DETERMINATION OF GENETIC MUTATIONS THAT EFFECT UMOD GENE IN CHRONIC RENAL FAILURE AT NINEVEH CITY, IRAQ

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Abstract

The current study was conducted at Al-Salam Teaching Hospital in Nineveh City for the period between 1/2/2018 and 1/5/2018. It aimed at identifying the mutations that cause chronic kidney failure in the UMOD gene in patients. This study included Follow-up of 95 patients according to a questionnaire containing the information for each patient, after which the DNA was extracted from the blood samples of patients with chronic renal failure and by using the technique of heterogeneity restriction for polymerase Chain Reaction Restriction Fragment Length Polymerase (RFLP-PCR), genetic polymorphism was determined. For the UMOD gene, a Nucleotides sequence determination technique was used of the enlarged segment using the Sequencing nucleotide reader device. The results showed that no interruption by means of categorical enzyme (THIII) occurred. As for the results of sequencing, a number of genetic variations of the enlarged segments were determined by polymerase chain reaction

Keywords: Chronic Kidney Failure, UMOD Gene, Uromodulin, PCR, Sequence Determination.

Introduction

Kidney diseases are among the most common causes leading to death in many countries around the world. Kidney diseases can be divided into two types: Acute Renal Failure (AFR) and Chronic Renal Failure (CRF) (Al Hilali, 2018). Chronic kidney failure leads to a gradual loss of kidney function over a long period of time that may be a month or years. CRF is also known as the continuous destruction of renal functional units (Nephrons), coinciding with the symptoms of unspecified kidney degradation until advanced stages are reached up to the fifth stage which is known as the End Stage of Kidney Disease (ESKD). It is largely synonymous with the term chronic kidney failure (KDiGo 2009; 2013; National kind. 2002; Henry, 2001). The prevalence of this disease is increasing in the world and this increase exceeds those reported by various countries, where huge and rapid changes in lifestyle, massive population growth, rapid rise in life expectancy and the great urbanization occurred in recent years have made various cases of chronic kidney disease. It is expected that this disease will be associated with the spread of several diseases that have an effect such as a risk factor such as diabetes, hypertension and obesity (Ahmed et al., 2014a; 2014b; Al-Sayyari & Shaheen 2011).

Genetic analysis demonstrated that a mutation in the UMOD gene caused a prevalent physical disease condition diagnosed by finding cysts in the region of the kidney core, inflammation of the inner tubules, high level of uric acid and it was accompanied by gout. The disease progresses to the last stage of chronic kidney disease, which is kidney failure (Sian et al., 2009; Lens et al., 2005; Ramopoldi et al., 2003). This gene is known by other names: ADMCKD2, FJH, HNFJ, HNFJ1, MCKD2, THGP, THP (Ramopoldi et al., 2003).

The UMOD gene is made up of 11 Axon encoded pieces. Exons are (2-11) encoded (Pennica et al., 1987) to produce Uromodulin (UMOD). Uromodulin, also called Tamm Horsfall, is the most abundant protein in the urine. Under natural conditions, this protein was first purified in the 1950's, and since then it has been highlighted, but only slightly, on the importance of this protein. However, so far, the remainder of the physiological functions of this protein has not been identified. This protein consists of 640 amino acids, including 24 amino acids containing peptide at the nitrogenous end, 48 cysteine and 8 amino ends for association with glycated groups (Pennica et al., 1987; Tamm-Horsfall, 1952) seven of which are related (Sian et al., 2009; Kammarr, 1990). The amino acid sequence indicates the possibility that uromodulin may contain growth factors in the form of specific clusters, of which the second and third contain calcium binding factors. The region of the second cysteic acid (D8C) is within the zinc rich region and the Zona Pellucida (ZP) region which is responsible for the polymerization of extracellular proteins into helical strands (Yong et al., 2009; Serafin et al., 2003).

Materials and Methods

During this study, (95) blood samples were collected from males with chronic kidney failure whose ages ranged between (15-70) years and who were subjected to hemodialysis weekly. They also proved their pathological condition through conducting functional tests for the kidney and periodically reviewing the department of kidney failure at Al-Salam Teaching hospital in the province of Nineveh. As 1.5 ml of blood was taken, and it was placed in container tubes on an anticoagulant (EDTA), and then the DNA was extracted. The DNA was isolated from the blood for all the samples included in the study, which are 95 samples depending on the method modified by (Iranpur and Esmailizadeh, 2010).

DNA extraction

DNA was extracted from the blood of all the samples included in the study using the method modified by (Iranpur & Esmailizadeh, 2010).

The solutions used in the extraction were:

1. Ethylene Diamine Tetracetic Acid EDTA solution with a concentration of 0.5M and of 8.0. Weighing 186.1 g of EDTA powder and dissolved in 800 ml of distilled water. The acid function was set at pH 8, completed the volume to 1 liter of distilled water and sterilized the
solution in a sterile or autoclave at 141 °C and under pressure 1 for 15 minutes.

1. 2.Toris-HCl gear solution with a concentration of 1 molar and a 7.6 acidic switch, dissolved 121.1 g of Tris-HCl in 800 ml of distilled water and set the acidic function at 7.6 pH and completed the volume to 1 liter of distilled water. Then the solution was sterilized in the sterile or autoclave at a temperature of 121 °C and under 1 atmosphere for 15 minutes.

2. Red Blood Cell Lysis Buffer regulating solution consisting of Tris-HCl solution with a concentration of 0.01M, sucrose at a concentration of 320M, magnesium chloride 2 MgCl at a concentration of 25M and Triton -100X at a concentration of 1%. This solution was prepared by adding 10 mL of the buffer solution at a concentration of 0.01 M and adding 109.54 g of sucrose, 1.01 g of magnesium chloride and (10) mL of Triton-x 100 to 800 mL of distilled water. The acidic function was set at pH 8.0, and then completed the volume to 1 liter of distilled water, sterilized the solution using the reagent at 121 °C and press 1 atmosphere for 10 minutes.

3. Nucleic Lysis Buffer buffer solution. This solution consists of Tris-HCL solution with a concentration of 0.01M and sodium citrate at a concentration of 11.4M and EDTA at a concentration of 1M and Sodium Dodecyl Sulphate (SDS) at a concentration of 1%. This solution was prepared by adding 10 mL of Tris-HCl at a concentration of 0.01M, 3.75g of EDTA and 10g From SDS and 2.94 g of sodium citrate to 800 mL of distilled water. The acid function is set at pH 8.0 and volume is supplemented to liters with distilled water, sterilized the solution in the conduct or at 121 ° C and compressed for 15 minutes.

4. TE Buffer solution: this solution was prepared by withdrawing 5 mL of 1 Tris – HCl pH 7.6 and 2 mL of EDTA solution with a concentration of 0.5 and an acidic switch 8 and complete the volume to a liter of distilled water. The acid function was set at 8.0 and the sterility of the solution was kept at a temperature of 121 °C for a period of 15 minutes.

5. Chloroform

6. Ethanol (100%), -20

DNA extraction method

1. 500 µl of blood was placed in a 1.5 ml tube, and an additional 1 ml of regulating solution for the red blood cells analysis was added to it, and mixed quietly with the Vortex apparatus.

2. Centrifugation of the tube in the microfuge was performed at a speed of 7000 rpm for two minutes, the filtrate was eliminated, and the previous plan was repeated 1-3 times for eliminating hemoglobin and the tube was placed on the filter paper upside down for several seconds.

3. 400 µl of buffer solution for nucleic lysis was added to the tube and mixed quietly.

4. Added 100 µl of NaCl at a concentration of 5M saturated, add 600µl of chloroform to that tube and mix quietly, then centrifuge that tube in the Microfuge at 7000 rpm for two minutes.

5. Withdrew 400 µl of the filtrate, place in a clean and dry tube, add 800 µl of cold ethanol, and mix the contents quietly. In this stage, DNA bundles are obtained as white strands.

6. Centrifuge the tube in the Microfuge at a speed of 12000 rpm for one minute, then the leachate is removed and the tube was allowed to dry at room temperature.

7. 50 µl of the TE solution was added to the extracted DNA and mixed quietly and the sample was preserved at -20 °C until use.

Determination of extracted DNA purity and concentration

The purity and concentration of DNA extracted from blood samples was estimated in this study using Nano drop device.

Electrical migration

Solutions used in the electric relay process

1. TBE 10 solution with a power of X10: A TBE solution prepared from the English company Lonza consisting of one liter of X10 was used, using Tris-Base at a concentration of 0.89 molar, Boric acid at a concentration of 0.89 molar and Na2EDTA at a concentration of 0.02 molar. The acidic function of the solution was set at 8.3.

2. Loading Buffer with a concentration of X10, prepare by dissolving 0.25 g of bromophenol blue in the glycerol at a concentration of 50%, add EDTA at a concentration of 60 mmol and an acid exchange of 8.0 and completed the volume to 100 ml of distilled water.

3. Ethedium bromide dye was prepared by dissolving 100 mg of dye powder in 10 ml of distilled water and kept in a sterile vial at 4 ° C until use.

Preparation of the agarose gel and the electrophoresis process of DNA

To move and detect genomic DNA, the agarose gel is prepared with a concentration of 1%. To obtain this concentration, 0.5 g of agarose powder was dissolved in 50 ml of X10 TBE using a heat source with constant stirring until boiling and then left for cooling to a temperature of (60-50) ° C. The agarose gel solution was poured into the tray sink of the electrostatic relay device after the wells comb was installed at the edges of the gel and the casting was quietly to avoid bubbles formation, and that they should be removed using the pipette, then the gel was left for the purpose of hardening. The tray was placed in an electrostatic relay containing X10 TBE solution and then quietly raised the comb. Relay samples were prepared by mixing 5 µl of the DNA sample with 3 µl of loading solution. The relay is switched on by operating a voltage of 5 V \ V Cm and the process takes (2-1.5) hours. After completing the deportation process, the gel was transferred to a basin containing the dye of ethidium and left for 45 minutes with continuous stirring. Then the gel was imaged under ultraviolet radiation using a gel documentation device to be able to see the DNA bundles and the output of the PCR reaction.

PCR - REFLP interactions

The DNA concentration was adjusted in all samples studied by dilution by TE to obtain the required
concentration for conducting PCR reactions and was 25 ng/µl for each sample.

The Master Reaction main reaction mixture for each PCR reaction was prepared by mixing the DNA sample and the initiator of the UMOD Renal Failure gene with the Pri Mix components inside the 0.2 ml tube that is equipped by Promega USA. The reaction volume was then set to 20 µl with distilled water, and the mixture is put in a device Microfuge for a period of (3-5) seconds to complete the mixing of the reaction components. The tubes must be placed inside the ice during work, after which the reaction tubes were inserted into the Thermocycler to conduct the reaction using the special program for each reaction. The PCR reaction was incubated with the enzyme that breaks the mutation UMOD gene for half an hour at a temperature of 65 °C. Then the sample was loaded into the pre-prepared agarose gel at a concentration of 2% with the volumetric guide ladder DNA prepared from Biolaps. Then the samples were carried over by operating the electrophoresis device for a period of (75-90) minutes and then the gel was stained and immersed with the dye of ethidium bromide for a period of (30-45) minutes with stirring, and finally the gel was imaged using the Gel documentation device.

Detection of a mutation in the UMOD gene

The presence of the mutation was detected in 95 samples, as 100 ng of molded DNA and 10 micromoles of the initiator of the mutation supplied by Alpha were added to the Pri Mix contents.

<table>
<thead>
<tr>
<th>No.</th>
<th>Stage</th>
<th>Temperature</th>
<th>time</th>
<th>Cycle number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Initial denaturation</td>
<td>95</td>
<td>5 min.</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Denaturation</td>
<td>95</td>
<td>1 min.</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>Annealing</td>
<td>63</td>
<td>1.5 min.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Extension</td>
<td>72</td>
<td>1.5 min.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Final extension</td>
<td>72</td>
<td>7 min.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Stop reaction</td>
<td>4</td>
<td>5 min.</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 shows the output of the PCR of the UMOD gene, since M represents the volumetric evidence and the samples (1,2,3,4,5,6,7,8,9) represent the reaction result in a size of 110 bp while the samples (1a, 2a, 3a, 4a, 5a, 6a) represent the reaction product after being treated with the THIII categorical enzyme, as no cut of the amplification product of 110 bp has occurred. Which was carried over in the agarose gel at a concentration of 2%.

Results and Discussion

The results showed that the categorical enzyme (THIII) did not recognize the location of the mutation in the amplified segment, as the reaction result of 110BP before digestion and after digestion with the categorical enzyme was similar, as shown in Figure 1.

Figure 2: Shows the percentage of match between the samples on which the nucleotide sequence test was performed compared to the UMOD gene sequences in the NCBI.
In the current study, when comparing the sequence of the nitrogenous bases of the amplified segment and the sequences of the original gene, it was observed that these variations occurred in the sequence of the second intron site of the gene within the sequence (3420 >> 3360). The second intron sequences start in the gene from 2230 and end in 3666 sequence. Samples were taken for two patients who suffer from chronic kidney failure disease associated with kidney stones and high blood pressure. One of them has diabetes. Due to the lack of studies that link chronic kidney disease to the genetic factor and determine the mutations in the UMOD gene that cause UMOD-related kidney disease, it was not automatically agreed that any of the variants has any effect on the gene completely. Since the variance diagnosed in this study is of a single nitrogen base polymorphism (SNP) type. However, variations in the process of cloning into an RNA from the intron include formations or formulas. There are many ways to organize genes. The variations that occur in the sequence of the intron rules may affect the successive binding of the mRNA pieces (such as linking of receiving or donor sites, secondary structure, etc.). Variations in the sequence of intron bases can also act as enhancers, which can act on the gene in which they are present, or can enhance the expression of many genes. Therefore, you can look at the expected protein binding sites and see if there is any differential binding to a specific protein. But if there is no evidence to believe this intron has a regulatory effect, it is important not to assume that the SNP is effective. Remember that SNP can bind to some groups because it is bound to another SNP that has a physical effect on gene regulation. We can say that the occurrence of this heterogeneity in the intron region and its repetition has been attributed to the fact that this intron has a regulatory effect on the formation of the uromodulin protein, which causes the emergence of a condition of kidney disease that develops from hyperuricemia, kidney stones, high blood pressure and up to the last stage of this state, which is chronic kidney failure.

The genetic study relied on the UMOD gene sequence and mutation detection, with about 60 mutations described so far (Rampoldi et al., 2011; Bollee et al., 2001; Wolf et al., 2006; Lens et al., 2005). Since the majority of mutations are on the exons 3 and 4 of the gene, it is usually studied in most research, although mutations have also been described in other Exons, such as Exon 7. 19 No clear association has been found between phenotype and genotype. (Scolari et al., 2004; Lhotta et al., 2012; Bollee et al., 2011). Williams et al., 2009 indicated that 90% of mutations are in amino acid substitution and 62% of them alter cysteine sites, which leads to changes in the protein folding process. The UMOD gene is regulated by many transcription factors, including HNF1b, whose mutation is responsible for another disease of hyperuricemia that should be suspected in cases with clinical presentation consistent with chronic kidney failure (Faguer et al., 2011; Zaucke et al., 2010; Wolf et al., 2009). It causes mutations in the UMOD gene (chromosome region 16) two types of disorders are tubular nephropathy with a predominant autosomal genotype and type two cystic-mediated cystic kidney disease. With hereditary hyperuricemia, these disorders are currently included under the term “kidney disease caused by the UMOD gene mutation” (Ahmed et al., 2014; Ayasreh-Fierro et al., 2013; Bleyer et al., 2011;) Kidney disease caused by a mutation in the UMOD gene has a very low prevalence rate, less than 1% of end-stage kidney disease (ESKD) in adults and this proportion corresponds to a lack of diagnosis of this relationship (Coto Garcia, 2011). Ayasreh-Fierro et al., 2013 indicated that the proportion of people with a mutation in the UMOD gene in their study sample of patients with uric acidosis was 12.5% of cases. Uromodulin deficiency causes tubular re-absorption deficiency causing increased urea and hyperuricemia (Moro et al., 1991), in kidney disease associated with UMOD gene mutations Kidney failure develops slowly, generally reaching the final stage between the fourth and sixth decades of life (Bollee et al., 2001; Scolari et al., 2004;) Inherited cases of MCKD1-associated kidney failure usually have a higher level of hyperuricemia and increased early and recurrent gout attacks, and ultimately lead to end-stage renal failure. Early in life, although there are various causes of chronic kidney disease and failure for chronic renal hereditary factors are often excluded as chronic kidney disease is a lifelong disorder that is associated with the disease. And the death of large numbers of people around the world is due to the complications of gradual loss of

![Fig. 3](image)

*Fig. 3: shows the frequency of the variation in the sequence 3262 from the nitrogenous base cytosine C to thymine T.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nucleotide</th>
<th>Location</th>
<th>Sequence ID</th>
<th>Gaps</th>
<th>Expect</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T → A</td>
<td>3423</td>
<td>NG-008151.1</td>
<td>1e11</td>
<td>3e-10</td>
<td>93%</td>
</tr>
<tr>
<td>2</td>
<td>A → T</td>
<td>3429</td>
<td>NG-008151.1</td>
<td>1e11</td>
<td>1e-10</td>
<td>95%</td>
</tr>
<tr>
<td>3</td>
<td>C → T</td>
<td>3462</td>
<td>NG-008151.1</td>
<td>0e45</td>
<td>1e-11</td>
<td>98%</td>
</tr>
</tbody>
</table>
kidney function and eventual kidney failure. Early diagnosis and detection of the causative factors are important to improve the health outcomes of patients with chronic kidney disease. Mutations in the gene that encodes uromodulin protein lead to prevalent and rare physical diseases, collectively referred to as kidney disease related to uromodulin. They are characterized by gradual tubular damage, impaired kidney ability to increase urine concentration, hyperuricemia, renal cysts, and progressive kidney failure. Recent studies indicate that the accumulation of mutant uromodulin within the cells of the living body is a major cause of chronic kidney failure. Recently, genomic correlation studies have identified Uromodulin as a risk factor for chronic kidney disease (CKD) and hypertension (Fugiel et al., 2013). Several studies were conducted around the world about the extent of this gene's association with chronic kidney disease syndrome, and among these studies was a study by Ahmed and others 2017 on 103 Saudi kidney failure patients who reached an important conclusion, which is that the rate of mutation recurrence in the UMOD gene in patients with kidney failure was 9.7 % of these patients.

**Conclusions**

Mutations in the UMOD gene cause a disease characterized by excess uric acid, gout, and kidney disorders. The proportion of cases where mutations were detected was 2% of the total in the second intron, which could be considered a regulatory intron for the gene to function.

**References**


