Biochem. Cell. Arch. Vol. 20, Supplement 2, pp. 000-000, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

STUDY OF SOME PHYSIOLOGICAL VARIABLES RELATED TO GOUT AND THE ROLE OF POLYMORPHISM OF THE GLUT9 GENE IN DISEASE ETIOLOGY

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(received 18 November 2019, Revised 21 February 2020, Accepted 16 March 2020)

ABSTRACT : Gout is a disease caused by a disorder in the Purines metabolism and consequently an increase in uric acid and needle crystals from urate crystal that precipitates inside the joints causing severe pain.Gout is one of the most common autoimmune diseases and may be deposited monosodium urate crystal (MSU) crystals in the surrounding tissues and fluids. The current study aimed to demonstrate the relationship of gout to some physiological and genetic variables. Samples collected from private laboratories and external medical clinics for the period from 1/July/2018 to 1/September/2018. Samples consisted of 70 patient with high uric acid in the blood and the control group consisting of 30 volunteers did not have clinical evidence of a previous infection with a high level of uric acid. The age of patients and control groups ranged (20-70) years old. The study indicated significant differences in blood urea level between patients group and healthy group and serum creatinine and also indicated a positive correlation relationship $P \leq 0.05$ between uric acid and both urea (Creatinine r = 0.2532), (TCHO r = 0.241) and (TG r = 0.2736). The association was negative between HDL and uric acid (HDL r = - 0.3028). In addition, the level of vitamin D decreased in the patient's group compared to the healthy group and the relationship was negative with uric acid (Vit. D3 r = - 0.4162). The results of the genetic analysis of the SNPs of the *SLC2A9* gene (rs2276961 rs2280205 rs3733591) showed two alleles (A, G) and three genotypes (AA, GG and AG). The single nucleotide polymorphism appeared rs2280205 with one genotype of all samples (100% of all samples), while the AA and GG genotypes did not appear in all study samples, whereas rs3733591 with three genotypes appeared in the G alleles the highest frequency in the patients and healthy subjects samples. As for rs2276961, allele A appeared at the highest frequency in the patient sample compared to the healthy sample.

Key words : Gout, uric acid, GLUT9, SLC2A9, rs 2280205, rs3733591, rs2276961.

INTRODUCTION

Gout is one of the most common autoimmune diseases. It results from chronic elevation of uric acid levels to the threshold level (saturation), which causes Monosodium urate crystal (MSU), which in turn deposits in the peripheral joints and surrounding tissues (Kuo *et al*, 2015; Chhana *et al*, 2019). The main cause of gout may result of a disorder in Purines metabolism, as it results in the process of demolishing urine uric acid, which leads to the formation of needle crystals from urate crystal salts that are deposited inside the joints, which results in Crystal arthritis infections and severe pain (Martillo, Nazzal and Crittenden, 2014). MSU crystals may also precipitate in surrounding tissues and fluids forming chalky calcifications (Singh, 2014). Several studies have proven that gout is associated with age and sex, as it affects males more than females, where the infection rate increases in males in an intermediate stage of life and rarely occurs in young people, while the rate of infection among women increases after menopause (Trifirò *et al*, 2013). Eating foods rich in purines such as Red meat, seafood, and alcohol intake lead to the production of large amounts of uric acid to the extent that the body faces difficulty in getting rid of it, causing hyperuricemia and then gout (Li, Yu and Li, 2018). It was also found that gout is caused by many disorders resulting from many conditions such as metabolic syndrome, Hypertension, Diabetes and Dyslipidemia resulting from obesity, in addition to the increased risk of cardiovascular disease risk (Sharaf El Din Salem and Abdulazim, 2017). Uric acid is one of the main antioxidants, as there is a strong relationship between it and the longevity of the cell, as it has the ability to disassemble and inhibit the free radicals in the plasma (Song *et al*, 2019). Serum urea and gout is considered to be genetic traits. Despite the fact that dietary factors affect the level of urea in the blood, however, the development of the level of uric acid is mainly due to genetic factors (Krishnan *et al*, 2012). The topic of this study was chosen to determine the relationship

between gout with some biochemical and genetic variables related to the events of this disease due to the increased prevalence of gout and the problems it causes to the health of individuals and society.

MATERIALS AND METHODS

The study samples were collected from private laboratories and external medical clinics in Anbar Governorate for the period from 1/June/2018 to 1/ September/2018. Sample consisted of 70 patients with high uric acid in the blood and the rate of 50 males and 20 females whose ages ranged between 70-20 years old, and the control group consisted of 30 healthy volunteers with the same age group.

The concentration of uric acid, blood urea, creatinine concentration and serum lipid profile was estimated for all samples according to the method attached with the measurement kit prepared by Linear Chemicals, of Spanish origin and Vitamin D3 concentration was measured for all study samples by following the steps attached with their ready-made analysis kit as per instructions Manufacturer based on an immunoassay known as Enzyme-linked immunosorbent assay (ELISA)

DNA extraction

Genomic DNA has been extracted from the blood of people who suffer from a high level of uric acid in their blood, who number 70 samples, as well as members of the control sample (30) according to the method of extraction approved by the equipped company (Geneaid American). The extracted DNA and PCR product were detected by using agarose gel electrophoresis. The molecular weights of the extracted DNA were estimated compared to the volumetric index of the known nucleic acid (Genomic marker 1Kbp). As for the molecular weights of the pieces resulting from the PCR technique It was estimated, in comparison with a volumetric guide for PCR (DNA Ladder 100bp)

The reaction of PCR

The PCR reaction was conducted using the PCR-Premix kit prepared by the Korean intron company at a final volume of 25ìL and a pair of primer was used for each of SNP designed by the researcher according to the tetra-primer ampliûcation refractory mutation system–polymerase chain reaction T-ARMS–PCR system. The prefixes were prepared lyophilized from the American Bioneer company and dissolved in distilled water. A final concentration of 10 picomols/µl was prepared as the reaction solution.

The PCR product of the SLC2A9 gene (rs2280205, rs3733591 and rs2276961) was sent to the Korean Macrogen Company to determinate the sequences of genetic area in order to confirm the results of the tetra-primer ARMS–PCR technique. The results of the sequences were identical to the results of tetra-primer ARMS-PCR, after the results of the sequences were analyzed and matched with The National Center for Biotechnology Information NCBI. The Mega X-version 10.0.5 program was used in the analysis to do the Alignment matching and alignment of the *SLC2A9* gene polymorphism with what is in the database of NCBI on the fourth human chromosome, and registered with the site entry number (NC_000004.12).

Statistical analysis

The data were analyzed statistically according to the Complete Randomized Design (CRD) model using the Statistical Package for Social Science-version 24 (SPSS) and using the T-test to compare the mean at a significant

Table 1 : Primers prefixes used in the current study with their sequences and expected size.

Primer		Sequence	Tm (^{0}C)	GC (%)	Prod. size
Inner*rs 2280205*	F	5'-CTCAAGGTGACGTATGGGATCTTTGAC - 3'	64.2	50	**426 bp**
	R	5'- CTTTGGAAAAGCTGGGATCCCGCT- 3'	64.5	54.2	
Outer*rs 2280205*	F	5'-CACAAATGATGTCTGGGTTCTCCCCAT - 3'	64	48.2	
	R	5'- GCCTCTCCACCTTGGGACTCTGAGTTTA- 3'	66.2	53.6	
Inner*rs3733591*	F	5'-GGTAGAGGAGGTCCTGGCTGAGAGACG - 3'	67.7	63	**342 bp**
	R	5'- CAGGCGGATGCTCCTCTGCAAGT- 3'	66.1	60.9	
Outer*rs3733591*	F	5'-GACTCCATAGTTTCAAAGGCCCCTGCC - 3'	66.3	55.6	
	R	5'- AGGACTCACTGCATTGAGGCCACAGAG- 3'	67.1	55.6	
Inner*rs2276961*	F	5'-CCTCACAGATGACACCAGCCACGACG - 3'	68	61.5	**333 bp**
	R	5'- GCCCTCCCTGGCCCTGGAGGACT- 3'	70.6	73.9	
Outer*rs2276961*	F	5'-GGAAATGCCTTGGCAGAGTCTGGGGTC - 3'	68.1	59.3	
	R	5'- TCAAAGCCCCAGATCCCCCAGCTACAC- 3'	68.6	59.3	

level (P≤0.05). The variables were linked with an examination Uric acid in the form of a linear correlation coefficient. The association was measured by the Person's moment correlation and the correlation coefficient was tested in terms of Significance of correlation coefficient, and the frequency and alleles of the genotypes, the odd ratio and the duration of the Confidence Intervals were tested. Using Fisher-Test and Hardy-Weinberg equilibrium,the significance between the data values understudy was tested using the Duncan's test, wherever possible, under different significance levels (P ≤ 0.05).

RESULTS

The results of the current study shown in Table 2 indicated that there were significant differences P ≤ 0.05 in uric acid mean levels between the patients and control groups (8.141±0.1331 and 4.393±0.1273 mg/dl), respectively. In addition, significant differences were found in the mean level of blood urea, as the urea was 44.5±1.778 and 29.67±0.6403 mg/dl for both patients and control groups, respectively. A positive relationship was found between urea and uric acid (r=0.2554). The creatinine mean of patients and control groups was 1.079±0.06855 and 0.7633±0.01694 mg/dl respectively, and the creatinine relationship with uric acid was positive (r=0.2532). In addition, a positive relationship was found between TCHO and uric acid (r=0.2241), as the mean TCHO concentration in the patient and control group were 218.7±3.716 and 165.1± 3.3 mg/dl, respectively. As shown in Table 3 the relationship of TG with Uric acid is positive (r=0.2736), the mean of TG was 209.8±5.732 and 132±3.359 mg/dl for patient and control group concentration. Table 3 indicated an inverse relationship between HDL and uric acid (r = -0.3028), wherethe mean of HDL in the patient's group was 22.74±1.14 mg/dl, and 44.5±0.6446 mg/dl in the control group. The results indicated a negative relationship between Vitamin D3 and uric acid (r=-0.4162) as in Table 3. The mean of Vitamin D3 among the patient's group was 18.55±1.06pg/ml, while in the control group was 40.74±1.089pg/ml.

The current study indicates that there was no discrepancy in the site rs2280205 between the study and control samples, as all samples appeared in genotype (AG). Table 4 showed that there was a difference in the genotypes in the site rs3733591 between the study and control samples by (36%, 17% and 47%) forthe patient sample and (13%, 3% and 84%) for (AG, AA, GG) genotypes, respectively. The results of the Frequency distribution of alleles A and G of rs3733591 for the *SLC2A9* gene by using Hardy-Weinberg equilibrium indicate a difference in the Frequency distribution between the sample of those with high uric acid and the control sample. In the patient's sample, alleles A was recorded 24 compared to the allele G which repeats46. As for control sample, the allele A was recorded in the 3 compared to the allele G, which was recorded 27 (Table 3).

As for rs2276961, the study indicated that there was a difference in the genotypes ratios for (AG, AA, GG) genotypes by (76%, 23% and 1%) in the patient sample and (60%, 7% and 33%) in the control sample respectively (Table 4). The study showed that the frequency distribution of A and G alleles of patient control samples was significant at P≤0.01, as allele A appeared in the patient sample42 times compared to the allele G which The frequency of its was 28 times, while the allele A was recorded 11 in the control sample compared with the allele G, which was recorded 19 as shown in Table 3.

Table 2 : Mean and standard deviation of physiological and hormonal tests in the serum of samples understudy.

Parameter	Groups		*p-Value*
	Gout disease (No. 70) Mean (± S.D)	**Control (No. 30) Mean (± S.D)**	
B. urea	44.5(±1.778) mg/dl	29.67(±0.6403) mg/dl	0.0001*
Creatinine	1.079 (± 0.06855) mg/dl	0.7633 (± 0.01694) mg/dl	0.0036*
Uric Acid	8.141(±0.1331) mg/dl	4.393 (± 0.1273) mg/dl	0.0001*
Vit. D3	18.55(±1.06) pg/ml	40.74 (± 1.089) pg/ml	0.0001*
TCHO	218.7 (± 3.716) mg/dl	165.1 (± 3.3) mg/dl	0.0001*
TG	209.8 (± 5.732) mg/dl	132 (± 3.359) mg/dl	0.0001*
HDL	22.74 (± 1.14) mg/dl	44.5 (± 0.6446) mg/dl	0.0001*
LDL	154 (± 3.235) mg/dl	96.76 (± 3.267) mg/dl	0.0001*

NS = Non-significant, * significant at p-value ≤ 0.05

Table 3 : Correlation and probability coefficient values for physiological and hormonal examinations with uric acid for the samples understudy.

Parameter	Serum uric acid	
	***p*-value**	***r* value**
B. urea	0.049*	0.2554
S. creatinine	0.034*	0.2532
Vitamin D3	0.001*	-0.4162
TCHO	0.044*	0.241
TG	0.022*	0.2736
HDL	0.011*	- 0.3028
LDL	0.016*	0.2865

NS=Non-significant, * significant at p value ≤ 0.05

Table 4 : Genotypes of the SNPs for SLC2A9 gene.

SNP 2 rs3733591 Genotype Frequency (%)					
Genotype	**Control n=30**	**Cases n=70**	**P-value**	**Odds Ratio**	**95% Cl**
AA	**1 (3%)**	**12 (17%)**	**0.101 N**	**6.00**	**0.80 to 265.1**
AG	**4 (13%)**	**25 (36%)**	**0.030 ***	**3.61**	**1.06 to 15.67**
GG	**25 (84%)**	**33 (47%)**	**0.001****	**0.18**	**0.05 to 0.56**
Chi-squa.	**2.0165 NS**	**3.2379 NS**			
P Value	**0.3649**	**0.1981**			
Allele frequency (%)					
Allele	**Control n=30**	**Cases n=70**	**P-value**	**Odds Ratio**	**95% Cl**
A	**0.10 (3)**	**0.35 (24)**	**0.0002****	**0.2063**	**0.08286 to 0.5139**
G	**0.90 (27)**	**0.65 (46)**			
SNP 3 *rs2276961* Genotype Frequency (%)					
Genotype	**Control n=30**	**Patient n=70**	**P-value**	**Odds Ratio**	**95% Cl**
AA	2 (7%)	16 (23%)	0.086 NS	4.15	0.86 to 39.3
AG	18 (60%)	53 (76%)	0.149 NS	2.08	0.75 to 5.66
GG	10 (33%)	1 (1%)	< 0.0001 **	0.03	0.00 to 0.23
Chi-squa.	2.555 NS	24.133**			
P value	0.6999	< 0.0001			
Allele frequency (%)					
Allele	**Control n=30**	**Patients n=70**	**P-value**	**Odds Ratio**	**95% Cl**
A	0.37 (11)	0.6 (42)	0.0020**	0.3746	0.2005 to 0.7000
G	0.63 (19)	0.4 (28)			

DISCUSSION

Urea is the main nitrogenous substance from metabolic wastes that mainly form in the liver and is release through the kidneys. The formation of uric acid in the liver depends on the effectiveness of the hepatic enzyme xanthine oxidase then the blood transports it to the kidneys, where 75% of it excreted. The reason for the presence of uric acid in the urine may sometimes be attributed to its excretion by the active cells that lininig the renal tubules of human kidney, any defect or lack of kidney function leads to a lack of urea put out of the body, leading to collect and accumulate in the blood concentration rises (Arif and Ali, 2014).

Measuring serum creatinine concentration is a health indicator for measuring kidney function. Creatinine level in the blood serum is more stable and it is the least altered nitrogenous material. Creatinine is formed in the muscles as a product of vital activities and is associated with muscle mass in the body. The reason for high creatinine concentration in a patient's serum may be due indicates that creatinine is a metabolic nitrogenous residue that is excreted naturally by Urination.Creatinine concentration is inversely proportional to Glomerular Filtration Rate (GFR) (Eren, 2014).

Several previous studies have shown that high levels of triglycerides TG are associated with hyperuricemia in the blood (Clausen *et al*, 1998; Conen *et al*, 2004). The possible mechanism can be explained on the basis that the construction of TG accelerates de-synthesis process (de novo) from ribose-5-phosphate to phosphoribosyl pyrophosphate (PRPP) through the metabolic pathway NADP-NADPH. As a result, uric acid production increases (Matsuura *et al*, 1998). A study of Peng *et al* (2015) indicated that the construction of TG requires NADPH and this will lead to increased uric acid production. Although, many studies have shown an increase in uric acid levels in obese individuals (Choi and Ford, 2007; Kim *et al*, 2012). Meanwhile, mature adipose cells and adipose tissue are produced and excrete uric acid. Thus, it is possible that this tissue contains all the enzymes needed for purine metabolism. Interestingly, the visceral fat region correlates positively with uric acid levels in the blood, indicating hyperuricemia. In addition, obesity increases the expression of mRNA, xanthine oxidoxase activity and uric acid secretion of adipose tissue (Cheung *et al*, 2007; Tsushima *et al*, 2013). On the other

hand, in the traditional lipid pathway, triglycerides (TG) are a direct product of fructose metabolism by making multiple enzymes including italdolase B (Aldo B) fatty acid synthase (FAS).

Several studies indicated that there was an inverse (negative) relationship between uric acid and vitamin D in postmenopausal women (Lu *et al*, 2009; Peng *et al*, 2013). The study of Abokhosheim *et al* (2013) indicated the same previous results in Egyptian older adults. Moreover, Vitamin D supplementation may be used as an alternative treatment for uric acid hyperemia and results indicate that the reason for this negative adverse relationship maybe that uric acid in the blood can inhibit the enzyme 1-á hydroxylase, which is the required enzyme to convertvitamin D 25 (OH) into its active form vitamin D 1,25 (OH) 2 (Mehta *et al*, 2015) and to confirm the results support the idea that the vitamin D3 state it was inversely related to high uric acid, as it was found that Allopurinol (Zyloric), which is the most common treatment that leads to a decrease in the level of uric acid, leads to a decrease in uric acid in patients with gout, and in turn leads to an increase in Vitamin D3 (Takahashi *et al*, 1998; Zhang, 2006).

Arg265His (rs3733591) variant of SLC2A9 has been shown to have a connection with gout in different populations. In Japanese men, rs3733591 showed association with gout (Urano *et al*, 2010), rs3733591 association of gout in Han Chinese, Solomon Islanders, New Zealand and Malay (Wan Rohani *et al*, 2018).

Genetic variation in *SLC2A9* gene may be one of factor that causes gouts. SLC2A9 is expressed in the cartilage of human articular cartilage. The minor allele rs3733591 may be impact on the activity of SLC2A9 in articular cartilage cells and increases the risk of MSU crystallization and tophi formation in joint structures (Hollis-Moffatt *et al*, 2011). Another issue is that host factors other than hyperuricemia may contribute to the development of tophi; quantitative analysis of tophus tissues indicates that unlike innate immune responses that are activated in acute gout, both innate and adaptive immunity are involved in the development of Tophus (Dalbeth *et al*, 2010). Thus, different immune responses to MSU crystals may lead to different manifestations of the disease (Hollis-Moffatt *et al*, 2011).

CONCLUSION

The study indicated significant differences in blood urea level between patients group and healthy group and serum creatinine and also indicated a positive correlation relationship. The association was negative between HDL and uric acid. In addition, the level of vitamin D decreased in the patient's group compared to the healthy group and the relationship was negative with uric acid. The results of the genetic analysis of the SNPs of the *SLC2A9* gene (rs2276961 rs2280205 rs3733591) showed two alleles (A, G) and three genotypes (AA, GG, and AG). The single nucleotide polymorphism appeared rs2280205 with one genotype of all samples (100% of all samples), while the AA and GG genotypes did not appear in all study samples, whereas rs3733591 with three genotypes appeared in the G alleles the highest frequency in the patients and healthy subjects samples. As for rs2276961, allele A appeared at the highest frequency in the patient sample compared to the healthy sample.

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