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**Constituents of Urinary Calculi, some Metabolic  
Screening, and Detection of Polymorphism in  
SLC3A1 gene in Sudanese Children with Cystine  
Calculi**

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# **Dedication**

Dedicated with love to

My Mother & husband

Our kids (Hiba, Esra, & Abdelrahman)

Sisters & brothers

# Abstract

The objectives of this study were to insight the light in urinary calculi constituents in Sudanese children, evaluation of some metabolic risk factors and detection of polymorphism in exon 8 at SLC3A1 gene in patients with cystine stones. One hundred and seventy five urinary calculi from pediatric patients which were removed surgically in Soba Teaching Hospital at Khartoum state Sudan were analyzed during the period October 2005- May 2009. The patient's age was between 3 months- 16 years with mean of (4.96± 4 years).

The stones were analyzed by semi quantitative chemical method. Using a kit supplied by DiaSys Diagnostisic systems GmbH. (Germany). The stone was powdered and the standard both was analyzed for calcium, oxalate, ammonium, phosphate, magnesium, uric acid, and cystine.

One hundred and thirty blood samples (80 patients, 50 controls) were collected from patients to screen some metabolic risk factors, serum calcium, serum phosphate, serum uric acid, and serum creatinine. Fifty samples, of 24 hours urine collection (30 patients, 20 controls) were collected to determine the excretion of urine calcium, phosphate and uric acid. It was analyzed by auto analyzer Cobs Integra 400 Plus.

Ten blood samples were collected in EDTA container from patients with cystine stones, DNA was extracted by phenol chloroform isoamyle alcohol method, then the exon 8 of SLC3A1 gene were amplified by using thermo cycler machine (X Bioer, China), and the exon 8 was sequenced by Genetic auto analyzer AB3130 ( Applied Biosystem).

Urinary calculi were more predominant in male than female ratio 2.8:1 .The stones were located in the upper urinary tract in 75%, lower urinary tract in 25% and 1.1% in both upper and lower tract. On presentation 66.8% had flank pain 27.4% had hematuria, 49.1% had urinary tract infection (UTI), and 6.2% had renal failure, 20% had positive family history of stones formation. The age group 2-6 years was the most frequent of stones onset for all types of stones

except cystine stone in which the age group was found to be less than two years. 20% had recurrent stones formation.

Calcium oxalate stone were the commonest constituents (55.4%), followed by ammonium urate (48.6%), Struvite (15.9%), calcium phosphate (11.4%), uric acid stones (8%), and cystine stones (5.7%). The components of the upper urinary tract calculi were calcium oxalate (40%), ammonium urate (35.4%) and calcium phosphate (21%), whereas the main components of the lower urinary tract calculi were ammonium acid urate (23%), struvite (9.1%), and calcium oxalate (10.4%).

There was significant association between upper urinary tract and calcium phosphate stones, cystine stones  $P < 0.028$ ,  $0.054$  respectively, and lower urinary tract with Struvite stones  $P < 0.0001$ . Cystine stones were more common in patients with positive family history  $P < 0.000$ .

This study showed that there was no difference between the mean of total serum calcium, phosphate, serum uric acid, and serum creatinine in patients compared to control group, the means of total calcium for patients were ( $9.6 \pm 0.85$  mg/dL) , and control ( $9.5 \pm 0.7$  mg/dL),  $P < 0.56$ , mean of serum phosphate for patients ( $4.3 \pm 0.4$  mg/dL), and control ( $4.2 \pm 0.4$  mg/dL),  $P < 0.345$ , mean of serum uric acid in patients were ( $3.5 \pm 1.1$  mg/dL), control ( $3.5 \pm 1$  mg/dL)  $P < 0.979$ , and serum creatinine in patients were ( $0.46 \pm 0.24$  mg/dL), control ( $0.4 \pm 0.1$  mg/dL),  $P < 0.089$ .

There was a reduction in urine volume per 24 hours in patients with renal stones ( $675 \pm 200$  mL/day) compared to control group ( $898 \pm 350$  mL/day),  $P < 0.011$ . There was increase in urine calcium excretion mg/kilogram/day mean ( $1.98 \pm 0.8$ ) mg/kg/day compared to control ( $1.4 \pm 0.5$ ) mg/Kg/day  $P < 0.009$ , urine uric acid excretion in patients were ( $1.0 \pm 0.5$ ) mg/Kg/day, and control ( $0.6 \pm 0.4$ ) mg/Kg/day  $P < 0.013$ , and no significant variation between the mean of urine phosphate excretion in patients ( $5 \pm 1.9$ ) mg/Kg/day, and control ( $5.2 \pm 1.2$ ) mg/kg/day  $P < 0.627$ .

The mutation which was found in exon 8 at SLC3A1 gene was M467K, due to substitution of one base thymine (T) to adenine (A), which affects the transport function of cystine and dibasic amino acids in the kidney and small intestine.

هدفت هذه الدراسة الى تسليط الضوء علي مكونات حصاوي المجاري البولية عند الاطفال السوداني المصابين بحصاوي الجهاز البولي مع تقييم بعض العوامل الايضية المؤدية الي تكوينها ثم تحديد الطفرات الوراثية علي جين  $SLC_3A_1$  عند الاطفال المصابين بحصاوي حمض السستين الاميني فقط . تم تحليل 175 حصوة استخرجت من الاطفال المصابين الذين اجريت لهم عمليات جراحية في مستشفى سوبا الجامعي (الخرطوم-السودان) في الفترة ما بين اكتوبر 2005- مايو 2009 تتراوح اعمارهم بين ثلاثة اشهر- 16 سنة بمتوسط (  $4.96 \pm 4$  سنة).

تم تحليل كل الحصاوي البولية بالاضافة الي عينه الضابطة الموجبه بواسطة التحليل شذبه الكمي الكيمائي باستخدام محاليل شركة DiaSys الالمانية لقياس مستوي كل من الكالسيوم- الاكسالات- الامونيات- الفوسفات- الماغنيزيوم- الحامض البولي وحمض السستين الاميني . ايضا تم سحب 130 عينه دم (80 عينة من الاطفال المصابين و 50 من الاصحاء كعينة ضابطة) في حاوية خاليه من مادة مانعة التجلط بغرض الحصول على مصد الدم لقياس مستوي كل من الكالسيوم الكلي - الفوسفات غير العضوي- الحامض البولي- والكرياتين في مصد الدم ايضا تم جمع 50 عينة بول لكي لمدة 24 ساعة (30 عينة من الاطفال المصابين بالحصاوي الكلوية فقط و 20 من الاصحاء كعينة ضابطة) في حاوية تحتوي على مادة حافظه ( Thymol Auto) بغرض قياس تركيز كل من الكالسيوم - الفوسفات غير العضوي- الحامض البولي التي خرجت مع البول في 24 ساعه بواسطة جهاز التشغيل الذاتي analyzer Cobas Integra 400 Plus .

سحبت 10 عينات دم في حاوية تحتوي مادة مانعه للتجلط EDTA من الاطفال المصابين بحصاوي حمض السستين الاميني بغرض الحصول على الحامض الذووي مذروع الاكسجين (DNA) باستخدام الفينول كلورفورم ايزواميل الكحول ثم تم اخضاعه الي تقنيتي التفاعل السلسلي المتبلر (PCR) لزياده كمية  $SLC_3A_1$  جين والتسلسل الذاتي لا قواعد النيروجينية باستخدام جهاز التشغيل الذاتي Genetic analyzer AB 3031 لمعرفة تسلسل الا قواعد النيروجينية على  $SLC_3A_1$  جين .

اظهرت النتائج أن الحصاوي اكثر شيوعا عند الذكور من الاناث بنسبه 2.8:1 نجد ان 75% من الحصاوي قد تكونت في الجزء العلوي من الجهاز البولي و 24.8% في المثانة البولية و 1.1% في كل من اعلي واسفل الجهاز البولي.

الاعراض المصاحبه للحصوه تتمثل في 66.8% ألم جانبي 27.4% بول دموي 49.1% التهابات في المجاري البولية (UTI) و 6.2% اصيبو بالفشل الكلوي. نجد ان الاصابه بالحصاوي اكثر شيوعا في الفئة العمريه 2-6 سنة لكل انواع الحصاوي ما عدا حصاوي حمض السستين الاميني في الفئة اقل من سنتين .

عندما تم تحليل مكونات الحصاوي وجد أن 55.4% اكسالات كالسيوم, 48.4% يورات امونيا, 15.9% استرفات , 11.4% فوسفات الكالسيوم 8% حمض بولي و 5.7% حمض السستين الاميني. نجد ان اكثر مكونات الحصاوي في الجزء الاعلى من الجهاز البولي هي اكسالات كالسيوم تليها يورات امونيا ثم فوسفات الكالسيوم بينما الاكثري الجزء الاسفل هي يورات امونيا ثم استرفات. هناك علاقه ذات دلالة احصائية بين تكوين حصاوي فوسفات الكالسيوم و حصاوي حمض السستين الاميني في الجزء الاعلى من الجهاز البولي ب قيمه احتماليه اكبر من 0.028 و 0.054 علي التوالي وكذلك بين الجزء الاسفل من الجهاز البولي وحصاوي استرفات ب قيمه احتماليه اكبر 0.0001 .

عندما تم قياس مستوى كل من الكالسيوم الكلي - الفوسفات غير العضوي- الحامض البولي والكرياتنين في مصل الدم لم يوجد هناك فرق ذو دلالة احصائية , حيث ان متوسط الكالسيوم الكلي عند المرضي ( $0.85 \pm 9.6$  ملجم/دل) والعينه الضابطه ( $9.7 \pm 0.7$  ملجم/دل) ب قيمه احتماليه اكبر من 0.56, وان متوسط الفوسفات غير العضوي عند المرضي ( $0.4 \pm 4.3$  ملجم/دل) والعينه الضابطه ( $0.3 \pm 4.3$  ملجم/دل) ب قيمه احتماليه اكبر من 0.345, و ان متوسط الحمض البولي عند المرضي ( $1.1 \pm 3.5$  ملجم/دل) والعينه الضابطه ( $1 \pm 3.5$  ملجم/دل) ب قيمه احتماليه اكبر من 0.979, وان متوسط الكرياتنين عند المرضي ( $0.24 \pm 0.46$  ملجم/دل) والعينه الضابطه ( $0.1 \pm 0.4$  ملجم/دل) ب قيمه احتماليه اكبر من 0.089 .

وجد ان هناك انخفاض في متوسط كمية البول التي تم اخراجها خلال 24 عند المرضي الحصاوي ( $200 \pm 675$  مل/24 ساعه) مقارنة بالمجموعه الضابطه ( $898 \pm 350$  مل/24 ساعه) ب قيمه احتماليه اكبر من 0.012 . وجد ان هناك زياده في كمية الكالسيوم البولي التي تم اخراجها خلال 24 عند المرضي ( $0.8 \pm 1.98$  ملجم/كجم/24 ساعه) والعينه الضابطه ( $1.4 \pm 0.5$  ملجم/كجم/24 ساعه) ب قيمه احتماليه اكبر من

0.009 وايضا هناك زيادة في كمية الحامض البولي التي تم اخراجها في 24 ساعة عن طريق البول عند المرضى ( $0.5 \pm 1$  ملجم/كجم/24 ساعة) والعينه الضابطه ( $0.6 \pm 0.4$  ملجم/كجم/24 ساعة) ب قيمه احتماليه اكبر من 0.013 لا فرق بين متوسط الفوسفات التي تم اخراجها عن طريق البول في 24 ساعة للمرضي بمتوسط ( $5 \pm 1.9$  ملجم/كجم/24 ساعة) والعينه الضابطه ( $5.2 \pm 1.2$  ملجم/كجم/24 ساعة) ب قيمه احتماليه اكبر من 0.627 .

عندما تم ترتيب القواعد النيتروجينية وجد ان احلال لقاعدة الثيمين (T) (thymine) الي ادنين (A) (adenine) أدت الي تغير الحامض الاميني (M) (Methionine) الي (M467K) (Lysine (K)). تؤثر هذه الطفره الوراثيه علي نقل كل من حمض سستين الأميني و الأحماض الأمينيه ثنائية القاعدة في الكلية والأمعاء الدقيقة.



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