# Clinical features and outcome of children with hereditary spherocytosis

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March 30, 2022

**Abstract**

Objective: Hereditary spherocytosis is the most common erythrocyte membrane disorder characterized by splenomegaly, jaundice and anemia. The aim of this study was to evaluate the demographics, clinical and laboratory findings and treatment responses of patients with hereditary spherocytosis. Material and Method: Data of patients under the age of 18 with a diagnosis of hereditary spherocytosis between 1989-2018 were examined retrospectively. Diagnosis was based on clinical history, physical examination, family history, presence of spherocytes in peripheral smear and osmotic fragility test. Demographic, clinical and laboratory features, family history, complications, and history of splenectomy and cholecystectomy were evaluated. Results: One hundred and one patients were included. The median (range) age at diagnosis was 38.0 (1-188) months. Mild, moderate and severe forms of hereditary spherocytosis were present in 29 (28.7%), 15 (14.9%) and 57 (56.4%) patients, respectively. Family history was investigated in 73 (72.3%) and 56 (76.7%) had a family history of hereditary spherocytosis. Ninety one patients had available physical examination results; of these 79 (86.8%) had splenomegaly, 53 (58.2%) pallor and 16 (17.6%) jaundice. Forty-five (44.5%) patients needed regular transfusions and most (78.9%) of these had severe spherocytosis. Although most patients did not require transfusion post-splenectomy, two of 45 (4.4%) patients continued to require transfusion. Transfusion dependence was significantly ($p<0,001$) higher in patients with severe spherocytosis. Conclusions: In hereditary spherocytosis splenomegaly, pallor and jaundice are the most common physical findings. Splenectomy is effective in reducing hemolysis and virtually abolishes further requirement for transfusion. Key words: hereditary spherocytosis, anemia, hemolytic anemia, children

## Introduction

Hereditary spherocytosis (HS) is the most common red cell membrane disorder and is characterised by anaemia, jaundice, and splenomegaly. It is reported worldwide and is the most common inherited anaemia in individuals of northern European ancestry (1). Clinical severity is variable although in most individuals the condition is mild, requiring no therapy. In severe cases there is severe haemolytic anaemia requiring transfusion. The primary lesion in hereditary spherocytosis is loss of membrane surface area, leading to reduced deformability. This is due to defects in one of the erythrocyte membrane proteins ankyrin, band 3, beta spectrin, alpha spectrin, or protein 4.2. Morphologically, spherocytes are rounded red blood cell which have lost their ability to change shape and as a result are trapped and destroyed in the spleen leading to anemia. Common complications of HS with severe phenotype are cholelithiasis, haemolytic episodes,

and aplastic crises. Splenectomy is curative but should be undertaken only after careful assessment of the risks and benefits because splenectomy is associated with an increased risk for infections with encapsulated bacteria.

The diagnosis of HS is accomplished with basic, well-established tests, which include identification of spherocytes in a peripheral blood film smear, reticulocyte count, and measurement of osmotic fragility (OF). OF is a labor-intensive and time-consuming test to perform and has low sensitivity and specificity. The osmotic fragility test (OFT) is used for diagnosis of several erythrocyte disorders, such as hemolytic anemia, hereditary spherocytosis, elliptocytosis and thalassemia (2). Recently, new methods have been developed for diagnosis of HS including the use of flow cytometry (3). However, flow cytometers are not available in all routine diagnostic laboratories. With the use of genetic diagnostic technologies, many novel mutations have been identified in HS-related genes, including *SPTA1* , *SPTB* ,*ANK1* , *SLC4A1* , and *EPB42* that are involved in the interaction between the erythrocyte membrane and the lipid bilayer (4). Unfortunately, in many areas of the globe, including in those where higher rates of consanguinity where congenital hemolytic anaemias are more common, access to these molecular tests are less easy. In these areas clinicans still rely on the less specific and sensitive clinical and basic laboratory findings to diagnose and treat patients with HS.

The aim of this study was to examine the demographic characteristics, clinical features, laboratory findings, complications and treatment outcomes of children with HS in order to describe our experience which may be of benefit to clinicians in areas where there are still diagnostic and treatment challenges.

## Materials and methods

Medical files of 1700 patients, under the age of 18, attending the Pediatric Hematology and Oncology Clinic and diagnosed with anemia between 1989 and 2018, were interogated. All patients with anemia due to hereditary spherocytosis and who attended follow-up visits regularly were included in the study. Patient data were analyzed retrospectively from patient files and the computer information system. The demographic characteristics, medical histories, physical examination and laboratory findings of the patients were evaluated. Patients diagnosed with hemolytic anemia whose family member(s) had a history of splenomegaly, gallstones, jaundice, and/or splenectomy were considered to have a positive family history. Perfusion information of the patients was scanned from medical records. The use of iron binders with transfusion dependence was evaluated.

Hereditary spherocytosis was diagnosed by clinical history, physical examination findings, family history, the presence of spherocytes in peripheral smear and OF test. Since data about reticulocyte counts was not available for the majority of patients, patients were sub-divided into three HS severity groups based on their hemoglobin concentration: mild (11-15 gr/dL), moderate (8-11 gr/dL), and severe ($<$8 gr/dL).

Statistical evaluation was performed using the Statistical Package for the Social Sciences (SPSS), version 15.0 (IBM Inc., Armonk, NY, USA). The fitness of variables to normal distribution was examined with histograms and the Kolmogorov-Smirnov test. Descriptive analyzes were presented as mean, standard deviation and median values. Mann-Whitney U and Kruskal-Wallis Tests were used when evaluating nonparametric groups. While making comparisons between categorical data, Pearson Chi Square and Fisher Tests were used. Results with $p$ values below 0.05 were evaluated as statistically significant.

The study was evaluated by the Clinical Research Ethics Committee on 25.05.2018 and received ethical approval (Subject No: KAEK / 2018.5.02).

## Results

Of the 1700 patients under the age of 18 years with anemia, one hundred and one (5.9%) patients were identified with HS. The median (range) age at diagnosis of the HS patients was 38.0 (1-188) months. Ten (9.9%) patients were siblings. Family history was available for 73 (72.3%) patients and 56 of these (76.7%) were found to have a family history of HS. When the complications of the patients were examined, 29 of 88 patients (33.0%) evaluated by ultrasonography had gallstones. Cholecystectomy was performed in 21

(20.8%) and splenectomy in 51 (50.5%) patients. Forty-five (44.5%) patients needed regular transfusions and transfusion requirement persisted after splenectomy in two (4.4%) of them. while the remainder did not need transfusion after splenectomy. Erythropoietin was given to one patient, but was stopped because there was no decrease in the need for transfusion. Table 1 summarises the demographic and clinical data of the HS patients.

Of the 82 patients with available indications for admission, 52 (63.4%) presented with pallor, 12 (14.6%) with jaundice and eight (9.7%) with malaise. Patients were referred for further investigation because of increased spleen size in 26 (31.7%) cases and gallstones in nine (10.9%) patients. The findings on physical examination, obtained from patient files, showed splenomegaly in 79 (86.8%), paleness in 53 (58.2%) and jaundice in 16 (17.6%) of 91 patients. Laboratory findings of the patients are presented in Table 2.

Severity of HS was assessed by patient hemoglobin concentration. This resulted in 29 (28.7%), 15 (14.9%) and 57 (56.4%) patients being designated as mild, moderate and severe HS, respectively (see Table 3). Demographic and clinical characteristics of the three severity sub-groups was then compared. The median age at diagnosis of mild HS was found to be significantly older than those with moderate and severe HS ($p$ <0.001). Interestingly, gallstones were significantly less common in patients with moderate severity (6.7%) when compared with mild (24.1%) and severe (36.8%) forms of the disease ($p$ <0.05). Transfusion dependence (78.9%) was significantly greater in those with severe HS compared to the mild and moderate groups ($p$ <0.001). None of the patients with mild and moderate forms of HS were transfusion dependent. The rate of splenectomy (78.9%) in those with severe form of HS was significantly higher than in the other severity groupings ($p$ <0.001).

Splenectomy was not performed in any patient with moderate form of HS although six (20.7%) of patients with mild disease did undergo splenectomy. The cholecystectomy rate (33.3%) was significantly higher in those with severe HS compared to the mild and moderate groups ($p$ <0.05). The use of iron binders (12.2%) in those with severe HS was significantly more frequent than in mild or moderate severity patients ($p$ <0.05) as none of the patients with mild and moderate HS were prescribed iron binders.

Laboratory results of HS patients were compared by disease severity (Table 4). The mean red cell distribution width (RDW) in severe and moderate forms of HS was significantly higher than that of mild HS ($p$ <0.001). The mean value of the mean corpuscular hemoglobin concentration (MCHC) in severe cases was significantly lower than in those with moderate and mild severity ($p$ <0.05). The mean lactate dehydrogenase (LDH) and the mean total bilirubin concentrations in the severe form of HS was significantly higher than in the mild and moderate severity groups ($p$ <0.001 and$p$ <0.05, respectively).

**Discussion**

Hereditary spherocytosis is usually inherited in an autosomal dominant fashion accounting for 75% of cases, while in 25% it is inherited by autosomal recessive or *de novo* mutations and there is no gender bias (5). In a Brazilian study of 63 children with HS, 44.4% of the cases were girls and 55.6% were boys and in addition, the family history was positive in 57% of the cohort (6). Ayhan *et al.*investigated erythrocyte membrane disorders in Turkish patients and reported that in 50 HS patients 42% were boys and 58% were girls (7). In our study, also from Turkey, the gender ratio was almost exactly 50/50 and three-quarters of the patients had a positive family history of HS.

Although HS is frequently diagnosed in childhood, it may be diagnosed at any stage of life (8). In the present study the median age of diagnosis was 38 months while Güngör *et al* . recently evaluated 65 Turkish patients with HS and reported the median age of diagnosis to be later at 48 months (9). A further Turkish study by Konca *et al* . performed with 68 HS children found the age of diagnosis was between 3 and 216 months (18 years) with a median of around 67 months (10). The relatively earlier median age of diagnosis in our study is possibly due to the higher number of patients with severe HS.

MCHC is generally found to be increased in hereditary spherocytosis (11). Christensen *et al* . investigated the frequency of hereditary spherocytosis in newborns with hyperbilirubinemia, and reported that, especially

in the neonatal period, MCHC value [?]36 g/dL was a useful indicator for the diagnosis of HS, with 82% sensitivity and 98% specificity (12). Michaels *et al* . found the mean MCHC value to be 35.9 g/dL in a study evaluating the red blood cell indices in 112 HS patients aged between 12 months and 19 years. This MCHC value in the HS patients was significantly higher than the control group consisting of healthy children. They also reported that an MCHC value of >35 g/dL had a 70% sensitivity and a 86% specificity for HS (13). In our study, the mean MCHC value of patients was found to be 35.1+-1.7, 35.4+-1.4 and 33.9+-2.2 in patients with mild, moderate and severe forms of HS, respectively, and the MCHC was [?]35 g/dL in 65% of patients.

Gallstones are among the most common complications of hereditary spherocytosis. Tamary *et al* . found the frequency of cholelithiasis to be 41% in their study of 44 patients with HS (14). In contrast Das *et al* . reported a much lower frequency of cholelithiasis of 26% in 62 patients with HS diagnosed by ultrasonography (15). The frequency in the present study falls between these two figures at 33%, and 72% of these patients underwent cholecystectomy.

Based on hemoglobin concentrations, the proportions of severe, moderate and mild HS in our cohort were 56.4%, 14.9% and 28.7%, respectively. Rocha et al. (16) reported 17.5%, 37.5% and 45% of patients having severe, moderate and mild HS while these same proportions in the cohort of Oliveira *et al* were 20.6%, 54% and 25.4% (6). Once again, the reason for the differences in proportions of disease severity in our cohort is the nature of our center, where severe cases of HS are referred for treatment.

Since abnormal erythrocytes are retained in the spleen in HS where they are removed from the circulation, splenectomy is an effective treatment method (5). In our study splenectomy was performed in 50.5% of the patients diagnosed with HS. Rocha *et al* . investigated erythrocyte membrane protein defects and the clinical outcomes of having defective proteins and reported that 32.5% of patients diagnosed with HS underwent splenectomy (16). In contrast, Konca *et al* . reported a splenectomy rate of only 7.3% (10). The rate of splenectomy in our study was higher than previous reports as our cohort was attending a tertiary referral center and many patients were referred specifically in order to undergo splenectomy.

Splenectomy is an effective method in reducing hemolysis. When considering splenectomy, the general condition of the patient and accompanying complications should be taken into account. Performing splenectomy in severe forms of HS significantly reduces complications such as anemia and gallstones (5). Therefore, splenectomy is usually performed in patients with severe HS. In moderate HS, the decision is made according to the clinical condition of the patient whereas in mild HS, splenectomy is generally not required (5). In a study by Rocha et al; splenectomy was performed in 60.7% of the patients with severe, 48% of moderate, and 8.3% of mild forms of HS (16). In a study conducted by Oliveira et al., it was reported that splenectomy was performed in 46.2% of the patients with severe, 38.2% of moderate 18.8% of mild forms of HS (6). In our study, splenectomy was performed in 78.9% of patients with severe forms of HS while this proportion was 60.7% and 46.2% in the studies of Rocha *et al* and Oliveira*et al* , respectively (16, 6). Although it has been reported in the literature that splenectomy in mild form of HS is not generally required, in our study, 20.7% of patients with mild form of HS were splenectomized while this proportion was 8.3% and 18.8% in the Rocha*et al* and Oliveira *et al* studies (16, 6).

Classifying severity of HS based on hemoglobin concentration or based on reticulocyte count can result in differences in the proportions of patients designated severe, moderate or mild in the same cohort. Roy*et al* . investigated 64 patients who underwent splenectomy for HS. When patients were classified only according to hemoglobin level, the patients were found to have mild (25%), moderate (59%) and severe (10%) HS. However, when the same patients were classified according to reticulocyte count these proportions were mild (5%), moderate (27%) and severe (66%) HS (17). Unfortunately reticulocyte counts were not available for most of our patients, their classification was based only on individual hemoglobin concentration which might have affected our classification.

Splenectomy usually eliminates hemolysis and associated signs and symptoms in most patients with HS, except for some autosomal recessive variants (18). In our study, in only two patients with severe HS the need for regular transfusions continued after splenectomy. These two patients were siblings and their diagnoses

were confirmed at another center. Unfortunately no genetic analysis was performed. In both, the presence of accessory spleen was investigated but not detected.

Erythropoiesis decreases physiologically in the first months of life. In patients diagnosed with HS, an adequate erythropoietin response to increased rates of erythrocyte destruction is not possible in the first months, and 70-80% of cases require blood transfusion early in life. After this period, less than 30% of patients usually need regular transfusions. Successful results have been reported with recombinant human erythropoietin (rHu-Epo) treatment as an alternative to blood transfusion in patients with HS during the first year when the need for transfusion is high (19). In our study, rHu-Epo was used in one patient because of the need for transfusion during infancy. The patient received rHu-Epo 300 units/kg/day, three times a week for four months. However, this therapy did not decrease the need for transfusion and the rHu-Epo therapy was not continued further.

It was a single-center study with a retrospective design, and the medical records of some patients were not available. In addition, the study was conducted in a tertiary health center which might be expected to introduce some bias into the severity of the cases encountered, and thus some results are likely to vary when compared with those reported in the literature. As a result, we think that the results we obtained will contribute to the literature in terms of demographic, clinical and laboratory features and treatment responses of this common disease.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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