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Genetic analysis of *ABCG2* gene polymorphism and its association with some biochemical aspects in a sample of Iraqi gout patients

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Abstract
Gout is the most common type of inflammatory arthritis that is caused by the deposition of monosodium uric acid crystals in and around peripheral joints. This study was carried out to investigate biochemical and genetic aspects of gout arthritis. Two approaches were adopted, the first included the study of some biochemical parameters (serum uric acid, creatinine and WBC count) in gout patients and the second comprised the study of the role of gene in the variation site (rs2231142) in the incidence of gout.
Blood samples were collected from 25 gout patients and 25 healthy subjects as control group, during the period from December 2020 to May 2021, at the Rheumatology Department of Clinic Consultant at Al-Yarmouk Hospital. Personal information for each patient was obtained. Uric acid and creatinine were measured to study relationship between kidney function and the disease. White blood cells were counted to investigate the inflammation status. The results revealed that Both serum uric acid and serum creatinine concentration were significantly higher in gout patients as compared to controls. There is a positive correlation between gout and WBC count because of inflammation.
The genetic study included an extraction of genomic DNA and PCR technique, To detect the role of ABCG2 gene in the variation site (rs2231142) in the incidence of gout. The results of mutational analysis showed there is a number of a substitution mutation in ABCG2 gene that may be lead to gout progression. The percentage of substitution mutation was 40%**.** The Frequency distribution of rs2231142 genotype showed that, the mutant homozygous (TT) was (48.0%) associated with gout susceptibility. It is concluded that gout was associated with increased serum uric acid creatinine and white blood cell count also there is a number of mutations in ABCG2 gene that may be lead to progression of gout.

Key words: Genetic Polymorphism, ABCG2 gene, gout arthritis

Introduction

Gout is a type of inflammatory arthritis, arises after chronic hyperuricemia that permits the deposition of monosodium urate in and around joints with the subsequent painful recurrent flares and tissue damage (Zhu *et al.*, 2011; Vargas-Santos and Neogi, 2017) .

It is the common form of inflammatory arthritis and thought to affect men more than women. In general, women become more susceptible to gout after the menopause (Zhu *et al.*, 2011).

Gout usually occurs as recurrent attacks resulting in painful, red and swollen joints, the big toe joint is commonly affected, but other joints including fingers, knees, heels, and wrists may also be affected (Schlesinger, 2010).

The clinical features of gout are caused by the inflammatory response to monosodium urate crystals (Schlesinger, 2017). They induce episodes that are initially infrequent, affecting the foot joint, and well responsive to anti-inflammatory medications such as colchicine and non-steroidal anti-inflammatory drugs (Moolenburgh *et al.*, 2006). However, these do not prevent urate deposition from progressing, so more frequent and widespread attacks may develop, permanent joint damage may result from gouty erosions, and tophi formation may occur. Thus, optimal management is needed to provide a gradient for resorption of the crystals (Wong, 2005).

Several genetic variant loci in various genes associated with serum urate concentrations (Dong *et al.*, 2018). Urate transporter-coding genes such as ABCG2, SLC2A9 and SLC22A12 showed a strong

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association with both the serum urate concentration and progression from hyperuricemia to gout and could influence the risk of gout and some of the other urate associated genes, that did not code urate transporters, were found to affect the development of gout (Dong *et al.*, 2015; Beydoun *et al.*, 2018). ATP-binding cassette subfamily G member 2 (ABCG2) gene variant, which is encoded by the single nucleotide polymorphism (SNP) rs2231142, was identified to take an essential part in gouty arthritis (Shih-Tsung Cheng. *et al.*, 2017).

This study was designed to investigate the role of ABCG2gene variant in the incidence of gout. And the association between kidney function and white blood cell count with gout arthritis.

Methods

Patient selection

Twenty-five gout patients (22) male and (3) female participated in this study during their attendance in the Rheumatology Department of Clinic Consultant at Al-Yarmouk Hospital. All cases were investigated by rheumatologist. The period of study was from December 2020 to May2021. Their ages ranged between 25 and 70 years. The study also included 25 gout free Healthy controls with ages ranging between 28 to 70years. Personal information for each patient was obtained, including name, age, weight, height, medications, chronic diseases.

Biochemical assay

Serum was separated from peripheral venous blood samples obtained from each participant and stored at –70°C for the clinical chemistry assays. Serum uric acid and creatinine concentrations were measured with the abbot ARCHITECT system.

Uric acid (Tietz, 1995)

The Uric Acid assay is designed for *in vitro* diagnostic use only for the quantitation of uric acid in human serum, plasma, or urine on ARCHITECT c Systems.

Creatinine (Young DS, 2000)

The Creatinine assay is designed for *in vitro* diagnostic use only for the quantitation of creatinine in human serum, plasma, or urine on ARCHITECT c Systems.

Identification of single nucleotide polymorphisms

Polymerase chain reaction (PCR)-direct sequencing was performed to detect SNP rs2231142 Location on Exon 5 of ABCG2 gene and nearby regions was amplified by PCR. The products were sequenced sent Company bioneer for DNA sequencing in Korea.

Table 1. The sequences of the primers used

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTGTGATGGGCACTCTGACG	Plus	20	618	637	60.32	55.00	3.00	2.00
Reverse primer	GTGGGGGAGGAGCATAAGGA	Minus	20	1041	1022	60.69	60.00	2.00	0.00
Product length	424								

Results and Discussion

This study was conducted to investigate the relationship between genetic polymorphism in ABCG2 gene with susceptibility to gout in a sample of Iraqi patients and determination the association between kidney function and the gout arthritis.

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Participant demographic and clinical characteristics

The main anthropometric characteristics of gout patients and healthy control subjects are summarized in Table (2).

Table (2) Anthropometric Indices of gout patients and controls.

Variables		Gout Patients (n=25)	Control (n=25)
Gender	Male	22 (88%)	16 (64%)
	Female	3 (12%)	9 (36%)
Age (Years)	(25-49)	10 (40%)	14 (56%)
	(50- >70)	15 (60%)	11 (44%)
BMI (Kg/m^2)	Normal (18.5-24.9)	4 (16%)	12 (48%)
	Overweight (25-30)	10 (40%)	10 (40%)
	Obese (>30)	11 (44%)	3 (12%)
Hypertension prevalence	Yes	16 (64%)	12 (48%)
	No	9 (36%)	13 (52%)

Table 2. Reveals that the study included males more than females in both gout patients and controls. In addition, two ranges of ages were collected (25-49 years) and (50->78 years). The frequency of the elderly gout patients is higher than that of the young gout patients. Also, it appears that the obese category is common among gout patients' group (44%) followed by the overweight category (40%). Moreover, the prevalence of hypertension increases among gout patients (64%) when compared to the control (48%). No significant differences between the two groups in their age, gender and in the prevalence of hypertension are observed except for BMI, (P-value = 0.05). Gout is high frequent in old ages, Gout is uncommon in young adults and children. The common of the study participants (88%) were male and 12% female suffering from gout. Men are more probable to develop gouty arthritis than women at the ages of 40 and 50 years , Women are infrequently develop the condition before the onset of menopause Estrogens have a uricosuric result, making gout very uncommon in younger women. However, urate levels rise, after the menopause and gouty arthritis becomes prevailing. (Roddy *et al.*, 2014). Aging is an vital risk factor in both men and women, probably because of multiple factors including: an increase in serum uric acid levels (chiefly due to reduced renal function); increased use of diuretics and additional medication that raise serum uric acid age-related changes in connective tissues, which can promote crystal formation (Roddy *et al*., 2007). The risk of gout increased among men and elderly (Roddy *et al.*, 2014). The current results confirmed association of the variation SNP rs2231142 with UA levels and hyperuricemia in Iraqi population, and found it mostly in males, these result is consistent with result of (Shih-Tsung Cheng *et al.*,2017).

Biochemical Parameters

Serum uric acid concentration and creatinine concentration and white blood cell status

(Table 3).shows serum uric acid& creatinine concentrations and white blood cell (WBC) count in both gout patients and healthy.

Serum uric acid concentration has been measured in gout patients (6.85 ± 0.25mg/dl) and controls (4.25 ± 0.10 mg/dl), there is a significant increase in serum uric acid in gout patients as compared to control.

Serum creatinine concentration has been measured in gout patients (1.85 ± 0.22 mg/dl) and control (0.70±0.22 mg/dl), there is a significant increase in serum uric acid in gout patients as compared to control.

White blood count has been measured in gout patients (9120.35 ± 228.25) and control (6250.00 ± 215.22), there is a significant increase in serum uric acid in gout patients as compared to control($P<0.01$).

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Table 3. Serum uric acid concentration and creatinine concentration and WBC count among gout patients and control

Type of Groups	No	Serum uric acid Con. (mg/dl)	Serum creatinine Con. (mg/dl)	White blood cell count(cell/µl)
Control	25	4.25 ± 0.10	0.70±0.22	6250.00 ± 215.22
Patients	25	6.85 ± 0.25	1.85 ± 0.22	9120.35 ± 228.25
LSDvalue	----	0.522 **	2.231 **	458.20 **
P-value	----	0.001	0.001	0.0001

Table 3 shows increase in serum uric acid concentration and creatinine concentration and WBC count in gout patients in comparison with the control (P<0.01). The results of the present study showed a significant increase in serum uric acid concentration among gout patients when compared to the control. This is because high concentrations of uric acid gout are the essential predisposing factor for the development of gouty arthritis that causes acute inflammation of the joints. (Roddy and Doherty, 2010).Hyperuricemia arises from excess nutritional purine or alcohol drinking, decreased renal excretion of uric acid , or from cancer lyses in lymphoma, leukemia, or solid tumors (Kutzing *et al.* , 2008). earlier genome-wide association studies have found that the ABCG2 (SNP) rs2231142 is an vital genetic factor for increased uric acid (UA) levels, and the degree of association between rs2231142 and hyperuricemia is affected by both sex and ethnicity (Shih-Tsung Cheng *et al.* ,2017).

Shih-Tsung Cheng *et al.* ,(2017) confirmed association of the SNP rs2231142 with UA levels and hyperuricemia in a Taiwanese cohort, and found it predominantly in males. These results were consistent with the current study results.

Roddy and Doherty, 2010 showed that, a significant increase in serum uric acid concentration among gout patients , This is because high concentrations of uric acid gout are the essential predisposing factor for the development of gouty arthritis that causes acute inflammation of the joints. In this study, serum creatinine concentration show significant change between gout patients and control. However, Grassi *et al.* (2013) reported that uric acid concentration is correlated with a serum creatinine concentration. creatinine contributes to the assessment of comorbid disorders in gout patients especially measurement for chronic kidney disease (Dalbeth *et al.*, 2017). It was proved that hyperuricemia improved 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)

Molecular detection of gout by PCR technique.

Genomic DNA was extracted from all blood samples of gout patients and healthy controls. There are clear DNA bands obtained after DNA extraction and electrophoresis on a 0.7% agarose gel Figure (1). The concentration of DNA extracted from all samples ranged between 200-400 µg/ml and purity ranged between 1.5-2.0. The PCR products of 25 patients were screened by sequencing, The results were compared with the control NCBI nucleotide blast.

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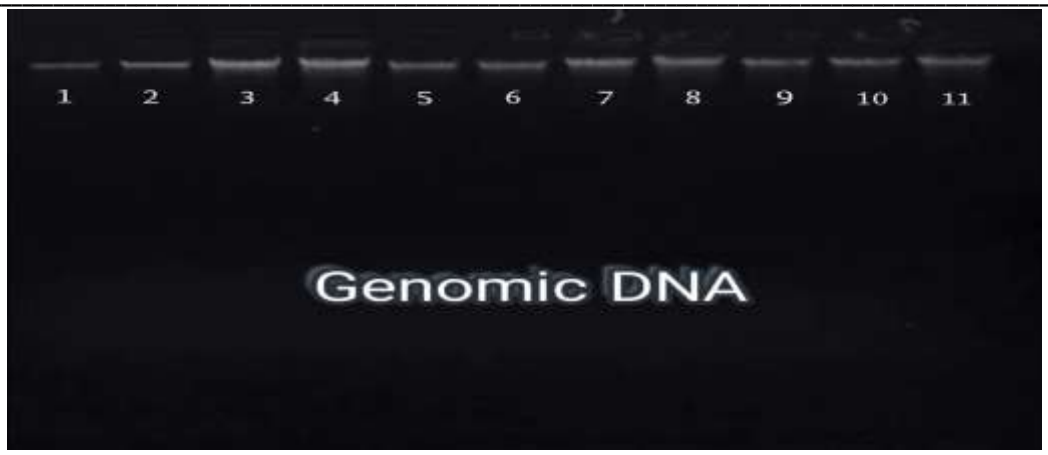

Figure 1. genomic DNAfrom Gout patients.
Gel electrophoresis on a (0.7%) agarose gel for 1 hour at 50 V. Lanes 1-10: Gout patients.

Type of mutations

In order to study the effect of the genetic variation of ABCG2 in susceptibility to gouty arthritis in the Iraqi population, The presence of mutations in several evaluated in individuals with point mutations detecting in gout patient sample Table 4.

Out of 25 samples, only ten that sequenced gave substitution mutation; the type of substitution is Missense . The percentage of substitution mutation were 40% .

Table 4. Represents the statistical analysis of Type of Mutation.

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Table 4: SNP analysis in all samples with their allele specification

No. of sample	Type of substitution	Nucleotide	Allele	New Possible SNP	Allele
1	Missense	G>T	TT	C>T+C	CT
2	Missense	G>T	TT	C>T+C	CT
3	None	G>G	GG	C>C	CC
4	None	G>G	GG	C>T+C	CT
5	None	G>G	GG	C>C	CC
6	Missense	G>T	TT	C>C	CC
7	None	G>G	GG	C>T+C	CT
8	None	G>G	GG	C>T+C	CT
9	None	G>G	GG	C>T+C	CT
10	Missense	G>T	TT	C>T	TT
11	None	G>G	GG	C>T+C	CT
12	Missense	G>T	TT	C>T+C	CT
13	None	G>G	GG	C>C	CC
14	Missense	G>T	TT	C>C	CC
15	None	G>G	GG	C>T+C	CT
16	None	G>G	GG	C>T	TT
17	Missense	G>T	TT	C>C	CC
18	None	G>G	GG	C>T+C	CT
19	None	G>G	GG	C>T+C	CT
20	Missense	G>T	TT	C>C	CC
21	None	G>G	GG	C>T+C	CT
22	Missense	G>T	TT	C>C	CC
23	Missense	G>T	TT	C>C	CC
24	None	G>G	GG	C>C	CC
25	None	G>G	GG	C>C	CC
Control					
A	None	G>G	GG	C>C	CC
B	None	G>G	GG	C>C	CC
C	None	G>G	GG	C>C	CC
D	None	G>G	GG	C>C	CC
E	None	G>G	GG	C>C	CC

Table (5): Allele frequencies of rs2231142 among all case and control samples. The SNP position is 105152 bp of the (ABCG2) Reference gene

Genotype	Case 25	Control 25		
rs4714210 (G>C,T) Non Coding				
GG	13 (52.0%)	25 (100%)		
TT	12 (48.0%)	0		
GT	0	0		

ABCG2 belongs to the G-family ABC transporters. The point of association between ABCG2 rs2231142 SNP and gout risk has been found to differ with ethnicity (Dong Z *et al*., 2015).

Allele frequency was defined as the percentage of the individuals carrying the allele among the total number of the individuals; The SNP nomenclature used in this study was based on the Human Genome

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Variation Society recommendations and the National Center for Biotechnology Information SNP database. Result in a table (5) documented , The Frequency distribution of rs2231142 genotype showed that, the mutant homozygous (TT) was (48.0%) in the case group compared to the control group and associated with gout susceptibility and the allele (T) may be a risk factor for the development of disease in Iraqi population. These findings are similar to those reported previously. Yun Sung Kim *et al*., 2015 reported that. A strong association of rs2231142 in the *ABCG2* gene with gouty arthritis in Korean population.

These findings were consistent with the result of the current study. Many studies have shown association of the ABCG2 (SNPs) with various phenotypes, include UA levels, gout, and low-density lipoprotein cholesterol levels. C-to-A mutation rs2231142, which results in a Q-to-K substitution at position 141, has been associated with hyperuricemia and gout (Shih-Tsung Cheng *et al.*,2017) **(**Woodward OM, *et al*. 2009) **(**Wang F, *et al*. 2011)(Yang Q, *et al*. 2010) (Stark K, *et al*. 2009). In another study Yusur Alnaqashli.(2020), observed that, No clear impact of SNP rs16890979 (C/T) in SLC2A9 on the susceptibility to Iraqi gout patients. In fact, the small sample size could affect the consequence of the study and give conflicting and inconclusive results.

We recognized novel polymorphisms in the position 105503 bp during the assess the genetic associations of these SNP and nearby regions with gout (table 6).

Table (6): New Possible SNP (C>T) in the position 105503 bp of the (ABCG2) Reference gene.

Genotype	**Case** **25**	**Control** **25**
CC	11 (44.0%)	25 (100%)
TT	2 (8.0%)	0
CT	12 (48.0%)	0

Increasing the sample size will provide more visible results about the effect of ABCG2 gene on gout. in addition to studying the role of lifestyle and genetic factors on the susceptibility of gout.

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