Hereditary Spherocytosis: Evaluation of 68 Children

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Abstract To determine the clinical and hematologic features of 68 children with hereditary spherocytosis (HS). In this retrospective study, we analyzed recorded information of 68 HS patients diagnosed between March 1997 and March 2007, including clinical manifestations at admission, gender, median age at diagnosis, family history, hematologic and biochemical data, patient management, complications, median age of splenectomy, and median follow-up time. Sixty-eight patients with HS (36 male and female) were investigated. The median age at diagnosis was 5.6 years (range 3 months to 18 years). Twenty-seven (39.7 %) had parents with consanguineous marriages, and 20 (29.4 %) had parents with first-degree consanguinity. Predominant clinical manifestations at admission were anemia in 59 patients (86.76 %), splenomegaly in 49 (72.05 %), and jaundice in 33 (48.52 %). Patients were classified as mild, moderate, or severe in 29.4, 61.7, and 8.8 % of patients, respectively. Five patients (7.3 %) underwent splenectomy. Major complications of HS were hemolytic, aplastic, and megaloblastic crises and cholelithiasis in 7 (10.2 %), 1 (1.4 %), 7 (10.2 %), and 6 (8.8 %) of patients, respectively. There were no deaths during

follow-up. HS should be considered in evaluating possible diagnoses in patients with hemolytic anemia. In this study, the clinical course of patients with HS was relatively benign, with low proportions of patients having splenectomized and aplastic crises.

Introduction

The Hereditary spherocytosis (HS) is a worldwide disease. In the Northern European population, it is the most-common, inherited form of anemia, affecting approximately 1 in 1,000–2,500 individuals. In nearly 75 % of cases, the inheritance has an autosomal dominant pattern, and the other 25 % of cases present recessive forms and de novo mutations [1–3]. HS syndromes are a group of inherited disorders characterized by the presence of spherical-shaped erythrocytes on the peripheral blood smear [4].

Clinical findings of HS are variable and include jaundice, splenomegaly, gallstones, and hemolytic anemia, which can be compensated or severe and sometimes requires exchange transfusion at birth and/or repeated blood transfusions [5, 6].

The primary molecular abnormality of HS may affect several membrane proteins, such as spectrin, ankyrin, band 3 and, rarely, protein 4.2 [7]. These proteins are employed in the attachment of the cytoskeleton to the membrane integral domain, so any deficiency or dysfunction in them results in a loss of surface area and leads to spheroidal, osmotically fragile erythrocytes [8, 9]. It is impossible for these spherocytic, fragile erythrocytes to pass through the splenic sinusoids easily. Thus, these erythrocytes are selectively trapped in the spleen. Family history and typical clinical and laboratory findings make diagnosis easy, without requiring additional investigation [10].

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The purpose of this study was to determine clinical and hematologic features of 68 children with HS who had been diagnosed and followed up in the Dicle Medical Faculty Pediatric Hematology Department between March 1997 and March 2007.

Materials and Methods

In this retrospective study, 68 HS patients were evaluated. All were under age 18 at diagnosis and were followed between March 1997 and March 2007 by the Pediatric Hematology Department of Dicle University (Southeastern Anatolia, Turkey), where city hospitals and primary health centers refer patients. This retrospective investigation was performed through their charts. Data gathered included clinical manifestation of patients at admission, gender, median age at diagnosis, family history, hematologic and biochemical data, patient management, complications, median age of splenectomy, and median follow-up time.

HS was diagnosed on the basis of clinical history, physical examination, and laboratory test results, including complete blood count, blood smear, reticulocyte count, bilirubin concentration, positive osmotic fragility test (OFT), and negative direct antiglobulin (Coombs') test [11]. An automated hematology analyzer, the Abbott Cell-dyn 3700 (USA), was used to determine hematologic parameters, and the Abbott ARCHITECT C-16000 (USA) was used for bilirubin determination. In our hospital, there is no opportunity for molecular genetic analysis or quantitative erythrocyte-membrane protein-level analysis. To detect gallstones, we performed an abdominal ultrasound every third to fifth year and before splenectomy.

Folate supplements administered to patients with moderate and severe HS were 2.5 mg/day up to the age of 5 years and 5 mg/day thereafter. Clinical presentation at baseline was classified retrospectively as mild, moderate, or severe based on the modified criteria of Eber et al. [2]. Patients who underwent splenectomy received immunization for pneumococcus, haemophilus, and meningococcus before the procedure. Oral or intramuscular penicillin was recommended as a lifelong prophylaxis. Reference values for children were taken from Nathan and Oski's *Hematology of Infancy and Childhood*, 7th ed., Philadelphia, PA, 2009 [12].

Statistical analysis was performed using the Statistical Package for Social Science (SPSS), Version 15.0 (SPSS, Inc., Chicago, IL). Mean, median, minimum, and maximum values and standard deviations were calculated for numerical parameters. The Mann–Whitney U test and the Kruskal–Wallis test were used to compare groups. Cross-table statistics were used to compare categorical variables (Mantel–Haenszel). A *p* value less than 0.05 was

Table 1 Clinical manifestation of patients at admission

Clinical features	<i>N</i>	%
Anemia	59	86.7
Splenomegaly	49	72.1
Jaundice	33	48.5
Growth failure	10	14.7
Gallstones	1	1.4

Table 2 Hematologic and biochemical data of HS patients

	<i>N</i>	Mean	SD
MCV (fL)	68	73.6	15.5
MCHC (g/dL)	68	35.9	4.7
Hb (g/dL)	68	10.2	2.1
HTC (%)	68	19.5	7.5
RDW (%)	68	19.8	4.6
Reticulocytes (%)	68	6.4	4.8
Spherocytes (%)	56	8.6	2.2
Unc. Bilirubin (mg/dL)	36	2.3	0.8

considered statistically significant. Because it is retrospective, this study does not conflict with the Declaration of Helsinki.

Results

The patients comprised 36 males (52.9 %) and 32 females (47.1 %). The median age at diagnosis was 5.6 years (range 3 months to 18 years). The median follow-up time was 5.4 years. Predominant clinical manifestations at diagnosis were anemia in 59 (86.76 %) patients, splenomegaly in 49 (72.05 %), and jaundice in 33 (48.52 %). One patient had gallstones (Table 1).

Baseline morphologic examination of peripheral blood smears from the 68 patients showed that 56 (82.3 %) patients had spherocytic red cells. In all 68 patients, mean Hb concentration, MCV, MCHC, and RDW values were 10.2 g/dL, 73.6 fL, 35.9 g/dL, and 19.8, respectively. The mean, indirect bilirubin concentration for 36 patients was 2.3 mg/dL. In all 68 patients, the mean reticulocyte count was 6.4 ± 4.8 % (Table 2).

When patients were classified according to modified Eber criteria and hematologic and clinical features, 20 (29.4 %), 42 (61.7 %), and 6 (8.8 %) patients were classified as mild, moderate, and severe, respectively. There were no significant differences among the three groups regarding gender and family history. There was significant difference regarding median age at diagnosis. Patients classified as severe had a median age at diagnosis of 6.6 months, whereas those classified as mild or moderate

Table 3 Clinical and demographic characteristics of patients according to modified criteria of Eber ($n = 68$) (12)

	Mild ($n = 20$) (29.4 %)	Moderate ($n = 42$) (61.7 %)	Severe ($n = 6$) (8.8 %)	<i>P</i> value
Gender, n (%)				
Male	11 (55 %)	21 (50 %)	4 (66.7 %)	0.865
Female	9 (45 %)	21 (50 %)	2 (33.3 %)	
Median age at diagnosis (months)	124.1	62.1	6.6	<0.001
Family history, n (%)				
Yes	11 (55 %)	15 (35.7 %)	4 (66.7 %)	0.732
No	9 (45 %)	27 (64.3 %)	2 (33.3 %)	
First case n (%)				
Yes	11 (55 %)	8 (19.0 %)	1 (16.7 %)	<0.01
No	9 (45 %)	34 (81.0 %)	5 (83.3 %)	
Degree of parent's consanguineous				
First	1 (5 %)	17 (40.4 %)	2 (33.3 %)	0.022
Second	1 (5 %)	1 (4.7 %)	0 (0 %)	
Third	5 (25 %)	0 (0 %)	0 (0 %)	
Median age of splenectomy (years)	No one	12.4	6.5	<0.001
Complications				
Cholelithiasis	No one	5 (11.9 %)	1 (16.6 %)	<0.001
Haemolytic crises	No one	5 (11.9 %)	2 (33.3 %)	<0.001
Megaloblastic crises	1 (5 %)	4 (9.5 %)	3 (50 %)	<0.001
Aplastic crises	No one	No one	1 (16.6 %)	<0.001
Treatment, n (%)				
Splenectomy	No one	2 (4.8 %)	3 (50 %)	0.001
Folate supplementation	No one	42 (100 %)	6 (100 %)	<0.001
Transfusion	No one	5 (11.9 %)	5 (83.3 %)	<0.001
Iron supplementation	No one	No one	No one	
Erythropoietin treatment	No one	No one	No one	

were approximately 124 and 60 months old, respectively ($p < 0.001$). Twenty patients, (11 (55 %) in the mild group, 8 (19.0 %) in the moderate group, and 1 (16.7 %) in the severe group) were considered the first cases in their families ($p < 0.01$). Twenty-seven (39.7 %) had parents with consanguineous marriages, and 20 (29.4 %) had parents with first-degree consanguinity. Of those with parents who had first-degree consanguinity, 1, 17, and 2 were in the mild, moderate, and severe categories, respectively. A positive family history was recorded for 30 patients (44.1 %), and 20 (29.4 %) were considered the first cases in their families (Table 3).

One patient had cholelithiasis at admission, and 5 (7.4 %) developed cholelithiasis, identified by gallbladder ultrasonography, during follow up. Median age was 11 years (range 5.2–17.6 years) at diagnosis of gallstones. One patient underwent cholecystectomy, due to symptomatic gallstones, at 12 years old. No splenic sequestration was seen during follow up. Aplastic crisis was seen in 1 patient with severe HS. Megaloblastic crises developed in the mild, moderate, and severe groups at the rates of 1 (5 %), 4 (9.5 %), and 3 (50 %), respectively (Table 3).

Transfusions were required in 5 (11.9 %) moderate and 5 (83.3 %) severe cases of HS. The need for transfusions was significantly greater among patients classified as severe ($p < 0.001$). Five patients (7.3 %) underwent splenectomy. The most common indication for splenectomy was transfusion-dependency. The median age at splenectomy was 8 years (range 11 months to 18 years). There was statistical difference among splenectomized patients regarding median age at splenectomy ($p < 0.001$). Folate supplements were administered to 42 (100 %) moderate and 6 (100 %) severe HS patients. No patients received iron supplementation or erythropoietin treatment (Table 3). There were no deaths during follow up.

Discussion

HS is a worldwide disease and the most common cause of inherited, chronic hemolysis in Northern Europe and North America, where it affects about one person in 2,000. The incidence of HS in Turkey is unknown. This study may help in understanding clinical and hematologic features of

children with HS in Turkey. Although HS often is diagnosed in childhood or young adulthood, it may be diagnosed at any time in life, including old age [11, 13]. About 75 % of cases have a family history of HS [14]. Oliveira et al. [15] reported that gender was not a statistically significant factor in risk for the disease, that median age at diagnosis was about 5 years, and that positive family history was detected in 57 % of cases. In the current study, median age at diagnosis was 5.6 years (range 3 months to 18 years), and a positive family history was recorded for 30 patients (44.1 %), who had 12 siblings, 8 parents, and 10 distant relatives who were diagnosed with HS. Twenty patients (29.4 %) were considered the first cases in their families.

HS is usually diagnosed based on a combination of clinical and family histories, physical examination (for splenomegaly or jaundice), and laboratory data (full blood count, especially red blood cell indices and morphology, and reticulocyte count) [16]. It has been reported that a combination of MCHC exceeding normal range and RDW > 15 % is highly related to HS diagnosis [17]. The MCV may be low, or high if there is substantial reticulocytosis [18]. Eberle et al. [19] compared 94 patients with HS to an equal number of healthy children. They found that MCHC and RDW were significantly higher in HS patients than in normal, control subjects, and they reported that the combination of the two tests is an excellent predictor for HS. In our study, MCHC and RDW values were 35.9 ± 4.7 g/dL and 19.8 ± 0.6 %, respectively, consistent with findings in the literature.

Clinical features are very heterogeneous, ranging from asymptomatic to hydrops fetalis, or stillbirth [20, 21]. Clinical findings show variability according to quantitatively and functionally inadequate protein types and mutation types. In children, anemia is the most frequent sign (50 % of cases), followed by splenomegaly, jaundice, and a positive family history [22]. In a study, the predominant clinical manifestations at diagnosis were reported as splenomegaly in 44 (69.8 %) cases, anemia in 40 (63.5 %), and jaundice in 24 (38 %) [15]. In this study, the predominant clinical manifestations at diagnosis were anemia in 59 (86.76 %) patients, splenomegaly in 49 (72.05 %), jaundice in 33 (48.52 %), and gallstones in one patient.

Severity of hereditary spherocytosis is classified as mild, moderate, moderately severe, or severe, according to several common clinical laboratory variables, including hemoglobin and bilirubin concentrations and reticulocyte count [2]. In a study of 468 Italian children with HS, only 7 % were classified as severe, while 21 % were mild and 72 % were moderate [23]. In another study, patients were classified as mild, moderate, or severe, 25.4, 54, and 20.6 %, respectively [15]. In another study, patients were

classified as trait, mild, moderate, and severe in 42, 38, 11, and 9 % of spectrin/ankyrin-deficient patients, and in 50, 30, 16, and 4 % of band 3-deficient patients, respectively [13]. In the current study, patients were classified according to modified Eber [2] criteria as mild, moderate, or severe in 29.4, 61.7, and 8.8 % of patients, respectively.

The major complications of HS are aplastic and megaloblastic crises, hemolytic crisis, severe neonatal hemolysis, cholecystitis, and cholelithiasis. Chronic hemolysis leads to formation of bilirubinate gallstones. The best method of detecting gallstones is ultrasonography [24]. Gallstones are noted in at least 5 % of children under age 10 [23]; the proportion increases to 40–50 % in the second to fifth decades, with most stones arising between 10 and 30 years of age [24]. In a study, gallstones were noted in 27 % of patients, with the average age at diagnosis being 10 years [15]. Hemolytic crises are the most common, often triggered by viral illnesses and typically arising in childhood. Hemolytic crises are generally mild, and most patients need only supportive care; red blood cell transfusions are needed only if hemoglobin concentrations are greatly reduced [22]. Aplastic crises generally are less common and nearly always follow virally induced bone-marrow suppression. The most common causative agent is parvovirus B19 [25]. Another type of crisis is megaloblastic, which occurs when dietary intake of folic acid is inadequate for the increased needs of erythroid hyperplasia. In the current study, the major complications of HS were hemolytic, aplastic, and megaloblastic crises and cholelithiasis in 7 (10.2 %), 1 (1.4 %), 7 (10.2 %), and 6 (8.8 %) patients, respectively.

Management of patients with HS is supportive. Folate supplements are recommended in moderate and severe HS but not necessarily in mild HS if the diet is adequate [26]. Red cell transfusions may be needed in severe cases, but unnecessary transfusions may lead to iron overload [14]. Iron supplementation should not be given unless there is additional evidence of deficiency. In the first year of life, erythropoietin treatment may be beneficial and may lower transfusion requirements [27, 28]. Splenectomy is very effective in reducing hemolysis, leading to significant prolongation (although not necessarily to normal) of the red cell lifespan [29, 30]. Splenectomy should be performed in children with severe HS, considered in those who have moderate disease, and probably not performed in those with mild disease [14, 26]. Mean age at splenectomy has been reported as from 6.6–13 years [24, 31]. The proportion of splenectomized patients reported in a study was 36 % [15] and in another, 38.5 % [23]. In our study, folate supplements were administered in moderate and severe cases of HS. Although 10 (14.7 %) patients needed transfusion support, none received erythropoietin. Five (7.3 %) patients underwent splenectomy.

Prior to splenectomy, all individuals should be vaccinated against encapsulated organisms, according to national guidelines [26, 32, 33]. Although repeat pneumococcal vaccination is recommended at five-year intervals, there is no clear evidence for it in the literature [11]. Despite post-splenectomy penicillin prophylaxis often is advised, there is controversy regarding duration of treatment [32–35]. In our study, all patients were vaccinated against encapsulated organisms, and antibiotic prophylaxis was administered for life.

If parents were tested for incubated OFT and their hemogram results were analyzed, it would be interesting to determine the inheritance pattern in some patients. But, the major limitations of our study were that it was retrospective and that it did not consider parents' hematologic data.

Conclusion

HS should be considered in evaluating possible diagnoses in patients with hemolytic anemia. In this study, the clinical course of patients with HS was relatively benign, with a low proportion of patients with splenectomized and aplastic crises. Prospective studies with long-term follow-up periods are needed to determine the clinical course of patients with HS.

Conflict of interests The authors declare that they have no competing interests.

References

1. Tse WT, Lux SE (1999) Red blood cell membrane disorders. *Br J Haematol* 104:2–13
2. Eber SW, Ambrust R, Schroeter W (1990) Variable clinical severity of hereditary spherocytosis: relation to erythrocytic spectrin concentration, osmotic fragility, and autohemolysis. *J Pediatr* 117:409–416
3. Miraglia del Giudice E, Nobili B, Francese M, D'Urso L, Iolascon A, Eber S, Perrotta S (2001) Clinical and molecular evaluation of non-dominant hereditary spherocytosis. *Br J Haematol* 112:42–47
4. Eber S, Lux SE (2004) Hereditary spherocytosis-defects in proteins that connect the membrane skeleton to the lipid bilayer. *Semin Hematol* 41:118–141
5. Hassoun H, Palek J (1996) Hereditary spherocytosis: a review of the clinical and molecular aspects of the disease. *Blood Rev* 10:129–147
6. Gallagher PG (2005) Red cell membrane disorders. *Hematology Am Soc Hematol Educ Program*, pp 13–8
7. Palek J, Jarolim P (1993) Clinical expression and laboratory detection of red cell membrane protein mutation. *Semin Hematol* 30:249–283
8. Cooper RA, Jandl JH (1969) The role of membrane lipids in the survival of red cells in hereditary spherocytosis. *J Clin Invest* 48:736–744
9. Da Costa L, Mohandas N, Sorette M, Grange MJ, Tchernia G, Cynober T (2001) Temporal differences in membrane loss lead to distinct reticulocyte features in hereditary spherocytosis and in immune hemolytic anemia. *Blood* 98:2894–2899
10. Hug S, Pietroni MA, Rahman H, Alam MT (2010) Hereditary spherocytosis. *J Health Popul Nutr* 28(1):107–109
11. Bolton-Maggs PH, Stevens RF, Dodd NJ, Lamont G, Tittensor P, King MJ (2004) Guidelines for the diagnosis and management of hereditary spherocytosis. *Br J Haematol* 126:455–474
12. Brugnara C, Oski FJ, Nathan DG (2009) Nathan and Oski's hematology of infancy and childhood, 7th edn. WB Saunders, Philadelphia
13. Mariani M, Barcellini W, Vercellati C, Marcello AP, Fermo E, Pedotti P, Boschetti C, Zanella A (2008) Clinical and hematologic features of 300 patients affected by hereditary spherocytosis grouped according to the type of the membrane protein defect. *Haematologica* 93:1310–1317
14. Bolton-Maggs PH, Langer JC, Iolascon A, Tittensor P, King MY (2012) Guidelines for the diagnosis and management of hereditary spherocytosis-2011 update. *Br J Haematol* 156:37–49
15. Oliveira MC, Fernandes RA, Rodrigues CL, Ribeiro DA, Giovanardi MF, Viana MB (2012) Clinical course of 63 children with hereditary spherocytosis: a retrospective study. *Rev Bras Hematol Hemoter* 34(1):9–13
16. Discher DE, Carl P (2001) New insights into red cell network structure, elasticity, and spectrin unfolding: a current review. *Cell Mol Biol Lett* 6:593–606
17. Eber S, Ehninger G, Goede J, Gassmann W et al (2014) Hereditary spherocytosis (Spherocytic Anemia) guideline: recommendations from the society for diagnosis and therapy of haematological and oncological diseases. <http://www.onkopedia.guidelines.info/en/onkopedia/guidelines/hereditary-spherocytosis.pdf>. Accessed 17 Feb 2014
18. Kanellopoulou T, Kontopidou FN, Dourakis SP (2011) Hereditary spherocytosis in a young male report of an unusual case. *Archives Hellenic Med* 28(6):814–818
19. Eberle SE, Sciuccati G, Bonduel M, Díaz L, Staciuk R, Torres AF (2007) Erythrocyte indexes in hereditary spherocytosis. *Medicina (B Aires)* 67(6 Pt 2):698–700
20. Arcasoy MO, Gallagher PG (1995) Hematologic disorders and nonimmune hydrops fetalis. *Semin Perinatol* 19(6):502–515
21. Whitfield CF, Follweiler JB, Lopresti-Morrow L, Miller BA (1991) Deficiency of alphaspectrin synthesis in burst-forming unit-erythroid in lethal hereditary spherocytosis. *Blood* 78(11):3043–3051
22. Perrotta S, Gallagher PG, Mohandas N (2008) Hereditary spherocytosis. *Lancet* 372:1411–1426
23. Pinto L, Iolascon A, Miraglia Del Giudice E, Materese MR, Nobili B et al (1995) The Italian survey on hereditary spherocytosis. *Int J Pediatr Hematol Oncol* 2:43–47
24. Tamary H, Aviner S, Freud E (2003) High incidence of early cholelithiasis detected by ultrasonography in children and young adults with hereditary spherocytosis. *J Pediatr Hematol Oncol* 25:952–954
25. Brown KE (2000) Haematological consequences of parvovirus B19 infection. *Baillieres Best Pract Res Clin Haematol* 13(2):245–259
26. Bolton-Maggs P (2012) Comments on 'clinical course of 63 children with hereditary spherocytosis: a retrospective study'-with the particular question: 'should HS be treated the same way throughout the world?'. *Rev Bras Hematol Hemoter* 34(1):3–8
27. Iolascon A, Perrotta S, Stewart GW (2003) Red blood cell membrane defects. *Rev Clin Exp Hematol* 7:22–56
28. Bianchi P, Fermo E, Vercellati C, Marcello AP, Porretti L, Cortelezzi A et al (2012) Diagnostic power of laboratory tests for hereditary spherocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics. *Haematologica* 97(4):516–523

29. Chapman RG (1968) Red cell life span after splenectomy in hereditary spherocytosis. *J Clin Invest* 47:2263–2267
30. Baird RN, Macpherson AI, Richmond J (1971) Red-blood-cell survival after splenectomy in congenital spherocytosis. *Lancet* 298(7733):1060–1061
31. Abdullah F, Zhang Y, Camp M, Rossberg MI, Bathurst MA, Colombani PM et al (2009) Splenectomy in hereditary spherocytosis: review of 1,657 patients and application of the pediatric quality indicators. *Pediatr Blood Cancer* 52(7):834–837 *Pediatr Blood Cancer*. 2009;52(7):753–754
32. (BCSH) (1996) Guidelines for the prevention and treatment of infection in patients with an absent or dysfunctional spleen: working party of the British Committee for Standards in Haematology (BCSH) clinical haematology task force. *Br Med J* 312:430–434
33. Davies JM, Barnes R, Milligan D (2002) Update of guidelines for the prevention and treatment of infection in patients with an absent or dysfunctional spleen. *J R Coll Physicians Lond* 2(5):440–443
34. Davies JM, Lewis MP, Wimperis J, Rafi I, Ladhani S, Bolton-Maggs PH (2011) Review of guidelines for the prevention and treatment of infection in patients with an absent or dysfunctional spleen: prepared on behalf of the British Committee for Standards in Haematology by a working party of the Haemato-Oncology task force. *Br J Haematol* 155(3):308–317
35. Reid MM (1994) Splenectomy, sepsis, immunisation, and guidelines. *Lancet* 344:970–971