Fourier Transform Infrared Analysis of Urinary Calculi and Metabolic Studies in a Group of Sicilian Children

Maria Michela D'Alessandro,[1] Giuseppe Gennaro,[2] Pietro Tralongo,[3] Silvio Maringhini[1]

[1]UO Nefrologia e Dialisi Ospedale "G Di Cristina" ARNAS Palermo, Palermo, Italy
[2]Dipartimento STEBICEF, sezione "Chimica", Università degli Studi di Palermo, Palermo, Italy
[3]Università degli Studi di Palermo, Corso di Medicina e Chirurgia, Palermo, Italy

Keywords. urolithiasis, oxaluria, cytraturia, spectrometric analysis

Introduction. Prevalence of urinary calculi in children has been increasing in the past years. We performed an analysis of the chemical composition of stones formers of the pediatric population in our geographical area over the years 2005 to 2013.
Materials and Methods. Fourier transform infrared spectroscopy was employed for the determination of the calculus composition of a group of Sicilian children, and metabolic studies were performed to formulate the correct diagnosis and establish therapy.
Results. The prevalence of stone formation was much higher for boys than for girls, with a sex ratio of 1.9:1. The single most frequent component was found to be calcium oxalate monohydrate, and calcium oxalates (pure or mixed calculi) were the overall most frequent components. Calcium phosphates ranked 2nd for frequency, most often in mixed calculi, while urates ranked 3rd. The metabolic disorder most often associated with pure calcium oxalate monohydrate calculi was hypocitraturia, while hyperoxaluria was predominantly associated with calcium oxalate dihydrate calculi.
Conclusions. Mixed calculi had the highest prevalence in our pediatric population. Our data showed that Fourier transform infrared spectroscopy was a useful tool for the determination of the calculi composition.

IJKD 2017;11:209-16
www.ijkd.org

Original Paper

INTRODUCTION

Urolithiasis in pediatric age is estimated to have a 2% to 3% incidence against a 10% to 15% in adults[1]; there is a growing evidence that urolithiasis in children is rising.[1,2] Both genetic and environmental factors have a role in the formation of urinary calculi in the pediatric age.[3] Many studies on the composition of the urinary calculi and on the associated metabolic disorders or environmental factors causing them in pediatric age have been reported.[3-7] Very few studies, however, have exploited the great potential of Fourier transform infrared (FTIR) spectroscopy for the analysis, qualitative and possibly quantitative, of pediatric urinary calculi.[8] We report our data on FTIR spectroscopy in this prospective study, compared with metabolic studies in children followed at our center. Urinary calculus disease is unfortunately a largely diffuse one and is, moreover, progressively rising throughout the world. Furthermore, even after symptomatic treatment and elimination of the calculi, recurrence rates might be unacceptably high if appropriate metabolic investigations and follow-up are not performed.

Such a knowledge can first of all help in choosing the best method for the removal of the calculi, since, for instance, one component might be more susceptible to a particular treatment than another. The second point of interest is the possibility to associate the composition of the calculus with

the underlying metabolic disorder or abnormal urinary composition, allowing correct medical diagnosis and proper long-term follow-up; the calculus composition analysis should, however, be accompanied by blood and urine analysis for the assessment of the proper treatment.

MATERIALS AND METHODS

Patients

In the past 8 years, we diagnosed urolithiasis in 252 children with a mean age of 9 years (range, zero to 18 years). Only 85 children (56 boys and 29 girls) eliminated the calculi, and a complete metabolic study was available only in 29 of these children.[9]

The inclusion criterion was the diagnosis of urolithiasis with the ultrasonography; there were no exclusion criteria. The diagnosis of urolithiasis was based on anamnesis, medical examination, blood and urine laboratory tests, instrumental determinations (plain abdominal radiography, ultrasonography, and computed tomography) and FTIR calculus analysis, when the calculi were recovered by surgery or by expulsion.

All of the 252 children underwent investigations that included blood test for blood urea nitrogen; serum creatinine, uric acid, and serum levels of sodium, potassium, chloride, calcium, phosphorus, magnesium, and bicarbonate; urinary sediment; urine pH; and urine levels of creatinine, uric acid, sodium, potassium, chloride, calcium, and phosphorus measured in the urine collected over 24 hours. For children aged less than 3 years, we used the first morning urine determination of the ratio between solutes and creatinine (spot urine).

For 85 children, the following analyses were additionally performed: blood test for parathyroid hormone, vitamin D, and amino acids; 24-hour urine examination for creatinine, glucose, proteins, oxalic acid, citrate, and amino acids; and fasting urine dosage of creatinine, oxalic acid, tubular enzymes, and microalbumin. Only 29 patients performed these investigations: oxalemia, hematic, and urinary glycolic acid, and genetic analysis (distal renal tubular acidosis; Bartter syndrome; Gitelman syndrome; 1st, 2nd, and 3rd types hyperoxaluria; cystinuria; and loss-of-function mutations of *CYP24A1*).

Children with urolithiasis documented by ultrasonography, two months after their hospitalization for an acute event, and in the absence of any pharmacological or diet therapy, were firstly tested for the first-level investigations, then for oxaluria, citraturia, calciuria, and creatininuria in the 24-hour urine collection (or spot urine for children aged less than 3) and in fasting urine. The same tests were performed in 100 healthy control children, matched for age and sex; the control group was randomly selected from 2 schools (age range, 5 to 13 years). The reference pediatric 24 hour urinary and haematic parameters were used according to the Western literature.

Instrumental Equipment and Methods

The infrared spectra were recorded with a Jasco 420 instrument, exploring the spectral range from 4000 cm^{-1} to 200 cm^{-1} and collecting the spectra in the absorbance mode. The samples were prepared as potassium bromide pellets mixing in an agate mortar a weighed amount (90mg to 100 mg) of potassium bromide with 1 mg to 2 mg of the calculus under examination, previously powdered. Potassium bromide (Sigma-Aldrich, FTIR-grade) was previously dried for at least 12 hours in an oven at 120°C and then stored under vacuum in a desiccator over silica gel. The finely ground powder thus obtained was then transferred to the appropriate die (Specac Atlas evacuable pellet die), routinely kept under vacuum in a desiccator, and made into a 13-mm diameter transparent pellet by application of a 10-tons/cm^2 pressure by means of a manual hydraulic press (Specac Atlas Series) for about 10 minutes.

The Abbot C8000 automatic chemistry analyser was employed for enzymatic or colorimetric determination of hematochemical and chemicoclinical urinary parameters. The Roche Modular ES100 was used for the parathyroid hormone dosage by electrochemiluminescence. The 25-hydroxyvitamin D dosage was performed on a Pantec microplate by an enzyme-linked immunosorbent assay test kit. Oxaluria and citraturia were determined by the HPLC (Agilent UP 1200 Series) and blood acid-base equilibrium by hemogasanalysis (Instrumentation Laboratory GEM Premier 4000).

RESULTS

Only 85 (56 boys and 29 girls) of the 252 children followed for urolithiasis expelled the calculi, which

were analyzed only by FTIR spectroscopy. The results obtained are summarized in Table 1. For 29 of the 85 patients, it was possible to perform a complete metabolic study and the prevalence of the different calculus components is summarized in Table 2.

We observed a strict connection between the levels of oxalate and citrate in the urine and the formation of oxalate calculi. The 29 patients, with calculi of different types, showing one or the other of the three metabolic behaviors, are reported in Figures 1, 2, and 3. In Figures 1 and 2, the control groups are the same as those from the literature). Two cases were worth of special mention. A girl eliminated calculi that were analysed both by wet chemical analysis and by FTIR spectroscopy. The chemical analysis gave the following results for calculus composition: calcium oxalate and uncertain traces of cystine, while spectroscopy indicated that the only component of the calculi was cystine. The FTIR spectrum of the calculus in potassium bromide pellet was compared with that of pure cystine and they were perfectly superimposable, both as wavenumbers and intensity of the signals. On the other hand, a potassium bromide pellet prepared with a mixture of calcium oxalate dihydrate (COD), also known as weddellite, and traces of cystine gave a completely different spectrum (Figure 4).

Another interesting case was a 1-year-old child with acute abdominal pain who had a bladder calculus, which was surgically removed later. The composition of the calculus, as determined by FTIR spectroscopy, appeared to be struvite with approximately 5% of carbapatite; no urinary tract infection was reported, but only of a mild febrile episode at the age of 6 months, resolved with antipyretic. The FTIR result was confirmed by preparing a potassium bromide pellet with a mixture of 95% magnesium ammonium phosphate hexahydrate and 5% carbapatite. The real calculus and the mixture spectra were practically superimposable (Figure 5). Cystography revealed a 4th degree vesicoureteral reflux on the right side.

Table 1. Composition of 110 Urinary Calculi in 85 Children as Determined by Fourier Transform Infrared

Calculus Components	Number of Calculi (%)		
	Boys (n = 56)	Girls (n = 29)	All (n = 85)
Calcium oxalates	41 (73.2)	21 (72.4)	62 (72.9)
Calcium oxalate monohydrate	19 (33.9)	6 (20.7)	25 (29.4)
Calcium oxalate dihydrate	8 (14.3)	2 (6.9)	10 (11.8)
Calcium oxalate monohydrate and dihydrate	5 (8.9)	1 (3.4)	6 (7.1)
Mixed*	9 (16.1)	12 (41.4)	21 (24.7)
Calcium phosphates†	12 (21.4)	15 (51.7)	27 (31.8)
Uric acid and urates†	10 (17.9)	2 (6.9)	12 (14.1)
Struvite†	2 (3.6)	0	2 (2.4)
Cystine	0	2 (6.9)	2 (2.4)
Calcium carbonate	2 (3.6)	3 (10.3)	5 (5.9)
Other	1 (1.8)	2 (6.9)	3 (3.5)

*Variably made of proteins, carbapatite and oxalates, carbapatite, and uric acid.
†Both pure and mixed calculi are included.

Table 2. Patients' and Calculi Characteristics of 29 Children With Complete Metabolic Analysis as Compared With the Control Group

Characteristic	Patients With Calculi			Control Group	*P*
	Boys	Girls	All		
Age, y	…	…	9.1 ± 4.4	10.1 ± 2.9	> .05
Sex					
Male	…	…	19	68	
Female	…	…	10	32	> .05
Calculus component					
Calcium oxalate monohydrate	3	2	5	…	
Calcium oxalate dihydrate	3	0	3	…	
Cystine	0	2	2	…	
Uric acid	2	0	2	…	
Calcium carbonate	2	1	3	…	
Mixed	9	5	14	…	…
Urine oxalate-creatinine ratio, μmol/mmol		…	57.3 ± 32.2	43.2 ± 9.4	< .001
Urine citrate-creatinine ratio, μmol/mmol	…	…	0.10 ± 0.12	0.27 ± 0.32	.06
Urine calcium-creatinine ratio, μmol/mmol	…	…	0.21 ± 0.07	0.05 ± 0.03	< .001
Urine uric acid-creatinine ratio, μmol/mmol	…	…	0.88 ± 0.36	0.84 ± 0.28	> .05

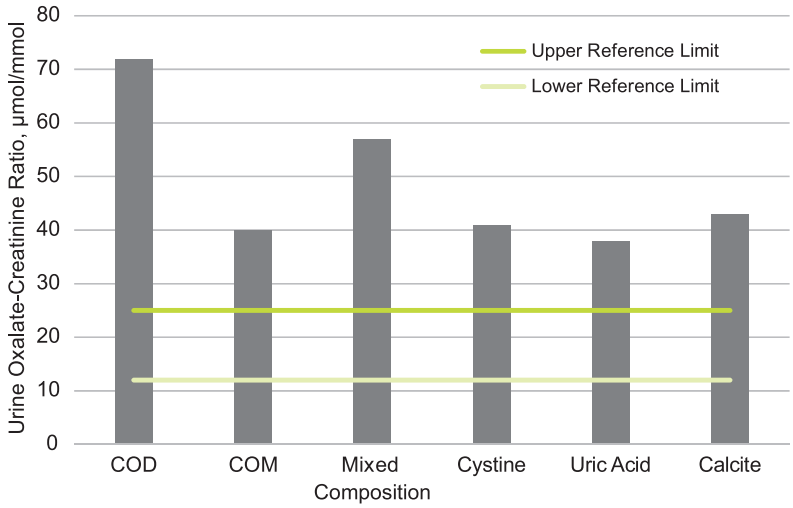

Figure 1. Urinary calculus types and 24-hour urine oxalate-creatinine ratio. COD indicates calcium oxalate dehydrate and COM, calcium oxalate monohydrate.

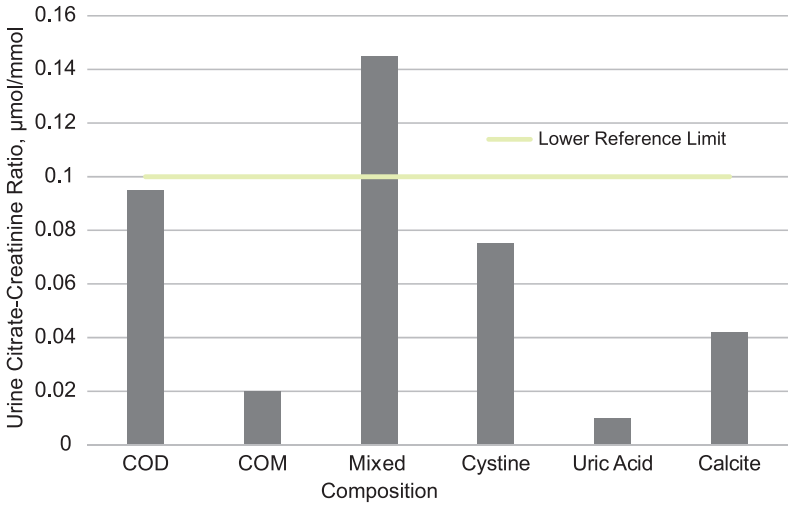

Figure 2. Urinary calculus types and 24-hour urine citrate-creatinine ratio. COD indicates calcium oxalate dehydrate and COM, calcium oxalate monohydrate.

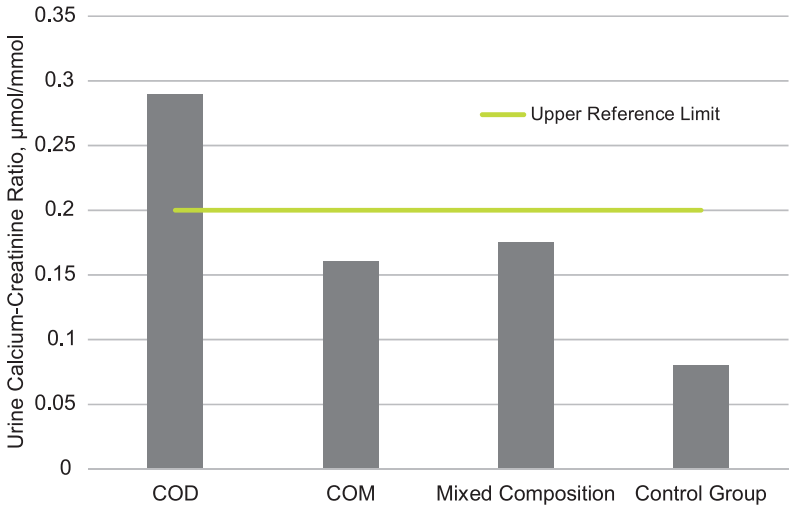

Figure 3. Urinary calculus types and 24-hour urine calcium-creatinine ratio. COD indicates calcium oxalate dehydrate and COM, calcium oxalate monohydrate.

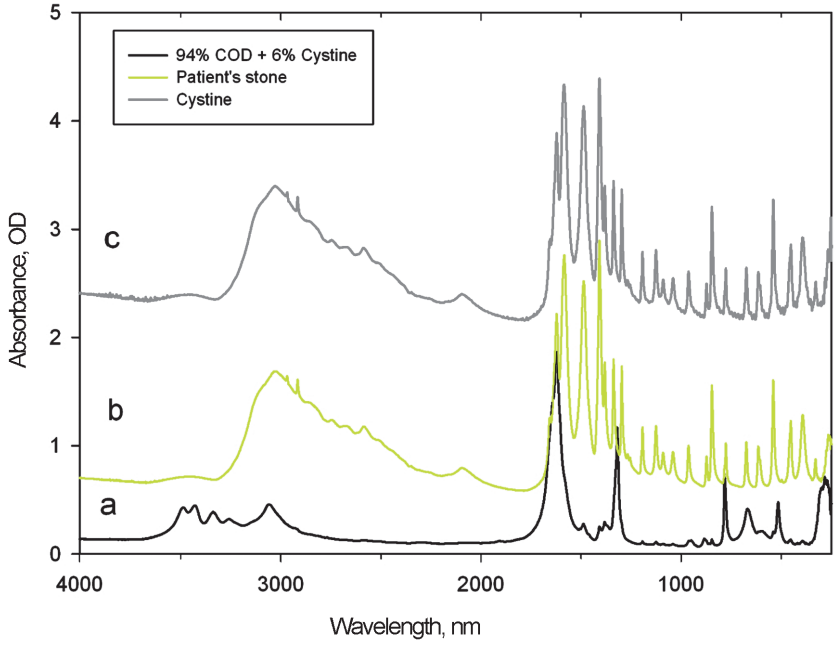

Figure 4. Fourier transform infrared spectra (potassium bromide pellet) of a 94% calcium oxalate dihydrate (COD) plus 6% cysteine mixture, patient's calculus, and pure cysteine. Spectra for the latter two have been displaced by 0.5 and 2.5 OD units, respectively, along the vertical axis for clarity.

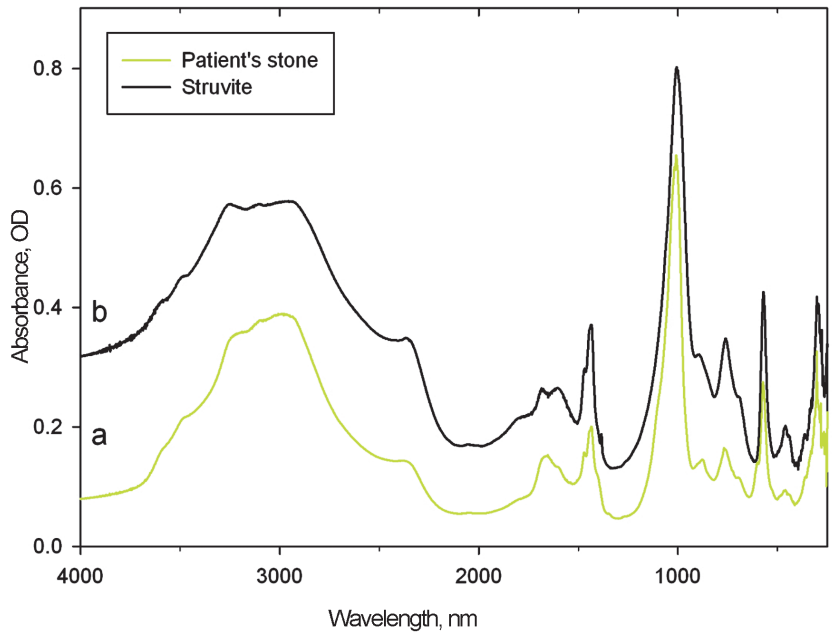

Figure 5. Fourier transform infrared spectra (potassium bromide pellet) of patient's calculus and pure struvite. Spectra for the latter has been displaced by 0.3 OD units along the vertical axis, for clarity.

DISCUSSION

Urinary calculus disease is largely diffuse and is progressively rising throughout the world.[1,2] The incidence of pediatric urolithiasis is significantly increasing over the years.[4] Surgical treatment is less cumbersome than in the past, but recurrence rates might be unacceptably high if appropriate metabolic investigations and follow-up are not performed.[10] A step of primary importance in the management of nephrolithiasis, and in the prevention of recurrences, is to determine the qualitative, and possibly quantitative, composition

of the calculi. Such information may help in choosing the best method for the removal of the calculi.[11] Furthermore, the composition of the calculus and the underlying metabolic disorder allows correct medical diagnosis and proper treatment.[11,12]

Many different techniques are available for the qualitative, and in some cases quantitative, analysis of kidney calculi, that include classical wet chemical analysis and sophisticated techniques such as X-ray diffraction crystallography or polarized microscopy. An overview of the different methods of calculus analysis, with clear comments on their relative advantages and disadvantages, can be found in 2 rather recent reviews.[13,14] Routine calculus analysis in hospital laboratories is mainly performed by traditional wet chemical methods that are easy and low cost. However, chemical analysis has rather poor performance, requires large amounts of material, is time consuming, and often overlooks rare components. Much more reliable results can be obtained by FTIR spectroscopy, which is a rapid, sensitive, and versatile technique, requiring relatively small amounts of material. Fourier transform infrared spectroscopy, at a moderate cost, allows the identification of organic and mineral components, of crystalline and noncrystalline substances and can even quantify the relative proportions of different constituents of a single heterogeneous calculus.[13,14] We used FTIR for analysis of the urinary calculi. This allowed an easy distinction between calculi composed of COD or calcium oxalate monohydrate (COM), and of different types of urate (anhydrous and dihydrate uric acid and sodium or ammonium acid urate).[15] Indeed, each of these compounds, as well as the other possible constituents of urinary calculi, has a typical infrared signature,[15,16] allowing not only unambiguous assignments but also the detection of mixed calculi. Calcium oxalate was the main component of the calculi we examined by FTIR; 48.2% of the children eliminated calculi of pure oxalate (COM, COD, or both) and the presence of oxalate was observed in a total of 72.9% (Table 1). As shown in some cases of our cohort, it has already been reported that results from wet chemical analysis of calculi containing cystine alone or in mixture with uric acid or COM can be different from that of FTIR analysis; however, control measurements by a completely independent technique (scanning electron microscopy with energy dispersive X-ray analysis) invariably confirmed the FTIR results.[17]

These findings strongly resemble those recently reported for countries where childhood lithiasis is endemic, while oxalate calculi appear less frequent in industrialized and developing countries (Table 3).[18-24] Calcium phosphate calculi rank 2nd for frequency (31.8%, including pure and mixed calculi) in our children, in line with the results reported for industrialized countries[25]; developing and "endemic" countries, on the other hand, show a markedly lower incidence of phosphocalcic calculi (5% to 15%). Purine calculi, ie, uric acid, urates and mixed, represent the 14.1% of the cases we examined, with a frequency comparable to that reported for developing countries (Table 3).[21] These figures are significantly higher than those observed

Table 3. Composition of Urinary Calculi in Pediatric Populations from Industrialized, Developing, and "Endemic" Countries*

Country or Area	Main Component, %					
	Calcium Oxalates	Purines	Calcium Phosphate	Struvite	Cystine	Mixed and Other
General statistics[18]	45 to 65	5 to 10	14 to 30	5 to 10	5 to 10	4
The Netherlands (1996-2010)[19]	59†	8.0	...	25.0	8.0	...
United Kingdom (1997-2001)[20]	50†	...	...	29.0	3.0	18.0
France (1991-2000)[21]						
Boys	31.8	5.2	45.1	8.1	3.0	6.8
Girls	48.8	4.3	20.9	5.3	7.9	12.8
Developing countries (1991-2000)[21]						
Boys	52.6	21.3	8.3	11.8	3.0	3.6
Girls	67.8	13.6	15.3	3.4	0	0
Turkey (endemic; 1996-2005)[22]	74	1.5	12	8	3	1.5
Tunisia (endemic; 1990-2004)[23]	71.5	9	5	12	3	4.5
Monastir (Tunisia; 1996-2009)[24]	54.7	20‡	14.6	7.8	1.5	1.5

*Pure and mixed calculi are included in the statistics.
†The figure includes calcium phosphate.
‡Predominant component; presence of urates in 60% of the calculi

in France,[24] other industrialized countries,[26] or "endemic" countries,[23,24] even though a recent account from a Tunisian hospital reported purine calculi as predominant in 20% of the cases, and the presence of purines in 60% of the calculi.[24] Interestingly, we found purine calculi mainly in male patients (Table 1), and a markedly higher frequency of purine calculi in male children was also found in developing countries (Table 3).

Examination by FTIR in our study shows that calcium oxalate calculi (pure or mixed) are the most frequent; oxalate pure calculi are more frequently found in boys, while mixed calculi in girls. Metabolic risk factors, for both sexes, were found to be mainly hyperoxaluria in the case of COD calculi and hypocitraturia in the case of mixed calculi (Figures 1 and 2). Hyperoxaluria was not frequently associated with enzymatic disorders, but probably related with the diet of Sicilian children, rich of sodium and animal proteins.[21,27]

CONCLUSIONS

Our data shows that FTIR spectroscopy is a crucial tool for the determination of the calculi composition; indeed, the traditional wet chemical analysis, even if easy and low cost, has a too high rate of erroneous results, as already observed by many research groups.[13,28] However, to formulate a correct diagnosis and establish a correct therapy, parallel metabolic analysis is highly recommended.[20,25,29,30]

CONFLICT OF INTEREST

None declared.

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Correspondence to:
Maria Michela D'Alessandro, MD
Via Sant'Antonio, 21 Bagheria, Palermo (PA), Italy
E-mail: marielladalessandro@alice.it
Tel: +390 9196 2452
Fax: +390 9196 4333, +390 9166 66078

Received June 2016
Revised November 2016
Accepted December 2016