ROLE OF HEPATOBILIARY SCINTIGRAPHY & BILIRUBIN PROFILE IN EARLY DIAGNOSIS OF BILIARY ATRESIA IN CHILDREN WITH PERSISTENT NEONATAL JAUNDICE–A REVIEW OF 8 YEAR EXPERIENCE

Muhammad Inamullah, Mohammad Omair Riaz*, Fida Hussain

Armed Forces Institute of Pathology/ National University of Medical Sciencs (NUMS) Rawalpindi Pakistan, *Combined Military Hospital Lahore Pakistan

ABSTRACT

Objective: To evaluate the role of Hepatobiliary scintigraphy and bilirubin profile in early diagnosis of biliary atresia in children with persistent neonatal jaundice in resource constrained settings.

Study Design: Cross sectional observational study including retrospective data evaluation.

Place and Duration of Study: Nuclear Medical Center (NMC), AFIP Rawalpindi, from Jul 2009 to Jun 2017 over a period of 8 years

Material and Methods: Neonates/infants referred for Hepatobiliary scintigraphy were included in the study with a diagnosis of cholestatic liver disease along with conjugated hyperbilirubinemia. Age in days, gender and total/ direct bilirubin levels were documented for each case at presentation. Patients were classified into three diagnostic subgroups on the basis of hepatobiliary scintigraphy into biliary atresia, normal and neonatal hepatitis in both genders. The mean of direct/total bilirubin in all gender specific subgroups was calculated and differences in similar groups across genders tested for significance by students' t-test. Differences in age and gender specific frequency of bilary atresia were tested by chi square Fischer's exact test.

Results: Total 106 patients comprising 69 males and 37 females having persistent neonatal jaundice were included. Among them, those with a final diagnosis of biliary atresia, normal and hepatitis were 33, 27, 9 in males and 24, 10 and 3 in females respectively. Biliary atresia was distinguishable from normal cases in male infants through bilirubin profile whereas it provided insignificant help in such cases in female infants. With an earlier mean age at presentation as compared to males (69 vs 80 days), this underscored the importance of early HIDA scan.

Conclusion: In clinically equivocal cases, early hepatobiliary scintigraphy helped by excluding biliary atresia definitively.

Keywords: Biliary atresia, Hepatobiliary scintigraphy, Neonatal jaundice.

INTRODUCTION

Persistent neonatal cholestasis is a major clinicopathological concern affecting almost[1] in 2500 infants and is an important diagnostic challenge[1]. Clinically, it presents as jaundice, pale stools, dark urine, pruritus, and steatorrhea while biochemically conjugated hyperbilirubinemiais its identifying hallmark[2]. Cholestasis is generallydue to underlying neonatal hepatitis or biliary atresia and atimely diagnosis of these two conditions critically impactsclinical outcomes[3,4]. Biliary atresia (BA) is the most common cause of cholestasis in infants and most frequent indication for paediatricliver transplantation. Theincidence of BA is 0.5 to 3.2 per 10,000 live births, depending on geographyand ethnicity[5,6]. Pathologically, BA is characterized by progressive inflammation and fibrosis ofthe bile ducts, resulting in progressive obliteration of the extra-hepatic and variably intrahepatic bile ducts[5,7,8]. Uncorrected biliary atresia is invariably fatal[9,10]. Because early surgical intervention is critical for positive outcome in biliary atresia, it is imperative to differentiate it from other causes of choles-tatic jaundice[11]. Timely evaluationof persistent neonatal jaundice in resource constrained settings in third world countries provide a unique set of problems such as delayed initial presentation,

Correspondence: Dr Muhammad Inamullah, Armed Forces Institute of Pathology Rawalpindi Pakistan
Email: inamfarsi786@hotmail.com
Received: 20 Mar 2018; revised received: 21 Mar 2018; accepted: 22 Mar 2018

lack of hospital followup, absence of population based screening and non availability of cost effective universal healthcare. The crux is to replace, long treatment plans requiring frequent healthcare visits by a realistic shorter plan of treatment which attempts to readily & effectively "rule out the dangerous diagnosis first!". This essentially means reframing the question. In place of an early identification of the underlying cause of neonatal cholestasis, we posit that an early negative diagnosis of biliary atresia as the target is more easily achievable as borne out by our experience. In this context, hepatobiliary scintigraphy comes across as peculiarly useful and itshigh negative predictive value can help in triaging the patients with persistent neonatal jaundice for diagnosing biliary atresia versus other causes ofneonatal jaundice[12]. The present study was carried out at the Nuclear Medical Centre, AFIP, Rawalpindi from 2009-2017 in order toevaluate the role of Hepatobiliary Scintigraphy& bilirubin profile in early diagnosis of biliary atresia in children with persistent neonatal jaundice & to formulate recommen-dations for resource constrained settings on the basis of review of our 8 year experience.

MATERIAL AND METHODS

It was a cross sectional observational study and also included retrospective evaluation of patient records and was carried out at the nuclear medical center (NMC), AFIP Rawalpindi from July 2009 to June 2017. A total of 106 patients presenting at NMC for hepatobiliary scintigraphy were included in the study by non-random consecutive sampling. The sample size was also calculated by WHO Sample size determination for health studies calculator by utilizing 0.5625 as anticipated population proportion (P) as found in our previous study on the subject. A sample size of 95 yielded an absolute precision of d=0.10 (90%) with a confidence level of 95% (1-α). The neonates and young infantsreferred for Hepatobiliary Scintigraphy from various hospitals of Rawalpindi/Islamabad and upper part of the country were included in the study with a diagnosis ofcholestatic liver disease alongwith conjugated hyperbilirubinemia as defined by the consensus North American & European Guidelines on the subject[12]. The test procedure was followed as per the standardized protocol of Society of Nuclear Medicine Practice Guideline for Hepatobiliary Scintigraphy[13]. The preparation for the test included cessation of breastfeeding for at least 4 hours before the test. A 2 mCi dose of 99mTc-DISIDA/99mTc-BrIDA with was injected intravenously. Anterior abdominal 60-sec images were obtained at 1-min, 5-min, 10-min, 15-min, 30-min, 1-hour, 2-hour, 3-hour till biliary excretion was demon-strated or up to a maximum of 24 h post-injection and where appropriate, additional delayed views were obtained to optimize visualization of radionuclide intestinal transit. The criteria for interpretation used by two independent consul-tants comprised presence or absence of biliary excretion based on visualization of tracer activity in the intestinal tract till 24 hours post injection and evaluation of hepatic uptake by visually comparing liver radioactivity to cardiac-blood-pool radioactivity in the five-minute image[13,14]. Prior clinical diagnosis was confirmed by parent interviews, clinical examination and laboratory investigations where possible and by scrutiny of medical records where appropriate. Clinical and pathologic features were documented on a specially designed form. The study was approved by the ethical review committee at AFIP. Age in days, gender and total and direct bilirubin levels

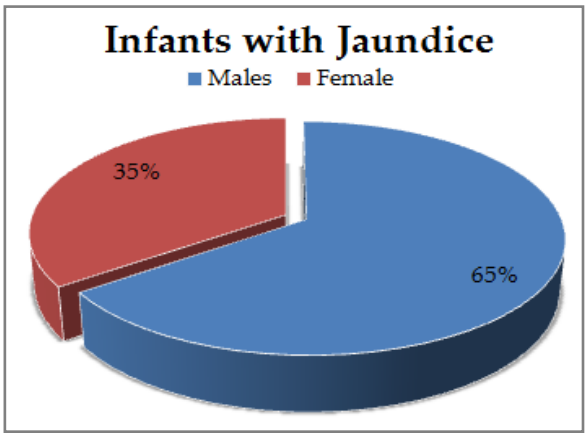

Figure: Infants with Jaundice divided by gender.

were documented for each case at presentation. Subsequently the patients were classified into three diagnostic subgroups on the basis of hepatobiliary scintigraphy including biliary atresia, normal and neonatal hepatitis. Microsoft Excel 2010 was used for data entry and Graph Pad Software tool was utilized for analysis of categoric& numeric variables[15]. Frequency and percentages were calculated for gender as well as three diagnostic subgroups. Mean ± standard deviation (SD) was calculated for total and direct bilirubin levels. Both male and female patient subsets were divided into three diagnostic subgroups and comparison of means of total and direct bilirubin was carried out by paired student's t-test. Chi square was used to test for significance in the prevalence of biliary atresia in malesvs females with neonatal cholestasis as well as between males and females in comparable age related groups.

RESULTS

A total of 106 patients were included in the study having persistent neonatal jaundice. A total of 57 patients were found to have biliary atresia out of 106 (53.7%). Out of 106 patients, 69 were male & 37 were female (n=106, Males=69, Females=37) as shown in figure. Out of 69 Male patients, 33 were found to have biliary atresia (47.8%). Out of 37 Female patients, 24 were found to have biliary atresia (64.8%).

The proportion of biliary atresia in male versus female was compared by chi square fischer's exact test and the difference was found to be insignificant as shown in table-I (Male vs Female 33/69 vs 24/37, p=0.1060). On the basis of

Table-I: Gender based diagnosis of biliary atresia in infants with persistent neonatal jaundice.

	Total Patients (n)	Patients with Biliary Atresia	Percentage of Patients with Biliary Atresia	2 tailed p*
Total	106	57	53.7	Male vs Female 33/69 vs 24/37 p-value = 0.1060
Male	69	33	47.8	
Female	37	24	64.8	

*Chi-Square (Fischer's Exact test)

Table-II: Causes of persistent neonatal jaundice in infants.

	Total	Biliary Atresia (Gp 1)	Normal (Gp 2)	Hepatitis (Without biliary atresia) (Gp 3)
Male	69	33	27	9
Female	37	24	10	3
	106	57	37	12

Table-III: Male patients with primary diagnosis & Total/direct bilirubin levels.

Gps	Diagnostic subgroups	Total with neonatal jaundice	Patients with lab tests	Total Bilirubin (A)	Direct Bilirubin(B)
1	With Biliary atresia	33	22	190.95 ± 64.05	85.77 ± 49.76
2	Normal	27	21	142.49 ± 87.66	49.64 ± 35.22
3	With Hepatitis	9	7	201.71 ± 109.93	83.5 ± 49.03

Table-IV: Comparison of means of Total & Direct Bilirubin levels in male patients in different diagnostic sub groups.

Gps	n of 2 groups	Comparison of gps as per table-III	p-value*
1vs 2	22 vs 21	(Gp 1A vs Gp2A) 190.95 ± 64.05 vs 142.49 + 87.66	0.0312
1 vs 2	22 vs 21	(Gp 1B vs Gp2B) 85.77 ± 49.76 vs 49.64 + 35.22	0.0026
1 vs 3	22 vs 7	(Gp 1A vs Gp 3A) 190.95 ± 64.05 vs 201.71+ 109.93	0.71
1 vs 3	22 vs 7	(Gp 1B vs Gp 3B) 85.77 ± 49.76 vs 83.5 + 49.03	0.45
2 vs 3	21 vs 7	(Gp 2A vs Gp 3A) 142.49 ± 87.66 vs 201.71 + 109.93	0.55
2 vs 3	21 vs 7	(Gp 2B vs Gp 3B) 49.64 ± 35.22 vs 83.5 + 49.03	0.22

*(two tailed p calculated by paired students t-test)

subsequent diagnosis, the patients were divided into three groups; with biliary atresia (Gp-1), normal (Gp-2) and hepatitis without biliary atresia (Gp-3). Subject groups were formed in both male and female patient subsets as given in table-II. In case of male patients, out of 69, 33 had biliary atresia, 27 were normal and 9 had hepatitis whereas in female patients out of 37, 24 had biliary atresia, 10 were normal while only 3 had hepatitis.

Out of a total of 106 patient records included in the study, a total of 73 patients had laboratory investigations carried out including total and direct bilirubin levels at the time of presentation. This comprised 50 males and 23 female patients. On the basis of available lab investigations in various diagnostic sub groups, 3 separate patient groups were formed for males as per table-III. Mean and SD of total Bilirubin (A) as well as direct bilirubin (B) was determined for each diagnostic subgroup for males as shown in table-III. The means of total and direct bilirubin were compared for various diagnostic sub groups in males by applying students t-test and calculating two-tailed P. Results are as shown in table-IV. Out of a total of 106 patient records included in the study, 37 were female out of which lab investigations including total and direct bilirubin were available in 23 cases. On the basis of available lab investigations in various diagnostic sub groups-3 separate patient groups were formed for females as per table-V. Mean and SD of Total Bilirubin (A) as well as direct bilirubin (B) was determined for each diagnostic subgroup for females as shown in table-VI. The means of total and direct bilirubin were compared for various diagnostic sub groups in females by applying students t-test and calculating two-tailed P. Results are as shown in table-VI. Age at presentation provided curious insight (table-VII). In case of males age range was from 30-1000 days with 94 days being the mean age. Removing the single outlier value of 1000 corrected the mean age at presentation to 80.94 days for males. In case of females, age at presentation ranged from 8-700 with a mean of 86.05. Removing the single outlier value of 700 corrected the mean age at presentation to 69 days for females. As we evaluated this difference in mean age at presentation between males and females, we found it statistically significant as shown in table-VIII. A comparison of prevalence of biliary atresia in male and female infants with persistent jaundice in age related sub groups

Table-V: Female patients with primary diagnosis & Total/direct bilirubin levels.

Gps	Diagnostic subgroups	Total with neonatal jaundice	No of patients with lab tests	Total Bilirubin (A)	Direct Bilirubin(B)	Remarks
1	With Biliary atresia	24	17	169.30 ± 119.97	69.96 ± 56.2	
2	Normal	10	5	144.4 ± 145.53	47 ± 45.86	
3	With Hepatitis	3	1	420	203	(single value)

Table-VI: Comparison of means of Total & Direct Bilirubin levels in female patients in different diagnostic sub groups.

Gps	n of 2 gps	Comparison of gps as per table-III	*p*-value*	Remarks
1vs 2	17 vs 5	(Gp 1A vs Gp2A) 169.30 ± 119.97vs 144.4 ± 145.53	0.5742	
1 vs 2	17 vs 5	(Gp 1B vs Gp2B) 69.96 ± 56.2 vs 47 ± 45.86	0.42	
1 vs 3	17 vs 1	(Gp 1A vs Gp 3A)	-	Comparison of means not possible due to single value of A and B in Gp 3 (table-V)
1 vs 3	17 vs 1	(Gp 1B vs Gp 3B)	-	
2 vs 3	5 vs 1	(Gp 2A vs Gp 3A)	-	
2 vs 3	5 vs 1	(Gp 2B vs Gp 3B)	-	

*(two tailed *p* calculated by paired students t-test)

was carried out. Difference in subject prevalence between two genders was evaluated by chi square (Fisher's exact test). It was observed that an increased number of female infants >30 ≤60 days of age at presentation had underlying biliary atresia as compared to male patients. This difference was found to be statistically significant (16/23 vs 11/33, *p*-value = 0.0137).

DISCUSSION

Population based data on the prevalence of biliary atresiaamong patients with persistent neonatal jaundice in Pakistan is not available. However, an earlier single center multi-disciplinary study by the principal author incorporating a comparison with eleven similar national, regional & international studies had attempted to provide a fair assessment of the diagnostic problem at hand[14].

In the present study, out of all the patients presenting with persistent jaundice, a proportionally higher number of males as compared to females were normal on subsequent investigations. However, this was not significant. (27/69 vs 10/37, Chi-Square Fischer's Exact-test 2-tailed *p*-value=0.2857). Whether this also translates into an increased overall incidence of neonatal jaundice in males is unknown and maybe studied separately. The incidence of biliary atresia in males versus females was compared but the difference was not statistically significant as well (27/69 vs 10/37, Chi-Square Fischer's Exact test 2-tailed *p*-value=0.2857). It appears that in Pakistani infant patients with persistent neonatal jaundice, males & females had an equal predilection to be suffering from biliary atresia as the primary cause.

As per the results, there was significant difference in the means of total bilirubin levels in patients with biliary atresia in males as compared to those with a subsequent diagnosis of being normal (190.95 ± 64.05 vs 142.49 ± 87.66, *p*-value=0.0312). Similarly, significant difference was also observed in the means of direct bilirubin

Table-VII: Age range & mean age at presentation & Comparison of gender specific age related means at presentation.

	Males	Females	Remarks
Mean	80.94	69.00	80.94 ± 54.12 vs 69.00 ± 41.55. *p* value* = 0.0004
SD	54.12	41.55	
n (corrected)	68		
Age range (Days)	30-1000	8 -700	
Mean (Days)	94	86.05	
Corrected mean	80.94	69	

* Two tailed *p*, paired students t-test

Table-VIII: Comparison of prevalence of biliary atresia in male and female infants with persistent jaundice in age related sub groups.

Age Group	Age (in days) [Less than or equal to]	Females		Males		Remarks
		Total	BA	Total	BA	
1	30	3	0	6	3	Nil*
2	60	23	16	33	11	16/23 vs 11/33 *p*-value = 0.0137
3	90	5	4	15	9	4/5 vs 9/15 *p*-value=0.61
4	120	3	3	5	4	Nil*
5	150	1	1	5	3	
6	180	0	0	2	1	
7	>180	2	0	3	2	
		37	24	69	33	

* Chi square could not be applied due to zero values in at least one cell of 2x2 contingency table.

levels in patients with biliary atresia in males as compared to those with a subsequent diagnosis of being normal (85.77 ± 49.76 vs 49.64 ± 35.22, *p*-value=0.0026). Consequently higher mean & total total bilirubin levels at presentation served as a good surrogate marker for underlying biliary atresia in male infants presenting with persistent neonatal jaundice.

Comparison of means of Total and Direct Bilirubin levels in female patients in different diagnostic sub groups reveals that there is no difference among the patients with or without biliary atresia and those with hepatitis. While this is surprising, there are two possible explanations. It might be an artifact of low sample size, 23 in this case and the differences may pan out over a bigger sample size. Secondly, it maybe due to an earlier age of female patients at presentation in our case as shown in table-VII & VIII. It is possible that with time, biochemical derangements might become more specifically indicative of the diagnostic sub groups in female patients as well. The female patients presented 11 days earlier than male patients on average as shown in table-VIII and the diagnostic subgroups were not identifiable on the basis of differences in mean levels of total and direct bilirubin. This presents a diagnostic red flag which is relevant to our healthcare settings. In male infants presenting with persistent jaundice, the diagnostic sub groups (biliary atresia, normal, hepatitis) are identifiable on the basis of statistically significant difference of means for all 3 groups. However, in female infants this difference is not significant. Theoretically, this implies that a biochemical picture of normal or hepatitis in female patients may mask an underlying diagnosis of biliary atresia and clinical suspicion MUST NOT BE reduced in view of unsuggestive biochemical profile including total and direct bilirubin.

CONCLUSION

Delayed diagnosis of biliary atresia significantly increases mortality associated outcome due to late surgical intervention & bilirubin profile provides insignificant help in diagnostic sub groups in female infants to allow a timely diagnosis of bilary atresia. In clinically equivocal cases, early hepatobiliaryscintigraphy may help by excluding biliary atresia.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Poddar U, Thapa BR, Das A, Bhattacharya A, Rao KL, Singh K (2009) Neonatal cholestasis: Differentiation of biliary atresia from neonatal hepatitis in a developing country. Acta Paediatr 98: 1260-64.
2. Fischler B, Lamireau T. Cholestasis in the newborn and infant. Clin Res Hepatol Gastroenterol 2014; 8(3): 263-7.
3. Kwatra N, Shalaby-Rana E, Narayanan S, Mohan P, Ghelani S, Majd M. Phenobarbital-enhanced hepatobiliaryscintigraphy in the diagnosis of biliary atresia: Two decades of experience at a tertiary center. Pediatr Radiol 2013; 43(10): 1365-75.
4. Dolgin SE. Answered and unanswered controversies in the surgical management of extra hepatic biliary atresia. Pediatr Transplant 2004; 8: 628-31.
5. Lane E, Murray KF. Neonatal Cholestasis. Pediatr Clin North Am 2017; 64(3): 621-39.
6. McKiernan PJ, Baker AJ, Kelly DA. The frequency and outcome of biliary atresiain the UK and Ireland. Lancet 2000; 355(9197): 25-9.
7. Garcia-Barcelo MM, Yeung MY, Miao XP, Tang CS, Cheng G, So MT, et al. Genome-wide association study identifies a susceptibility locus for biliary atresia on 10q24.2. Hum Mol Genet 2010; 19(14): 2917-25.
8. Mack CL. The pathogenesis of biliary atresia: evidence for a virus-induced autoimmune disease. Semin Liver Dis 2007; 27(3): 233-42.
9. Feldman AG, Mack CL. Biliary Atresia: Clinical Lessons Learned. J Pediatr Gastroenterol Nutr 2015; 61(2): 167-75.
10. Makin E, Davenport M Biliary atresia. Curr Paediatr 2006; 16: 59–63.
11. Nio M, Wada M, Sasaki H, Tanaka H. Effects of age at Kasai portoenterostomy on the surgical outcome: A review of the literature. Surg Today 2015; 45(7): 813-18.
12. Fawaz, R, Baumann U, Ekong U, Fischler B, Hadzic N, Mack CL, et al. Guideline for the Evaluation of Cholestatic Jaundice in Infants: Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). J Pediatr Gastroenterol Nutr 2017; 64: 154-68.
13. Tulchinsky M, Ciak BW, Delbeke D, Hilson A, Holes-Lewis KA, Stabin MG, et al. SNM practice guidelines for hepatobiliary-scintigraphy. J Nucl Med Tech 2010; 38(4): 210-18.
14. Inamullah M, Ali MK, Mirza TM, Riaz MO. 99mTc-labelled hepatoiminodiacetic acid(HIDA) scintigraphic evaluation of prevalence of biliary atresia in children with persistent neonatal jaundice: A comparison with national, regional & international studies. Pak J Nucl Med 2014; 4: 40-5.
15. 15.Graph Pad Software, Inc. T test Calculator [Internet] La Jolla, CA: 2014.