

Urinary tetrahydroxy bile acids investigation in infantile cholestasis

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To Cite:

Al-Bassam M. Urinary tetrahydroxy bile acids investigation in infantile cholestasis. *Medical Science* 2022; 26:ms293e2283.

doi: <https://doi.org/10.54905/disssi/v26i125/ms293e2283>

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Peer-Review History

Received: 11 May 2022

Reviewed & Revised: 14/May/2022 to 12/July/2022

Accepted: 14 July 2022

Published: 20 July 2022

Peer-review Method

External peer-review was done through double-blind method.

URL: <https://www.discoveryjournals.org/medicalscience>



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ABSTRACT

Background: Tetrahydroxy bile acids present at negligible or undetectable levels in healthy human adults but present at elevated levels in urine of infants with cholestasis clinically are the most valued marker of cholestasis next to serum tests. **Methods:** Twenty subjects with cholestasis, age range between seven days and one year, the study measured urinary tetrahydroxy bile acids for all cases. **Results:** Study found the presence of urinary tetrahydroxy bile acids in new-borns and infants with infantile cholestasis. New-borns and infants with a good prognosis had higher tetrahydroxy bile acids levels than those with a poor prognosis according to their outcomes. **Conclusion:** The study found the incidence of urinary tetrahydroxy bile acids in new-borns and infants with infantile intra-hepatic and extra-hepatic cholestasis. Extra-hepatic cholestatic patients had higher tetrahydroxy bile acids urine level than intra-hepatic, Cytomegalovirus and idiopathic neonatal hepatitis patients had a lesser tetrahydroxy bile acids level than in patients with genetic cholestasis. Tetrahydroxy bile acids play a role in clinical managing and treatment, in addition to their possible defensive properties against hepatic injury.

Keywords: Urinary Tetrahydroxy bile acids, Genetic Cholestasis, Intrahepatic Cholestasis, Extrahepatic Cholestasis.

1. INTRODUCTION

Bile acid is a detergent that is produced in the hepatic part and released into the gastrointestinal tract to facilitate absorption of lipid-derived nutrients; it reabsorbs in the gut part, organizing with nutrients, and returns to the hepatic part via the portal vein; high levels of bile acid are highly toxic, and both entero-hepatic circulation and hepatic manufacture highly organized; additionally, bile acids detected by a nuclear bile acid receptor called the farnesoid X receptor (FXR), which encoded by the NR1H4 gene, and elevated bile acid levels activate FXR, enhancing a program that inhibits hepatocyte bile acid biosynthesis and uptake while increasing exportation (Chiang & Ferrell, 2022).

Cholestasis is caused by any level of deactivation of this bioprocess, which means that the regular elimination of bile from the hepatic part is blocked,

which leads to the abnormal accumulation of bilirubin, lipids, and bile salts in the hepatic and blood (Amirneni et al., 2020). Cholestasis is not the same as conjugated hyperbilirubinemia when the bilirubin is held in the liver and the blood, it divides into:

- (1) Hepatocellular, caused by an infection, damage to the bile transport, and genetic or metabolic problems.
- (2) Biliary, which means there are obstruction and structural problems with extra-hepatic or intra-hepatic bile ducts (Petrescu & DeMorrow, 2021).

Galactosemia is an inborn error of galactose metabolism, resulting from a lack of the enzyme uridine diphosphate galactose-4-epimerase, galactose-1-phosphate uridylyltransferase or galactokinase, galactose-1-phosphate uridylyltransferase deficit is the most recurrent goal for galactosemia and causes the failure to metabolize galactose molecules into glucose-1-phosphate molecules inside the body, so it increases the chance for infants with galactosemia to develop gram-negative sepsis, therefore; it may show sincerely with sepsis and associated jaundice (Lane & Murray, 2017; Teke Kisa et al., 2019).

Tyrosinemia type (1) is a condition of autosomal recessive type, generally; it exists in the neonatal period and may involve in the differential of neonatal hepatic failure, as well acute hepatic failure, infants and neonates with tyrosinemia may exist with ascites, vomiting symptoms, failure to thrive, clinical laboratory hyperbilirubinemia, and clinical laboratory hypoglycemia, in older infants, a more chronic presentation characterizes by neurologic manifestations, growth-failure, and Fanconi-syndrome may be established (Yildiz & Sivri, 2020).

Biliary atresia is a biliary obstruction that starts from progressive fibrosis of intra-hepatic and extra-hepatic bile ducts with unidentified pathogenesis, frequently shows with cholestasis between two and five weeks of life, the presence of acholic stools maybe confirmation and determine biliary obstruction; so, onset usually tracks the onset of jaundice, therefore; if the affected infant has a previous history of physiological jaundice, the growth of cholestasis may go unremarkable and retard the suitable evaluation and management (Ortiz-Perez et al., 2020). Besides, numerous genetic syndromes can have biliary atresia including Alagille-syndrome (Van Tung et al., 2021).

Biliary hypoplasia is the other type of obstructive jaundice with biliary atresia (Fried et al., 2020). The most common reasons for cholestatic liver disease in infancy are alagille syndrome and biliary atresia (Wang et al., 2021). Intra-hepatic cholestasis involves liver disease in an alagille-syndrome, which is of autosomal dominant type, affected by a defect in the Notch signalling pathway that disturbs many organ systems with a phenotypic variability, considered by ductopenia (Kohut et al., 2021). However, ductular proliferation may also be prominent in early infancy and can refer to a dilemma. Proofs of massive duct biliary obstruction raise the concern for biliary atresia (Ahmed et al., 2021).

Progressive familial intra-hepatic cholestasis: separate monogenic disorders results from gene mutation concerns in canalicular hepatobiliary transport, causes liver injury and progressive cholestasis (Fawaz et al., 2017). Studies have revealed the persistent gathering of bile acids in the liver fibrosis and the growth of portal hypertension and cirrhosis, finally requiring liver transplantation or sometimes dangerous hemorrhage secondary to vitamin K deficiency (Lane & Murray, 2017). Idiopathic neonatal hepatitis: prolonged neonatal intra-hepatic cholestasis in the first six months of life, characterized by the presence of "giant cells" beside the absence of other reasons of type infectious, genetic, or obstructive causes (Misra et al., 2021).

Bile acids molecules are necessary for the absorption of lipid-soluble vitamins and lipids. Bile acids homeostasis is closely controlled by nuclear receptors and is directly connected with glucose and lipid metabolism (Evangelakos et al., 2021). In the human body, two primary bile acids, chenodeoxycholic and cholic acids, secrete into a part called the duodenum, then converted into minor bile acids (lithocholic and deoxycholic acids) by gastrointestinal microbiota; other bile acids also exist in human body unlike the recent one they are fewer concentrations than primary (major) and secondary (minor) types (Kriaa et al., 2022).

Hepatic bile secretion and production depend on canalicular or sinusoidal transporters, bile acid metabolic enzymes, and linked membrane proteins; in cholestasis patients, the bile acid profile and bile acid metabolism are changed, leading to the gathering of bile acids with cytotoxic effects, resulting in death by severe liver injury. The cytotoxicity of bile acids relates to their structures; bile acids with advanced toxicity will be with superior hydrophobicity. Both hydroxylation and conjugation detoxify bile acids by increasing their solubility (Boyer & Soroka, 2021; Lee et al., 2017).

Tetrahydroxy bile acid is a hepatoprotective agent in alleviating cholestatic stress and of hydrophilic nature and low toxicity and exists with a most negligible concentration in adults, unlike in infants with intra-hepatic cholestasis, which have a high level in urine; it has been suggested to investigate the incidence of Tetrahydroxy bile acids in neonates and infants with infantile intra- and extra-hepatic cholestasis and tetrahydroxy bile acids association with their outcomes (Sheps et al., 2021).

2. MATERIALS AND METHODS

The study was carried out during the term from January 2022 to March 2022 study, it involves 20 subjects with cholestasis diseases like (Intra-hepatic, Extra-hepatic Cholestasis, Idiopathic Neonatal Cholestasis, Cytomegalovirus, and Tyrosinemia Type I) of age between seven days and eleven to twelve months. The exclusion criteria were characterized by select neonates and infants who did not suffer from diabetes mellitus and dyslipidemia and must not use any therapy such as ursodeoxycholic acid and must stop it two weeks before tetrahydroxy bile acid analysis. All urine samples were collected in Baghdad from Child's Central Teaching Hospital, Digestive Center at Medical City, and Al-Imameen Al-Kademen Medical City. The container was labelled and returned as instructed, then frozen at -20 °C until investigation for determination of bile acid profile by GC-MS. The human urinary tetrahydroxy bile acids profile was obtained from GC-MS Model TQ-8030 Shimadzu Company by Smart Lab. Group in Amman.

The procedure that is followed for the analysis of total plus individual bile acids in bile and urine is:

1. Two micrograms of internal standard (3,12 diol 7-one 5 β -cholanic acid) are added to urine (200 μ l) and bile (15 μ l).
2. 0.15M sodium hydroxide (2ml) was added to each sample, and then heated at 64 °C for 5-10 min.
3. Then, these samples were subjected to a solid-phase extraction using a Bond-Elute C18 cartridge. The C18 cartridge was preconditioned before loading the samples with successive elution of 2 ml of chloroform-methanol (2:1 v/v), methanol, and HPLC water solutions.
4. After loading the samples, the column was washed with 2 ml of HPLC water and n-hexane.
5. The column was left for 10 mins to remove any excess solvents.
6. Bile acids recovered from the cartridge by elution with methanol (5 ml).
7. Then, they hydrolyzed and derived for gas chromatography mass spectrometry (GC-MS) analysis.

3. RESULTS

There is an essential proportion of urinary bile acids in neonates and infants with infantile cholestatic hepatic disease, the patients with a good prognosis had a significantly higher proportion Tetrahydroxy Bile Acids, as this study recommended that the percentage of Tetrahydroxy Bile Acids from total bile acids 30% - 60% is a good prognosis disease, which mean slower disease progression according to their clinical follow up and investigations, in present study there were 20 patients had intra-hepatic, extra-hepatic cholestasis, Cytomegalovirus, Tyrosinemia Type 1, Idiopathic Neonatal Hepatitis experienced urinary Bile Acids profile analysis, 8 of them had more than 30% Tetrahydroxy Bile Acids from total bile acids according to their good outcomes as shown in table (1) and the average of percentage represents in figure (1) which suggested they were in good prognosis of disease because when Tetrahydroxy Bile Acids are highly hydrophilic and excrete with urine, according to (Lee et al., 2017) and (Sheps et al., 2021) studies, this present study divides the value of tetrahydroxy bile acid to urine creatinine and divides the total bile acid to urine creatinine and divides the value of tetrahydroxy bile acid to total bile acid making as percentage values calculated by the following equation:

Actual Tetrahydroxy bile acid Conc. = Tetrahydroxy bile acid/urine creatinine Conc.

Actual Total Bile Acid Conc. = Total Bile Acid/urine creatinine Conc.

Percentage of prognosis = Actual Tetrahydroxy bile acid Conc./Actual Total Bile Acid Conc.*100 (Lee et al., 2017).

Table 1 Comparison of Urinary Bile Acids between cholestatic patients.

Type of Cholestasis Number (20)	Good Prognosis (30%-60%)	Poor Prognosis (< 30%)
Intra-hepatic Cholestasis Number (7)	30.40% 36.80% 31.10%	20.50% 21.00% 22.70% 23.00%
Extra-hepatic Cholestasis Number (5)	42.60% 43.50%	29.10% 27.70% 28.60%
Cytomegalovirus Number (3)	37.10%	24.00% 23.56%

Idiopathic Neonatal Hepatitis Number (2)	39.20%	25.90%
Tyrosinemia type 1 Number (3)	41.50%	27.10% 28.00%

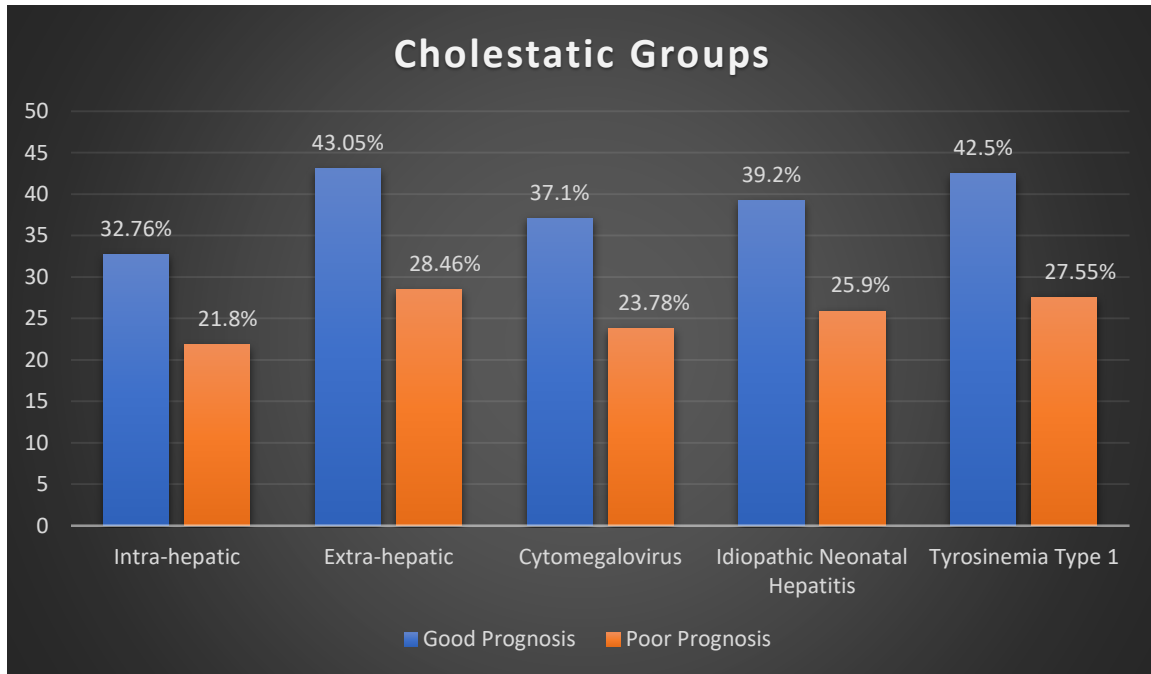


Figure 1 Average of Tetrahydroxy Bile Acids Percentage in Cholestatic Groups

4. DISCUSSION

The current study establishes the relationship of Tetrahydroxy bile acids concentration, which represents an essential proportion of urinary bile acids in neonates and infants with infantile cholestatic hepatic disease, the neonates and infants with a good prognosis had an essential higher proportion Tetrahydroxy bile acids, as this study recommends that the percentage of Tetrahydroxy bile acids from total bile acids 30%-60% is a good prognosis disease, which means slower disease progression, but the percentage decreases in some cases of intra- and extra-hepatic cholestasis as this study assumes to less than 30% as represent in table (1) due to the other clinical evaluation symptoms from the presence of acholic stool and dark urine in poor prognostic patients and the absence of these symptoms in good prognostic patients, Tetrahydroxy bile acids more than 30% from total bile acids suggests patients are in a good prognosis of disease because Tetrahydroxy bile acids are highly hydrophilic in nature and excrete with urine and exist in human body mainly during the neonatal period (Sheps et al., 2021).

Tetrahydroxy bile acids detect among urinary bile acids between seven days and one year after birth, with the highest concentration in the first month, and their concentrations stay steady from two to one year, as seen in the present study in cases of intra-hepatic and extra-hepatic cholestasis after one year of age the level of Tetrahydroxy bile acids decreases in compared to before one year old, which specifies that almost all neonates and infants with cholestasis capable of synthesizing a noteworthy amount of Tetrahydroxy bile acids, which have little toxicity, and may connects with a good outcome (Sato et al., 2020).

The study determines the average of Tetrahydroxy bile acids proportion in patients with different disease objects, neonates and infants with cholestasis caused by cytomegalovirus, idiopathic neonatal hepatitis tend to have a lesser ratio of Tetrahydroxy bile acids than a cholestatic genetic disease, as disagree with (Lee et al., 2017) and agree with (Wang et al., 2019) as shown in figure (1), because explanations for the variations of the patient's ability to create Tetrahydroxy Bile Acids are unknown, and the metabolic changes that happen during different growing stages, these variations may connect with differences in bile acid metabolic enzyme activities because of a genetic variation, differences in bile acid metabolism in other disease objects or discrepancies in the degree and type of injury to hepatocytes, clinically; it is easy to carry out the urine sample collection, and information is not influenced by

fasting conditions after correction by creatinine and investigates have high stability and low variability compared with investigates of serum bile acids levels (Di Ciaula et al., 2017).

The present study also suggests that Tetrahydroxy Bile Acids may have a therapeutic function. Administering adrenocorticotrophic hormones to humans fifty years ago is credited with encouraging the synthesis of tetrahydroxy bile acids. Some evidence suggests that taurine, phenobarbital, and rifampin induce the production of tetrahydroxy bile acids in humans (Gui et al., 2021). However, most have temporary effects. This study's findings suggest that humans can produce Tetrahydroxy Bile Acids under certain conditions. It would be of great clinical interest to further investigate inducible or exogenous tetrahydroxy bile acids as potential therapeutic agents.

5. CONCLUSION

The study reports the incidence of urinary tetrahydroxy bile acids in neonates and infants with infantile intra-hepatic and extra-hepatic cholestasis. Neonates and infants with a good prognosis have the highest tetrahydroxy bile acids levels than neonates and infants with a poor prognosis. The clinical proposals of the present study results influence patient diagnosis and management Firstly, urinary bile acid lab. The analysis isn't carried out in most hospitals; it must incorporate in the investigational study of chronic cholestatic neonates and infants with prolonged lists of metabolic and phenotypic defects linked with inborn errors of bile acid metabolism have previously stated.

Limitations

This research has many limitations. First, the exact cholestatic disorder unit and its pathophysiology directly impact the outcomes of neonates and infants. However, Tetrahydroxy Bile Acids may only play a minor role in the outcomes, or they may reveal an effect of a specific disease-induced hepatic injury. The study concludes that patients with less preserved liver function or more severe liver disease cannot produce Tetrahydroxy Bile Acids as a compensatory and protective mechanism during cholestasis. Determining the mechanism by which Tetrahydroxy Bile Acids are produced in patients with cholestasis is significant because it reveals the underlying association between the disease and the outcomes of neonates and infants; secondly, neonates and infants are a highly diverse group with many different diagnoses. Due to the scarcity of mutations, only a small number of neonates and infants are incorporated with each genetic defect. It is, therefore, necessary to evaluate a larger patient population to confirm the bile acid profile changes specific to each genetic and metabolic disease.

Compliance with ethical standards

The work has been cleared by the Ethics committee of institutional board review of medicine college, Alnahrain University with registration number 14/75/666/01102017.

Acknowledgments

From my deep heart special thanks to all staff members of Al- Emamain Al-Kadhemiyayn medical city, Child's Central Teaching Hospital and Digestive Center at Medical City in Baghdad and to Smart Lab. Group staff in Amman.

Funding

This study has not received any external funding.

Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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