ORIGINAL ARTICLE

Biological particularities of sickle cell syndrome
Particularités biologiques des syndromes drépanocytaires majeurs

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Résumé

Introduction

Les syndromes drépanocytaires majeurs représentent un problème de santé. Dans ce travail, nous nous proposons d'étudier les particularités biologiques de 66 malades en phase stationnaire.

Patients et méthodes

C'est une étude prospective, descriptive et transversale réalisée sur 66 patients présentant un syndrome drépanocytaire majeur en phase stationnaire (36 malades S/S, 18 malades S/β-thalassémiques, 7 malades S/C et 5 malades S/OArab) sur une période de deux ans (Janvier 2018-Décembre 2019) suivis à la consultation externe d'hémoglobinopathies de l'hôpital d'enfants de Tunis. Chaque patient a bénéficié d'un bilan hématologique et biochimique.

Résultats

La moyenne d'âge de notre population est de 15.5 ± 8.4 ans. L'anémie est normocytaire normochrome chez les S/S, S/OArab, et S/C et elle est microcytaire hypochrome chez les S/β-thalassémiques. L'hyperleucocytose et la thrombocytose ont été observées chez tous les patients à l'exception des patients S/C. Le taux de l'Hb F est relativement élevé chez notre population. L'hémolyse a été rapportée chez tous les malades mais de façon modérée chez les S/C avec une augmentation de la bilirubine totale et directe, de l'activité de la LDH et de l'ASAT qui sont plus marquées chez les S/S.

Conclusion

Notre étude a conclu que les données biologiques sont différentes d'un type de syndrome drépanocytaire à un autre ; le phénotype S/C étant la forme la mieux tolérée.

Mots clés : *Syndromes drépanocytaires majeurs, Signes biologiques, hémolyse.*

Abstract

Introduction

Sickle Cell Syndrome is a real health problem because of its frequency and chronic evolution. In the present work, we propose to study the biological particularities of a population of 66 patients.

Patients and methods

This is a descriptive and cross-sectional prospective study carried out on a population of 66 patients with major sickle cell disease in the steady state (36 S/S, 18 S/β- thalassémics, 7 S/C and 5 S/OArab) over a two-year period (January 2018-December 2019) followed at the outpatient haemoglobinopathy clinic of the children's hospital in Tunis. Each patient was given haematological and biochemical test.

Results

The population studied is composed of 66 patients with sickle cell syndrome with an average age of 15.5 ± 8.4 years. The anemia is normocytic normochromic in S/S, S/OArab and S/C. On the other hand it is microcytic hypochromic in S/β-thalassemics. Hyperleukocytosis and thrombocytosis were found in all but not in S/C patients. The rate of HbF is relatively high in our population. Hemolysis has been reported in all phenotypes but moderately in S/C patients with increases in total and direct bilirubin, LDH activity and AST activity which are more marked in S/S patients.

Conclusion

Our study concluded that the biological data are different from one type of syndrome to another; the S/C phenotype being the best tolerated form.

Key Words: *Sickle Cell Syndrome, Biological Signs, Hemolysis.*

INTRODUCTION

Sickle cell syndrome (SCS) is an autosomal recessive genetic disorder. They represent a real health problem due to their frequency and chronic evolution [1]. SCS include: homozygous sickle cell disease βS/βS and double heterozygous βS/βX; it is the association of the heterozygous state of hemoglobin S with a lesion of the other gene β (β- thalassemia or other abnormal hemoglobins, hemoglobin C, hemoglobin OArab...). Hemoglobin S (HbS) is the result of a single point mutation in the 6th codon of the β gene located on chromosome 11. It is due to the replacement of glutamic acid by a valine. Hemoglobin C results from a point mutation leading to the replacement of glutamic acid by lysine at position 6, while hemoglobin OArab results from the replacement of glutamic acid by lysine at position 121 on the same chromosome. As for the β-thalassemia trait, it results from a broad mutation spectrum that can reach more than 200 worldwide. In Tunisia, 31 mutations have been identified, the most frequent of which are: cd 39 C/T and IVSI-110 G/A [1, 2].

Sickle cell disease is particularly frequent in Sub-Saharan Africa, around the Mediterranean (Maghreb, Southern Italy, Greece), in the Middle East, on the Indian continent and in the migration areas of all these populations [3]. The homozygous S/S form accounts for about 70% of SCS. The S/β-thalassemic form accounts for 15% of SCS [4]. It is the most common form in the eastern Mediterranean and in India [5,6]. The S/C form presents 25 to 30% of cases of sickle cell disease of African origin [3,7]. The S/OArab form is an infrequent form, found in North Africa, the Middle East and the Balkans [5].

In Tunisia, the most frequently encountered haemoglobin abnormalities are β-thalassaemia with a prevalence of 2.21% and sickle cell disease with a prevalence of 1.89%. While Hb C and Hb OArab are rarer, their prevalence is around 0.37% [6].

SCS predominate in North-Western Tunisia (59.7%) and North-Eastern Tunisia (19.46%). Relatively high rates have been recorded in the South-West (6.9%) in Kebeli but especially in Tozeur [8].SCS are characterized by biological variations responsible for great heterogeneity in their phenotypic expressions, with only 15% of patients developing severe disease [9].In the present work, we propose to study the biological variations in a population of 66 patients with SCS in the steady state.

PATIENTS AND METHODS

This is a descriptive and cross-sectional prospective study conducted on a population with SCSin the steady state over a two-year period from January 2018 to December 2019.

Patients

Our study population consists of 66 patients with SCS in the steady state, followed regularly during quarterly visits to the outpatient haemoglobinopathy clinic at the Bechir Hamza Children's Hospital in Tunis. The steady state is defined by the absence of any fever, vaso-occlusive attacks or hemolysis crises. They are divided into 36 homozygous sickle cell patients S/S, 18 patients S/β-thalassemic, 7 heterozygous composite S/C patients and 5 heterozygous composite S/OArab patients.

Methods

Epidemiologic data : The current age, age of diagnosis, origin and inbreeding were collected from the patients' medical records.

Haematological study : Each patient was given an EDTA tube sample for complete blood count (CBC) and a hemoglobin study.

The CBC was performed by flow cytometry on an automated Beckman LH750TM Hematology analyzer (Bechman Miami, FL, USA). Hemoglobin fraction analysis was performed by High Performance Liquid Cation Exchange Chromatography (HPLC) using the Variant II Hemoglobin Analyzer (Bio-rad Laboratories, Hercules, CA, USA).

Biochemical study : five milliliters of whole blood was collected in heparin tube to measure biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total bilirubin (TB), direct bilirubin (DB), haptoglobin, iron, and creatinine. Microalbuminuria was measured on 24 hoursurine . These parameters were measured on the Cobas c501 analyzer. Ferritin was determined by electrochemiluminescence (ECLIA) on the Cobas e411 analyzer.

Genotyping data: The molecular study of the β gene was used in the case of a differential diagnosis between homozygous and S/β-thalassemic, in the absence of a family hemoglobin study.

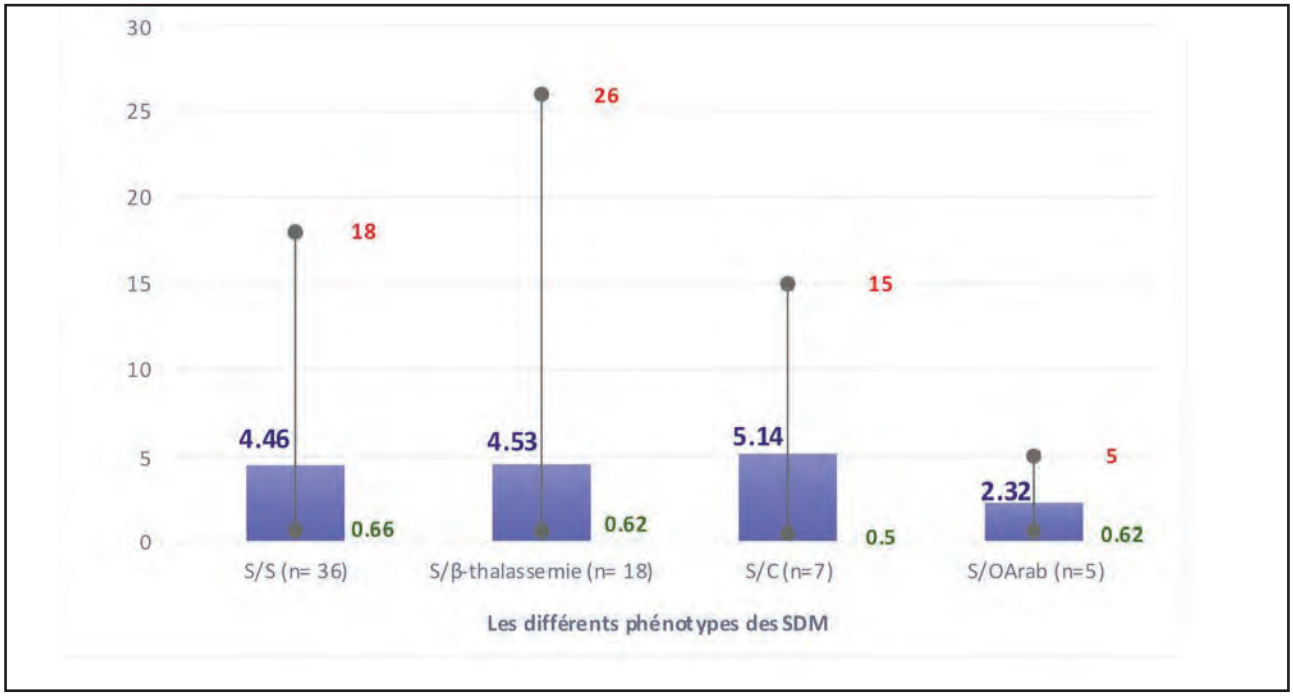
Statistical analysis : The data collected was processed using SPSS software. version v20.0 (Statical Package for Social Sciences). Results for quantitative variables are expressed in terms of means ± standard deviation. If the standard deviation is greater than the mean, the results are expressed in terms of the median [minimum-maximum].The STUDENT t-test was used to compare the means of the quantitative variables. Comparison of the means of the different variables with small numbers ($n<10$) was tested by analysis of variance (Mann Whitney). The significance level for the statistical tests was set at $p < 0.05$.

RESULTS

Epidemiological characteristics

Our population includes 66 patients, 39 (59%) female and 27 (40%) male, for a sex ratio of 0.69. The average age is 15.5 ± 8.4 years with ages ranging from 1 year to 49 years. In this population, 36 patients (55%) come from a consanguineous marriage and 35 patients (53%) have siblings with SCS. The majority of patients are from the North-West of the country (80%).The average age at diagnosis is 3.8 ±2.6 years, ranging from 7 months to 23 years. Thirty three of our patients (50%) were diagnosed before the age of 2 years. Among them, 25 patients (37.87%) (14 S/S patients, 7 S/β-thalassemics, 2 S/C patients and 2 S/OArab patients) were diagnosed before the age of one year, of which 10 patients were identified by neonatal screening. The average age of diagnosis of SCS patients by phenotype is shown in Figure 1.

Figure 1 : Age of diagnosis of SCS **patients by phenotypeIn blue :** Average age of diagnosis, **In green** : Minimum age of diagnosis, **In red :** Maximum age of diagnosis, **n :** Number of patients



Biological data

The sample was taken during the steady state without any vaso-occlusive crisis or infection.

Hematological parameters

The hematological data include the study of the hemogram and analysis of hemoglobin fractions.The values retained for the CBC is the average over the last year. The hemogram data are shown in Table I. For each patient, the hemoglobin fractions were studied at the time of diagnosis. The results of this study are reported in Table II. The Hb F level in patients whose age at diagnosis was under or equal to 5 years (n=50) is 21.7±15.61% while the Hb F level in patients whose age at diagnosis was upper than 5 years (n=16) is 14.28±9.69% (p=0.0928).

Biochemical parameters

The biochemical parameters for hemolysis and cytolysis in SCS patients are shown in Table III.The mean sideremia in SCS patients is normal (16.11±5.64 g/L). However, hyposideremia was noted in 8 patients (6 S/S patients and 2 S/β-thalassemic patients). The median ferritinemia is normal 124.6 µg/L [11.85-3752] in SCS patients. Hyperferritinemia ranging from 394.2 to 3752 µg/L, was noted in 7 patients (4 S/S patients and 3 S/β-thalassemic patients). No pathological plasma creatinine levels were observed (mean=33.22±10.03 µmol/L). Microalbuminuria (> 30mg/24h) was reported in 17 patients SCS (10 S/S patients and 7 S/β- thalassemic patients).

Table I: Hematologic Parameters of SCS Patients

	S/S	S/β-thalassemia	S/C	S/O_{Arab}	P
Basic hemoglobin (g/dL)	8±1.1	8.2±1.2	11±1.4	9.4±1.2	P1 : 0.26 **P2: 0.022** **P3: 0.028** **P4: 0.015**
MCV (fl)	90.6±10.6	69.8±5.1	81.1±4.4	85.6±9	P1: 0.12 **P2: 0.033** **P3: 0.017** P4: 0.187
MCH (pg)	30.8±4	22.5±1.2	27.7±2	30±3.3	P1: 0.237 **P2: 0.019** **P3: 0.037** P4: 0.67
MCHC(%)	33±1.7	30±2.3	34.7±1.2	34.6±0.8	P1: 0.345 P2: 0.001 P3: 0.002 P4: 0.55
Hematocrit (%)	23.6±5.7	26.1±3.2	32.8±3.8	27.8±3.5	P1: 0.236 **P2: 0.014** **P3: 0.004** **P4: 0.048**
Red cells (x1012/L)	2.8±0.6	4±0.8	4±0.5	3.1±0.2	**P1: 0.029** **P2: 0.041** P3: 0.567 P4: 0.191
Leucocytes (elements/mm3)	13 598 ± 3 200	11 100 ± 4 600	7 775 ± 2 800	14 00 ± 2000	**P1: 0.007** P2: 0.467 P3: 0.17 **P4: 0.0221**
Platelettes (103/µL) *150-400 103/µL*	470 ± 172	400 ± 210	205.2 ± 94	480 ±140	P1: 0.69 **P2: 0.012** **P3: 0.048** **P4: 0.042**

MCV: Mean Corpuscular Hemoglobin; **MCH:** Mean Corpuscular Hemoglobin Content;
MCHC: Mean Corpuscular Hemoglobin Concentration.

P1 = Significance rating of the comparison of means between the **S/S** and S/**β-thalassemia** groups
P2 = Significance rating of the comparison of means between **the S/S and S/C** groups
P3 = Significance rating of the comparison of means between the **S/β-thalassemia** and **S/C** groups
P4 = Significance rating of the comparison of means between the **S/C and** S/O_{Arab} groups

Table II : Data from the study of hemoglobin fractions of SCS patients practiced at the time of diagnosis.

	S/S	S/β-thalassemia	S/C	S/OArab	P
Hb A2 (%) Mean ± standard deviation	3±1.7	4.3±1.2	2.7±0.7	2.2±0.6	P1: 0.23 P2: 0.14 **P3: 0.017** P4: 0.489
Hb F (%) Mean ± standard deviation	15.1±8.1	20.3±10.1	10.8 [5.7-36.7]	20.7±12.9	**P1: 0.037** P2: 0.65 P3: 0.015 P4: 0.847
Hb S (%) Mean ± standard deviation	75.8±10	70±10.5	43.7±10	31.6±11.3	P1: 0.297 P2: 0.34 P3: 0.71 P4: 0.567
Hb C (%) Mean ± standard deviation	--	--	42.2±6.1	--	
Hb OArab (%) Mean ± standard deviation	--	--	--	35.2±8	

P1 = Significance rating of the comparison of means between the **S/S** and **S/β-thalassemia** groups
P2 = Significance rating of the comparison of means between the **S/S** and **S/C** groups
P3 = Significance rating of the comparison of means between the **S/β-thalassemia** and **S/C** groups
P4 = Significance rating of the comparison of means between the **S/C and S/O$_{Arab}$** groups

Table III : Biochemical Variations of Cytolysis and Hemolysis in SCS Patients

	S/S	S/β-thalassemia	S/C	S/OArab	P
AST (UI/L) Mean ± standard deviation	40,04±13.88	30,46±13.1	23,98±9	37,95±6.24	**P1: 0.008** **P2: 0.019** P3: 0.254 **P4: 0.042**
ALT (UI/L) Mean ± standard deviation	19,44±8.53	15,95±7.8	13,03±3.8	14,35±2.11	P1: 0.13 **P2: 0.048** P3: 0.44 P4: 0.448
BT (µmol/L) Mean ± standard deviation	47,29±34.59	33,74±18.1	25,26±15.78	44,12±27.42	P1: 0.12 **P2: 0.012** P3: 0.25 P4: 0.23
BD (µmol/L) Mean ± standard deviation	11,69±3.59	9,45±2.88	8,54±2.53	8,27±2.26	P1: 0.056 **P2: 0.027** P3: 0.4 P4: 0.84
LDH (UI/L) Mean ± standard deviation	496,09±196.95	444,85±244.34	252,14±76. 54	460,5±176.04	P1: 0.24 **P2: 0.0005** **P3: 0.018** **P4 : 0,1**
Haptoglobin (g/L) Mean ± standard deviation	0.088±0.052	0.11±0.054	0.074±0.04	0.082±0.043	P1: 0.17 P2: 0.497 P3: 0.114 P4: 0.759

P1 = Significance rating of the comparison of means between the S/S and S/β-thalassemia groups
P2 = Significance rating of the comparison of means between the S/S and S/C groups
P3 = Significance rating of the comparison of means between the S/β-thalassemia and S/C groups
P4 : Significance rating of the comparison of means between the S/C and S/OArab groups

DISCUSSION

1. Epidemiological characteristics

The age of our study's population ranges from 1 to 49 years with a median of 16 years. Patientsover the age of 18 years refuse to be transferred to adult haematology consultations and continueto be followed in our consultation by the same medical and paramedical staff with whom they have established a relationship based on trust and understanding, thus emphasizing the psychological aspect in the management of SCS. The study of the geographic origins of our patients has shown that the North-West region (80%) of the country, is the most concerned.

This predominance of SCS in North-West of Tunisia has been reported in the literature [6]. Only 3 SCS patients are from the South, however, this number does not reflect the incidence of sickle cell disease in this region. In fact, a relatively large number has been recorded in the South-West at the cities of Kebeli and Tozeur [8].

This discrepancy can be explained by the fact that these patients are followed either in their localities or at the University Hospital Hedi Chaker of Sfax (CHU), a town in the South East of Tunisia. Inbreeding increases the risk of sickle cell disease. It is about 55% in our cohort whileconsanguinity in the Tunisian population is 32% and can reach up to 60% in rural areas [10]. This result is close to the rate reported in the Tunisian study by Mseddi S (44%) [8]. In our study, the number of patients with affected siblings is relatively high (n=35) at 53%. This is due either to a lack of knowledge of the disease in the first affected child, or to genetic counselling not received or poorly assimilated, and therefore prenatal diagnosis (PND) not carried out, or to the refusal of therapeutic termination of pregnancy (TTP) in case the foetus issick [11]. The average age of diagnosis in our population is 3.8 years. This result is similar to that found in a study conducted in the Congo, which reports a mean age of diagnosis equal to 3.2 years [11]. Half of our patients (50%) were diagnosed before the age of 2 years. This is partly related to the severity of sickle cell disease in Tunisia characterized by a Beninese haplotype in 95% of cases [6]. The family surveys carried out systematically in the siblings of patients already followed and the neonatal screening carried out in 10 of our patients are also responsible for the early diagnosis of the disease. This is also the case for S/C patients diagnosedrelatively early despite having a moderate and well tolerated form and therefore should be diagnosed late.

Biological data

In our study, normocytic normochromic anemia was noted in most SCS patients. In S/S, S/OArab and S/C patients, the anemia was normocytic normochromic with mean baseline hemoglobin levels of 8 g/dL, 9.4g/dL and 11g/dL, respectively. However, the microcytosis seen in these S/S, S/OArab and S/C patients is due to iron deficiency confirmed by martial assessment. The macrocytosis observed in some S/S patients could be explained by hyperhemolysis with associated hyper reticulocytosis, which itself is responsible for folic acid deficiency.

The mean MCV rate of S/S subjects was 90.6±10.6 fl and that of S/C subjects was 81.1±4.4 fl. This difference was statistically significant (p2= 0.033). Indeed, 42.85% of S/C subjects had microcytosis compared to 50% of S/S subjects. Our results are similar to those of Mounkaila [12]. However, anemia in S/β-thalassemics is microcytic hypochromic with a basal hemoglobinlevel of 8.2g/dL. In our study, hemoglobin S/C disease is distinguished from other SCS by moderate, or in some cases absent, anemia (mean baseline Hb of 11 g/dL up to 13 g/dL). These S/C patients have a statistically significant higher baseline hemoglobin level than S/β-thalassemics (p3=0.028), S/O_{Arab} (p4=0.015) and S/S (p2=0.022). This has been confirmed in some studies reporting near-normal hemoglobin levels in S/C patients [10,12,13]. Static phasehyperleukocytosis free of infections was noted in S/S, S/β-thalassemics and S/OArab. It was statistically significant more in S/S than in S/β-thalassemics (p1=0.0075) and in S/O_{Arab} than in S/C (p4=0.042). This hyperleukocytosis is absent in S/C. Similar results have been reported inthe literature [14-17]. Indeed, the mechanism underlying this increase is due to the chronic inflammation that characterizes sickle cell disease, This inflammation promoted by adhesive interactions between various cells and the production of pro-inflammatory cytokines [17]. The platelet count is statistically significantly higher in S/S, S/β-thalassemics and S/O_{Arab} than in S/C with p2=0.0125, p3=0.048 and p4=0.042 respectively. This can be explained in part bythe fact that 16 patients (7 S/S patients and 9 S/β-thalassemics) were splenectomized with thrombocytosis while no splenectomy was reported in S/C patients, most of whom still have splenomegaly responsible for decreased platelet counts [7]. It has been reported in the literaturethat activated platelets secrete the thrombospondin (TSP) involved in red cells-endothelium bypass and participate in

the hypercoagulable state of sickle cell disease contributing to the occurrence of vaso-oclusive crises [18]. SCS are always marked by the presence of Hb S but at variable rates depending on the phenotype. This rate is high in S/S and S/β-thalassemics with average rates of 75.8 ± 10% and 70 ±10.5% respectively.

The average Hb S rate is lower in S/OArab (31.6±11.3%) and S/C (43.7±10%). Hb A2 is normalin S/S, S/OArab and S/C patients, but is elevated in S/β-thalassemic patients due to the presenceof the thalassemic trait. Concerning Hb F and since we only have the values obtained at the time of diagnosis, a non-significant difference in the Hb F rate was observed (p= 0.0928) between patients diagnosed before the age of 5 years and those diagnosed after this age. Indeed,the Hb F rate decreases progressively from birth and stabilizes after the age of 5 years [19].

In addition, relatively high Hb F levels have been observed in the different phenotypes SCS: 15.1±8.1% for S/S, 20.3±10.1% for S/β-thalassemics, 10.8% [5.7-36.7] for S/C and 20.7±12.9% for S/OArab, which can be explained by the fact that 37.87% (n= 25 patients) of ourpopulation was less than one year old at the time of diagnosis. It should be noted that Hb F plays a role in the level of hemolysis and the occurrence of complications of . A high level of Hb F is a protective factor that reduces the onset of painful vasoocclusive attacks, and also reduces the occurrence of leg ulcers, osteonecrosis and acute chest syndrome, thus allowing better tolerance of the disease [20,21]. The highest levels of Hb F were found in S/β-thalassemic patients compared to S/S patients (p1=0.03) and S/C patients (p3=0.015) suggesting that they would have less severe clinical manifestations and complications than the others. These high Hb F levels are inconsistent with the fact that 95% of sickle cell patients in Tunisia have a Beninese haplotype [6] which is associated with a low Hb F level of around 7% [22]. It may be necessary to look for the presence of other genetic markers that have an implication on the Hb F expression rate such as those located at the level of genes: BCL11A, HBS1L-MYB, SAR 1 and γ-globin(XmnI) [23]. Our study shows that the majority of our SCS patients have hemolysis that varies according to genotype with increased levels of total bilirubinemia predominantly unconjugated total bilirubinemia, LDH activity and AST. The total bilirubin level was 25.26 µmol/L in S/C versus 47.29 µmol/L in S/S.

Our results were higher than those of Coulibaly [24] who found 12.1 mg/L in S/S. For free bilirubin, the mean level was 11.69 µmol/L in S/S versus 8.54 µmol/L in S/C in our sudy. Thisstatistically significant difference supports the hypothesis of greater hemolysis in S/S versus S/C subjects. The increase in LDH activity was more marked in S/S (496.09±196.95UI/L) and S/β-thalassemics (444.85±244.34UI/L) than in S/C (252.14±76.54UI/L) with respective p values of p2=0.0005 and p3=0.018. Elevated LDH activity has been shown to be associated with some complications (leg ulcer, priapism and pulmonary arterial hypertension) [25]. AST activity is higher in S/S (40.04±13.88UI/L) than in S/β-thalassemics (30.46±13.1UI/L) and S/C (23.98±9UI/L) with p1=0.0008 and p2=0.019, respectively, but still less than two times normal.The literature has reported a moderate increase of AST activity in SCS patients outside of CVO but is less frequent in S/C [26].

In addition, haptoglobin levels is collapsed in all phenotypes, indicating chronic intravascular hemolysis, which by definition is present in SCS. In our study, microalbuminuria greater than 30 mg/24h was found in 10 S/S patients and 7 S/β-thalassemic patients. Creatinemia was normalin all patients. Indeed, microalbuminuria is systematically performed every year for all our sickle cell syndrome patients in order to detect in time a sickle cell nephropathy. It is an early and sensitive biological marker of sickle cell nephropathy, which prevelance increases with age, thus constituting a chronic and frequent complication of SCS [27]. Mean serum iron and ferritinemia values are normal in all patients. However, hyposideremia was noted in 8 patients(6 S/S patients and 2 S/β-thalassemic patients) related to iron deficiency. The increase in ferritinemia observed in 7 of our patients (4 S/S patients and 3 S/β-thalassemics) is related to blood transfusions.

CONCLUSION

Sickle cell syndrome include homozygous S/S, as well as compound heterozygous forms : S/β- thalassemics, S/OArab and S/C. They are characterized by chronic hemolysis anemia in the steady state, the evolution of which is marked by multiple acute and chronic complications. Ourstudy concluded that the biological data are different from one phenotype to another but always dominated by the anemia which is normocytic normochromic in S/S, S/OArab and S/C patients and microcytic hypochromic in S/β-thalassemics. This anemia is less pronounced or absent in S/C patients. Biological signs of hemolysis were reported in all patients studied, but moderatelyin S/C patients; making this S/C phenotype the best tolerated form.

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