EXPLORING URINARY BILE ACIDS AS POTENTIAL MARKERS OF METABOLISM: REFERENCE VALUES IN CHILDREN BY TARGETED LC-MS/MS

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INTRODUCTION

Bile acids (BA) are C47 steroids synthesized in liver from cholesterol1. They can be conjugated by amidation2 and sulfation3. While BA’s role as emulsifiers has been known for long, their additional endocrine functions have lately aroused interest2. In comparison to BA in blood, it is surprising that hardly any data exist on BA in the most accessible human biofluid urine – especially when it comes to children.

AIM

• Development and validation of a targeted LC-MS/MS method for measurement of 18 urinary BA
• Establishment of reference values for urinary BA in healthy children aged 3-18 years

METHOD

2 mL of 24-hour urine were used for sample preparation comprising protein precipitation (acetone- ZnSO4) and solid phase extraction (C18 cartridges). Reversed-phase liquid chromatography was done on a phenyl-hexyl column, followed by tandem mass spectrometry with a triple quadrupole mass spectrometer using electrospray ionization (ESI) in the negative mode.

RESULTS

The method achieved good linearity (R2 > 0.99) and recovery (90.49% - 113.99%). Intra-day/inter-day precision and accuracy ranged from 0.42% to 11.47% and 85.75% to 110.93%, respectively. No significant matrix effect was observed. CA (median: 55.2 ng/mL) and GCA (48.9 ng/mL) were the two dominant non-sulfated BA. However, sulfated BA showed much higher concentrations, with GCDC-S (337.5 ng/mL) showing the highest levels among all BA, followed by GLCA-S (197.4 ng/mL) and GDCA-S (183.2 ng/mL). In total, 86.5% of quantified BA were sulfated. The total concentrations of glycine amidated BA measured were higher than taurine amidated and non-amidated ones. No obvious trends between urinary BA and age or sex, respectively were observed.

CONCLUSIONS

• A new method for measuring 18 BA by targeted LC-MS/MS was successfully developed, validated and applied to 24-hour urine samples of 80 healthy children.
• Urinary BA concentrations neither changed with age nor showed a sex difference.
• BA were mostly present in their sulfated form in children’s urine, indicating hepatic sulfation to be a major metabolic pathway for urinary BA excretion in humans.

REFERENCES


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