Diagnostic Evaluation Of Cholestasis In Infants And Young Children In Alexandria

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Citation

Abstract
Cholestasis includes retention of conjugated bilirubin, bile salts and other components of the bile. It is not a disease; rather, it is a symptom of many diseases. This study aimed at finding out the etiological diagnosis of chronic cholestatic liver diseases among cases of cholestasis admitted to El-Shatby Children Hospital-University of Alexandria, Egypt during a 2 years period. Seventy infants and children with their ages ranging from 1 to 36 months were included in the study. Various diagnostic modalities were used to establish their diagnosis.

Patients were diagnosed as neonatal hepatitis (n=29, 41.4%), biliary atresia (n=17, 24.3%), and a miscellaneous group (n=24, 34.3%).

The conclusion is that in Alexandria neonatal hepatitis is the commonest cause of infantile cholestasis and biliary atresia is the second common cause.

INTRODUCTION
Cholestasis includes retention of conjugated bilirubin, bile salts and other components of the bile. It is not a disease; rather, it is a symptom of many diseases therefore, it is a signal that disease exists. The mechanisms by which diseases produce cholestasis can be classified as either hepatocellular or obstructive cholestasis. (1)

The first diagnostic concern of cholestatic disorders should be the differentiation of hepatocellular from obstructive cholestasis, because it represents the differentiation between medical versus surgical disorders. All disorders that deserve intervention in the first few months of life are obstructive disorders, and their timely identification can improve outcome. (2)

Because of the frequent lack of distinctive clinical features leading to differentiation between hepatocellular and obstructive disorders, cholestatic infants require a stepwise, comprehensive evaluation. Diagnostic schemata should incorporate clinical, biochemical, radiological and histological features. However, at any point during the process of evaluation, a serologic test or imaging study may establish the probable cause of cholestasis. (3)

The aim of the present study was to find-out the etiological diagnosis of chronic cholestatic liver diseases among cases of cholestasis presented to the pediatric outpatient clinic at El-Shatby Children Hospital-University of Alexandria over a 24 months period.

PATIENTS AND METHODS
Seventy patients (36 females and 34 males) with their ages ranging from 1 to 36 months were enrolled in the study They were fulfilling the clinical and laboratory criteria of chronic cholestasis, that is cholestasis persisting beyond two weeks of age in neonates, cholestasis which is expected to persist in infants, or cholestasis lasting for four weeks or more in children. (4) An informed consent was taken from the parents of all patients included in the study. Patients with the classical clinical picture of acute hepatitis and those with cholestasis showing signs of end stage liver failure were excluded from the study.

Every patient was subjected to a full history taking stressing on the age , sex, birth weight ,onset and duration of jaundice,
colour of urine and stool as well as serological evidence of maternal infectious diseases. Also every patient was subjected to thorough clinical examination, laboratory investigations including total and direct serum bilirubin, serum bile salts, the cholestatic enzymes alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) aspartate transaminase (AST), alanine transaminase (ALT), prothrombin time and activity, serum albumin, fasting blood glucose, urine examination for bilirubin, bile salts and reducing substances.

Serological tests for hepatitis B surface antigen, hepatitis C virus IgG antibodies, Toxoplasma, Rubella, Cytomegalovirus (CMV) and herpes virus (HSV) i.e. TORCH IgM antibodies were done using ELISA technique. Alpha fetoprotein was measured by chemiluminescence and alpha 1 antitrypsin level by immunoturbidimetry. Moreover, alpha 1 antitrypsin phenotyping by isoelectric focusing was done for deficient cases.

Radiological imaging (abdominal ultrasound seeking for the gall bladder and Triangular cord sign) and radionuclide imaging (Tc-99m- HIDA scintigraphy). Lastly, percutaneous liver biopsy was done using Manghini technique, a small part of the biopsy sample was immersed in gluteraldehyde for transmission electron microscopy and the rest of the sample was fixed in 10% buffered formalin and is processed in the ordinary paraffin embedding method, serial sections 3 μm thick were made and stained by hematoxyline and eosin, trichrome, reticulin, periodic acid schiff (PAS), and PAS after diastase.

RESULTS

All cases had jaundice and dark urine, the onset of jaundice ranged from one day to 20 months after birth and continued till the time of presentation except in one case where the jaundice appeared at the age of 20 months and took an intermittent course.

Patients were classified according to the various diagnostic tests into 3 groups (fig 1):

Group I: Neonatal hepatitis (41.4%), with transaminase level more than 5 folds elevated and GGT less than 5 folds elevated.

Group II: Biliary atresia (24.3%), with transaminase level less than 5 folds elevated and GGT more than 5 folds elevated.

Group III: Miscellaneous (34.3%).

Table 1 shows the etiological diagnosis of neonatal hepatitis cases, while table (II) shows the final diagnosis of all cholestatic patients included in the study.

![Figure 1](image1.jpg)

Figure 1: The 3 major groups of cholestatic patients in the study

Among the clinical parameters used in this study only birth weight, age of onset of jaundice and presence of splenomegaly were found to be useful. Infants with neonatal hepatitis were found to have low birth weight and later onset of jaundice than those having biliary atresia table (I).

Moreover splenomegaly was found in higher percentage in infants with neonatal hepatitis compared to biliary atresia cases Table (I). Anthropometric data and onset of jaundice:
Diagnostic Evaluation Of Cholestasis In Infants And Young Children In Alexandria

Figure 3
Table 2: Clinical findings in the studied groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>x2(x2df)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>55</td>
<td>53</td>
<td>46</td>
<td>x2=2.392</td>
<td>0.502</td>
</tr>
<tr>
<td>Primritus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>97</td>
<td>76.5</td>
<td>91.7</td>
<td>x2=0.262</td>
<td>0.608</td>
</tr>
<tr>
<td>Small</td>
<td>3</td>
<td>23.5</td>
<td>8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver span</td>
<td>3.4</td>
<td>5.6</td>
<td>8.3</td>
<td>x2=0.094</td>
<td>0.394</td>
</tr>
<tr>
<td>Normal</td>
<td>31</td>
<td>70.6</td>
<td>45.8</td>
<td>x2&lt;0.05</td>
<td>0.016*</td>
</tr>
<tr>
<td>Large</td>
<td>69</td>
<td>29.4</td>
<td>54.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant x2 test at P value ≤ 0.05

All cases had elevated serum levels of total bilirubin (10.8±3.6mg/dl) with elevated direct fraction (9.4 ±3.4 mg/dl).

In 65 infants, elevated levels of serum transaminases (reference value is up to 40 u/L) were found, of those 7 patients had markedly elevated level (>200 u/L), 58 had moderate elevation between 70 and 200 U/L. The remaining 5 patients had normal transaminases level.

Serum alkaline phosphatase level (reference value 3-13 K&A) was high in all infants with a mean of 90±30 k&A. Gamma glutamyl transpeptidase level was normal in 3 infants (10-40 U/L), low in 2 (<10 U/L) and high in 65 infants (> 40 U/L). In those with elevated levels, 18 had GGT greater than five fold elevation (>200 U/L) and 47 had GGT less than five fold elevation (< 200 U/L).

In patients who had normal and low GGT (5 cases), 4 had elevated serum bile salts level, while the 5th had normal serum bile salts (< 9.8 ?mol/L) as well as normal GGT, ALP, AST and ALT levels.

Impaired synthetic liver function (reflected by serum albumin and prothrombin activity) were detected in the majority of cases. Fasting blood glucose level was normal in all cases except two who had hypoglycemia.

Urine tests for conjugated bilirubin and bile salts were done for all cases and revealed positive results. On the other hand, urine samples were negative for reducing substances.

By abdominal ultrasound examination, two patients were proved to have choledochal cysts.

Table (III) show the etiological diagnosis of neonatal hepatitis cases, while table (IV) show the final diagnosis of all cholestatic patients included in the study.

Figure 4
Table 3: Etiology of neonatal hepatitis in group 1

<table>
<thead>
<tr>
<th>Neonatal hepatitis</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>12</td>
<td>17.14</td>
</tr>
<tr>
<td>HSV</td>
<td>3</td>
<td>4.29</td>
</tr>
<tr>
<td>CMV+HSV</td>
<td>6</td>
<td>8.57</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>2</td>
<td>2.86</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>6</td>
<td>8.57</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>41.43</td>
</tr>
</tbody>
</table>

Figure 5
Table 4: Final diagnosis of all studied cases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal hepatitis</td>
<td>29</td>
<td>41.43</td>
</tr>
<tr>
<td>Biliary Atresia</td>
<td>17</td>
<td>24.3</td>
</tr>
<tr>
<td>Byler's disease</td>
<td>3</td>
<td>4.29</td>
</tr>
<tr>
<td>Intrahepatic hypoplasia</td>
<td>4</td>
<td>5.71</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>4</td>
<td>5.71</td>
</tr>
<tr>
<td>benign recurrent intrahepatic cholestasis</td>
<td>1</td>
<td>1.43</td>
</tr>
<tr>
<td>a-antitrypsin deficiency</td>
<td>1</td>
<td>1.43</td>
</tr>
<tr>
<td>Tyrosinemia</td>
<td>1</td>
<td>1.43</td>
</tr>
<tr>
<td>Congenital hepatic fibrosis</td>
<td>1</td>
<td>1.43</td>
</tr>
<tr>
<td>Glycogen storage</td>
<td>2</td>
<td>2.86</td>
</tr>
<tr>
<td>Neimann-Pick</td>
<td>1</td>
<td>1.43</td>
</tr>
<tr>
<td>Biliary cirrhosis</td>
<td>4</td>
<td>5.71</td>
</tr>
<tr>
<td>Choleodochal cyst</td>
<td>2</td>
<td>2.86</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100</td>
</tr>
</tbody>
</table>

Cytomegalovirus was the commonest congenital infection reported. It constituted 52.2% of infants with congenital infection while herpes simplex virus constituted 13%, and combined infection of both occurred in 26.1% while congenital toxoplasmosis constituted 8.7% of congenital infectious agents.

The miscellaneous group included 22 (31.4%) patients who were diagnosed as Neimann-Pick disease (1.43%), Glycogen storage disease(2.86%) “Figure 2”, Congenital hepatic fibrosis(1.43%), tyrosinemia (1.43%), alpha-1- antitrypsin deficiency (1.43%), recurrent benign intrahepatic cholestasis (1.43%) ,Byler's disease(4.29%) “Figure 3”, non syndromic intrahepatic bile duct paucity(5.71%), Biliary cirrhosis(5.71%) , and Idiopathic “ with equivocal liver biopsy”(5.71%).
We compared different diagnostic modalities in the diagnosis of neonatal hepatitis and biliary atresia. (Table V & VI).

The clinical tool was more specific in diagnosing biliary atresia than in diagnosing neonatal hepatitis (86.8% versus 29.3%), also more accurate (81.4% versus 50%) but less sensitive (64.7% versus 79.3%).

Transamiasies were specific in differentiating biliary atresia from neonatal hepatitis or other causes of cholestasis (85.4% versus 36.1%), also more accurate (80% versus 57%) but less sensitive (64% versus 82.8%). Serum GGT level is the only biochemical test found to be of discriminating value. GGT values less than 200 U/L correlated with the diagnosis of hepatocellular cholestasis while GGT values more than 200 U/L favored the diagnosis of biliary atresia (table VII). However, low or normal GGT level was found in patients with Byler’s disease and benign recurrent intrahepatic cholestasis.

Serologic tests were specific for congenital infection. They should be done with histopathological diagnosis of neonatal hepatitis to confirm diagnosis. In this study the presence of CMV- IgM in the serum of cholestatic infants didn't exclude biliary atresia.

Abdominal ultrasonography is an excellent test in excluding
choledochal cyst as a cause of infantile cholestasis. In this study abdominal ultrasound was found to be more specific for the diagnosis of biliary atresia (86.2% versus 30.9%) and more accurate (82.3% versus 52.9%) but less specific (64.7% versus 82.8%). On the other hand hepatobiliary scintigraphy is a sensitive but less specific test in differentiating biliary atresia from intrahepatic cholestasis.

DISCUSSION

Cholestasis develops in response to a wide variety of causes. Irrespective of the etiology, its pathophysiological consequences on liver cell function are the same and early recognition and treatment could prevent permanent liver damage.

Even when treatment is not available or effective, infants with progressive liver disease usually benefit from efforts to provide optimal nutritional support and medical management of complications.

Several works had been done to study the etiological incidence of cholestasis and reported variable results with the neonatal hepatitis remaining the commonest etiology of cholestatic syndromes ranging from 38% to 79%.

CMV infection was found to be the commonest cause of neonatal hepatitis by some workers, as proved in the present study. However, we did not detect cases with Rubella, hepatitis B, hepatitis C or HIV infection in our patients.

Danks et al (1977) and Dick et al (1985) suggested idiopathic hepatitis as the main cause of neonatal hepatitis, but their studies antedate the descriptions of recently recognized metabolic causes of cholestasis. On the other hand advances in preventive medicine may result in the lower incidence of congenital infections compared to idiopathic hepatitis in some recent studies.

Biliary atresia was the second common cause of cholestasis in the present study (24% of cases), this agrees with studies made by Dick et al (1985) 20.4%, while Danks et al (1977) found extrahepatic biliary atresia in 32.2% of cholestatic cases.

Two recent Indian studies reported biliary atresia in 34% and 19.4% in infants with cholestatic syndrome respectively. Early diagnosis and surgical correction is of at most benefit for such patients.

The low incidence of choledochal cyst in the present study (2.9%) coincides with reports of Howard et al (1990) and Yachha et al (1990) 3% and 4% respectively of their cholestatic cases.

Metabolic liver diseases in the form of tyrosinemia, α-antitrypsin deficiency, Niemann Pick disease, congenital hepatic fibrosis and glycogen storage disease were diagnosed in 8.57% of our cholestatic cases. Several reports of variable incidence and types of metabolic liver diseases in cases of cholestasis are present. An Indian study reported 4% of cases while Howard et al diagnosed α-antitrypsin deficiency in 17.4% of cases with infantile cholestasis. Other studies conducted in London, Oslo and Australia reported α-antitrypsin deficiency as an etiological cause in 13%,6.5% and 4.7% of cases with infantile cholestasis respectively.

The variable incidence of metabolic liver diseases reported in different studies may be attributed to underlying genetic factors.

Byler’s syndrome has a world wide distribution and occurs most commonly in societies with high rate of consanguinity. Two out of three patients with Byler’s disease in this study had consanguinity between their parents. High incidence of the disease was reported in the United States among descendants of Jacob Byler (Amish community). In India, Yachha reported relatively low incidence of Byler’s disease among infants with cholestasis « 8.3% ».

The incidence of non-syndromic bile duct paucity in this study was 2.8% while the syndromic bile duct paucity (Alagille’s syndrome) was not found. These findings agree with the results of the John's study but others reported higher incidence of Allagille’s syndrome might be due to different genetic background.

CONCLUSION

From the present study we can conclude that:

Neonatal hepatitis is the commonest cause of infantile cholestasis and biliary atresia is the second common cause.

Routine laboratory tests as well as tests for specific hepatic disorders are important for the diagnosis and evaluation of cholestasis in pediatric age.

Histological study is a valuable tool in etiological diagnosis of cholestasis provided that it is done by an expert hands and lead to no complications.

Metabolic liver diseases are clinically excellent simulators to
the common pediatric illnesses and should be considered and searched for during the work up of cholestatic disease diagnosis in pediatrics.

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References
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