Journal of Pediatric Urology (2014) 10, 1267–1272

Journal of Pediatric urology

A correlation study between macro- and micro-analysis of pediatric urinary calculi

Mahmoud Abdel-Gawad [a,*], Bedieier Ali-El-Dein [b], Sanjeev Mehta [c], Khaled M. Al-Kohlany [d], Emad Elsobky [e]

[a] *Department of Urology, Emirates International Hospital, Al Ain, United Arab Emirates*
[b] *Mansoura Urology and Nephrology Center, Mansoura University, Egypt*
[c] *National Laboratory for Stone Analysis, Urolab, Ahmedabad, India*
[d] *General Military Hospital, Sana'a, Yemen*
[e] *Al-Noor Hospital, Abu Dhabi, United Arab Emirates*

Received 6 March 2014; accepted 22 June 2014
Available online 7 August 2014

KEYWORDS
Urolithiasis;
Children;
Heavy metals;
Calcium;
Microelements

Abstract *Objective:* The aim was investigate the relationship between macro- and micro-compositions of pediatric urinary stones by using two combined analytical techniques: Fourier transform infrared spectroscopy (FT-IR) and inductively coupled plasma-optical emission spectrometry (ICP-OES).
Materials and methods: A total of 74 consecutive urinary calculi were collected from children. Each stone was divided into two equal portions. One part was analyzed by FT-IR to determine mineralogical composition. The second part underwent analysis by ICP-OES to determine the heavy metals and trace elements contents. The association between mineralogical components and elemental contents was evaluated.
Results: The percentages of mineralogical components of the stones were 78.3% calcium oxalate monohydrate, 63.5% calcium oxalate dihydrate, 24.3% ammonium urate, 13.5% uric acid, 10.9% dahllite, 12.1% brushite, 8.1% ammonium calcium phosphate, 8.1% struvite, 4.5% cysteine, and 2.7% were xanthine. There were seven elements with significant different high concentrations; magnesium, sulfur, strontium, lead, chromium, calcium, and phosphorous. High calcium-containing stones had significant higher contents of magnesium, lead, strontium, and zinc ($p < 0.05$) than low calcium-containing stones. Phosphate stones had significant contents of magnesium, strontium, zinc and chromium when compared to other stones ($p < 0.05$).
Conclusions: Pediatric urinary stones have variable biochemical structures. The stones contained many significant heavy metals and trace elements in different concentrations, and phosphate stones enclosed most of the heavy and trace elements.

* Corresponding author. Emirates International Hospital, P.O. Box: 18088, Al Ain, United Arab Emirates. Tel.: +971 507417673; fax: +971 37636555.
E-mail address: naggarm@yahoo.com (M.Abdel-Gawad).

http://dx.doi.org/10.1016/j.jpurol.2014.06.022
1477-5131/

Introduction

Over the last decade, there have been many studies that indicated an increased incidence of pediatric urolithiasis [1,2]. The identification of urinary stones composition is essential as it provides information about the possible underlying causes of stone formation and, therefore, will be useful in stone therapy. Urinary stones may be regarded as an example of biomineralization, which involves the formation of inorganic minerals by living organisms [3]. However, kidney stones are a pathological manifestation of the phenomenon exhibiting features typical of uncontrolled biomineralization [4]. The exact underlying factors that have led to the increment in pediatric urinary stones have not yet been defined but could be attributed to many possibilities. Changes in the nutritional habits and environmental pollution could have a role in addition to increased pediatric obesity [5,6]. Unfortunately, stone composition studies in the pediatric population are, like adults, limited to routine biochemical analysis using Fourier transform infrared spectroscopy (FT-IR), which cannot detect micro-components of the stones such as heavy metals and trace elements [7]. There are many recent studies that have investigated and supported the association between heavy metals content and formation of adult urinary calculi [8–10]. Currently, no study has attempted to evaluate the concentrations of heavy metals and trace elements in pediatric urolithiasis even though it is a recurrent disease. Nevertheless, quantification of heavy metals and trace elements in urinary stones by routine laboratory methods is limited and incomplete [11]. Alternatively, inductively coupled plasma-optical emission spectrometry (ICP-OES) is a technique that has the capability to detect low levels of major and minor trace elements along with macro-components, such as phosphorous, magnesium, calcium, and potassium, in urinary stones [11,12]. The aim of this study was to analyze the mineralogical macrocomposition of urinary stones in children by using FT-IR, and identification and quantification of heavy metals and trace elements using ICP-OES. The possible interrelationship between different elements was assessed and correlated.

Materials and methods

Patient population

This study was approved by the local scientific ethics committee. A total of 74 consecutive urinary calculi were collected from children below the age of 12 years after open surgery and endoscopic procedures. All patients underwent a preoperative general physical examination, and body mass index was recorded. Initial routine laboratory investigations included urine analysis, culture, and serum uric acid and electrolytes. All patients were primary stone formers. Children with secondary systemic causes such as prematurity, Crohn's disease, cystic fibrosis, renal failure, renal tubular acidosis, and urinary diversion were excluded. Insufficient stone samples for analytical techniques were discarded.

Analytical procedures

The retrieved stones were washed with distilled water to remove loose debris such as blood and mucous, and casts were rewashed with ethanol and air-dried. Stones were stored at ambient conditions until analysis. Each stone was pulverized and weighed and divided into two equal portions. One portion was analyzed by infrared spectroscopy to determine biochemical components using a Nicollet Avatar 330 FTIR spectrophotometer (Thermo Electronic Corporation, Waltham, MA, USA) in the frequency range 600–4000 cm^{-1}. All stones were analyzed using the well-validated method described by Soloway and Wu [12] for gall bladder stone analysis.

The second portion of each stone underwent simultaneous multi-elemental analysis (22 elements) using ICP-OES (ICP-OES Optima 5300DV, Perkin Elmer, MA, USA) to determine heavy metal and trace element contents. In this process, samples were microwave digested: 8.0 mL of hydrochloric acid and 2 mL of nitric acid were added to about 0.25 g of each sample. The mixture was heated up to 180 °C under pressure for 30 min. The digested solution was transferred to a 50-mL volumetric flask and filled up to the mark using deionized water. The final solution was injected to ICP to determine the metal and mineral concentrations. The level of Ca (calcium), Na (sodium), K (potassium), P (phosphorus), Zn (zinc), Mg (magnesium), Fe (iron), Cu (copper), Sr (strontium), S (sulfur), Mn (manganese), Cr (chromium), Mo (molybdenum), Co (cobalt), Cd (cadmium), Ba (barium), Al (aluminum), Pb (lead), B (boron), Se (selenium), Ni (nickel), and As (arsenic) were determined in these stones. The limit of detection (LOD) was >5 mg/kg for As, Cd, Co, Mo, Ni, Se, Sr, and Pb; >20 mg/kg for Al, B, Ba, Cu, Mn, and Zn; and >50 mg/kg for Ca, Fe, K, Mg, Na, P, and S.

Accuracy of the ICP-OES method of analysis was controlled with two supplied certified materials (ICP-OES multi-element solutions IV and VI), which were injected and analyzed before each measurement series of the stones.

Statistical methods

The stones were divided into three groups for analytical purposes based on the mineralogical compositions results of FT-IR: urate stones (low Ca and P), oxalate group (high Ca and low P), and phosphate group (high Ca and P). For association between heavy metals and calcium, all stones were divided into high (Ca $>$ 10,000 ppm) and low calcium-containing stones (Ca $<$ 10,000 ppm). Statistical analysis using the non-parametric Mann–Whitney *U* test and the Kruskal–Wallis test for correlation between different minerals and elements. SPSS 15.0 software was used for descriptive statistics. The difference was considered statistically significant if $p < 0.05$.

Results

Clinical and radiological results

There were 61 (82.4%) stones retrieved from males and 13 (17.6%) from females. Thirty-nine (52.7%) patients were below the age of 5 years and 35 (47.3%) were over 5 years. The associated genitourinary abnormalities were five (6.75%) patients had primary vesicoureteral reflux, nine (12.1%) had urinary tract infection, six (8.1%) patients had pelviureteral junction obstruction with simultaneous renal