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Genetic Polymorphism in SCL22A12 Gene Associated with Gout in a Sample of Iraqi Patients

A Thesis
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جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة النهرين

كلية العلوم

قسم التقانة الاحيائية

التباين الوراثي في جين SCL22A12 المصاحب لمرض النقرس في عينة من المرضى العراقيين

رسالة ماجستير

مقدمة الى مجلس كلية العلوم /جامعة النهرين كجزء من متطلبات نيل درجة الماجستير في التقانة الاحيائية

من قبل

اطياف عدنان نايف

بكالوريوس علوم تقانة احيائية /النهرين / ٢٠١٣

باشراف

د.وليد حميد يوسف

استاذ

شوال ١٤٣٦

تموز ٢٠١٦

2. Materials and Methods.

2.1. Materials.

2.1.1. Apparatus.

Various apparatuses were used in this study as listed in Table (2-1)

Table (2-1): Apparatuses used in this study and their company and origin

Apparatus	Company / country
Analytical balance	FC-400 / China
Autoclave	HIRAYAMA
Bench centrifuge	Uni-Media / Korea
Electrophoresis equipment	JUNY1-JY200C
Gel –documentation	Bio-Red / U.S.A
Heater – magnetic stirrer	Stuart / England
Microcentrifuge	MIKRO120 – Hettich
Microwave oven	LG / Korea
Nanodrop spectrophotometer	Techne / U.K.
Oven	Sanyo / Japan
Gradient PCR thermal cycler	Techne / U.K.
Conventional PCR	Techne / U.K.
pH meter	Martini / Japan
Sensitive balance	Mettlev / Switzerland
U.V. Transilluminator	Flowgen / U.K.
Vortex mixer	Scientific industries / U.S.A.
Water bath	Memmert / Germany

2.1.2. **Chemicals** and biological materials

Various chemicals were used in the study as listed inTable (2-2).

Table (2-2): chemicals and biological materials used in this study and their company and origin

Chemicals	Company / country
Absolute ethanol	Phamacia / sweeden
Agaros	Biobasic / Canada
DNA ladder	Promega / USA
Deionized water	Bioneer / korea
Ethedium bromide	Biobasic / Canada
Loading dye	Biobasic / Canada
master mix	Bioneer / korea
Primers	Bioneer / Korea
Tris Borate EDTA (TBE)	Biobasic / Canada

2.1.3. Kits.

Table (2-3): the kits used in the study and their company and origin are listed in Table 2-3

Kits	Company	Origin
DNA Extraction Mini Prep System	Geneaid	Taiwan
Creatinine determination kit	Linear chemicals	Spain
Uric acid determination kit	Linear chemicals	Spain

2.1. 4. Primers .

2.4 the sequences of the primers used.(Li T, *et al ,.2004)*

No.	Name	Oligonucleotides	Tm	GC%	Product size	Sequence (5'-3')
1	EX1-F	Forward primer	55.4	40.9 %	600bp	5-TTC CAT GGC ATT TTC TGA ACT C-3
	EX1 –R	Reverse primer	56.1	55.0%	-	5-CCA CCG TGA TCC ATG ACT GA-3
2	EX2-F	Forward primer	55.6	60.0	400	5-CAA CCT CCT GAG CTC AGC CT-3
	EX2-R	Reverse primer	57.7	43.5	-	5-ACT TGG TTT TGT GAG GCT GGT TT-3
3	EX3-F	Forward primer	62.8	65.0	300bp	5-TGG CAA GCC ACA GAC CCT GC-3
	EX3-R	Reverse primer	63.6	65.0%	-	5-AGC CCT GGA GCC TGC ATG GA-3
4	Ex4 –F	Forward primer	63.6	65.0%	253bp	5-TCC ATG CAG GCT CCA GGG CT-3
	Ex4-R	Reverse primer	55.3	60.0%	-	5-GGC AGG ATC TCC TCT GAG GA-3
5	Ex5-F	Forward primer	61.0	65.0%	269bp	5-GCC ACA GGC AAT GAC CCC TC-3
	Ex5- R	Reverse primer	56.8	60.0%	-	5-ACC TTC TTC CCA GGG AGC TG-3

2.2. Study subjects.

The study included 35 patients suffering from gout during the period from January 2015 to June 2015 at Al- Karamah and Al-Yarmouk teaching hospitals . All cases were investigated by rheumatologist Dr. Ahmed Abdulbari .20s healthy subject were selected to represent the control group. The mean ages of the patients

were 34 -63 years and the mean ages of the control were 25 - 40 years The personal informations for each patient were obtained , including name , age , weight , length , medications and family history of gout.

2.3. Blood samples collection.

Five ml of blood collected, two ml kept in EDTA tube and three ml in tube , serum was separated and stored at -20 until use

2.4. DNA Extraction ‘Mini Prep System kit.

The Geneius ™ Micro g DNA extraction kit , from Geneaid, Taiwan , ready to use that contained the following Component:

- S1 Buffer
- Proteinase K Solution (PK)
- Carrier RNA
- W1 Buffer
- Wash Buffer
- Elution Buffer
- GD Columns
- 2ml Collection tubes

2.5. Master Mix.

AccuPower ® PCR(polymerase chain reaction) Master Mix is a premixed, ready to use solution containing Top DNA polymerase , each dNTP (dATP, dCTP,dGTP ,dTTP),Tris- HCl (pH9.0),KCl, $MgCl_2$ and ,Stabilizer and tracking dye

2.6. DNA ladder (100 bp).

DNA ladder 100 - 3000 bp containing 13 fragments supplied in storage buffer 10mM Tris-HCL pH (7.5), 0.1 mM EDTA.

2.7. Uric Acid determination kit (appendix 1)

The components of kit are

R1 Monoreagent phosphate buffer,100mmol/L , pH7.8 uricase >50U/L ,peroxidase >1KU/L, ascorbic oxidase >0.1 KU/L , 4- aminoantipynine mmol/L, DCPS 2 mmol/L nonionic tensionactive 2 g/l

CAL Uric acid standard . uric acid 6 mg /dL(357µmol/L)

2.8. creatinine determination kit(appendix 2)

The components of kit are

R1 Picric acid : picric acid 25 mmol/L

R2 Alkaline buffer :phosphate buffer 300mmol/L

CAL Creatinine standard : creatinine 2 mg /dL (177µmol/L).

2.9. Methods.

2.9.1. Sterilization methods.

- Autoclaving: Buffers and solutions were sterilized by pressure vessel (autoclave) at 121^{0}C and 15 bar for 15 minutes.
- Dry heat: A laboratory oven was used for glassware sterilization. Glassware was placed in the oven at 200^{0}C for 2 hours.
-

2.9.2determination of uric acid (Tietz, 1995)

2.9.2 .1Test Principle

Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide . in the presence of peroxidase (POD), a mixture of dichlorophenol sulphonate (DCPS) and 4- aminoantipynine (4-AA)is oxidized by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of uric acid in the sample

Uric acid $+O_2$ +2 H_2O $\underline{URICASE}$ Allantoin $+H_2O_2$

4-AA + DCPS

2.9.2.2Procedure

1- Aliquot of one ml of R1reageant was added to a tube containing 25 µl of sample , mixed well and let the tube stand 10 minutes at room temperature or 5 minutes at 37 ºC

2- Aliquot of one ml of R1reageant was added to a tube containing 25 µl of standard, mixed well and let the tube stand 10 minutes at room temperature or 5 minutes at 37 ºC

3- Aliquot of one ml of R1reageant was added to a tube and let the tube stand 10 minutes at room temperature or 5 minutes at 37 ºC to used as blank

4-The absorbance of the sample and standard, against the blank was read at520 nm ,the color should be stable for at least 30 minutes protected from light

5-The uric acid concentration mg/dL was calculated as follow

Uric acid concentration(mg/dl) $=\frac{absorbance\ of\ sample}{absorbance\ of standard}\times$standard concentration (6mg/dL)

2.9.3 Determination of creatinine (Young DS, 2000)

2.9.3.1 test principle

Creatinine under alkaline condition reacts with picrate ions forming reddish complex . The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample

Creatinine + picric acid $\xrightarrow{pH>12}$ red addition complex

2.9.3.2 procedure

1- Aliquot of Five hundred µl of R1 reagent and 500 µl of R2 reagent was added into tube containing 100 of sample , mixed well and let the tube stand 10 minutes at room temperature or 5 minutes at 37 ºC

2- Aliquot of five hundred µl of R1 reagent and 500 µl of R2 reagent was added into tube containing 100 of standard , mixed well and let the tube stand 10 minutes at room temperature or 5 minutes at 37 ºC

3- Aliquot of five hundred µl of R1 reagent and 500 µl of R2 reagent was added into tube used as blank , mixed well and let the tube stand 10 minutes at room temperature or 5 minutes at 37 ºC

4- The absorbance(A) at 510 nm was recorded after 30 seconds (A_1) and after 90 seconds (A_2) of the sample or standard addition.

5- The creatinine concentration was calculated as fallow

$$\text{Creatinine concentration(mg/dl)} = \frac{(A2-A1)Sample}{(A2-A1)standard} \times \text{C standard}$$

2.9.4 White blood cells counting

1- Aliquot of twenty µl of total blood was mixed with 380 µl of leucocytes diluent

2- the mixture was left at room temperature for 5 minutes

3- One drop of the mixture was applied on the stage of hemocytometer neurbaeur slide containing chamber under the cover slip

4-the slid was left for 3 minutes to settle the cells

5- The leucocyte was counted in 4 large (each with 16 small squares)

6- The total count of leucocytes was calculated as follow :

$$total\ count\ (cell/\mu l.blood) = \left(\frac{number\ of\ cell\ counted}{4}\right) \times 10 \times 20$$

2.9.5. DNA Extraction from blood samples. (Appendix 3)

2.9.5.1 Procedure:

The Extraction was briefly carried out as follow:

1. Aliquot of one µl of carrier RNase solution and 200µl of S2 buffer per sample was transferred into RNase-free 1.5 ml microcentrifuge tube and vortex shortly to mix
2. Aliquot of one hundred µl of whole blood was transferred into 1.5 ml microcentrifuge tube and S1 buffer was added to make the final volume 200µl and 20µl of proteinase K then mixed by vortex
3. The mixture was incubated at 60°C for 5 minutes to lyse the sample
4. Aliquot of two hundred µl of S2 buffer was added and mixed by vortex ,the mixture was incubated at 60ºCfor 5 minute
5. Aliquot of two hundred µl of elution buffer was transferred to 1.5 ml microcentrifuge tube and heated to 60ºC to be used in elution step
6. Aliquot of two hundred µl of Absolute ethanol was added into sample lysate ,mixed thoroughly by vortex for 10 second
7. GD column was placed on a 2 ml collection tube
8. All mixture was transferred to the GD column , centrifuged at 14000xg for 1 minute . the 2ml collection tube was discarded and GD column was placed in a new collection tube
9. Aliquot of four hundred µl of W1buffer was added to the GD column , centrifuged at 14,000xg for 30 seconds , the flow – through was discarded then GD column placed back into 2ml collection tube .
10. Aliquot of six hundred µl of Wash Buffer was added to GD column .centrifuge at 14,000xg for 30 seconds then GD column was placed in the 2ml collection tube .centrifuged again for 3 minutes at 14000xg to dry column matrix
11. The dried GD column was transferred to a clean 1.5 ml microcentrifuge tube
12. Aliquot of one hundred µl of pre-heated elution buffer was added into the center of the column matrix

13. The mixture was left for at least 3 minutesto allow elution buffer to be completely absorbed , centrifuged at 14000xg for 1 minute to elute the purified DNA .

2.9.5.2. Measurement of extracting DNA concentration and purity.

Ananodrop spectrophotometer (Techne / U.K) was used to estimate the concentration and purity of the extracted DNA (from blood of patient and control) according to the following procedure:

2.9.5.2.1. Sample Measurement.

1- Two µl of the sample was pipetted on to the measurement pedestal while the sample arm is opened.

2- The sampling arm was laid down and the solution was observed bridging the gap between both optical fibers.

3- The button (Measure) was clicked and the measurement result appeared on the screen within 3 sec.

4- When any sample gives purity less than 1.5 and /or concentration less than 10 ng/µl DNA extraction from the blood of that sample re-performed until the desired purity and concentration were obtained.

2.9.6. PCR Protocols.

Extracted DNA from blood samples and healthy was used in PCR for amplification of (Exon1 ,Exon2 ,Exon3 ,Exon4 , Exon 5)

2.9.6.1.PCR Amplification.

For PCR amplification of SCL22A12 gene the following PCR protocols was followed:

1. The AccuPower ® Master (Bioneer / Korea) Mix was thawed at room temperature. The master mix was mixed by vortexing then it was spine briefly in a microcentrifuge.

Components of Taq® Master mix are illustrated in table (2-5)

Table (2-5) components of Taq® Master mix.

NO.	Components	concentration
1-	*Taq* polymerase	2.5 µl
2-	dNTP(dATP, dCTP, dGTP, dTTP)	250 µM
3-	Tris – HCl (pH 9.0)	10 mM
4-	KCl	30mM
5-	Mg Cl_2	1.5mM

2. The reaction mixture was prepared by adding the components listed in table (2-6)

Table (2-6): The optimal values of various steps in the amplification.

Components	Volume(µl)	Concentration
Master Mix	25	1x
Forward primer	0.2	0.2 µM
Reverse primer	0.2	0.2 µM
DNA template	5	> 250 ng
Nuclease free water	19.6	-
Final volume	50µl	

3.The mixture was put in microcentrifuge and spinned for better mixing.
4. After mixing the master mix tubes were transferred to the thermocycler (Techne 32 thermal block / UK) which was previously programmed with the above protocol according to the *SCL22A12*gene.

2.9.6.2. Optimal protocol of PCR amplification.

For PCR amplification of number gene (Ex1 ,Ex2 ,Ex3 ,Ex4 , Ex 5)) annealing temperature in optimizing the following PCR protocols as in tables 2.7 ,2.8.2.8, 2.10and 2.11

Table 2.7 .PCR Amplification using Ex1 primer

Thermal cycler protocol	No. of cycle	Temperature(°C)	Time (Min)
Initial Denaturation	1	94	5
Denaturation	35	94	1
Annealing		60	1
Extension		72	1
Final Extension	1	72	10

Table 2.8. PCR Amplification using Ex 2primer

Thermal cycler protocol	No. of cycle	Temperature(°C)	Time (Min)
Initial Denaturation	1	94	5
Denaturation	35	94	1
Annealing		64	1
Extension		72	1
Final Extension	1	72	10

Table 2.9. PCR Amplification using Ex3primer

Thermal cycler protocol	No. of cycle	Temperature(^{0}C)	Time (Min)
Initial Denaturation	1	94	5
Denaturation	35	94	1
Annealing		67	1
Extension		72	1
Final Extension	1	72	10

Table 2.10.PCR Amplification using Ex4 primer

Thermal cycler protocol	No. of cycle	Temperature(^{0}C)	Time (min.)
Initial Denaturation	1	94	5
Denaturation	35	94	1
Annealing		58	1
Extension		72	1
Final Extension	1 cycle	72	10

Table 2.11.PCR Amplification using Ex5primer

Thermal cycler protocol	No. of cycle	Temperature(^{0}C)	Time (min.)
Initial Denaturation	1	94	5
Denaturation	30cycle	94	1
Annealing		62	1
Extension		72	1
Final Extension	1 cycle	72	10

2.10. Agarose gel electrophoresis (Sambrook,2002).

1. Two percent of agarose gel was prepared by mixing: 100ml of 1X TBE buffer and 2 gm agaros in a glass bottle. A glass bottle was heated in a magnetic stirrer with heater until the components were dissolved.
2. This solution was cooled to 70°C, 2 µl ethidiumbromides was added from stock solution and mixed thoroughly.
3. The clean glass tank (17X12X4 cm) was set on a horizontal section of the bench. The comb was set in position 0.5-1.0 mm above the surface of the tank so that a complete well was formed when agarose was added.
4. The warm agarose – solution was poured into the mold.
5. After the gel was completely set (20-30 min. at room temperature), the comb was carefully removed and the gel mounted in the electrophoresis tank which contain previously small amounts of 1X TBE buffer.
6. A volume of 600ml 1X TBE was added to cover the gel in depth about 1mm.
7. A volume of 10 µl the sample of DNA (PCR product) was added slowly into the slots of the submerged gel using an automatic micropipette.
8. A volume of 5 µl DNA marker was mixed with 1 µl of loading buffer. The mixture was set slowly into the slots of the submerged gel using an automatic micropipette.
9. The gel was subjected to electrophoresis at 70 volts until the bromophenol blue tracking dye, migrated at least two-thirds of the way down the gel.
10. The gel was examined by ultraviolet light using a UV transilluminator then photographed.

2.11. DNA sequencing.

Polymerase chain reaction products of *SLCA22A12* gene (Ex1, Ex2,Ex3,Ex4,Ex5) 20 blood samples were sent Company bioneer for DNA sequencing in Korea. The obtained sequences were aligned using NCBI software with normal sequence from NCBI GenBank and examined of the presence of SNPs.

2.12. Statistical Analysis.

The Statistical Analysis System- SAS (2012) program was used to determine of difference factors in study parameters . Least significant difference –LSD test was used to significant compare between means in this study(SAS. 2012)

Appendix

Chapter one

Introduction and literature review

1.Introduction and literature review

1.1 Introduction

Gouty arthritis (also called as podagra when it involves the big toe) is a medical condition that characterized by recurrent attacks of acute inflammatory arthritis—a red, tender, hot, swollen joint. The metatarsal-phalangeal is a joint at the base of a big toe is the most affected (approximately 50% of infected people). Although , it may also present as tophi, kidney stones, or urate nephropathy. It is caused by high levels of uric acid in the blood. The uric acid accumulated and crystallizes, and the crystals deposit in joints, tendons, and surrounding tissues .Gout can cause: pain, swelling , redness , heat, and stiffness in joints(Brookhiser *et al*., 2008).Symptoms of gout include Hyperuricemia (elevated level of uric acid in the blood) , uric acid crystals at joint fluid , more than one attack of intense arthritis , arthritis that develops in one day, producing a swollen, red, and warm joint ,and attack of arthritis in only one joint, usually the toe, ankle, or knee(Chen *et al* .,2008). Gout is a common shape of inflammatory arthritis - a condition affecting the joints and musculoskeletal system. It is the commonest form of inflammatory arthritis in men, and though it is more likely to affect men, women become more susceptible to it after the menopause .(Hyon Choi,.2011)

There are a number of different factors that may increase uric acid in the body, including higher production of uric acid and the reduced elimination of uric acid by the kidney(Francis.,2008)

Not necessarily everyone with high levels of uric acid, however, will develop gout. Several factors can be associated with high uric acid or gout:

1. Genetic problem of uric acid metabolism (rare) : There may be a family history of gout associated with these rare defects.(Terkeltaub *et al*.,2003)

2. Some of drugs , which may include water pills taken for edema (e.g. heart failure) may block the excretion of uric acid in the kidney .(Klippel .,2001)

3. Foods with high purines contents , which are the building blocks of uric acid in the cell, may raise levels of uric acid. Purine-rich foods include certain sea foods and red meat. Alcohol and fructose found in soft drinks may also increase amounts of uric acid. (Shoji et al., 2004)

4. Obesity, hypertension (high blood pressure) and diabetes may be associated with elevated uric acid levels and gout The most majority of people with hyperuricemia do one condition that can sometimes be confused with gout is pseudo gout. The symptoms of pseudo gout are very similar to those of gout, the difference being that the joints are infected by calcium phosphate crystals rather than urate crystals .This means that pseudo gout requires different treatment to gout.(Tausche *et al* ., 2009)

The majority of gout cases are treated with medication. Medication was used to treat the symptoms of gout attacks, prevent future flares and reduce the risk of gout congealment such as kidney stones and the development of tophi.(Firestein *et al* ., 2008)

This study aimed to

1- to investigate the relationship between single nucleotide polymorphism in SCL22A12 gene and the incidence of gout

2- Study the relationship between kidney function and the gout

1.2 literature review

1.2.1 definition of gout

Gout is an inflammatory type of arthritis caused by uric acid crystals. Painful attacks is occur when uric acid crystallizes in the joints (Hyon *et al* ; 2008).Gout is derived from *gutta* (Latin for drop), as it was believed in the thirteenth century that poison falling in drops into the affected joint caused gout(Fam(2002).Gouty arthritis occurs more often in men than women. It affects about two per cent of both men over age thirty and women over age fifty in Canada, it usually, but not always, develops in people over the age of forty . Men are more likely to get gout, but women become increasingly susceptible to gouty arthritis after menopause. Gouty arthritis is strongly associated with obesity, hypertension, diabetes, and genetic factors (Weaver, (2008) .The ability to maintain uric acid in dissolvable form is an important body function; people with gouty arthritis may not be able to do this(Hyon *et al* ., 2008). Gouty arthritis results from an innate immune reaction to monosodium urate crystals deposited in the joints of individuals with elevated serum urate levels (hyperuricaemia). Urate, the primary cause of gouty arthritis , is a metabolite with useful properties(Merriman *et al*; 2013).The most common sign of gouty arthritis is a night time attack of swelling, tenderness, redness, and sharp pain in the big toe , foot, ankle, or knees, or other joints . (Murray *et al* ; 2006)

Cases of gouty arthritis have increased in recent years, This increase is likely due to an aging population, dietary and lifestyle changes, greater use of medicines, such as diuretics medication , all of which can lead to a high uric acid level in the body (Lebiedz-Odrobina *et al* .,2010).

Recent studies have provided information on dietary risk factors for gouty arthritis : higher intakes of red meat, fructose and beer are independently associated with

increased risk, whereas higher intakes of low-fat dairy products and vitamin C are associated with lower risk(Zhang *et al ;*(2006).

1.2.2 Stages of Gouty arthritis

The progression of gouty arthritis is divided into following the four stages

1.Stage one : Asymptomatic Hyperuricemia

During this stage, a person has no symptoms of gouty arthritis, but uric acid levels are above 6.0 mg/dL (Roubenoff *et al .,* 2005). The body will already have excess amounts of uric acid in the blood there's no discomfort or outward evidence of disease but gouty arthritis is beginning to form(Becker *et al .,*(2009)

2. Stage two: Acute gout

During this stage, crystals have been depositing in the joints activate and cause episodes of intense pain and swelling in the joint. The pain will subside, even without treatment, within three to ten days (Moyer *et al .,*(2003). it is at this point that gout starts to do damage to the body. The buildup of uric acid causes crystals to form in the joints causing a sudden onset of severe pain. An acute attack of gouty arthritis is typically evident upon awakening from sleep and residual discomfort may longer for more than a week. Subsequent episodes of gouty arthritis may not occur for months or even years; however, over time attacks can last longer and recur more frequently.(Abrams 2005).

3. Stage Three: Interval Gout

This stage is similar to stage one in that the symptoms of gouty arthritis are not present except for the high levels of uric acid in the blood.(Richette *et al.,*(2010).During this stage, a person is in between gouty arthritis flares. it's a symptom-free time, when their joints are functioning normally. Although, even when symptoms are not present , ongoing deposits of uric acid crystals continue to

accumulate, silently. Additional and more painful attacks of gouty arthritis are likely to continue unless the uric acid is lowered to below 6.0 mg/dL(Terkeltaub 2003).

4. Stage Four: Chronic Gout

In this final gouty arthritis stage, the gouty arthritis attacks are more frequent and the attacks themselves are longer .This is the most debilitating form of gouty arthritis . It usually takes a long time to develop—up to 10 years—and is most common in those whose gouty arthritis is not treated. (Haines,2013).

At this stage the disease has caused permanent damage to the affected joints producing persistent joint inflammation, joint deformity, and deposits of crystallized uric acid (tophi). Tophi can cause chronic pain, joint destruction, damage surrounding tissues, and cause deformities(Zhang , *et al.,* 2006).

1-2-3 Uric acid

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $C_5H_4N_4O_3$.(McCrudden *et al* .,2009).

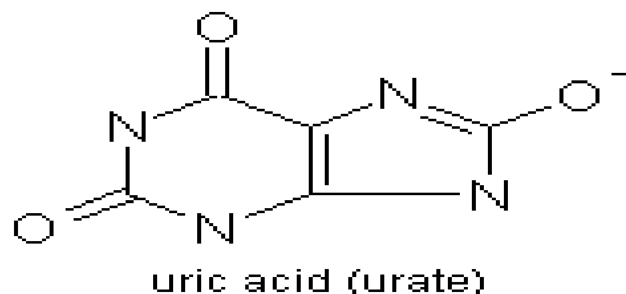


Figure 1.1 – chemical structure of uric acid (Richette P *et al*.,2010)

Uric acid is a product of purine metabolism generated throughout the breakdown of nucleic acids (DNA and RNA) and ATP(adenosine tyriphosphat) , and uric acid can also be generated from proteins . (Lonardo *et al* .,2002). Serum uric acid levels may

be vary greatly in humans, and can range from 2.5 to 12 mg/dl or more .(Weiner *et al* .,2008).

Uric acid is the end product of purine metabolism. Its immediate precursor, xanthine, is converted to uric acid by an enzymaticly reaction involving xanthine oxidoreductase (Baillie *et al* .,2007)It has been hypothesized that uric acid (UA) provides an antioxidant defense in humans, so it might be protective against oxidative stress in cardiac, vascular, and neural cells (Hayward *et al.,* 2008)

The levels of uric acid in the blood depend up on two factors. The first is the rate of uric acid synthesis in the liver. Because of uric acid results from purine degradation, its levels are influenced by both the amount of purines synthesized in the body, also the amounts of purines absorbed from the diet (Richette 2010). The second determinant of blood uric acid levels is the rate of uric acid excretion from the kidneys. Excretion has the highest effect on blood uric acid levels, with about 90% of hyperuricemia cases attributed to impaired renal excretion (Choi, 2005).

Impaired excretion is most often because of abnormalities in the kidney urate transporter (called URAT1) or organic ion transporter (OIT), both of which control the movement of uric acid out of proximal kidney tubules and into urine (Enomoto 2002).

The ability of humans and primates to preserve blood levels of uric acid (because of slow kidney filtration and lack of a uricase enzyme) was probably advantageous to our evolution, by increasing antioxidant capacity of the blood (Alvarez-Lario and Macarrón-Vicente ,2011).The rate of urate deposition and, consequently, the rate of tophi formation, correlate with the duration and severity of hyperuricemia (Medellin *et al* .,1997)The use of genome-wide association scanning has identified 28 loci that control serum urate levels. Predominant among these are loci containing uric acid

transporter genes involved in renal and gut excretion of uric acid. (Arromdee *et al* .,2002)

Havelock Ellis proposed in the early 1900s that uric acid might have a role in intelligence. He noted that gouty arthritis was frequently a disease of the educated peoples , and that many famous philosophers and scientists from the 1800s had gout. This concept was reintroduced in a letter to Science in the 1950s by Orowan, who noted that uric acid has chemical characteristics similar to caffeine (Orowan ,1955)

1.2.4Hyperuricemia

Hyperuriemia is a case that based upon the solubility limit of urate in serum. A concentration of >7 mg per dL. (in each sexes) is taken to denote hyperuricemia, the concentration above which urate saturation occurs . (Campion *et al* .,1987)

Hyperuricemia results from insufficient renal excretion or urate overproduction from purines, or both (Emmerson ,*et al ;*(1992).As local serum uric acid concentrations rise above their limit of solubility, monosodium urate can begin to precipitated out of the blood, forming needle-like crystals preferentially in cartilage and fibrous tissues. Here, the crystals might reside for years without causing any problems (Doherty 2009).

Approximately 70% of the urate that produced daily is excreted out by the kidneys, while the rest is eliminated by the intestines. However, during renal failure, the intestinal contribution of urate excretion increases to compensate for the decreased elimination by the kidneys. (Kim *et al* .,2009) .

Urate excretion does appear to correlate with the serum urate concentration due to a small increase in the serum concentration results in a marked increasing in urate excretion. (Mayor , *et al ;* (2006). Hyperuricemia may occur because of

low excretion (underexcretors), increased production (overproducers), or a combination of these two mechanisms (Inoue *et al* .,2004) . It is a very common condition, being sometimes caused by an unhealthy lifestyle that is mainly represented by a poor diet exceeding in purine nucleotides, protein, alcohol, and carbohydrates intake. (Johnson *et al* .,2004) .

Asymptomatic hyperuricemia is a term traditionally applied to settings in which the serum urate concentration is elevated but in which neither symptoms nor signs of monosodium urate (MSU) crystal deposition disease, such as gout, or uric acid renal disease, have occurred.(Sampat *et al* .,2011).

1.2.5 Gouty arthritis epidemiology

Several British and American surveys have estimated the prevalence of gouty arthritis to be 2.6–8.4 per 1000 in adults, with the prevalence increasing with age to rates of 24 per 1000 in men and 16 per 1000 in women aged 65–74 years. Gouty arthritis has a predilection for the first metatarsophalangeal joint , with as many as 50–70% of first gouty arthritis attacks occurring here (Wright, *et al* ., 2007)

The National Health and Nutrition Examination Survey (NHANES) data from 2007 to 2008 showed a hyperuricemia (serum urate ⩾7 mg/dl) prevalence of 21.1% in men and 4.7% in women (Zhu, *et al* ., 2011)

The epidemiology of gouty arthritis is difficult to quantify precisely due to variations in methodology between studies, including differences in case definition and in the means of estimating incidence and prevalence. Although, there is no doubt that it is a very common condition and studies from several countries, particularly in the USA, report an increase in prevalence in recent decades (Roddy , *et al* ., 2007).

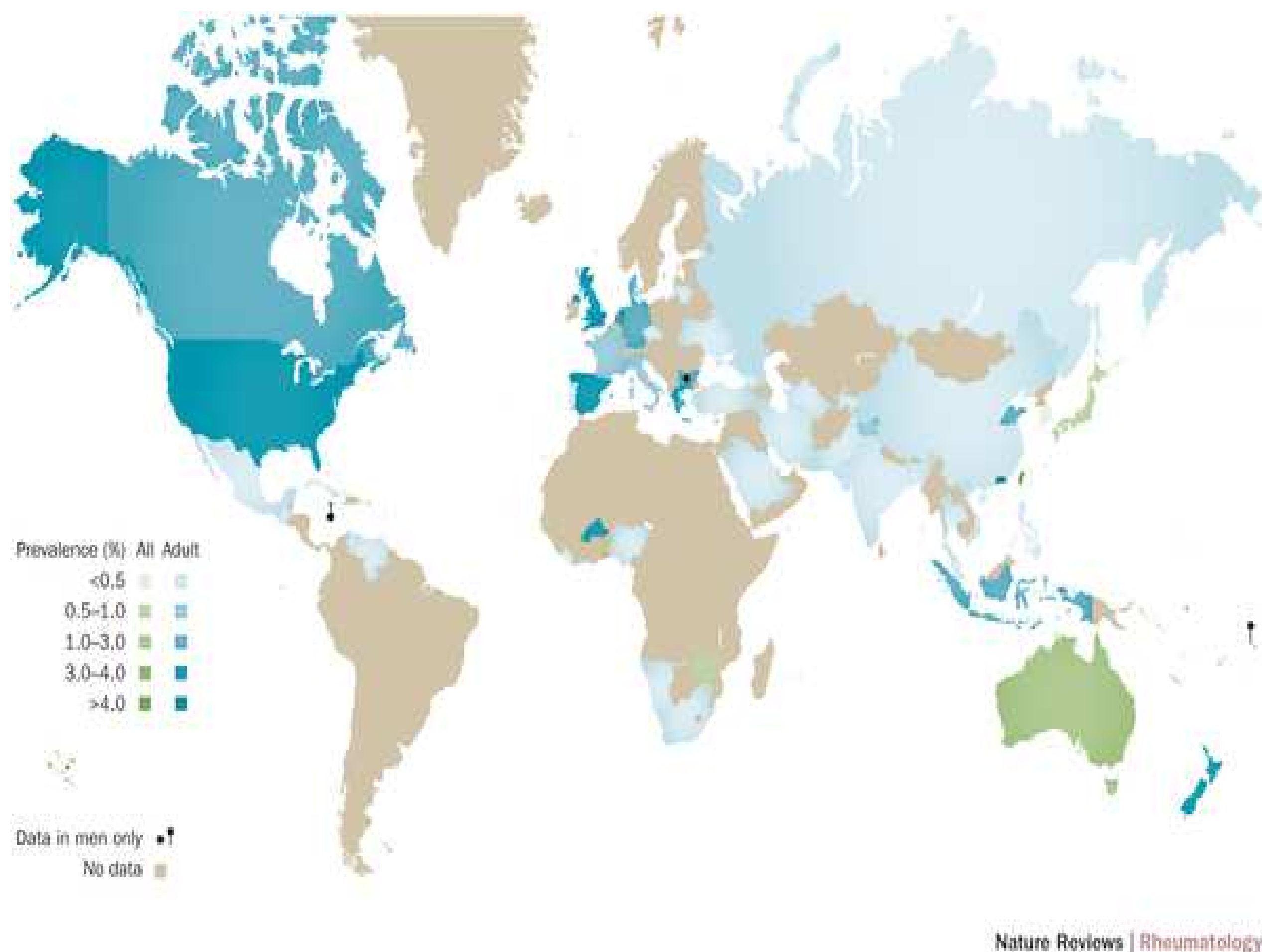


Figure 1.2 Gouty arthritis epidemiology (Kuo, C. F., *et al* ,.2014)

1.2.6Gouty arthritis symptoms

Warmth, pain, swelling, and extreme tenderness in a joint, usually a big toe joint . This symptom are called podagra (Pizzorno *et al* .,2006) .Gouty arthritis usually becomes symptomatic suddenly without warning, often in the middle of the night. The main symptoms are intense joint pain that subsides to discomfort, inflammation and redness. Gouty arthritis frequently affects the large joint of the big toe, but can also commonly affect the ankles, knees, elbows, wrists and fingers (Wu *et al* .,2008).Another symptom of gouty arthritis is the presence of tophi. A tophus is a

hard nodule of uric acid that deposits under the skin. Tophi can be present in various locations in the body, commonly on the elbows, upper ear cartilage, and on the surface of other joints. When a tophus is present, it indicates that the body is significantly overloaded with uric acid. (*Lin et al ., 2000*)

Other symptoms may seldom occur along with the joint pain, including fatigue and a high fever . (Eggebeen , (2007)Some people may not experience gouty arthritis as many painful attacks. Instead they have gout nearly all the time (chronic gout). Chronic gouty arthritis in older adults may be less painful and can be confused with other forms of arthritis . (Bhole *et al*., (2010).Gouty arthritis may first appear as nodules (tophi) on the hands, elbows, or ears. The person may not have any of the classic symptoms of a gouty arthritis attack . (Comeau *et al*., (2011).

1-2-7 Pathogenesis

The exact cause of gouty arthritis is not known, although it may be linked to a genetic defect in purine metabolism (Weaver ,2008) The pathogenesis of gouty arthritis starts with the crystallization of urate within the joint, bursa, or tendon sheath, which leads to inflammation as a result of phagocytosis of monosodium urate crystals; the disease is usually associated with an elevated concentration of uric acid in the blood(Dore,2008). These crystals then trigger local immune mediated inflammatory reaction, with one of the key proteins in the inflammatory cascade being interleukin 1β. An evolutionary loss of uricase, which breaks down uric acid, in humans and higher primates has made this condition common (Richette *et al.,*2010).

Other factors believed important in triggering an acute episode of arthritis include cool temperatures, rapid changes in uric acid levels, acidosis, articular

hydration, and extracellular matrix proteins, such as proteoglycans, collagens, and chondroitin sulfate (Moyer *et al.*,2003).

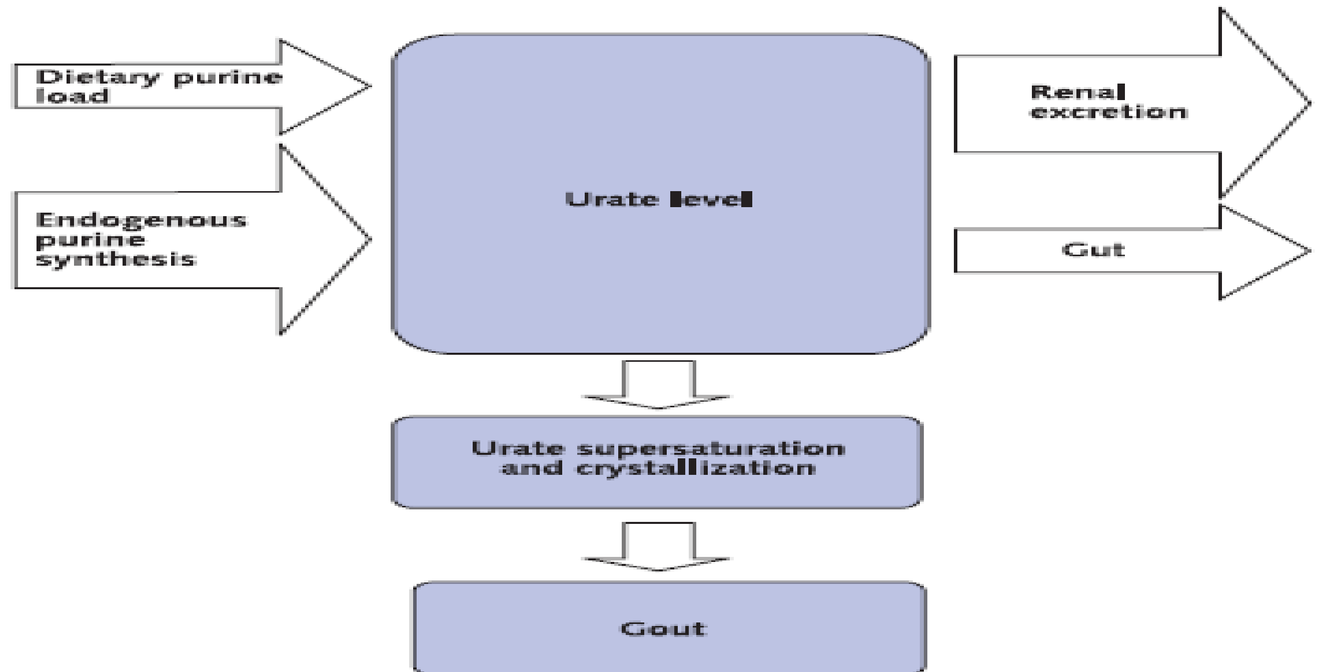


Figure 1.3 Overview of the pathogenesis of gout (Hochberg , *et al* .,2003)

1.2.8 Causes of Gouty arthritis :

Gouty arthritis is caused by too much uric acid in the blood (hyperuricemia). The exact cause of hyperuricemia sometimes isn't known, although inherited factors (genes) seem to play a role (Bhole *et al*., (2010).This can occur for a number of reasons, including diet, genetic predisposition, or under excretion of urate, the salts of uric acid (Chen *et al*.,2008).

Normally, uric acid dissolves in the blood and is excreted from the body in urine via the kidneys. If too much uric acid is produced or not enough is excreted then it can build up and form the needle-like crystals that cause inflammation and pain in the joints and surrounding tissue(James 2014).

When uric acid levels in the blood are high, it is called hyperuricemia. Most people with hyperuricemia do not develop gout. But if excess uric acid crystals form in the body, gouty arthritis can develop.(Choi *et al* .,2005)

Certain high-protein foods can make the body produce too much uric acid, triggering gout. Beverages such as tea, coffee, cocoa, and especially alcohol in any form lead to extra water loss from the body, which can cause an attack (Pizzorno *et al* .,2006) .

Hyperuricemia can have many causes. Serum uric acid levels become elevated in any disorder that results in the proliferation of cells or the excessive turnover of nucleoproteins. Hyperuricemia can also occur with decreased renal function and in genetic disorders that increase the production or limit the excretion of uric acid (Wortman ,1998).

There are a number of factors that can increase the likelihood of hyperuricemia, and therefore gouty arthritis : Age and gender , Genetics , Lifestyle choices , Lead exposure , Medications , Weight ,and other health problems (Weaver, 2008)

1.2.9 Gout Risk Factors

The genes that inherited, male gender, kidney function, and nutrition (alcoholism ,obesity) play key roles in the development of gouty arthritis . (William,2014).

1.2.-9.1 Age

Children

Gouty arthritis in children is uncommon except for rare inherited genetic disorders that cause hyperuricemia.(Keenan *et al.,*2012).

Middle-Aged Adults.

Gout usually occurs in men in the mid-40s. Men of this age group who have gouty arthritis are often obese, have high blood pressure, unhealthy cholesterol levels, and drink large amounts of alcohol(Doherty *et al* .,2009).

Elderly.

Gouty arthritis occurs equally in men and women. In this group, gouty arthritis is most often associated with kidney problems and the use of diuretics medications . It is less often associated with alcohol use.

1.2.9.2Gender

Clinically, gouty arthritis is often considered a male disease. Although gouty arthritis prevalence has increased in both sexes, among patients younger than 65 years men have a fourfold greater prevalence than women (Wallace *et al.,*2004).In men, uric acid level normally rises at puberty(Lebiedz-Odrobina *et al.,*2010)Men get gouty arthritis more than women at younger ages. The onset of gout among men is typically between the ages of 40-60 years, although it may begin earlier for those with a genetic predisposition .Most women experience gouty arthritis after menopause (Roddy *et al* .,2005).

1.2.9.3 Genetics

About 20% of persons with gouty arthritis have family histories of this condition. Several genes are linked to uric acid metabolism and gouty arthritis . Some people have a defective protein (enzyme) that interferes with the way the body degradation purines (Zhu *et al* .,2012).

Three genes called *SLC2A9*, *SLC22A12*, and *ABCG2* have been found commonly to be associated with gouty arthritis, and variations in them can approximately double the risk (Merriman *et al* .,2011).

Genome-wide association studies (GWAS) are a developed research technique to identify DNA(Deoxyribo nucleic acid) polymorphisms distributed across different large populations, and they permit the determination of common genetic factors that influence health and disease. Since 2008, different GWAS identified nine DNA loci associated with serum urate concentration, with four linked to gouty arthritis in several populations (Dehghan et al .,2008) .

1.2.9.4 Lifestyle choices

Alcohol consumption interferes with the removal of uric acid from the body. Eating a high-purine diet also increases the amount of uric acid in the body (Neogi ,(2011) Increased meat and seafood intake were associated with 1.41-fold and 1.51-fold increases in risk for gouty arthritis , respectively, for the highest versus the lowest quintiles of intake(Arromdee E *et al ;* (2002) Weight gain and obesity plays a huge role in the development of gouty arthritis .When the body becomes too heavy, a lot of weight is being put onto the kidneys, so they won't be able to function correctly ,Data suggest that obesity increases serum urate by eliciting both increased production and decreased renal excretion of urate (Dessein *et al* .,2000)

1.2.9.5Medical conditions

Medical conditions that can increase your risk of developing gouty arthritis include:

- high blood pressure (hypertension)
- Diabetes mellitus – both types
- kidney disease
- Hyperlipidemia
- osteoarthritis (Karl , (2007)

1.2.9.6 Medications

Gouty arthritis is associated with a number of different medications, including diuretics, low-dose aspirin, and drugs that are commonly used in organ transplantation (immunosuppressant drugs) (Abdelrahman *et al* .,2002) .

The treatment of certain types of cancer can cause gouty arthritis because of high levels of uric acid released when cells are destroyed(cytotoxic drugs)(Sulem *et al* .,2011)

1.2.9.7Renal and other major organs transplants

Hyperuricemia and gouty arthritis are common complications of renal and other major solid-organ transplants. Gouty arthritis joint damage is accelerated in transplant patients with hyperuricemia compared with patients with primary hyperuricemia, and gouty arthritis is frequently poly articular (Baroletti *et al* .,2004)

1.2.9.8Lead Exposure

Long-term exposure to lead is associated with buildup of uric acid and a high incidence of gouty arthritis. (Krishnan *et al* .,2012)

1-2-10Diagnosis

The history and physical examination is important, but the only sure way to confirm a diagnosis of gouty arthritis is to test for uric acid crystals in the synovial fluid or tophi under polarizing microscopic examination. These appear as needle and rod shapes and have strong negative birefringence. These must be differentiated from pseudo-gout crystals (calcium pyrophosphate), which appear differently under polarized light and are more rhomboid rods, squares or irregular in shape, and are weakly positively and bluish in coloration(Krishnan, *et al* ., 2008)

The differential diagnosis for symptoms suggesting an acute gouty arthritis attack includes acute pseudo gout, septic arthritis, inflammatory arthritis, cellulitis, and trauma. Because these diagnoses may have similar presentations, it is important to establish a correct diagnosis, as the treatment implications are significant (Roberts ,*et al* ., 2009)

Exams and tests that may help with diagnosis and treatment of gout include:

1-A joint fluid analysis (arthrocentesis) to see whether uric acid crystals are present. This is the only certain way to diagnose gout.

2-A medical history and physical exam,

3-A test to measure levels of uric acid in blood. This may be done if the doctor cannot safely get fluid from the affected joint ,A test to measure levels of uric acid in urine,

4-X-rays of extremities (hands and feet) are sometimes useful in the late stages of the disease . (Comeau *et al* .,2011)

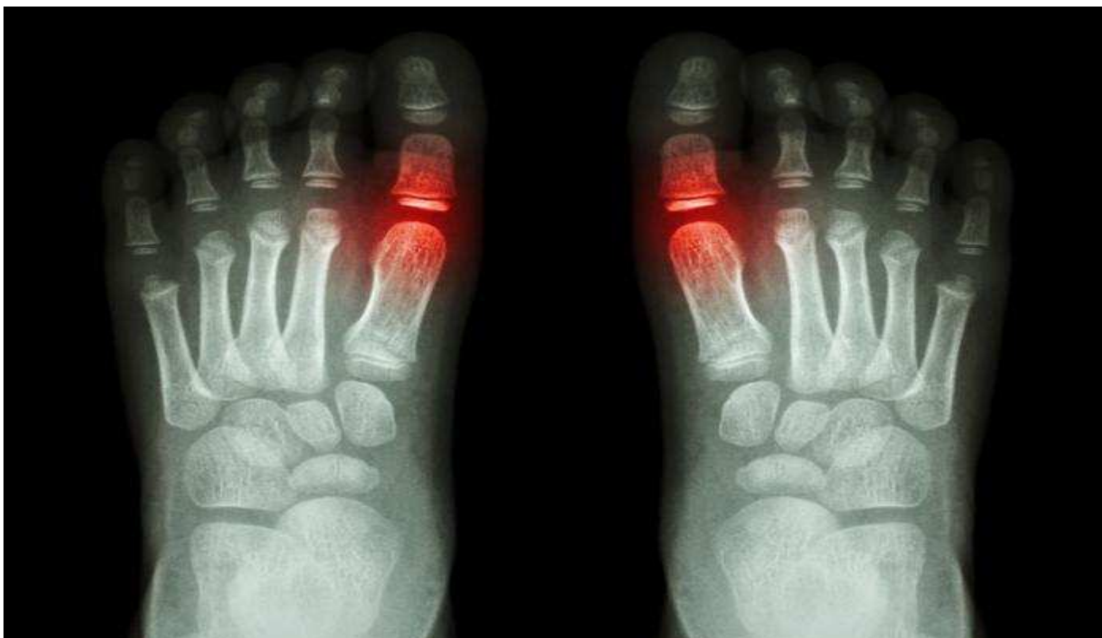


Figure 1.4: x- ray of inflamed big toe (james ,2015)

Blood tests may reveal an abnormally high level of uric acid. Having a higher level of uric acid in the blood alone does not mean a gouty arthritis. Many people with elevated levels of uric acid do not develop gouty arthritis. A definitive diagnosis is made by inserting a needle into the joint and taking a sample of fluid, which, when examined properly, reveals the classic appearance of uric acid crystals .(Terkeltaub , 2010).

1-2-10-1Synovial fluid

A definitive diagnosis of gouty arthritis is based upon the identification of monosodium urate crystals in synovial fluid or a tophus .(Schlesinger , 2010). To confirm the presence of gouty arthritis , a doctor or health care professional may insert a needle into the inflamed joint and draw a sample of synovial fluid, the liquid that provides lubrication for each joint. This joint fluid is placed on a slide and examined beneath a microscope in search of uric acid crystals. (Steele ,1999).

1.2.10.2Blood tests

Hyperuricemia is a classic feature of gouty arthritis , but it occurs nearly half of the time without hyperuricemia, and most people with raised uric acid levels never develop gouty arthritis . Thus, the diagnostic utility of measuring uric acid level is limited. (Tausche *et al* .,2009)

In acute attack , blood test can reveal elevated white blood cell count and elevated erythrocyte sedimentation rate . (Mandel *et al* .,2007)

1.2.11Gout prevention

Both lifestyle changes and medications can decrease uric acid levels .(Chen *et al* .,2008)

Medications and lifestyle changes, such as reducing alcohol consumption, limiting intake of meats and fish rich in purines, and maintaining a healthy weight can help to lower uric acid levels in the body. However, in most cases, medication is require(Elme 2006)

1.2.12 Gouty arthritis treatment

The goals of treatment for gouty arthritis are fast pain relief and prevention of future gouty arthritis attacks and long-term complications, such as joint destruction and kidney damage . (Wortmann ,2009).Through proper treatment and understanding of the disease, patients can avoid painful episodes and long-term joint damage and disability due to gouty arthritis , and maintain their normal lifestyle as well.(Huang *et al* .,2005)

1-212.1Treatments to Relieve Pain and Reduce Swelling in Acute Gout

1-nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are the usual first-line treatment for gouty arthritis, and no specific agent is significantly more or less effective than any other-Improvement may be seen within four hours, and treatment is recommended for one to two weeks. (Richette *et al* .,2010).

2-Colchicine

Colchicine is an alternative for those unable to tolerate NSAIDs. At high doses, side effects (primarily gastrointestinal upset) limit its usage. At lower doses, which are still effective, it is well tolerated. . (Laubscher *et al.,*2009)

3-Glucocorticosteroids (cortisone)

Taken orally or injected, cortisone quickly inhibit the inflammation of an acute attack.(Firestein *et al* .,2008)

1.2.12.2Treatments to Lower Uric Acid Levels and Prevent Future Attacks

Patients who have repeated gouty arthritis attacks, abnormally high levels of blood uric acid, or tophi or kidney stones should strongly consider medicines to lower blood uric acid levels (Hayden MR *et al* .,2004). These medications do not help the painful flares of acute gouty arthritis , so most patients should start taking them after acute attacks subside. The drug most often used to return blood levels of uric acid to normal is allopurinol (Lopurin, Zyloprim).

1.2.13. Molecular Genetics of Hyperuricaemia and Gout

The use of genome-wide association scanning has identified 28 loci that control serum urate levels (Batt C *et al* ,.2013).

Predominant among these are loci containing uric acid transporter genes involved in renal and gut excretion of uric acid. The SLC2A9 (GLUT9) and ABCG2 genes have particularly strong effects on serum urate and risk of gout (Caulfield MJ et al ,. 2010).The SCL22A11 and SCL22A12 genes (encoding organic aniotransporter 4 (OAT4) and urate transporter 1 (URAT1), respectively) are located together on chromosome 11. Variants in each of the genes have previously been associated with serum urate concentration in Caucasian (Dalbeth N *et al*.,2013).

Nowadays, URAT1 is considered the most important element in the mechanisms involved in the reabsorption and urinary excretion of uric acid. URAT1 action is immediately modified by organic anions (i.e. lactate) and several uricosuric or antiuricosuric drugs including probenecid, benzobromarone, sulfinpyrazone, phenylbutazone, non-steroidal anti-inflammatory drugs, losartan and diuretics(Enomoto A *et al* ., 2002)

URAT1 is encoded by the SLC22A12 gene (11q13), which is constituted by 2642 base pairs in 10 exons. While several SLC22A12 gene mutations have been found in

Japanese patients with idiopathic renal hypouricaemia (IRH), the healthy Japanese also have various polymorphisms of the same gene(Ichida K *et al* ., 2004)

The locus most strongly linked to gout corresponds to the glucose transporter 9 (GLUT9), also known as the solute carrier 2A9 (SLC2A9). In contrast to the three other loci that are linked to an increased risk of gout, several variants of GLUT9 are linked to a reduced risk (Wallace C *et al* ., 2008).The second locus linked to gout corresponds to the gene coding the urate transporter 1, URAT1 (also named solute carrier 22A12). The gene was cloned in 2002 and immediately identified as a urate-organic anion exchanger, re-absorption being triggered by high intracellular loads of lactate and several other organic anions (Enomoto A *et al* ., 2002)The third locus linked to gout encodes the renal sodium phosphate transport protein 1 (NPT1, or solute carrier17A1), which is localized at the apical membrane of renal proximal tubules(Iharada M *et al*., 2010)

The last locus linked to gout corresponds to the ABCG2 gene, a transporter initially known for its involvement in resistance to chemotherapy (Noguchi K *et al* ., 2009)

Chapter three

Results and discussion

3. Results and discussion

3.1.Study subject

The study included 35 patients suffering from gout during the period from January 2015 to June 2015 at Al- Karamah and Al-Yarmouk teaching hospitals . All cases were investigated by rheumatologist Dr. Ahmed Abdulbari .20s healthy subject were selected to represent the control group. The mean ages of the patients were 34 -63 years and the mean ages of the control were 25 - 40 years The personal informations for each patient were obtained , including name , age , weight , length , medications and family history of gout.

3.2. Serum uric acid and creatinine concentration and white blood cell count :

The relationship between gout, uric acid , creatinine and white blood cell(WBC) count is shown in Table 3.1 and Figures 3.1, 3.2 . and 3.3 ,

Table 3.1. Serum uric acid and creatinine concentration and WBC count in control subjects and pateints with gout

Groups	No	Serum uric acid concentration (mg/dl)	Serum creatinine Concentration (mg/dl)	White blood cell count(cell/µl)
Control	20	4.39 ± 0.14	1.845 ± 0.06	6375.00 ± 237.62
Patients with gout	35	7.57 ± 0.28	20.61 ± 1.29	9488.57 ± 214.69
LSD value	---	0.787 **	3.451 **	674.61 **
P-value	---	0.0001	0.0001	0.0001
** (P<0.01), NS: Non-significant.				

Values are mean ±SE

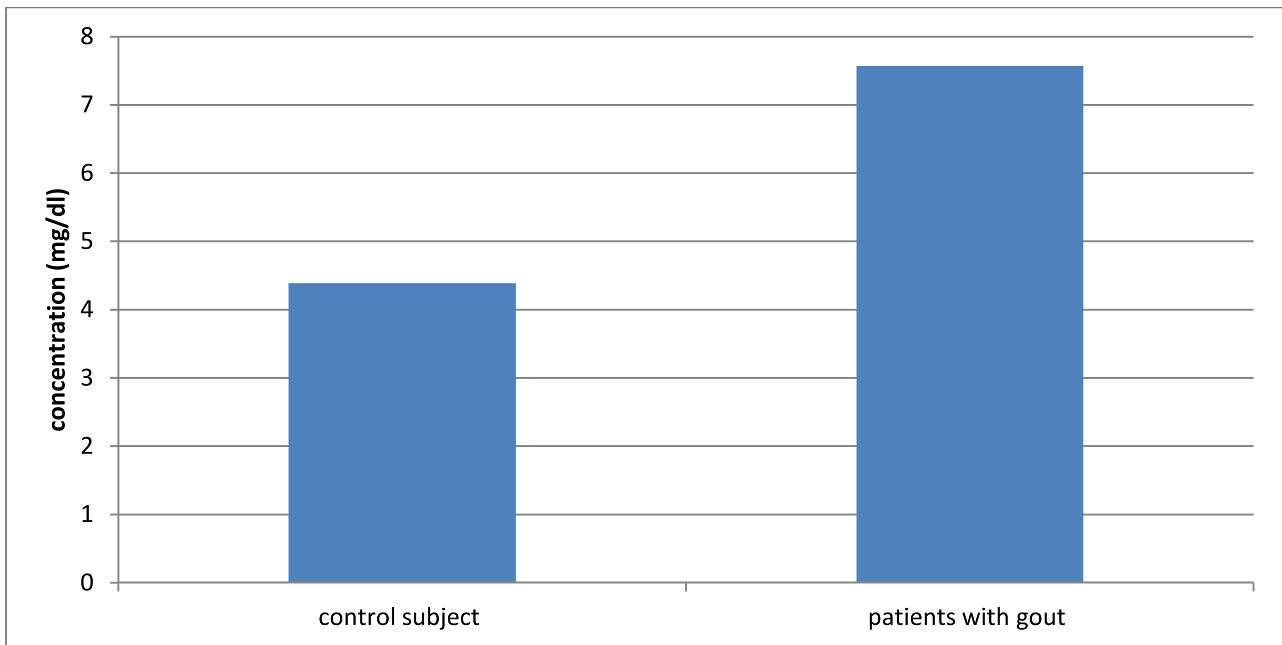


Figure 3.1. Serum uric acid concentration in control subjects and patients with gout

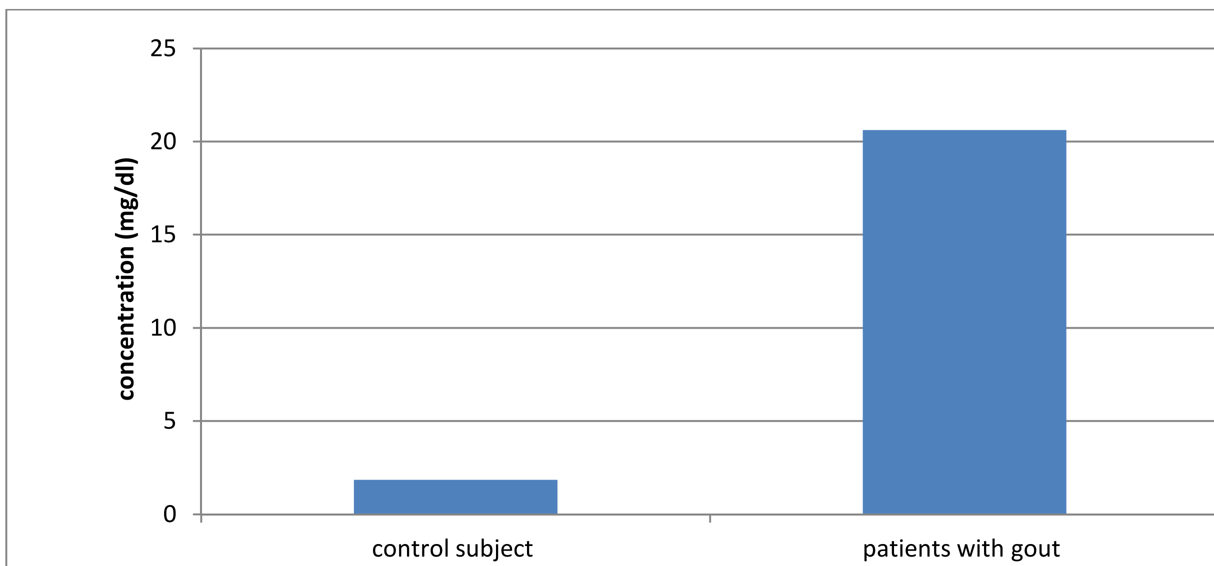


Figure 3.2. Serum creatinine concentration in control subjects and patients with gout

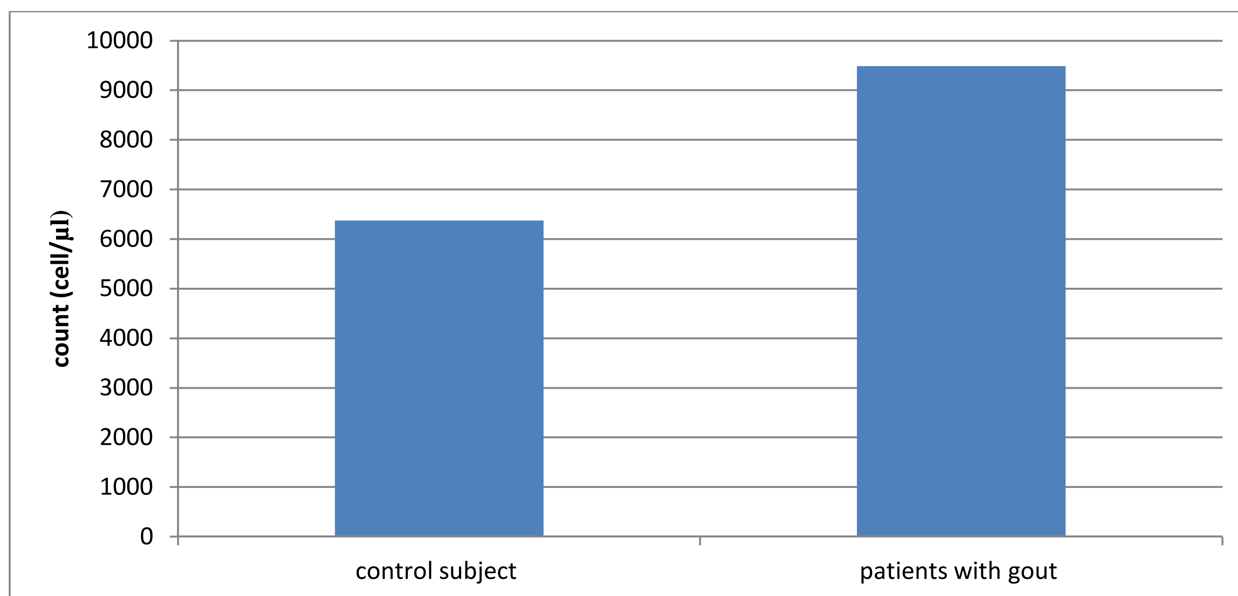


Figure 3.3.White blood cell count in control subjects and patients with gout

Table 3.1 shows a significant (P<0.01) increase in serum uric acid and creatinine concentrations and WBC count in patients with gout in comparison with the control. Hyperuricemia arises from excess dietary purine or alcohol intake, decreased renal excretion of uric acid , or from cancer lyses in lymphoma, leukemia, or solid tumors (Kutzing *et al.* , 2008).

Serum uric acid and creatinine concentrations and WBC count in male and female patients with gout and control subjects with different age groups are shown in table 2.3 and 3.3

Table 2.3.Serum uric acid and creatinine concentration and WBC count in male and female in patients with gout

Gender	No.	Serum uric acid concentration (mg/dl)	Serum creatinine concentration (mg/dl)	White blood cell count(cell/µl)
Male	26	7.70 ± 0.34	23.24 ± 1.90	9334 ± 282.41
Female	9	7.12 ± 0.42	18.46 ± 1.78	9933 ± 489.75
LSD value	---	1.1078 *	5.654 **	1041.40 NS
P-value	---	0.0239	0.0021	0.1060
* (P<0.05), ** (P<0.01), NS: Non-significant.				

Values are mean± SE

Table 3.3. Serum uric acid and creatinine concentration and WBC count in gout patients with different age groups

Age groups	No.	Serum uric acid concentration (mg/dl)	Serum creatinine concentration (mg/dl)	White blood cell count (cell/µl)
Less than 40	10	7.97 ± 0.50	27.74 ± 3.07	7822.22 ± 451.11
40-50	9	7.46 ± 0.42	20.46 ± 2.87	8241.18 ± 495.12
More than 50	16	7.51 ± 0.50	18.09 ± 1.77	8935.00 ± 392.96
LSD value	---	1.321 NS	6.744 NS	1242.20 NS
P-value	---	0.3074	0.4839	0.1883
** (P<0.01), NS: Non-significant.				

Mean ±SE

Tables 2.3. and 3.3. shows a significant ($p<0.05$) increase and in serum uric acid and creatinine concentration and WBC count in male patients as compared to female. Gout is rare in children and young adults. Men are more likely to develop gouty arthritis than women at the ages of 40 and 50 years , women are rarely develop the condition before the onset of menopause. Men have higher uric acid concentration than women with an increased prevalence of gouty arthritis at all ages, though less marked in older age(Roddy *et al.*, 2007). Estrogens have a uricosuric result, making gout very rare in younger women. However, urate levels rise , after the menopause and gouty arthritis becomes prevalent. Aging is an important risk factor in both men and women, possibly because of multiple factors including: an increase in serum uric acid levels (mainly due to reduced renal function); increased use of diuretics and other medication that raise serum uric acid age-related changes in connective tissues, which can encourage crystal formation (Roddy *et al.*, 2007).

The Correlation coefficient between in serum uric acid, creatinine and white blood count in patients with gout is shown in table 3.4

Table 3. 4. Correlation coefficient between studied parameters in patients with gout

Parameters	Correlation coefficient (r)	Level of significance
Serum uric acid & Creatinine	0.68	**
Serum uric acid and white blood cell count	0.63	**
white blood count and serum creatinine	0.60	**
		** (P<0.01).

There is a positive correlation between parameters in patients with gout. uric acid may be an active player in the pathogenesis of renal disease that cause endothelial dysfunction intra renal vascular disease and renal impairment that lead to increase in creatinine level (Kanellis *et al* .,2004). It was proved that hyperuricemia enhanced the production of pro-inflammatory mediators and in turn high the effect of endotoxin, which resulted in more exacerbation of systemic inflammatory response in other mean increase in white blood cell count (Karapinar *et al* ., 2009)

3.3. Molecular detection of gout by PCR technique.

3.3.1 SLC22A12 (URAT1) gene

The *SLC22A12* (URAT1) gene is located on the chromosome (11q13.1). The protein encoded by this gene is a member of organic ion transporter (OIT) family, acting as transporter for the urate and contribute in regulation of urate concentration in blood. This (protein) is an integral membrane protein primarily found in epithelial cells of the proximal tubule of excretory . An elevated level of serum urate, hyperuricemia, is related with increased incidence of gouty arthritis. (Enomoto A, *et al*., 2002)

3.3.2. Concentration and purity of DNA extracted from blood samples

Genomic DNA was extracted from all samples of patients using genomic DNA miniprep extraction kit for frozen blood see table (3-6).

Type of patient sample	DNA conc.	DNA purity
Frozen blood	50.8- 120.2 ng/ µl	1.7-2.0

3.3.3. Primers set.

Indentification of nucleotide changes in 1 ,2 ,3 ,4 , and 5 exons performed in all samples with the primers used in the current study.

3.4. Molecular analysis of *SCL22A12* gene.

Direct sequencing of the *SCL22A12*gene from patients with gout was carried out using primers EX1,EX2,EX3,EX4 and EX5

The first primer set employed in this PCR technique(Ex-1) amplifies exon (1) of *SCL22A12* gene from NCBI with product size(600bp) which is shown in Figure (3-4)

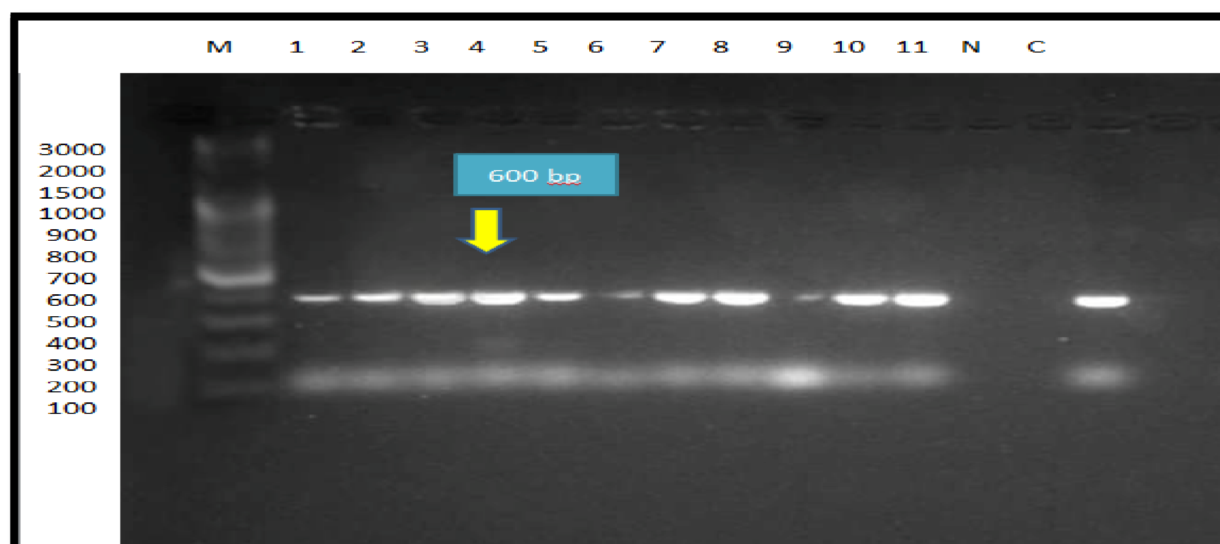


Figure 3.4 PCR product for Ex-1 primer, agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltages and then lowered to 70 Volts, 80minutes). Visualized under U.V light after staining with ethidium bromide lane M: 100bp marker,lane1-10: from patients with gout , lane C: from control (healthy) subjects and lane N: from negative control.

The second primer set employed in this PCR technique (Ex-2) amplifies exon (2) of *SCL22A12* gene from NCBI with product size (400bp) which is shown in the figure (3-5).

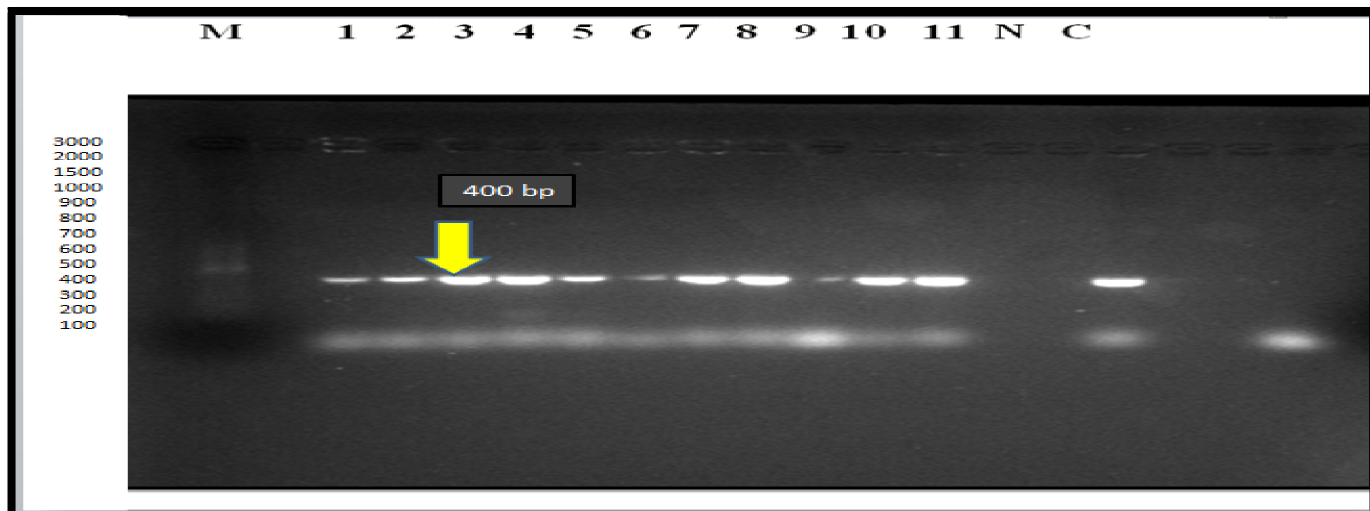


Figure 3.5 PCR product for Ex-1 primer, agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltages and then lowered to 70 Volts, 80minutes). Visualized under U.V light after staining with ethidium bromide lane M: 100bp marker,lane1-10: from patients with gout , lane C: from control (healthy) subjects and lane N: from negative control.

Primer (Ex-2) used in this study was previously designed to amplify *SCL22A12*gene, giving a band in PCR product (400bp).

The third primer set employed in this PCR technique (Ex-3) the amplifies in exon (3) of *SCL22A12* gene from NCBI with product size (300bp)which is shown in Figure (3-6).

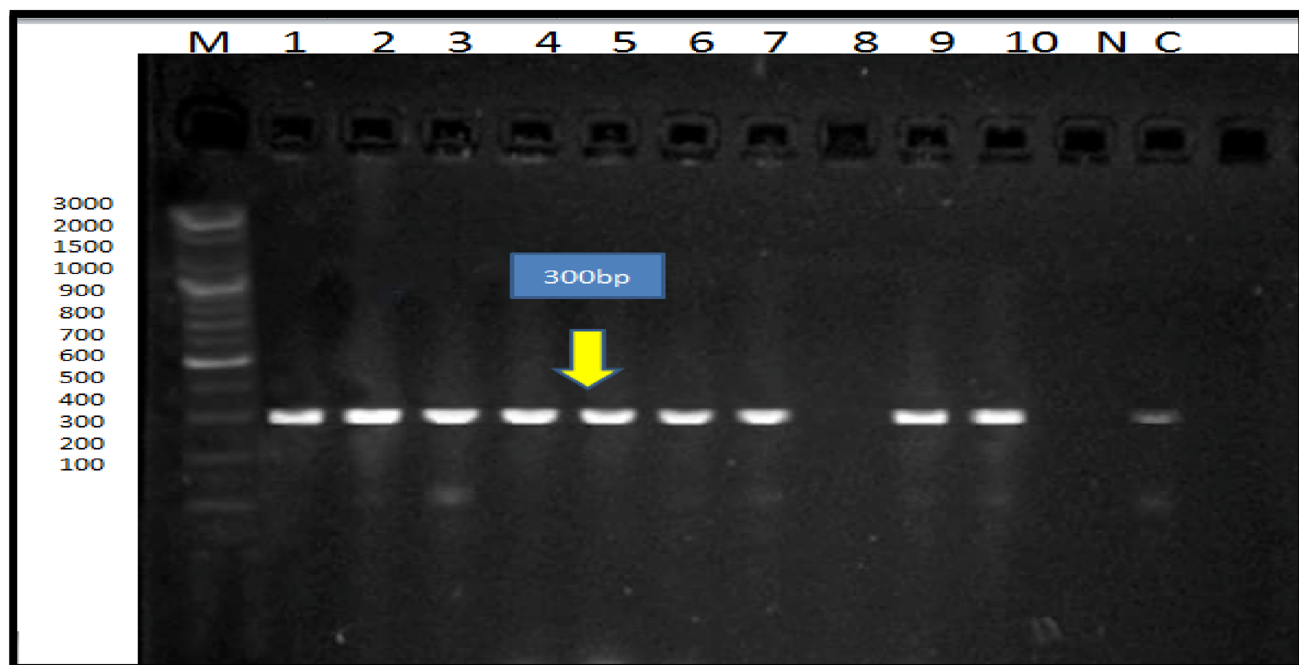


Figure 3.6 PCR product for Ex-3 primer, agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltages and then lowered to 70 Volts, 80minutes). Visualized under U.V light after staining with ethidium bromide lane M: 100bp marker,lane1-10: from patients with gout , lane C: from control (healthy) and lane N: from negative control.

Primer (Ex-3) employed in this study was previously designed to amplify the *SCL22A12* gene(exon 3) giving a band in PCR product (300bp).

The fourth primer set used in this PCR technique (EX) the amplifies in exon (4) of *SCL22A12* gene from NCBI with product size (bp)which is shown in the figure (3-7)

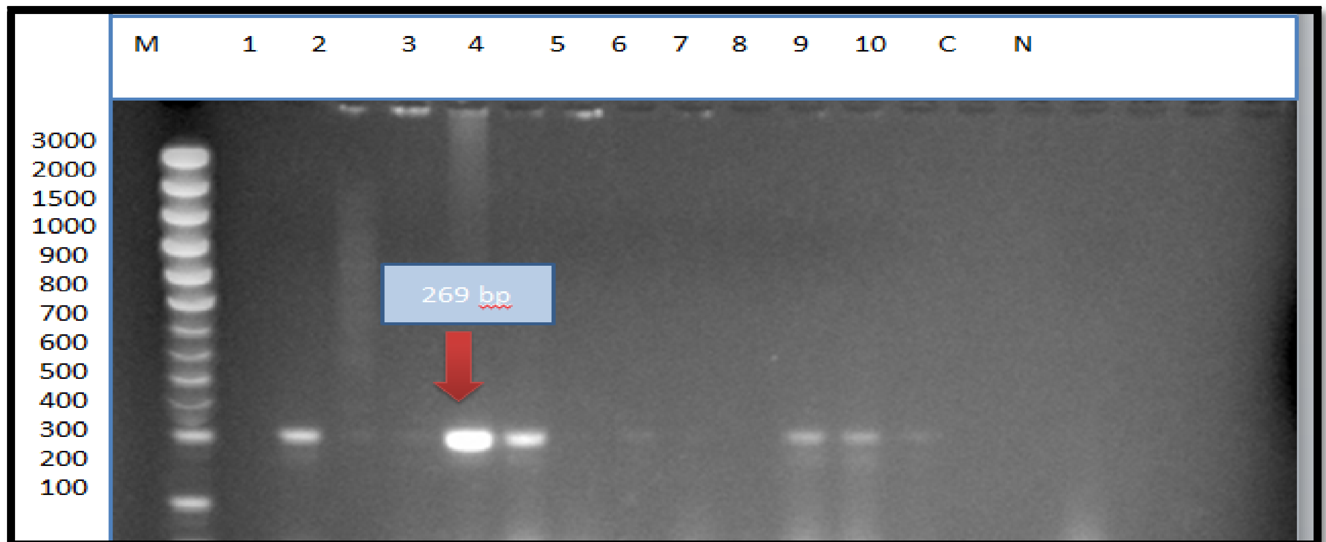


Figure 3.7 PCR product for Ex-4 primer, agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltages and then lowered to 70 Volts, 80minutes). Visualized under U.V light after staining with ethidium bromide lane M: 100bp marker,lane1-10: from patients with gout , lane C: from control (healthy) and lane N: from negative control.

Primer (Ex-4) used in this study were previously designed to amplify the *SCL22A12* gene, giving a band in PCR product (269bp).

The fifth primer set used in this PCR technique (Ex-5) the amplifies in exon (5) of *SCL22A12*gene from NCBI with product size (269bp) which is shown in the figure (3-8)

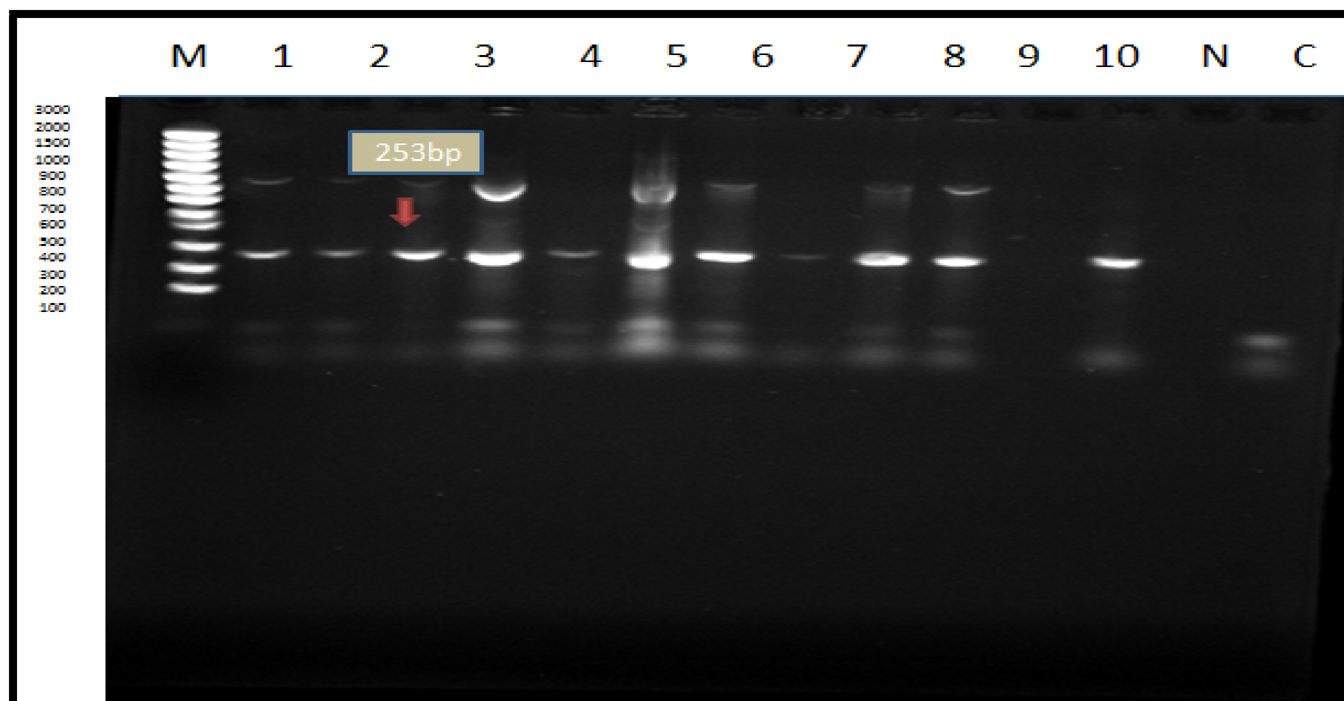


Figure 3.8 PCR product for Ex-5 primer, agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltages and then lowered to 70 Volts, 80minutes). Visualized under U.V light after staining with ethidium bromide lane M: 100bp marker,lane1-10: from patients with gout , lane C: from control (healthy) and lane N: from negative control.

Primer(Ex-5) used in this study were previously designed to amplify the *SCL22A12* gene, giving a band in PCR product (253bp).

3.4.1. Type of mutations:

The PCR products of gouty arthritis patients were screened by sequencing. The results were directly compared with the control (NCBI nucleotide blast) and Mega 6 program .In the present study forward primer was used for sequencing the *SCL22A12* gene in 20 patients with gout compared with control NCBI nucleotide blast .

The presence of mutations in several main exons of SLC22A12 gene was evaluated in individuals with gout .

The First DNA sequence of the *SLC22A12* gene located in exon 1from patient with gout and NCBI nucleotide blast is shown the Figures (3-9A,B and C)

```
Homo sapiens solute carrier family 22 member 12 (SLC22A12), RefSeqGene on chromosome 11
Sequence ID: ref|NG_008110.1| Length: 18539 Number of Matches: 1

Range 1: 5744 to 6176 GenBank Graphics            Next Match  Previous Match
Score           Expect  Identities      Gaps        Strand
784 bits(424)   0.0     431/434(99%)    2/434(0%)   Plus/Minus

Query  12    CTCGAGTGGCTCTGGGGGAGGCCCTACCTTGGCCACGATTGTGGAGGTGAAGATGCTGCG  71
Sbjct  6176  CTCGAGTGGCTCTGGGGGAGGCCCTACCTTGGCCACGATTGTGGAGGTGAAGATGCTGCG  6117
Query  72    GTCATAGACCCAGCCATCCACACACGGCTCCGTGTCGGCCTCGCTCCAGCTGGTGGCCGT  131
Sbjct  6116  GTCATAGACCCAGCCATCCACACACGGCTCCGTGTCGGCCTCGCTCCAGCTGGTGGCCGT  6057
Query  132   GGCATTGGGGTCCAAGAGCTGCCACTGTGGCTGGCGGAAGCGGCGGCACTGATGGGGCCT  191
Sbjct  6056  GGCATTGGGGTCCAAGAGCTGCCACTGTGGCTGGCGGAAGCGGCGGCACTGGTGGGGCCT  5997
Query  192   CTGGTTGGGGCCCGGCGGGATGGAAATAGCCAGGAGGGCCTCAGGACTCAAGCTCCCTAG  251
Sbjct  5996  CTGGTTGGGGCCCGGCGGGATGGAAATAGCCAGGAGGGCCTCAGGACTCAAGCTCCCTAG  5937
Query  252   GATGCTGGCCTGAGCCGTGCTGTTGTCCAGGAGGGGTGCCCAGCAGCGGTGGCTGGGCAC  311
Sbjct  5936  GATGCTGGCCTGAGCCGTGCTGTTGTCCAGGAGGGGTGCCCAGCAGCGGTGGCTGGGCAC  5877
Query  312   GGCGGCCGAGAAGTTCTCCAGCATGCTCTGGGTACACAGCCACATGATGGAGACCATCAG  371
Sbjct  5876  GGCGGCCGAGAAGTTCTCCAGCATGCTCTGGGTACACAGCCACATGATGGAGACCATCAG  5817
Query  372   AGCCATCGTCTGGAGAACCTGGAACCTGCCCAGGCCACCCACGAGGTCCAGGAGTTCAGA  431
Sbjct  5816  AGCCATCGTCTGGAGAACCTGGAACCTGCCCAGGCCACCCACGAGGTCCAGGAGTTCAGA  5757
Query  432   AA-TGCCCATGGAA  444
Sbjct  5756  AAATGCC-ATGGAA  5744
```

Figure (3-9A):The automated sequencing of *SCL22A12* gene show a substitution in (6006/A/G) , deletion in (5754/5756) and insertion in (5749/c)base pair in Exon (1) of the *SCL22A12* gene as compared with the NCBI nucleotide blast.

A sample(I) of primer (EX-1) showed a substiutation in nitrogen base (6005/A/G), in exon (1) that caused change in amino acid aspartic acid to glycine codon , deletion in nitrogen base (7555/C)that cause change in amino acid asparagine to lysine and insertion in nitrogen base(5760/A)that cause change in amino acid cysteine to methionine . These results are in agreement with Zammiti *et al*.(2006) who demonstrated the presence of insertion mutation but in exon 11 of *SCL22A12* gene .

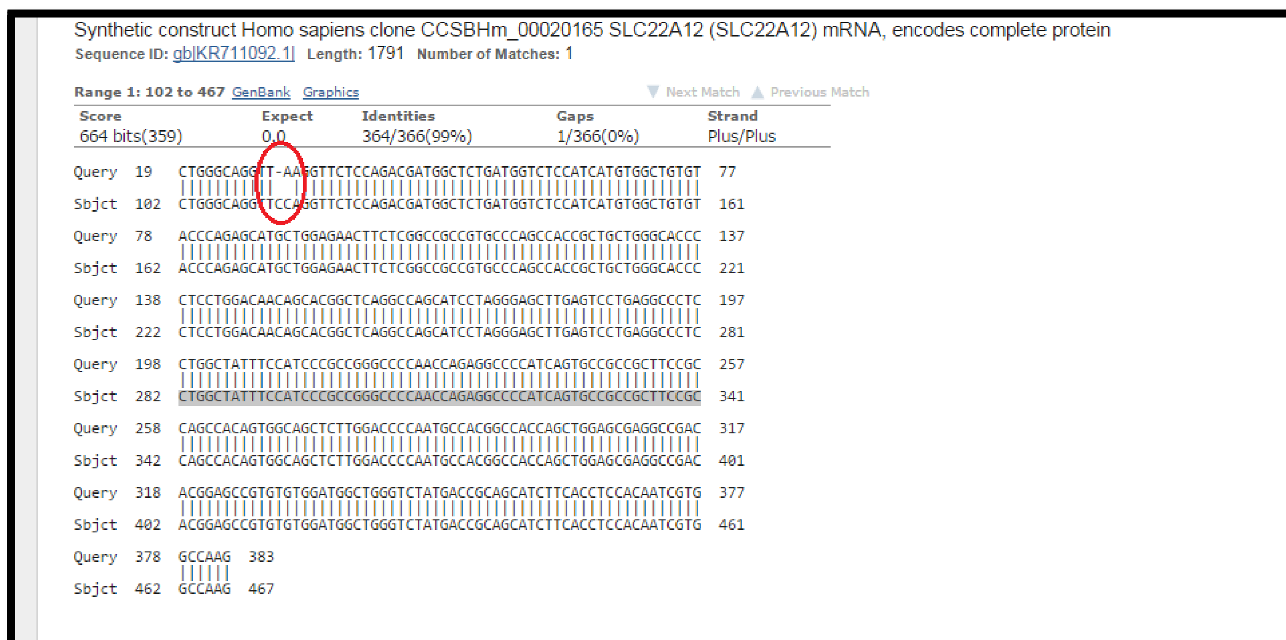
The Second DNA sequence of the *SCL22A12* gene located in exon 1 from patient with gout and NCBI nucleotide blast is shown in Figure (3-13A,B and C).

```
Homo sapiens genomic DNA, chromosome 11 clone:RP11-727K3, complete sequence
Sequence ID: dbj|AP001092.5| Length: 160739 Number of Matches: 1

Range 1: 34523 to 34952 GenBank Graphics          ▼ Next Match ▲ Previous Match
Score           Expect   Identities       Gaps         Strand
773 bits(418)   0.0      427/431(99%)     1/431(0%)    Plus/Plus

Query  16     CTGGGCAGGTTCCAGGTTCTCCAGACGATGGCTCTGATGGTCTCCATCATGTGGCTGTGT  75
Sbjct  34523  CTGGGCAGGTTCCAGGTTCTCCAGACGATGGCTCTGATGGTCTCCATCATGTGGCTGTGT  34582

Query  76     ACCCAGAGCATGCTGGAGAACTTCTCGGCCGCCGTGCCCAGCCACCGCTGCTGGGCACCC  135
Sbjct  34583  ACCCAGAGCATGCTGGAGAACTTCTCGGCCGCCGTGCCCAGCCACCGCTGCTGGGCACCC  34642

Query  136    CTCCTGGACAACAGCACGGCTCAGGCCAGCATCCTAGGGAGCTTGAGTCCTGAGGCCCTC  195
Sbjct  34643  CTCCTGGACAACAGCACGGCTCAGGCCAGCATCCTAGGGAGCTTGAGTCCTGAGGCCCTC  34702

Query  196    CTGGCTATTTCCATCCCGCCGGGCCCCAACCAGAGGCCCATCAGTGCCGCCGCTTCCGC  255
Sbjct  34703  CTGGCTATTTCCATCCCGCCGGGCCCCAACCAGAGGCCCACCAGTGCCGCCGCTTCCGC  34762

Query  256    CAGCCACAGTGGCAGCTCTTGGACCCCAATGCCACGGCCACCAGCTGGAGCGAGGCCGAC  315
Sbjct  34763  CAGCCACAGTGGCAGCTCTTGGACCCCAATGCCACGGCCACCAGCTGGAGCGAGGCCGAC  34822

Query  316    ACGGAGCCGTGTGTGGATGGCTGGGTCTATGACCGCAGCATCTTCACCTCCACAATCGTG  375
Sbjct  34823  ACGGAGCCGTGTGTGGATGGCTGGGTCTATGACCGCAGCATCTTCACCTCCACAATCGTG  34882

Query  376    GCCAAGGTAGGGCCTCCCCCAGAGCCACTCGAGTCCCACCACCTTGGAGGTCAGTCATGA  435
Sbjct  34883  GCCAAGGTAGGGCCTCCCCCAGAGCCACTCGAGTCCCACCACCTTGGAGGTCAGTCATGG  34942

Query  436    TTCCACGGTGG  446
Sbjct  34943  ATC-ACGGTGG  34952
```

Figure (3-10A):The automated sequencing of *SCL22A12* gene display a subsitation in (34745/ T/C,34942/A/G and 34943/T/C) and deletion (34946/C)base pair in Exon (1) of the *SCL22A12* gene when compared with the NCBI nucleotide blast.

In the sample (II)of DNA from patients of gout of primer (EX-1). It was show a substitution in (34745/ T/C)that cause change in amino acid isoleucine to threonine ,substitution in base pair (34942/A/G) and substitution in base pair (34943/T/C)that cause change in amino acid isoleucine to aspartic acid and deletion (34946/C)base pair that cause change in amino acid prolin to stop codon in Exon (1) of the *SCL22A12* gene Taniguchi et al ., (2005) studied subsitution mutatin but in exon 12 of *SCL22A12* gene

The Third DNA sequence of the *SCL22A12* gene located exon 13 from patient and NCBI nucleotide blast is shown in Figure (3-11A,B and C).



.

Figure (3-11A):The automated sequencing of *SCL22A12* gene display a substitution in (114/C/A) base pair in Exon (1) and deletion in (113/C) base pair in Intron (1) of the *SCL22A12* gene as compared with the NCBI nucleotide blast.

A sample of DNA from patients with gout of primer (Ex-1)demonstrated the presence of substitution in (114/C/A) base pair in Exon (1) and deletion in (113/C) base pair in exon (1) of the *SCL22A12* gene in exon (1) that caused deletion in amino acid Leu/Phe

A mutation in which a part of a chromosome or a sequence of DNA is lost during DNA replication. Any nucleotide from a single base to an entire piece of chromosome can be deleted (Lewis, 2004).

The Fourth DNA sequence of the *SCL22A12* gene is located in exon2 from patient and NCBI nucleotide blastis show n in Figure (3-12A,B and C)

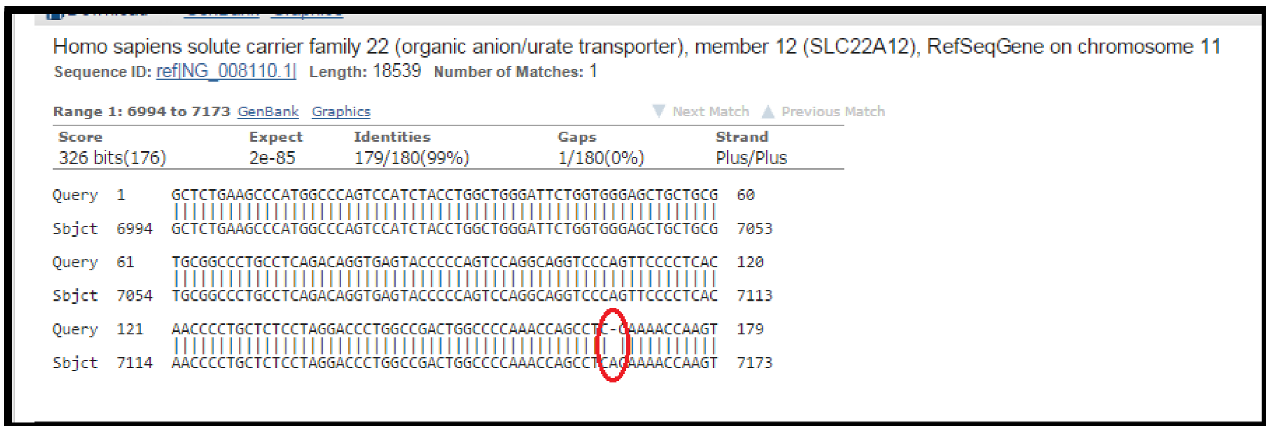


Figure (3-12A): The automated sequencing of *SCL22A12* gene display a deletion in (7160/ A) base pair in Exon(2)of the *SCL22A12* gene whecompared with the NCBI nucleotide blast.

A sample of primer (Ex-2) showed a deletion in nitrogen base 7160/A, in exon (2) that caused deletion in amino acid Proline to Threonine this results in agreement withZammiti *et al*.,2006).

Deletion of a number of base pairs that's not evenly divisible by 3 will lead to a frame shift mutation , causing all of the codons occurring after the deletion to be scanned incorrectly during translation, producing a severely altered and potentially non functional protein. On the contrary , a deletion that is evenly divisible by 3 is called The fifth DNA sequence of the *SCL22A12* gene . an in-frame deletion (Ren , 2005) located in exon 3 from patient with gout and NCBI nucleotide blast is shown in Figure (3-14A,B and C).

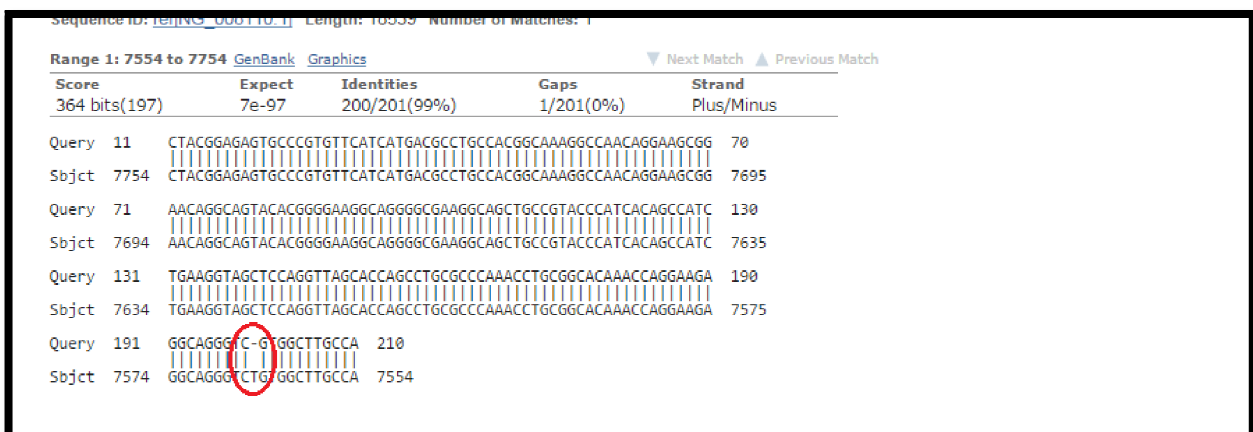


Figure (3.13A) the automated sequencing of *SCL22A12* gene display a deletion in (7543/ T) base pair in Exon(3)of the *SCL22A12* gene when compared with the NCBI nucleotide blast.

In the sample (I) of primer (Ex-3) show deletion in nitrogene base(7543/T), in exon (3) that caused no change in amino acid resulting in silent mutation
Different types of mutation of one or more than founded in gene region. However point mutation ,substitution, insertion and deletion affected the *SCL22A12* gene in Iraqi patients. as show in Table**(3.6)**

Table (3-6):point mutations detecting in gout patient sample

Name of Primers	Wild type	Mutant type	Change in amino acids	Site Of N.A.	Type of Mutation	Effect on Translation
Ex-1	GAT TGC	GGT ATG	Gly _ substitution Met – substitution	183 343	Substitution Insertion	Missense Frame shift
Ex-1	TCC ATT CCA	ACC GAT C-A	Thr _ substitution Asp- substitution Termination	137 435 440	Substitution Substitution Deletion	Missense Missense None sense
Ex-2	ACA	-CA	- Thr	168	Deletion	Missense
Ex-3	TCT	TC-	Val-val	200	Deletion	Missense

Different genetic variants among a species are termed as alleles, and so a new mutation is said to create a new allele. Every allele is characterized by a selection coefficient, which measures the expected change in an allele's frequency over time (Wielgoss *et al*., 2011).

The direct sequencing of the of *SCL22A12* gene located in exon 1, exon 2 ,exon 3, exon 4 and exon 5 change the position in 183,343,137, 435, 440, 168 and 200 These

different types of mutations caused the *SCL22A12* gene . This result agree with J. Va´ zquez-Mellado, *et al.*(2007), Zhao-Wei Zhou, *et al* .,(2015) that found a novel homozygous single base pair insertion within codon 227 (680insG) producing a premature stop codon;11 patients harbored a novel heterozygous missense mutation within codon 284 (C850G) resulting in a substitution of glycine instead of arginine; (iii) two patients had a novel heterozygous missense mutation within codon 305 (T914G) producing a
substitution of serine instead of isoleucine and (iv) one patient showed a novel heterozygous missense mutation within codon 297 (C889G) resulting in a substitution of glutamate instead of glutamine

3.4.2. Percentage of mutations.

The rate at which various types of mutations occur over time. Mutation rates are typically given category for a specific class of mutation, for instance point mutations, small or large scale insertions or deletions. The rate of substitutions can be further subdivided into a mutation spectrum which describes the influence of genetic context on the mutation rate (Ossowski *et al*., 2010).

Analysis of *SCL22A12* gene by sequencing for Iraqi patients exhibited the existence of many genetic variation. three types of mutations namely deletion , insertion and substitution were present. The percentage of mutation types28.57 % for deletion , 42.86% for substitution and 28.57% for insertion. as shown in table (3-7).

Table (3-7): percentage of mutation type.

Type of Mutation	*Percentage%*
Substitution	42.86%
Insertion	28.57%
Deletion	28.57%

3.4.3.Effect of mutations.

Mutation can result in several different types of change in sequences of *SCL22A12* gene. point mutations typically refer to alterations of single base pairs of DNA or to a small number of adjacent base pairs. Point mutations are classified in molecular biology , which shows the main types of DNA changes and their functional effects at the protein level (Freeman and Company,2000).

Table (3-8) shows that the substitution mutation was a missense (42.86%) causing impact on phenotype that lead to replacement in number of amino acid. The deletion and insertion mutations lead to frame shift which represented 57.14% .This frame shift usually insert premature stop codons as well as lots of amino acid changes.

Table (3-8) : percentage of mutation effect .

Effect of mutation	*Percentage%*
Frame shift	**57.14%**
Missense	**42.86%**

If a mutation changes a protein produced by a gene, the result is likely to be harmful, with an estimated 70 percent of amino acid polymorphisms have adverse effects, and the remainder being either neutral or weakly beneficial (Sawyer *et al.*, 2007).

Chapter two

Materials and methods

Committee certification

we , the examining committee certify that thesis entitled (**Genetic Polymorphism in SCL22A12 Gene Associated with Gout in a Sample of Iraqi Patients**) and examined the student (**Atyaf Adnan Naif**) in its contents and that in our opinion, it is accepted for the degree of Master of Science in Biotechnology .

Signature:

Name:

Scientific Degree:

Date : / / 2016

(Chairman)

Signature:	Signature:
Name:	Name:
Scientific Degree:	Scientific Degree:
Date:	Date:
(member)	(member)

Signature:

Name: Dr. Waleed H. Yousif

Scientific Degree: Professor

Date: / /2016

I, here certify upon the decision of the examining committee.

Signature

Name: Hadi M.A. Abood

Scientific Degree: Assistant Professor

Title: Dean of College of Science

Conclusions and Recommenda tions

4 . conclusions and recommendations

4.1 conclusions

1- There is a positive correlation between gout and uric acid and creatinine levels in blood

2- There is a positive correlation between gout and WBC count because of inflammation

3-Gout disease is high frequent in male and in female after menopause

4-gout is high frequent in old ages

5- PCR was rapid , sensitive ,and useful for diagnosing gout by using Ex primers

6-point mutations detection in *SCL22A12* gene including deletion ,substitution , and insertion causing missense , silent none sense and frame shift

4.2 Recommendations

1- Studying expression of *SCL22A12* gene in gout patients by studying the intron by RT PCR technique

2- Studying other gene that may be responsible for gout

List of abbreviation

4- AA	4- amino antipyrine
ASA	Acetyl salicylic acid (Aspirin)
ATP	Adenosine triphosphate
bp	Base pair
DCPS	Di chloro phenol sulphate
DNA	Deoxyribonucleic acid
dsDNA	Double stranded deoxyribonucleic acid
EDTA	Ethylene di amine tetra acetic acid
GLUT9	Glucose transporter 9
GWAS	Genome wide association studies
IRH	Idiotype renal hypouriceamia
MSU	Monosodium urate
NCBI	National center for biotechnology information
NHAHES	National –health and nutrition examination survey
NSAID	Non –steroidal anti-inflammatory drug
OIT	Organic ion transporter
PCR	Polymerase chain reaction
POD	Peroxidase
PK	Proteinase K solution
RNA	Ribonucleic acid
Rpm	Round per minute
SNP	Single nucleotide polymorphism
TBE	Tris-borate EDTA
UA	Uric acid
URAT1	Urate transporter 1
UV	Ultraviolet

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بسم الله الرحمن الرحيم

﴿قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العظيم

سورة البقرة (٣٢)

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Summary

The current study aimed to investigate the molecular defect causing the manifestation of gouty arthritis , the relationship between kidney function and the gout and , study the relationship between white blood cell count and gout in a sample of Iraqi patients .The study included 35 patients suffering from gout during the period from January 2015 to June 2015 at Al- Karamah and Al-Yarmouk teaching hospitals .20 healthy subjects were selected to represent the control group. The mean ages of the patients were 34 -63 years and the mean ages of the control were 25 - 40 years The personal information's for each patient was obtained , including name , age ,gender, weight , length , medications and family history of gout . Uric and creatinine tests were performed to study relationship between the disease and kidney function .White blood cells was counted to investigate the inflammation status .DNA extraction was performed from whole blood samples. After DNA quantification, the DNA samples were used for polymerase chain reaction (PCR) amplification .Agarose gel electrophoresis of PCR product showed the presence of band with size of 300 bp, 400bp, 600bp, 253bp and 296bp. After amplification of *SCL22A12* gene, good quality products were selected to be sequenced .the result of Ex-1 primer sequencing showed substitution in (6006/A/G) , deletion (5754/5756) a subsitation in (34745/ T/C,34942/A/G 34943/T/C) a substitution in (114/C/A) , deletion in (113/C) and deletion in (34946/C)base pair and insertion in (5749/c)base pair in Exon (1) of the *SCL22A12* gene, while the result of Ex-2 primer sequencing show **a** deletion in (7160/ A) base pair in Exon(2)of the *SCL22A12* gene**.**And results of Ex-3 primer sequencing showed a deletion in (7543/ T) base pair in Exon(3)of the *SCL22A12* gene **.**Out of 35 samples , only twenty Iraqi patients that sequenced ,six only gave positive results when compared to

healthy control; the type of mutation is deletion, substitution and insertion. The percentage of mutation types were substitution mutation42.86% and deletion mutation 28.57% and insertion mutation 28.57%. In conclusion there is a positive correlation between gout and uric acid, creatinine and white blood cell count also there is a number of mutation in *SCL22A12* gene that lead to gout.

Supervisor certification

I, certify that this thesis entitled **(Molecular Aspects of Gout in a Sample of Iraq Patients)** was prepared by (**Atyaf Adnan Naif)** under my supervision at the College of Science /Al-Nahrain University as a partial fulfillment of requirements of the Degree **of Master of Science in Biotechnology** .

Signature:

Name : Dr . Waleed H. Yousif

Scientific Degree : Professor

Date : / /2016

In view of available recommendation , I forward this thesis for debate by examining committee

Signature :

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Date : / /2016

الخلاصة

الدراسة الحالية تهدف إلى التحري عن المظاهر الجزيئية التي تسبب مرض النقرس والعلاقة بين وظائف الكلى و النقرس ، و دراسة العلاقة بين عدد خلايا الدم البيض و النقرس في عينات من المرضى العراقيين. الدراسة تضمنت عينات دم من ٣٥ مريض يعانون من مرض النقرس يرتادون مشتشفى اليرموك والكرامة للفترة من شهر كانون الثاني لشهر حزيران من عام ٢٠١٥ . وكذلك ٢٠ عينه من الاشخاص الاصحاء يمثلون مجموعة السيطرة. تراوح متوسط اعمار المرضى بين ٣٤-٦٣ سنه و متوسط عمر مجموعه السيطرة بين ٢٥ -٤٠ سنة . المعلومات الشخصية لكل مريض المتضمنة الاسم والعمر و الجنس لوزن وطول والعلاج الذي يتناوله و التاريخ المرضي للعائلة .تم اجراء فحص حامض اليوريك و الكرياتنين للمرضى و لمجموعة السيطرة لدراسة العلاقة بين المرض و وظائف الكلى . فضلا عن حساب لخلايا الدم البيض لدراسة علاقة المرض بالالتهاب . بعد ذلك تم استخلاص الحامض النووي(الدنا) من المرضى ومجموعة السيطرة وبعد قياس نقاوته و تركيزه بعدها اجريت عملية تضخيم الدنا باستخدام تفاعل البلمرة المتسلسل و اظهر الترحيل الكهربائي وجود حزمه بحجم ٦٠٠ قاعدة نتروجينية باستخدام البادئ الاول و حزمه بحجم ٤٠٠ قاعدة نتروجينية باستخدام البادئ الثاني و حزمة بحجم٣٠٠ قاعدة نتروجينية باستخدام البادئ الثالث و حزمه بحجم ٢٥٣ قاعدة نتروجينية باستخدام البادئ الرابع و حزمه بحجم ٢٦٩ قاعدة نتروجينية باستخدام البادئ الخامس وبعد تضخيم الجين (*SCL22A12*)تم اختيار افضل النتائج لعمل تتابع للقواعد النتروجينية. و قد اظهر تسلسل البادئ الاول وجود حالات الاستبدال في (A / G / ٦٠٠٦)، الحذف (٥٧٥٦/٥٧٥٤) الاستبدال في (T/C / ٣٤٧٤٥، A / G 34943 / ٣٤٩٤٢) إجراء تبديل في (C / A / ١١٤)، والحذف في (G/ ١١٣) والحذف في (C / ٣٤٩٤٦) والإدراج(الادخال) في (C/ ٥٧٤٩) في اكسون (١) من هذا الجين SCL22A12، في حين أن نتيجة تسلسل البادئ الثاني اظهرت حالات الحذف في (A/٧١٦٠) في اكسون (٢) من نتائج الجين SCL22A12 اما تسلسل البادئ الثالث فقد اوضح الحذف في (T / ٧٥٤٣) زوج قاعدة في اكسون (٣) من هذا الجين SCL22A12. وان ستة عينات من عشرين عينة من المرضى الواتي ارسلن لاجراء التعاقب لهن لقد اظهرت نتائج ايجابية بالمقارنة مع الأصحاء. وان نوع الطفرات هو الحذف، الإستبدال، والإدخال . وكانت النسبة المئوية للأنواع طفرة استبدال 42.86٪ وطفرة حذف ٢٨,٥٧٪ و طفرة الإدراج ٢٨,٥٧٪. وقد استنتج من الدراسة بان هناك علاقة إيجابية بين

النقرس وحامض اليوريك والكرياتينين وعدد خلايا الدم البيض كما يوجد عدد من الطفرات في الجين SCL22A12 التي تؤدي إلى النقرس.