Original Article

Correlation of Bilirubin and Alkaline Phosphatase in Infantile Patients with Cholestasis

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Abstract

Background: Early and successful individualized management of infantile cholestasis depends on the time of diagnosis. **Objective:** The objective of the study is to assess the clinical value of serum total bilirubin, direct bilirubin, and alkaline phosphatase in children with cholestasis. **Materials and Methods:** Sixty patients with diseases and 25 healthy controls distributed into three groups – total/direct bilirubin, and alkaline phosphatase were measured for all case and control studies by enzyme-linked immunosorbent assay. **Results:** The results showed serum total, direct bilirubin, and alkaline phosphatase have a highly significantly elevated in intra- and extra-hepatic cholestasis groups when compared with control ($P < 0.001$). There was a significantly elevated serum total and direct bilirubin when compared the extrahepatic cholestatic group with intrahepatic cholestatic group ($P = 0.002$ and 0.017), and there was no statistical difference between the intrahepatic cholestatic patients and extrahepatic cholestatic group when serum alkaline phosphatase measured ($P = 0.610$). **Conclusion:** From these findings, it was concluded that the level of direct bilirubin in sera of extrahepatic cholestasis group when compared with intrahepatic cholestasis group is elevated, serum total bilirubin, and alkaline phosphatase levels are good markers in determining the poor prognosis of cholestasis disease.

Keywords: Alkaline phosphatase, direct bilirubin, extrahepatic cholestasis, intrahepatic cholestasis, total bilirubin

Introduction

In neonate, the jaundice is common, usually secondary to unconjugated or indirect hyperbilirubinemia, conjugated hyperbilirubinemia ensures proper evaluation as the timing of the invention in some cases directly affects the clinical outcome, bile consists mainly of bile acids, bilirubin, and fats, is formed in the liver, and is secreted into the canaliculus, from the canaliculus, bile flows into biliary ducts from where it is eventually secreted into the intestine after temporary storage within the gallbladder.[1] They are reabsorbed in the gut together with the nutrients and backed to the liver through the portal vein, too much bile acid levels are toxic.[2,3] Disabling of this process at any level leads to cholestasis, cholestasis is the end effect of obstruction of the normal excretion of bile from the liver, giving rise to the abnormal accumulation of bile salts, bilirubin, and lipids in liver and the blood, the abnormal retention of bilirubin, high serum levels in cholestasis, excretion of bile acids in urine, and wide availability of testing make serum-conjugated bilirubin clinically the more useful marker of cholestasis next to urine test, clinically, the cholestasis in the infant may present as jaundice, pruritus, and fat-soluble vitamin deficiency.[4] In case of jaundice in infants is usually clinically evident when the total serum bilirubin level exceeds 2.5–3.0 mg/dl (42–51 mmol/L) and is seen as scleral icterus or yellowing of the oral mucosa, conjugated hyperbilirubinemia in an infant (direct bilirubin levels >1.0 mg/dl or >17 mmol/L, or >15% of total bilirubin) is never normal and indicates hepatobiliary abnormality, secondary laboratory evaluations after cholestasis is determined may include serum alkaline phosphatase.[1]

Biliary atresia shows commonly with cholestasis between 2 and 5 weeks of life, acholic stools may be show and determine biliary obstruction; so as cholestasis and chronic inflammation lead to malabsorption, many infants with biliary atresia showed insufficient weight gain and are characterized as failure to thrive;[5,6] early path of disease, infants with biliary atresia usually reveal conjugated hyperbilirubinemia

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(direct bilirubin 2–7 mg/dl with total bilirubin levels between 5 and 12 mg/dl), with increase in transaminases and gamma-glutamyltransferase.[1]

Alkaline phosphatase is glycosylphosphatidylinositol-anchored ecto-phosphomonoesterases with different tissue expressions and multiple isoforms.[7-9] Patients with liver disease only hepatic alkaline phosphatase will be found in their serum, particularly cholestatic patients, mild-to-moderate increasing of the enzyme (two to three times the higher limits of normal) can be seen in all types of liver disorders, significant increasing (more than three times the higher limits of normal) are mainly seen in diseases associated with intra- or extra-hepatic cholestasis, the amount of elevation does not differentiate between intra- and extra-hepatic cholestasis.[10,11] Proof indicates that serum alkaline phosphatase activity offers helpful data for the long-term prognosis of primary biliary cirrhosis.[12] In liver diseases, the marked increasing of serum alkaline phosphatase activity is an indication of cholestatic conditions; in cholestasis, elevated serum alkaline phosphatase activity is primarily resulted from elevated hepatic synthesis and the following release of liver alkaline phosphatase in the sinusoidal blood flow.[13]

The aim of this study was to assess the clinical value of serum total bilirubin, direct bilirubin, and alkaline phosphatase in children with cholestasis.

Materials and Methods

The study was executed during the term from January 2018 to June 2018, this study included 60 patients with diseases and 25 healthy controls and distributed into three groups: First group includes 40 patients with intrahepatic cholestasis diseases, second group includes 20 patients with extrahepatic cholestasis diseases, and third group: represents the apparently healthy control group which comprises of 25 individuals who do not suffer from liver disease or any chronic illness and matched with age and gender to patient groups. All samples were collected from Al-Imameen Al-Kademen Medical City, Digestive Center at Medical City, and Child's Central Teaching Hospital. The exclusion criteria were representing by selected the patients who were do not suffer from dyslipidemia and diabetes mellitus or any other disease that may interfere with the study and patients who were not underwent any type of therapy such as ursodeoxycholic acid and were stopped the treatment 2 weeks before investigations. The preparation of blood samples was done by collected about 5 mL of blood that obtained from veins of patients having cholestasis and healthy controls. Blood samples were left for 20 min at room temperature. After coagulation, sera were separated by centrifugation at 3000 rpm. For 10 min, aspirated and stored at 20 until assayed for total/direct bilirubin and alkaline phosphatase. They were measured using enzyme-linked immunosorbent assay kits. The human total/direct and human alkaline phosphatase kits were obtained from Kono Biotech Company.

Results

Serum bilirubin

The median of serum total bilirubin in the control, intra- and extra-hepatic cholestasis groups was (0.54, 5.05, and 15.48) mg/dl, respectively, has a highly significantly elevated in an intra- and extra-hepatic cholestasis groups when compared with control ($P < 0.001$). This is s a significant difference between groups according to the Mann–Whitney test as shown in Table 1.

The median of serum direct bilirubin in the control, intra- and extra-hepatic cholestasis groups was (0.05, 3.55, and 10.88) mg/dl respectively, has a highly significantly elevated in an intra- and extrahepatic cholestasis groups when compared with control ($P < 0.001$). This is a significant difference between groups according to the Mann–Whitney test as shown in Table 1.

Serum alkaline phosphatase

The median of serum alkaline phosphatase in the control, intra- and extra-hepatic cholestasis groups was (88, 559, and 527) U/L respectively, has a highly significantly elevated in an intra- and extra-hepatic cholestasis groups when compared with control ($P < 0.001$). This is a significant difference between groups according to the Mann–Whitney test as shown in Table 1.

Comparison between intra-and extrahepatic cholestasis groups

There was no statistical difference between the intrahepatic cholestatic patients and extrahepatic cholestatic group in terms of age, weight, weight for age, height, stature for age, and serum alkaline phosphatase ($P = 0.368$, 0.235, 0.059, 0.281, 0.368, and 0.610), respectively, but there was a highly significantly elevated serum total bilirubin and direct bilirubin when compared the extrahepatic cholestatic group with intrahepatic cholestatic group ($P = 0.002$ and 0.017), respectively, and these are significant differences between intrahepatic cholestasis and extrahepatic cholestasis groups according to the Mann–Whitney test as shown in Table 2.

Table 1: Comparison between control versus three study groups by Mann-Whitney test

Parameters	Control ($n=25$)	IHC ($n=40$)	EHC ($n=20$)
STB (mg/dl)			
Median	0.54	5.05	15.48
Range	0.25-1.1	1.4-33.0	7.0-37.38
P		<0.001	<0.001
SDB (mg/dl)			
Median	0.05	3.55	10.88
Range	0.0-0.1	0.4-14.5	3.0-28.95
P		<0.001	<0.001
ALP (U/L)			
Median	88.0	559.0	527.0
Range	39.0-411.0	198.0-2278.0	175.0-841.0
P		<0.001	<0.001

IHC: Immunohistochemistry, EHC: Enzyme histochemistry, STB: Serum total bilirubin, SDB: Serum direct bilirubin, ALP: Alkaline phosphatase

Table 2: Comparison between intra and extrahepatic cholestasis groups by Mann-Whitney test

Parameters	IHC ($n=40$)	EHC ($n=20$)	*P*
Age (m)			
Median	34.0	8.0	0.368
Range	1.5-180.0	1.3-132.0	
Weight (kg)			
Median	12.0	5.75	0.235
Range	1.4-50.0	2.8-26.5	
Weight for age (%)			
Median	10.0	2.0	0.059
Range	0.0-38.0	0.0-10.0	
Height (cm)			
Median	89.5	63.0	0.281
Range	48.0-166.0	49.5-131.0	
Stature for age (%)			
Median	12.5	2.5	0.171
Range	0.0-50.0	0.0-10.0	
SBT (mg/dl)			
Median	5.05	15.48	0.002
Range	1.4-33.0	7.0-37.38	
SDB (mg/dl)			
Median	3.55	10.88	0.017
Range	0.4-14.5	3.0-28.95	
ALP (U/L)			
Median	559.0	527.0	0.610
Range	198.0-2278.0	175.0-841.0	

IHC: Immunohistochemistry, EHC: Enzyme histochemistry, STB: Serum total bilirubin, SDB: Serum direct bilirubin, ALP: Alkaline phosphatase

Discussion

Cholestasis is the end effect of obstruction of the normal excretion of bile from the liver, giving rise to the abnormal accumulation of bile salts, bilirubin, and lipids in liver and the blood[1] and has a wide variety of etiologies, including infection-related neonatal hepatitis, progressive familial intrahepatic cholestasis, inborn errors of bile acid metabolism, and other metabolic or toxic insults.[14-16]

Patients with a family history of cholestasis, especially in a first-degree relative (brother, mother, father, and sister) and second degree (uncle and aunt) have increased risk of developing cholestasis themselves. The study showed that the rates of family history obtained in intra- and extra-hepatic cholestasis patients were 37% and 75%, respectively. These results are in agreement with the thesis that obtained from.[17-19]

The clinical evaluation of symptoms of cholestasis may suggested by the presence of dark urine or acholic stools. In the present study, there were 32 patients with acholic stool and 38 patients with dark urine from 40 patients of intrahepatic cholestasis group, while in patients of extrahepatic cholestasis group, there were 6 patients with acholic stool and 8 with dark urine. These results were also confirmed by several previous studies.[19,20]

The median of total bilirubin of the present study in sera of intrahepatic cholestasis 5.05 mg/dl with range 1.4–33 mg/dl, extrahepatic cholestasis 15.48 mg/dl with range 7–37.38 mg/dl, and control 0.54 mg/dl with range 0.25–1.1 mg/dl, total bilirubin showed an elevation in patients with intra-and extra-hepatic cholestasis groups when compared with control group. As shown in Table 1, this study revealed that high levels of total bilirubin was associated with poor prognosis of cholestasis disease, which agree with the finding of the significant association between the high levels of total bilirubin and cholestasis disease may be attributed to impairment one of the pathway of bile secretions from the hepatocytes into the canaliculi or obstruction of bile duct.[20]

The median of direct bilirubin of the present study in sera of intrahepatic cholestasis 3.55 mg/dl with range 0.4–14.5 mg/dl, extrahepatic cholestasis 10.88 mg/dl with range 3–28.95 mg/dl, and control 0.05 mg/dl with range 0–0.1 mg/dl, direct bilirubin showed an elevation in patients with intra- and extra-hepatic cholestasis groups when compared with control group [Table 1]. Furthermore, the present study showed an elevated level of direct bilirubin in sera of extrahepatic cholestasis group when compared with intrahepatic cholestasis group which agree with the result of previous studies.[20,21] This study revealed that high levels of direct bilirubin was associated with poor prognosis of cholestasis disease, which agree with the finding of the significant association between the high levels of direct bilirubin and cholestasis disease may be attributed to impairment in bile flow and may be caused by either an intrahepatic or extrahepatic disorder by obstruction of bile duct.[1,20,22]

Alkaline phosphatase is glycosylphosphatidylinositol-anchored ecto-phosphomonoesterases with different tissue expressions and multiple isoforms, alkaline phosphatase is able to hydrolytic transphosphorylase and phosphatase activity on host-derived nucleotides such as ATP, ADP, and uridine diphosphate.[7-9] The median of alkaline phosphatase of the present study in sera of intrahepatic cholestasis 559 U/L with range 198–2278 U/L, extrahepatic cholestasis 527 U/L with range 175–841 U/L, and control 88 U/L with range 39–441 U/L, alkaline phosphatase showed an elevation in patients with intra- and extra-hepatic cholestasis groups when compared with control group [Table 1].

This study revealed that high levels of alkaline phosphatase were associated with poor prognosis of cholestasis disease, which agree with the finding of the significant association between the high levels of alkaline phosphatase and cholestasis disease may be primarily resulted from elevated hepatic synthesis and the following release of liver alkaline phosphatase in the sinusoidal blood flow as shown in previous studies.[20,23]

Patients with the liver disease only hepatic alkaline phosphatase will be found in their serum, particularly cholestatic patients. Mild-to-moderate increasing of the enzyme (two to three times the higher limits of normal) can be seen in all types of liver

disorders. Significant increasing (more than three times the higher limits of normal) are mainly seen in this study groups of intra- or extra-hepatic cholestasis. The amount of elevation does not differentiate between intra- and extra-hepatic cholestasis.[10,11]

Limitations

One of the most difficult issues is the number of patients that suffer from cholestasis in Iraq and their knowledge how to get early to specialist centers for diagnosis when they suffer from jaundice in the long term which may be having cholestasis in fact.

Conclusion

There was an elevated serum total/direct bilirubin in patients with extrahepatic cholestasis when compared with patients of intrahepatic cholestasis.

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Conflicts of interest

There are no conflicts of interest.

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