Pakistan J. Zool., pp 1-4, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20240114052214

Short Communication

Haematological and Biochemical Indices of Neonatal Intrahepatic Cholestasis Caused by Alagille Syndrome

Liu Nie

Medical Office, West Hospital, Wuhan Children's Hospital, Wuhan 430000, Hubei Province, China.

ABSTRACT

The aim of this study was to determine the blood routine and biochemical indices of Alagille syndrome (ALGS) and to explore the changes of the indicators of neonatal intrahepatic cholestasis caused by ALGS. This study was conducted on data of thirty-two neonatal intrahepatic cholestasis caused by ALGS treated from June 2017 to January 2020 and were randomly selected as a study group (ALGS group). Thirty-six children with unexplained cholestasis in the same period were selected as a control group. The incidences of cardiovascular malformations, special faces, vertebral changes and ocular abnormalities in the ALGS group were greater than those in the control group before and after treatment ($P<0.05$). There was no significant difference in WBC, RBC and PLT count and absolute value of neutrophil (Neu) between two groups before and after treatment ($P>0.05$). The absolute value and percentage of Neu and the ratio of Neu to lymphn ocytes (LYM) in the ALGS group were raised compared to the control group before and after treatment ($P<0.05$). There was no significant difference in the levels of GOT, TG, HDL, and Alb between the two groups at before and after treatment ($P>0.05$). The levels of γ-GT, DBil, TBA, GPT, TC, LDL, TP and GLB in the ALGS group were raised compared to the control group at before and after treatment ($P<0.05$). The various clinical manifestations, haematological and biochemical changes are of great significance over time in judging neonatal intrahepatic cholestasis caused by ALGS.

Article Information
Received 14 January 2024
Revised 08 March 2024
Accepted 26 March 2024
Available online 25 July 2024
(early access)

Key words
Alagille syndrome, Neonatal intrahepatic cholestasis, Blood routine, Biochemical index, Longitudinal study

Cholestasis is the primary cause of hospitalization and treatment in pediatric liver diseases, and it is also an important cause of death or disability (Feldman and Sokol, 2021). According to the etiology of cholestasis, intrahepatic cholestasis is due to abnormal structure and function of hepatocytes or capillaries, which leads to the disorder of bile secretion and excretion, so that substances that should be excreted bile accumulate in the liver and serum (Zhou *et al*., 2017). Intrahepatic cholestasis is not a single disease, abnormal secretion and excretion of bile can occur in the development of various liver diseases, resulting in cholestasis. Long-term cholestasis of hepatocytes and bile duct will cause varying degrees of cell damage, but if not treated in time, intrahepatic cholestasis will gradually cause liver fibrosis, cirrhosis and even liver failure, which has a serious impact on the quality of life and health of patients (Hao *et al*., 2017). In recent years, with the continuous development of molecular biology and liver biopsy technology, patients with such diseases caused by related pathogenic gene mutations or genetic factors have been continuously discovered and recognized. However, due to the broad clinical manifestations of the disease, and many multi-system diseases are more common in early liver disease or without cholestasis; in addition, the prognosis of patients with cholestasis caused by different etiology is not the same (Siede and Seiffert, 1983; Trauner *et al*., 2017). Therefore, it is important to identify the causes of cholestasis early according to its clinical manifestations, blood routine and biochemical indices (Nguyen *et al*., 2014). Alagille syndrome (ALGS) is a multi-system disease mainly caused by Notch signal failure, which can affect the liver, kidney and central nervous system of patients, and may also form characteristic faces (Zhang *et al*., 2019). However, some early clinical features of neonatal intrahepatic cholestasis caused by ALGS are not obvious, which aggravates the difficulty of early diagnosis. This study aims to explore and analyze the clinical manifestations, blood routine and biochemical indexes of neonatal intrahepatic cholestasis caused by ALGS.

Materials and methods

Thirty-two cases of neonatal intrahepatic cholestasis caused by ALGS treated in Wuhan Children's Hospital from June 2017 to January 2020 were randomly selected as the study group (ALGS group). All patients who

* Corresponding author: yuanxilan326@163.com
0030-9923/2024/0001-0001 $ 9.00/0

signed the informed consent form, met the diagnosis and treatment criteria of neonatal intrahepatic cholestasis newborns or infants, andwith clinical manifestations of intrahepatic cholestasis were included in the study. Those with a biliary atresia detected by B-ultrasound or nuclear magnetic resonance, with toxic liver disease caused by breast milk jaundice, congenital choledochal cyst, liver tumor and other organs of toxic drugs were excluded. Thirty-six children with cholestasis of unknown origin in the same period were selected as the control group. Blood samples were collected before and after treatment. The serum was separated by centrifugation.

Blood parameters white blood cell (WBC), red blood cell (RBC), platelet (PLT), lymphocyte (Lym), Neu/Lym ratio and monocyte (MO) were analyzed by automatic blood routine analyzer (Wuhan Kelda Medical Technology Co., Ltd., model: RT-7200).

The blood serum was analyzed by automatic biochemical analyzer (Beijing Tailin Oriental Trading Co., Ltd., model: 7600) for measuring γ-glutamyl transpeptidase (γ-GT), conjugated bilirubin (DBil), total bile acid (TBA), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), total protein (TP), albumin (Alb), globulin (GLB).

SPSS22.0 software package was used to analyze the statistical data. The counting data were all tested by χ^2test. Analysis of variance was used to compare the measurement data between groups, and t-test was used to compare the measurement data within groups. $P<0.05$ was considered statistically significant.

Results and discussion

From the 162 neonatal intrahepatic cholestasis caused by ALGS treated, 68 patients were included in the study. The gender distribution was 26 female and 42 male patients aged between 0.85 and 1.60 years, with a M(SD) age of 1.23(0.41) years. Supplementary Table I shows that there are no significant differences between the two groups.

For clinical manifestations, cholestasis was found in both groups, and the incidences of acute cardiovascular disease, special face, vertebral changes and ocular abnormalities in the ALGS group were raised than those in the control group after the treatment ($P<0.05$) (Table I). There were statistically significant differences in cardiovascular malformation, special face, vertebral body change and ocular abnormality among groups after the treatment.

Table II shows the hematological parameters and biochemical indexes of the two groups before and after treatment. There was no significant difference over time in WBC, RBC, PLT count and MO count between the two groups ($P>0.05$). The Neu and the ratio of Neu to Lym were raised in the ALGS group than those in the control group ($P<0.05$). Moreover, there was no significant difference over time in the levels of GOT, TG, HDL and Alb between two groups ($P>0.05$). The levels of γ-GT, DBil, TBA, GPT, TC, LDL, TP and GLB in the ALGS group were raised over time than those in the control group ($P<0.05$).

Table I. Clinical manifestations related indices between groups after treatment.

Index	Control group (n=36)	ALGS group (n=32)	χ^2	P
Manifestation of cholestasis	36	32	-	1.000
Cardiovascular malformation	6(16.67)	24(75.00)	12.285	<0.001
Special face	15(41.67)	25(78.13)	5.347	0.021
Vertebral body change	3(8.33)	17(53.13)	7.243	0.007
Ocular abnormality	3(8.33)	17(53.13)	7.243	0.007
Kidney injury	6(16.67)	4(12.50)	0.129	0.720
Acidosis	21(58.33)	14(43.75)	0.744	0.388
Underdevelopment	30(83.33)	24(75.00)	0.345	0.557

For details of group, see Table I.

Hepatocytes are a kind of polar cells, which can be divided into hepatic sinusoid surface and bile tubule surface. There is an important molecular pathway regulating bile secretion and excretion on the hepatic sinusoid surface of the hepatocyte membrane, which can pump bilirubin and other bile components into the hepatocytes (Pan *et al*., 2018). In the study of animal cholestasis, some scholars have found that the expression of bile transporter in specific hepatocytes is decreased or missing in animal cholestasis model, and these abnormalities damage the function of bile transporter protein and lead to the decrease of bile flow. and then develop into cholestasis (Mahdi *et al*., 2016). Cholestatic liver disease has a wide range of etiology, and the diagnosis requires detailed medical history, comprehensive clinical examination and laboratory testing. The etiology of cholestasis is gradually clear, and the proportion of genetic metabolic diseases such as ALGS is gradually increasing. It is often characterized by neonatal cholestasis, and many systems such as heart, bone, eye, face and kidney can be involved, which threatens the health of patients (Mouzaki *et al*., 2016).

Patients with neonatal intrahepatic cholestasis caused by ALGS often have typical deformities such as butterfly vertebrae, saddle nose, pointed chin, sunken eyes etc. In addition, there are many kinds of eye dysfunction, among which the posterior corneal embryonic ring is the

Table II. Haematological and biochemical indices of the two groups before and after treatment.

Indicator	Control group (n=36)		ALGS group (n=32)		P
	Before treatment	After treatment	Before treatment	After treatment	
Haematological parameters					
WBC ($\times10^9$/L)	11.89±1.74	11.81±1.87	11.76±1.66	12.23±0.18	0.208
RBC ($\times10^{12}$/L)	4.32±0.41	4.14±0.48	4.21±0.52	3.94±0.42	0.183
PLT ($\times10^9$/L)	420±118.2	419.13±120.13	423±116.6	379.68±94.85	0.260
Absolute value of Neu ($\times10^9$/L)	2.22±0.61	2.13±0.78	2.18±0.69	5.16±0.32	<0.001
Percentage of Neu (%)	22.16±4.98	20.31±5.64	21.27±5.59	41.62±0.25	<0.001
Absolute value of Lym ($\times10^9$/L)	8.44±1.87	8.39±1.92	8.41±1.55	5.46±0.20	<0.001
Lym percentage (%)	69.49±7.45	68.78±7.86	70.71±6.86	46.37±0.15	<0.001
Neu/Lym ratio	0.23±0.12	0.24±0.03	0.23±0.16	0.97±0.06	<0.001
Absolute value of MO ($\times10^9$/L)	1.02±0.44	1.03±0.43	1.03±0.41	1.06±0.43	0.838
Biochemical indices					
γ-GT (U/L)	140.12±59.2	138.00±66.08	141.32±61.1	440.25±56.84	<0.001
DBil (μmol/L)	73.44±28.16	70.00±34.96	72.28±33.26	116.88±54.82	0.009
TBA (μmol/L)	94.29±39.55	95.70±40.60	94.41±45.33	135.32±15.53	<0.001
GPT (U/L)	156.42±80.22	161.75±79.15	159.11±78.71	234.29±47.87	0.001
GOT (U/L)	183.36±58.11	179.50±61.96	180.11±60.73	146.82±52.84	0.089
TC (mmol/L)	4.11±1.21	4.56±1.17	4.33±1.62	7.88±1.47	<0.001
TRIG (mmol/L)	1.32±1.23	1.54±1.07	1.44±1.09	2.43±1.79	0.115
LDL (mmol/L)	2.66±0.89	2.53±0.91	2.60±1.01	4.25±1.09	<0.001
HDL (mmol/L)	0.89±0.51	0.95±0.46	0.92±0.64	1.57±1.04	0.054
TP (g/L)	52.12±4.48	55.95±5.41	54.36±5.16	68.78±2.13	<0.001
Alb (g/L)	40.18±4.15	42.83±3.77	41.62±3.89	44.56±2.31	0.072
GLB (g/L)	12.16±3.49	13.12±3.14	13.41±3.89	24.74±2.03	<0.001

WBC, white blood cell; RBC, red blood cell; PLT, platelet; Neu, neutrophil; Lym, lymphocyte; MO, monocyte; γ-GT, γ-glutamyl transpeptidase; DBil, conjugated bilirubin; TBA, total bile acid; GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase; TC, total cholesterol; TG, triglyceride; LDL, low density lipoprotein; HDL, high density lipoprotein; TP, total protein; Alb, albumin; GLB, globulin. Control group: children with unexplained cholestasis; ALGS group: children with neonatal intrahepatic cholestasis caused by Alagille syndrome.

most common eye dysfunction, and the incidence rate can be as high as 95% (Andersson *et al*., 2017; Huppert, 2016). This study found that the incidences of cardiovascular malformation, special face, vertebral body changes and eye abnormalities in neonatal intrahepatic cholestasis caused by ALGS were 74.19%, 77.42%, 51.61% and 51.61%, respectively, which were raised than those in neonatal intrahepatic cholestasis caused by Citrin deficiency. It is suggested that the above manifestations can be used as important manifestations in the diagnosis of ALGS.

For patients whose clinical manifestations are not obvious, blood routine and laboratory biochemical tests will also provide critical clues for the cholestasis diagnosis (Xu *et al*., 2017). In this study, neutrophils, lymphocytes, and their ratio play critical role in the diagnosis of intrahepatic cholestasis caused by ALGS. Immune cells are a group of cells that interact with each other. Lymphocytes and neutrophils are both important cellular components of the body's immune response function. Among them, neutrophils are the most important components of leukocytes in the blood. They are rich in lysosomes, can release a variety of inflammatory factors, and playcritical role in the body's non-specific immunity. It is found that when intrahepatic cholestasis occurs, the proportion of lymphocyte subsets is out of balance and the immune function is significantly decreased, thus aggravating the patient's condition (Pushpam *et al*., 2019). The levels of neutrophils and lymphocytes play critical role in reflecting the progression of liver disease. Among biochemical indices, γ-GT is most expressed in the liver during the embryonic stage, and is an important index to reflect the severity of the disease (Lipiński *et al*., 2018). The normal secretion and fluidity of bile mainly depends on the ATP-dependent pumps and secretory proteins of hepatocytes and bile duct epithelial cells. However, Jag1 gene mutations in patients with ALGS can cause functional impairment of small bile ducts, which can easily lead to cholestasis, while cholestasis and poisoning

can damage hepatocytes, lead to damage to liver repair ability, and then aggravate cholestasis and enter a vicious circle (Ataalla *et al*., 2016). TP and Alb which reflect the state of liver disease and nutritional status of the body will be significantly decreased of the body is malnourished (Togawa *et al*., 2016). The results show that biochemical indexes such as γ-GT, conjugated bilirubin, TBA, TP and GLB play critical role over time in distinguishing intrahepatic cholestasis caused by ALGS.

DECLARATIONS

Acknowledgments

Thanks are due to the members from Wuhan Children's Hospital. They collected samples, obtained data, and provided theoretical guidance.

Ethical approval

The study was carried out in compliance with guidelines issued by ethical review board committee of Wuhan Children's Hospital, China. The official letter would be available on fair request to corresponding author.

IRB approval

This study was approved by the Advanced Studies Research Board of Wuhan Children's Hospital, Hubei Province, China.

Ethical statement

Ethics Committee approval was obtained from the Institutional Ethics Committee of "Wuhan Children's Hospital" to the commencement of the study.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20240114052214

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Andersson, E.R., Chivukula, I.V., Hankeova, S., Sjöqvist, M., Tsoi, Y.L., Ramsköld, D., Masek, J., Elmansuri, A., Hoogendoorn, A., Vazquez, E., Storvall, H., Sjöqvist, M., Tsoi, Y.L., Ramsköld, D., Masek, J., Elmansuri, A., Hoogendoorn, A., Vazquez, E. and Storvall, H., 2018. *Gastroenterology*, **154**: 1080-1095. https://doi.org/10.1053/j.gastro.2017.11.002

Ataalla, W.M., Ziada, D.H., Gaber, R., Ossman, A., Bayomy, S. and Elemary, B.R., 2016. *J. Matern. Fetal Neonatal Med.*, **29**: 1445-1450. https://doi.org/10.3109/14767058.2015.1051020

Cho, S.J., Perito, E.R., Shafizadeh, N. and Kim, G.E., 2021. *Hum. Pathol.*, **112**: 102-115. https://doi.org/10.1016/j.humpath.2020.12.007

Feldman, A.G. and Sokol, R.J., 2021. *NeoReviews*, **22**: e819-e836. https://doi.org/10.1542/neo.22-12-e819

Hao, H., Cao, L., Jiang, C., Che, Y., Zhang, S., Takahashi, S., Wang, G. and Gonzalez, F.J., 2017. *Cell Metab.*, **25**: 856-867. https://doi.org/10.1016/j.cmet.2017.03.007

Huppert, S.S., 2016. *Hepatology*, **63**: 365-367. https://doi.org/10.1002/hep.28338

Lipiński, P., Jankowska, I. and Liberek, A., 2018. *Pediatria Polska*, **93**: 94-97. https://doi.org/10.5114/polp.2018.74767

Mahdi, Z.M., Synal-Hermanns, U., Yoker, A., Locher, K.P. and Stieger, B., 2016. *Mol. Pharmacol.*, **90**: 23-34. https://doi.org/10.1124/mol.116.103390

Mouzaki, M., Bass, L.M., Sokol, R.J., Piccoli, D.A., Quammie, C., Loomes, K.M., Heubi, J.E., Hertel, P.M., Scheenstra, R., Furuya, K. and Kutsch, E., 2016. *Liver Int.*, **36**: 755-760. https://doi.org/10.1111/liv.12920

Nguyen, K.D., Sundaram, V. and Ayoub, W.S., 2014. *World J. Gastroenterol.,* **20**: 9418. https://doi.org/10.3748/wjg.v20.i28.9418

Pan, H., Li, R., Li, Y., Lan, H. and Zheng, X., 2018. *Pakistan J. Zool.*, **50**: 159-164.

Pushpam, D., Chopra, A., Sreenivas, V., Kumar, R. and Bakhshi, S., 2019. *Indian Pediatr.*, **56**: 381-383. https://doi.org/10.1007/s13312-019-1534-2

Sadiku, E., Taci, S., Dibra, A., Nela, E. and Babameto, A., 2015. *Alban med. J.*, (Vol?): 72-82.

Siede, W.H. and Seiffert, U.B., 1983. *Clin. Chem.*, **29**: 698-700. https://doi.org/10.1093/clinchem/29.4.698

Togawa, T., Sugiura, T., Ito, K., Endo, T., Aoyama, K., Ohashi, K., Negishi, Y., Kudo, T., Ito, R., Kikuchi, A. and Arai-Ichinoi, N., 2016. *J. Pediatr.*, **171**: 171-177. https://doi.org/10.1016/j.jpeds.2016.01.006

Trauner, M., Fuchs, C.D., Halilbasic, E. and Paumgartner, G., 2017. *Hepatology*, **65**: 1393-1404. https://doi.org/10.1002/hep.28991

Xu, L., Wu, L., Liu, T., Xing, W., Cao, X., Zhang, S. and Su, Z., 2017. *J. Phys. Ther. Sci.*, **29**: 1573-1577. https://doi.org/10.1589/jpts.29.1573

Zhang, W., Zhao, X., Huang, J., Ou, X. and Jia, J., 2019. *Rev. Esp. Enferm. Dig.*, **111**: 323-326. https://doi.org/10.17235/reed.2019.5679/2018

Zhou, H.Q., Liu, W., Wang, J., Huang, Y.Q., Li, P.Y., Zhu, Y., Wang, J.B., Ma, X., Li, R.S., Wei, S.Z. and Li, K., 2017. *Biomed. Pharmacother.*, **89**: 696-704. https://doi.org/10.1016/j.biopha.2017.02.084

Pakistan J. Zool., pp 1-4, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20240114052214

Supplementary Material

Haematological and Biochemical Indices of Neonatal Intrahepatic Cholestasis Caused by Alagille Syndrome

Liu Nie

Medical Office, West Hospital, Wuhan Children's Hospital, Wuhan 430000, Hubei Province, China.

Supplementary Table I. General and clinical information of the two groups in this study.

Index	Control group (n=36)	ALGS group (n=32)	χ^2	P
General information				
Age (years)	1.25±0.43	1.22±0.37	0.232	0.817
Gender				
Male	24	18	0.392	0.531
Female	12	14		
Full-term child	27	26	0.210	0.647
Underdevelopment	16	25	1.303	0.200
Jaundice symptoms	36	32	-	1.000

Control group: children with unexplained cholestasis; ALGS group: children with neonatal intrahepatic cholestasis caused by Alagille syndrome.

* Corresponding author: yuanxilan326@163.com
0030-9923/2024/0001-0001 $ 9.00/0