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Elevated Bilirubin Level Increase the Risk of Gallstone Disease in Pediatric Hereditary Spherocytosis Patients: A Case Report

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ABSTRACT
Disorders of gall bladder are common and remain as one of the main patients' chief complaints that might lead to surgical interventions. High level of bilirubin causes gallstones in patients with hereditary spherocytosis (HS). It is often misdiagnosed, because these deposits of bilirubin can be easily broken down in the gallbladder. Gallstones develop in less than half of patients with mild HS and do not always cause symptomatic biliary tract disease. HS is a genetic defect in red blood cells, caused by defects in structural membrane proteins. It is characterized by a decrease the surface area to volume ratio in erythrocytes due to the heterogeneous changes in the various genes that encode for proteins. A 14-year old male was brought to Medical Park hospital/Turki at the emergency room with a sudden onset of pain in the right upper quadrant, vomiting, anemia and jaundice. The patient was diagnosed with gallstone due to obstruction in common bile duct (CBD),with elevated bilirubin as a result for mild HS. This case report study highlight on the role of hereditary spherocytosis in forming many abdominal problem such as gallstone due to high level of heamolysis and elevated of bilirubin.

Keyword: Gallstone, Hereditory spherocytosis, liver function tests, cholelithiasis.

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INTRODUCTION

Biliary bilirubin has an important role in the formation of gallstones, and is one of the most common and costly gastrointestinal tract diseases. Biliary bilirubin and calcium can combine together to form calcium bilirubinate salts, which may later turn into symptomatic as pigment gallstones (Vitek and Carey, 2012). Increased bilirubin production is associated with an increased risk of gallstone disease (Buche*tal*.,2010). Arrange of hemolytic conditions, which are characterized by high levels of plasma secondary bilirubin and which lead to the breakdown of free hemoglobin, have been linked significantly to an increased risk of gallstones.

Hereditary spherocytosis (HS) is a genetic defect of red blood cells, a potentially life-threatening hematological disease (Sociaetal,2018). HS was first described in 1871. It was found in increasing numbers among persons of northern European origin (Shah andVega,2019). It has a variety of mutations that result in defects in red blood cell membrane proteins (Socia et al, 2018).

Morphologically, spherocytes are round red cells that have lost the ability to change shape. Shah and Vega (2019) They mentioned that chronic hemolysis is a hallmark of the HS. Usually, there are four forms of HS: mild, moderate, moderate to severe and sharp, all of which depend on the severity of the signs and symptoms (Barcellini *et al*.,2011). In most individuals, the condition is mild and does not require specific treatment. In severe cases, it leads to severe anemia, splenomegaly, and jaundice. Splenectomy is sometimes recommended as treatment for severe cases and this can improve disease. On the other hand, patients who have undergone splenectomy are at increased risk of infection with encapsulated bacteria (Socia e*t al*, 2018).

This hematological disorder contains at least five genetic mutations that cause the red blood cell membrane to distort and harden, and instead of a flat disc, the cell shape is spherical (Diez-Silva *et al*., 2010). The main mechanism of HS signs and symptoms includes that when red blood cells reach the bloodstream, due to their inability to change their shape, they are removed from the blood circulation and transferred to the spleen for destruction, shortage of red blood cells in the bloodstream and the presence of a large number within the spleen, where they undergo hemolysis (Gallagher, 2013). Increased bilirubin along with anemia, jaundice is another important sign of hereditary spherocytosis.

Several researchers in basic science have discovered that abnormalities in many red cell membrane proteins can lead to typical clinical manifestations of the HS. Regardless of the molecular basis of the HS case, the common denominator of spectrin deficiency results in an unstable red cell membrane. Unkerine disorders, band 3, or other structural proteins lead to minor defects common to spectrin assembly, leading to an unstable red cell membrane. Lipids are lost from the bilateral layer as microvesicles. When the affected cell gradually loses surface area, it changes from a biconcave disc to a sphere. As a result of these changes in shape, red cells lose their ability to circulate freely through the narrow capillaries in the body.

The resulting spherocytes become trapped in the pathway of the spleen asthey course through the sinuses, and the red cells are engulfed by macrophages. Hemolysis also causes elevated unconjugated bilirubin and the development of gallstones (Shah andVega,2019).

Often, this high level of bilirubin leads to gallstones in patients with HS. It is often misdiagnosed, because these deposits of bilirubin can be easily broken down in the gallbladder. Gallstones develop in less than half of patients

with mild HS and do not always cause diseases of the bile duct (Socia et al, 2018).

However, whether elevated levels of bilirubin in the plasma are associated with an increased risk of gallstone disease in the general population, it remains unknown. This question is important to be answer by study specific cases.

METHODS AND RESULTS (CASE PRESENTATION)

A 14-year-old Iraqi pediatric male presented to Medical Park hospital /Turki at the emergency room in 12 Nov. 2019 with sudden onset of pain in the upper right quadrant, vomiting, anemia and jaundice.

The first Ultrasonography(1st week) showed gallbladder was normal in size and contains few small stones with debris, largest stone was 4.0mm, mildy dilated common biliary duct (CBD) 6.0mm diameter(figure 1).with normal size and texture for pancreas and spleen. The results of complete blood count , total serum bilirubin ,liver function test, HBs Ag titer and HCV Ab titer were illustrated in table (1).The patient was supposed to have obstruction in CBD due to gallstone because of , ultrasonography ,elevated liver enzymes and bilirubin .

The clinical staff decided to delay the surgery of cholecystectomy to begin treatment (Cefixime, isosorbidedinitrate , and co-codamol) and Diets from fats and meat of all kinds. The following laboratory examinations were performed after one week(2nd week) the ultrasonography showed GB was normal in size and wall thickness filled with sludge and seveal tiny stones (in the range of 3.0mm)and normal CBD 4.0 mm liver function test showed normal levels, with normal size and texture for pancreas and spleen(figure 2),the level of liver enzymes were decreased to the normal rang and total bilirubin decreased also, but not to the normal range. The Magnetic resonance cholangiopancreatography (MRCP) showed distended with thick sludge GB, no stone, normal intra and extra hepatic biliary passages and normal pancreatic duct measuring , with no filling defect.

In the 3rd week, the ultrasonography showed normal CBD. GB was normal in size and wall thickness filled with sludge and seveal tiny stones , Biochemical test for liver enzymes showed decreased in level and total serum bilirubin was high (2.63 mg/Dl), hematological test showed WBC(8.13K/µL),elevated of RDW(15.3K/µL) than normal(table3,figure 3).

The patient had undergone laparoscopic cholecystectomy in surgical room of Medical park hospital/Istanbul. On the 3rdday post-operation, the patient transfer to Medipol University hospital for detection of hereditary spherocytosis according to some pediatric hematology tests.Glucose-6-phosfatase and Hb electrophoresis showed normal value (270pU/RBC)and(table5), respectively. Reticulocyte value (RET%) elevated. Osmotic fragility of patient was increased compared with normal erythrocytes(table 4).finally the patient had mild HS according to some laboratory finding (Hemoglobin, total bilirubin ,Reticulocyte ,and MCHC) as described by Gungor *et al*.(2018).

DISCUSSION

To our knowledge, this is the first description of the correlation between the elevated level of bilirubin in the plasma and the potential risk of gallstone due to undiagnosed HS. Our initial clinical impression was a blockage in CBD due to gallstones, and acute obstructive suppurative cholangitis caused by a combination of high fever, jaundice and elevated liver enzymes. If HS had not recognized, an emergency operation(cholangiopancreatography) might have been done. Zare *et al* .(2011) assessed the role of liver function testing in diagnosing common bile duct stone in patients with cholecystitis and assisting in their management. Results of the study showed that liver enzymes In patients with dilated CBD diameter, serum level of ALP had no significant difference between patients with or without CBD stones. Penget al.(2005)compared liver enzymes between acute and chronic cholesistitis patients and reported that there was no significant difference between them. Kaldoretal. (2006) reported determined predictability of liver enzymes in patients with cholesistitis who were undergone laparoscopy for cholecystectomy. Evaluating role of liver enzymes in prediction of extra liver obstruction in patients with delay diagnosis of CBD stone. He found that serum level of bilirubin was the best predictor CBD stenosis due to stone or malignancy Karavone et al.(2006).

Possible links between the level of bilirubin in plasma and gallstones were previously described. Bilirubin is a major component not only of pigment gallstones (primarily composed of calcium bilirubinate salts)..Unrelated bilirubin in the bile combines with calcium to form calcium bilirubinate salts. A range of hemolytic plasma bilirubin level will increase the risk of gallstone disease. Several previous studies reported an increased risk of gallstone disease, as it reduced hepatic bilirubin association due to a genetic variation in the gene that encodes the conjugated bilirubin enzyme UGT1A1(Wasmuth *et al.(*2006) and Tsezou *et al*.(2009).

Hereditary spherocytosis(HS) is a dominant hereditary autosomal dominant disease and is the most common cause of hereditary hemolysis in Northern Europe and the United States (Bolton-Maaggs,2004), it is characterized by a decrease in the ratio of area to volume in the erythrocytes due to the heterogeneous changes in the various genes that symbolize the proteins participating in the vertical assemblies that tie the membrane skeleton to the lipid bilayer(Bolton-Maggs *et al*.,2012). Hereditary spherocytosis is diagnosed frequently in childhood and early adulthood, as well as it could be diagnosed at any age. In Gungor et al.,2018)study, the age at diagnosis was ranging from 15 days to 17 years; and 20% were diagnosed during neonatal period. Patients with severe HS were diagnosed at an earlier age. The triad of anemia, splenomegaly and jaundice was not present in most neonates with HS. Neonatal red blood cells show altered response to osmotic stream due to decreased membrane surface area. However, incubated OFT was successfully used for the diagnosis of neonatal HS(Gungor *et al*.,2018).

Hereditary spherocytosis was classified as mild, moderate and severe according to Hb, bilirubin and reticulocyte values(Lux *et al*., 2015). The most significant finding in HS

is the appearance of spherocytes in peripheral blood blood smear is completely normal or a few spherocytes can be seen and the most frequently observed complication is cholelithiasis in HS.The incidence of bilirubin gallstone was reported as 21-63%(Segel, 2007).

It is known that splenectomy, removing the main site of red cell destruction, decreases hemolysis and reduces anemia by extending the life of the erythrocytes(Lux,2015).

CONCLUSION

Hereditary spherocytosis was a benign disease and anemia, jaundice and hepato/ splenomegaly were the most common clinical features. Gallstone is the main risky of elevated bilirubin in HS. The diagnosis of HS is based on a combination of the clinical and laboratory findings such as spherocytes on peripheral smear, increased MCHC values and osmotic fragility tests. Routine ultrasonographies should be performed in all HS patients, to exclude the gallbladder and common bile duct stones. Clinical parameters such as splenectomy and transfusion requirement and laboratory findings such as Hb/MCHC and Hb/RDW ratios might be helpful for classifying the clinical severity.

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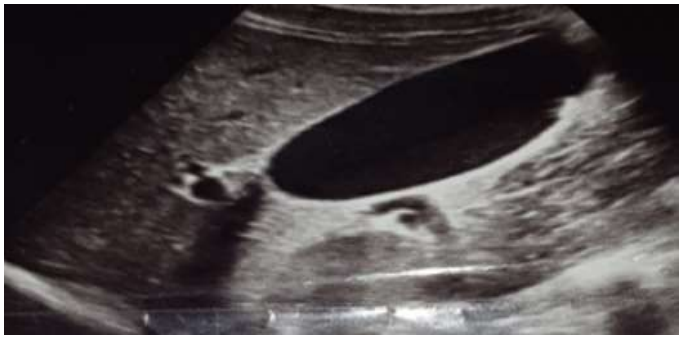

Fig.1: Ultrasonography in 1st week.

Fig.2: Ultrasonography in 2nd week.

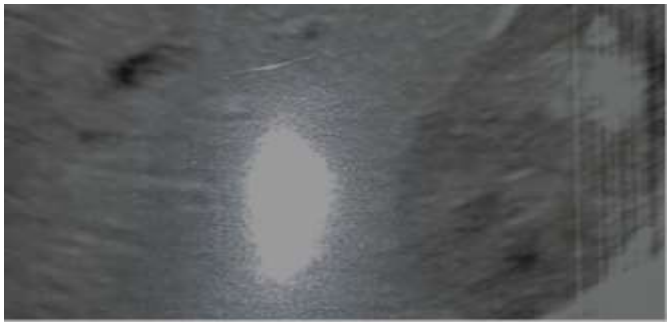

Fig.3: Ultrasonography in 3rd week.

Table 1: Hematological, Biochemical, Serological results in the 1st week of case.

Hematology test			
Parameter	Result	Unit	Ref.Range
WBC	6.98	10^9/L	4.00-11.00
RBC	4.53	10^{12}/L	3.50-5.50
HGB	11.6	g/dL	11.0-16.0
Neu%	60.7	%	50.0-70.0
Lym%	26.4	%	20.0-40.0
Mon%	7.2	%	3.0-12.0
Eos%	5.3 ↑	%	0.5-5.0
Bas%	0.4	%	0.0-1.0
MCV	84.9	fL	80.0-100.0
MCH	25.6	pg	27.0-34.0
MCHC	30.2	g/dL	32.0-36.0
RDW	16.0		11.0-16.0
Biochemical test			
Total serum bilirubin (TBIL)	6.4 ↑	Mg/dl	≤ 1

Direct (DBIL)	4.6	Mg/dl	≤ 0.45
Indirect(IBIL)	1.8 ↑	Mg/dl	≤ 1
Serum GOT(AST)	266	U/L	≤ 31
Serum GPT(ALT)	290	U/L	≤ 42
GGT	47	U/L	≤ 50
Alkaline phosphatase (ALP)	221	U/L	45-150
Serological test			
HBs Ag titer	0.03(negative)	iu / ml	> 1
HCV Ab titer	0.2 (negative)	iu /ml	> 1

Table 2: Hematological, Biochemical, Serological results in the 2nd week of case.

Hematology test			
Parameter	Result	Unit	Ref.Range
WBC	6.88	10^9/L	4.00-11.00
RBC	4.48	10^{12}/L	3.50-5.50
HGB	11.3	g/dL	11.0-16.0
Neu%	60.5	%	50.0-70.0
Lym%	26.2	%	20.0-40.0
Mon%	7.2	%	3.0-12.0
Eos%	5.2↑	%	0.5-5.0
Bas%	0.2	%	0.0-1.0
MCV	84.7	fL	80.0-100.0
MCH	25.4	pg	27.0-34.0
MCHC	30.0	g/dL	32.0-36.0
RDW	16.0		11.0-16.0

Biochemical test			
Total serum bilirubin (TBIL)	2.12↑	Mg/dl	≤ 1
Direct (DBIL)	0.39	Mg/dl	≤ 0.45
Indirect(IBIL)	1.73↑	Mg/dl	≤ 1
Serum GOT(AST)	19	U/L	≤ 31
Serum GPT(ALT)	18	U/L	≤ 42
GGT	43	U/L	≤ 50
Alkaline phosphatase (ALP)	92	U/L	45-150

Serological test			
HBs Ag titer	0.02(negative)	iu / ml	> 1
HCV Ab titer	0.2 (negative)	iu /ml	> 1

Table 3: Hematological, Biochemical, Serological results in the 3rd week of case.

Hematology test			
Parameter	Result	Unit	Ref.Range
WBC	8.13	k/uL	4-10
RBC	4.24	M/uL	4-6
HGB	10.8	g/dl	10.3-14.9
Neu%	61.7	%	34-67.9
Lym%	26.6	%	21.8-53.1
Mon%	6.2	%	5.3-12.2
Eos%	5.3	%	0.8-7
Bas%	0.2	%	0.2-1.2
MCV	80	fl	79-92.2
MCH	25.5 ↓	pg	25.7-32.2
MCHC	31.9 ↑↑	g/dl	26-36
RDW	15.3 ↑	%	11.0-16.0

Biochemical test			
Total serum bilirubin (TBIL)	2.63↑	Mg/dl	≤ 1

Direct (DBIL)	0.41	Mg/dl	≤ 0.45
Indirect(IBIL)	1.6 ↑	Mg/dl	≤ 1
Serum GOT(AST)	20.5	U/L	≤ 31
Serum GPT(ALT)	9.5	U/L	≤ 42
GGT	41	U/L	≤ 50
Alkaline phosphatase (ALP)	95	U/L	45-150
Serological test			
HBs Ag titer	0.413	iu / ml	> 1
HCV Ab titer	0.029	iu /ml	> 1

Table 4: Results for Specific erythrocyte membrane analysis for detection Hereditary Spherocytosis.

Parameter	Result	Unit	Ref.Range
Glucose -6-phosphatdehidroginase	270	Pu/RBC	146-376
Reticulocyte Count (unadjusted)			
RET%	3.52 H	%	0.8-2.2
RET#	0.1524H	$10^{6/}$ uL	0.046-0.121
IRF	30.1 H	%	1.6-10.5
LFR	69.9 L	%	87.2-97.9
MFR	22.2 H	%	3.2-11.3
HFR	7.9 H	%	0.2-1.4
RET-He	28.8 L	pg	31.2-36.2
Erythrocyte osmotic resistance (osmotic fragility)			
%0.55 NaCl	1.8	%	0-0%
%0.50 NaCl	2.8	%	0-6%
%0.45 NaCl	24.0	%	5-45%
%0.40 NaCl	84.0	%	50-95%
%0.35 NaCl	95.0	%	90-99%
%0.30 NaCl	96.0	%	97-100%
Incubation(24h.37C)			
% 0.85 NaCl	0	%	
% 0.80 NaCl	0.6	%	
%0.65 NaCl	5.0	%	0-10 %
% 0.60 NaCl	13.0	%	10-40 %
%0.55 NaCl	53.0	%	15-70 %
% 0.50 NaCl	74.0	%	40-85 %
% 0.45 NaCl	82.0	%	55-95 %
% 0.40 NaCl	85.0	%	65-100 %
% 0.35 NaCl	88.0	%	75-100 %
% 0.30 NaCl	89.0	%	80-100 %

Table 5: Hb electrophoresis result.

Type of bands	Concentration
Hb A	96.7 %
Hb A2	1.9 %
Hb F	1.4 %
Others	0 %
conclusion	NormalHb-Electrophoresis