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ROLE OF LIVER BIOPSY IN THE DIAGNOSIS OF PROLONGED CHOLESTASIS IN INFANTS

Original Research

Abdullah Akram[1]*, Sadaf Nawaz[1], Manal Zahid[1], Naeem Farid[1], Waseem Ahmed[1], Muhammad Shahan Anwar[1], Sher Afgan Raisani[2]
[1]CMH Gujranwala, Pakistan.
[2]Provincial Manager TB Control Program Baluchistan, Pakistan.
Corresponding Author: Abdullah Akram, CMH Gujranwala, Pakistan. abdullah.akram002@gmail.com
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ABSTRACT

Background: Prolonged neonatal cholestasis is a critical condition requiring early differentiation between biliary atresia and neonatal hepatitis to ensure timely intervention. Biliary atresia necessitates surgical correction, whereas neonatal hepatitis is managed medically. Accurate diagnosis is essential to prevent delays in treatment and avoid unnecessary surgical procedures. Although hepatobiliary iminodiacetic acid (HIDA) scanning is widely used, its availability is limited in many settings. Liver biopsy with histopathological evaluation is considered a more definitive diagnostic tool. This study aims to compare the sensitivity, specificity, and diagnostic accuracy of HIDA scanning versus liver biopsy in infants with prolonged cholestasis.

Objective: To evaluate and compare the diagnostic accuracy of liver biopsy and HIDA scanning in differentiating biliary atresia from neonatal hepatitis in infants with prolonged cholestasis.

Methods: This prospective observational study was conducted at the Pediatric Department of Combined Military Hospital, Gujranwala, from January to December 2024. A total of 90 infants aged 15–180 days with prolonged cholestasis were included. All patients underwent both HIDA scanning and percutaneous liver biopsy. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy of both modalities were calculated and compared. Statistical analysis was performed using SPSS version 26.0.

Results: The sensitivity of the HIDA scan for differentiating biliary atresia from neonatal hepatitis was 84.3%, specificity was 80.0%, PPV was 93.7%, and NPV was 59.3%, with an overall diagnostic accuracy of 87.7%. In comparison, liver biopsy demonstrated a sensitivity of 92.9%, specificity of 90.0%, PPV of 97.0%, and NPV of 78.3%, with a diagnostic accuracy of 94.4%.

Conclusion: Liver biopsy with histopathological confirmation exhibited superior diagnostic accuracy compared to HIDA scanning for differentiating biliary atresia from neonatal hepatitis. Given its higher sensitivity and specificity, liver biopsy should be prioritized, particularly in settings where HIDA scanning is unavailable or delayed.

Keywords: Biliary atresia, Cholestasis, Diagnostic accuracy, HIDA scan, Histopathology, Infant, Liver biopsy.

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INTRODUCTION

Cholestasis is characterized by a decrease in bile secretion or flow through the hepatobiliary system, posing a significant concern when it persists beyond two weeks after birth during the first three months of life (1). It is clinically defined by an increase in conjugated bilirubin exceeding 1 mg/dL when total serum bilirubin remains below 5 mg/dL (2). The incidence of neonatal cholestasis is approximately 1 in 2500 live births worldwide, with regional variations (3). Persistent neonatal jaundice is always pathological and necessitates early diagnosis and intervention to mitigate complications and reduce mortality. Studies indicate that neonatal cholestasis carries an estimated post-treatment mortality rate of 10.8% within the first year, underscoring the need for timely and accurate diagnosis (4). The etiology of neonatal cholestasis is broad, encompassing hepatocellular, obstructive, and idiopathic causes. Among these, biliary atresia and neonatal hepatitis account for more than 80% of cases (5). Despite their similar clinical presentations, these conditions require fundamentally different management approaches. While neonatal hepatitis can often be managed medically, biliary atresia necessitates prompt surgical correction (6). Delayed diagnosis can result in irreversible liver damage, increased morbidity, and adverse long-term outcomes. Therefore, early differentiation between these conditions is crucial to ensuring timely medical intervention for neonatal hepatitis and appropriate surgical decisions for biliary atresia.

Diagnostic modalities such as hepatobiliary iminodiacetic acid (HIDA) scans have gained prominence in detecting functional and structural abnormalities of the liver, gallbladder, and biliary tree (7). Studies suggest that HIDA scans offer good sensitivity and acceptable specificity in distinguishing biliary atresia from neonatal hepatitis. However, in resource-limited settings where HIDA scans are unavailable, alternative diagnostic methods must be explored to ensure accurate clinical decision-making. Histopathological evaluation of liver biopsy specimens, particularly through scoring systems designed to differentiate biliary atresia from neonatal hepatitis, has been proposed as a viable alternative (8,9). Given the limited accessibility of advanced imaging in many healthcare settings, the diagnostic utility of liver biopsy in infants with prolonged cholestasis warrants further investigation. This study aims to compare the sensitivity and specificity of HIDA scan results with liver biopsy findings in differentiating biliary atresia from neonatal hepatitis in infants with prolonged cholestasis. The findings will provide critical insights into the diagnostic accuracy of liver biopsy, potentially offering a reliable alternative for clinical decision-making in settings where advanced imaging modalities are unavailable.

METHODS

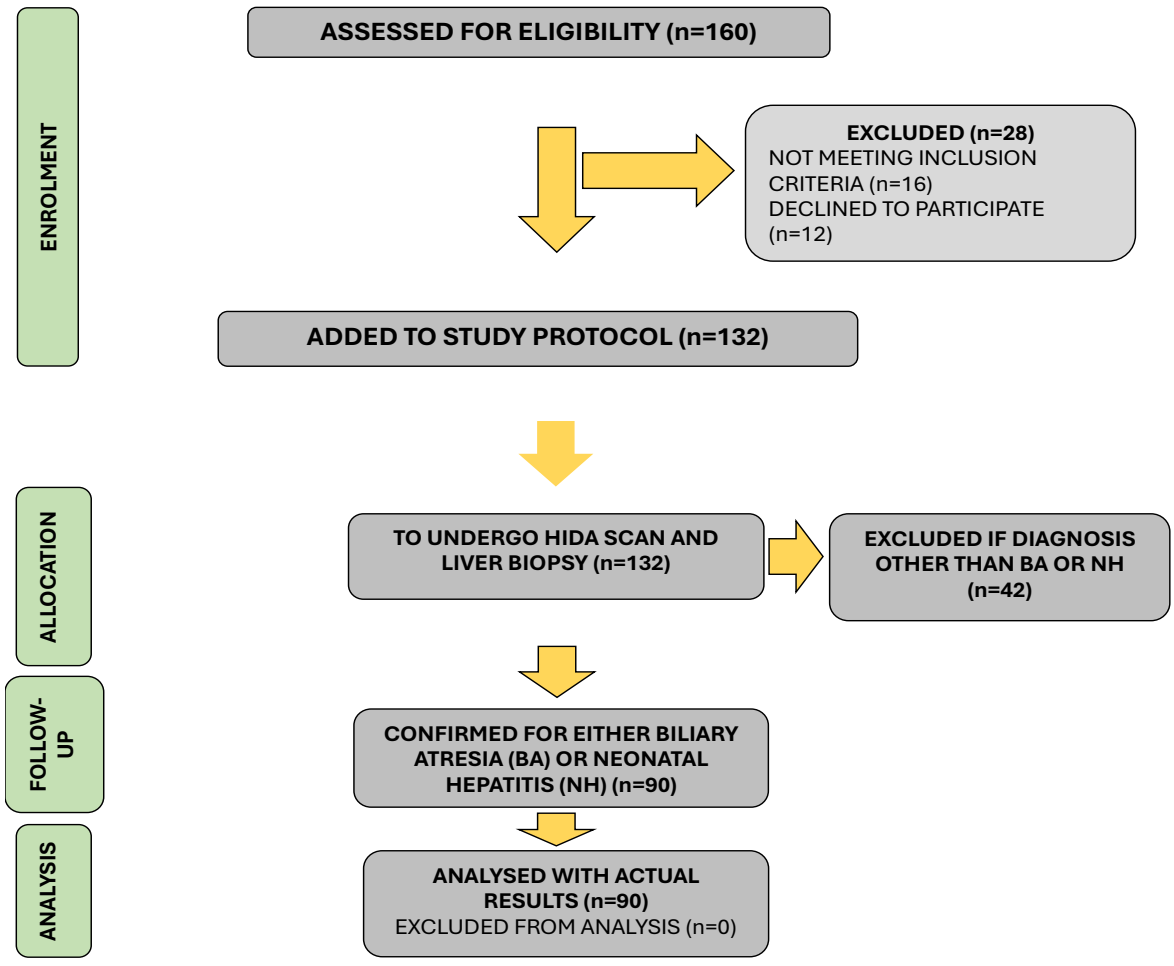
This prospective observational study was conducted at the Department of Pediatrics, Combined Military Hospital, Gujranwala, from January to December 2024. The sample size was determined based on a 95% confidence interval, a 5% margin of error, and an anticipated specificity of the HIDA scan versus liver biopsy in diagnosing biliary atresia and neonatal jaundice at 80% and 95%, respectively (10,11). The minimum required sample size was calculated to be 73 patients; however, a total of 90 patients were included in the final study to enhance the reliability of the findings. Patients aged between 15 to 180 days, presenting with prolonged cholestasis, defined as conjugated bilirubin levels exceeding 1 mg/dL with a total bilirubin level of less than 5 mg/dL, were eligible for inclusion. Patients were excluded if their final diagnosis differed from biliary atresia or neonatal jaundice after workup, if they had inconclusive HIDA scan or biopsy results, if they were lost to follow-up, if they had a documented allergy to the HIDA scan contrast, or if parental or guardian consent was not obtained for study participation. Ethical approval was obtained from the Institutional Review Board (IRB) before the commencement of the study, and informed written consent was secured from the parents or legal guardians of all enrolled patients. Ethical concerns, including confidentiality and voluntary participation, were fully addressed.

A detailed clinical history and physical examination were performed by a consultant pediatrician for all patients meeting the inclusion criteria. Demographic variables, including age, weight, and gender, were recorded. Baseline investigations included serum bilirubin (total, unconjugated, conjugated), complete blood count, coagulation profile, and international normalized ratio (INR). HIDA scans were performed after pre-treatment with oral phenobarbital (5 mg/kg/day) for at least four to five days to enhance hepatic enzyme activity and bile flow, minimizing false-positive results. The procedure was conducted under sedation with anesthetic supervision following a fasting protocol. Patients were positioned supine with arms secured in an outstretched position to optimize imaging. The imaging sequence included one frame per second for 90 seconds, followed by static images at 2, 4, 6, 12, and 24 hours post-tracer injection (200 µCi/kg). The final interpretation was independently conducted by two nuclear medicine consultants blinded to the study protocol and

clinical history to prevent bias. A diagnosis of biliary atresia was confirmed if there was no visualization of the gallbladder and/or bowel at any time within the 24-hour imaging sequence.

Liver biopsy was performed percutaneously on all patients who underwent HIDA scanning. Biopsy specimens were sent for histopathological analysis, where features suggestive of biliary atresia included bile ductular proliferation, porto-portal bridging, and bile plugs in the ductules, while neonatal hepatitis was diagnosed based on lobular and portal inflammation, hepatocellular necrosis, lymphocytic and neutrophilic infiltrates, giant cell transformation, and hepatocellular swelling. Two independent histopathologists, blinded to patient history and HIDA scan results, reviewed and categorized the histological findings as either biliary atresia or neonatal hepatitis. A standardized 15-point histopathological scoring system was used, with a score of seven or higher indicating biliary atresia (9). Patients diagnosed with conditions other than biliary atresia or neonatal hepatitis were excluded from the final analysis. Data were compiled for both diagnostic modalities, and their results were subsequently compared with surgical outcomes in cases suspected of biliary atresia. Descriptive statistics were reported as mean and standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. Independent samples t-tests were used for the comparison of mean values, while the chi-square test was employed for categorical variables. The Mann-Whitney U test was used to compare median values. Sensitivity, specificity, and diagnostic accuracy were calculated for both procedures. A p-value of ≤0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 26.0.

FIGURE-I: PHASES OF THE STUDY



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RESULTS

A total of 160 patients were initially assessed for inclusion in the study, out of which 132 met the eligibility criteria and underwent both HIDA scanning and liver biopsy. After excluding cases with diagnoses other than biliary atresia or neonatal hepatitis, 90 patients were included in the final analysis for comparison with actual clinical outcomes. The mean age of the study population was 69.01±6.42 days, and the mean weight was 2.08±0.17 kg. The gender distribution comprised 69 males (76.7%) and 21 females (23.3%). Mean serum total bilirubin levels were recorded at 8.03±0.82 mg/dL, while serum conjugated bilirubin levels averaged 2.17±0.12 mg/dL. The mean hemoglobin level was 10.48±1.35 g/dL. Clinically, dark urine was observed in 24 patients (26.7%), and an abnormal liver span on examination was noted in 46 patients (51.1%). The diagnostic performance of HIDA scanning in distinguishing biliary atresia from neonatal hepatitis revealed a sensitivity of 84.3%, specificity of 80.0%, a positive predictive value of 93.7%, and a negative predictive value of 59.3%, yielding an overall diagnostic accuracy of 87.7%. In contrast, histopathological evaluation via liver biopsy demonstrated a sensitivity of 92.9%, specificity of 90.0%, a positive predictive value of 97.0%, and a negative predictive value of 78.3%, with an overall diagnostic accuracy of 94.4%. The results indicated superior sensitivity, specificity, and diagnostic accuracy of liver biopsy over HIDA scanning in differentiating biliary atresia from neonatal hepatitis.

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF THE STUDY GROUP (n=110)

Variable	Study Group
Mean Age (Days)	69.01±6.42
Mean Weight (Kg)	2.08±0.17
Gender	
Male	69 (76.7%)
Female	21 (23.3%)
Mean Serum Total Bilirubin (Mg/Dl)	8.03±0.82
Mean Serum Conjugated Bilirubin (Mg/Dl)	2.17±0.12
Mean Hemoglobin (G/Dl)	10.48±1.35
DARK URINE (N %)	24 (26.7%)
ABNORMAL LIVER SPAN (N %)	46 (51.1%)

TABLE 2: SENSITIVITY, SPECIFICITY AND DIAGNOSTIC ACCURACY OF HIDA SCAN VERSUS LIVER BIOPSY

	Hida Scan	Liver Biopsy
Sensitivity	84.3%	92.9%
Specificity	80.0%	90.0%
Positive Predictive Value	93.7%	97.0%
Negative Predictive Value	59.3%	78.3%
Diagnostic Accuracy	87.7%	94.4%

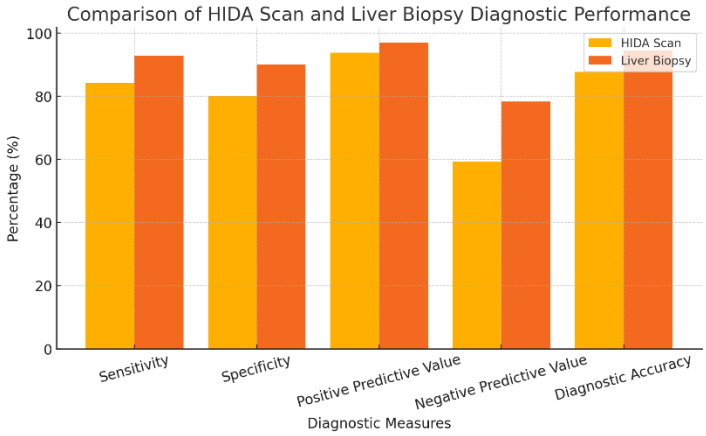

Figure 1 Comparison of HIDA Scan and Liver Biopsy Diagnostic Performance

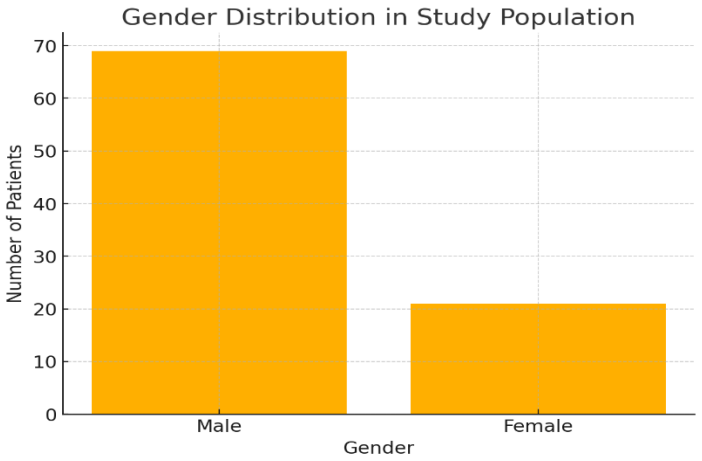

Figure 2 Gender Distribution in Study Population

DISCUSSION

The findings of this study demonstrated that liver biopsy, with histopathological confirmation, exhibited superior sensitivity, specificity, and overall diagnostic accuracy compared to the HIDA scan in differentiating biliary atresia from neonatal hepatitis in neonates with prolonged cholestasis. The results align with previous national and international studies that have evaluated the diagnostic performance of these modalities. Several studies have reported that neonates presenting with elevated conjugated bilirubin levels, clay-colored stools, and dark urine were commonly diagnosed with biliary atresia or neonatal hepatitis, reinforcing the clinical relevance of these parameters in early screening. While the HIDA scan has been acknowledged as a useful screening tool, its lower sensitivity and specificity limit its reliability as a standalone diagnostic test, particularly in settings where timely surgical intervention is crucial (12). Further comparative analysis with existing literature reveals that biliary atresia is more prevalent in term neonates, with a male predominance observed in both biliary atresia and neonatal hepatitis. Multiple studies have corroborated the superior diagnostic accuracy of liver biopsy, reporting sensitivity and specificity exceeding 85% and 92%, respectively, findings consistent with the present study (13). However, some reports have recommended prioritizing the HIDA scan due to concerns about iatrogenic injury associated with percutaneous liver biopsy and prolonged turnaround times for histopathological analysis in certain healthcare settings. Despite these concerns, limited access to nuclear medicine facilities in many regions, coupled with the potential for delays in HIDA scanning, can result in missed diagnoses and unnecessary surgical interventions. These factors highlight the necessity of considering liver biopsy as the primary diagnostic tool in resource-limited settings where nuclear imaging is not readily available (14,15).

Nationally, research has further validated the superiority of liver biopsy, with studies incorporating biochemical markers such as gamma-glutamyl transferase (GGT) to enhance diagnostic precision for biliary atresia. Findings have consistently demonstrated male predominance, increased liver span in a significant proportion of cases, and a correlation between dark urine and both biliary atresia and neonatal hepatitis, reinforcing the clinical utility of these signs in early disease recognition (16,17). As international guidelines continue to support both HIDA scanning and liver biopsy as standard diagnostic approaches, the need for protocol standardization based on regional healthcare resources is imperative. Given the current limitations in the accessibility of HIDA scans, histopathological confirmation through liver biopsy remains the most viable option for early and accurate diagnosis in many settings. Until nuclear imaging services become more widely available, histopathological evaluation should be prioritized as the first-line diagnostic approach in neonates with prolonged cholestasis (18). Despite the strengths of this study, including a well-defined cohort and the use of both diagnostic modalities in direct comparison, several limitations must be acknowledged. The study was conducted at a single center, which may limit the generalizability of the findings to a broader population. Additionally, the availability and cost of HIDA scans remain significant barriers in regions where healthcare services are not universally accessible, potentially restricting the ability to conduct further large-scale comparative studies. Future research should focus on multicenter trials incorporating larger sample sizes to validate these findings further and refine diagnostic algorithms for neonatal cholestasis. Expanding access to nuclear medicine services and integrating additional biomarkers may enhance the precision of early diagnosis and improve clinical outcomes.

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CONCLUSION

Liver biopsy with histopathological confirmation has proven to be a more reliable diagnostic tool than the HIDA scan in accurately differentiating biliary atresia from neonatal hepatitis, ensuring timely and appropriate treatment decisions. Given its superior diagnostic performance, liver biopsy should be prioritized in clinical settings where nuclear imaging is limited or delayed. This study reinforces the need for accessible, accurate, and efficient diagnostic pathways for neonates with prolonged cholestasis, ultimately improving early intervention strategies and patient outcomes.

AUTHOR CONTRIBUTIONS

Author	Contribution
Abdullah Akram*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Sadaf Nawaz	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Manal Zahid	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Naeem Farid	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Waseem Ahmed	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Shahan	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Sher Afgan Raisani	Contributed to study concept and Data collection Has given Final Approval of the version to be published

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