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FULL PAPER

CD4+, CD8+, CD4+Th1, and CD4+Th2 counts in biliary atresia

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Biliary atresia (BA) is a disease characterized by a gradual inflammatory process resulting in fibrosis and total obstruction of the bile ducts. This can gradually lead to biliary cirrhosis, portal hypertension, liver failure, and mortality. The pathogenesis is still not fully understood. It is suggested that viral infection of the bile duct epithelium leads to cholangiocyte apoptosis, which is then followed by an immunologic response involving autoreactive T cells. This study compares CD4+, CD8+, CD4+Th1, and CD4+Th2 counts between biliary atresia and non-biliary atresia in neonatal cholestasis infants. A cross-sectional study was performed for six months. Diagnosis of biliary atresia was confirmed through percutaneous liver biopsy, along with the analysis of CD4+, CD8+, CD4+Th1, and CD4+Th2 cell counts using immunohistochemistry. Statistical analysis was performed using an Independent sample t-test, Mann-Whitney U test, and Kruskal-Wallis test. A total of 20 biliary atresias and 14 non-biliary atresias were included in this study. Specific immune cell counts were higher in the group with biliary atresia than non-biliary atresia. This difference was observed in CD4+, CD8+, CD4+ Th1, and CD4+ Th2 cells with specific counts being [16 (14-18) vs. 11.5 (9-12.25)], [10.5 (9-12) vs. 3 (2-4)], [10 (9-12.75) vs. 6 (5-7)], and [11.5 (9.5-14) vs. 2 (1-3)] respectively with $p<0.05$. Patients with biliary atresia showed elevated number of CD4+, CD8+, CD4+ Th1, and CD4+ Th2 cells. Clinical evidence suggests the potential involvement of these specific markers, particularly CD4+ Th2 cells, along with possible autoimmune mechanisms in the pathogenesis of biliary atresia.

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KEYWORDS

Biliary atresia, neonatal cholestasis, CD4+, CD8+, CD4+Th1, CD4+Th2.

Introduction

Biliary atresia (BA) causes biliary cirrhosis, portal hypertension, hepatic failure, and death in the first two years of life due to increasing inflammation, fibrosis, and bile duct obliteration. A liver transplant remains necessary for the majority of patients with biliary atresia, even after completing the Kasai hepatoportoenterostomy procedure [1,2].

Biliary atresia is the major cause of cholestasis in children. The occurrence of biliary atresia in the world can vary significantly, with an estimated rate of 1 case

for every 10-19,000 births [3,4]. The pathogenesis of biliary atresia remains unclear. The hypothesis suggests that viral infection of the epithelial cells in the bile duct leads to the apoptosis of cholangiocytes, which is then followed by an immune response including autoreactive T cells [1,5]. Further research is needed to fully understand the contribution of T lymphocytes, including CD4+, CD8+, CD4+ Th1, and CD4+ Th2 cells, to the pathological process of biliary atresia [6].

The experimental research with animals tries to demonstrate the role of CD4+ Th1, CD4+ Th2, and CD8+ on the occurrence of biliary atresia with varied results. Infant rhesus rotavirus-induced mice (RRV) as a model of biliary atresia showed an increase in CD4 + Th1 and proinflammatory cytokines such as IFN, IL2, and osteopontin on the 7th day, followed by an increase in CD8 + T cells and macrophages on the 14th day. Another study showed that depletion of CD4 + does not prevent biliary atresia whereas depletion of CD8 + prevents biliary atresia. Studies to prove the role of CD4+ Th2 by eliminating Stat -/- mice in animal experiments Th1 phenotype produces cystic biliary atresia [7,8].

Studies on liver biopsies of patients with biliary atresia compared to cholestasis due to other causes found a dominant and varied lymphocyte deposit, consisting of CD4+, CD8+, and CD4+ Th1 proinflammatory cytokines such as IL12, IFN, TNF-α. This indicates the involvement of CD4+ Th1 cells in the pathogenesis of biliary atresia. The presence of CD8+ and NK cells was found to be higher in biliary atresia patients compared to other neonatal cholestasis infants. This study found that CD8+ does not function as CD8+ cytotoxic T cells [8,9]. Accordingly, further research is needed to compare the number of CD4+ Th1, CD4+ Th2, CD8 +, and the ratio of CD4+/CD8 + in neonatal cholestasis infants with biliary atresia and non-biliary atresia to understand the pathogenesis of biliary atresia. This study aims to compare the number of CD4+, CD8+, CD4+ Th1, and CD4+ Th2 between biliary atresia and non-biliary atresia in neonatal cholestasis infants.

Experimental

Study design

This study was conducted for six months, with neonatal cholestasis for 1-12 months as subjects. The study took place in a tertiary health center. Diagnosis of biliary atresia was confirmed through percutaneous liver biopsy and staining with hematoxylin-eosin. Cell counts for CD4+, CD8+, CD4+ Th1, and CD4+ Th2, were analyzed using flow cytometry with CD markers, and the presence of IL-4 and IFN-γ was detected through immunohistochemistry. The statistical analysis was conducted using the Mann-Whitney U Test, Kruskal-Wallis Test, and Independent Sample T-test.

Results and discussion

The study included a total of 34 subjects, with 20 subjects diagnosed with biliary atresia and 14 subjects were non-biliary atresia. The characteristics of these subjects are shown in Table 1. Immunohistochemical examination of CD4+ Th1, CD4+ Th2, CD4 +, and CD8 + was performed on the biliary tract with the following results in Table 2. CD4+ Th1 counts higher (mean 11 ± 3.15, median 10 interquartile (9-12.75) in the group of biliary atresia compared to non-biliary atresia (mean 5.9 ± 1.4, median 6 interquartile 5-7) ($p<0.05$).

TABLE 1 Subject characteristics between the two groups

Variable	Biliary Atresia (N=20)	Non-Biliary Atresia (N=14)	*P*-value
Sex			
Male (%)	6 (30%)	9 (64.3%)	0.048
Female(%)	14 (70%)	5 (35.7%)	
Age (months), median (*IQR*)	8(5.25-9.75)	5.5(3-9)	0.186
Body Weight(kg), mean (±SD)	5.93(±1.19)	4.58(±1.67)	0.009
Height (cm), median (*IQR*)	63.5(58-67.75)	56(50.75-60.25)	0.005
Nutritional Status	3 (15%)	4(28.6%)	
Severely wasted (%)	4 (20%)	2(14.3%)	
Wasted (%)	13(65%)	7(50%)	
Normal			
Overweight	0	1(7.1%)	
Duration of sickness (months)	7(5-9)	5.5(3-9)	0.341
Acholic stool N (%)	15 (75%)	8 (57,1%)	0.458
Dark Urine N (%)	15 (75%)	10 (71,4%)	1.000
Hepatomegaly N (%)	20 (100%)	13 (92,9%)	0.412
Direct Bilirubin (mg/dl), median (*IQR*)	11.09 (8.53-13.17)	9.63(7.73-12.6)	0.227
Indirect Bilirubin (mg/dl), median (*IQR*)	14.49 (10.78-23.60)	11.32(6.72-16.32)	0.054
AST (mg/dl), median (*IQR*)	264 (159.8-357)	204 (126.8-275)	0.142
ALT (mg/dl), median (*IQR*)	185 (111-334.3)	151 (103-232)	0.421
Platelet (x1000 cell/mm^3), mean (±SD)	335(±235)	344(±193)	0.945

TABLE 2 Immunohistochemical examination of CD4+, CD8+, CD4+ Th1, and CD4+ Th2 on biliary tract

	Biliary Atresia median (*IQR*)	Non-Biliary Atresia median (*IQR*)	*P*-value
CD4+	16 (14 – 18)	11.5 (9-12.25)	<0.001
CD8+	10.5 (9 – 12)	3 (2 - 4)	<0.001
CD4+Th1	10 (9 – 12.75)	6 (5 – 7)	<0.001
CD4+Th2	11.5 (9.5 – 14)	2 (1 – 3)	<0.001

p <0.05: Statistically significant

In this study, CD4 + Th1 counts higher in the group of biliary atresia compared to non-biliary atresia. This is consistent with previous research on the analysis of the liver biopsy patients with biliary atresia obtained deposit CD4+ and has been shown to secrete cytokines cellular Th1 including IFN, IL-2, and TNF-α, which only exist in biliary atresia and was not found to the disease other neonatal cholestasis. The mechanistic mice models induced RRV is used to explain the role of cellular immunity in damage to the bile ducts as a model of biliary atresia associated with an increase of CD4+ on the portal tract secrete IFN and TNF-α 1 week after infection RRV, followed by infiltration of CD8+ T cells and macrophages at 2 weeks of age obtained expression of CD4+ at day 7, 14, and 21 after the induction and expression of IFN-γ and IL-2 is higher compared with controls and the highest value of IFN-γ occurred on the 14th day showed the role of CD4 + Th1 on the occurrence of biliary atresia [10].

The study found that there was a notable increase in CD4+ Th2 numbers in the group of subjects with biliary atresia, as compared to those with non-biliary atresia. This is in contrast to previous research which found CD4+ Th1 dominance in immunohistochemical examination of liver tissue taken during surgery Kasai. After analysis by age category obtained at the age of 3 months ≤ obtained CD4+ Th1 dominance following a previous study on liver biopsy tissue during surgery patients with biliary atresia Kasai where his age ≤ 3 months. While in this study age > 3 months obtained CD4 + Th2 dominance. The previous study showed that screening serum biliary atresia patients during surgery in a minority of subjects Kasai 2 of 11 patients had increased expression of marker genes Th2 and Th2 cytokine marker gene which is IL4. The combination of these findings with increased concentrations of IL4 patients with biliary atresia at 3 months and 6 months later shows potential transition dominance of Th1 to Th2 pathway profibrogenic in some patients who already displayed upon diagnosis of liver fibrosis [11].

This study found that CD8+ counts were significantly higher in the biliary atresia group as compared to the non-biliary atresia group. A previous study on liver biopsy of biliary atresia patients aged 12-79 days found increased expression of CD8+ compared to patients with choledochal cysts and fetal liver. Other studies have found an increased expression of CD8+ and IL-2 but have not found an increased expression of CD4+ compared to other patients with cholestasis. Mechanistic studies on mice-induced infant rhesus rotavirus (RRV) followed by depletion of CD4+ and CD8+. The results showed depletion of CD4 + phenotype remains biliary atresia, while depletion of CD8+ did not show any phenotype, and their biliary atresia IFN, itself does not show any phenotype of biliary atresia. This shows that acts as an effector in biliary epithelial cells are CD8+ [12].

The group with non-biliary atresia had significantly higher amounts of CD4+ compared to the group with biliary atresia. This is consistent with the previous study on liver biopsy patients with biliary atresia compared to cholestasis due to other causes found dominant deposit lymphocytes varied consisting of CD4+, CD68+ in patients with biliary atresia compared to neonatal cholestasis infants with other causes. Mechanistic studies on mice-induced infant rhesus rotavirus (RRV) were also associated with an increase in CD4+ 7 days after injection RRV and CD8+ 14 days after injection showed CD4+ obtainment role in the pathogenesis of biliary atresia [12]. Studies on RRV induced in mice in Indonesia found expression in CD4+ biliary atresia models on days 7, 14, and 21 after induction [13].

This study found additional evidence of the role of the cellular adaptive immune system in biliary atresia characterized by higher numbers of CD4+, CD8+, CD4+ Th1, and CD4+ Th2 compared to non-biliary atresia cholestasis. The presence of adaptive immune

system in biliary atresia with a CD4+/CD8+ ratio >1 indicates a more dominant role of T helper cells [14]. In this study, the difference in the number of CD4+ Th2 between the two groups was more dominant than other immunohistochemical variables. This indicates the role of CD4+ Th2 and the suspected autoimmune process in biliary atresia.

Conclusion

Higher numbers of CD4+, CD8+, CD4+Th1, and CD4+Th2 cells were observed in patients with biliary atresia. The difference in CD4+ Th2 counts between the two groups was the most significant immunohistochemical variable in this study. There is evidence suggesting the involvement of CD4+ Th2 cells and a potential autoimmune mechanism in the development of biliary atresia. Since this study was conducted in a single center with a relatively small population, a larger follow-up study is needed.

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Authors' Contributions

The author stated no conflict of interest in this study.

Conflict of Interest

The author was involved in all aspects of the study, including writing, collecting, analyzing, and approving the manuscript.

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