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ARTICLE

EVALUATION OF LABORATORY TESTS IN DIAGNOSIS OF BILIARY ATRESIA IN THE PEDIATRIC WARDS OF HOSPITALS AFFILIATED TO SHIRAZ UNIVERSITY OF MEDICAL SCIENCES FROM 2007 TO 2013

Mozhgan Zahmatkeshan[1], Vahideh Modabber[2] , Seyyed Ahmad Razavizadegan[3*], Bita Geramizadeh[4]

[1,2,3]Department of pediatrics , School of medicine, Shiraz University of Medical Science, Shiraz, IRAN
[4]Department of Pathology, school of medicine, Shiraz University of Medical Science, Shiraz, IRAN

ABSTRACT

Background: *Biliaryatresia (BA) is a complex idiopathic disease with external hepatic bile duct obstruction. Surgical intervention is the only functional treatment available. For BA patients early diagnosis plays an important role in having a better outcome. Laboratory tests are easy and fast so we designed a study to see if there is a significant abnormality in the results of the patients with BA.* ***Methods and materials:*** *In this retrospective study we evaluate the medical records of all the patients with confirmed BA referring to hospitals affiliated to Shiraz University of medical sciences. Laboratory results were extracted and then sent to a biostatistician for further analyses.* ***Results:*** *At the end 73 cases were evaluated in the study. Fibrosis was present in 54(74%) patients. Twenty (27.4 %) patients were positive for cirrhosis, 39 patients had high INR. Patients affected by cirrhosis had significantly higher INR than the ones without cirrhosis (P<0.05). We recorded an increase in the blood concentration of amino transferases and alkaline phosphatase in almost all the patients. Total and direct bilirubin was higher than in our patients.* ***Conclusion:*** *BA directly and indirectly affects the results of the laboratory tests. Although we can predict the test results of the patients with BA, we still can't rely on these tests to establish a strong screening system.*

KEY WORDS
Biliary atresia, laboratory tests, bilirubin, aminotransferases, alkaline phosphatase

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***Corresponding Author**
Email:
razavisa2@gmail.com

INTRODUCTION

Biliary atresia (BA) is a gradual and mostly idiopathic disease with extra-hepatic bile duct obstruction and is exclusively seen in neonates [1].

This is not a common disease regarding an incidence rate of 1 in 10000 to 20000 births [2]. BA is known for causing jaundice which is persistent and won't respond to common medical treatments. Most of the liver transplantations performed during childhood are due to BA. Up to 85% of the infants affected, have BA without any other malformations or anomalies. This type is also called perinat al BA. Clay-color stool and jaundice is observed in the first two months' of their life [3]. About 10 to 15 percent of the cases have BA in association with laterality malformations. These may include asplenia, polysplenia, malrotation, situs inversus and other anomalies. Infants with this type of BA have poorer outcome [4]. The last type of BA is associated with other congenital malformations like cardiac and renal malformations [5].

Etiological definition of BA is complex. The exact cause of BA is still unknown. However, many mechanisms are thought to play an important role in formation of BA, in other words it is an anatomical abnormality which may be the phenotype of many different congenital or acquired problems. One theory points to some viruses as the cause of BA which isn't proved yet [6, 7]. Genetics may have an impact on the onset of this disease. Some genes may cause the carrying person to be more prone to developing BA [8]. Other hypotheses suggest immunologic and toxic causes but they are still under investigations[9].

Jaundice, which is nonresponsive to common treatments and phototherapy, pale stool and dark urine are other important signs of BA which can be identified by the parents with appropriate information[10]. Conjugated bilirubin's level is usually raised in BA [11]. The mean serum level of aminotransferases and gamma-glut amyl transpeptidase is mildly elevated in BA patients. Although these may indicate BA they aren't cost-effective and aren't constant enough to use them as the screening tool of BA.

It is very important to diagnose BA as fast as possible because late diagnosis has poorer outcomes [12]. There is a list of diseases with similar signs and symptoms which will make fast diagnosis of BA a challenge. Diseases like Alagille Syndrome or alpha-1-antitrypsin deficiency are included in that list.

After observing the signs, first line diagnosis is to use ultrasound imaging to investigate the anatomical structure of the liver and bile ducts. It is beneficial to omit other anatomical anomalies that cause similar signs. Radiologists look for certain signs in the images to confirm BA [13]. After sonography liver biopsy is done to study the liver tissue for further decision.

As mentioned there isn't a fast way to diagnose BA, so we designed this study to examine and evaluate the laboratory test results of the patients with BA to see if these tests can be used as screening tests?

METHODS AND MATERIALS

It was a retrospective cross sectional study which was conducted between March 2007 and March 2013. This study was reviewed by the ethical committee of Shiraz University of Medical sciences. After the approval of all the related health authorities we started to study the cases. Our primitive population was defined as all the patients diagnosed with BA in pediatric wards of the hospitals affiliated to Shiraz University of Medical Sciences. Only the patients who were hospitalized in the wards and the diagnosis of BA was claimed by pathologists were included. Then we retained all their medical records and sheets from the medical records library of Shiraz university of Medical Sciences. Then the patients with insufficient information in their sheets were excluded from the study. BA was confirmed for all the patients with ultrasound and liver biopsy. At the end after all the exclusions 73 cases remained in the study aged 1 week to 12 yrs. With this amount of cases we could achieve a study power of around 80%. The laboratory and pathology test results of the patients were extracted and then evaluated closely. The test results which we collected included international normalized ratio [INR] , Hemoglobin [HB] , Platelet Count (PLT) , alkaline phosphatase (ALK.PH) , Pro-thrombin Time (PT) , AST , ALT , Albumin (ALB) , Total bilirubin (TB) and Direct bilirubin (DB) .From the pathology reports we collected the presence of cirrhosis and fibrosis .

For statistical analyses SPSS 15.0 was used. Independent t-test was used and P value under 0.05 was defined meaningful.

RESULTS

At the bottom line, 73 cases remained in the study. Regarding pathological reports of the cases, Fibrosis was present in 54(74%) patients, 20(27.4 %) patients were positive for cirrhosis, 21 (28.8%) patients had received liver transplantation.

Mean international normalized ratio (INR) was 1.89±1.95, 34 patients had normal INR and 39 had high INR. Patients affected by cirrhosis had significantly higher INR than the ones without cirrhosis ($P<0.05$); 69(94.5 %) of the patients had haemoglobin below the healthy range. The mean HB level was 9.86±2.01 g/dl; 66(90.4%) patients had elevated ALK.PH level and the remaining 9.6% had ALK.PH below healthy range. The mean ALK.PH was 1367.36±819.70 IU/L.

AST levels were high in the patients with the mean of 218.14±245 U/L; 63(86.3%) patients had high AST levels; ALT was very high in the patients too with the mean of 150.45±174.91 U/L; 60 (82.1%) patients had high ALT levels; 59 (80.8%) patients had abnormal AST, ALT, and ALK.PH results. Mean albumin level was 3.72±2.24 g/dL and most of the patients were in normal albumin range.

Total bilirubin was abnormally high in 69(94.5%) patients. The mean TB was 12.59±7.46 mg/dL. Almost all the patients had high level of direct bilirubin (unconjugated bilirubin); the mean DB was 5.91±4.28 mg/dL .

Table 1: Laboratory findings of the patients affected by biliary atresia

Test type	Mean	Normal range
INR	1.89±1.95 IU/L	0.8-1.2 IU/L
AST	218.14 ±245 U/L	8 - 48 U/L
ALT	150.45 ± 174.91 U/L	7 - 55 U/L
ALK.PH	1367.36±819.70 IU/L	45- 115 U/L
ALB	3.72 ± 2.24 g/dL	3.5 - 5.0 g/dL
HB	9.86 ±2.01 g/dl	11-13 g/dl
TB	12.59±7.46 mg/dL	0.3-1.9 mg/dL
DB	5.91±4.28 mg/dL	0 - 0.3 mg/dL

Table 2: Pathological findings of the patients affected by biliary atresia

Pathology	Positive (%)
Cirrhosis	27.40%
Fibrosis	74%

DISCUSSION

Laboratory tests that evaluate the functions of liver show abnormal results in the patients with BA. This is mostly due to the liver trauma caused by cholestasis and excess bile in the liver. Based on our results 74% of the patients had fibrosis in their liver which is the onset for liver cirrhosis. Although surgical procedure can help those with BA, Hepatic fibro genesis will continue and the cause of this is still unknown [14]. Fibrosis is the main reason that we should diagnose BA fast because if BA remains undiagnosed, fibrosis will extend to a dangerous level and then liver transplantation may be the only choice [15]. Balistreri *et al.* investigated the responsible cells for this excessive fibrosis. They concluded that hepatic stellate cells play the main role there with creating excessive collagen. They also found that bile duct epithelial cells, hepatic stellate cells, and hepatocytes all produce cytokines that initiate and continue the fibrosis process [16]. Investigations on how to stop these cells and slow the fibrosis process is suggested.

This indicates that most of the BA patients suffer from severe liver, injury and this can easily be traced in blood tests. ALT and AST are the most important indications for the liver injury. Our patients had severely high levels of these enzymes in their blood. This indicates that most of our patients had been diagnosed slowly so they had already developed a great liver injury. This is very important and shows the urge to find faster ways to diagnose this disease as the current data suggest that we lack this required haste. Though we can't use these 2 factors as screening tests for BA for two reasons; the first is because they indicate many hepatic related diseases and the second reason is higher than normal levels of these enzymes are usually traced in the blood when the liver is already damaged so again we lose the precious time to help those suffering from BA.

We recorded a moderate elevation in alkaline phosphatase in the blood and this elevation was far more than AST and ALT. This is a great indication for biliary obstruction. In severe hepatic injuries like hepatitis C virus infection AST and ALT get elevated far more than ALK.PH [17]; but when we are talking about the obstruction it's completely vice versa and ALK.PH get elevated a lot more than AST and ALT [18].

BA will significantly affect the appetite and as a result some of the patients have lower levels of albumin but it is not a very consistent sign as we didn't observed any meaningful abnormality in our samples regarding albumin concentration. Lykavieris *et al.* followed 63 children with BA for 20 years. They also didn't find a huge abnormality in albumin concentration in this 20 years [19]. So based on what mentioned we can easily drop the albumin from our diagnostic tools.

We observed a mild decrease in HB count of our cases. This is due to the fibrosis and cirrhosis of the liver but yet not a great screening measure for BA. Our patients had higher INR than normal. Liver plays an important role in the vital mechanisms of the body like blood coagulation. Coagulation is a process which requires many different minerals, factors, vitamins and proteins and liver produces some of the key factors. Based on this when liver is damaged, body will experience a drought regarding those factors so the whole coagulation metabolism will be affected [20]; So it isn't a wonder that why we recorded higher INR.

One of the most significant things in the lab tests was the elevation of total and direct bilirubin. Harpavat *et al.* evaluated the birth time bilirubin of the neonates affected by BA and they found that all of them had higher amounts of direct bilirubin in comparison with the control group who were healthy neonates [11]. By using this we can obtain a blood sample from all the newborns and then follow the ones with elevated direct bilirubin as they are more susceptible to BA, but it isn't something functional due to the costs of the tests and the rare incidence of BA.

Overall, we found that some special characteristics can be found in the lab test results of the patients affected by BA. But yet these are not enough to create a good, fast and functional screening test for BA.

CONFLICT OF INTEREST
None

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FINANCIAL DISCLOSURE
None

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