Dicle Tıp Dergisi /
Dicle Medical Journal

2016; 43 (2): 199-204
doi: 10.5798/diclemedj.0921.2016.02.0667

ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Ultrastructural Analysis of Urinary Stones by Microfocus Computed Tomography and Comparison with Chemical Analysis

Üriner Sistem Taşlarının Mikrofokus Bilgisayarlı Tomografi ile Ultrastrüktürel Yapısının Analizi ve Kimyasal Analizle Karşılaştırılması

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ABSTRACT

Objective: To investigate the ultra-structure of urinary system stones using micro-focus computed tomography (MCT), which makes non-destructive analysis and to compare with wet chemical analysis.

Methods: This study was carried out at the Ankara Training and Research hospital. Renal stones, removed from 30 patients during percutaneous nephrolithotomy (PNL) surgery, were included in the study. The stones were blindly evaluated by the specialists with MCT and chemical analysis.

Results: The comparison of the stone components between chemical analysis and MCT, showed that the rate of consistence was very low ($p<0.001$). There was no significant relationship between the components of stone and its heterogeneity or homogeneity. No significant difference was found between the stones with single component and those with multiple components in terms of its 3D structure being homogenous or heterogeneous and the presence of voids ($p>0.05$). It was also seen that there was no significant relation between its 3D structure being heterogeneous or homogenous.

Conclusion: The stone analysis with MCT is a time consuming and costly method. This method is useful to understand the mechanisms of stone formation and an important guide to develop the future treatment modalities.

Key words: Computed tomography, micro, urinary stone analysis, urolithiasis

ÖZET

Amaç: Mikro bilgisayarlı tomografi (MCT) yöntemiyle üriner sistem taşlarının non- destruktif olarak ultrastrüktürel yapısının incelenmesi ve kimyasal analiz ile karşılaştırılması amaçlanmıştır

Yöntemler: Bu çalışma Ankara Eğitim ve Araştırma Hastanesinde etik kurul onamını takiben yapıldı. Perkutan nefrolitotomi sırasında 30 hastadan çıkarılan intakt taşlar çalışmaya dahil edildi. Taşlar MCT ve takiben kimyasal analiz yöntemleriyle analiz edildi.

Bulgular: Kimyasal analiz ile elde edilen taş komponentleriyle MCT ile tespit edilen taş komponentleri arasında bire-bir uyum değerlendirildiğinde uyum oranının istatistiksel olarak çok düşük olduğu görülmüştür ($p<0{,}001$). MCT yöntemine göre taşların içerdiği her bir bileşen yönünden taşın üç boyutlu (3D) yapısının homojen veya heterojen olma sıklığında istatistiksel olarak anlamlı değişimin olmadığı görülmüştür. Tek ve çoklu içeriğe sahip olan taşlar arasında taşın üç boyutlu yapısının homojen/heterojen olma sıklığında ve içerdikleri havada boşluk olup olmaması oranlarında istatistiksel olarak anlamlı farklılık bulunmamıştır ($p>0{,}05$). Ayrıca taşın üç boyutlu yapısıyla içeriğinin homojen ya da heterojen olması arasında bağlantı saptanamamıştır.

Sonuç: Mikro bilgisayarlı tomografi ile taş analizinin oldukça zaman alıcı ve çok yüksek maliyetli bir yöntem olduğu görülmektedir. Bu yöntemin taş oluşum mekanizmaları ve gelecekte yapılabilecek tedavi yöntemlerine faydası olabileceği düşünülmektedir.

Anahtar kelimeler: Bilgisayarlı tomografi, mikro, üriner taş analizi, ürolitiyazis

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Geliş Tarihi / Received: 16.03.2016, Kabul Tarihi / Accepted: 01.04.2016

INTRODUCTION

Methods, used in the analysis of urinary system stones, are mostly destructive. The stones are classified according to type of mineral, which is most abundant in them [1]. The most abundant mineral leads to the evaluation of a stone, which has actually a complex structure, as a homogenous single type stone and to its treatment accordingly. There is not an established method showing all of the elements in the structure of heterogeneous stones and their distribution. Therefore, stones as are referred to with terms such as calcium oxalate, uric acid, apatite, struvite and cystine stones. This tends to underestimate the complexity of an individual's stone history as, indeed, it has been determined that the vast majority of stones actually contain more than one type of mineral.

The mostly used urinary stone analysis methods are spectroscopy, X-ray diffraction, chemical analysis, mass spectrometry, and laser induced breakdown spectroscopy. X-ray diffraction and IR spectroscopy are reference techniques for stone analysis [2]. The chemical method can fairly identify the small amounts of an element but cannot usually identify a compound as such, and in stones of mixed composition, the results merely indicate which ions and radicals are present. Unfortunately, chemical methods are destructive and need several milligrams of the sample, so small stones cannot be analyzed with chemical methods [3].

The fragility of the stones and the factors influencing fragility are not known completely. However, there are some clues. For example, cystine stones with rough surface are easily broken whilst those with smooth surface are more difficult to break [4]. Some stones contain radiolucent areas; it is thought that these defects are the weak points of stones. Hence, the fragility of a stone is directly related to its inner structure [5].

The aim of the present study is to analyze the ultra-structure of urinary system stones with MCT method.

METHODS

Following the approval of local ethics committee, renal stones, removed from 30 patients during percutaneous nephrolithotomy (PNL), were included in the study. The samples were selected from stones that were not destroyed with any kind of lithotripter. The stone fragility wasn't detected by any lithotripter to keep the stones intact.

Stones were numbered and collected in containers. Then, the stones were screened non-destructively with Skyscan 1174 micro-focus computed tomography device and the images were processed. In view of the previous literature, they were analyzed according to tomography units. Afterwards, they were submitted to wet chemical analysis.

Wet Chemical Analysis Method

This is the most widely employed technique for stone analysis in routine clinic laboratories. The principal of this method is the color indicators such as addition of ammonium molybdate and 1-amino-2-naphthol-4-sulphonic acid solution. A blue color shows the presence of phosphate.

Wet chemical analysis (WCA) detects calcium and oxalate separately and therefore cannot differentiate crystalline types of Calcium oxalate (CAOx) as Calcium oxalate monohydrate (COM) or calcium oxalate dihydrate (COD). Cystine stones may easily be confused with urate stones if submitted to chemical analysis only.

Micro-focus Computed Tomography Method

MCT obtains sectional images using x-rays and reconstructs 3D images of the objects by combining these images. The term micro is based upon the expression of sectional images obtained by the device in micrometers. MCT allows a spatial resolution of less than 10 mm corresponding to a voxel (three dimensional counterpart of pixel) size of almost 1x106 mm3. As in "Macro" CT screening devices, they can be reconstructed and analyzed without destroying internal structure. In the analysis of urinary stones, 2D and 3D images are obtained with measurements at 6-30 µm voxel size and 3D images are obtained at multiple planes with image averaging ratio 3.

All samples were scanned using a desktop x-ray micro-focus computed tomography scanner (Skyscan 1174, Skyscan, Aartselaar, Belgium) at Department of Anatomy, Faculty of Medicine, Hacettepe University, Ankara, Turkey. MCT scanning technique was applied to the stones ex-vivo. Scanning time was 60 to 120 minutes. The scanning procedure was completed using 50 kV x-ray tube voltages, 800 μA anode current. There were 120-180 panoramic images with 3 degree rotation step, resulting in a pixel size of 10 to 18 μm.

This digital data is further elaborated with a reconstruction software (NRecon) for attenuation measurement and 3D model creative software (CTan) for surface rendering. The evaluated parameters with MCT were presence or absence of homogeneity, heterogeneity and internal voids in ultra-structural body of stones. In the transverse sections; we defined homogeneous stones, which have only one density, and heterogeneous stones have more than two densities. Homogeneous pattern was accepted as a smooth type, which could be broken with difficulty, while heterogeneous pattern was accepted as a rough type that could be broken easily (Figure-1). We investigated the stones three dimensionally from the top to the bottom in sequential horizontal sections. The region of interest of our sections is whole stone that can be seen in sections.

Attenuation values of minerals were determined in Hounsfield Unit (HU) shown with table-1. The concordance of our results was criticized with the results of the conventional and MCT studies from the literature [5-7].

Statistical Analysis

Analysis of the data was made in SPSS for Windows 11.5 program. Descriptive statistics were expressed as the number of observations and percent (%) was investigated whether the results of stone analysis carried out with MCT were in accord with the results of chemical analysis. In order to determine consistency in each variable, sensitivity, specifity, positive and negative predictive values and diagnostic accuracy rates were calculated. Nominal variables were evaluated with Pearson's chi-square or Fisher's exact chi-square test. P value of <0.05 was considered significant for all results.

RESULTS

In MCT, stones were classified according to their attenuation values, but some stones remained in transition zones due to their close values and it was not possible to decide on their exact category.

In the analysis performed with MCT method, there were more than one components in 11 of 30 stones (37%), 2 (6%) contained cystine, 6 (20%) uric acid, 1 (3%) hydroxyapatite, 22 (73%) calcium oxalate monohydrate (COM) and 5 (16%) Calcium oxalate dehydrate (COD) while in 3 (10%) mineral could not be classified.

While in chemical analysis method, hydroxyapatite was found in 24 of 30 stones (80%), in MCT 3 hydroxyapatite was analyzed normally, hydroxyapatite stones are normally expected to occur at the rate of 2%. Their being seen at the rate of 80% in chemical analysis suggests false positivity.

When the consistency between stone components found with chemical analysis and those found with MCT was evaluated, the rate of consistency was found to be very low. ($p<0.001$)

Table 1. Attenuation values of minerals in Hounsfield Unit (HU)

Stone Type	Attenuation value (HU)
Hydroxy-Apatite	620
COM	380-560
COD	300-370
Cystine	250-290
Uric acid	190-260

COM: Calcium oxalate monohydrate, COD: Calcium oxalate dihydrate

Table 2. The relation between 3D structure and the components of the stones

Variables	Heterogeneous n (%)	Homogeneous n (%)
Single component	2 (10)	18 (90)
Multiple component	4 (40)	6 (60)
Internal voids	3 (50)	9 (37.5)

The efficacy of MCT method in correctly classifying stones with CaOx or uric acid content was compared to that of chemical analysis and it was established that MCT method was 100% successful in differentiating stones with CaOx than those without but, MCT was found to have low sensitivity and positive predictive value in detecting uric acid.

According to MCT data, there was no significant relationship between the component type in the stone and its' heterogeneity or homogeneity (p>0.05). No significant difference was found between stones with single component and multiple components in terms of its 3D structure being homogenous or heterogeneous and the presence of voids. (p> 0.05). It was established that there was no significant relation between the presence of void in the stone and its 3D structure (p= 0.660) (Table 2).

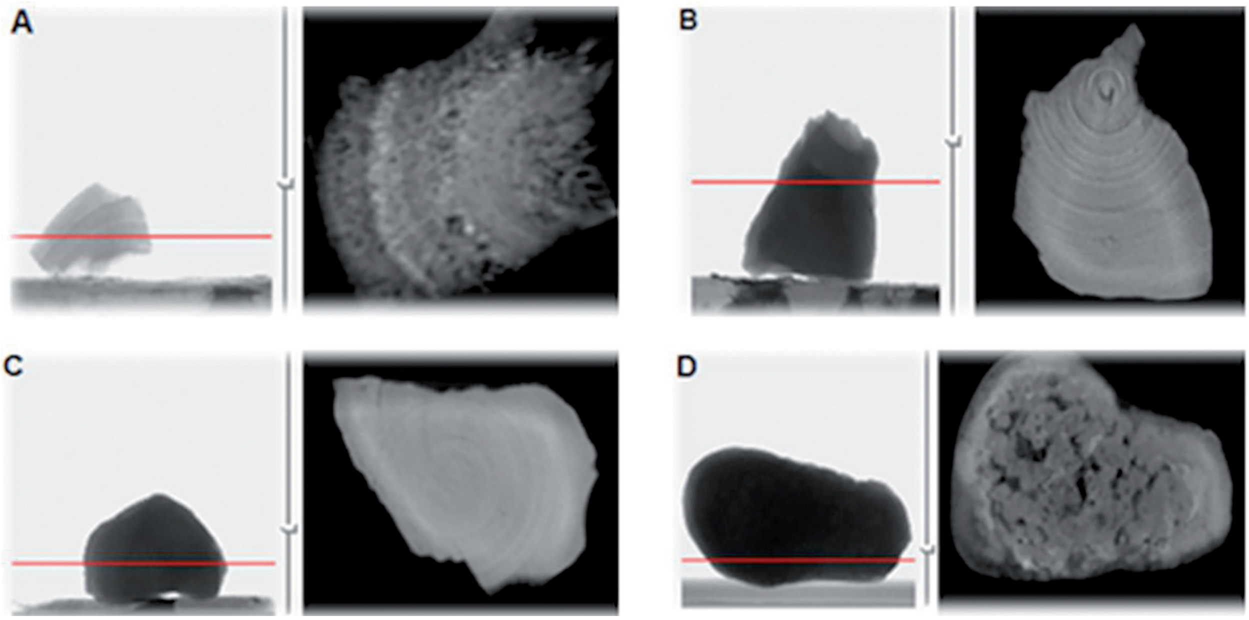

Figure 1. Micro-focus computed tomography images of the stones. The red line shows the cross section of the stones. A: Heterogeneous in appearance but homogeneous in content uric acid stone. B: Homogeneous in appearance but heterogeneous in content uric acid and Calcium oxalate monohydrate (COM) stone. C: homogeneous in both appearance and content a COM stone. D: A highly heterogeneous mixed COM and Calcium oxalate dihydrate stone includes many internal voids.

DISCUSSION

The analysis of urinary system stones use techniques that destroying the structure and termed according to the mineral, which is most abundant in the stone [8]. Chemical analysis is not accurate and can lead to clinically significant errors [9].

The variability in stone fragility may be related to the differences in stone structure [10,11]. To support this hypothesis, Leger et al. [12] reported that stones that were highly organized in their crystalline structure broke more easily than those that were less organized. Additionally, Williams et al. [13] hypothesized that the presence of voids and/or apatite regions could correlate with altered matrix protein content on MCT evaluation. Alexander Randall in the late 1930's proposed that stones grow on the renal papilla attached to underlying interstitial apatite deposits [14]. The common idiopathic calcium oxalate stone former most stones do indeed grow attached to the papillae, on plaque. In our study we haven't see the evidence of Randalls' plaque maybe because the stones were collected randomly and most of them were not attached to the papilla.

MCT shows detailed internal structure of the stones as seen in our study. The chemical component of a stone is not related with its 3D structure. A chemically homogeneous stone may be heterogeneous in 3D structure. Huri et al. [5] showed that the fragility of the stone is related to its heterogeneity and internal voids. 3D structure may explain why the same chemical structure shows different fragility.

In the view of information with using this method, we can evaluate the existence of other minerals in its content in addition to the dominant mineral, describe the nucleus of the stone and the structures extending from the nucleus to the outer surface and store the obtained images as creating an archive which has contribution to the future studies. Zarse et. Al. [1] investigated stones with MCT in according to their minerals' attenuation values respectively uric acid 3515 – 4995, Struvite 7242 -7969, Cystine 8619 – 9921, COD 13815 – 15797, COM 16297 –18449 and hydroxy apatite 21144 – 23121. Our results are consistent with these data.

MCT shows detailed analysis of a stone but forming the 3D structure is still unknown. Analysis of more stone with MCT and in vitro fragmentation methods will give us more information about stone fragility. In the future 3D structure would provide a new classification to determine stone fragility instead of chemical composition.

Motley et al. [15] used non contrast computed tomography as a diagnostic tool for the identification of the stones and found that the mean HU values of 87 calcium stones (440±262), 7 uric acid stones (270±134), 4 struvite stones (401±198), and 2 cystine stones (248±0).

Clinical helical CT is increasingly used in clinical practice and yields better images with high resolution [16]. Higher quality images were obtained especially with the development of multi-detector helical CT [17]. Williams et al. [18] used four-row multi-detector CT to show that some degree of internal structure can already be seen in urinary stones. Hillman et al. [19] could differentiate pure COM, UA, and struvite calculi using absolute HU values. Mostafavi et al. (20) using the absolute HU values at 120 kV, could differentiate the three pure types (Uric Acid, COM and struvite). Since Helical multi-detector CT makes 3D reconstruction possible as MCT does, with its development, more in vivo information can be obtained on the structure and fragility of stones and their differentiation and management can be planned accordingly.

The analysis of the stone with MCT is a method, which we can rely on in the evaluation of the mineral composition and the structure of the stone. No direct relation was found between the chemical structure of the stone and its three dimensional structure as seen in the present study. No convincing hypothesis has been put forward to explain how the three dimensional structure develops, why some stones are homogenous while others are heterogeneous and why there are voids in some of them.

In conclusion, although stone analysis with MCT is a time consuming and costly method, it gives us detailed information about three 3D structures of the stones. We think that MCT method will shed more light on mechanisms of stone formation and treatment approaches through larger and more detailed studies.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

Financial Disclosure: No financial support was received.

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