Vol. 4 No. 3 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Stool Color Card: A Screening Tool to Predict Biliary Atresia - Experience at a Teaching Hospital in Pakistan

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Keywords: Stool Color Card, Cholestasis, Acholic Stools, Biliary Atresia.

Received on 10 Apr 2025
Accepted on 30 Jun 2025
Published on 14 July 2025

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Abstract

Objective: To determine the diagnostic accuracy of Stool Color Card (SCC), as a screening tool in predicting Biliary Atresia (BA), in cholestatic infants up to 3 months (90 days) of age.

Study Design: Prospective cohort study

Place and Duration of Study: Department of Pediatric Gastroenterology and Hepatology, University of Child Health Sciences, Lahore; 10th March 2024 to 28th August 2024.

Materials & Methods:
Infants up to 3 months of age, presenting with cholestatic jaundice, were enrolled. Mothers of these infants, were asked to fill the stool color card, by matching the stool color of their baby. These infants were divided into group A (abnormal stool color) and group B (normal stool color). Both groups were followed up, for a final diagnosis of Biliary Atresia. Infants who screened as true positive and true negative by the stool color card were noted; sensitivity, specificity of the stool color card was calculated.

Results: Out of total 56 patients enrolled, 33 were male and 23 were female. 46/56 infants had abnormal (acholic) stool color - group A. Whereas, 10/56 babies had a

normal (pigmented) stool color - group B. It was found that 22/56 babies in group A were diagnosed as Biliary Atresia. All 10 patients in group B, did not have biliary atresia. Sensitivity of SCC was 47.8%, Specificity was 100%, PPV was 100% and NPV was 29.4% according to our study.
Conclusion: Stool color remains the most practical means of screening for biliary atresia in terms of its ease to use and its economic value. It requires little expertise for the screening purpose, while having a high specificity.

Introduction

Biliary Atresia (BA) is one of the major causes of end-stage liver disease in infants, and the most common indication of pediatric liver transplant.[1] It presents with cholestatic jaundice and leads to 100% mortality by three years of age, without any surgical intervention. Kasai Hepato-Porto-Enterostomy (HPE) is the first-line treatment option for biliary atresia. A delay in Kasai procedure leads to early cirrhosis; requiring early liver transplant as the definitive treatment.[2] HPE has a better outcome if performed before 46 days of life, however few studies also document a good 5-year survival rate of HPE, if performed before 90 days of life. Liver transplant is ultimately needed in BA patients; however, HPE delays its need for a few years.

Early intervention is of prognostic significance and there is a narrow treatment window. Effective treatment of BA requires early screening of the condition. Late diagnosis and late referral of BA is a universal problem. Average age of diagnosis of BA in Canada is 55 days (3), in US and Germany it is 60 days, in China it is 75 days.[3] According to a study, native 4-year liver survival is 50%, if HPE performed at <30 days of life, whereas it is 36%, if performed between 31-90 days of life.[3] If Kasai is performed within the first 60 days of life, approximately 70% of patients will establish bile flow, if it is performed beyond 90 days of life, only <25% of patients will have effective bile flow.[4] In cholestatic infants, the major diagnostic challenge is to distinguish BA from other diseases like genetic cholestatic syndromes, metabolic diseases, infections and syndromic causes. Timing for the presentation of all these diseases overlaps with physiological jaundice as well. BA is the most important thing to be ruled out as it is a surgical emergency.[5]

Many screening tools have been used worldwide for early detection of the condition; including GGT levels, serum bile acid levels, Stool Color Cards, Ultrasonographic findings including abnormal gall bladder, triangular cord sign, Hepatobiliary scintigraphy, etc. Each parameter has been found to be of significant utility in diagnosis of the condition.[6] However, stool color remains the most practical means of screening for biliary atresia in terms of its ease to use and its economic value. It requires little expertise and little to no cost for the screening purpose.[7,8] Tseng et al demonstrated that the introduction of SCC screening resulted in a decline in the median age at first presentation of patients with suspected BA; and the percentage of delayed referral decreased from 9.5% to 4.9%. The median age of performing HPE decreased from 51 to 48 days; and rate of HPE increased from 68.9% to 73.6% within the first 60 days of life.[9]

The rationale for this study was to determine the diagnostic accuracy of Stool Color Cards as a screening tool in early detection of Biliary Atresia in infants; which is required for an early referral for the HPE, so that their long-term survival can be improved. It is important to conduct this study in a developing country like ours, with a low literacy rate and very few resources, because its use does not require a high level of education or expertise.

Objective:
The objective of this study was to determine the diagnostic accuracy of Stool Color Card (SCC), as a screening tool in predicting Biliary Atresia (BA), in cholestatic infants up to 3 months (90 days) of age.

Materials & Methods:
This Prospective Cohort Study was conducted at the Department of Pediatric Gastroenterology & Hepatology, University of Child Health Sciences, Lahore. It was conducted from 10th March 2024 to 28th August 2024. After taking approval from the Institutional Review Board; and taking the informed consent from parents, infants presenting with cholestatic jaundice to the inpatient, as well as outpatient department, were enrolled in the study. A total of 56 patients fulfilling the inclusion and exclusion criteria were enrolled. Inclusion criteria included all infants less than 3 months of age, belonging to both genders, presenting with cholestatic jaundice. We defined cholestatic jaundice as prolonged jaundice, with pale stools and/or dark colored urine, with conjugated hyperbilirubinemia >20 % on veinous sample. Exclusion criteria included those infants who had diarrhea, sepsis, chest infection, were critically ill, or admitted in HDU.
Mothers of all infants were given stool color cards and they were asked to match the color of their infant's stool, with the appropriate color on the stool color card for three consecutive days. This color matching process was supervised by a doctor as well. The Stool color card used in our study was originally designed by the Health Promotion Administration of the Republic of Taiwan and subsequently developed by the Perinatal Services BC (British Columbia; located in Vancouver, Canada), which is an agency of the Provincial Health Services Authority.[10] The card contains nine colored photos of stools. The first six are ranked as abnormal stool colors and the last three are ranked as normal stool colors **(Figure 1).**
These infants, were then divided into two groups; A and B. Group A comprised of infants who had abnormal stool color according to the Stool Color Card. Whereas, Group B had infants who had normal stool colors according to the Stool Color Card. Both groups were followed up, for definite diagnosis, whether positive or negative for Biliary Atresia. Hence, the infants who screened as true positive and true negative by the stool color card were detected; sensitivity and specificity of the stool color card was calculated.
Demographic details were noted. Confounding factors like type of feed, number of stools passed per day and any medication being taken by infant including supplements and antibiotics was noted. Positive cases of Biliary Atresia were confirmed by per-operative cholangiogram ± liver biopsy. Final diagnosis of babies, who were not diagnosed as biliary atresia, were also recorded after further workup.

Data Analysis:
Data was analyzed using SPSS version 26. Demographic details were collected including gender, age at presentation, age at confirmation of diagnosis and final diagnosis. Percentage of mothers who were able to correctly match their infant's stool color on the Stool Color Card was recorded. Data related to mother's education status, and family socioeconomic background was recorded. True and false positive cases, as well as true and false negative cases were identified. Diagnostic accuracy of the screening tool (SCC) was calculated in terms of sensitivity, specificity, Positive Predictive Value and Negative Predictive Value. Normality of data was calculated using the Shapiro-Wilk test. Median ± IQR were calculated for the quantitative variables; whereas, frequencies and percentages were calculated for the qualitative

variables. Chi-square and Fischer's exact test were applied for the categorical data; and t-test applied for continuous variables. For all statistical tests, p value of less than 0.05 was considered as statistically significant.

Results:

Out of 56 patients, 33 patients were male and 23 patients were female. 41 patients belonged to lower socioeconomic status; whereas, 15 patients belonged to middle socioeconomic group. The maternal educational status was noted, as described in

Table-I, along with other demographic details.

It was found that 46/56 infants had abnormal (acholic) stool color, as indicated by the stool color card, and they were assigned to group A. Whereas, 10/56 babies had a normal (pigmented) stool color according to the stool color card; these babies were assigned group B. These patients were then followed for their final diagnosis **(Figure 2).** It was found that 22/46 (47.8%) babies in group A with acholic stools were finally diagnosed as Biliary Atresia (as confirmed by per-operative cholangiogram and liver biopsy). So, the sensitivity of the stool color card in predicting Biliary Atresia in infants presenting with cholestatic jaundice was 47.8%. In group B, patients with pigmented stools, all 10 patients were followed for a final diagnosis. All 10 patients did not have biliary atresia. 4 of them had PFIC, 1 patient had hemolytic anemia and 1 patient was diagnosed as neonatal hepatitis. So, the specificity of Stool Color Card in predicting Biliary Atresia was found to be 100% according our study data. PPV and NPV were calculated to be 100% and 29.4%, respectively. **(Table II).**

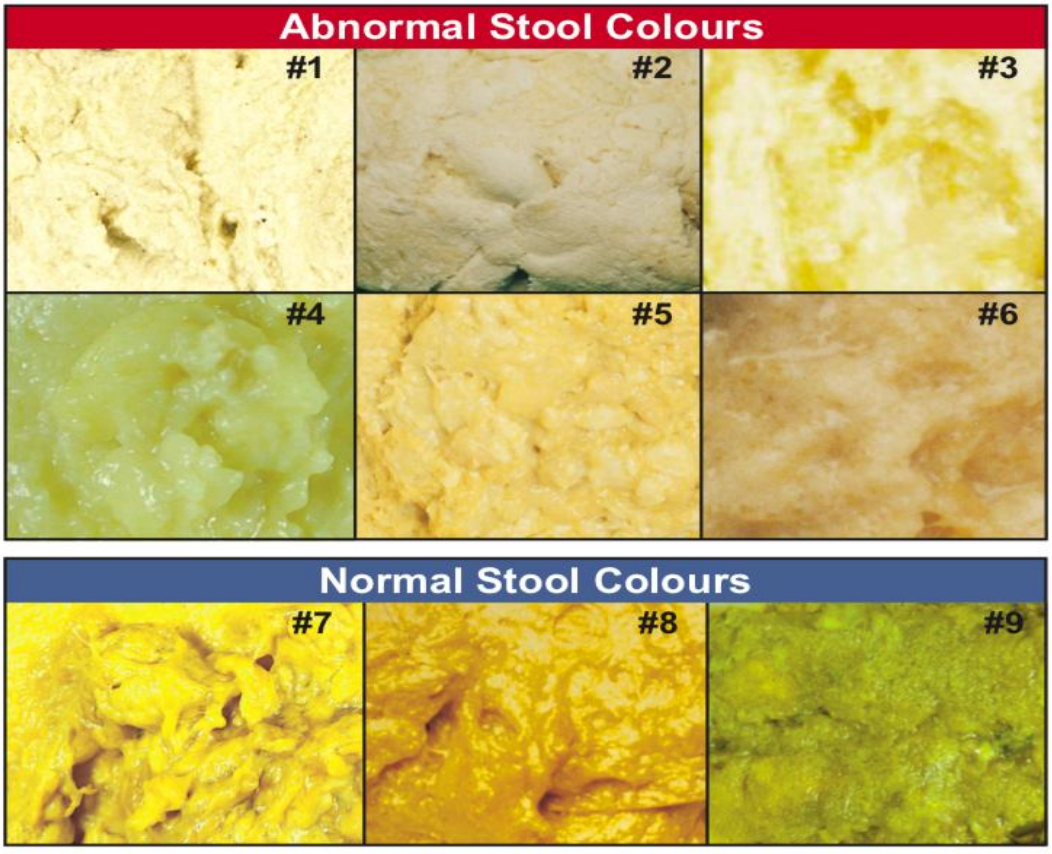

Figure 1: Stool Color Card- showing 6 abnormal stool colors and 3 normal stool colors (10).

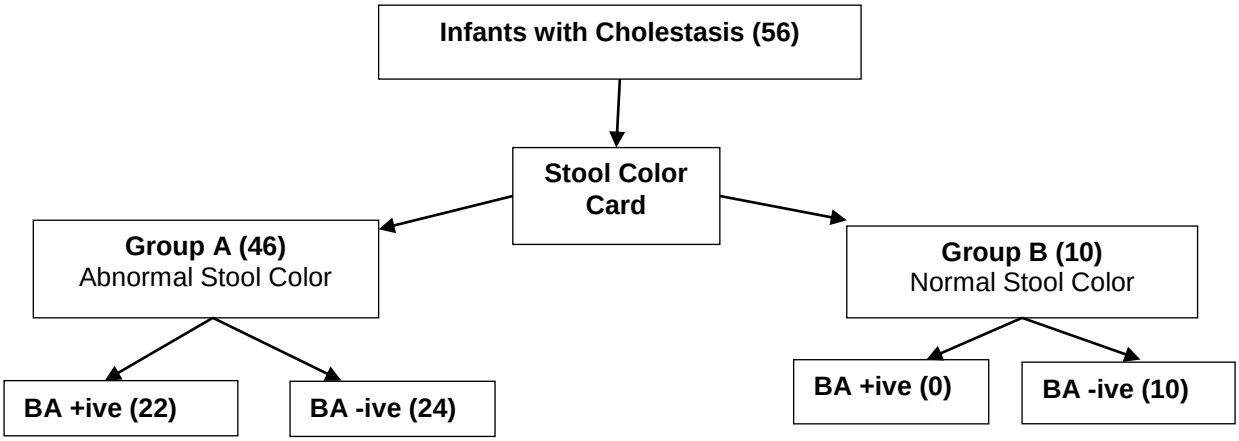

Figure 2: Flow Chart showing patient enrolment

Table I: Demographic details (n=56)

	Group A	**Group B**	**p-value**
Gender			
Male	24 (52.2%)	9 (90%)	0.036
Female	22 (47.8%)	1 (10%)	
Socioeconomic Status			
Middle class	13 (28.3%)	2 (20%)	0.713
Lower Class	33 (71.7%)	8 (80%)	
Maternal Literacy Rate			
Uneducated	9 (19.6%)	3 (30%)	0.634
Primary Education	8 (17.4%)	2 (20%)	
Secondary Education	14 (30.4%)	4 (40%)	
Inter	7 (15.2%)	0 (0%)	
Graduation	5 (10.9%)	0 (0%)	
Higher Education	3 (6.5%)	1 (10%)	

Table-II: Diagnostic accuracy of Stool Color Card (SCC) as a screening tool for Biliary Atresia (n=56)

		Confirmed cases of BA*		**Total**
		Positive	**Negative**	
Stool color according to SCC	**Acholic**	22 (47.8 %)	24 (52.2 %)	46 (100%)
	Pigmented	0 (0%)	10 (100%)	10 (100%)

*By taking cholangiogram as a gold standard

** **Sensitivity: 47.8%, Specificity: 100%, PPV: 100%, NPV: 29.4%, Prevalence: 39.3%**

Table-III: Confounding Factors (n=56)

	Group A N= 46 (Acholic Stools)	**Group B N= 10 (Pigmented Stools)**	**p-Value**
Type of Feed			
Breast Feed	19 (41.3 %)	5 (50 %)	0.608*
Formula Feed	13 (28.3 %)	4 (40 %)	
Cow's Milk	1 (2.2 %)	0 (0%)	
Combination	13 (28.3 %)	1 (10 %)	
Supplements Taken			
No Supplements	43 (93.5%)	10 (100%)	1.000*
Iron Supplements	3 (6.5%)	0 (0%)	
No. of Stools/Day			
1-2	11 (23.9%)	2 (20%)	0.792*
3-4	24 (52%)	7 (70%)	
5-6	8 (17.4%)	1 (10%)	
7-9	3 (6.5%)	0 (0%)	

*By applying Pearson's chi-square and Fischer's exact test, where applicable, p-Values are insignificant, which means that the confounding factors did not have any effect on the stool color

In the study, record was also made of several confounding factors, to see their effect on the stool color and whether they could alter our results. These confounding factors included type of feed the baby was taking, number of stools passed per day and any iron supplements taken (**Table III).**

Table IV: Analysis for the quantitative and qualitative variables (n=56)

Analysis of quantitative variables			
	Group A N = 46	**Group B N = 10**	**p-Value**
Age at presentation – Age^{p} (in days) Median (IQR)	25 (7 – 45)	20 (5 – 45)	0.619
Age at diagnosis – Age^{d} (in days) Median (IQR)	75 (60-90)	75 (60 – 90)	0.407
Δ Age = Age^{d} – Age^{p} (in days) Median (IQR)	45 (25 - 65)	40 (30 - 73)	0.676
GGT** (IU/L) Median (IQR)	477 (141 – 679)	90 (48- 160)	0.002*

*****p-value = significant

**This is different from mean ± SD GGT value in infants with BA vs non-BA.

Discussion:

Stool color card was first introduced in Japan in 1994 for screening and early detection of biliary atresia, so that infants could timely be referred for hepatic portoenterostomy (HPE), thus decreasing the burden on liver centers, related to hepatic failure and liver transplant. Persistent acholic stool is a major and the earliest sign of BA. In few developed countries, the Stool Color Cards are distributed among the mothers of newborn babies, along with their birth/vaccination certificate, and mothers are required to fill it and submit at their 1-month baby visit to the hospital. The infants with abnormal stool color are picked up and directly referred to a specialist center, where all other investigations are completed for the confirmation of BA.

Uniformity of this screening system, which targets the families of newborns is cost-effective as well as time-saving. The SCC increases awareness of the masses and has a high diagnostic accuracy. A cohort study in Japan found its sensitivity to be 76.5% and specificity of 99.9%.[11]

While another study from Bangladesh showed SCC to have sensitivity of 83 % and specificity of 78.9%.[5] In our study population, sensitivity of SCC was 47.8%, specificity was 100%, PPV was 100% and NPV was 29.4 %. The reason for a low sensitivity level may be due to a small sample size. However, a high specificity of the test is very helpful to rule in the diagnosis of biliary atresia. However, our study population mostly belonged to lower socio-economic group, with a lower maternal literacy rate. Still all the mothers, except one of them, correctly matched the stool color of their baby with the SCC.

Another point worth-noting in our study is that median ages of presentation to healthcare facility and median ages of diagnosis are similar in both the groups (acholic vs pigmented), with a p-value= 0.619 **(Table IV).** The reason for this is self-explanatory. These infants were brought to the hospital on clinical detection of jaundice, instead of on noting their stool colors. It is due to unawareness among general population, about the abnormal stool colors in infants and its significance. We are of the view that if awareness is created in general population and screening method is implemented on national level, these median ages of presentation and diagnosis can further be reduced for the acholic group.

This concept of stool color card may seem familiar to the pediatricians, especially pediatric gastroenterologists, but according to literature, this concept was new to many referring pediatricians/ general practitioners. According to a Turkish study, the knowledge regarding SCC was tested in a total of 724 medical students and 88 primary health care physicians. They were shown the same color card used in our study, containing 9 stool colors; 6 acholic and 3 pigmented. Out of them, 24% students and 11.4% of PHCP answered correctly to all of the stool colors. The rate of correct answers to acholic stool colors were approximately 43.9%-87.6% and 23.9%-86.4% for students and PHCP, respectively. Whitish acholic stool colors were better recognized than mild yellowish pale stool colors.[4] In another study conducted on 254 pediatric residents, approximately 54.7% of the residents did not know about SCC. 71% of the participants properly identified the first three normal photographs of stool color out of the six photos in the SSC example. Nevertheless, only 13% of the participants correctly identified the final three abnormal images. The majority of residents (84.6%) had the knowledge of when to refer the patients to gastroenterologists.[12]

Stool color cards are standardized for every population and different countries have tried to establish their own stool color cards, in pursuit of developing a referral system for infants with BA. An example is the Indian Integrated Stool Color Card, which incorporated the urine and stool colors of infants with cholestasis. This Card was established by a standardized and validated methodology.[13] It is easy to match one's

own baby's stool color from a pictorial array, and parents need not even be educated. In our study, we collected data from our patients by providing them the Indian SCC, in addition to the Canadian SCC, keeping in mind the geographical and ethnic similarities our infants with the Indian population. It was observed, that three infants whose stools were acholic according to the Canadian SCC were labelled as pigmented as per the Indian SCC, and vice versa.

The Stool Color Cards were adapted by other developed countries as well, including Taiwan, China, United States and Canada.[14] Stool Color Card Screening method is now a part of the Newborn Screening Programs in many developed countries like Switzerland, Taiwan, Japan and Canada.[12] Studies are also being conducted in low-resource countries to determine the effectiveness of Stool Color Cards.[5,15] As the technology has made progress, various mobile phone software apps have been launched for the early detection of abnormal stool color in developed countries.[16-18]

The idea of early screening and detection of BA is really helpful to decrease the morbidity and mortality of patients with biliary atresia.[19] A few studies have used SCC in combination with measurement of direct bilirubin level in order to get better results with higher sensitivity and specificity.[20] However, stool color card is more effective in early sensitization of the mothers, as it does not involve invasive testing or require a high literacy rate; and is also cost-effective overall.

Conclusion:

This pilot study has demonstrated that screening with SCC can help to predict BA at an early stage. It can sensitize mothers even before the jaundice is visible clinically. We recommend that awareness must be created about the importance of SCC and its implementation should be done at state level.

Acknowledgments:

The authors acknowledge the contributions of parents, of all the patients, who consented to be enrolled in the study for research purpose.

Conflict of Interest:

The authors declared no conflict of interest.

Funding:

None

References

Lendahl U, Lui VCH, Chung PHY, Tam PKH. Biliary Atresia – emerging diagnostic and therapy opportunities. EBioMedicine. 2021 Dec 1; 74: 103689. DOI: 10.1016/j.ebiom.2021.103689

Obatake M. Screening and Stool Color Card System of Biliary Atresia. Introduction to Biliary Atresia [Internet]. 2021 [cited 2023 Feb 12];81–6. DOI: 10.1542/peds.2010-3495

Schreiber RA, Barker CC, Roberts EA, Martin SR, Alvarez F, Smith L, et al. Biliary Atresia: The Canadian Experience. J Pediatr. 2007 Dec 1;151(6):659-665.e1. DOI: 10.1016/j.jpeds.2007.05.051

Gürcan Kaya N, Sarı S, Dalgıç B. Are Medical Students and Primary Health-care Professionals Aware of Neonatal Cholestasis and Acholic Stool. Turk Arch Pediatr. 2024 May 1;59(3):283–8. DOI: 10.5152/TurkArchPediatr.2024.23316

Alam R, Nahid KL, Faruk MO, Rasna EH, Rukunuzzaman M. Use of stool color card as screening tool for biliary atresia inresource-constraint country.

Gastroenterol Hepatol Bed Bench 2024;17(2):146-150. DOI: 10.22037/ghfbb.v17i2.2931

Liu X, Peng X, Huang Y, Shu C, Liu P, Xie W, Dang S. Design and validation of a noninvasive diagnostic criteria for biliary atresia in infants based on the STROBE compliant. Medicine (Baltimore). 2019 Feb;98(6):e13837. DOI: 10.1097/MD.0000000000013837

Borgeat M, Korff S, Wildhaber BE. Newborn biliary atresia screening with the stool colour card: A questionnaire survey of parents. BMJ Paediatr Open. 2018 Jan 1;2(1). DOI: 10.1136/bmjpo-2018-000269

Cai H, Chen H, Gao Y, Huang Q, Lv C, Cang X, et al. A preliminary study of further attempt at the development, testing and application of an independent primary screening stool card. Sci Rep. 2022 Dec 1;12(1). DOI: 10.1038/s41598-022-26649-2

Tseng JJ, Lai MS, Lin MC, Fu YC. Stool color card screening for biliary atresia. Pediatrics. 2011 Nov;128(5):e1209-15. DOI: 10.1542/peds.2010-3495

BC INFANT STOOL COLOUR CARD © SCREENING PROGRAM FOR BILIARY ATRESIA CHECK YOUR BABY'S STOOL COLOUR EVERY DAY FOR THE FIRST MONTH AFTER BIRTH TO SCREEN FOR BILIARY ATRESIA. [cited 2023 Feb 22]; Available from: www.perinatalservicesbc.ca

Gu YH, Yokoyama K, Mizuta K, Tsuchioka T, Kudo T, Sasaki H, et al. Stool Color Card Screening for Early Detection of Biliary Atresia and Long-Term Native Liver Survival: A 19-Year Cohort Study in Japan. J Pediatr. 2015 Apr 1;166(4):897-902.e1. DOI: 10.1016/j.jpeds.2014.12.063

Shaibo, R. S., Mohammed, M. A., & Hamad, H. A. Pediatricians perception of stool color cards as a screening tool for biliary atresia: a cross-sectional survey in Sudan. Alexandria Journal of Medicine. 2024; 60(1): 60–64. https://doi.org/10.1080/20905068.2024.2320038

Yachha SK, Das MC, Kumar P, Sharma L, Singh SK, Sen Sarma M, Kumar A, Srivastava A, Poddar U. Development of integrated neonatal cholestasis card for early recognition and referral of neonatal cholestasis. Indian J Gastroenterol. 2020 Dec;39(6):584-590. DOI: 10.1007/s12664-020-01094-z

Schreiber RA, Harpavat S, Hulscher JBF, Wildhaber BE. Biliary Atresia in 2021: Epidemiology, Screening and Public Policy. J Clin Med. 2022 Feb 14;11(4):999. DOI: 10.3390/jcm11040999

El-Shabrawi MH, Baroudy SR, Hassanin FS, Farag AE. A pilot study of the value of a stool color card as a diagnostic tool for extrahepatic biliary atresia at a single tertiary referral center in a low/middle income country. Arab Journal of Gastroenterology. 2021 Mar 1;22(1):61–5. DOI: 10.1016/j.ajg.2020.12.004

Angelico R, Liccardo D, Paoletti M, Pietrobattista A, Basso MS, Mosca A, Safarikia S, Grimaldi C, Saffioti MC, Candusso M, Maggiore G, Spada M. A novel mobile phone application for infant stool color recognition: An easy and effective tool to identify acholic stools in newborns. J Med Screen. 2021 Sep;28(3):230-237. DOI: 10.1177/0969141320974413

Hoshino E, Hayashi K, Suzuki M, Obatake M, Urayama KY, Nakano S, Taura Y, Nio M, Takahashi O. An iPhone application using a novel stool color detection algorithm for biliary atresia screening. Pediatr Surg Int. 2017 Oct;33(10):1115-1121. DOI: 10.1007/s00383-017-4146-8

Franciscovich A, Vaidya D, Doyle J, Bolinger J, Capdevila M, Rice M, Hancock L, Mahr T, Mogul DB. PoopMD, a Mobile Health Application, Accurately Identifies Infant Acholic Stools. PLoS One. 2015 Jul 29;10(7):e0132270. DOI: 10.1371/journal.pone.0132270

Isa HM, Irshad Z. Clinical Characteristics and Outcome of Infants with Biliary Atresia in Bahrain. Oman Med J. 2023 Mar 31;38(2):e485. DOI: 10.5001/omj.2023.64

Hoshino E, Moriwaki K, Morimoto K, Sakai K, Shimohata N, Konomura K, Urayama KY, Suzuki M, Shimozuma K. Cost-Effectiveness Analysis of Universal Screening for Biliary Atresia in Japan. J Pediatr. 2023 Feb;253:101-106.e2. DOI: 10.1016/j.jpeds.2022.09.028