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Research article

Effectiveness of oral methylprednisolone as adjuvant therapy for clinical improvement, biochemical markers, and inflammation in infants with cholestasis

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ABSTRACT

Aims: This study analyzed the effectiveness of methylprednisolone in improving jaundice, bilirubin levels, liver function tests, and inflammatory biomarkers in infants with cholestasis.
Methods: The randomized, actively controlled, parallel-group trial (ISRCTN45080388 registry) was conducted from November 2022 to May 2023 in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, on infants with cholestasis. The ethics committee of Dr. Soetomo General Academic Hospital, Surabaya approved the study protocol. Infants 14 days to 3 months old, with cholestasis followed by acholic stool, dark urine, and hepatomegaly were included in the trial. Participants were randomly assigned to methylprednisolone 2 mg/kg/day twice daily or to placebo twice daily for two weeks. Ursodeoxycholic acid (10 mg/kg) was administered to all patients thrice daily. Clinical examination and laboratory measurements (direct and total bilirubin, Aspartate aminotransferase (AST), Alanine transaminase (ALT), Gamma-glutamyl transferase (GGT), and inflammatory biomarker) were performed at baseline and after 2-week treatment. Measurement of inflammatory biomarkers (IL-2, IL-4, IL-6, IL-10, IFN-γ, TGF-β, and ANCA) was performed using enzyme-linked immunoassays. Data distribution was checked for normality. Analysis was carried out using SPSS ver. 21 with p significant <0.05.
Results: In total, 40 participants were randomized to methylprednisolone ($n = 20$; mean age 8.39 $\pm$ 3.11 weeks) and placebo ($n = 18$; 2 drop out; mean age 8.98 $\pm$ 2.80 weeks) groups. At baseline, the methylprednisolone treatment and placebo groups significantly differed in gender ($p = 0.02$) but not in clinical, laboratory examination, or inflammatory biomarker levels. The methylprednisolone group had direct bilirubin 8.36 $\pm$ 4.84 mg/dL; total bilirubin 10.40 (2.70–33.25) mg/dL; AST 187.05 (42.00–911.00) U/L; ALT 170.43 $\pm$ 134.43 U/L; IL-2 171.29 (73.70–378.57) ng/L; IL-4 119.57 $\pm$ 59.69 ng/L; IL-6 71.74 $\pm$ 29.83 ng/L; IL-10 138.15 $\pm$ 70.62 ng/L; IFN-γ 42.54 $\pm$ 12.17 ng/L; TGF-β 316.58 (163.68–606.16) ng/L; ANCA 1.70 (0.66–3.25) ng/L. After two weeks of treatment, direct bilirubin, total bilirubin, AST, IL-10, and IFN-γ levels were significantly lower in the methylprednisolone group ($p < 0.05$) than those in the placebo group. No serious adverse events were reported.
Conclusion: Methylprednisolone was efficacious in reducing 2-week bilirubin levels. These results support the hypothesis that the immunological process is involved in cholestasis. Further studies with larger sample sizes are needed to confirm the bile duct anti-inflammatory effect of

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methylprednisolone in cholestasis as an opportunity for new therapies to prevent the immunopathological process of cholestasis to biliary atresia.

1. Introduction

Cholestasis is a condition that involves a disruption or obstruction in the regular movement of bile. Possible factors contributing to cholestasis involve blockages within the bile excretory system or problems with the hepatocytes' ability to secrete bile [1]. Approximately 1 in every 2500 term newborns experience cholestatic jaundice during infancy, which is often overlooked by primary care physicians when it presents alongside normal jaundice. Pathologic cholestatic jaundice is a clear indication of liver and bile duct problems. During the early months of life, cholestatic jaundice is most commonly caused by biliary atresia, accounting for 25–40 % of cases. Biliary atresia is a specific, severe form of cholestasis that occurs due to the congenital or early-life obstruction of bile ducts, leading to significant liver dysfunction if not promptly treated. The etiology of biliary atresia is not known clearly, but the hypothesis states that a viral infection is involved in an autoimmune mechanism in the biliary tract [2,3].

Inflammation often occurs alongside cholestasis, particularly in cases where immune-mediated factors or damage to liver cells and bile ducts are involved. Gaining insight into the effects of inflammatory processes on the liver is essential for understanding the progression of cholestasis and the resulting tissue damage and disease advancement. Furthermore, inflammation can trigger the release of substances that promote inflammation, further cause liver cell damage, and activate the immune system [4].

The management of cholestatic jaundice is profoundly affected by the cause and type of cholestasis. Typically, biliary decompression is the primary treatment for obstructive cholestasis. Despite advancements in research on the disease and its management, the outcome of cholestasis continues to pose a significant challenge. It is crucial to explore alternative treatments to prevent additional bile duct obstruction, which can ultimately lead to biliary atresia, and to enhance the prognosis [1]. Although recent studies have increased the understanding and management of the disease, the outcome of biliary atresia remains a significant challenge that requires investigation of alternative treatments to prevent continued bile duct obstruction and improve prognosis. The provision of targeted therapy to slow disease progression and eliminate the need for liver transplantation is urgently needed, especially in countries where liver transplantation remains limited, such as Indonesia, and further understanding of the pathogenesis of biliary atresia mediated by the immune system is therefore required.

Based on the current pathogenesis theory, this study sought to analyze the effectiveness of steroids in treating cholestasis to suppress the occurrence of the obstructive process, such as in biliary atresia. This randomized controlled trial (RCT) was designed to compare two weeks of treatment with methylprednisolone versus placebo in improving clinical, laboratory outcomes, and inflammatory markers in infants with cholestasis.

2. Methods

2.1. Study design

An Randomized Controlled Trial (RCT) (ISRCTN45080388 registry) was conducted from November 2022 to May 2023 at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The study protocol was approved by the ethical committee of Dr. Soetomo General Academic Hospital, Surabaya (No. 0468/KEPK/VIII/2022). Written informed consent to participate in the study was provided by the parents of all patients.

This study was a randomized, double-blind, placebo-controlled study of oral methylprednisolone as adjuvant therapy in cholestasis infants over a 2-week randomized treatment. Participants were randomly assigned to placebo and methylprednisolone groups. Methylprednisolone was administered at 2 mg/kg/BW divided into two doses. Ursodeoxycholic acid (10 mg/kg) was administered to both groups three times daily. Clinical monitoring and laboratory evaluation of the participants were performed before the intervention and at two weeks of treatment.

2.2. Study population

Infants aged above 14 days to 3 months eligible for the trial were recruited. The inclusion criteria were patients suffering from cholestasis and permission to participate in the study. Patients with congenital abnormalities, such as heart defects, and syndromes, such as Down syndrome, hemodynamic instability, or sepsis, were excluded from the study. Cholestasis is defined as an increase in the direct bilirubin level of >1 mg/dL (if the total bilirubin level is < 5 mg/dL) or >20 % of the total bilirubin (if the total bilirubin level is > 5 mg/dL [1].

2.3. Intervention and follow up

Participants were randomly divided into two groups and provided with either placebo or methylprednisolone at a dose of 2 mg/kg/BW divided into two doses to be taken daily at 08:00 and 16:00. Both groups were administered ursodeoxycholic acid 10 mg/kg/BW in divided doses every 8 h. After two weeks of treatment, the participants underwent clinical evaluation and laboratory examination. Each participant was monitored for any potential side effects that may occur through a "self-assessment card" to assess whether

gastrointestinal disorders (vomiting, constipation, diarrhea), moon face, seizures, or the presence of fever. Information on drug use, side effects, and medication adherence was monitored every three days.

2.4. Clinical and laboratory measurements

Participants underwent anamnesis and physical examination before and two weeks after the intervention was given. Clinical examination of jaundice and physical examination of the abdomen were performed. Stool color was examined periodically weekly with photographs and local stool color cards. Before and after the intervention, laboratory tests were conducted, including direct bilirubin levels, total bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), and measurement of inflammatory biomarkers in serum. AST, ALT, and bilirubin levels were measured using enzyme-linked immunoassay (ELISA) at baseline and 14 days. The levels of inflammatory biomarkers interferon-gamma (IFN-γ), interleukin (IL)-2, IL-4, IL-6, IL-10, transforming growth factor-beta (TGF-β), and antineutrophilic cytoplasmic antibody (ANCA) were measured using ELISA at baseline and 14 days.

2.5. Statistical analysis

Data was statistically analyzed using SPSS ver. 21, including normality and homogeneity test ($p > 0.05$). The Shapiro–Wilk normality test was used because the sample size was <50. An independent sample *t*-test was used if data were normally distributed and homogeneous; the Mann–Whitney *U* test was used for numeric data variables. Fisher exact and Chi-square tests were used for nominal or ordinal data. The paired sample *t*-test was used for normally distributed and homogeneous data, otherwise Wilcoxon rank for numerical data variables. Significance was set at a p-value of <0.05.

3. Results

Out of 53 infants with cholestasis presented to the pediatric hepatology department, 13 were ineligible to participate in the study. Forty participants met the inclusion criteria and were randomly assigned into two groups: methylprednisolone (n = 20; mean age 8.39

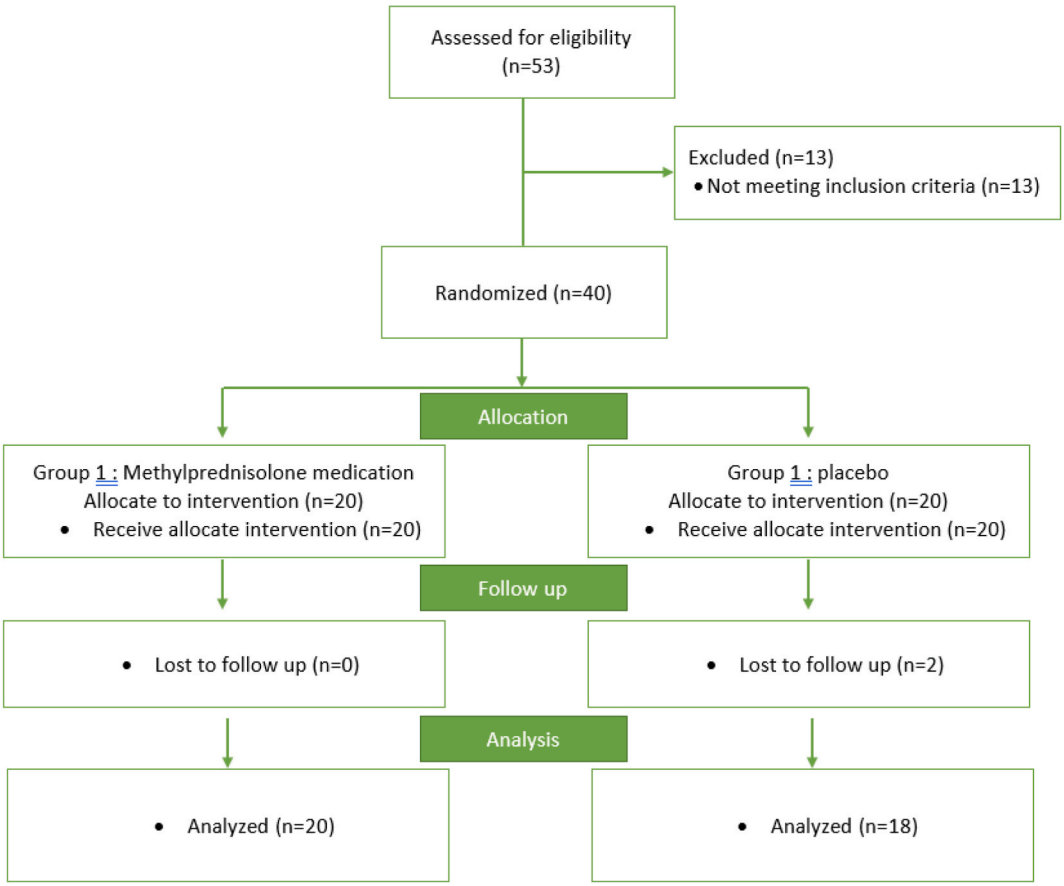

Fig. 1. Subject recruitment.

± 3.11 weeks) or placebo (n = 18; 2 dropouts; mean age 8.98 ± 2.80 weeks) (Fig. 1). Table 1 shows the baseline characteristics of the 38 randomized participants.

The gender distribution and mean age of the participants were analyzed. In the methylprednisolone group, 17 (85.0 %) participants were male, with a mean age of 8.39 ± 3.11 weeks while in the placebo group, eight (44.4 %) participants were male, with a mean age of 8.98 ± 2.80 weeks. A significant difference was present based on gender ($p = 0.02$) while no significant differences were present in age, disease duration, disease onset, or natal history (birth weight, gestational age, and method of delivery) between the groups ($p > 0.05$) (Table 1).

On physical examination, hepatomegaly was found in all participants in both groups. A total of two (10.0 %) participants in the methylprednisolone group and three (16.7 %) participants in the placebo group had splenomegaly. In the two-phase abdominal ultrasound examination, two (10.0 %) patients in the methylprednisolone group and four (22.2 %) in the placebo group showed triangular umbilical cord sign/contracted gallbladder. Examination of liver biopsies was only conducted on 27 participants as permission was refused for several participants. On liver biopsy examination, extrahepatic cholestasis was detected in six (42.9 %) and four (33.3 %) participants in the methylprednisolone and placebo groups, respectively (Table 1). All participants were tested for HBsAg with nonreactive results.

In this study, the mean/median levels of direct bilirubin, total bilirubin, ALT, AST, and GGT were measured in the methylprednisolone and placebo groups. No significant differences were present in the levels of Hb, WBC, platelets, direct bilirubin, total bilirubin, AST, ALT, GGT, coagulation factor, or albumin between the placebo and methylprednisolone groups before intrevention ($p < 0.05$) (Table 2).

The levels of inflammatory biomarkers such as mean/median levels of IL-2, IL-4, IL-6, IL-10, IFN-γ, TGF-β, and ANCA were examined in the methylprednisolone and placebo groups. The two groups had no significant difference in inflammatory biomarkers before intervention ($p > 0.05$) (Table 3).

In this study, we found that there was a significant reduction in direct bilirubin levels in both groups before and after the intervention. Results showed that there was a decrease in direct bilirubin of 7.30 (1.70–23.29) to 3.45 (0.90–27.64) mg/dL ($p = 0.01$) (Table 4, Fig. 2) in the methylprednisolone group greater than the placebo group of 7.67 ± 4.46 to 5.96 ± 3.53 mg/dL ($p = 0.04$) (Table 4, Fig. 3). The reduction in total bilirubin levels in the methylprednisolone group was greater than in the placebo group, namely 10.40 (2.70–33.25) to 5.17 (1.30–30.06) mg/dL ($p = 0.00$) (Table 4, Fig. 4) vs. 11.57 ± 7.29 g/dL to 8.23 ± 5.02 mg/dL ($p = 0.01$) (Table 4, Fig. 5).

There was a significant decrease in serum AST levels in both groups before and after intervention, namely 206.90 (29.90–759.50) to 176.30 (50.00–604.30) U/L, ($p = 0.02$) vs. 249.12 ± 199.89 to 149.42 ± 84.57 U/L, ($p = 0.01$) (Table 4). There was a significant difference in serum GGT levels in the methylprednisolone group before and after the intervention, namely 170.00 (58.00–563.00) to 208.50 (40.00–1119.00) U/L, ($p = 0.03$) (Table 4, Fig. 6).

For inflammatory biomarkers, a significant decrease was found in the methylprednisolone group with IL-10 levels (133.08 (37.34–315.55) to 100.67 (11.58–264.25) ng/L; $p = 0.02$) and at IFN-γ (42.54 ± 12.17 to 33.36 ± 15.37 ng/L; $p = 0.04$) (Table 4). However, in the placebo group, no significant reduction in inflammatory markers was found before or after treatment ($p > 0.05$) (Table 4).

4. Discussion

Cholestasis is a condition characterized by the disruption of bile flow from the liver to the duodenum, resulting in the buildup of bile acids in the liver and bloodstream. The symptoms of cholestasis are often associated with a buildup of bile acids and other substances that are normally excreted through bile. Common symptoms are jaundice, itching, dark urine, pale stools, enlarged liver to

Table 1
Basic characteristics of study participants.

Characteristics	Methylprednisolone (n = 20)	Placebo (n = 18)	P
Age, week	8.39 ± 3.11	8.98 ± 2.80	0.54[a]
Onset of jaundice, week	1 (1–7)	2 (1–8)	0.27[b]
Male, n (%)	17 (85.0)	8 (44.4)	0.02[c]
Duration of illness, week	6.24 ± 3343	6.09 ± 3.010	0.89[a]
Birth weight, g	3000 (1500–3700)	3000 (1700–4200)	0.72[b]
Gestational age, week	38 (33–41)	38 (35–41)	0.92[b]
Preterm	4 (20)	3 (16.7)	1.00[d]
Cesarean section	8 (40)	6 (33.3)	0.93[c]
Hepatomegaly	20 (100.0)	18 (100.0)	1.00[d]
Splenomegaly	2 (10.0)	3 (16.7)	0.65[d]
Triangular cord sign/Contracted gall bladder	2 (10.0)	4 (22.2)	0.39[d]
Extrahepatic cholestasis	6 (42.9)	4 (33.3)	0.69[d]

[a] Independent sample *t*-test.
[b] Mann–Whitney Test.
[c] Chi-square tests.
[d] Fisher's exact test; p significant <0.05.

Table 2
Basic laboratory measurements before intervention.

Variable	Methylprednisolone	Placebo	p
	Mean ± SD	Mean ± SD	
Hb (g/dL)	11.48 ± 2.077	11.53 ± 3.342	0.95[a]
WBC (10^3/μL)	11.09 ± 3.21	12.52 ± 5.53	0.34[a]
Platelet (10^3/μL)	393.40 ± 141.98	386.61 ± 175.34	0.89[a]
Direct bilirubin (mg/dL)	8.36 ± 4.84	7.67 ± 4.46	0.65[a]
ALT (U/L)	170.43 ± 134.43	205.46 ± 125.06	0.41[a]
Albumin (g/dL)	3.65 ± 0.56	3.48 ± 0.60	0.37[a]
	Median (min–max)	**Median (min–max)**	
Total bilirubin (mg/dL)	10.40 (2.70–33.25)	9.58 (2.50–34.06)	0.91[b]
Indirect bilirubin (mg/dL)	2.90 (0.17–9.96)	2.80 (0.81–12.01)	0.80[b]
AST (U/L)	187.05 (42.00–911.00)	206.90 (29.90–759.50)	0.82[b]
GGT (U/L)	170 (58.00–563.00)	144.50 (37.02–1481.1)	0.53[b]
APTT (s)	33.19 (16.704–7.70)	34.35 (26.60–70.60)	0.39[b]
PTT (s)	11.55 (9.69–13.90)	11.80 (9.90–35.00)	0.70[b]

SD: Standard deviation; min: minimum; max: maximum; Hb: Hemoglobin; WBC: White Blood Cell; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; APTT: Activated Partial Thromboplastin Time; PPT: Plasma Prothrombin Time.
[a] Independent sample *t*-test.
[b] Mann–Whitney Test; p significant <0.05.

Table 3
Basic inflammatory biomarker levels before intervention.

Variable	Methylprednisolone	Placebo	p
IL-2 (ng/L)	171.29 (73.70–378.57)	179.89 (12.19–1762.67)	0.70[b]
IL-4 (ng/L)	119.57 ± 59.69	117.99 ± 65.78	0.94[a]
IL-6 (ng/L)	71.74 ± 29.83	85.89 ± 42.88	0.24[a]
IL-10 (ng/L)	138.15 ± 70.62	218.88 ± 138.36	0.03[a]
IFN-γ (ng/L)	42.54 ± 12.17	38.17 ± 18.88	0.39[a]
TGF-β (ng/L)	316.58 (163.68–606.16)	316.583 (162.14–2481.35)	0.78[b]
ANCA (ng/L)	1.70 (0.66–3.25)	1.58 (0.66–2.48)	0,81[b]

SD: Standard deviation; Min: minimum; Max: maximum; IL-2: Interleukin-2, IL-4: Interleukin-4; IL-6: Interleukin-6; IL-10: Interleukin-10; IFN-γ: Interferon-gamma, TGF-β: Transforming Growth Factor-Beta; ANCA: Antineutrophilic Cytoplasmic Antibody.
[a] Independent sample *t*-test.
[b] Mann–Whitney test; p is significant <0.05.

more serious complications like liver scarring and liver failure [5].

Cholestatic jaundice is a definitive sign of hepatobiliary dysfunction and should be evaluated by a medical professional. Based on its location, it can be classified into intrahepatic and extrahepatic categories. Intrahepatic cholestasis is caused by conditions that impact the liver cells or bile canaliculi. Some common causes of liver damage are viral hepatitis, alcoholic liver disease, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and drug-induced liver injury. Extrahepatic cholestasis occurs when the bile ducts outside the liver become obstructed. Typical causes consist of gallstones, strictures, cholangiocarcinoma, and biliary atresia in infants. This condition, known as biliary atresia, leads to cholestasis and results in the gradual inflammation and fibrosis of both the extrahepatic and intrahepatic bile ducts [6,7]. Biliary atresia is the main cause of liver damage in children [8].

The results of this study showed that clinical manifestations in the form of hepatomegaly were found in most subjects in both groups before the intervention (Table 1). The cause of cholestasis is reduced or blocked bile flow from the liver. This can result in a build-up of bile acids and other substances in the liver, leading to inflammation and an increase in liver size [1]. In both groups, a minor fraction of the study participants had splenomegaly prior to the intervention (Table 1). Massive hemolysis or portal hypertension can lead to the presence of splenomegaly [1].

Laboratory results showed that there were no significant differences in the levels of Hb, WBC, platelets, direct bilirubin, total bilirubin, AST, ALT, GGT, blood coagulation factors, and albumin in both groups before intervention (Table 2). When assessing a newborn with possible cholestasis, various methods are used, such as laboratory tests, imaging, and liver biopsy. Various tests are used to assess liver function, including measurements of bilirubin (both direct and total), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and transaminases (ALT and AST). Neonatal cholestasis is a severe condition characterized by high levels of direct bilirubin, usually exceeding 1.0 mg/dL [1,9,10].

A two-phase abdominal ultrasound examination showed signs of a constricted umbilical cord/gallbladder in a part of the subjects in both groups. This triangular cord sign is a distinctive feature observed during ultrasonography in the porta hepatis. It is a fibrous tissue cord that resembles a triangle or tube and is believed to be the remnant of the extrahepatic bile duct. Its presence is often indicative of biliary atresia, making it a valuable diagnostic tool. This sign is helpful in evaluating infants with cholestatic jaundice, aiding in distinguishing between biliary atresia and neonatal hepatitis [11].

Table 4
Laboratory measurements and inflammatory markers before and after intervention.

Variable	Group	Pre	Post	p
Direct bilirubin (mg/dL)	**Placebo**	7.67 ± 4.46	5.96 ± 3.53	0.04[a]
	Methylprednisolone	7.30 (1.70–23.29)	3.45 (0.90–27.64)	0.01[b]
Indirect bilirubin (mg/dL)	**Placebo**	2.80 (0.81–12.01)	2.10 (0.06–7.30)	0.00[a]
	Methylprednisolone	2.90 (0.17–9.96)	1.35 (0.40–14.35)	0.03[b]
Total bilirubin (mg/dL)	**Placebo**	11.57 ± 7.29	8.23 ± 5.02	0.01[a]
	Methylprednisolone	10.40 (2.70–33.25)	5.17 (1.30–30.06)	0.00[b]
AST (U/L)	**Placebo**	206.90 (29.90–759.50)	176.30 (50.00–604.30)	0.02[b]
	Methylprednisolone	249.12 ± 199.89	149.42 ± 84.57	0.01[a]
ALT (U/L)	**Placebo**	205.45 ± 125.06	203.30 ± 111.35	0.94[a]
	Methylprednisolone	170.43 ± 134.43	182.16 ± 140.21	0.64[a]
GGT (U/L)	**Placebo**	144.50 (37.00–1481.00)	248.50 (62.00–1189.00)	0.18[b]
	Methylprednisolone	170.00 (58.00–563.00)	208.50 (40.00–1119.00)	0.03[b]
IL2 (ng/L)	**Placebo**	179.89 (12.19–1762.67)	159.39 (9.66–1827.33)	0.11[b]
	Methylprednisolone	177.05 ± 83.36	149.43 ± 92.00	0.21[a]
IL4 (ng/L)	**Placebo**	117.99 ± 65.58	132.72 ± 93.61	0.58[a]
	Methylprednisolone	122.41 (20.16–218.46)	68.48 (24.68–252.10)	0.10[b]
IL6 (ng/L)	**Placebo**	85.89 ± 42.88	79.67 ± 38.47	0.51[a]
	Methylprednisolone	71.74 ± 29.83	76.54 ± 34.77	0.65[a]
IL10 (ng/L)	**Placebo**	196.42 (44.46–580.87)	139.68 (40.43–395.04)	0.12[b]
	Methylprednisolone	133.08 (37.34–315.55)	100.67 (11.58–264.25)	0.02[b]
IFN-γ (ng/L)	**Placebo**	38.17 ± 18.88	33.35 ± 10.95	0.24[a]
	Methylprednisolone	42.54 ± 12.17	33.36 ± 15.37	0.04[a]
TGF-β (ng/L)	**Placebo**	316.58 (162.14–2481.35)	280.76 (74.59–1774.12)	0.19[b]
	Methylprednisolone	327.68 ± 118.50	304.29 ± 106.12	0.44[a]
ANCA(ng/L)	**Placebo**	1.56 ± 0.49	1.57 ± 0.51	0.94[a]
	Methylprednisolone	1.70 (0.66–3.25)	1.67 (0.48–3.09)	0.86[b]

AST: Aspatate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; IL-2: Interleukin-2, IL-4: Interleukin-4; IL-6: Interleukin-6; IL-10: Interleukin-10; IFN-γ: Interferon-gamma, TGF-β: Transforming Growth Factor-Beta; ANCA: Antineutrophilic Cytoplasmic Antibody.

[a] Paired smple *t*-test.

[b] Wilcoxonsigned ranks test; p is significant <0.05.

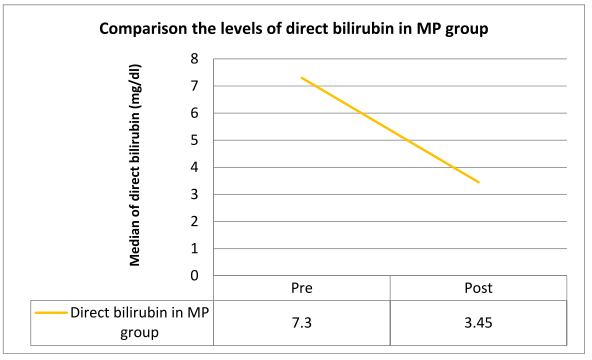

Fig. 2. Comparison level of direct bilirubin in methylprednisolone group before and after intervention (MP = Methylprednisolone).

During the liver biopsy examination in this study, it was found that 42.9 % of the subjects in the methylprednisolone group and 33.3 % of the subjects in the placebo group had extrahepatic cholestasis (Table 1). Liver biopsy is a crucial diagnostic procedure for evaluating cholestasis, especially when non-invasive examination like blood test and imaging studies do not provide a clear understanding of the underlying cause. Imaging studies like abdominal ultrasound, hepatobiliary scintigraphy, and occasionally magnetic resonance cholangiopancreatography (MRCP) can be used to visualize the biliary tree. Histological examination through liver biopsy allows for the identification of specific liver pathology and the evaluation of the degree of liver damage [5,12].

In laboratory evaluation before and after the intervention showed that decrease in direct bilirubin levels in the methylprednisolone group by 7.30 (1.70–23.29) to 3.45 (0.90–27.64) mg/dL (p = 0.01) (Table 4, Fig. 2) was greater than the placebo group 7.67 ± 4.46 to 5.96 ± 3.53 mg/dL (p = 0.04) (Table 4, Fig. 3). Direct bilirubin, or conjugated bilirubin, is a type of bilirubin that undergoes liver processing and is typically eliminated through bile excretion. When there is a disturbance in the flow of bile, direct bilirubin may build up in the bloodstream, suggesting a potential case of cholestasis. A recent study revealed that direct bilirubin levels in newborns can serve as a reliable indicator for cholestatic liver disease, offering both sensitivity and specificity. Screening newborns for direct bilirubin levels could potentially lead to earlier detection of biliary atresia and other cholestatic liver diseases. In some studies, it has been

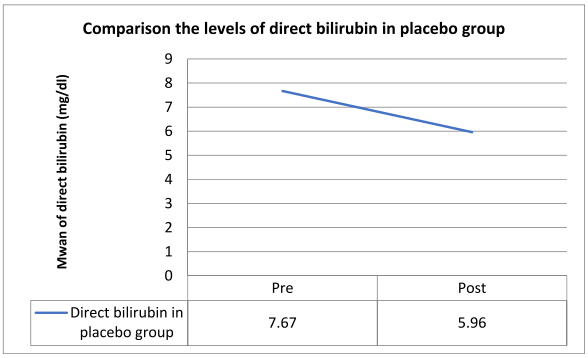

Fig. 3. Comparison level of direct bilirubin in placebo group before and after intervention.

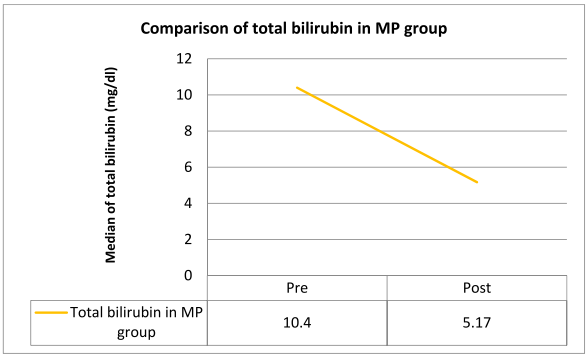

Fig. 4. Comparison level of total bilirubin in methylprednisolone group before and after intervention (MP = Methylprednisolone).

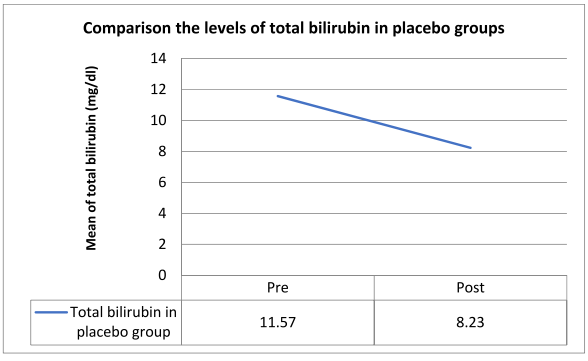

Fig. 5. Comparison level of total bilirubin in placebo group before and after intervention.

found that the timing of the Kasai procedure does not have a significant impact on the long-term survival of children with their native liver. However, it is important to note that early intervention may still have positive outcomes in certain cases [13].

The decrease in serum AST levels in the methylprednisolone group was greater than in the placebo group, namely 249.12 ± 199.89 to 149.42 ± 84.57 U/L ($p = 0.01$) vs 206.90 (29.90–759.50) to 176.30 (50.00–604.30) U/L ($p = 0.02$) (Table 4). Elevations in serum aspartate aminotransferase enzyme (AST) and its isoenzymes are commonly observed in various liver diseases. The measurement of total AST activity in human serum has proven to be a valuable tool in diagnosing and evaluating liver necrosis, as well as determining prognosis [14]. A recent study revealed that patients with ALT levels ≥30x the upper limit of normal (ULN) experienced a significantly higher spontaneous survival (SS) rate when treated with corticosteroids compared to those who did not receive steroid treatment. Specifically, the steroid use group had a survival rate of 13.33 % (4/30), indicating a positive effect of corticosteroids on liver failure severity. There was a notable difference in the SS rate between patients who used steroids and those who did not, specifically in patients with AST levels ≥30x the ULN [15].

Inflammatory biomarkers examined in this study were IL-2, IL-4, IL-6, IL-10, IFN-γ, TGF-β, and ANCA. The results showed that both groups did not have significant differences in inflammatory biomarkers before intervention ($p > 0.05$) other than IL-10 ($p < 0.05$)

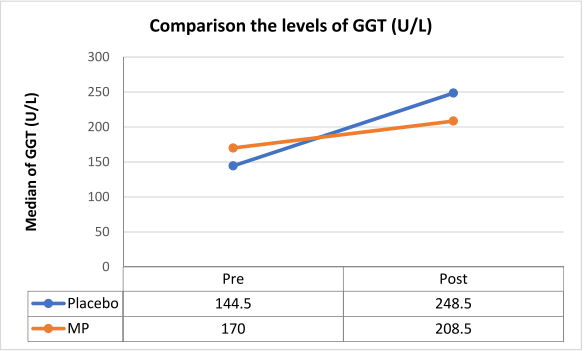

Fig. 6. Comparison level of GGT in both groups before and after intervention (MP = Methylprednisolone).

(Table 3). A significant decrease was found in the methylprednisolone group, namely on the measurement of IL-10 levels of 133.08 (37.34–315.55) to 100.67 (11.58–264.25) ng/L ($p = 0.02$) and at IFN-γ of 42.54 ± 12.17 to 33.36 ± 15.37 ng/L ($p = 0.04$). However, in the placebo group, there was no significant reduction in inflammatory markers before and after treatment ($p > 0.05$) (Table 4). A study revealed the significance of Interleukin (IL)-10, a crucial immunoregulatory cytokine that is synthesized by various cell populations. Multiple studies indicate that IL-10 has a significant impact on chronic liver diseases. IL-10 is also produced by different cell types in other organs, such as the liver. IL-10 production has been observed in various cells within the liver, including hepatocytes, sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and liver-associated lymphocytes [16].

Research findings from animal model results and patient clinical data, cytokines linked with inflammation, including pro-inflammatory cytokines like TNF-α and TGF-β as well as anti-inflammatory cytokines like IL-10, play a role in the progression of liver injury. There has been a growing focus on the impact of cytokine levels on inflammatory and immune responses. This could explain the variations seen in the outcomes of chronic liver diseases, including HBV and HCV infection, alcoholic liver disease, and autoimmune hepatic disease. However, there have been limited studies on the relationship between IL-10 and cholestasis [17].

Viral infections, such as cytomegalovirus (CMV), can trigger clonal expansion of virus-specific $CD4^+$ T cells, causing biliary tract injury. Th1 cell differentiation activates macrophages, which release proinflammatory cytokines such as IL-2 and TNF-α, while cytotoxic $CD8^+$ T cells directly invade the epithelium and B cells produce antibodies. Over time, the apoptotic proteins of the bile duct epithelium are seen as "foreign bodies" and trigger an autoreactive T cell-mediated inflammatory response that causes bile duct injury [18]. Many studies of biliary atresia have linked an autoimmune and antigen-recognizing error that causes a repetitive process that culminates with biliary atresia. The biliary atresia process is not considered to occur directly due to viral infection as viruses do not infect bile; however, inherited maternal antibodies may not correctly recognize antigens. Several studies show that the antigens evoked by CMV are similar to those produced by afferent nerves. This causes impaired bile secretion. The immune system cannot recognize antigens properly because the ability of regular T cells to recognize non-self-cells has not been fully formed [19,20].

Previous studies have shown that serum levels of IL-18 and IFN-γ in biliary atresia patients are higher than normal controls, as well as IFN-γ [21,22]. In this study, there is a decrease in direct bilirubin, total bilirubin, and AST levels in the methylprednisolone group compared to placebo, with a decrease in direct bilirubin being greater than the placebo group (Table 4). At week 2, IL-10 level and IFN-γ significantly reduced from baseline by methylprednisolone, p 0.02 and $p = 0.04$ (Table 4). Steroid administration was shown to suppress and ultimately stop this inflammatory process, and then further damage to the bile duct epithelium, which causes fibro-obliterative processes in the bile ducts, does not occur.

Understanding the pathophysiology of cholestasis requires a thorough examination of the factors that affect bile production, secretion, or flow. This is a frequently observed occurrence in the liver, often resulting from exposure to harmful substances and various liver disorders. The production of bile by hepatocytes is crucial for the proper digestion and absorption of fats and fat-soluble vitamins. When bile flow is blocked, bile acids build up in the liver, leading to damage and inflammation of the liver cells. If not addressed, this can result in ongoing liver damage and fibrosis [23].

The accumulation of bile acids in the liver is believed to be a significant factor in causing liver damage during cholestasis, potentially resulting in liver fibrosis and cirrhosis over time. Therefore, the current treatment approach for chronic liver diseases with significant cholestasis focuses on enhancing choleretic to prevent the buildup of bile acids. Several diseases exhibit autoimmunity before the development of cholestasis, suggesting that inflammation might play a crucial role in the progression of the condition. In addition, there is an accumulation of cytotoxic inflammatory mediators during cholestasis that can worsen liver damage. There have been limited successes in clinical trials with anti-inflammatory biologics and small molecules for these diseases. Therefore, the effectiveness of targeting inflammation to treat cholestatic liver injury is still a topic of debate. Timely identification and intervention play a vital role in effectively addressing the root causes and mitigating potential long-term harm to the liver. Continued research and advancements in diagnostic techniques are crucial for enhancing outcomes in affected infants [1,23].

To the best of our knowledge, steroids have only been used as adjuvant therapy in patients undergoing Kasai surgery and have never been used in patients with cholestasis in the early stages. The current steroid studies are only performed as postoperative adjuvant therapy. Postoperative adjuvant steroid therapy can significantly improve bile flow if Kasai is performed at ≤70 days of age and maintain long-term native liver survival but has no significant effect on the short-term recovery of native liver survival and

postoperative cholangitis [24]. No significant difference has been found in administering steroids to jaundice-free patients or in mortality rates after Kasai surgery [25]. The current study aimed to evaluate the efficacy of methylprednisolone on clinical manifestations, liver function levels, and inflammatory biomarkers in infants with cholestasis. We hypothesized that administration of methylprednisolone could improve jaundice, liver function, and immunological markers. The study results show that the immunological parameters improved after administration of methylprednisolone, which suppressed inflammatory mechanisms in the bile ducts in the early phase to prevent a bile duct obstruction, which is a sign of biliary atresia.

In cholestasis management, the main focus is on identifying and treating the root cause, if possible, while also addressing bothersome symptoms such as itching and fatigue. As someone well-versed in this area, it is standard procedure to use ursodeoxycholic acid (UDCA) as the first-line therapy for primary biliary cholangitis. This medication has been found to improve liver function and effectively slow down the progression of the disease [26].

Based on this hypothesis, therapy focuses not on treating biliary atresia but on preventing this from occurring. Currently, the treatment of biliary atresia only focuses on improving the extrahepatic condition, although intrahepatic fibrosis will still occur and cause cirrhosis. Early detection of biliary atresia, through early detection of cholestasis or an increase in direct bilirubin levels accompanied by viral infection, predominantly via positive CMV serology and subsequent treatment by inhibiting inflammation (giving anti-inflammatory drugs) is still challenging. In addition to stopping the process of cholangiocyte and hepatocyte damage, the administration of anti-inflammatory drugs can help activate regulatory T cells for better recognition. Preventing cholestasis into biliary atresia can only be achieved by administering anti-inflammatories with multiple initial doses and tapering off while waiting for the immune system to mature.

In this study, levels of direct bilirubin, total bilirubin, and AST were shown to be lower in the methylprednisolone group than in the placebo group after intervention, with the decrease in direct bilirubin being larger. The levels of IL-10 and IFN-γ were significantly reduced from baseline by methylprednisolone treatment at week two. Thus, steroid administration may suppresses the inflammatory process to avoid further damage to the bile duct epithelium and prevent fibrotic processes in the bile ducts.

5. Conclusion

This RCT study is the first to be performed to evaluate the effectiveness of methylprednisolone in improving jaundice, bilirubin levels, liver function tests, and inflammatory biomarkers in infants with cholestasis. The study supports the role of adaptive immune response and autoimmunity in the occurrence of immune-mediated and autoimmune bile duct injury in biliary atresia. The results of this study demonstrate opportunities for the development of new medical interventions aimed at suppressing specific immune responses, reducing inflammatory damage to the bile ducts, and preventing the need for liver transplantation. However, additional multicenter studies are required considering the small number of laboratory values, lack of clinical and histological data, and the short follow-up time in this study.

Data Availability statement

Data available at https://doi.org/10.6084/m9.figshare.24954198.v1.

CRediT authorship contribution statement

Bagus Setyoboedi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Martono Tri Utomo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rendi Aji Prihaningtyas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sjamsul Arief:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Bagus Setyoboedi reports financial support was provided by Indonesia's Ministry of Education, Culture, Research, and Technology (Kemendikbudristek).

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