GALLSTONES IN PATIENTS WITH INHERITED HEMOLYTIC DISEASES

UDAY Y. H. ABDULLAH[1*], HAITHAM M. JASSIM[2], ATIF A. BAIG[1], RANA M. KHORSHEED[1], ALI M. H. AL-KHAYAT[1], AHMAD F. B. SULONG[3], NAGHAM F. ABED[1], WISAM A. YASSIN[1]

[1]Faculty of Medicine, University Sultan Zainal Abidin (Uni SZA), Jalan Sultan Mahmud 20400, Kuala Terengganu, Malaysia, [2]Intensive Care Medicine Department, Al-Wakra Hospital, Hamad Medical Corporation (HMC), Doha, Qatar, [3]Kulliyyah of Medicine, International Islamic University of Malaysia (IIUM), Kuantan, Malaysia
Email: Al_ubaydiuy@yahoo.com

ABSTRACT

The purpose is to provide an overview on the incidence of gallstone disease in patients with various types of inherited (chronic) hemolytic diseases at risk of cholelithiasis/choledocholithiasis with particular emphasis on its pathogenesis, genetic, risk factors and management. A detailed electronic literature search to determine the source of materials for this review article was done. The reported incidences of gallstones and choledocholithiasis vary according to the different types of inherited hemolytic diseases and the ethnicity of the studied populations. To date, no review article summarises the incidences of cholelithiasis in patients with various inherited haemolytic diseases was published. Regular ultrasound examination for the presence of gallstones recommended in patients with inherited haemolytic anaemias, particularly those with additional risk factors recommended. Further studies for evaluating the reasons for the higher incidence of cholelithiasis in thalassemia major and sickle cell anemia compared to hereditary spherocytosis; the effect of co inheritance of alpha thalassaemia on decreasing bilirubin level in patients with sickle cell disease and beta thalassaemia; the effect of the co inheritance of *UGT1A1* and *ABCG8* gene mutation on the incidence of gallstones in other blood diseases such as Hb-H disease, autoimmune haemolytic anaemias, congenital dyserythropoietic anaemia, hereditary elliptocytosis, Southeast Asian Ovalocytosis, glucose-6-phosphate and pyruvate kinase deficiency are recommended. Evaluation of the potential role of the solubility of the mutant proteins and haemoglobin subunit in the red blood cells as an additional mechanism for the development of gallstones in patients with inherited haemolytic anaemias recommended.

Keywords: Gallstones, Cholelithiasis, Choledocholithiasis, Haemolytic diseases.

INTRODUCTION

In reviewing the literature through 2014, the reported incidences of gallstones (cholelithiasis) and stones formed in the hepatic ducts (choledocholithiasis) vary according to the different types of inherited (chronic) hemolytic diseases and the ethnicity of the studied populations. The reported incidences of gallstones in different types of inherited hemolytic diseases are; 4-85 % in Sickle Cell Anaemia (Hb-SS), 29 % in Sickle Cell trait (Hb-AS), 23 % in Sickle Cell Hemoglobin-C (Hb-SC), 12 % in Hb S-B-Thalassaemia, 4-61 % in Hereditary Spherocytosis, 30->80 % in β-thalassaemia Major, 23-57 % in β-thalassaemia Intermedia, 21 % in β-thalassaemia Minor (Trait). Chronic hemolysis leads to increased bilirubin excretion and pigment gallstones formation, thus patients who are homozygous have the higher incidence of gallstones development than those who are heterozygous for that particular gene defect and disease, and there is a general trend in this direction. The incidence of gallstones increased in the last years [1]. To date, no review article summarises the incidences of cholelithiasis in patients with various inherited haemolytic diseases. We aim to increase the awareness of the incidence of gallstone disease with particular emphasis on its pathogenesis, genetics and risk factors and management in patients with various inherited chronic haemolytic diseases. A detailed electronic literature search to determine the source of materials for this report was done. Keywords used for websites searching were "Gallstones, cholelithiasis, choledocholithiasis, hemoglobinopathies and haemolytic disease". The Pro Quest Hospital Collection and NCBI search were the principal sources of information. All searches limited to research published in English. In addition, the Google Scholar search engine used in the search. The resulting literature involved over 700 peer-reviewed journal articles. Out of the total 100 reviewed and analysed articles, 62 selected articles were included in this report. In addition, review articles were included in the references because they provided contextual material for the review.

Aetiology and pathogenesis

The pathogenesis of biliary calculi in the various inherited chronic haemolytic anaemias is not understood clearly. In haemolytic anemias, the liberated hemoglobin is broken down, and its heme component is eventually, degraded into bilirubin by the liver. The development of pigmented gallstones due to chronic hemolysis is age-dependent. Etiologically, cholelithiasis is divided into three groups; hemolytic, other known etiology, and idiopathic. A 30% of all gallstones in children are due to haemolytic diseases such as sickle-cell disease, hereditary spherocytosis and thalassemia. In about 30% of cases, gallstones are due to another known etiology such as cholestasis, chronic liver disease, prolonged fasting and ileal disease or resection. In 40% of cases, gallstones are idiopathic mainly in adults and adolescent girls [1]. Cholesterol gallstones (green, white, or yellow stones) (pure and mixed) that are made mostly of cholesterol (70-80%), plus calcium salts and bilirubin compounds (20-30%). The pigmented stones (black or brown) contain less than 20% cholesterol and are composed mainly of bilirubin and calcium salts. Mixed gallstones composed of a mixture of the two gallstone types (fig. 1).

Mixed gallstones are the commonest stones in adults, and the pigment stones are more common in children. Cholesterol supersaturation of bile together with biliary stasis predisposes to cholesterol gallstone formation. Pigmented stones formed due to supersaturation of bile with calcium bilirubinate as in hemolytic disorders. Biliary sludge composed of mucin; calcium bilirubin and cholesterol crystals commonly associated with sickle-cell disease (fig. 2). The biliary sludge may resolve spontaneously or may progress to gallstone development. Persistent sludge gives rise to biliary complications (such as obstruction or infection). Bilirubin converted in the hepatocytes to bilirubin monoglucuronide or di glucuronide by several classes of the enzyme glucuronosyl transferase.

The poorly soluble bilirubin is frequently present in the cholesterol stones as the bilirubin precipitates in the gallbladder and forms bilirubin stones (fig. 3). Unconjugated as well as defectively conjugated bilirubin enhances the cholesterol crystallization by direct interaction with biliary lipid or indirect alteration of biliary lipid or both [2].

Fig. 1: (A) Cholesterol, (B) pigmented (Source: www. med. umich. edu) and (C) mixed stones (Source: health-fts.blogspot.com)
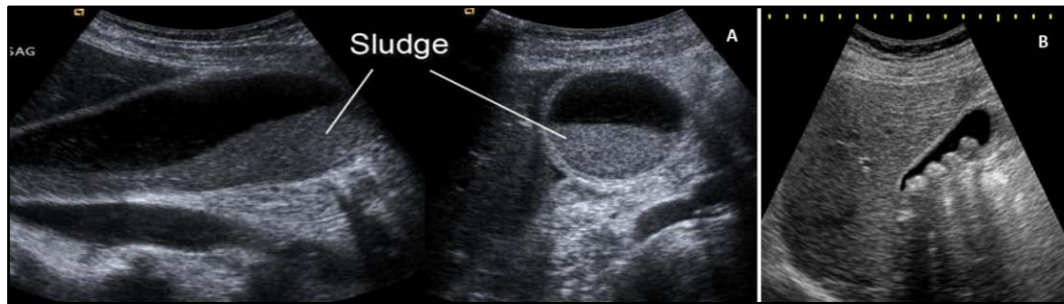

Fig. 2: (A) Biliary sludge (Source: http://www.radiologytutorials.com), (B) Multiple gallstones (Source: http://www.rcstn.net/marisa.bush)

It has formulated by Dewey KW and Grossman H. in 1970 [3] that the incidence of cholelithiasis in hereditary spherocytosis is higher compared to beta thalassemia major and sickle cell anemia. The proposed higher rate attributed to the concentration of hemoglobin and total hemoglobin mass. In hereditary spherocytosis, an abnormality in red cell shape is secondary to changes in the cell membrane. The spherocytic cells despite their relatively small size, contain normal to increased amounts of hemoglobin. In both thalassemias major and sickle-cell anemia, the primary defect is failure to synthesize normal adult haemoglobin either by the inability to produce beta chains or by abnormal amino acid substitutions, respectively. As a result, the red blood cells in thalassemia major are hypochromic and microcytic, thereby containing less hemoglobin; whereas in sickle-cell anemia, the total cell mass may be less because of the abnormal red cell shape. Thus, for each red blood cell hemolyzed, there is a greater amount of bilirubin produced from the cell of hereditary spherocytosis [3]. This proposal is suboptimal to explain the higher reported incidences of gallstone disease in beta thalassemia major and sickle cell anemia compared to that in hereditary spherocytosis. Moreover, genetics and other risk factors are proven to contribute toward the development and incidences of gallstone disease in various inheritedchronichemolytic disease. However, the practice of using the non-invasive and sensitive detection technique (ultrasound scan) and the longer survival of patients with inherited hemolytic diseases might explain the higher incidences of gallstone disease in beta thalassemia major and sickle cell anemia compared to that in hereditary spherocytosis. According to the physiology of macrophages and their role in the cellular protein catabolism, unstable globin is polyubiquitinated and degraded via the proteasome. If ubiquitin-proteasome activity is insufficient, unstable globin forms insoluble aggregates that serve as a substrate for macro autophagy. Chaperones are involved in refolding unstable globin or in targeting unstable globin for degradation. Accumulation of unfolded proteins activates autophagy (fig. 3). The complete breakdown of proteins requires the action of proteinases capable of degrading proteins to small peptide fragments, and peptidases that hydrolyze these peptides to amino acids [4]. However, does gallstone disease is pertinent to the broader framework of protein-aggregation or metabolism disorders?. This question requires to know the sequence of proteolytic events in detail particularly in hematological diseases with insoluble/unstable globins [5, 6].

Additional studies both in laboratories and in the clinics will be necessary to appraise with accuracy the role of hemoglobin degradation products (such as hemorphins) in physiology or pathology [7]. Evaluating the role of the altered solubility of the mutated proteins and haemoglobin subunit in the red blood cells could provide additional information for the mechanism of the gallstones development in patients with inherited hemolytic diseases.
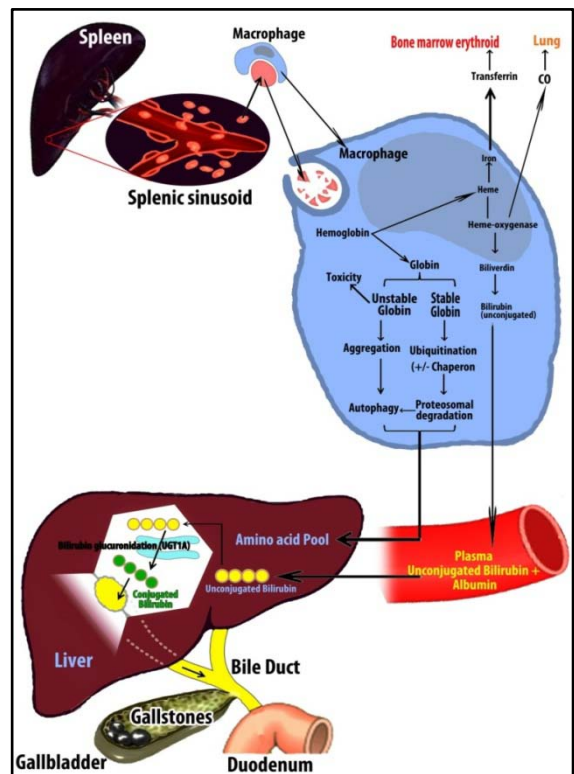

Fig. 3: Mechanism of the extravascular hemolysis and gallstones development

Genetic and risk factors

Up to one-third of gallstones may be related to genetic factors.

1- Ethnicity: it is significant risk factors mainly in the paediatric population.

2- Mutation in the uridine diphosphate-glucuronosyltransferase gene (*UGT1A*): This gene is a nested gene complex comprising nine transcriptional units, each encoding a specific isoform of the enzyme. *UGT1A1* is responsible for bilirubin glucuronidation. The 2-bp (TA) insertion within the promoter of the *UGT1A1*, is associated with Gilbert syndrome. The promoter polymorphisms in the *UGT1A1* gene resulted in excessive bilirubin production and associated with unconjugated hyperbilirubinemia. The latter appear to be a risk factor for symptomatic gallstones in subjects with sickle cell anemia (SCA) and hereditary spherocytosis [8]. It is reported that, children with SCA and the 7/7 promoter *UGT1A1* genotype had a significantly higher mean bilirubin level than those with the 6/6 or 6/7 genotypes and were more likely to have had a previous cholecystectomy [9,10]. The presence of the 7/7 *UGT1A1* genotype may also adversely affect the ability of hydroxyurea to prevent gallstone formation in SCA. Carriers of the 7/7 genotype had bilirubin levels more than 3 mg/dL despite full-dose hydroxyurea therapy while those with the 6/6 genotype had normal bilirubin levels following treatment with this agent [11]. Coinheritance of Gilbert syndrome increases the risk of developing gallstones in patients with hereditary spherocytosis. *UGT1A1* gene promoter polymorphism is also a major genetic risk factor modifying the frequency and age-at-onset of cholelithiasis in patients with SCA [12]. The individual susceptibility to form gallstones from TA insertion in the TATA-box of the *UGT1A1* promoter should be considered during the follow-up of patients with different forms of inherited (eg, thalassemia, intraerythrocytic enzymatic deficiency) or acquired (eg, autoimmune hemolytic anemia, hemolysis from mechanical heart valve replacement) chronic hemolysis. Homozygosity of the (TA)7 allele (also known as *UGT1A1*28*) has been implicated in Gilbert's syndrome and the unusually high unconjugated bilirubinemia observed in individuals with various forms of inherited chronic hemolysis: heterozygous β-thalassemia, β-thalassemia intermedia, neonatal jaundice associated with glucose-6-phosphate dehydrogenase deficiency, hereditary spherocytosis and sickle cell anemia [12].

3- Mutation in the ATP-binding cassette transporter sub-family C member 8 (*ABCG8*) gene significantly increases the risk of gallstones by causing the cholesterol pump to work continuously at a high rate. A single gene, however, does not explain the majority of cases, so multiple genes and environmental factors play an intricate role.

4- Multiple blood transfusions: In patients who periodically receive blood transfusions, hemolysis of the transfused cells may supplement the chronic endogenous blood destruction as a factor in the formation of gallstones [13].

5- High rates (20% by age of four years) of recurrent biliary tract obstruction in children with the sickle cell disease reported [14].

6- Obesity: With the prevalence of childhood obesity on the rise, there is a need to be more aware of obesity-related comorbidity including gallbladder disease. No clear link between the diet and risk for cholelithiasis, though foods high in cholesterol and low in fiber may increase the risk [15].

7- Inefficient and infrequent gallbladder contractions, which allow bile to sit in the gallbladder for long periods of time, resulting in an over concentrated bile that is conducive to stone formation [16].

8- Other risk factors include liver cirrhosis and biliary tract infections, trisomy 21 in patients with sickle cell disease and extensive small bowel resection requiring total parenteral nutrition [13].

Epidemiology

A prominent feature of children with chronic haemolytic anemia is the development of premature bilirubin gallstone disease and biliary tract inflammation. Of interest is the variation with the age of incidence of cholelithiasis in the haemolytic anemias(table 1). As patients with hemolytic anemia grow older, gallstones are more likely to develop.

Table 1: Prevalence (%) of gallstones according to the type of inherited hemolytic diseases, age group and population

Type of inherited hemolytic diseases [%]	Age group	Population	Gallstones (%)	Ref.
Sickle Cell Anaemia (Hb-SS) [4-85]	Children	Jamaican	30/226 (13)	14
		Brazilian	72/143 (50)	48
		USA	(34-70)	17
		African	(4-25)	17
		KSA	(8)	17
		KSA	60/305 (20)	47
		Sudan	30/261 (11)	22
		USA	4/47 (9)	3
		Italy	(15)	50,51
		Italy/Cuba	17/27 (63)	53
	Adults	Jamaican	96/311 (31)	13
		UK	55/95 (58)	17
		KSA	90/170 (53)	56
		Italy	(80)	50,51
		India	8/80 (10)	54
		USA	11/42 (26)	34
		USA	(30-70)	46
	Both	USA	15/36 (42)	3
		Nigeria	30/133 (23)	62
		USA	13/35 (37)	57
		KSA	(50-85)	23
		Nigeria	23/140 (16%)	61
Sickle Cell Trait (Hb-SA) [0-29]	Children	Jamaican	00/25	14
	Adults	Cameron	26/90 (29)	21
	Both	USA	3/15 (20)	57
Sickle Cell Hemoglobin-C (Hb-SC) [3-23]	Children	Jamaican	4/138 (3)	14
		Brazilian	10/44 (23)	47
		USA	3/15 (20)	34
	Adults	Jamaican	18/167 (11)	13
	Both	UK	24/95 (17)	17

Disease	Age group	Country		
		Nigeria	8/24 (33)	62
		Nigeria	1/10 (10%)	61
Hb S-B-Thal [12]	Adults	USA	1/8 (12)	34
Hereditary Spherocytosis [4-61]	Children	Brazil	17/63 (27)	41
			18/44 (41)	50
		Italy/Cuba	7/27 (26)	53
		USA	1/25 (4)	3
		USA	6/13 (46)	3
	Adult	USA	45/74 (61)	3
	Both	India	26/70 (37)	38
β-thalassaemia Major [30->80]	Children [11-50]	Italy	257/858(30)	27
		Italy	(50)	52
		Italy/Cuba	3/27 (11)	53
		USA	7/56 (13)	3
	Adults	Italy	(>80)	52
		USA	6/26 (23)	3
		USA	6/11 (55)	3
	Both	Italy	52/261 (20)	55
		Italy	257/858 (30)	59
β-thalassaemia Intermedia [23-57]	Adults	Sri Lanka	18/79 (23)	29
	Both	Italy	148/261 (57)	55
β-thalassaemia Minor (Trait) [21]	Adults	Italy	30/143 (21)	27

1-Sickle Cell Disease (SCD): Most patients are asymptomatic. Symptomatic biliary tract disease is difficult to diagnosis in patients with SCD, who frequently can have acute abdominal pain, fever and jaundice, as symptoms of vaso-occlusive crisis. Distinguishing acute cholecystitis from the sickle hepatic crisis may be difficult because of their similar clinical presentations. Early recognition of this entity is essential to avoid life-threatening complications. The prevalence of pigmented gallstones in SCD directly related to the rate of hemolysis [17].

a- Sickle Cell Anaemia (Homozygous): Pigmented gallstones are seen in two third of patients, particularly in young children. Gallstones in children as young as 3-4 years of age found in about 70 percent of patients (table 1)[18-23]. In a study of 509 adult patients with SCD, 48 percent of patients aged 18 to 47 years and 69 percent of those with more than 47 years of age had undergone cholecystectomy [24]. However, the frequency of gallstones in patients with sickle cell anaemia varies according to other different studies [25-29].

b- Sickle Cell Trait (Heterozygous): The reported incidence of gallstones in patients with sickle cell trait is low (table 1)[20].

c- Hb-SC and Hb-S B-Thalassaemia syndrome: The risk of gallstones is low in patients with HbSC disease and HbSß-thalassaemia [21].

d- Coinherited alpha thalassaemia causes decreased bilirubin level in patients with sickle cell disease. The incidence of gallstones and bilirubin levels strongly associated with the number of the *UGT1A1*promoter (TA) repeats [22, 23].

2-Beta-Thalassaemias: Hemolysis and extra medullary erythropoiesis lead to the more prominent hepatomegaly in beta thalassaemias than in other causes of inherited haemolytic anemia.

a- Beta-Thalassaemia Major. Two-thirds of patients have multiple, calcified bilirubin stones by the age of 15. However, cholecystitis or cholangitis are rare. Adult patients with beta-thalassaemia major have gallbladder dysmotility with delayed small bowel transit and autonomic dysfunction that together with hyperbilirubinemia contribute to the pathogenesis of pigment gallstones/sludge in beta-thalassemia major [30].

b- Beta Thalassaemia Minor (Trait): Characterized by chronic ineffective erythropoiesis and variable levels of serum bilirubin. Thalassaemia minor represent a risk factor for gallstones, but the associated gilbert mutation further increases this risk. Carriers of beta thalassaemia havesignificantly higher risk of gallstone formation than the normalpopulation [31].

c- Beta-Thalassaemia Intermedia (Hb-E β-Thalassaemia): Chronic hyper bilirubinaemia, gallstones formation and gallbladder disease are unusually frequent in people with haemoglobin E β-thalassaemia.

This category is the most common of beta-thalassaemia of intermediate severity in the world and represents an important health problem for populations of the Indian subcontinent and Southeast Asia [32, 33].

3-Hereditary Spherocytosis (HS): Bilirubin stones found in 50 % of patients with HS between 30 and 50 years of age, even in those with very mild forms of the disease. Gallstones have reported during infancy, but they are uncommon in very young children. Pigmented gallstones form as early as 4-5 years old children, and at least 50 % of unsplenectomized patients ultimately form gallstones, although they may be asymptomatic. The incidence of gallstones is uncommon before age ten but is present in at least one-half of adults with more severe hemolytic disease [34]. The development of jaundice due to gallstones improves the hemolytic process and normalizes osmotic fragility [35]. The increased membrane surface area due to the abnormal plasma lipid profile normalizes the previously reduced surface area-to-volume ratio due to loss of membrane surface area in the spherocytes. The incidence of gallstones is increased further if the patient with HS co-inherits the genetic defect resulting in the diminished activity of the bilirubin conjugating enzyme uridine diphosphate-glucuronyltransferase (i.e., Gilbert syndrome). In a study of 102 children from southern Italy with mild to moderate HS, gallstones were found in 12, 26, and 48 percent of children who were normal, heterozygous, or homozygous for the Gilbert syndrome mutation, respectively. However, no significant difference in the age of onset of gallstones or the degree of hemolysis, as measured by average reticulocyte count [36].

4-Other chronic haemolytic anemias: The gallstones could also occur in patients with other inherited haemolytic anaemia such as Hb-H disease, autoimmune haemolytic anaemias, congenital dyserythropoietic anaemia, hereditary elliptocytosis, Southeast Asian Ovalocytosis and pyruvate kinase deficiency [8,9]. Prevalence of gallstones in these blood disorders in not well known.

Management

Silent gallstones require no intervention. Laparoscopic cholecystectomy can be safely performed, but associated common bile duct stones first require endoscopic retrograde cholangiopancreato

graphy (ERCP) [37-39]. In hereditary spherocytosis, cholecystectomy is indicated for symptomatic gallstones with recurrent cholecystitis or biliary colic. Combined prophylactic splenectomy and cholecystectomy provide a substantial gain in life expectancy for young patients and adults with mild HS and gallstone [40, 41]. However, the use of concomitant splenectomy is controversial and considered on an individual basis, weighing the severity of haemolytic anemia versus the post-splenectomy risks [42-45]. Elective cholecystectomy considered in patients with sickle cell disorders and gallstones-related symptomatology [46]. After a decision is made to perform cholecystectomy, the gold standard surgical strategy is the laparoscopic procedure. Laparoscopic cholecystectomy is recommended because it associates with a shorter hospital stay and lower rates of postoperative complications. Prior to surgery, blood transfusion therapy is recommended and aimed to get the hemoglobin up to 100 g/l to prevent potentially life-threatening Intra-and postoperative complications [47]. A multicenter, randomized trial compared observation with blood transfusion therapy no more than ten days prior to surgery [48]. In the latter multicenter study, a) out of the 70 enrolled patients, complications were more common in observation group than in transfusion group (39 versus 15 %), b) severe complications were more common in the observation group (30 versus 3 percent), c) acute chest syndrome occurred in nine of the participants in the observation group and one in the transfusion group, d) hemoglobin-S decreased to at least 30 % and total haemoglobin be increased at least 110 g/l to avoid sickling during the postoperative period. Blood transfusion considered for individuals with Hb-SS and Hb less than 90 g/l undergoing cholecystectomy. For individuals with Hb-SS or other types of SCD who have baseline hemoglobin greater than 90 g/l, partial exchange transfusion and adequate hydration are considered to avoid the development of hyperviscosity syndrome. Red blood cell pheresis designed to decrease the Hb-S level to<30 percent does not confer any advantage over simple blood transfusion therapy and preferably avoided, except under special circumstances [49-54]. During the surgery and the recovery period, hypotension, dehydration, hypoxia, hypothermia and acidosis should be prevented [55]. Elective cholecystectomy is recommended for patients with hepato biliary symptoms and biliary sludge only [56]. Any symptomatic gallstone should be treated by cholecystectomy because the accelerated hemolysis in beta thalassaemia leads to the formation of gallstones. Since cholecystitis is life-threatening in any splenectomized patient, inspection of the gallbladder in any patient who is undergoing splenectomy recommended [31].

Conclusions and recommendations

1. Regular ultrasound examination for the presence of gallstones recommended in patients with inherited haemolytic anaemias, particularly those with additional risk factors.

2. Further studies for evaluating the reasons for the higher incidence of cholelithiasis in beta thalassemia major, sickle cell anaemia compared to hereditary spherocytosis recommended.

3. Evaluation of the potential role of the solubility-changing mutations expressing low-solubility haemoglobin subunit in the red blood cells as an additional mechanism for the development of gallstones in patients with inherited haemolytic anaemiasisrecommended.

4. The question whether gallstone disease is pertinent to the protein-aggregation or metabolism disorders requires toknowthe sequence of proteolytic events in detail particularly in hematological diseases with unstable globins.

5. Additional studies both in laboratories and in the clinics will be necessary to appraise the role of haemoglobin degradation products (such as hemorphins) in physiology or pathology recommended.

6. The effect of coinheritance of alpha thalassaemia decreasing bilirubin level in patients with sickle cell disease and beta thalassaemiarequire further evaluation.

7. The effect of the coinheritance of uridine diphosphate-glucuronosyltransferase and ABCG8 gene mutation on the incidence of gallstones in other blood diseases (such as Hb-H disease,

autoimmune haemolytic anaemia, congenital dyserythropoietic anaemia, here ditary elliptocytosis, Southeast Asian Ovalocytosis, glucose-6-phosphate dehydrogenase and pyruvate kinase deficiency) need to be formulated.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Poddar U. Gallstone Disease in Children. Indian Pediatr 2010;47:945-53.
2. Carpenter SL, Lieff S, Howard TA, Eggleston B, Ware RE. UGT1A1 promoter polymorphisms and the development of hyperbilirubinemia and gallbladder disease in children with sickle cell anemia. Am J Hematol 2008;83:800.
3. Dewey KW, Grossman H. Cholilithiasis in thalassaemia major. Radiology 1970;96:385-8.
4. Khandros E, Thom CS, D'Souza J, Weiss MJ. Integrated protein quality-control pathways regulate free α-globin in murine β–thalassemia. Blood 2012;119:5265-75.
5. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132:27-42.
6. Botbol V, Scornik OA. Intermediates in the degradation of globin. J Biol Chem 1979;254:11254-57.
7. Fruitier I, Garreau I, Lacroix, Cupo A, Piot JM. Proteolytic degradation of hemoglobin by endogenous lysosomal proteases gives rise to bioactive peptides. Hemorphins 1999;447:81-6.
8. Haverfield EV, McKenzie CA, Forrester T, Bouzekri N, Harding R, Serjeant G, *et al.* UGT1A1 variation and gallstone formation in sickle cell disease. Blood 2005;105:968-72.
9. Vasavda N, Menzel S, Kondaveeti S, Maytham E, Awogbade M, Bannister S, *et al.* The linear effects of alpha-thalassaemia, the UGT1A1 and HMOX1 polymorphisms on cholelithiasis in sickle cell disease. Br J Haematol 2007;138:263.
10. Passon RG, Howard TA, Zimmerman SA, Schultz WH, Ware RE. Influence of bilirubin uridine diphosphate-glucuronosyltransferase 1A promoter polymorphisms on serum bilirubin levels and cholelithiasis in children with sickle cell anemia. J Pediatr Hematol Oncol 2001;23:448-51.
11. Fertrin KY, Melo MB, Assis AM, Saad ST, Costa FF. UDP-glucuronosyltransferase 1 gene promoter polymorphism is associated with increased serum bilirubin levels and cholecystectomy in patients with sickle cell anemia. Clin Genet 2003;64:160-2.
12. Chaar V, Kéclard L, Diara JP, Leturdu C, Elion J, Krishnamoorthy R, *et al.* Association of UGT1A1 polymorphism with prevalence and age at onset of cholelithiasis in sickle cell anemia. Haematologica 2005;90:188-93.
13. Mehta S, Lopez ME, Chumpitazi BP, Mazziotti MV, Brandt ML, Fishman DS. Clinical characteristics and risk factors for symptomatic pediatric gallbladder disease. Pediatrics 2012;129:82-8.
14. Amoako MO, Casella JF, Strouse JJ. High rates of recurrent biliary tract obstruction in children with sickle cell disease. Pediatr Blood Cancer 2013;60:650–2.
15. Maher MM, Mansour AH. Study of chronic hepatopathy in patients with sickle cell disease. Gastroenterol Res 2009;2:338-43.
16. Portincasa P, Moschetta A, Berardino M, Di-Ciaula A, Vacca M, Baldassarre G, *et al.* Impaired gallbladder motility and delayed orocecal transit contribute to pigment gallstone and biliary sludge formation in beta-thalassemia major adults. World J Gastroenterol 2004;10:2383-90.
17. Walker TM, Hambleton IR, Serjeant GR. Gallstones in sickle cell disease: observations from the jamaican cohort study. J Pediatr 2000;136:80-5.
18. DK Webb, JS Darby, DT Dunn, SI Terry, GR Serjeant. Gall stones in Jamaican children with homozygous sickle cell disease. Arch Dis Child 1989;64:693.

19. Sarnaik S, Slovis TL, Corbett DP, Emami A, Whitten CF. Incidence of cholelithiasis in sickle cell anemia using the ultrasonic gray-scale technique. J Pediatr 1980;96:1005-8.

20. Trevor N Golding, Adeleke Adeyemo, Lewis Kurtz. The Incidence and surgical significance of Cholelithiasis in sickle cell anemia and sickle cell trait. J Natl Med Assoc 1973;65:410-4.

21. LR Bond, SR Hatty, ME Horn, M Dick, HB Meire, AJ Bellingham. Gall stones in sickle cell disease in the United Kingdom. Br Med J 1987;295:234-6.

22. Chaar V1, Kéclard L, Etienne-Julan M, Diara JP, Elion J, Krishnamoorthy R, *et al.* UGT1A1 polymorphism outweight the modest effect of deletional (-3,7 kb) alpha-thalassaemia on chlelithogenesis in sickle cell anaemia. Am J Hematol 2006;81:377-9.

23. Haidar MZ, Ashebu S, Aduh P, Adekile AD. Influence of alpha thalasaaemia on cholilithiasis in SS patients with elevated Hb-F. Acta Hematol 1998;100:147-50.

24. Adam S, Jonassaint J, Kruger H, Kail M, Orringer EP, Eckman JR, *et al.* Surgical and obstetric outcomes in adults with sickle cell disease. Am J Med 2008;121:916-21.

25. Billa RF, Biwole MS, Juimo AG, Bejanga BI, Blackett K. Gall stone disease in African patients with sickle cell anaemia: a preliminary report from Yaounde, Cameroon. Gut 1991;32:539-41.

26. Attalla BAI, Karrar ZA, Ibnouf G, Mohamed AO, Abdelwahab O, Nasir EM, *et al.* Outcome of cholelithiasis in sudanese children with sickle cell anaemia (SCA) after 13 years follow-up. Afr Health Sci 2013;13:154–9.

27. Al-Mulhim AS, Al-Mulhim AA. Laparoscopic cholecystectomy in 427 adults with sickle cell disease: a single-centerexperience. Surg Endosc 2009;23:1599–602.

28. Giuseppe Curro, Anna Meo, Daniela Ippolito, Anna Pusiol, Eugenio Cucinotta. Asymptomatic cholelithiasis in children with sickle cell disease early or delayed cholecystectomy? Ann Surg 2007;245:126–9.

29. Tripathy D, Dash BP, Mohapatra BN, Kar BC. Cholelithiasis in sickle cell disease in India. J Assoc Physicians India 1997;45:287-9.

30. Pignatti CB, Gamberini MR. Complications of thalassemia major and their treatment. Expert Rev Hematol 2011;4:353-66.

31. Borgna-Pignatti C, Rigon F, Merlo L, Chakrok R, Micciolo R, Perseu L, *et al.* Thallassaemia minor, the Gilbert mutation and the risk of gallstones. Haematologica 2003;88:1106-9.

32. Musallam KM, Taher AT, Rachmilewitz EA. β-Thalassemia intermedia: a clinical perspective. Cold Spring Harb Perspect Med 2012;2:a013482.

33. Premawardhena A, Fisher CA, Fathiu F, de Silva S, Perera W, Peto TE, *et al.* Genetic determinants of jaundice and gallstones in haemoglobin E beta thalassaemia. Lancet 2001;357:1945-6.

34. Kar R, Rao S, Srinivas UM, Mishra P, Pati HP. Clinico-hematological profile of hereditary spherocytosis: experience from a tertiary care center in North India. Hematology 2009;14:164-7.

35. Cooper RA, Jandl JH. The role of membrane lipids in the survival of red cells in hereditary spherocytosis. J Clin Invest 1969;48:736-44.

36. Del Giudice Em, Perrotta S, Nobili B, Specchia C, D'urzo G, Iolascon A. Coinheritance of gilbert syndrome increases the risk for developing gallstones in patients with hereditary Spherocytosis. Blood 1999;94:2259-62.

37. AlFadhli S, Al-Jafer H, Hadi M, Al-Mutairi M, Nizam R. The Effect of UGT1A1 Promoter Polymorphism in the Development of Hyperbilirubinemia and Cholelithiasis in Hemoglobinopathy Patients. PLoS ONE 2013;8:e77681. doi: 10.1371/journal. pone.0077681.

38. Al-Salem AH, Nourallah H. Sequential endoscopic/laparascopicmanagment of cholilithiasis and choledocholithiasis in children who have sickle cell disease. L PaediatrSurg 1997;32:1432-35

39. Rennel MD, Dunne MG, Grossman NJ, Schwartz AD. Cholilithiasis in patients with major sickle hemoglobinopathies. Am J Dis Chil1984;138;66-67.

40. Marchetti M, Quaglini S, Barosi G. Prophylactic splenectomy and cholecystectomy in mild hereditary spherocytosis: analysing the decision in different clinical scenarios. J Inter Med 1998;244:217-26.

41. Al-Salem AH, Al-Abkari H, Al-Jishi L, Al Jam'a A, Yassin YM. Experience of concomitant splenectomy and cholecystectomy in patients with sickle cell disease. Annals of Saudi Medicine 1997;18:266-68

42. Krueger HC, Burgert EOJ. Hereditary spherocytosis in 100 children. Mayo ClinProc 1966;41:821.

43. Alizai NK, Richards EM, Stringer MD. Is cholecystectomy really an indication for concomitant splenectomy in mild hereditary spherocytosis? Arch Dis Child 2010;95:596-99.

44. Kar R, Rao S, Srinivas UM, Mishra P, Pati HP. Clinico-hematological profile of hereditary spherocytosis: experience from a tertiary care center in North India. Hematology 2009;14:164-7.

45. Oliveira MC1, Fernandes RA, Rodrigues CL, Ribeiro DA, Giovanardi MF, Viana MB. Clinical course of 63 children with hereditary spherocytosis: a retrospective study. Rev Bras Hematol Hemoter 2012;34:9-13.

46. Malone BS, Werlin SL. Cholecystectomy and cholelithiasis in sickle cell anemia. Am J Dis Child 1988;142:799-800.

47. Vichinsky EP, Haberkern CM, Neumayr L, Earles AN, Black D, Koshy MM, *et al.* A comparison of conservative and aggressive transfusion regimens in the perioperative management of sickle cell disease. Preoperative transfusion in sickle cell disease study group. N Engl J Med 1995;333:206-14.

48. Goers T, Panepinto J, Debaun M, Blinder M, Foglia R, Oldham KT, *et al.* Laparoscopic versus open abdominal surgery in children with sickle cell disease is associated with a shorter hospital stay. Pediatr Blood Cancer 2008;50:603-6.

49. Howard J, Malfroy M, Llewelyn C, Choo L, Hodge R, Johnson T, *et al.* The transfusion alternatives preoperatively in sickle cell disease (TAPS) study: a randomised, controlled, multicentre clinical trial. Lancet 2013;381:930-8.

50. Haberkern CM, Neumayr LD, Orringer EP, Earles AN, Robertson SM, Black D, *et al.* Cholecystectomy in sickle cell anemia patients: perioperative outcome of 364 cases from the national preoperative transfusion study. Blood 1997;89:1533-42.

51. Al-Salem AH, Qaisruddin S. The significance of biliary sludge in children with sickle cell disease. Pediatr SurgInt 1998;13:14-6.

52. Gumiero APS, Bellomo-Brandão MA, Costa–Pinto EAL. Gallstones in children with Sickle cell disease followed up at a brazilianhematologycenter. Pediatr Gastroenterol 2008;45:313-8.

53. Attalla BAI, Karrar ZA, Ibnouf G, Mohamed AO, Abdelwahab O, Nasir EM, *et al.* Outcome of cholelithiasis in sudanese children with sickle cell anaemia (SCA) after 13 years follow-up. Afr Health Sci 2013;13:154-9.

54. Curro G, Meo A, Ippolito D, Pusiol A, Cucinotta E. Asymptomatic cholelithiasis in children with sickle cell disease early or delayed Cholecystectomy? Ann Surg 2007;245:126–9.

55. Hanna T, Shraga A, Enrique F, Hagit M, Tatyana K, Michael S, *et al.* High incidence of early cholilithiasis detected by ultrasonography in children and young adults with hereditary spherocytosis. J Pediatr Hematol Oncol 2003;25:952-4.

56. Hendricks-Ferguson V, Nelson MA. Treatment of cholelithiasis I children with sickle cell disease. AORN J 2003;77:1170-82.

57. Curro G, Iapichino G, Lorenzini C, Palmeri R, Cucinotta E. Laparoscopic cholecystectomy in children with chronic haemolytic anemia. Surg Endosc 2006;20:252–5.

58. Esposito C, Gonzalez Sabin MA, Corcione F, Sacco R, Esposito G, Settimi A. Results and complications of laparoscopic cholecystectomy in childhood. Surg Endosc 2001;15:890-2.

59. Tripathy D, Dash BP, Mohapatra BN, Kar BC. Cholelithiasis in sickle cell disease in India. J Assoc Physicians India 1997;45:287-9.

60. Galanelo R, Barella S, Leoni GB, Cipollina MD, Persue L, Cao A. Cholilithiasis and Gilbert's syndrome in homozygous beta thalassaemia. Br J Haematol 2011;115:926-8.

61. Maher MM, Mansour AH. Study of chronic hepatopathy in patients with sickle cell disease. Gastroenterol Res 2009;2:338-43.

62. Golding TN, Adeyemo A, Kurtz L. The incidence and surgical significance of cholelithiasis in sickle cell anemia and sickle cell trait. J Natl Med Assoc 1973;65:410-4.

63. Suell MN, Horton TM, Dishop MK, Mahoney DH, Olutoye OO, Mueller BU. Outcomes for children with gallbladder abnormalities and sickle cell disease. J Pediatr 2004;145:617-21.
64. Origa R, Galanello R, Perseu L, Tavazzi D, Domenica Cappellini M, Terenzani L, *et al.* Cholelithiasis in thalassemia major. Eur J Haematol 2008;82:22-5.
65. Agholor CA, Akhigbe AO, Atalabi OM. The prevalence of cholelithiasis in nigerians with sickle cell disease as diagnosed by ultrasound. Br J Med Med Res 2014;4:2866-73.
66. Durosimi MA, Ogunseyinde AO, Olatunji PO, Esan GJ. Prevalence of cholelithiasisin Nigerians with sickle cell disease. Afr J Med Sci 1989;18:223-7.