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Investigating the Diagnostic Value of Interleukin 33 Biomarker in the Diagnosis of Biliary Atresia in Infants with Cholestasis Admitted to Akbar Hospital in Mashhad

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Abstract

Background: Neonatal cholestasis is one of the important liver diseases in children; and infants are more prone to it due to the immaturity of the liver. It appears due to several reasons and its treatment is determined based on its cause. If the cause is not diagnosed in time and rapid treatment is not adopted, it will result in irreparable complications. Biliary atresia is the main cause of this disease and the most common symptom of the need for liver transplantation in children, which requires early diagnosis as a prognostic factor of the disease and its immediate differentiation from other causes of cholestasis. Considering the use of new diagnostic biomarkers, the present study was conducted with the aim of investigating the diagnostic value of serum interleukin 33 (IL-33) in infants with cholestasis and its relationship with clinical and laboratory data.

Methods: This research is a cross-sectional study, conducted on infants referred to the gastroenterology clinic of Akbar Hospital in Mashhad during the years 2021 and 2022. According to the entry and exit criteria, cholestatic infants were included in the study in two groups with and without biliary atresia along with the control group (healthy infants). The basic information, clinical manifestations, and laboratory findings of infants were collected through a researcher-made checklist; and recorded in SPSS software version 24. Data description and analyses were done using descriptive indices, statistical tests, regression methods, and rock curve at a significance level of less than 0.05.

Results: The participants included 78 infants with an average age of 2.80 ± 1.42 months, enrolled in three groups (26 cases each). The intervention groups consisted of patients with cholestasis with and without biliary atresia; and there was one group of healthy infants as the control group. The serum level of IL-33 biomarker was significantly higher in patients with biliary atresia than in patients without atresia (p=0.014); and in both groups, it was higher than that in the control group (p=0.001). The serum level of IL-33 had a significant positive correlation with laboratory indicators of AST, ALT and GGT (p<0.05). Interleukin-33 serum level at the cut-off point of 275 pg/ml can diagnose biliary atresia with a sensitivity of 84.6 and a specificity of 92.3%, so that the probability of biliary atresia in infants with IL-33 ≥ 275 is reported to be twice as often higher than that in infants with A serum levels lower than this value (p=0.011).

Conclusion: The serum level of interleukin 33 was significantly higher in patients with biliary atresia than in patients without atresia as well as in healthy infants; and at the determined cut-off point, it had a favorable diagnostic value for identifying infants with biliary atresia.

Key Words: Biliary atresia, Diagnostic value, Interleukin 33 serum level, Neonatal cholestasis.

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1- INTRODUCTION

Cholestasis is defined as a significant reduction in bile secretion and its flow, which may arise from functional deficiencies of liver cells in bile secretion or from obstruction at any level of the biliary pathways (1). It can occur in all age groups, but infants are more susceptible to this condition due to immature liver function (2). Neonatal cholestasis can arise from various causes, and its treatment is directed towards the underlying cause (3). It is primarily due to extrahepatic causes, including anatomical lesions and biliary atresia, or intrahepatic causes, including damage to liver cells (viral, metabolic, and idiopathic) and injury to the biliary ducts (hypoplasia or reduction of intrahepatic bile ducts) (1). Additionally, other factors such as genetic/metabolic causes, infections, and immune-related causes have also been reported (4). In clinical experience, neonatal cholestatic disease, which occurs as a cholestatic liver condition at birth, typically manifests within the first two months of life (5). However, there are studies reporting the diagnosis of cholestasis in infants after two months of age as well (6). Neonatal cholestasis, reported with varying prevalences across different regions of the world, is not highly common; however, its diagnosis is crucial and vital because it is never physiological and often indicates a hepatic biliary or metabolic problem that always requires an immediate evaluation to determine its specific cause (7). Neonatal cholestasis, due to various reasons, usually presents with similar clinical symptoms, including jaundice, acholic stools, and dark urine, hepatosplenomegaly, and growth disturbances (8). The main goal of evaluating neonatal cholestasis is to differentiate between intrahepatic and extrahepatic disorders (9), and the diagnosis involves patient history, clinical findings, physical examinations, laboratory results, imaging studies, and liver biopsy (4). Biliary Atresia (BA), the most common surgical cause in cholestatic infants (10), occurs with a frequency of only one case in every 8,000 to 18,000 live births in the United States (11), one case in every 10,000 to 19,000 live births in Europe and North America (12), and 1.7 to 3.7 per 10,000 live births in East Asia. It is a rare hepatic disease with ambiguous causes, leading to complete obstruction of part or all of the intrahepatic and extrahepatic biliary ducts (13). This condition is the leading cause of neonatal cholestasis and often results in end-stage liver disease within the first two years of life; it is also the most common indication for liver transplantation in children, with 40 to 50 percent of transplanted children having this symptom (7). On the other hand, early treatment of biliary atresia in newborns can delay or even prevent the need for liver transplantation; however, treatment is often delayed as diagnosing biliary atresia in its early stages is challenging (11).

Early diagnosis of biliary atresia is essential as a prognostic factor for the disease and for its immediate differentiation from other causes of cholestasis, as early surgical intervention is necessary to prevent progression to failure or serious extrahepatic manifestations (7) and ultimately improves disease outcomes (14, 15).

Among the diagnostic methods for cholestasis caused by various reasons, liver biopsy holds significant importance in differentiating between internal and external causes; studies have shown it to be preferred over traditional methods such as biliary imaging, duodenal intubation, ultrasound, and cholangiography (8), with approximately 90 percent accurate diagnoses reported (15).

Studies have been conducted and results are reported regarding the use of new biomarkers for the diagnosis or monitoring of infants with cholestasis. One finding from a study indicated that elevated serum levels of interleukin 33 (IL-33) in infants with cholestasis due to biliary atresia were associated with disease progression compared to patients without atresia (5).

The IL-33 biomarker is a recently identified cytokine from the IL-1 family, which, according to immunohistochemical studies, is expressed in various tissues including the liver, blood vessels, lymph nodes, and platelets (16). Additionally, results from two other studies have shown that in patients with biliary atresia, the expression of mRNA for the inflammatory cytokine interleukin 33 (IL-33) and its receptor (ST2) was increased in the liver, and serum levels of IL-33 were elevated (17, 18).

Considering the aforementioned points and the necessity for rapid diagnosis and differentiation of biliary atresia from other causes of cholestasis, as well as timely intervention for therapeutic or surgical management in these patients, the present study aims to investigate the diagnostic value of serum interleukin 33 (IL-33) levels in infants with cholestasis and its correlation with clinical and pathological data.

2- MATERIALS AND METHODS

This research is a cross-sectional study conducted on infants visiting the gastroenterology clinic of Akbar Hospital in Mashhad during the years 2021 and 2022. The inclusion criteria were defined as the -range of one to six months, while the exclusion criteria included those infected with hepatotropic viruses and other chronic diseases (such as respiratory allergy, bronchopulmonary dysplasia, and sepsis) with elevated serum levels. After the study protocol was approved with ethical code 1401.38.IR.MUMS.MEDICAL.REC, the eligible infants were enrolled in the study with written informed consent obtained from their parents or legal guardians, and they were divided into three study groups.

2-1. Study Population

The study population consisted of two groups of infants with neonatal cholestasis:

a) Biliary Atresia Group: This group included 26 infants diagnosed with neonatal cholestasis due to biliary atresia, confirmed based on biopsy, ultrasound, and cholangiography findings (absence of contrast material in the intraoperative cholangiogram after ruling out other obstructive causes). These infants underwent Kasai surgery.

b) Non-Biliary Atresia Group: This group comprised 26 infants with neonatal cholestasis without biliary atresia, diagnosed based on conducted examinations.

Additionally, the control group included 26 healthy infants, referred to Akbar Hospital in Mashhad for elective surgeries (hernia repair and circumcision).

2-2. Information Reviewed

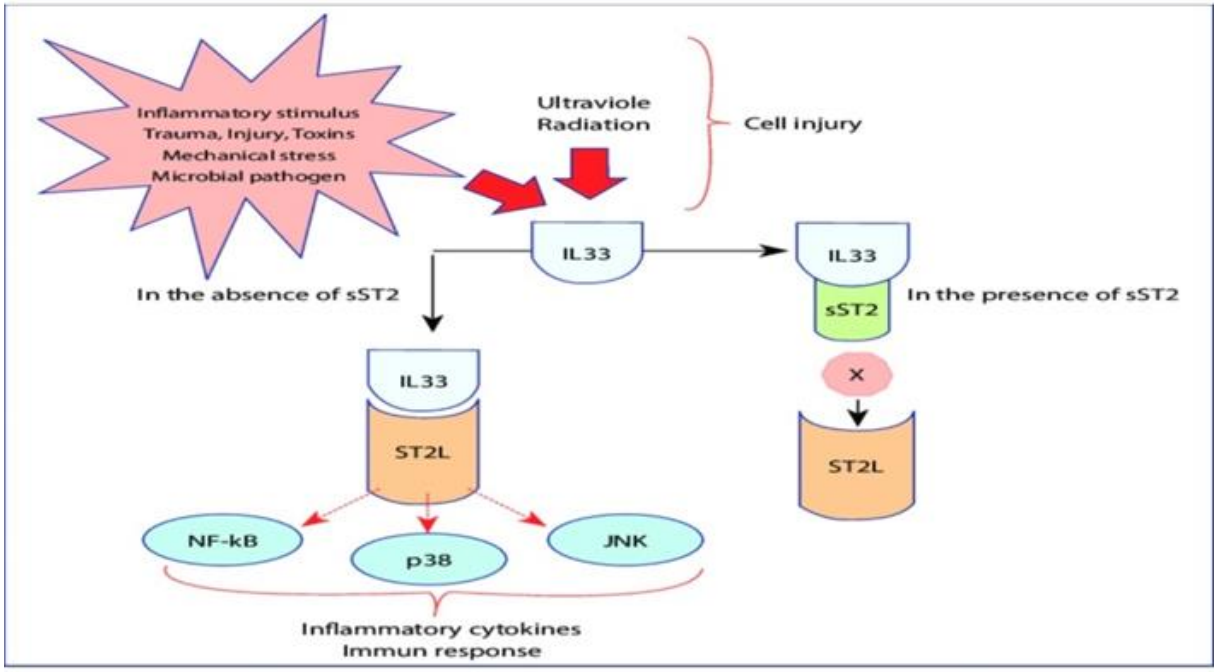
The following information was evaluated for all infants included in the study using a researcher-developed checklist:

a) Basic Information: This included age (in months) and gender of the infant, as well as anthropometric measurements such as weight and head circumference.

b) Clinical Manifestations: These included organomegaly, jaundice, ascites, bleeding, and acholic stools, based on medical history and physical examinations.

c) Laboratory Findings: This included liver function indicators such as PT, GGT, ALT, AST, albumin, as well as serum levels of interleukin-33. These were measured using the ELISA method in coordination with the laboratory and with a

specific kit for both patients and the control group.



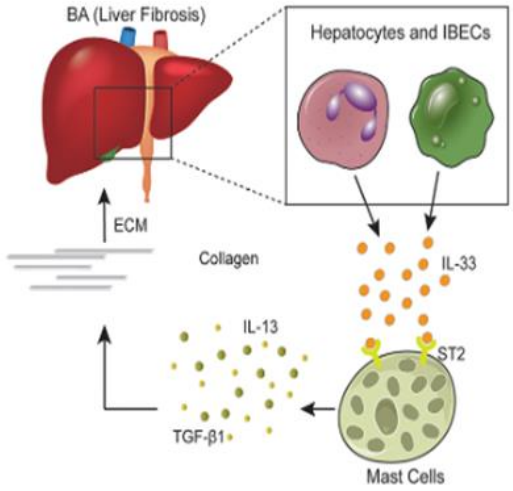


FIGURE 6. IL-33/ST2 signaling axis. IL-33 is secreted mostly by hepatocytes and intrahepatic biliary epithelial cells in the liver. After combining with ST2 receptor expressed on mast cells and other cells, IL-33 induces mast cells to release inflammation and fibrotic cytokines such as IL-13 and TGF-β1. IL-13 and TGF-β1 mediates the production of collagen and extracellular matrix, which could contribute to liver fibrosis in BA patients. BA, biliary atresia; IBECs, intrahepatic biliary epithelial cells; ECM, extracellular matrix.

Fig. 1: Inflammatory stimulus and serum levels of IL-33 elevated (19)

To assess the laboratory findings, approximately 5 cc of blood was collected from all patients via venipuncture under sterile conditions. One cc was used for CBC evaluation, while the remaining blood was centrifuged and stored at -20°C in a refrigerator. After collecting all samples, serum levels of interleukin-33 were evaluated using the ELISA method in coordination with the laboratory and with a specific kit.

The statistical analysis of the collected data was described and analyzed using SPSS software version 24. Quantitative variables were described using mean and standard deviation, while qualitative variables were described using frequency and percentage.

The normality of quantitative variables was assessed using the Kolmogorov-Smirnov test, and due to non-normality, the comparison of quantitative variables in

two and three independent groups was performed using the Mann-Whitney and Kruskal-Wallis non-parametric tests, respectively. The relationships between qualitative-qualitative variables were analyzed using the Chi-square test or Fisher's exact test, and the relationships between quantitative-quantitative variables were analyzed using Spearman's correlation coefficient. The serum levels of the response variable in the three studied groups were described using box plots, and to predict it based on variables with significant relationships at the 0.05 level, multiple linear regression was fitted. The cutoff point for interleukin-33 with the best sensitivity and specificity for diagnosing biliary atresia was determined using the ROC curve. To predict biliary atresia based on serum IL-33 levels at the established cutoff point, logistic regression was used among infants in the study.

2-3. Method of Calculation and Measurement of IL-33

After sampling, the samples and reagents were prepared. 40 microliters of serum sample were combined with 10 microliters of IL-33 antibody, 50 microliters of standard, and 50 microliters of streptavidin-HRP. The resulting solution was incubated for 60 minutes at 37 degrees Celsius for the reaction to occur. The plates were then washed five times with 300 microliters of buffer solution. 100 microliters of chromogenic solution were added and incubated for 10 to 20 minutes at 37 degrees Celsius to allow color development. Finally, 50 microliters of stop solution were added, and the Optical Density (OD) was read over a period of 10 minutes at a wavelength of 450 nanometers for each sample, allowing for the calculation of interleukin-33 levels.

Samples were coded based on the diagnosis, and the tester was unaware of the diagnoses and defined codes. The levels of interleukin-33 in patients and the control group were measured and compared. The collected data were entered into SPSS software version 24 for statistical analyses.

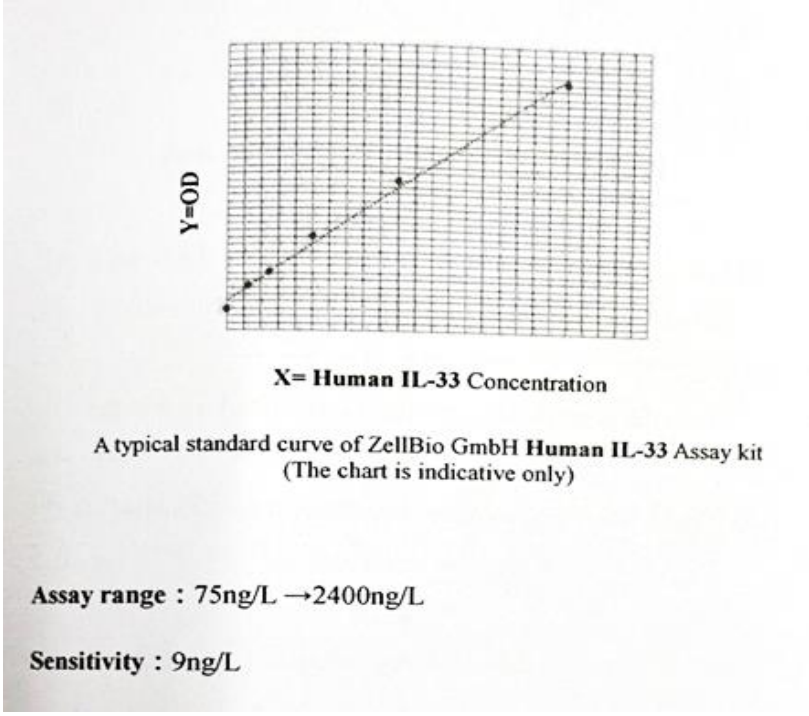


Fig. 2: Standard curve IL33 assay kit (figure from user guide for diagnostic kit of IL33)

3- RESULT

In this study, data related to 78 infants with neonatal cholestasis aged between 1 to 6 months, with a mean age of 2.80 months and a standard deviation of 1.42, were examined. Among the infants, 42 (53.85%) were males and 36 (46.15%) were females. The infants were divided into three groups of 26 each: patients with cholestasis due to biliary atresia, patients with cholestasis from causes other than biliary atresia, and healthy infants (control group). The normality of quantitative variables was assessed using the Kolmogorov-Smirnov test, which indicated a significant result ($p < 0.05$). Baseline data, clinical symptoms, and laboratory findings of the infants were described and compared (Table 1). The results showed that the mean age of healthy infants was significantly higher than that of infants with cholestasis in both the biliary atresia and non-biliary atresia groups ($p = 0.001$), suggesting that neonatal cholestasis occurs at a younger age. Additionally, the mean age of patients without biliary atresia was significantly higher compared to infants with biliary atresia ($p = 0.001$).

The mean weight of infants with cholestasis also showed a significant difference compared to healthy infants, and significant differences were observed in both the biliary atresia and non-biliary atresia groups ($p = 0.001$). The difference in head circumference among the infants across the three groups was not significant ($p = 0.150$), nor was it significant in the two groups of patients with and without biliary atresia ($p = 0.056$).

The results indicated that the frequency of clinical manifestations in healthy infants (the control group) was zero, and except for bleeding, the frequency of other clinical signs showed a significant difference among the three groups ($p < 0.001$). The clinical sign of jaundice was reported in all sick infants, including those with and without biliary atresia. Symptoms of organomegaly and ascites were significantly more prevalent in the cholestasis group without atresia compared to the group with atresia, with p-values of 0.001 and 0.008, respectively. The highest cases of acholic stools were significantly associated with patients having biliary atresia ($p < 0.001$), and bleeding was also more observed in patients without atresia, although the difference between the two patient groups was not significant ($p = 0.158$). According to the findings related to laboratory indices, sick infants with and without biliary atresia were significantly different from the healthy infants in all laboratory indices ($p < 0.05$). The serum level of AST was higher in patients with biliary atresia than in those without, but the difference was not significant ($p = 0.589$). The levels of GGT and albumin were significantly higher in patients with biliary atresia compared to the group without atresia, with p-values of 0.001 and 0.048, respectively.

3-1. Laboratory findings

There was no significant difference between cholestasis with and without atresia regarding the two indices of ALT and PT ($p > 0.05$). The three groups were graphically compared based on serum interleukin-33 levels (Fig. 3). As shown in Figure 3, there was a significant difference among the groups, with significantly higher serum interleukin-33 levels observed in patients with biliary atresia compared to those without ($p2 = 0.014$) and in both groups compared to the control group ($p1 = 0.001$).

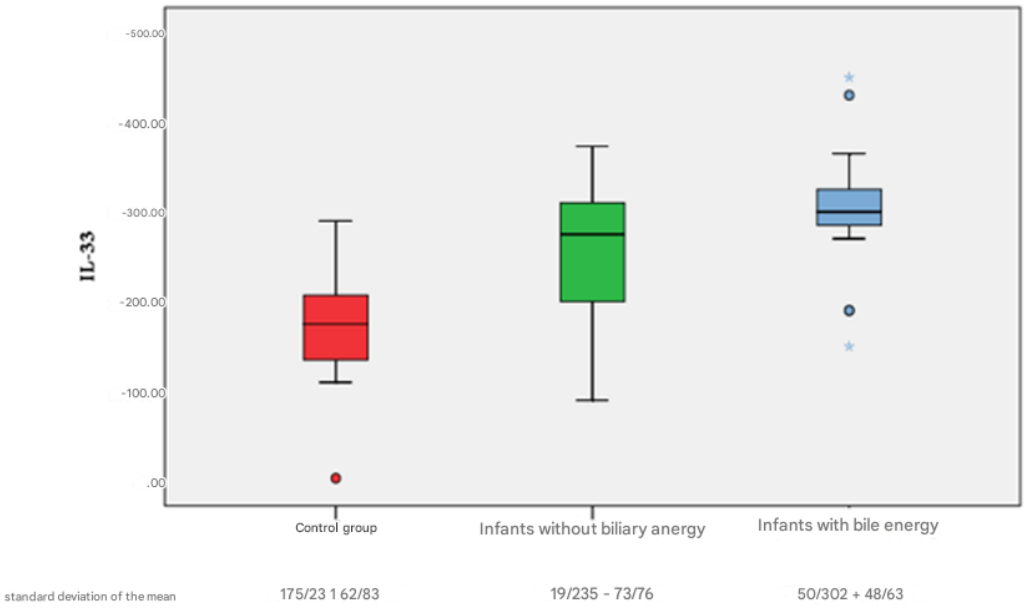
Table-1: Description and comparison of baseline information, clinical symptoms, and laboratory finding by the study groups

Variable			Cholestasis		Control (26=n)	p_1	p_2
			Biliary atresia (26=n)	Non biliary atresia (26=n)			
Information	Age (Month)		1.64 ± 2.15	0.84 ± 2.80	1.39 ± 3.46	0.001 [a]	0.001 [b]
	Gender	Male	16 (61.54)	12 (46.15)	14 (53.85)	0.001 [c]	0.001 [c]
		Female	10 (38.46)	14 (53.85)	12 (46.15)		
	Weight (kg)		0.43 ± 3.46	0.85 ± 4.60	1.39 ± 4.46	0.001 [a]	0.001 [b]
	Head circumference (cm)		0.96 ± 37.84	0.96 ± 38.17	1.56 ± 37.69	0.150 [a]	0.056 [b]
Clinical symptoms	Organomegaly	yes	4 (15.38)	17 (65.38)	-	<0.001 [d]	<0.001 [d]
		no	22 (84.62)	9 (34.62)	26 (100.00)		
	Icter	yes	26 (50.00)	26 (50.00)	-	<0.001 [d]	-
		no	-	-	26 (100.00)		
	Ascites	yes	2 (7.69)	10 (38.46)	-	<0.001 [d]	008/0 [d]
		no	24 (92.31)	16 (61.54)	26 (100.00)		
	Bleeding	yes	1 (3.85)	4 (15.38)	-	0.062 [d]	0.158 [d]
		no	25 (96.15)	22 (84.62)	26 (100.00)		
	Acholic stool	yes	26 (100.00)	13 (50.00)	-	<0.001 [d]	<0.001 [d]
		no	-	13 (50.00)	26 (100.00)		
Lab tests	AST, mg/dl		65.71 ± 176.76	27.55 ± 152.61	3.66 ± 30.07	0.001 [a]	0.589 [b]
	ALT, mg/dl		41.61 ± 146.03	29.39 ± 147.88	30.12 ± 30.19	0.001 [a]	0.990 [b]
	GGT, u/l		61.30 ± 253.26	10.23 ± 65.75	1.65 ± 32.75	0.001 [a]	0.001 [b]
	Albumin g/dl		0.47 ± 3.31	0.33 ± 3.01	0.50 ± 3.50	0.024 [a]	0.048 [b]
	PT, sec		1.07 ± 14.03	1.57 ± 14.80	0.50 ± 11.57	0.001 [a]	0.090 [b]

p_1: Comparing three groups of infants
p_2:With and without biliary atresia; comparing two groups of infants
[a] Kruskal Wallis test
[b] Mann Whitney test
[c] Chi Square test
[d] Fisher Exact test

The mean serum level of interleukin-33 was described and compared across different qualitative variables (infant gender and clinical signs) (Table 2). The results indicate that the serum level of interleukin-33 in male infants was significantly higher than that in female infants ($p = 0.020$). Considering that the clinical manifestation of jaundice was reported in all sick infants, the findings from this table show that the serum level of interleukin-33 in sick infants was significantly higher than that among the patients without this manifestation ($p < 0.001$). Although the levels of this index were consistently higher in infants with other clinical signs, no significant difference was observed ($p < 0.05$).

The relationship between serum levels of interleukin-33 and quantitative variables was described and tested using the Spearman correlation coefficient (Table 3). Based on the findings, the correlation of the variables age, weight, and laboratory indices ALT, AST, and GGT with serum levels of interleukin-33 was found to be significant. Specifically, interleukin-33 showed a negative correlation with age ($p < 0.001$) and weight ($p = 0.030$) of infants, while it exhibited a positive correlation with the indices ALT, AST, and GGT ($p > 0.001$).



P1=0.001, p2=0.014

Fig. 3: Comparing the three groups based on serum interleukin-33 levels

Biopsy results indicated a cholestatic pattern in all patients in the atresia group and steatosis in all patients in the cholestasis group, while patients in the control group did not have biopsy samples. Additionally, ultrasound results demonstrated atresia in all patients in the atresia group, while the other two groups were free of atresia (p = 0.001).

To identify the predictive variables for interleukin-33, variables that had a significant relationship or correlation with serum levels of IL-33 were entered into a multiple linear regression model (Table 4).

Table-2: Comprision of the average serum level of IL33in relation to basic (qualitative) variable and clinical symptom

Variable		Serum level IL-33 standard ±mean	p*
Gender	Male (42 people, 53.85 percent)	264.52 ± 81.28	0.020
	Female(36 people, 46.15 percent)	219.28 ± 82.11	
Organomet organomegali	Yes (21 people, 26.92 percent)	328.75 ± 24.95	0.933
	No (57 people, 73.08 percent)	52.243 ± 87.95	
Icter	Yes (52 people, 66.67 percent)	277.85 ± 72.54	< 0.001
	No(26 people, 33.33 percent)	175.23 ± 61.84	
Ascites	Yes (12 people, 13.79 percent)	260.00 ±77.84	0.434
	No (66 people, 84.62 percent)	240.66 ± 85.58	
Bleeding	Yes (5 people,6.41 percent)	301.40 ± 53.05	0.140
	No (73 people, 59.93 percent)	239.68 ± 84.77	
Acholic stool	No (39 people, 75.00 percent)	-	-
	No (13 people, 25.00 percent)	-	

* Mann Whitney Test

Table-3: Correlation of serum interleukin-33 levels with basic quantitative variables and laboratory findings.

Variable	Serum level IL-33 Correlation index	p
Age (Month)	-0.386	< 0.001
Weight (kg)	-0.246	0.030
Head circumference (cm)	-0.120	0.296
AST (mg/dl)	0.565	< 0.001
ALT (mg/dl)	0.439	< 0.001
GGT (u/l)	0.642	< 0.001
Albumin (g/dl)	-0.004	0.974

Table-4: Multiple regression analysis to predict serum level il33

variable	Regression index β	Standard deviation β	Confidence interval β (Upper limit, lower limit)	Statistics t	p-value
Age	-5.25	7.31	(9.32, -19.82)	-0.718	0.475
Gender	-42.38	17.74	(-12.99, 71.77)	-2.876	0.005
Weight	1.97	279	(20.46, -16.51)	0.213	0.832
Icter	-94.75	49.97	(4.91, -194.41)	-1.896	0.062
AST	0.01	0.20	(0.40, -0.39)	0.028	0.977
ALT	-0.21	0.27	(0.34, -0.75)	-0.762	0.449
GGT	0.21	0.10	(0.40, 0.02)	2.229	0.029

The results showed that with a regression model fit of R^2 equal to 0.474, only two variables—infant gender (p = 0.005) and the clinical index GGT (p = 0.029)—were significant predictors for serum levels of interleukin-33.

Using the ROC curve (Fig. 4), it was determined that serum levels of interleukin-33 at a cutoff point of ≥ 275 picograms per milliliter (pg/ml) had a sensitivity of 84.6%, specificity of 92.3%, positive predictive value of 91.6%, negative predictive value of 85.7%, and an AUC of 0.697, indicating that it could be a predictor for biliary atresia (p = 0.015).

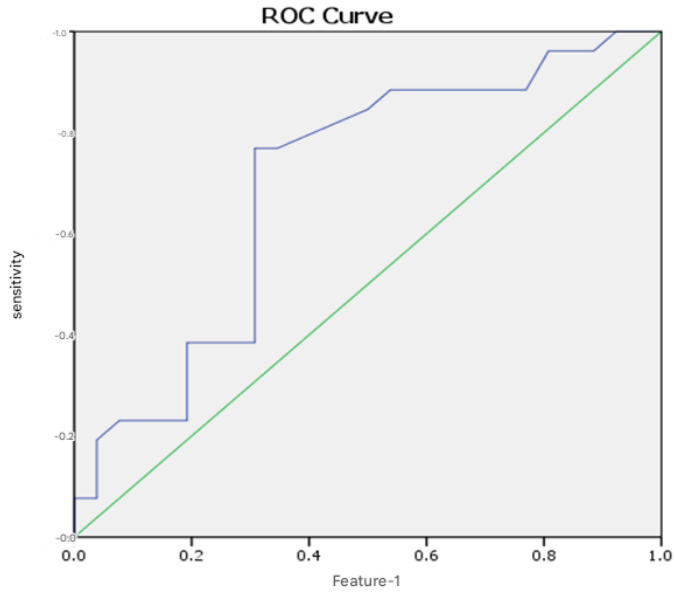


Fig. 4: Rock curve to determine theIL33 cutoff point in the diagnosis of biliary atresia

According to the results of Logistic regression analysis in patients, interleukin-33 at values greater than or equal to 275 pg/ml indicates biliary atresia among infants with cholestasis (p = 0.011). The likelihood of developing biliary atresia in infants with interleukin-33 levels ≥ 275 pg/ml is 5.5 times higher compared to that among infants with serum levels less than 275 pg/ml (Table 5).

Table-5: Regression analysis to predict biliary atresia in cholestatic patients

Variable	Regression index β	Standard deviation β	OR	Confidence iterat OR (Upper limit, lower limit)	p-value
275 ≤ IL-33	1.71	0.67	5.50	(20.46, 1.48	0.011

3-2. Discussion and Conclusion

In this study, aimed at evaluating the diagnostic value of the biomarker interleukin-33 in diagnosing biliary atresia in infants with cholestasis, the results indicated that serum levels of interleukin-33 were significantly higher in patients with biliary atresia compared to those without atresia, as well as in both patient groups compared to healthy infants (controls). Additionally, a cutoff level above 275 pg/ml for serum IL-33 was found to be a significantly acceptable predictor for diagnosing atresia in the patients of this study, with a sensitivity of 84.6% and specificity of 92.3%. It can be claimed that IL-33 levels ≥ 275 pg/ml correctly identified biliary atresia in 84.6% of cases and non-atresia in 92.3% of cases. Similarly, in the study by Behairy et al. (5) conducted in 2020 to assess the clinical value of serum IL-33 levels in 60 cholestatic infants with and without biliary atresia, along with a control group of 30 healthy infants, there was a significant difference in the levels of this biomarker among the groups. In their study, serum IL-33 levels were higher in the 60 infants with biliary atresia compared to those without atresia and healthy infants. Using the ROC curve for diagnosing biliary atresia, they found a cut-off for interleukin-33 at 20.8 pg/ml, with a sensitivity of 96.7%, specificity of 95%, positive predictive value of 90.6%, and negative predictive value of 98.3%. Compared to the current study, their findings demonstrated a greater accuracy in correctly diagnosing infants with atresia as well as those without atresia.

In 2019, Liu et al. (19) examined the correlation of the interleukin ST2/33 receptor with the progression of liver fibrosis in 36 patients with biliary atresia and 8 cholestatic infants (controls). Consistent with the current study, they reported significantly higher gene expression levels of IL-33 in infants with biliary atresia compared to the control group. In a study conducted by Chen et al., in 2018 (20), to investigate the increased serum levels of interleukin 33 and soluble ST2, which are co-expressed in relation to cytomegalovirus infection in infants, the biomarker IL-33 was reported with a cutoff of 2.04 pg/ml, showing a sensitivity of 95% and specificity of 68.7%, indicating a favorable diagnostic value for predicting liver fibrosis in the studied infants. In a meta-analysis in 2021, He et al. reviewed 51 eligible studies to evaluate the diagnostic and prognostic biomarkers in the realms of diagnosing biliary atresia and predicting fibrosis/cirrhosis (21); they reported the sensitivity and specificity of interleukin 33 to be calculated as 77% and 85%, respectively. This indicates a favorable sensitivity and specificity for interleukin 33; however, compared to the current study, the diagnostic power of interleukin 33 for correctly diagnosing atresia and non-atresia was assessed to be lower in this analysis.

In the present study, multiple linear regression analysis was performed to identify the predictors of IL-33, and the results indicated that with a determination coefficient of 0.474, only two variables—infant gender and clinical GGT index—were significant predictors. Behairy et al. (5) also utilized multiple linear regression in their study to determine predictors of IL-33, reporting that with a determination coefficient of 0.464, only the effects of two clinical indices, AST and bilirubin, were significant in predicting interleukin 33.

In the current study, logistic regression was fitted to use the cut-off for IL-33 as a predictor of biliary atresia. The odds of developing biliary atresia in infants with interleukin levels above 275 were estimated to be 5.5 times greater than that in infants with serum levels below 275. Consistent with this finding, Behairy et al. (5) also reported that serum levels of IL-33 at the determined cut-off indicated biliary atresia in cholestatic infants, such that the odds of biliary atresia in patients with IL-33 levels greater than or equal to the cut-off were several times higher compared to those with levels below it.

The findings of this study revealed that serum levels of interleukin 33 had a significant positive correlation with the clinical indices of ALT, AST, and Sun B, and a significant negative correlation with the demographic variables of age and weight of the infant. In line with the approach of this study, GGT et al., in 2021, conducted a review study examining recent advances in laboratory screening, diagnostic, and prognostic methods for biliary atresia. They discussed serum levels of ALT, AST, and GGT alongside some other serum values that are often measured in patients being evaluated for liver diseases. They found that GGT could be assessed alongside AST and ALT levels, which increase due to the loss of integrity of liver cells, as non-specific indicators for biliary atresia and cholestatic liver diseases (22). Similarly, in the study by Behairy et al., (5), serum levels of IL-33 showed a significant positive correlation with both AST and ALT indices, while lacking a significant correlation with GGT, age, and weight of the infant. Additionally, in the article by Dong et al., in 2013, which specifically examined the relationship between IL-33 gene expression and GGT in patients with biliary atresia, it was determined that serum levels of interleukin 33 in these infants correlated with GGT levels, suggesting that GGT levels may provide a new marker for diagnosing biliary atresia (23).

In the study by Sun et al. (24), which examined the levels of interleukin 33 and disease progression in patients with liver disease, no significant correlation was reported between serum levels of IL-33 and the levels of ALT, AST, and GGT in the studied patients. No similar study, investigating the relationship between serum levels of interleukin 33 and clinical manifestations in infants with neonatal cholestasis, was found indicating that the relationship between serum levels of interleukin 33 and liver findings is of greater interest.

In the present study, serum levels of AST and GGT liver indices were higher in patients with atresia compared to those without atresia; and overall, they were also higher than those of the patients in the control group. The difference in AST among the three groups was significant, and the difference in GGT between the two patient groups was also significant. ALT showed no significant difference among the three groups, while albumin exhibited a significant difference both among patients and across the three groups.

Behairy et al. (5) reported significant differences in the levels of ALT, AST, and GGT among three groups of infants, which

aligns with the results of the present study. However, in contrast, the difference in albumin among the three groups was not significant. In the referenced study, higher levels of GGT and albumin were observed in patients with atresia compared to those without atresia, while lower AST levels were found in the atresia group compared to the non-atresia group, which is contrary to the current study's findings. Liu et al. (19), in their study including a group of patients with biliary atresia and a control group of cholestatic patients—similarly found only two indices, GGT and albumin, showing significant differences between the two groups. In their study, ALT, AST, and albumin levels were lower in patients with atresia, which contradicts the current findings. However, the GGT levels in patients with atresia were reported to be higher than those in cholestatic patients, similar to this study.

In this study, the most common clinical manifestation was jaundice (clinical icterus), which was observed in all patients. This was followed, in order, by acholic stools, organomegaly, ascites, and bleeding. The frequency of clinical manifestations such as organomegaly, ascites, and bleeding was higher in patients without biliary atresia, while the higher frequency of the clinical manifestation of acholic stools was found in patients with biliary atresia. The manifestation of jaundice was observed in equal numbers in both groups of patients with and without atresia. Behairy et al. examined these findings in their study, and similarly indicated that jaundice, acholic stools, and ascites had the highest prevalence among all patients. Additionally, in comparison with the current study's results, it was consistent that the clinical manifestation of ascites was more prevalent in patients without atresia, while the non-consistent findings showed that organomegaly and acholic stools were more prevalent in patients with and without biliary atresia, respectively. The clinical sign of bleeding was not reported in the study by Behairy et al. (5)

In this study, the majority of the infants under investigation were male, and the gender distribution of the infants showed a significant difference. The average age of patients without atresia was significantly higher compared to that of infants with atresia, and the healthy infants. This suggests that neonatal cholestasis due to biliary atresia occurs at younger ages. On the other hand, the average weight of patients without atresia was significantly greater than that of healthy infants, and both groups had a higher average weight as compared to the patients with atresia; this indicates that cholestatic newborns due to biliary atresia, typically, have lower average weights. The head circumference index of infants showed no significant difference when comparing the three groups studied, nor when differentiating between patients with and without atresia. Behairy et al. (5) noted in their study that the gender distribution of infants in their study groups did not show a significant difference, which contrasts with findings of the current research. However, similar to this study, the average age of infants with atresia was lower than that of patients without atresia and healthy infants. In the study by Liu et al. (19), unlike the current research, the gender distribution of infants did not show a significant difference. However, regarding the average age, consistent with this research, the average age of cholestatic infants with biliary atresia was reported to be significantly lower than that of the infants without atresia.

4- CONCLUSION

The results of this study indicated that the serum level of biomarker 33-IL is significantly higher in infants with biliary atresia, as compared to cholestatic infants without biliary atresia, and to their healthy counterparts. This suggests that it may

have a potential role in the diagnosis and prognostic assessment of biliary atresia, which could assist in disease management and improve long-term outcomes.

5- LIMITATIONS AND STRENGTHS

The small sample size of cholestatic patients with biliary atresia is a limitation of this study. Additionally, the control group sample size was also small, primarily due to difficulties in collecting liver tissue samples from healthy infants. Considering that no studies have been conducted in this area within the Iranian population and that overall studies are quite limited, focusing on the diagnostic value of the biomarker under investigation as a tool for early diagnosis and timely treatment is a strength of this study. Furthermore, Interleukin-33 as a biomarker is non-invasive, inexpensive, and quick in identifying patients with biliary atresia, making it potentially more effective than other diagnostic biomarkers. Its selection for evaluation in this study is another strength.

6- RECOMMENDATIONS

To achieve more comprehensive and optimal results, conducting a multicenter study with a larger sample size would be beneficial for a more precise assessment of the diagnostic value and determining an accurate cut-off point. It is also recommended to investigate and compare other biomarkers, such as GGT, which according to some studies could serve as a diagnostic biomarker for biliary atresia.

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