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THE CLINICAL AND PATHOLOGICAL CHARACTERISTICS OF NEONATAL CHOLESTASIS

Nizar Bakir Yahya[1]*, Ali Abdulghani Ramadhan[2]

1. *Department of Pediatrics, College of Medicine, University of Duhok, Duhok City, Iraq.*
2. *Department of Internal Medicine, College of Medicine, University of Duhok, Duhok City, Iraq.*

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ABSTRACT

Neonatal cholestasis (NC) initiates in the first trimester of a newborn, comprising extra and intrahepatic medical conditions, with a high risk of fatality warranting early diagnosis and treatment to prevent morbimortality. The differential diagnosis of NC is a challenge demanding an accurate diagnosis for disease detection. The current study evaluates NC's clinical symptoms and differential diagnosis using ultrasound, liver biopsy, histopathology, and biochemistry. Infant registry data from Hevi Pediatric Teaching Hospital (January 2016 - January 2022) were obtained and screened for subject selection. The inclusion criteria include infants with direct hyperbilirubinemia within an onset of 15 to 90 days of birth. As indicated, the recruited subjects underwent liver ultrasound, blood biochemistry, and liver biopsy. Seventy-two children presented with the criteria for inclusion in the study. The study found that ultrasound helped diagnose 43.1% of subjects for biliary atresia (BA) compared to 34.7% through histopathology. The histopathology confirmed 13 children (18.1%) having neonatal hepatitis. Test sensitivity of the ultrasound method for BA and neonatal hepatitis (NH) was 60% (40.74, 76.6) and 38.46% (17.71, 64.48), respectively. The study found both ultrasound and liver biopsy to be critical diagnostic methods to differentiate the etiology of NC. Ultrasound has a higher specificity and sensitivity for diagnosing BA than NH. Histopathology and blood biochemistry should be considered, too, for effective diagnosis. In the future, larger sample and multicenter studies should be conducted to develop practically implementable strategies.

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Introduction

Neonatal cholestasis (NC) is a relatively common clinical problem, representing a complex diagnostics challenge for doctors [1]. Neonatal cholestasis is characterized by conjugated hyperbilirubinemia in the newborn and young infant and is a sign common to over 100 hepatobiliary and/or metabolic disorders Neonatal cholestasis is generally defined as prolonged conjugated hyperbilirubinemia occurring in the neonatal period and lasting more than 2 weeks [2, 3]. Neonatal cholestasis was defined as serum direct bilirubin > 1.0 mg / dL according to a definition from the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition and the guidelines of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHN / ESPGHN) [4-6]. Cholestatic jaundice is highly prevalent in children, affecting one in 2,500 newborns. The signature characteristic of cholestatic jaundice is serum-conjugated bilirubin [7, 8]. However, neonatal jaundice manifestations are multifactorial, that include biliary atresia (BA), neonatal hepatitis (NH), metabolic disorders, infections, and other structural abnormalities [9, 10].

In developed countries, such as Germany and the United States, it is mainly diagnosed in children aged 60 days. Prompt assessment of its etiology is critical to quickly identify treatable causes, many of which usually benefit from early therapy, such as biliary atresia [11, 12]. Once the diagnosis of direct hyperbilirubinemia is established in children, the main diagnostic concern is to differentiate the hepato-cellular from obstructive cholestasis [13]. Though in rare cases, potentially very high levels of bilirubin have also been reported [14, 15]. However, the detrimental effect of increased bilirubin such as brain damage is limited to the lower- and middle-income countries. Higher-income countries have very few cases reported for brain damage in high bilirubin cases [16]. Therefore, in middle- and low-income countries, early detection, and therapy initiation is the key

Corresponding Author: Nizar Bakir Yahya; Department of Pediatrics, College of Medicine, University of Duhok, Duhok City, Iraq. E-mail: nizar.yahya@uod.ac.

to preventing liver damage, while improper management prone neonates to hepatic encephalopathy and end-stage hepatic damage.

In neonatal cholestasis, it is imperative for early diagnosis and surgical correction of medical conditions such as BA to prevent any fatal damage to the liver or any other organ. BA's signature clinical symptom is triangular cord sign (TC) which can be diagnosed through different diagnostic approaches. Ultrasound has been the standard diagnostic method to detect this fibrous ductal remnant that usually looks thick tubular echogenic density on the plate. It is usually located at the anterior region of the portal vein where; the vein has bifurcated into right and left branches. TC is a reliable and time-saving characteristic of BA that can be estimated through ultrasound. Anyway, apart from the TC and the physical examination, NC identification and management might be challenging, especially in middle-income countries [14, 16, 17]. It is well-known that changes in the panorama of diseases and population health needs have rendered the existing primary healthcare system often unable to treat health-related needs in Iraq. An early diagnosis of diseases such as NC might reduce healthcare costs and improve patient outcomes.

Thus, this study aims to describe the clinical manifestations, causes, and characteristics of NC, and the strategy to employ liver ultrasound and liver biopsy to make an early diagnosis in Hevi Pediatrics Teaching Hospital. It also represents one of the first few attempts at data collection and analysis over a long period in the Kurdistan Region and Iraq. Hevi Pediatrics Teaching Hospital is a small 190 beds hospital in Duhok Province in the northern Kurdistan Region of Iraq serving a large population of approximately 2.4 million, and an average daily visit of 150 cases. A continuous series of conflicts and wars have caused a ceaseless flow of refugees and internally displaced persons. In the last 10 years, more than one million persons have sought refuge in Duhok Province which counts as a local population. Early disease detection might properly meet patients' needs and healthcare system requests in a low-cost country income.

Materials and Methods

Study Design

A review of infants with NC who were admitted to Hevi pediatric teaching hospital in Duhok City from January 2016 to January 2022 was conducted.

Setting and Sample

The Hevi Pediatric Teaching Hospital has 190 beds: 29 for the Intensive Care Department, 111 for the Pediatric Department, 26 for the Emergency Department, and 24 for the Surgical Department. Hevi is the only third-level pediatric hospital in the entire Duhok province, thus all critically ill or life-threatening patients are transferred to it, which is the referral hospital for about one million people. Pediatric patients hospitalized at Hevi Hospital and aged from 15 to 90 days were included in this study. The inclusion included children presenting jaundice, clay-colored stools, colored urine, hepatosplenomegaly, history of prolonged direct hyperbilirubinemia (> 2 mg/dL), family history of liver disease, and underwent medical assessment, blood analysis, and liver ultrasound.

A liver biopsy was performed when NC could not be diagnosed through ultrasound. All children presented with direct hyperbilirubinemia due to hemolytic diseases were excluded from the study. In addition, children who underwent liver biopsies for reasons other than determining the etiology of cholestasis were excluded from the study.

Data Collection

Baseline assessments, involving demographic characteristics, were performed for all enrolled cases. Exposure factors focused mainly on the socio-economic family background, and the course of mothers' pregnancy, including infection, disease, or consanguinity. Furthermore, children's signs and symptoms and the type of milk taken were recorded. Blood analysis results were reordered. Moreover, collected data included indications for liver biopsy, date, children's age, and laboratory results before liver biopsy.

Procedure

All patients who were selected to participate in the study underwent physical assessment, blood tests, and liver ultrasound. To perform a physical assessment, children's clothes were taken off in a lighted environment and digital pressure was performed to eliminate erythema. The degree of jaundice was measured and the jaundice intensity was scored using the Kramer score. Kramer score is a tool that allows healthcare professionals to assess jaundice severity by examining the spread of the jaundice dermally in the body. The Kramer score defines the entire body in six scoring zones. The zone from head to the neck is scored as 1, score 2 is given for trunk to the umbilicus, score 3 is given for the groin that also includes upper thighs, 4 score for extremities from knees to ankle, elbows to the wrist, and lastly scores 6 for hands and feet that is inclusive of soles and palms [18].

All blood tests and metabolic screens were analyzed by the same laboratory technician. To perform a liver ultrasound, not any pre-test patient preparation was performed. The ultrasound exam was performed with a high-frequency transducer. Siemens Acuson S1000 ultrasound machine was used; all ultrasound scans were performed by the same technician. During the ultrasound, the presence of the gallbladder, portal vein, sign of the triangular cord, and intrahepatic bile ducts were observed.

Liver biopsy has been considered the most accurate, specific, and sensitive diagnostic testing in NC and can help differentiate NC from other etiologists. Moreover, histopathology can indicate other specific diagnoses. The accuracy of liver biopsy histology for predicting the diagnosis of BA ranges from 85 to 95% [19]. Before performing the biopsies, the children's parents signed a consent to the procedure, and parents were asked to fast their child for 4 hours before the procedure.

All biopsies were performed under an aseptic technique and local anesthesia with lidocaine 2%. After skin cleansing was performed with 2% chlorhexidine, an 18 Gauge cannula was inserted into the liver through the skin of the right hypochondrium under an ultrasound guide. A liver biopsy specimen of approximately 1.5 cm in length and 1-2 mm in width was considered adequate. Vital signs were measured throughout the procedure. A compressive dressing was placed at the end of the procedures, and children were rolled on the right side and kept under observation for 4 hours. Though, as a precaution, fresh frozen plasma, vitamin K, and blood were prepared in case of any emergency.

Statistical Analysis

The first step is data collection and structuring for further analysis. Therefore, Microsoft Excel (the latest version) was used for data entry, data cleaning, and categorization. The data were cross-verified to ensure, the missing data and wrong data are eliminated before statistical analysis. IBM's statistical software, SPSS version 20.0 (IBM Corp., Armonk, NY, USA) was used for analyzing the data. We employed uni-variate analysis; the chi-square test was used for categorical variables. For the continuous variables, the Student's t-test/Wilcoxon test was used. Descriptive statistics were used to analyze the demographic variables and other parameters. The standard deviation (SD), medians (Interquartile ranges), and percentages have been used to present the means. The statistical significance was set t P value less than 0.05.

Ethical Considerations

The study followed international guidelines of ethics to ensure the privacy and confidentiality of the subjects. The participant data was appropriately stored, and access was restricted to only the authors of the study. Similarly, the data collected maintained anonymity by using numbers to designate the patients. Consent for the study was obtained from the Directorate General of Health (city name) for utilizing data in the present study (Protocol n. 1342022-2-14/13 April 2022), Consent from parents (guardians) was obtained for all infants.

Results and Discussion

A total of 4836 children have been hospitalized in the period January 2016- January 2022 at Hevi Pediatric Teaching Hospital. Of these children, 72 (1.5%) were eligible for inclusion in the study. Sample characteristics are described in **Table 1**. Patients were mainly females (56.9%), born at 38 weeks gestational age via vaginal delivery (75%). Their mean age in days was 45.11 ±18.87 and their weight in kilograms was 4.25 ± 1.32. In 90.3% of cases, children's mothers were not affected by any disease. Only 7 mothers had diseases such as hypertension (n= 4), kidney disease (n= 2), and diabetes mellitus (n= 1). Parents were blood relatives in 25% of cases, and the majority of children did not have a family history of jaundice. Finally, children were mainly fed with both breastfeeding and formula (81.9%). Signs and symptoms of patients are indicated in **Table 2**. Patients who underwent assessment presented with jaundice from 1 to 7 days. The jaundice onset in the most of children (48.6%) occurred within 3 days and 51.4% of them presented with a grade III jaundice. Stools appeared acholic in 36.1% of children, while urine was dark in 47.2% of them.

Table 1. Sample Characteristics

Parameters		N	%
Gender	Male	31	43.1
	Female	41	56.9
GESTATIONAL AGE (Weeks)	30-34	6	8.3
	34-36	8	11.1
	37- 40	58	80.6
Type of Delivery	Vaginal	54	75
	Cesarean	18	25
Mother's Diseases	No disease present	65	90.3
	Diabetes Mellitus	1	1.4
	Hypertension	4	5.6
	Kidney disease	2	2.8
Parent's Consanguinity	No	54	75
	Yes	18	25
Family History of Jaundice	No	64	88.9

	Yes	8	11.1
Type of Milk	Breastfeeding	9	12.5
	Formula	4	5.6
	Mixed	59	81.9

Table 2. Signs and Symptoms

Parameters		**No.**	**%**
Day of Jaundice Onset	1	2	2.8
	2	11	15.3
	3	35	48.6
	4	20	27.8
	5	3	4.2
	7	1	1.4
Grading of the Extent of Jaundice	Grade 1	4	5.6
	Grade 2	29	40.3
	Grade 3	37	51.4
	Grade 4	2	2.8
Stool Color	Normal	46	63.9
	Acholic	26	36.1
Dark Urine	No	38	52.8
	Yes	34	47.2

As reported in **Table 3**, only 20 patients (27.8%) presented with hepatomegaly at medical assessment. Splenomegaly was detected only in 15.3% of cases. Ultrasounds revealed the triangular cord sign in 31 children (43.1%). Biopsy was performed in 69 patients; in 3 patients the diagnosis of NC was performed with no further investigations apart from medical assessment and ultrasounds. When the biopsy was performed, the children's mean age was 35 ± 18 days. Biopsy showed BA in 26 patients (36.1%). In 22 patients (30.6%), the causes of signs and symptoms remained unknown, while neonatal hepatitis has been diagnosed in 13 children (18.1%). Chronic morbidity was the outcome in 43 children (59.7%). Blood test results are reported in **Table 4**. Sixty percent of children with histopathologically confirmed BA had triangular cord signs compared to 36.4% of children who had no BA on histopathology. This difference was statistically significant ($p = 0.05$). Triangular cord sign on ultrasound was found in 38.5% of children with confirmed NH compared to 46.4% of children with no NH histopathologically. This difference was not statistically significant ($p= 0.60$). The sensitivity and specificity of performed histopathology tests were calculated by using the Wilson score with a 95% confidence interval.

Table 3. Results from Medical Tests

Parameters		**No.**	**%**
Hepatomegaly on Clinical Examination	No	52	72.2
	Yes	20	27.8
Splenomegaly on Clinical Examination	No	61	84.7
	Yes	11	15.3
Biliary Tract Findings on Ultrasounds	Triangular cord sign	31	43.1
	No triangular cord sign	41	56.9
Histopathological Findings of Biopsy	Biliary atresia	25	34.7
	Neonatal hepatitis	13	18.1
	Intrahepatic bile duct paucity	1	1.4
	Genetic and metabolic cause	5	6.9
	Infective cause	2	2.8
	Unknown cause	22	30.6
	Missing	3	4.2
Outcome	Cured	16	22.2

	Chronic morbidity	43	59.7
	Death	13	18.1

Table 4. Blood tests results

	Mean	**Min.**	**Max.**	**Std. Dev.**
TSB in mg/dL	10.6	3.1	42	6.3
Direct serum bilirubin in mg/dL	5.98	2	30	3.96
Indirect serum bilirubin in mg/dL	4.7	1	20.00	3.7
Packed cell volume (Hematocrit) PCV	35.98	20	61.00	7.1
ALT in U/L	183.96	20	676.00	140.7
AST in U/L	177.86	23	700.00	132.4
ALP in IU/L	303.1	85	911.00	189
Haemoglobin in gm/dL	10.89	6	14.00	1.9
White blood cells per microliter	8467	2500	28000	4707
Platelet per microliter	193222	60000	665000	126814
Serum albumin in gm/L	3.5	2.3	4.20	.38
PT in seconds	16.5	11.7	45.0	6.5
aPTT in seconds	31.9	16	98.0	11.6
TSH in mU/L	3.5	1	35.00	4

TSB= Total serum bilirubin; ALT= Alanine aminotransferase; AST= Aspartate transaminase; ALP= Alkaline phosphatase; PT= Prothrombin time; aPTT= Partial thromboplastin time; TSH= Thyroid stimulating hormone

Table 5 shows the validity of triangular ultrasound signs on ultrasound to detect biliary atresia. It has a sensitivity of 60% (40.74, 76.6), a specificity of 70% (57.17, 80.86), a positive predictive value of 48% (31.97, 65.16), a negative predictive value of 79% (65.74, 88.27), a likelihood ratio for positive test of 2.025(1.642 - 2.497), and a likelihood ratio for negative test of 0.5684(0.4572 - 0.7067). The diagnostic accuracy was found to be 67% (56.15, 76.45).

Table 5. Screening Test Evaluation (ultrasound) for Biliary Atresia

	Biliary atresia		
		Positive	**Negative**
Positive triangular cord sign		15	16
Negative triangular sign		10	28
Parameter	Estimate	Lower - Upper 95% CIs	Method
Sensitivity	60%	(40.74, 76.6)	Wilson Score
Specificity	70.37%	(57.17, 80.86)	Wilson Score
Positive Predictive Value	48.39%	(31.97, 65.16)	Wilson Score
Negative Predictive Value	79.17%	(65.74, 88.27)	Wilson Score
Diagnostic Accuracy	67.09%	(56.15, 76.45)	Wilson Score
Likelihood ratio of a Positive Test	2.025	(1.642 - 2.497)	
Likelihood ratio of a Negative Test	0.5684	(0.4572 - 0.7067)	

Suppl. **Table 1** shows the screening test employed (liver biopsy) for neonatal hepatitis with 85.7% sensitivity and 95% specificity.

Table 6 shows the validity of triangular ultrasound signs on ultrasound to neonatal hepatitis. It has a sensitivity of 38.46% (17.71, 64.48), a specificity of 53.57% (40.7, 65.98), a positive predictive value of 16.13% (7.093, 32.63), a negative predictive value of 78.95% (63.65, 88.93), a likelihood ratio for positive test of 0.8284(0.4103 - 1.673), and a likelihood ratio for negative test of 1.149 (0.8496 - 1.553). The diagnostic accuracy was found to be 50.72% (39.21, 62.2).

Table 6. Screening Test Evaluation (ultrasound) for Neonatal Hepatitis

		Positive	**Negative**
Positive triangular cord sign		5	26
Negative triangular sign		8	30
	Estimate	Lower - Upper 95% CIs	Method

Sensitivity	38.46%	(17.71, 64.48)	Wilson Score
Specificity	53.57%	(40.7, 65.98)	Wilson Score
Positive Predictive Value	16.13%	(7.093, 32.63)	Wilson Score
Negative Predictive Value	78.95%	(63.65, 88.93)	Wilson Score
Diagnostic Accuracy	50.72%	(39.21, 62.17)	Wilson Score
Likelihood ratio of a Positive Test	0.8284	(0.4103 - 1.673)	
Likelihood ratio of a Negative Test	1.149	(0.8496 - 1.553)	

Suppl. **Table 2** shows the screening test employed (ultrasound) for neonatal hepatitis with 23.3% sensitivity and 97.1% specificity.

Neonatal cholestasis implicates severe hepato-biliary disease that requires early assessment, recognition, and intervention to prevent serious fatalities, such as severe liver decompensation [20]. The evaluation of neonatal cholestasis is a challenging issue; therefore, the current study was carried out to evaluate liver biopsy and ultrasound for early diagnosis of the condition considering BA and NH as primary clinical manifestations to be recognized. The ultrasound technology could differentiate between BA and NH, the two most common etiology of NC. Similarly, the medical examination and assessment helped in confirming the clinical symptoms of NC [21].

The study sample has more females (58.9%), and the jaundice onset was observed among 49% approximate sample population, with 51.4% representing grade III jaundice. Research suggests male gender is more associated with neonatal cholestasis; however, the uneven gender-wise distribution does not seem to affect the sample [11, 22]. More than half of the population demonstrated grade II jaundice. The mean age of our sample was found to be 45.11 ± 18.87, and weight in kilograms was found to be 4.25 ± 1.32. Differing from other reported studies due to the difference in sample selection strategy [21]. Infant jaundice initiates from the second day onwards and achieves peak from the sixth to the fourteenth day, and our results are within the range period. Another factor contributing to the lesser mean age for jaundice onset is pre-term birth before 38 weeks [23, 24].

In our study sample, 27.8% and 15.3% presented with hepatomegaly and splenomegaly. Although our study findings differ from other studies reported in Iraq, Al-Azzawi *et al*. (2013) reported 77.1% hepatomegaly and 54.3% splenomegaly, and the trend is similar to hepatomegaly percentage is higher than splenomegaly. The percentage difference is due to the difference in the sample's age of onset, impacting the disease course. The clinical symptoms identified in the current sample are consistent with Azzawi *et al*. (2013) study that evaluated cholestasis in infants in Iraq [25, 26].

Diagnosing Neonatal cholestasis is challenging in clinical scenarios due to overlapping symptoms with other medical conditions and a lack of proper awareness. The gold standards for diagnosing BA and NH in the study sample are ultrasound and liver biopsy. The triangular cord sign is the signature clinical indicator of biliary atresia. The current study reported 31 children (43.1%) having triangular cord signs as a diagnostic of BA, consistent with Al-Azzawi's study (2011), where 44% of the sample population was found to have BA (ultrasound measured). However, liver biopsy diagnosed BA in 36.1% of the population. Contrasting to the present study, the previous study reported 22% BA in the sample population contributing towards NC. The high percentage of BA found in our study reconfirms that BA is the most common cause of neonatal cholestasis [25]. Ultrasound has been the baseline diagnostic method employed to differentiate etiologic causes (intra and extrahepatic) of NC. Triangular cord sign identification in diagnosing biliary atresia can be difficult in case of liver hilum inflammation that hides the triangular cord sign [27, 28]. Neonatal hepatitis diagnosis stood at 18.1% measured by liver biopsy, implicating that the characteristic changes of BA, which start after nine weeks of age, may not have been captured by the biopsy method. The study's chronic morbidity and mortality were 60% and 18%, consistent with the previous study. This suggests that late referral, convincing parents of the procedures, and time taken to carry out liver biopsy could be the contributing factors [28, 29].

The blood tests, irrespective of BA and NH, revealed that most blood parameters are above the normal range—the total serum bilirubin. Hyperbilirubinemia was observed in the present study sample, which can be due to increased bilirubin production, reduced uptake by the liver, and reduction in bile excretion that increases the levels of total serum bilirubin, conjugated and unconjugated bilirubin too. The increased indirect bilirubin level is due to the increased bilirubin production (<6mg/dl). Similarly, poor uptake and excretion results of bilirubin result in increased direct bilirubin levels observed in our study. The trends and patterns in bilirubin tests are comparable and consistent with other reported studies.

Similarly, the levels of hepatocyte enzymes alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase are elevated in cholestatic infants. The levels of these three hepatocyte enzymes obtained in our study are consistent with other reported studies [30, 31]. The PT test was carried out to evaluate the liver's synthesizing ability for Vitamin-K-dependent clotting factors and fibrinogen. Recent insights from research studies have shown that prolonged PT is due to fat-soluble vitamin insufficiency. The study found a reduction in serum albumin levels because of an increased volume of distribution. The other test results are comparable and consistent across the studies reported [11].

The histopathology findings revealed BA in sixty percent of the patient and 38.5% NH, comparable to other studies regarding the trend. Liver biopsy is higher sensitivity, specificity, and accuracy has been reported in different studies [30, 31].

Our study findings suggest the ultrasound method is a better diagnostic approach, with specificity and sensitivity of 70% and 60% for detecting biliary atresia compared to detecting NH, the sensitivity (53.57) and specificity (33.46%). Our study data is consistent and similar to previous studies reporting high specificity (76.1%) and sensitivity (52.6%) of BA detection through ultrasound. The trends for positive predictive value for biliary atresia with U/S are consistent with the previous study's reported data [32]. The value difference can be attributed to inter-professional error and machine manufacturing parameters. The specificity and sensitivity of U/S in diagnosing biliary atresia and neonatal hepatitis were comparable to previous studies, ranging from as low as 23 % -93% in sensitivity and specificity of 80%-98%. There is a paucity of data regarding U/S comparison with histopathology or liver biopsy in detecting BA and NH.

One of the limitations is that breastfeeding as a confounding factor has yet to be addressed in NC. Acholic stool, an early indicator of BA, could also result from breastfeeding. The second limitation is more consistency in the statistical methodology employed to evaluate ultrasound, liver biopsy, and histopathology. Another limitation is that blood biochemistry data should be collected differently for biliary atresia and neonatal hepatitis patient to understand the significance of each biochemical marker for early diagnosis. Single-center case study limits generalizing the finding of the study. In the future, comparative analysis of diagnostic approaches for Neonatal cholestasis detection, clinical manifestation identification, and cause differentiation through thorough statistical methods are required. Similarly, a multicenter study with large population size is also warranted to validate the results in a diverse patient category.

Conclusion

Neonatal cholestasis remains a diagnostic challenge for clinicians and associated healthcare professionals. The medical assessment should be followed as a first step towards proper identification and facilitated as early as possible. Diagnosing neonatal cholestasis needs a multi-approach involving ultrasound, liver biopsy, and histopathological data accompanied by medical assessment and examination. The study data suggest that ultrasound is a better diagnostic technology to differentiate the causal factor for neonatal cholestasis. However, that does not undermine the significance of liver biopsy, biochemical studies, and histopathological studies that can facilitate crucial information for a thorough diagnosis. In the future, more quantitative data analysis and greater sample size should be considered for evaluating the diagnostic approach for neonatal cholestasis diagnosis and treatment strategy development.

Limitations

The study has several limitations. First, the study was conducted in a single hospital. Second, there was no histopathology laboratory at Hevi Pediatric Teaching Hospital, and accordingly, samples were sent to a distance of 360 km in another city with the possibility of loss or change to the quality of the sample. Third, no genetic testing or hepatobiliary scan was performed to get a more accurate and earlier diagnosis. Fourth, no cholangiography facility is available for the time being, though before decades the diagnosis was dependent on open biopsy and laparotomy. Fifth, no GGT was available at the time of research conduction.

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