région des Hauts Bassins. Sur leur frottis sanguin des elliptocytes à UN pourcentage variable selon les individus ont été mis en évidence. Les mêmes images ont été retrouvées chez les descendants qui ont été examinés. Tous (les propositus) ont une hémolyse compensée ET les femme's avaient une des anomalies visuelles.

Matériels et méthodes

Les examens suivants ont été réalisés : hémogramme, Frottis sur sang frais, frottis après mise pendant 24heures à l'étuve du sang, bilan d'hémolyse pour certains, ektacytométrie, acuité visuelle, champ visuel. Certains facteurs de gravité ont été recherchés : recherche des anomalies de l'hémoglobine par électrophorèse capillaire et chromatographie liquide haute performance, bilan martial, bilan vitaminique.

Résultats: *Alor's que certain ont présenté une elliptocytose simple, les autres avaient une pyropoïkilocytose. Les femmes ont présenté pour certaines une baisse de l'acuité visuelle et pour d'autre une amputation du champ visuel en temporal. La relation entre la maladie hématologique et les troubles visuels n'a pas pu être réalisée parce que la biologie moléculaire n'a pas pu être réalisée.*

Mot's clefs: *Elliptocytose, frottis sanguin, ektacytométrie, troubles visuels, Biologie moléculaire*

Abstract: -

Elliptocytosis at CHU Souro Sanou.
This study is about elliptocytosis (anomaly of the Red Blood Cells membrane) in a family of the county of "Hauts Basins". On the members' blood smears, elliptocytes, at variable percentage according to the persons, have been found. The same images have been found among the descendants who have been examined. All (the propositus) have hemolysis offset and women had a visual defect.

Materials and Methods

The following examinations were made: CBC, smears on fresh blood, smears after 24 hours in a blood autoclave, results of hemolysis for some, ektacytometry, visual acuity, visual field. Some factors of significance were sought: Research of the anomalies of the Hemoglobin by capillary electrophoresis and high performance liquid chromatography, martial balance, balance sheet vitamin.

Results: *while some have presented a simple elliptocytosis, the other had a pyropoikilocytosis. The women presented for some a decrease in visual acuity and for other an amputation of the visual field in temporal.*
The relationship between the hematological's disease and visual disturbances could not be performed because the molecular biology has not been able to be carried out.

Keywords: *Elliptocytosis, blood smear, ektacytometry, visual disturbances, molecular biology*

INTRODUCTION

The elliptocytosis is a morphological erythrocytopathy due to a horizontal interaction abnormality of its skeletal proteins (1). The skeletal proteins involved are varied and their distribution is ubiquitous in the body. It can be asymptomatic, responsible for a hemolytic anemia or in rare cases a hydrops fœtalis (2). This clinical heterogeneity is linked to a genetic heterogeneity (3). Its frequency in the West African region is 2% and according to the ethnic groups 0.6 to1, 6% for some (4, 5,6). The objective of this study is to describe the clinical and hematological aspects and diagnosis in the context of our hospital.

Material and Methods

Materials

- Patients: A sibling of five women and three men.
- Hematology automaton Mindray BC-6800 and ADVIA 2120 NGS
- slides, color reagent: May Grünwald Giemsa
- Jermaks stove
- High Performance Liquid Chromatography: Bio-Rad V-variant II hemoglobin testing system
- Flow Cytometer: Beckman Coulter Navios flow cytometer.
- Eosin 5'Maléimide of the Laboratory Sigma-Aldrich
- Phosphate Buffered Saline (PBS)
- Bovine albumin (Invitrogen)
- Ektacytometry: PLC Ektacytometer Technicon
- Polyvinylpyrrolidone powder
- Table of reading for visual acuity, Biomicroscope Zeiss, Octopus 300 for the visual field. Methods

The five women have completed each: a visual acuity, a study of the retina, the crystalline lens and a study of the optic nerve

The different blood samples have been taken at the level of the central vein of the fold of the elbow and were routed in less than 24 hours at 4°C to the hospital Kremlin Bicêtre for the ektacytometry and the test to the eosin 5' maleimide (EMA).

For the test to the EMA, the witnesses are adult patients of the day of the examination with normal CBC without any abnormal sign, six witnesses have been taken. The erythrocytes' fluorescence of witnesses has been measured and the average of the six witnesses were computed to assess the status of patients.

For the ektacytometry, four witnesses were selected including two normal, one with a spherocytosis and another with a elliptocytosis. The curve has been established for each of them.

The phenotypes of the hemoglobin have been made by capillary electrophoresis (Sebia) and by high performance liquid chromatography.

-Thin blood smears with the blood maintained at the temperature of the laboratory or with the blood put in the stove at 37 °C for 24 hours, dried immediately and colorful with May Grunwald Giemsa

The different analyzes have been performed according to the Protocol of the devices.

Results

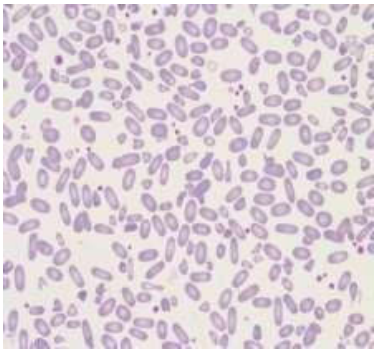
Table I: CBC of patients

Patient number	1	2	3	4	5	6	7	8
years	63	61	58	56	53	51	47	43
Sex	Male	Fem	Fem.	Fem.	Male.	Fem.	Fem.	Male.
Leukocytes 10^9/L	5.59	5.85	5.32	5.07	5.27	3.64	2.69	5.55
The RBCS 10^{12}/L	4 ,52	4.03	3.85	3.52	6.21	4.18	3.64	5.48
Hemoglobin g /DL	13.9	9.0	11.2	9.1	16.9	8.3	7.3	13.8
Hematocrit L/L	0.44	0.33	0.37	0.29	0.516	0.32	0.28	0.45
VGM FL	96.7	81.7	95.3	82.4	83.1	75.4	77.5	82.8
TGMH pg	30.8	22.4	29.2	25.8	27.2	19.8	20.1	25.1
CGMH g/dL	31.8	27.4	30.6	31.3	32.7	26.2	26	30.3
IDR%	14.6	16.5	20.3	17.6	14.2	38.4	26.3	
Platelet 10^9/L	209	421	242	315	272	436	177	285
VMP FL	11.2	9.4	10.1		10.3	9.5	11.6	
The RBCS hyperdenses%	0.70	3.10	2.00			1.8	1.10	2.2
Reticulocytes 10^9/L	47	162.6	23.9	164	31.7	192	37.7	65.7
Average volume reticulocyte FL	118	116	116	109	107	114	101	104

N°. 2, 4 normocytic hypochromic regenerative anemia.

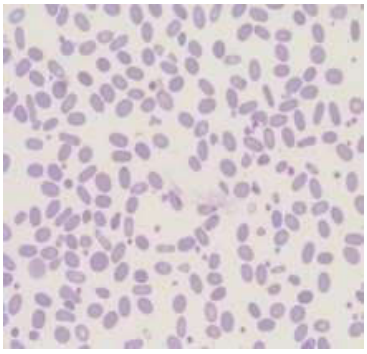
Nº 3 normocytic normochromic arégénérative anemia,
Nº 6 microcytic hypochromic regenerative anemia
Nº 7 microcytic hypochromic arégénérative Anemia.

A-Smear in a fresh state



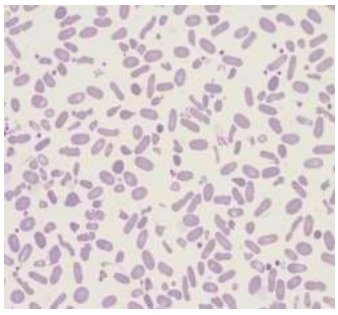
The smear A is the Nº.4.

B- smear in a fresh state

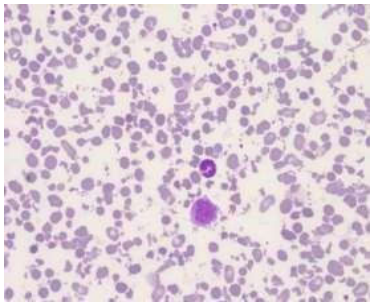


The smear B is the aspect presented by the Nº. 2.3, 6, and 7.

Smear C after the stove
Patient Nº.4



smears D of after the stove
patient Nº. 3, 4, 6, 7.



The Patients 1 and 5 have about 65% of elliptocytes on the fresh smears and many fragments after placing in the stove. On the smear of the patient N°.8 of elliptocytes have not been sighted in the fresh state.

Table II: phenotypes of Patients' hemoglobins

Hemoglobin fraction	1	2	3	4	5	6	7	8
Haemoglobin A2 %	3	2.6	3.1	3		3	3	2.8
Hemoglobin F%	<1	<1	<1	<1		<1	<1	0.5
Haemoglobin A1c %	5.3	04	4.5	4.7		4.2	3.9	05
Haemoglobin A%	86.2	91.9	89.5	92		92	91.1	87.2

Part glyquée of A2 and F have been omitted

Table III: Test of binding of the EMA

EMA	1	2	3	4	5	6	7	8
Fluorescence intensity witness %	61	61	61	61		62.7	61	61
Fluorescence intensity patient %	59.9	52	52.2	63.3		57.8 Double peak	64.1	59.6
Decreased in fluorescence intensity %	-2	-15	-14	4		-8	5	-2

Curves of the ektacytometry in osmolaire gradient

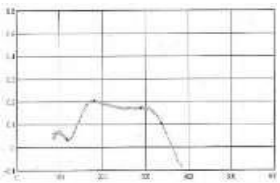


Fig N° 1: Witness Elliptocytosis

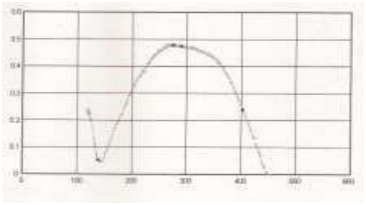


Fig N°2 : normal light

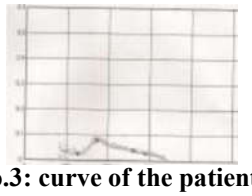


Fig No.3: curve of the patient No. 3

Classically X refers to the X axis and Y the ordered but here the laboratory has reversed this appellation.
X=ordered the hypotonic point, isotonic, hypertonic. Translated the deformability or stretching in these different points;
Y=the abscissa. It is the osmolality in mosmol /kg: The hypotonic point, isotonic, hypertonic.
Hypotonic Point: measure the point from which the cell no longer does hemolysis thus, the membrane fragility
Isotonic Point: measure the deformability of the cell to the isotonic point,
Hypertonic Point: measure the deformability of the cell to the hyperdense point

Max: point or the cell deforms to maximum
FigN^{0}1 witness simple elliptocytose
Max X=180 Max y=0.204; hypo X=117 Hypo y=0.036; Hyper X=336 Hyper y=0.102; ISO X=290 ISO y=0.174
Fig N°.2 normal witness
Hypo: x=0.058 Hypo Y=138. A move to the right reflects a membrane fragility
Iso Y=290 ISO x=0.475 a lowering of Y reflects a decrease of the deformability
Hyper Y=401 Hyper X=0.24 A decrease reflects an increase in the density intra-erythroid at concentrations osmolaires low.
Max: Y=274 x=0,479
FigN°3 Patient N°.3
Max: Y=174 x=0.08; Hypo: Y=127 x=0.025; Hyper: Y=261 x=0.04; ISO: Y=290 x=0.028

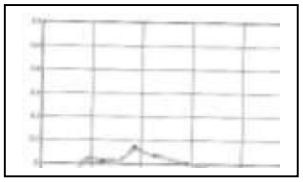
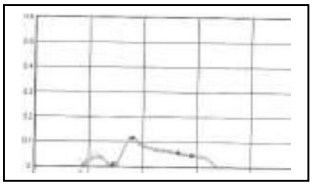
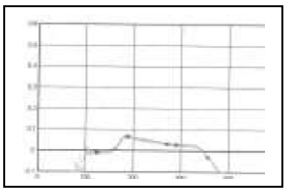




Fig N°. 4 patient N^{0}2 Fig N^{0}5 Patient N°. 4 Fig N°.6 Patient N^O.5

Fig No. 4 Patient No.2
Max: Y=185 x=0.072; Hypo: Y=121 x=0.011; Hyper: Y=227 x=0.036; Iso Y290 x0.002
Fig No.5 Patient No.4
Max: Y=180 x=0.115; Hypo: Y=144 x=0.01; Hyper: Y=264 x=0.058; ISO: Y=290 x=0.048
Fig No.6 Patient No.5
Max: Y=188 x=0.068; Hypo: Y=123 x=0.01; Hyper: Y=270 x=0.034; ISO: Y=290 x=0.023 Patient N^O.1
Max: Y=220 x=0.072; Hypo: Y=145 x=0.001; Hyper: Y=334 x=0.036; ISO: Y= 171 x=0, 024
Patient N°.6
Max: Y=174 x=0.08; Hypo: Y=127 x=0.025; Hyper: Y=261 x=0.04; ISO: Y=290 x=0.028
Patient No.7 (see curve in Annex)
Max: Y=177 x=0.072
Hypo: Y=145 x=0.001
Hyper: Y=255 x=0.036
Iso: Y=290 x=0.025
Patient No.8 (see appendix)
Max: Y=190 x=0.069 ; Hypo : Y=124 x=0.01 : Hyper : Y=269 x=0.033, ISO : Y=290 x=0.027

Table IV: Martial balance sheet and inflammatory

Patient	2	3	4	6	7
Serum ferritin ng/ml	< 10	73,47	90	14	< 10
CRP mg/ml	2,4	2	2,3	2,4	2

The Serum folate and erythrocyte folate of the patient N°3 is 17ng/ml and 200ng/ml.
Patient N°2 visual acuity: 4 /10 to the left and 2/10 to the right. Biomicroscopy: cataract bilateral nuclear scalable. Bottom of eye: absence of sign of retinopathy
Visual field: amputation of the visual field in temporal
Patient N°3: Visual field: amputation of the visual field in temporal.
Patient N° 4: Biomicroscopy: maculopathie bilateral. Visual field: nebulous
Patient N°6 bottom of eye: home of chorioretinitis. Visual field: amputation of the visual field in temporal
Patient N°7 visual disorders not otherwise specified, she did not come to the ophthalmologic consultation.

Discussion
The limits of our study are: non realization of hemolysis report and or absence of its repetition in all patients.
The biochemical study of the erythrocyte membrane and the molecular biology have not been performed.
According Soderquist C and Bagg A., to make the diagnosis of elliptocytosis, blood smear must be make in front of any anemia (7). They claim that there are asymptomatic forms, what must be done for these cases? We believe that a blood smear must be done systematically, especially in the presence of an ethnic group or according to the origin of the patient.
We have searched for biological factors of gravity such iron deficiency and vitamin deficiency that can explain the exaggeration of the Erythrocyte fragmentation. Patient number 2 has a deficiency martial with normocytic hypochromic regenerative anemia due to the Association of hemolysis. The patient number 7 has a deficiency martial with microcytic hypochromic arégénérative anemia which could increase the fragmentation. The patient number 6 has an iron deficiency with a microcytic hypochromic regenerative anemia with hemolysis and negative direct Coombs test. In addition to the

iron Deficiency, the microcytosis and the hypochromic could be related to a pyropoïkilocytose because of the schizocytose (8) or the association of a thalassemia. The iron deficiency decreased the synthesis of the hemoglobin A2. The results of the chromatography of patients 2.6 and 7 have not allowed us to exclude a thalassemia. Patients 3 and 4 were not of iron deficiency but had the same images of fragmentation that patients 2 ,6 and 7. It is to say that the iron deficiency is not the cause of the increase of the fragmentation of the erythrocytes of patients. The dry smear of the patient number 8 has presented rare poïkilocytes without elliptocytes while its curve of ektacytométrie has been that of a simple elliptocytose. A reading in phase contrast after a fixation by glutaraldehyde should have been made before to say the absence of elliptocytes (9)

The entire erythrocyte fragmentation at 37°C in the stove for 24 hours, we did not use the specific test for the study of the thermal stability of the membrane (10) Patients 2,5,6 have presented curves of ektacytométrie whose characteristics have been the following: the osmolarity of the hypo points were lower for the 2.5 and higher for the 6 compared to the normal. The stretch was at all points lower. The osmolarity to stretch were collapsed for the maximum stretch, the ISO point and hyper. The image of the N° 6 with an osmolarity greater than normal in point Hypo and a curve not so much as a trapezoidal the classical form, suggested a possible new form of elliptocytosis. The image of the curve of the patient N°1 has the same look like that of the patient N°. 5, but with values slightly above. The curves of the patients 3 and 7 are almost superimposable. Everything is collapsed except the osmolarity of the hypo point which was 145 while normal was to 138. The ISO point, the stretching is almost nil for the same osmolarity. The image of these two curves [2 and 7] is that described for the pyropoïkilocytose.
What has preceded, there is no correlation between the appearance of the dry smear and the ektacytometry in osmolaire gradient. The dry smear may not submit to elliptocytes and the ektacytometry highlights a curve of elliptocytosis. The aspect of the smear of several patients may be identical and the ektacytometry curves can be different: simple elliptocytose or pyropoïkilocytose.
In the hereditary elliptocytosis, there is a non-significant reduction in the intensity of fixing the eosin 5' maleimide to the band 3, that is to say less than 16% compared to that of the normal. (11). The Patient 1 presented a profile compatible with a simple elliptocytosis while the patients 2, 3 and 6 who have presented the most significant decreases of fluorescence (-15 and -14), had a profile compatible with a hereditary pyropoïkilocytose responsible for the increase of the fragmentation and this in a context of iron deficiency likely. It is to say that the test to the EMA only does not affirm the diagnosis of elliptocytosis when the decrease does not reach the defined threshold. When the decrease of the mounting reaches or exceeds the defined threshold, it means an association spherocytosis elliptocytose (11).
The profile of the other patients was compatible with a hereditary elliptocytosis associated with an alpha thalassemia. The context of work has not allowed us to make a myelogram to search for erythroblasts laminated glass which are elements of a suspected alpha thalassemia. The study of the alpha locus has not been achieved.
No sign of myélodysplasies has been found on the smears colorful devices to the gems of the patients. On the smears of the father of the siblings and children of patients 2 and 4, of elliptocytes were found respectively to rate of 10% and 25%. The patient N°.2 which was an Officer of Health, said present its signs since his young age. This has allowed us to eliminate the character acquired of their elliptocytosis (12,13)
In this siblings, the clinical presentation and the Biological phenotype is heterogeneous, reflecting a genetic heterogeneity. The electrophoresis of the membranes has not been carried out, but according to the literature, the violations of the protein 4.1 is to eliminate because it is specific to Caucasians and those of the spectrin Beta is rare. The deficit of the spectrin Alpha is the one who is encountered among the blacks in Africa (8.9)
By order of frequency, this family would be the carrier of a deficit spectrin Alpha. The Hereditary elliptocytose is due to a mutation heterozygous and the pyropoïkilocytose to a mutation homozygous or heterozygous composite. The composite heterozygosity could be due to two different mutations responsible for simple elliptocytosis one of which can be the alpha mutation Lely. The mutation alpha Lely can be null that is to say non- productive alpha channels and in this case the phenotype would be a pyropoikilocytosis or insufficiently productive and the phenotype would then be a simple elliptocytosis. The phenotype could therefore be a pyropoikilocytosis or a simple elliptocytosis in the case of composite heterozygosity with the alpha mutation Lely (14, 15, and 16).
The distribution of alpha channels being ubiquitous, (3) the amputation of the visual field among women of the siblings could be due to the influence of the mutation on another alpha gene located in the brain or is this a fortuitous association. If this is due to the influence of the mutation on the brain or to an influence on another alpha gene located in the brain, why it is among women only that this translates clinically. We have not identified the mutations responsible needed to make a good genetic Council. What are the shortcomings that we encounter in our context of work?

Conclusion

The diagnosis of hereditary elliptocytose is oriented guided by the blood smear, specified by the reading in phase contrast and confirmed by the ektacytometry and other examinations in the best case. The molecular biology gives the molecular lesion which allows a prenatal diagnosis and genetic counseling to avoid the complications of this erythrocytopathy.

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