Clinical course of 63 children with hereditary spherocytosis: a retrospective study

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Background: *Hereditary spherocytosis (HS) is an inherited hemolytic anemia that is caused by deficiency or dysfunction of erythrocyte cytoskeletal proteins.*

Aim: *The aim of this study was to describe the clinical course of hereditary spherocytosis in patients treated in the Pediatric Hematology Unit, Hospital das Clínicas, Universidade Federal de Minas Gerais.*

Methods: *Sixty-three under 16-year-old patients with hereditary spherocytosis were retrospectively evaluated between January 1988 and December 2007. Hereditary spherocytosis was diagnosed based on clinical history, physical examination and on a positive osmotic fragility curve. Patients underwent screening for cholelithiasis by ultrasonography. They were classified into three groups: mild, moderate and severe. The events of interest were need for blood transfusion, cholelithiasis, splenic sequestration, aplastic crisis, and splenectomy. Differences between subgroups were evaluated by the two-sided log-rank test.*

Results: *The mean age at diagnosis was 5.2 years and most patients were classified as moderate (54%). Patients with the severe form of the disease were younger (p-value = 0.001) and needed more blood transfusions (p-value = 0.004). Seventeen patients (27%) developed cholelithiasis, 14 (22.2%) splenic sequestration and three (4.8%) aplastic crises. Twenty-two patients (34.9%) were splenectomized with the main indication being splenic sequestration in nine patients (41%).*

Conclusion: *The clinical course of patients with hereditary spherocytosis in this study was relatively benign however cholelithiasis was a common complication.*

Keywords: *Spherocytosis, hereditary; Cholelithiasis; Splenectomy; Child; Retrospective studies*

Introduction

Hereditary spherocytosis (HS) is a common inherited hemolytic anemia that occurs in all racial groups but is particularly common in Caucasians from northern Europe and North America. The estimated prevalence ranges from 1:2000 to 1:5000.[1-3] The underlying primary molecular lesion is heterogeneous; it may involve several erythrocyte membrane proteins with ankyrin being the most commonly affected followed by spectrin, protein band 3, and, more rarely, protein 4.2.[4] The genetic defects identified in HS are encoded by five genes in chromosomes 1, 8, 14, 15 and 17. Approximately 75% of cases display an autosomal dominant pattern of inheritance; the others comprise of recessive forms and *de novo* mutations.[5,6] The deficiency or dysfunction of any one of these membrane components can weaken or destabilize the cytoskeleton whose role is to maintain the shape, deformability and elasticity of the red blood cell. This results in abnormal red cell morphology and a shorter lifespan.[6,7] The clinical manifestations of HS vary widely. The main clinical features are hemolytic anemia - which can range from compensated to a severe degree, sometimes requiring exchange transfusion at birth and/or repeated blood transfusions - variable jaundice, splenomegaly and gallstones.[3-8]

The aim of the present study was to describe the clinical course of HS in patients followed up in the tertiary Pediatric Hematology Center of Hospital das Clínicas, Universidade Federal de Minas Gerais (UFMG).

Methods

Sixty-three patients with hereditary spherocytosis (HS) were retrospectively evaluated. All were under the age of 16 years at diagnosis and were followed at Hospital of Clinics of UFMG from January 1988 to December 2007. HS was diagnosed on the basis of clinical history, physical examination and results of laboratory tests including complete

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blood count, blood smear, reticulocyte count, bilirubin concentration, positive osmotic fragility test and negative direct antiglobulin (Coombs') test. Red cell osmotic fragility was evaluated in tubes containing red blood cells washed in solutions of increasing higher sodium chloride (NaCl) concentrations with and without incubation for 24 hours.[3-9] The eosin-5'-maleimide flow cytometry test is not available in our laboratory.[10] Family studies were carried out for all patients. Follow-ups were scheduled at three-month intervals in our outpatient clinic or more frequently if considered necessary. Folic acid was prescribed to all patients. The clinical presentation at baseline was retrospectively classified as mild, moderate or severe based on the modified criteria of Eber et al.[11] using hematological parameters – hemoglobin (Hb) and bilirubin concentrations, and reticulocyte count. The patients were annually screened for cholelithiasis by ultrasonography starting at the age of 7 years old or at any age if the patient had symptoms of cholelithiasis. Patients submitted to splenectomy received immunization for *Haemophilus influenzae* and *Streptococcus pneumoniae* 15 days before the procedure. Oral or intramuscular penicillin as prophylaxis was recommended up to the age of 18 years old even though some authors recommend lifelong prophylaxis.[12]

The events of interest were transfusions, cholelithiasis diagnosed by the detection of gallstones at ultrasonography, spleen sequestration defined as an increase in the size of the spleen associated with a decrease in Hb and increased reticulocyte count, splenectomy, and aplastic crisis characterized by decreases in Hb concentration and reticulocyte count.

Statistical analysis

Data analyses were performed using SPSS version 11.5. The time to cholelithiasis was evaluated from the date the patient first frequented the hematology service until the date of ultrasound diagnosis. Differences between subgroups were compared by the two-sided log rank test. The cumulative proportion of events over time was estimated by one minus survival curve of Kaplan Meier. Differences in distributions of dichotomous variables were analyzed by chi-square or Fisher's exact test. The Kruskal-Wallis nonparametric test was used to compare values of non-Gaussian variables.

The study was approved by the Research Ethics Committee of UFMG.

Results

Baseline clinical and demographic characteristics of patients with hereditary spherocytosis according to severity classification are shown in Table 1.

The study group comprised 35 males (55.6%) and 28 females (44.4%) belonging to 52 unrelated families. The median age at diagnosis was 5.2 years (range: 5 months to 21

Table 1 - Baseline clinical and demographic characteristics of patients with hereditary spherocytosis according to severity classification (n = 63)

	Mild n = 16 (25.4%)	Moderate n = 34 (54%)	Severe n = 13 (20.6%)	p-value
Gender n (%)				
Male	7 (43.8)	19 (55.9)	9 (69.2)	
Female	9 (56.3)	15 (44.1)	4 (30.8)	0.38
Median age of diagnosis (years)	125.5	61.1	6.4	0.001
Family history n (%)				
Yes	10 (66.7)	19 (57.6)	7 (58.3)	
No	5 (33.3)	14 (42.4)	5 (41.7)	0.86
First case n (%)				
Yes	6 (9.5)	22 (34.11)	5 (7.9)	
No	10 (15.87)	11 (17.4)	7 (11.11)	0.8
Complications n (%)				
Transfusion	4 (25.0)	20 (60.6)	11 (84.6)	0.004
Cholelithiasis	5 (31.3)	11 (32.4)	1 (7.7)	0.2
Spleen sequestration	1 (6.3)	10 (32.3)	3 (23.1)	0.1
Aplastic crisis	0	2 (6.3)	1 (7.7)	0.5
Median age at gallstone detection (years)	11.6	10.66	#	0.2
Splenectomy n (%)				
Yes	3 (18.8)	13 (38.2)	6 (46.2)	
No	13 (81.3)	21 (61.8)	7 (53.8)	0.2

* Modified from references 1 and 11. Hemoglobin concentration was the main criterion for classification into mild ≥ 11 g/dL, moderate 8.1 - 10.9 g/dL, or severe ≤ 8 g/dL hereditary spherocytosis
# only one patient with severe HS had cholelithiasis

years). The median follow-up time was 4.6 years. The main reasons for referral to a specialist service were anemia of undetermined etiology in 16 patients (25%) and anemia that was unresponsive to iron supplementation in 8 (12.7%). The predominant clinical manifestations at diagnosis were splenomegaly in 44 patients (69.8%), anemia in 40 (63.5%) and jaundice in 24 (38%).

A positive family history was recorded for 36 patients (57%; 95% confidence interval: 44.9-69.4%), twenty-two patients (35%) were considered the first case in the family and for five children information was not recorded. Eleven fathers (17.5%) and 14 mothers (22.2%) had positive red cell osmotic fragility tests of which three fathers and three mothers were asymptomatic.

The baseline morphologic examination of peripheral blood smears of 51 patients showed that 23 patients (45%) had spherocytic red cells and the median Hb concentration was 10.0 g/dL. In 51 patients the median reticulocyte count was 6.3%, the indirect bilirubin concentration for 34 patients was 1.9 mg/dL and median lactate dehydrogenase (LDH) concentration was 667 IU for 13 patients.

Clinical characteristics according to the severity of clinical presentation are presented in Table 1. Sixteen (25.4%), 34 (54%) and 13 (20.6%) patients were classified as mild, moderate and severe, respectively. There was no significant difference regarding gender, family history and number of splenectomized patients between the three groups. However, age at diagnosis was significantly different. Patients classified as severe, had a median age at diagnosis of 0.5 years, whereas those classified as mild or moderate were approximately 10 and 5 years old, respectively (p-value = 0.001).

During the follow-up, 17 patients (27%) developed cholelithiasis as identified by gallbladder ultrasonography. The median age at diagnosis of gallstones was 10 years old (range: 4.8-18.3 years). Of these patients, 10 (15.9%), with a mean age of 12 years old, were submitted to cholecystectomy. There was no significant difference between groups regarding the probability of cholelithiasis. As estimated by survival analysis, the probabilities of cholelithiasis at 10 years for children with mild, moderate and severe HS were 15%, 24% and 10%, respectively. At 20 years, with a much smaller number of patients followed for this period, the probability was 40%, 80% and 10% for patients with mild, moderate and severe disease, respectively (Figure 1).

Other frequent complications were splenic sequestration and aplastic crisis in 14 (22.2%) and 3 (4.8%) patients, respectively. Thirty-five patients (55.6%) underwent blood transfusions during follow-up. There were no deaths during the follow-up. The need of transfusions was significantly more common for patients classified with severe disease (p-value = 0.004).

Twenty-two patients (36%) underwent splenectomy with the main indication for the procedure being spleen sequestration in 9 (14%) patients. The median age at splenectomy was 7 years (range: 9.8 months-21 years). The probability of splenectomy at 10 years of age for mild, moderate and severe disease was 9%, 38%, and 55%, respectively (p-value = 0.005). The median age for the severe group was about 6 and a half years old and the moderate, 12 years old (Figure 2).

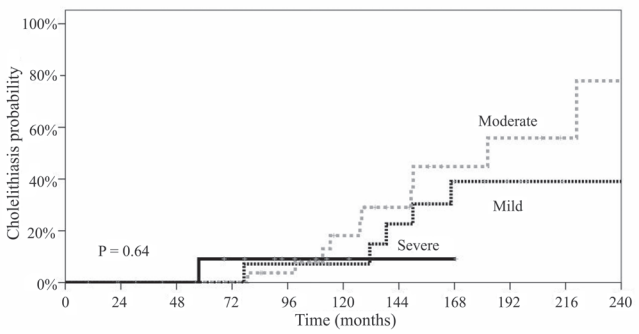

Figure 1 – Probability of having cholelithiasis according to the severity of the presentation

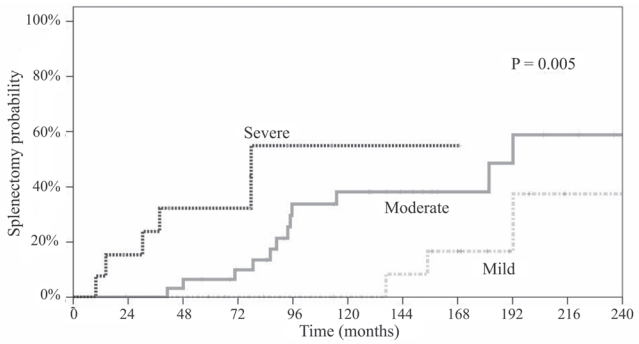

Figure 2 – Probability of having been submitted to splenectomy according to the severity of the presentation

Discussion

The commonest cause of anemia in children, worldwide, is iron deficiency. Hemolysis is a relative uncommon cause. In pediatrics, hemolytic anemia is commonly associated with hemoglobinopathies, erythro-enzymopathies or red cell membrane disorders, such as HS. HS has been reported in all ethnic groups, however, few cases have been reported in Black populations.[1,3,13] The incidence of HS is unknown in Brazil. Although HS is a relatively common cause of hemolytic anemia, there have been few clinical studies regarding its clinical course and management.

The diagnosis of HS is generally straightforward, based on a combination of the clinical examination and family history, and on physical and laboratory examinations.[1] In the current study the median age at diagnosis was about 5 years, ranging from 5 months to 21 years. As reported in the literature, the diagnosis of HS is often made in childhood

and during early adult life. However, depending on the severity of hemolysis, the disorder may be diagnosed at any age.[14] About 75% of cases have a family history of HS.[1] In this study only 57% had a positive family history but this feature was not mentioned in the medical files of 5 patients. Regarding the clinical features, patients have varying degrees of anemia and jaundice and most children have mild to moderate enlargement of the spleen. Around two thirds of these cases were anemic, 70% had varying degrees of splenomegaly and 38% were jaundiced at presentation.

The clinical feature is very heterogeneous ranging from asymptomatic to hydrops fetalis or stillbirth.[15,16] The clinical expression is relatively uniform within a given family but can vary considerably between families. In the present study, asymptomatic HS was diagnosed in three fathers and three mothers. The phenotypic variability of only one family was not uniform in its clinical presentation. In this family, the index case was classified as severe, transfusion-dependent, while his two siblings were classified as having mild disease.

It has been estimated that 20% of HS patients have mild disease with compensated hemolysis. Moderate HS represents the largest group of patients comprising about 70% of cases, while a small group of patients, 3-5%, have a severe form and require regular transfusions.[17] In the present study most patients were classified as moderate, but a fifth was classified as having a severe phenotype. These patients were younger and needed blood transfusions more frequently than patients with mild and moderate disease. In a series of 468 Italian children with HS, only 7% were classified as severe, while 21% were mild and 72% moderate.[18] A small proportion of patients with a severe degree was also found in another study of 44 patients; only 2% had severe HS.[19] The higher proportion of patients with severe HS found in the current study may be attributed to the fact that classification was retrospective, without a true knowledge of the clinical situation of the patient at diagnosis. It is known that, ideally, patients should be classified when clinically stable because a simple viral infection may lead to a severe transient anemia and so the severity of HS may be overestimated.[12] Furthermore, we were not able to assess the three laboratory variables – Hb, bilirubin, and reticulocyte count – for all patients, which may affect proper allocation of patients to risk groups.

The formation of bilirubin gallstones is one of the commonest complications in HS;[20] 6 to 50% of patients eventually develop symptomatic gallbladder disease at 10 to 30 years old and undergo cholecystectomy.[12,21,22] However, only 5 to 8% of children younger than ten have detectable gallstones.[18] In other words, the mean age at diagnosis of cholelithiasis is inversely proportional to the severity of the disease.[19] In the current series 27% of patients had gallstones, with the average age at diagnosis being 10 years old. Although it was possible to identify a tendency that the more severe forms of the disease are associated with a higher incidence of gallstones, the difference was not statistically significant. Possibly the relatively small number of patients with gallstones in this sample can explain this finding.

Other complications that occurred during the follow-up of these patients were acute spleen sequestration and aplastic crisis. Splenic sequestration was responsible for approximately 41% of indications for splenectomy. This finding supports results of the study of Rice et al.[23] in which 50% of splenectomies were indicated due to sequestration crises. Aplastic crises are generally less common and almost always following virally-induced bone marrow suppression. The most common causative agent is parvovirus B19, which confers life-long immunity.[24] As parvovirus serology was not available in this study, the etiology of aplastic crises could not be identified.

Splenectomy is generally indicated in patients with HS to alleviate symptoms related to hemolysis.[25] Previously, splenectomy was considered a "routine procedure" in HS patients. Although the risk of overwhelming post-splenectomy sepsis can be reduced by the use of immunizations as well as postoperative antibiotic prophylaxis, its risk is never eliminated.[26] Moreover, concern about incomplete protection by pneumococcal vaccinations, emergence of penicillin-resistant pneumococci and poor compliance to antibiotic prophylaxis still persists. These factors contribute by increasing the risk of post-splenectomy infection.[3,26] Nowadays, splenectomy is reserved for patients with severe HS or those who develop complications such as symptomatic gallstones and transfusion-dependency.[27] The mean age at splenectomy has been reported to be from 6.6 to 13 years of age.[19,28,29] The proportion of splenectomized patients in the present study (35%) was quite similar to that reported by others (38.5%).[18] As previously stated, the main reason for referring patients to the tertiary Pediatric Hematological Center at Hospital of Clínicas of UFMG was anemia of unknown etiology, followed by anemia unresponsive to iron supplementation. It is important to highlight that during the interview at UFMG many children had positive family histories for HS or clear-cut symptoms of hemolytic anemia. It is surprising that the diagnosis of HS had not been considered by the general practitioner as a cause of gallstones and splenomegaly in children.

The main limitation of this study is its retrospective design that results in limited control over the variables included in the analysis, especially regarding the classification of disease severity. In addition, molecular studies underlying the HS defect were not available and so its genetic distribution is not known.

In conclusion, the clinical course of patients with HS in this series was relatively benign with cholelithiasis being a common complication. Despite the high proportion of splenectomized patients in the current study, most of them (65%) were not submitted to splenectomy which corroborates the literature that recommends trying to postpone and, when

possible, to avoid splenectomy. Prospective studies with long-term follow-ups are needed to assess the risk of thrombotic events and pulmonary hypertension in patients submitted to splenectomy.

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