Detection of AGTX gene mutations of kidney stone patients in Tikrit city, Iraq

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Abstract

The present study was conducted at the Tikrit Teaching Hospital in Tikrit city of Iraq, during July 2018 - January 2019. Study based on 100 patients with kidneystones and 50 healthy, of both sexes, whose ages ranged from 20 to 60 years, and the most common type of stones are calcium oxalate. Physiological, biochemical and genetic parameters are considered indicators of the kidneystones status of patients, and the effect of the AGTX gene mutations in their composition. Calcium, Magnesium, Phosphorus, Potassium, Sodium, Urea and Creatinine were measured. Type 1 primary hyperoxaluria in the AGXT gene was studied, in addition to determining the nucleotide sequences of 14 patient samples. The results showed a significant difference in mutation between the patients and control, and most mutations were detected in exons 1 and 2 different mutations, transitions and transversion. A significant increase in the concentration of Calcium, Total Magnesium, Urea and Creatinine in mg/dl (17.9971, 7.4817, 10.082, and 3.2464) respectively and Sodium 174.8455 mmol/l. It also showed a significant decrease in the concentration of Potassium 4.7884 mmol/l and Total Phosphorus 3.2675 mg/dl.

Keywords: AGTX, Mutations, Kidney stones, Iraq


Introduction

Kidneystone disease or Nephrolithiasis is a common global problem and affects about 6-10% of the total population (Mittal and Kuma, 2004). [1]. The most common type of Kidney stone is calcium, which is 80% of the total. Calcium is often associated with oxalate and Phosphate. Calcium oxalate is the most common in patients due to metabolism defect of calcium and phosphorus in the body and increases in calcium and oxalate in urine (Moe,
Individuals of Arab origin, West India, West Asia, and Latin America are more likely to have kidneystones than those who European origins (Mente et al., 2007). Genetic factors play an important role in the causes of Nephrolithiasis as multi-gene or single-gene forms (Tanikawa et al., 2019). The causes of the stones formation may be primary kidney disease that affects the kidneys genetically or be congenital, including Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Medullary sponge kidney, this condition exists since birth and tubuli kidneys in the kidney is enlarged, leading to stagnation of urine and calcium precipitate, and then the formation of stones (Capone et al., 2017). Genetic defects of hypercalcemia have been discovered, such as the polymorphism of vitamin D receptors (VDR) (Bid et al., 2005), The nitrogenous bases substitution of the adenylate cyclase gene (Reed et al., 2002), Polymorphisms of the Calcitonin Receptor Gene (CALCR) (Chen et al., 2001) and a mutation in the calcium-sensing receptor (CASR) (Guarnieri et al., 2010).

AGTX is one of the genes of the human genome located on the second pair of chromosomes at position 37.3, consisting of 11 exon, which is 10kp long. Cyclic DNA (cDNA) contains an encoded area of 1179 nuclides that encodes 392 polypeptides (Takada et al., 1990). This gene encodes the alanine-glyoxylate transaminase enzyme (AGT), which depend on the pyridoxine 5-phosphate group and this enzyme is located in peroxisome cells in mitochondria in the liver (Hoppe et al., 2009). This enzyme acts on the glyoxylate metabolism, which converts the glyoxylate into two products are pyruvate and glycine. (Okuno et al., 1982) and when a mutation occurs in the enzyme, the glyoxylate accumulate and are converted into oxalates, which are secreted in the kidneys and crystallize into calcium oxalate, which leads to the formation of stones and thus leads to a decrease in the kidney function and kidney failure (Milliner et al., 2005).

Agt is in the form of two major gene variants either with proline (main allele) or leucine (secondary allele) in position 11 in the form of protein (Williams and Rumsby, 2007). An allele of Leu 11 encodes a protein of approximately 50% of the activity of the common pro 11 allele, while the leucine protein decreased the rate of dimer in high temperatures, and there is also evidence that the presence of leucine inhibits the effect of some mutations (Santana et al., 2003; Danpure and Lumb, 2000).

AGXT gene contains 50 mutations that corruption its function, characterized by point mutations, deletions, and substitution (Coulter-Mackie and Rumsby, 2004) the most common mutations are: (C.33-34insC >C.508G> A-C.731T> C), these mutations resulted in an increase of oxalate in urine by 34.5% in patients, and were found on
The oxons (1, 4 and 7). These mutations were detected by expression in alleles (main proline and secondary leucine) (Rumsby et al., 2004). [18].

The objective of this study was to determine most common types of kidney stones at Tikrit city of Iraq, determining the possible causes of their occurrence in patients, investigate the role of mutations type of AGTX gene as a biomarker in kidney stones patient, analysis of kidney stone composition and estimate the urinary value of the serum mineral elements.

Materials and methods

Sampling

One hundred urine and blood samples were collected from kidney stones and 50 healthy at Tikrit Teaching Hospital-Urology Department; from July-August 2018 and their ages range was 20-60 years.

Biochemical and determination of chemical analysis of stones

Calcium, creatinine and urea were determined using kit (Biolabo; France), magnesium and phosphorus (Linear; Espain), potassium and sodium (Spectrum; Germany) to measure the concentration in serum samples according to the manual instruction provided by the company. The concentration of kidney stone elements was estimated using Kit (Biolabo; France), based on the quantitative analysis method of kidney stone.

DNA extraction, genotype analysis and DNA sequencing

Genomic DNA was extracted from peripheral blood leukocytes using the DNA Blood Mini Kit (Bioneer, USA). The AGXT gene was amplified by polymerase chain reaction (PCR), using sequence specific primer (F: 5'-CCGCAGCACAAGCAGATAAG-3') (R: 5'-CCCCCGAGTGACCCCCACT-3') according to Monico et al., 2007 [19]. Components of PCR Interaction which was achieved in a 20μl mixture includes: 10μl Go Taq Green Master Mix (Promega), 2μl of each primer, 4μl DNase Free Water, and 4μl Template DNA. All reactions had an initial denaturation step of 5 min at 94°C followed by 35 cycles of denaturation at 94°C for 30s., annealing at 63°C for 30s. and 72°C for 30s., and a final elongation step at 72°C for 7min.

We sequenced exon 1 of primary hyperoxaluria type 1 (PH1) of the AGXT gene using specific primer. The sequence of the nucleotides of the upper strand was determined of 5' to 3' using the forward primer for each genetic sequencing at a concentration of 10 picomol. The results of the polymerization reaction (15μl) and 50μl of each primer were sent to Macrogen Co., USA, after obtaining the results, BLAST was used on the website of the gene.
Statistical Analysis

Statistical investigation was performed utilizing a two-followed t test using SPSS Statistics Version 21.0 software on windows 10. to dissect the difference between two nonstop factors. A p-value <0.05, 0.001 was expressed statistically significant.

Results and Discussions

A total of 100 patients with kidney stones were assessed (67 male and 33 female; Table 1). Males have more kidney stones than females, this is due to Testosterone, which increases the secretion of oxalate and deposition of crystals in the kidney, while females are the least, because that estrogen works to increase the citrate in the blood, this increase leads to reduced excretion of oxalate and lack of kidney stones (Strope et al., 2010)[20].

Table 1: Mean physiological and biochemical characteristics of kidney stones patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Kidney stones patients</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.0± 10.35</td>
<td></td>
</tr>
<tr>
<td>Male(%)</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td><strong>Stone composition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium(%)</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>Oxalate(%)</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Phosphate (%)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>3.24± 0.582*</td>
<td>0.6-1.2 mg/dl</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>10.08±1.046*</td>
<td>5.0-20 mg/dl</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>17.99±1.245*</td>
<td>8.5-10.5 mg/dl</td>
</tr>
<tr>
<td>Phosphors (mg/dl)</td>
<td>3.26±0.428</td>
<td>2.5-4.5 mg/dl</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>174.84±15.571*</td>
<td>135-145mEq/L</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.51±0.235</td>
<td>3.5-5.0mEq/L</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>7.48±1.144*</td>
<td>1.7-2.2 mg/dl</td>
</tr>
</tbody>
</table>

Serum values are presented as the mean ± Stander Division (SD), * Significant (p<0.01).

The mean age of patients was 36 years, the detection of kidney stones in more established people, can be trying due to atypical pain or nonappearance of agony, in addition to the presence of diseases such as diarrhea and urinary tract infection, which makes young people more age groups they have kidney stones (Krambeck et al., 2013)[21]. The chemical stones content indicates that most patients had high levels of calcium and oxalate (82%, 78%), the cause for the high rate of calcium and oxalate may be due to eating, drinks and weather. The information collected about this high percentage helps to know the reasons for its formation and the most prevalent. Numerous studies have indicated that most kidney stones are composed of calcium oxalate and in different parts of the
Fifty kidney stones were analyzed. The most common stones were calcium oxalate (82%), and were containing magnesium, oxalate and phosphate. Results indicated a significant increase in serum creatinine concentration (3.24) mg/dl at P≤0.01 compared with control group, while the concentration of urea calcium sodium and magnesium are (10.08, 17.99, 174.84 and 7.48) respectively, as well as phosphorus and potassium remained within the normal range (Table 1). The reason for the high level of creatinine and urea because of metabolic defect of the kidney resulting from the destruction of some glomerular filtration units as well as low glomerular filtration rate to precipitate calcium oxalate crystals causing the blockage of nephrons and increase acidity(Haley et al., 2016)[25].

Calcium is an important element in the cell life cycle to control cellular activity, low calcitonin in people with kidney stones leads to a decrease in the rate of calcium deposition in the bones and increases in the blood(Mittal et al., 2004).[26].The oxidative injury resulting from kidney stones and infections caused by pathogenic bacteria lead to epithelial tissue damage in the urinary system and increased by the presence of salts, the peroxidation of lipid phosphorylation of the cell membranes leads to the hardening of the membrane and thus affect the physiological functions of nephrons, especially epithelial cells in the renal glomerulus and convoluted tubule(Boonla, 2018)[27].Oxalates are natural substances found in many natural foods and are associated with calcium during digestion and absorption in the gastrointestinal tract and then leave the body with feces, however, oxalates that are not associated with calcium in the gastrointestinal tract are excreted through the kidneys with urine, therefore, the high levels of calcium in the kidneys lead to the association of oxalates with calcium to be crystals calcium oxalate that combine with each other to form kidney stones. This is one of the most common types of kidney stones, and this process is one of the reasons leading to the destruction of kidney tissues and low efficiency of the kidneys (Ratkalkar and Kleinman, 2011)[28].

There is a correlation between elevated serum calcium and sodium levels in patients with kidney stones, calcium and sodium are involved in the transportation of the kidneys. The reabsorption of calcium is parallel to the sodium in the convoluted tubule. So sodium affects either directly at the calcium level through tubular reabsorption or indirectly through a parathyroid hormone that stimulates calcium reabsorption(Seeger et al., 2017)[29]. Magnesium is found in the bones and soft tissues and is very low in the blood and is regulated by the
Magnesium prevents the calcium oxalate stones formation as magnesium correlates with oxalate in the gastrointestinal tract when eating foods containing it to prevent or reduce the absorption of oxalates in the blood and thus inhibits the calciumoxalate crystals formation in the kidney. Previous studies have shown a significant correlation between high magnesium level and lower risk of kidney stones formation (Negri 2013; Zerwekh 2007)[31,32].

Genotyping for exon 1 of AGXT gene was performed by PCR, the results of 100 patients and 50 control showed the DNA Bands Target With 360bp at concentration of 2% agarose gel electrophoresis (Figure 1). 15 samples (10 patients and 5 control) nucleotide sequences were analyzed to identify mutations in an AGXT gene. After comparing with the exon 1 of AGXT gene sequences found at www.ncbi.nlm.nih.gov, the results are shown in Table 2 and patient samples have alignment 96-98% while the healthy was the alignment between 99-100%.

**Figure 1.** Electrogam of PCR analysis of the AGXT gene. Agarose gel (2%) electrophoresis. DNA ladder (100-1000) lane M. lane 1-19, patient's samples were shown by the band of 360bp.

Gene sequencing was performed by the National Instrumentation Center for Environmental Management (NICEM) at the Biotechnology Laboratory, online at http://nicem.snu.ac.kr/main/?en_skin=index.html, and using DNA Sequencer 3730XL from Applied Biosystem, USA, alignment were performed using BLAST at NCBI and BioEdi software program to determine the mutation types and alteration. Table 2 shows the 60 alteration of 14 Transition mutation (purines to pyrimidine) and 9 Transversion mutation (point mutation indicated purines to purines A↔G or Pyrimidine to pyrimidine C↔G) and delete 37 nucleotides. To estimate the expected value to obtain the same similarity by chance and the lower value E, this indicates that the degree of similarity was high between sequences giving greater confidence, a value very close to zero means that these sequences are identical.
Table 2: Locations of variations when matched with global samples

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Substitutions</th>
<th>Mutation</th>
<th>Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.4992C&gt;G</td>
<td>Transversion</td>
<td>c.1245-&gt;G</td>
<td></td>
</tr>
<tr>
<td>c.1240G&gt;A</td>
<td>Transition</td>
<td>c.1553-&gt;T</td>
<td>Transition</td>
</tr>
<tr>
<td>c.1244G&gt;A</td>
<td>Transition</td>
<td>c.1406T&gt;C</td>
<td>Transition</td>
</tr>
<tr>
<td>c.1253-&gt;A</td>
<td></td>
<td>c.1245-&gt;G</td>
<td></td>
</tr>
<tr>
<td>c.1243G&gt;C</td>
<td>Transversion</td>
<td>c.1252-&gt;G</td>
<td></td>
</tr>
<tr>
<td>c.1253-&gt;A</td>
<td></td>
<td>c.1554A&gt;C</td>
<td>Transition</td>
</tr>
<tr>
<td>c.1277C&lt;-</td>
<td></td>
<td>c.1555C&gt;A</td>
<td>Transition</td>
</tr>
<tr>
<td>c.1243G&gt;C</td>
<td>Transversion</td>
<td>c.1559C&gt;G</td>
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<tr>
<td>c.1253-&gt;A</td>
<td></td>
<td>c.1303G&lt;-</td>
<td></td>
</tr>
<tr>
<td>c.4992-&gt;G</td>
<td></td>
<td>c.1411C&lt;-</td>
<td></td>
</tr>
<tr>
<td>c.5123G&lt;-</td>
<td></td>
<td>c.1474-&gt;C</td>
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<tr>
<td>c.5206G&lt;-</td>
<td></td>
<td>c.1501T&lt;-</td>
<td></td>
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<tr>
<td>c.5221-&gt;C</td>
<td></td>
<td>1516C&gt;T</td>
<td>Transition</td>
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<td>c.5227C&gt;A</td>
<td>Transition</td>
<td>c.1533G&lt;-</td>
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<td>c.5242-&gt;G</td>
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<td>c.1534A&lt;-</td>
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<tr>
<td>c.5261-&gt;G</td>
<td></td>
<td>c.1243-&gt;C</td>
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<tr>
<td>c.5262T&gt;G</td>
<td>Transition</td>
<td>c.1552-&gt;G</td>
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<tr>
<td>c.5263C&gt;T</td>
<td>Transition</td>
<td>c.1556C&lt;-</td>
<td></td>
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<tr>
<td>c.5281A&lt;-</td>
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<td>c.1556C&lt;-</td>
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<tr>
<td>c.1243-&gt;C</td>
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<td>c.1372A&gt;C</td>
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<td>c.1263C&gt;A</td>
<td>Transition</td>
<td>c.1388-&gt;A</td>
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<td>c.1289-&gt;T</td>
<td></td>
<td>c.1406T&gt;C</td>
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</tr>
<tr>
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<td></td>
<td>c.1407C&gt;G</td>
<td>Transversion</td>
</tr>
<tr>
<td>c.1555-&gt;A</td>
<td></td>
<td>c.1485C&gt;G</td>
<td>Transversion</td>
</tr>
<tr>
<td>c.1552C&lt;-</td>
<td></td>
<td>c.1553-&gt;T</td>
<td></td>
</tr>
<tr>
<td>1552A&gt;G</td>
<td>Transition</td>
<td>c.1556C&lt;-</td>
<td></td>
</tr>
<tr>
<td>1245-&gt;G</td>
<td></td>
<td>c.1245-&gt;G</td>
<td></td>
</tr>
<tr>
<td>c.1476C&lt;-</td>
<td></td>
<td>c.1246G&gt;C</td>
<td>Transversion</td>
</tr>
<tr>
<td>c.1537A&lt;-</td>
<td></td>
<td>c.1295C&gt;G</td>
<td>Transversion</td>
</tr>
</tbody>
</table>

Figure 2 A shows various mutations (missense) locus of patient samples, and B shows nonsense mutations in of healthy in the gene expression of AGXT gene, samples of patients with kidney stones showed a variation in the
locus of nitrogenous bases and alteration in amino acids with alignment 96-98%. While the healthy have alignment between 99-100%. The mutations in patients show kidney stones and a significant expectation unlike what is observed in healthy people, the effect of these mutations is in changes in the work of this gene, the effect of these mutations on changes in the work of this gene, when a mutation in the enzyme will accumulate glycosidic toxins and they transformed into oxalates and crystallize in the kidneys and are associated with calcium to produce calcium oxalate salts, which lead to the stones formation, causing reducing in rate of renal function, and failure of the oxalosis system (Milliner, 2005)[33]. The effect of these mutations on the amino acid change of the nucleotide chain of the gene, where the change of the nitrogen base as a result of an effective mutation will lead to the change of amino acid, while the ineffective mutation does not change the amino acid, while Figure (2 B) showed a match to a very high level reached 99-100% in healthy people, which indicates no genetic mutations.

Score: 584 bits (316); Expect: 6e-163; Identities: 322/326(97%); Gap: 0/326(0%)
Sbjct  1362  TTGCTGCGGACCATGGCCTCTCAAGCTGCTGGTGACCCCCCCGAAGGCCCTGCTCAA  1421
Score: 568 bits(307); Expect: 6e-158; Identities: 318/323(98%); Gap: 2/323(0%)

Query  307   AGGAGTGGGGG-TACCTCCGGGGG  328

Sbjct  1542  AGGAGTGGGGGCACTCCGGGGG  1564
Score: 544 bits(294); Expect: 1e-150; Identities: 311/318(98%); Gap: 6/318(1%)

Query  243   TCCTCGCATCATGGCAGCCGGGGGGTGAGTGGGGGCTGCAGATGATCGGGTCCATGAGCAAGGATATGG  302

Sbjct  1476  TCCTCGCATCATGGCAGCCGGGGGGGCTGCAGATGATCGGGTCCATGAGCAAGGATATGG  1533
Score: 577 bits(312); Expect: 4e-160; Identities: 322/326(97%); Gap: 2/326(1%)

Query  303   TACCAGTGGAGTAGGGGGG  320

Sbjct  1534  TACCAGTGGAGTAGGGGGG  1550
Score: 569 bits(308); Expect: 2e-158; Identities: 309/312(98%); Gap: 1/312(0%)

Query  6     AGGCA-GGCTGCCACGGAAGCCCATCCACCAATCCTCACCTCTCACCTCTGTGTCCGCCC  64

Sbjct  1240  AGGCAGGGCTGCCACGGAAGCCCATCCACCAATCCTCACCTCTCACCTCTGTGTCCGCCC  1299
Score: 579 bits(313); Expect: 6e-161; Identities: 319/323(98%); Gap: 2/323(0%)

Query  248   CATATGCCAGCCGCGGGGTGTCAGATGATCGGGCTGCAGAAAGTAATGTACCAGGT  307

Sbjct  1482  CATATGCCAGCCGCGGGGTGTCAGATGATCGGGCTGCAGAAAGTAATGTACCAGGT  1541
Query  308   AGGAGTGGGGGCACTCCGGGGG  329

Sbjct  1542  AGGAGTGGGGGCACTCCGGGGG  1563
Score: 569 bits(308); Expect: 2e-158; Identities: 309/312(98%); Gap: 1/312(0%)
**Fig. 2, A.** Various nucleotide locus of code number of amino acids and mutation type and effect in in-patient with kidney stones in exon 1 of **AGXT** gene

**Score:** 573 bits(310); **Expect:** 1e-159; **Identities:** 310/310(100%); **Gap:** 0/310(0%)

**Score:** 569 bits(308); **Expect:** 2e-158; **Identities:** 311/312(99%); **Gap:** 1/312(0%)

**Score:** 558 bits(302); **Expect:** 4e-155; **Identities:** 307/309(99%); **Gap:** 2/309(0%)

**Score:** 566 bits(306); **Expect:** 2e-157; **Identities:** 309/310(99%); **Gap:** 1/310(0%)

**Score:** 566 bits(306); **Expect:** 1e-157; **Identities:** 310/310(100%); **Gap:** 0/310(0%)

**Fig. 2B:** Various nucleotide locus of code number of amino acids and mutation type and effect in healthy with kidney stones in exon 1 of **AGXT** gene
Conclusion

The most common types of stones are calcium oxalate and a significant increase in the concentration of calcium ions, magnesium, sodium, urea and creatinine in patients compared to healthy. Mutations in Exon 1 for the AGXT gene have been shown to affect kidney stones, these mutations affected in the gene expression that lead to presence of transition and transversion mutations and transition mutations were more frequent than transversion.

Disclosures

None.

References


Punctuation Marks

Comma followed by space after each author do not write and before the last author

Full stop after the last author followed by space and then the article title

Singh JK, Bawa M, Kanojia RP, Ghai B, Menon P, Rao KL.

Idiopathic simultaneous intussusceptions in a neonate.


Full stop followed by space

Full stop at the end of the ref.

Hyphen to separate the page no.

Semicolon ; after the year without any space

Colon : after volume no. without any space

Single space between the journal abbreviation and the year, place no other punctuation marks