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Study of Gut Klebseilla in Biliary Atresia

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Abstract

Background: Biliary atresia (BA) is a neonatal cholestasis disease that is characterized by fibrosclerosing and inflammatory obliteration of the biliary tracts, which leads to progressive liver damage. It is the most common cause of cholestasis in the first 3 months of life presented by progressive conjugated jaundice, pale acholic stools and dark with later ones being, ascites, portal hypertension and splenomegaly. The recommended treatment is kasai portoenterostomy & liver transplantation. Characterization of gut klebsiella profile in infants with biliary atresia can provide valuable information and potential disease biomarkers.

Aim of Study: Our study aims to characterize the gut Klebsiellain infants with biliary atresia.

Material and Methods: This study was a case control study that included 10 infants diagnosed as BA&10 age-matched healthy infants as a control group.

Results: Increase level of Klebsiellain BA group more than control group. There was significant difference between BA group & control group (*p*-value >0.05).

Conclusion: Increased level of gut Klebsiella is associated with the incidence of biliary atresia.

Key Words: *Gut – Klebseilla – Biliary Atresia.*

Introduction

BILIARY atresia (BA) is a neonatal cholestasis disease that is characterized by fibrosclerosing and inflammatory obliteration of the biliary tracts, which leads to progressive liver damage **[1]**. It is the most common cause of cholestasis in the first 3 months of life and the most frequent pediatric indication for liver transplantation **[2]**.

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Biliary atresia is typically with progressive conjugated jaundice, pale acholic stools and dark urine. Other early presenting features may include a vitamin K dependent coagulopathy; with later ones being, ascites, portal hypertension and splenomegaly **[3]**.

The pathophysiology of the condition is not fully understood and proposed etiologic contributors include immunological factors, genetic predisposition, ischemia and infection **[4]**.

The recommended treatment of BA is sequential: in the first and second month of life, the Kasai portoenterostomy aims to restore the biliary flow to the intestine; in the case of failure of the operation and/or life-threatening complications of the biliary cirrhosis, liver transplantation may be needed. So early diagnosis of BA is very important **[5]**.

The gut-liver axis is a consequence of a close anatomical and functional, bidirectional interaction of the gastrointestinal tract and liver, primarily through a portal circulation. The symbiotic relationship between the gut microbiome and the liver is regulated and stabilized by a complex network of interactions that encompass metabolic, immune, and neuroendocrine crosstalk between them **[6]**.

Several studies have linked intestinal dysbiosis to the severity and progression of liver diseases, such as non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary sclerosing cholangitis, total parenteral nutrition-associated liver disease, and cystic fibrosis associated liver disease. However, there is limited information and interpretation with regard to how the microbiome could contribute to liver disease in the pediatric population **[7]**.

Stool anomalies have been consistently reported and may serve as a means to evaluate and manage BA **[8]**. Lien et al., demonstrated that the stool card

screening program for BA enhanced early diagnosis and increased the jaundice free rate at 3 months after the Kasai procedure **[9]**.

Understanding the role of the gut klebseilla in BA pathogenesis opens avenues for microbiome modulatory therapy. Current adjuvant therapy (antibiotics, steroids, and choleretics) for BA, as well as dietary manipulation, are likely to shape the microbiome **[10]**.

Patients and Methods

10 infants diagnosed as biliary atresia were recruited from Pediatric Hepatology, Gastroenterology and Nutrition Department, National Liver Institute, Menoufia University. 10 age matched healthy infants as a control group (they were recruited from infants attending health office for routine vaccination). The study was approved by the Research Ethical Committee of Menoufia University's National Liver Institute.

Consent:

A written informed consent was obtained from all the legal guardian of the participants before inclusion in the study, explaining the value of the study, plus the procedures that were commenced.

Inclusion criteria:

1- The clinical symptoms and imaging tests were in accordance with the diagnostic criteria for biliary atresia and also for non-biliary atresia group.

Exclusion criteria:

1- Antibiotic treatment within the preceding 2 weeks.
2- Manifestations of infection within the preceding 2 weeks.

All Patients were subjected to:

- Full history taking.
- Thorough clinical examination.
- The following investigations: Complete blood count, Liver function tests aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl-transferase (GGT), total bilirubin (TB) direct bilirubin (DB), total proteins (TP), Albumin (Alb) and prothrombin concentration (PT).
- TORCH screening.
- Abdominal ultrasound.
- Liver biopsy.
- Stool PCR for Klebsiella in stool sample.

Control group were subjected to:

- Full history taking.
- Thorough clinical examination.
- Stool PCR for Klebsiella in stool sample.

Isolation of gut microbiota:

1- Sampling: Fecal samples were obtained from baby diapers by means of a sterile swab in hospital & immediately frozen in sterile vial at –80°C until genomic DNA extraction.
2- Genomic DNA was extracted from frozen fecal samples using Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). To optimize the results and enhance the yield of DNA, a protocol modification derived from QIAGEN was implemented.
3- Real Time Quantitative PCR: This protocol outlines the steps for conducting qPCR experiments to amplify bacterial genomic DNA from human stool samples using Sybr Green as the detection method. The primer for the bacterial target was:

Klebsiella F - CGC GTA CTA TAC GCC ATG AAC GTA	[11]
R - ACC GTT GAT CAC TTC GGT CAG	

In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie the lower the Ct level the greater the amount of target nucleic acid in the sample). Quantitative analysis of Ct values was performed on the amplification curves generated during qPCR.

Results

10 infants with BA, 6 males & 4 females, had a mean age of (67.3) days and 10 infants as control group, 5 males & 5 females, had a mean age of (74.1) days. There was nostatistically difference between the two groups as regard weight, length & head circumference. 70% of BA infants were delivered by C-section while 80% of control infants were delivered by C-section. In BA group, all infants had increased level of serum TB, DB, AST, ALT & GGT. 40% of BA infants had hepatomegaly & 30% had splenomegaly.

Real time PCR founded that there was increase level of Klebsiella in BA group more than control group (p>0.05).

Discussion

Biliary atresia is the end result of a destructive, idiopathic and inflammatory process affecting both the intrahepatic and extrahepatic bile ducts, leading to fibrosis and obliteration of the biliary tract and to biliary cirrhosis. Clinical presentation is in the first few weeks of life with conjugated hyperbilirubine-

mia (dark urine and pale stools); other manifestations, such as failure to thrive, splenomegaly and ascites, appear only later, when surgery is unlikely to be successful. It is the most frequent surgically correctable liver disorder in infancy, as well as the most frequent indication for liver transplantation in pediatric age ***[12]***.

In our study there was no statistically difference between the two studied groups as regard age, sex distribution & mode of delivery.

Caesarean section (C-section) has a significant impact on the gut microbiota of infants, which is associated with a high abundance of opportunistic pathogens such as klebsiella ***[13]***. In this study, there was no significant difference between BA group and control group in the modes of delivery and, thus avoiding the impact of both on the composition of gut microbiota.

Bile acids are critically important for maintaining a healthy gut microbiota (GM). It interacts closely with the intestinal microbiota through the gut-liver axis. Bile drainage into the intestine can reduce the pH of the intestinal environment, inhibit the growth of pathogenic bacteria as klebseilla, maintain the balance of intestinal microorganisms, and affect the composition of GM ***[14]***.

Bile acids and the microbiome influence each other in the gut, where bacteria modify the BA profile, while intestinal BAs regulate the growth of commensal bacteria, maintain barrier integrity, and modulate ***[15]***.

In our study, Klebsiella mean CT was (39.7±1.97) in control group & (31.51±2.56) in BA group which means that there was increase level of Klebsiella in diseased group more than control group. There was significant difference between BA group & control group (*p*-value >0.05). This result agreed with Wang et al., 2020 who found that there was increase in Klebsiella level in BA ***[16]***.

Some studies have shown that there is an interaction between gut microbiota and bile acid homeostasis. Bile acid has a protective effect on intestinal mucosal barrier. However, the drainage of bile acid is limited in infants with BA, which may cause gut microbiota dysbiosis & increasing in pathological bacteria as klebsiella. Conversely, the imbalance of gut microbiota will also affect bile drainage, further affecting the postoperative outcome of BA infants ***[17,18]***.

Liu et al., 2024 also reported that, the infants with BA exhibited unique characteristics of gut microbiota. The composition of gut microbiota in BA patients was significantly different from that in normal children. Compared with the normal control group, the gut microbiota in BA group showed the numbers of Firmicutes and Proteobacteria increased, while the number of Bacteroidetes decreased in BA. Streptococcus and Klebsiella prolife rated in BA ***[17]***.

Limitations: The limitation in the current study is the relatively small sample size of patients & control group.

Conclusion:

Increased level of gut Klebsiella is associated with the incidence of biliary atresia.

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دراسة الكليبسيلا المعوية
فى مرض الرتق الصفراوى

رتق القناة المرارية هو مرض ركود صفروای وليدى يتميز بالتليف والتصلب الالتهابى للقنوات المرارية الصفراوية، مما يؤدى إلى تلف الكبد التدريجى وهو السبب الأكثر شيوعاً لحدوث الركود الصفراوى فى الاشهر الثلاثة الأولى من العمر، وتتمثل الأعراض الإكلينيكية فى زيادة اليرقان (البليروبين) المباشر، البراز الشاحب، البول الداكن، وتضخم الكبد، وفى وقت لاحق قد يحدث تضخم الطحال، الاستسقاء، ارتفاع ضغط الدم الوريدى البابى وعدم القدرة على النمو الطبيعى. ويوصى بإجراء جراحة كاساى وزراعة الكبد كعلاج للمرض. دراسة الكليبسيلا المعوية فى المواليد المصابين بمرض الرتق الصفراوى قد تعطى معلومات قيمة ومفيدة فى التعامل مع هذا المرض.

الهدف من هذه الدراسة: هو توضيف بكتيريا الكليبسيلا المعوية فى المواليد المصابين بمرض الرتق الصفراوى.

اشتملت الدراسة على مجموعة مكونة من ١٠ مواليد مرضى بمرض الرتق الصفراوى ومجموعة مكونة من ١٠ مواليد اصحاء.

النتيجة: ارتفاع مستوى الكليبسيلا المعوية فى مجموعة المرضى أكثر من مجموعة الاصحاء.

الخلاصة: ارتفاع مستوى الكليبسيلا مرتبط بحدوث مرض الرتق الصفراوى.