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Original Article

Hereditary Spherocytosis in an Asian Children's Hospital

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Abstract ***Purpose:*** Hereditary spherocytosis (HS) is a red cell membrane disorder less commonly seen in Asian populations. This study highlights the diagnostic features and complications of HS patients followed up in an Asian paediatric institution. ***Methods:*** Data was obtained from retrospective medical records of paediatric patients diagnosed with HS from 2006-2016. ***Findings:*** Thirty-one patients were identified with a mean age (MA) at diagnosis of 3.5 years (0-14.3). Sixteen patients (52%) had positive family history. Twelve patients (38.7%) presented early (<1 month), most with neonatal jaundice. Twenty-one patients (67.7%) required at least one transfusion. Gallstones were detected in 10 patients (32.3%) - 4 required cholecystectomy (MA 11.7 years). Six patients (19.4%) had splenectomy (MA 12.5 years), mostly due to frequent transfusions. ***Conclusions:*** Most paediatric patients in our institution are diagnosed early, with the majority requiring at least one transfusion. HS should be considered in neonates presenting with early jaundice requiring phototherapy.

Key words *Early jaundice; Haemolytic anaemia; Hereditary spherocytosis*

Introduction

Hereditary spherocytosis (HS) is a red cell membrane disorder affecting membrane proteins. The 5 genes associated with HS include SPTA1 (α-spectrin), SPTB (β-spectrin), ANK1 (ankyrin), SLC4A1 (band 3) and EPB42 (protein 4.2).[1,2] It is most commonly associated with dominant inheritance, although recessive inheritance has been described. It is the most common cause of haemolytic anaemia in individuals of Northern European ancestry with an incidence of 1 in 2000.[1,3] Although there are cases reported worldwide in all racial and ethnic groups, it is perceived to be less common in South-East Asia (SEA), with a reported frequency of 1.27 to 1.49 per 100 000 to 1 in 5000.[4,5] The heterogeneity of the condition results in individuals with differing severities varying from symptom-free carriers to severe haemolysis. The variable clinical severity suggests that the molecular defects are heterogeneous. In European and American patients, ANK1 mutation is the major cause of HS affecting 35-65% of patients, followed by band 3 mutations which affect 15-35% of patients. On the other hand, Japanese patients have primarily band 3 and protein 4.2 mutations.[1,6] The main clinical features of haemolysis are anaemia, jaundice and splenomegaly.[1,3,6] Biochemical features include reticulocytosis, raised unconjugated bilirubinemia and increased mean red cell haemoglobin concentration (MCHC).[1,3,6,7]

Individuals are classified as having mild, moderate, moderately severe or severe phenotypes depending on the baseline haemoglobin, bilirubin and transfusion

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requirements.[1] About 20-30% of patients have mild disease with compensated haemolysis and are largely asymptomatic. This can be left undetected till adulthood when complications of chronic haemolysis such as gallstones arise. The majority of patients have moderate disease (60-70%) and usually present in childhood with anaemia and sometimes jaundice in association with viral infections. Splenomegaly is often present in these patients. A small group of 10% have moderately severe disease characterised by low haemoglobin concentration (60-80 g/L) and intermittent need for transfusions while about 3-5% of patients with severe disease have life-threatening anaemia (<60 g/L) requiring regular transfusions. These patients usually have recessive inheritance and frequent transfusions place them at higher risk of side effects from chronic anaemia and iron overload such as growth retardation, delayed sexual maturation or extra-medullary haematopoiesis.

Splenectomy leads to improved red cell survival and is often considered curative when performed. However, the life-long risk of overwhelming sepsis, particularly with pneumococcal species, despite pre-operative vaccinations and post-operative antibiotic prophylaxis make routine splenectomy not advisable for all patients, particularly those with mild disease. Clinical size is not an indication for splenectomy and studies have shown that increased splenic size does not result in higher risk of rupture.[3] To date, no cohort study on paediatric patients from Singapore with a predominantly Asian population has been conducted. This study aims to characterise the diagnostic features and complications of paediatric patients diagnosed with HS in KK Women's and Children's Hospital (KKH), a tertiary academic centre which is the main paediatric hospital in Singapore seeing 73,500 inpatients and 160,000 emergency attendances a year.

Methods

This is a retrospective cohort study which looked at diagnostic features and complications of paediatric patients treated for or diagnosed with HS from 2006-2016 in KKH. Follow-up duration ranged from 4 months to 25 years (mean 7.75 years). Clinical data were obtained from hospital electronic records and clinical notes. Data collected included demographics, laboratory data at diagnosis (Haemoglobin (Hb), MCHC, serum bilirubin (SB), peripheral blood film findings), episodes of transfusion and complications.

All diagnoses of HS were confirmed with osmotic fragility (OF) or eosin-5-maleimide (EMA) binding tests. The osmotic fragility test (OFT) is a measure of the degree of haemolysis of red cells placed in serial solutions of saline at different concentrations. A normal curve is first obtained using normal red blood cells. A right shift in the curve indicating increased haemolysis of red cells compared to normal red cells at the same osmolar concentration is suggestive of red cell fragility due to a membrane disorder. Despite adequate sensitivity, the OFT has limited specificity and is time-consuming, and was replaced in our institution by the EMA flow cytometry test from 2012. The EMA dye is a fluorescent dye which binds to band 3 protein on the surface of red cells. The intensity of the fluorescent signal corresponds to the band 3 protein content on red cell membranes, and is both sensitive and specific for the diagnosis of HS when the mean fluorescent signal compared to normal controls is decreased.

Genetic testing was not routinely offered and was performed only in patients with an unusually severe phenotype, for example requiring frequent transfusions at a young age. Significant haemolysis was defined as a Hb drop of >2 g/dL with signs of clinical or biochemical jaundice while aplastic crisis was defined as a Hb drop of >2 g/dL with reticulocytopenia.[8] Routine ultrasound abdomens were performed from age 5 years for patients in this series, either every other year or more frequently where necessary. The study was approved by the Institutional Ethics Review Board of our institution. Data were analysed using SPSS Version 19.0 ((IBM, Armonk, New York, USA) with continuous variables such as Hb, MCHC and SB expressed as mean ± standard deviation (SD).

Results

There were 31 patients diagnosed or already on follow-up for HS from 2006 to 2016 in KKH. The clinical characteristics of these patients at diagnosis are shown in Table 1. There was a higher percentage of female patients (67.7%) and the ethnic distribution of patients was highest for Chinese (64.5%) followed by Malays (29%). The number of Malays with HS in our cohort was over-represented as compared to the typical ethnic distribution of the Singapore population which consists of 74.3% Chinese and 13.4% Malays.[9] Positive family history was present in 52% of the patients. The vast majority of patients were symptomatic at diagnosis (90.3%). The main presenting symptoms were anaemia/pallor (51.6%),

jaundice (48.5%) and hepatosplenomegaly/splenomegaly (22.6%). Three patients were asymptomatic, 2 had screening done due to a positive family history, 1 had spherocytes noted incidentally on peripheral blood film.

The mean age at diagnosis was 3.5 years (range 1 month to 14.3 years). Genetic analysis was performed on only 3 patients (9.7%), 2 had SPTB gene mutation which codes for β-spectrin and 1 had SLC4A1 gene mutation which codes for Band 3 protein.

The Hb level at diagnosis ranged from 5.4 g/dL to 17.5 g/dL with a mean of 9.7±2.5 g/dL and a median of 10 g/dL. Majority of patients (87.1%) had Hb <12 g/dL at diagnosis. The MCHC level ranged from 33% to 37.9% with a mean of 35.4±1.1%. Two patients had missing MCHC data. A significant percentage (67%) of patients required at least one transfusion, with a mean lowest Hb of 4.8±1.3 g/dL for those who were transfused as compared to 8.5±1.9 g/dL for those who were not transfused. Only 23 patients had SB data available, and it ranged from 9 to 168 umol/L, with a mean value of 68.2±41.4 umol/L. Two patients had missing peripheral blood film findings. Of the remaining, 16 patients had spherocytes noted in the initial blood film. The remaining majority had polychromatic macrocytes suggesting increased red cell production without mention of significant numbers of spherocytes.

Twelve patients (38.7%) were diagnosed before 1 month of age. A positive family history of HS was present in only 50% of the neonates. Four presented with neonatal jaundice (NNJ) requiring intense phototherapy, 2 required exchange transfusion, 2 with early NNJ presenting within 24 hours requiring less intense phototherapy, 1 with prolonged NNJ, 2 with neonatal anaemia and 1 was asymptomatic but screened due to a positive family history. None of the neonates with early NNJ had other contributory haematological conditions such as G6PD deficiency. All neonates requiring intense phototherapy or exchange transfusion presented early within 36 hours of life.

The complications from HS are shown in Table 2. Twenty-one patients (67.7%) required at least one transfusion during the period of follow-up, with 9 patients (29%) requiring ≥3 transfusions over the period of data collection. Splenectomy was performed in 6 (19.4%) patients at a mean age of 12.5 years. Indications for splenectomy included frequent transfusion requirements (12.9%), concurrent cholecystectomy (3.2%) and hypersplenism with pain (3.2%). The 4 patients who underwent splenectomy due to frequent transfusion requirements received a mean of 15.5±4.4 transfusions prior to splenectomy. None required chelation therapy.

Table 1 Clinical characteristics of patients at diagnosis

	No. of patients - 31 (%)
Gender	Female: 21 (67.7%) Male: 10 (32.3%)
Race	Chinese: 20 (64.5%) Malays: 9 (29%) Others: 2 (6.5%)
Mean age at diagnosis	3.5 years (birth to 14.3 years)
Positive family history	16 (52%)
Available genetic profiles	3 (9.7%) - 2 with SPTB gene - 1 with SLC4A1 gene
Haemoglobin (Hb) level at diagnosis (g/dL)	<6: 3 (9.7%) 6-7.9: 4 (12.9%) 8-9.9: 7 (22.6%) 10-11.9: 13 (41.9%) >12: 4 (12.9%) Mean Hb: 9.7±2.5
MCHC level (%)	Mean MCHC: 35.4±1.1
Total bilirubin (umol/L)	Mean bilirubin: 68.2±41.4
Presenting features	
Pallor or anaemia	16 (51.6%)
Jaundice	15 (48.4%)
Hepatosplenomegaly/ splenomegaly	7 (22.6%)
Peripheral blood film showing spherocytes	4 (12.9%)
Screening based on family history	2 (6.5%)

Table 2 Complications from HS

Transfusion	21 (67.7%) required at least one transfusion. 9 (29%) required (≥3 transfusions) ➢ 6 (19.4%) required frequent transfusions (at least once of >3 transfusions / year)
Splenectomy	6 (19.4%) Mean age at splenectomy: 12.5 years (range 7-19 years)
Gallstones	10 (32.3%) >3 required surgical removal
Haemolytic crisis	22 (71%) >8 (36.4%) had parvovirus infection
Aplastic crisis	3 (9.7%) >2 (66.7%) had parvovirus infection

Post-splenectomy, the Hb for the 5 patients increased by a mean of 5.6±1.3 g/dL, resulting in a mean post-splenectomy Hb of 14.3±0.4 g/dL. One of the patients experienced a rebound anaemia requiring repeated transfusions 4 years post-splenectomy for unknown reasons.

Ten patients (32.2%) developed gallstones on follow-up, with 3 (9.7%) requiring surgical removal. Most patients (77%) had at least one haemolytic crisis due to intercurrent illness, of which 7 out of 22 patients (31.8%) had parvovirus infection alone while 1 (4.5%) had a concomitant dengue infection. Parvovirus infection was noted in 2 out of 3 (66.7%) patients with aplastic crisis.

Discussion

The prevalence of HS in our institution is low with only 31 patients identified over 10 years. This does not represent the true population prevalence in Singapore as the study was conducted on paediatric patients in a single institution, hence patients with milder phenotype who present in adulthood will not be represented. The initial clinical presentation of our patients was similar to previous studies in children where anaemia was the most frequent complaint, followed by splenomegaly, jaundice and positive family history.[7,8] Mean age at diagnosis was 3.5 years, with 12 patients (38.7%) diagnosed before 1 month of age. Six out of 12 patients diagnosed before 1 month of age presented with NNJ requiring intensive treatment, suggesting that severe jaundice should prompt an investigation into congenital haemolytic conditions such as HS. This is similar to studies in Western populations where half of infants with HS developed severe NNJ compared to 8% of normal newborns.[10] Among neonates listed in the USA Kernicterus Registry, HS is the third most common underlying haemolytic condition after glucose-6-phosphate dehydrogenase deficiency and ABO haemolytic disease.[11] Positive family history was present in slightly over half (52%) of our patients which is less than the 75% reported in many studies.[3,12] This could be due to less screening in the older population if the parents are otherwise asymptomatic. This figure may change over time with increased awareness and screening of siblings of affected patients.

Confirmatory studies with OF or EMA were conducted for all patients, in contrast to recommendations stating that confirmatory testing is not necessary for patients with family history and typical clinical and laboratory features.[1,12] The diagnosis of HS at our institution is first suspected based on the clinical or family history and characteristic spherocytes noted on the peripheral blood film. Peripheral blood samples are then sent for the EMA test. EMA has replaced conventional OF in the screening of patients in our institution due to its higher sensitivity and specificity.[13]

Genetic studies are reserved mainly for patients with severe phenotype requiring multiple blood transfusions from an early age due to its significant cost. Hence, we are unable to determine the genotype for most of our patients. The 3 patients with genotyping done were aged 9 months to 3 years. Understanding their genotype can aid in the prognosis of the child as well as genetic counselling for their parents. There are comorbidities associated with each of the genotypes and understanding them would allow us to monitor for associated comorbidities. For instance, the SLC4A1 gene mutation is associated with distal renal tubulopathy which was screened for and detected in one of our patients.[14,15]

Transfusion was performed for patients with a mean lowest Hb of 4.8±1.3 g/dL as compared to 8.5±1.9 g/dL for those who were not transfused. This is close to the recommended practice to transfuse patients when Hb falls below 5-6 g/dL.[6,7] Patients were transfused when they were clinically symptomatic and not based on Hb alone, as per our hospital guidelines. Splenectomy was performed on 6 patients, with frequent transfusion requirements being the most common reason. According to guidelines, splenectomy is indicated in patients with moderate to severe HS who require frequent transfusions or those with complications such as gallstones or growth failure.[6,7] Splenectomy was delayed till after 6 years old in all patients as recommended.[7] No partial splenectomy was done in our institution. Amongst the 3 patients requiring surgical intervention for gallstones, 2 were due to symptomatic gallstones while one was due to concurrent splenectomy. The practice of cholecystectomy in patients with asymptomatic gallstones going for splenectomy is still controversial[7]. Similar to other studies, most patients (71%) experienced at least one haemolytic crisis, often triggered by viral infections.[1,6] Patients with HS may develop haemolytic or aplastic crisis with Parvovirus infection and serology for Parvovirus is routinely sent in our institution for patients presenting with either. The incidence of aplastic anaemia is low and if present is commonly associated with Parvovirus infection as shown in our study.

Conclusion

Most reports of HS are described in Caucasian populations. Our paper adds to the body of evidence describing the presentation of HS in a predominantly Asian population, which does not appear to differ from Caucasian populations. Most paediatric patients in our institution are diagnosed early, with the majority requiring at least one transfusion. Neonates with early jaundice requiring intensive therapy should be closely followed up and investigated for haemolytic conditions such as HS, even without a family history. Positive family history in a patient with clinical symptoms of jaundice or anaemia should certainly prompt for an investigation of HS.

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Declaration of Interest

The authors confirmed that there is no conflict of interest in this study and that no funding is given for this study.

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