POSTERS

BONE MARROW NECROSIS IN PATIENT WITH COMPOUND HETEROZYGOSITY FOR HBOARAB/HBS

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Introduction. Hemoglobin O_{Arab} is characterised by the substitution of lysine for glutamic acid at position 121 of the beta globin chain. It can be identified by electrophoresis on citrate agar at acidic pH. Diagnosis is confirmed with analysis by High-performance liquid chromatography (HPLC) or molecular tools using PCR and sequencing. Patients homozygous for O_{Arab} are clinically asymptomatic with a mild compensed hemolytic anemia. Heterozygotes are clinically and biologically asymptomatic. The association of O_{Arab} with HbS leads to a severe sickle cell disease comparable to Hb SD disease.
Case report. A 39-year-old Algerian man was admitted for hyperalgid lumbago and bilateral gonalgia with fever. Blood numeration revealed a mild microcytic regenerative anemia with signs of hemolysis without inflammatory syndrome. Blood smear showed target cells and poikilocytosis. Thoracic-abdominal-pelvic scan revealed a splenic atrophy. Bone marrow aspiration was totally necrotic. A total body RMI showed anomalies compatible with a sickle cell disease and Hb analysis using HPLC revealed the presence of hemoglobin S and OArab in the same proportion (40%). Molecular biology confirmed the Hb variant. The evolution was favourable after treatment by hydroxyurea.
Discussion. The usual severity of the association O_{Arab}/HbS is explained by three mechanisms: a stabilizing role of residue beta 121 of O_{Arab} in the HbS polymer structure, an increased RBC density possibly by alteration of cation transport leading to a dehydration of red cells and a lower oxygen affinity of erythrocytes containing both HbS and O_{Arab}. The history of this patient is badly known. He presents a mild clinical form of the disease, without clearly identified cause: its rate of HbF (7,6%) is relatively low to be protective; the presence of an associated alpha-thalassaemia was eliminated by molecular biology. He remains however at risk for the major complications associated with the sickle cell disease as testifies this episode of extensive bone marrow necrosis.

ERYTHROCYTE MEMBRANE PROTEINS ANALYSIS BY THE BIO-RAD'S EXPERION? PRO 260 AUTOMATED CAPILLARY ELECTROPHORESIS SYSTEM IN THE DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

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Aim of the study. The aim of our study was to evaluate the separation and quantification of the erythrocyte membrane proteins provided by the Experion instrument (BioRad), in order to detect protein deficiencies related to hereditary spherocytosis (HS). The Experion is an automated capillary gel electrophoresis (CGE) system using the microfluidics technology (Lab-on-a-Chip® Technology) to separate and analyse protein samples. Gel components and analytical conditions are constant. A fluorescent dye is incorporated into the gel and allows the proteins detection. At present, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is the method of choice to confirm the diagnosis of HS. However, this method is uneasy to implement, time-consuming and quite imprecise regarding quantification, which leads to a lack of diagnosis sensitivity.
Results. The major red cell membrane proteins (actin, protein 4.2, protein 4.1, band 3, ankyrin, α- and β-spectrin) were extracted and purified from membrane ghosts by centrifugation, immuno-precipitation and electro-elution. Their analyses were carried out by SDS-PAGE and CGE which allowed to establish a separation profile of the total ghosts by CGE. The best analysis conditions (no saturation, optimal peak resolution) were obtained thanks to successive dilutions of ghosts samples. Reproductibility (n=9) ranged from 6 to 20% for the proteins separated by CGE and from 6 to 60% by SDS-PAGE. Usual values, based on 12 healthy patients, gave an estimation of normal values but optimal reference values for a specific patient are the ones obtained for his parents/siblings as for the SDS-PAGE carried out in laboratory.
Conclusion. The separation and quantification of five major erythrocyte membrane protein fractions (actin, protein 4.2, band 3, α- and β-spectrin) were achieved with the Experion™ Pro 260 electrophoresis system. This system has an analytic sensitivity similar to the SDS-PAGE method but is easier to use and requires less time (3 hours for 10 samples instead of 2 days). Experion might thus be a very interesting diagnostic tool to detect membrane proteins abnormalities.

G6PD DEFICIENCY IN A DIABETIC PATIENT WITH A TURNER SYNDROME

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G6PD deficiency is a genetic disorder linked to X chromosome (Xq28); males and homozygous females express complete deficiency, while in heterozygous females, enzymatic expression is variable and dependent on X lyonisation. We report a case of a Mauritanian G6PD deficient diabetic patient with a Turner syndrome.

Patients and Methods. D.C. a 13 year-old girl was investigated for short stature and growth velocity decrease, obesity, delayed puberty. The assessment found low growth hormone level, hypogonadism, normal thyroid state, noninsulin-dependent diabetes. A karyotypic analysis, completed by fluorescent in situ hybridization (FISH) was performed, as well as HbA1c determination, study of hemoglobin (Hb) and G6PD activity assay. The sequencing of G6PD gene exons was carried out on PCR products by ABI 3130.

Results. Cytogenetic analysis showed a 46,X,i (X)(q10) karyotype corresponding to an isochromosome for the q arm of X chromosome. HbA1c level (5.4%; N: 4-6) was discordant with the patient glycemic status. G6PD activity was reduced (<1UI/g Hb; N≥8). Hb profile was normal. G6PD gene sequencing showed two mutations 376G/202A (African variant A-) and 949A (G6PD Kerala-Kalyan, Indian variant).

Discussion. In this African diabetic patient, HbA1c level shows a discrepancy with its glycemic state and has prompted us to seek a cause of underestimation of the Hb glycation, explained by high G6PD deficiency. Because of the presence of an isochromosome Xq and a normal X chromosome (trisomy q arm and monosomy for p arm), the patient presents 3 G6PD deficient genes including two identical on one chromosome, situation compatible with both mutations identified and enzyme activity. HbA1c level, an index of diabetes follow-up, has to be used with caution in this G6PD deficient diabetic patient.

SOCIAL ENVIRONMENT AND EDUCATION OF CONGOLESE SICKLE CELL DISEASE PATIENTS: A SURVEY IN KINSHASA AND LUBUMBASHI

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We report the case of 288 patients with sickle cell disease (SCD) followed up clinically in Kinshasa (two groups of 92 and 116 patients) and in Lubumbashi (80). The mean age was of 11.4 years (6-20) and the sex ratio 1.2. Most of them (58,6%) was from a family with 6 to 10 members and live in houses of 2-3 compartments and without ceiling. Two or more children use the same bed and only 11.2% use mosquito nets impregnated with insecticide. Most of them go to school on foot, 14.3% of them do it for more than 3 km. These conditions of the patients with SCD, associated to the clinical aspects affect negatively their school performances. Indeed, 86,3% in Lubumbashi and 65,2% in Kinshasa have not finished the elementary school and only 1,1% arrived at the university. Actually, the program of community education coupled with the early medical care seems to contribute to a better school application of the affected infants and allow them to be accepted in the society.

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SICKLE CELL DISEASE: THE BENIN EXPERIENCE

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Introduction. With 7 millions inhabitants, 3% population growth, Benin is considered as the most affected by hereditary hemoglobinic abnormalities amongst all Benin Gulf countries. Hemoglobin (Hb) Hb S prevalence is 24% versus 9% for Hb C. The rate of sickle cells disease (SCD) in the population is 4%.

Management of the disease. SCD management began in the seventies in free out-patients consultations. Antalgics, antipyretics, non steroid anti-inflammatory drugs, antibiotics, blood transfusion, folic acid supplementation and hydratation are usual treatments. SCD management got improvement since the National University Hospital (CNHU) created the Haematology Service in 1988. The SCD patients represent 65% of the work load and they consume 35% of blood collected by the Cotonou blood transfusion centre. Out of 519 patients hospitalized in two years, 63.6% were Hb SS and 14.8% Hb SC. Hb SS subjects were mainly hospitalized for anaemic syndrome (40.6%), hyperalgic attacks (34.5%) and infections (20.9%). Sustained follow-up increased patients'life quality. This results in a need of addressing their training, occupation and marriage issues. In 1991, patients were charged for the medical consultation; then paying professional services progressively reduces (60%) the Haematology Service attendance rate from 1991 to date.

Research. Traditional medicine, assessed through phytotherapy, seems to be only symptomatic. Other research topics concern psychosocial aspects, blood transmitted diseases, chronic complications, feasibility of physical activities as integration and self esteem factors. Documents are available to support fair information on SCD. SCD financial burden has been evaluated. A SCD Centre for children and pregnant women (1990) and a National Programme are available.

Associative activities. Associative activities are implemented to raise solidarity among patients, their relatives and the population. It's the main goal of Benin Sickle Cells Association (Association Béninoise de Lutte contre la

Drépanocytose, ABLD) created in 1990 of which some achievements are: National Days of fight against SCD, information and systematic SCD screening campaigns, contribution to the federation of the National Sickle Cells Diseases Associations in Africa in 1996, called FALDA (Federation des Associations de Lutte contre la Drépanocytose en Afrique) including 14 countries. Lack of resources is the main bottleneck of ABLD. Conclusion. Sickle cells disease is still deadly. The only alternative in the absence of curative care is a precocious multidisciplinary and decentralized management of the disease and a tight follow-up of the patients.

SPLEEN SEQUESTRATION IN CHILDREN WITH SICKLE CELL DISEASE

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Introduction. Acute spleen sequestration is a major cause of morbidity and mortality in children with sickle cell disease. The aim of this study was to evaluate prevalence and risk factors of splenic sequestration in a cohort of Tunisian patients with sickle cell disease.
Patients and methods. We performed a retrospective study including 45 children among 194 patients with sickle cell anemia (20 homozygous SS, 25 double composite heterozygous S-β thalassemia. We defined splenic sequestration as a an acutely enlarging spleen over 2 cm of steady state measurement with a fall in haemoglobin concentration over 20% of steady state with evidence of marrow activity (reticulocyte number >200000/mm^3). All patient aged under 5 years was placed on short term transfusion program. Total surgical removal of the spleen was recommended after 2 acute splenic sequestrations and over the age of 5 years.
Results. The prevalence of splenic sequestration found in our study was 23%. The mean age and hemoglobin concentration during the first episode were respectively 41months (range 3-124 months) and 4.9 g/dL (range 2.5-6.5 g/dL). Concomitant infection or painful episode were found in 7cases (15%). 31 patients presented more than two episodes of spleen sequestrations (69%). 6 patients were placed on program of monthly transfusions (13%). Among these 6 patients 2 patients had at least a recurrence of splenic sequestration. Splenectomy was performed in 29 patients at a mean age of 5 years.
Conclusions. Splenic sequestration is a common finding in children with sickle cell disease. Surgical removal of the spleen remains the radical treatment after the age of 5 years.

DELAYED PUBERTY IN TUNISIAN THALASSEMIC PATIENTS

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Introduction. Delayed puberty in patients with thalassemia major has become an issue of interest in the last few years since life expectancy was increased by hypertransfusion and iron chelating therapy. The aim of this study was to evaluate the prevalence, risk factors and causes of delayed puberty in a cohort of Tunisian transfusion dependant thalassemic patients.
Patients and methods. Forty six patients (25 males, 21 females) took part in the study. For each patient were identified: Haematological parameters (age at first blood transfusion, first iron chelation, mean ferritinemia values).Blood sample was obtained from each patient in order to measure glycaemia, T_4, PTH, IGF_1 and hypothalamic-pituitary-gonadal function. MRI study was performed to evaluate iron overload in hypothalamic-pituitary area. Statistical analysis was carried out by using χ^2 method and Fisher's exact test.
Results. Among the 46 studied patients, 23(50%) developed delayed puberty. Delayed puberty is more frequent in boys: 15 of 25 boys (60%) than in girls: 8 of 21girls (38%). Spontaneous induction of puberty was observed in 11 cases (4 females and 7 males). Hypogonadism was found in 9 patients. The risk factors for delayed puberty found in this study group were age of first iron chelation (p=0.002) and ferritinemia level (p=0.03).
Conclusions. Delayed puberty is very common in polytransfused thalassemic patients. Hypogonadism is the most frequent complication. Iron overload has been considered to be the major cause. It is mandatory to carefully monitor the pubertal development of these patients to detect abnormalities and to initiate appropriate iron-chelating agents before referring to substitutive hormones.

IDENTIFICATION OF A NOVEL DELETION-INSERTION IN THE β-GLOBIN GENE EXON 1 AT THE ORIGIN OF A MINOR β-THALASSAEMIA

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A minor β-thalassemia was diagnosed in a patient from Algeria in 1978. At the time, he presented a splenomegaly and a polycytemia (7,23.1012/L) without anemia (Hb 14 g/100 mL). There existed a microcytosis (61 fl) and a clear hypochromy (19.4 pg). Sideremy was normal. Hemoglobin electrophoresis of this patient showed the profile of a β thalassemia hétérozygote A/β^0: Hb

A_1=83.6%, Hb A2=5.1% et Hb F=11.3%. Sequencing of the β-globin gene reveals a novel mutation in exon 1. It is a 9 bp deletion of codons 2-5 (-AC CTG ACT C) associated to a 31 bp insertion at codons 10-11 (+TGA GGA GAA GTC TCC TGA GGA GAA GTC TGC C). Study of hemoglobin was realized in all the family. Only one girl among the five children presents the same mutation. This new mutation is almost similar in its structure to a mutation previously described (Novel and unusual deletion-insertion thalassemic mutation in exon 1 of the β-globin gene, C Badens *et al.* 1996). Only one nucleotide differs between this mutation described in 1996 and the mutation of this patient. The site of the 31 bp insertion is also different between the two mutations: between codons 4 and 5 in the mutation described by Badens *et al.*, and between codons 10 and 11 in our study.