



ABCG2 Polymorphism in Hyperuricemia associated Type 2 Diabetes and Hypertension Patients

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ABSTRACT

Hyperuricemia is one of those syndromes that is highly inherited similar to obesity and body weight. There are many ongoing types of research in connection to the genetic inheritance of hyperuricemia. Existing studies have found out that, abnormal production of SNP rs2231142 of ABCG2 gene, that belongs to the ATP-Binding cassette family, is responsible for the increased uric acid levels. Studies have proved that genetic polymorphism on the Q141K and V12M variants of the ABCG2 gene is responsible for the abnormal uric acid levels. Hence, people carrying these alleles are more likely to develop hyperuricemia. However, the extent of association of ABCG2 and hyperuricemia was found to vary with ethnicity. So, we have selected the same SNP rs 2231142 of the variants Q141K, in order to establish the theory of hyperuricemia variation with ethnicity. Here we want to study the significance of ABCG2 polymorphism in hyperuricemia patients suffering from type 2 diabetes and hypertension in the district of Srikakulam in South India. The aim of the present study was to find an association of novel ABCG2 gene with hyperuricemia in Srikakulam population attending GEMS&H, who are diagnosed as hyperuricemic and are suffering from type 2 diabetes and hypertension for more than 5 years. The study population is selected based on the disease conditions such as hypertension, hyperlipidemia, and type 2 diabetes. A total of 100 subjects were involved. Genomic DNA is extracted from the whole blood and gene polymorphism was identified by PCR and RFLP. Hyperuricemia was positively associated with age, obesity and alcoholism. The SNP locus rs2231142 of the ABCG2 gene was found to be associated with hyperuricemia and found significant in obese alcoholic men who are suffering from type 2 diabetes and hypertension.



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INTRODUCTION

Uric acid is an excretory product that is found in the blood. It is formed due to the breakdown of substances called "purines" that are part of a non-vegetarian diet. High amounts of uric acid in the blood can cause the formation of crystals in the joints, leading to gout. However, hyperuricemia, may be the result of a variety of metabolic and physiological disturbances (Seegmiller *et al.*, 1961). Hyperuricemia is one of those syndromes that is highly inherited similar to obesity and body

weight (Cheng *et al.*, 2017).

There are many ongoing researches in connection to the genetic inheritance of hyperuricemia.

Epidemiology

Both environmental and genetic factors are the causes of elevated serum uric acid levels. Prevalence increases with age and male gender and the duration of type 2 diabetes and hypertension (Billa *et al.*, 2018). From the literature, the pathological basis of hyperuricemia is due to gouty arthritis, renal failure, hypertension and coronary heart disease (Soltani *et al.*, 2013). Other factors that cause hyperuricemia include obesity and alcoholism (Shiraishi and Une, 2009).

Etiology

Hyperuricemia occurs when purine intake through food or the formation of endogenous purine due to cell turnover increases. It can also occur as a group of diseases that cause elevated uric acid in the blood or decreased uric acid output by kidneys, which is a type of metabolic syndrome (Bhole *et al.*, 2010).

The two main reasons of hyperuricemia are the rate of synthesis of uric acid in the liver and the rate of uric acid excretion from kidneys (Nath *et al.*, 2007). An abnormal uric acid excretion is mainly due to the abnormal expression of urate transporter in the kidneys. As updated recently, the genetic factors account for nearly 40-70% cause for hyperuricemia that alters the renal uric acid transport system (Matsuo *et al.*, 2009). Existing studies have found out that variation in SNP rs2231142 of the ATP-Binding cassette gene family ABCG2 is responsible for the increased uric acid levels that leads to hyperuricemia (Zhang *et al.*, 2013).

Genetics and Hyperuricemia

ABCG2 belongs to the ATP binding cassette G2 family of transporters that secrete and transport a wide variety of substrates, including Uric acid (Cleophas *et al.*, 2017).

ABCG2 was identified as the urate efflux transporter, along with a role in multidrug resistance (Robey *et al.*, 2007). The urate transporters of this family are widely studied and has been proved that the variants of this gene are responsible for the reduced expression of the ABCG2 gene leading to reduced ATPase activity and uric acid transport by 53% (Woodward *et al.*, 2013). Abnormalities in ABCG2 functionally imparts decreased uric acid excretion ratio and causes its elevation in the blood (Woodward *et al.*, 2009). It is predicted that single nucleotide polymorphism (SNP) of the ABCG2 gene displays a crucial role in abnormal uric acid

excretion.

In the present study, the polymorphism sites of the ABCG2 gene are studied in Srikakulam population who are under treatment for type 2 diabetes and hypertension for a period of more than 5 years.

MATERIALS AND METHODS

Clinical features of patients

Patients diagnosed with hyperuricemia were reported to the General medicine department of GEMS Medical College, Ragolu, Srikakulam. Inclusion criteria was met for all the patients diagnosed with hyperuricemia of age 40-70 years. There was no gender limitation. Cardiovascular, hematopoietic disease, kidney disease, mental illness, hypo and hyperthyroidism, smokers and patients who are all on treatment with drugs that cause decreased excretion of uric acid like, pyrazinamide, ethambutanol, nicotinic acid, cyclosporin, 2-ethylamino-1,3,4-thiadiazole and cytotoxic agents were excluded from the study. Blood pressure was checked using a sphygmomanometer and readings are noted. Medical history of subjects, along with investigations and treatment, has been collected in a questionnaire format. The patients were reviewed with three or more physicians to determine the condition. A total of 100 patients were included into the study. Patient informed Consent was obtained from all participants and ethical clearance was taken from the institutional ethics committee.

Fasting blood sample of 5 ml was collected from all the patients. Blood samples were estimated for fasting glucose, urea, uric acid, creatinine and lipid profile.

1. Serum Glucose is estimated by Hexokinase method,
2. Serum Urea is estimated by Urease method,
3. Serum Uric acid is estimated by Uricase enzymatic method and
4. Serum Creatinine is estimated by Jaffe's method
5. Serum Cholesterol and Serum Triglycerides were estimated by CHOD-POD and GPO-POD methods respectively on Biochemistry Analyzer (Roche Cobas c311)

Anthropometric measurements of all the participant patients were recorded. BMI of all participant patients was calculated using their height and weight measurements. BP was also recorded.

Serum Hyperuricemia diagnosis

Table 1: No. of participants with hyperuricemia

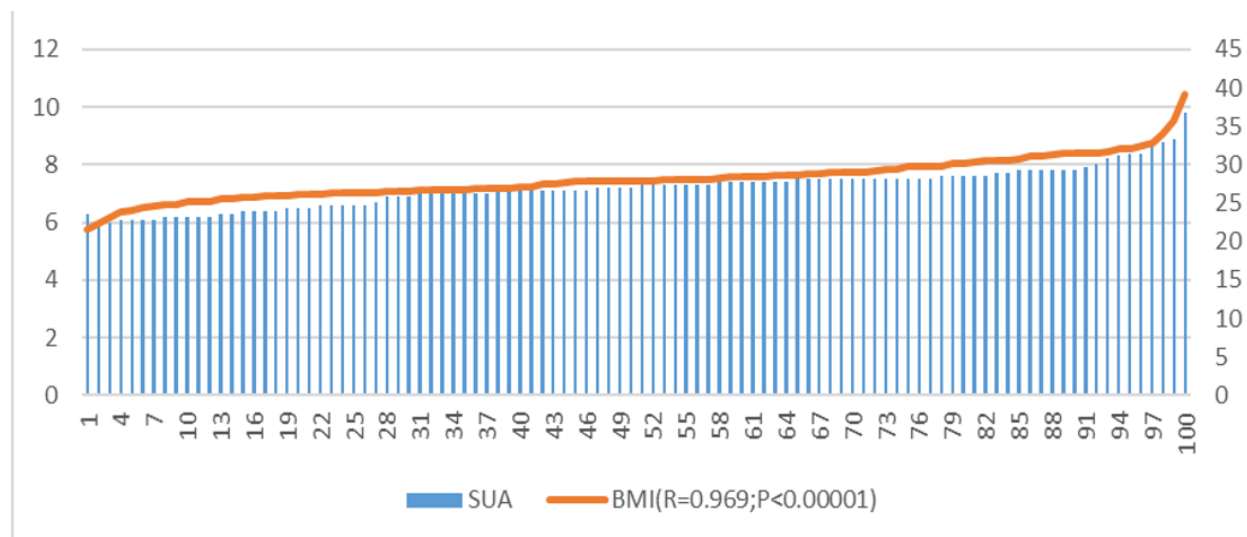
S.No	Gender	Diagnosed with Hyperuricemia
1.	Males	64
2.	Females	36

Table 2: Baseline characteristics of studied parameters in the studied population

Parameter	Mean	Standard Deviation
Age (Years)	56.08	11.08
Fasting Glucose (mg/dl)	242.15	25.45
Urea (mg/dl)	26.52	8.58
Uric acid (mg/dl)	7.12	1.51
Creatinine (mg/dl)	1.35	0.31
Systolic B.P (mm of Hg)	220	13.97
Diastolic B.P (mm of Hg)	94.15	9.78
Cholesterol(mg/dl)	196.25	18.38
Triglycerides(mg/dl)	246.29	16.97
BMI	28.16	2.19

Table 3: Hardy - Weinberg equilibrium of rs 2231142 on ABCG2 gene

Ethnicity	SNP type	Allele	HWP value
Srikakulam	rs2231142	G/T	0.62

**Figure 1: Prevalence of hyperuricemia in obese and non obese patients**

Detection of Hyperuricemia was done with uric acid levels in the blood as > 7 mg/dl in males and > 6 mg/dl in females. The number of males diagnosed with hyperuricemia is 64 and that of females is 36 (Table 1).

DNA isolation was performed by Salting out method

3 ml of the blood sample was collected in Ethylenediaminetetraacetic acid (EDTA) vacutainer and stored

at 4°C until processing. DNA was isolated from the peripheral blood lymphocytes using the salting-out method. The purity of the sample was determined by Ultraviolet Spectrophotometry (Systronics Spectrophotometer 119).

Genotyping ABCG2 polymorphism

Genomic DNA was amplified by Polymerase chain reaction on Prime Thermo Cycler. The primers used for rs2231142 were GCCTTAAGATGTTGTGAT

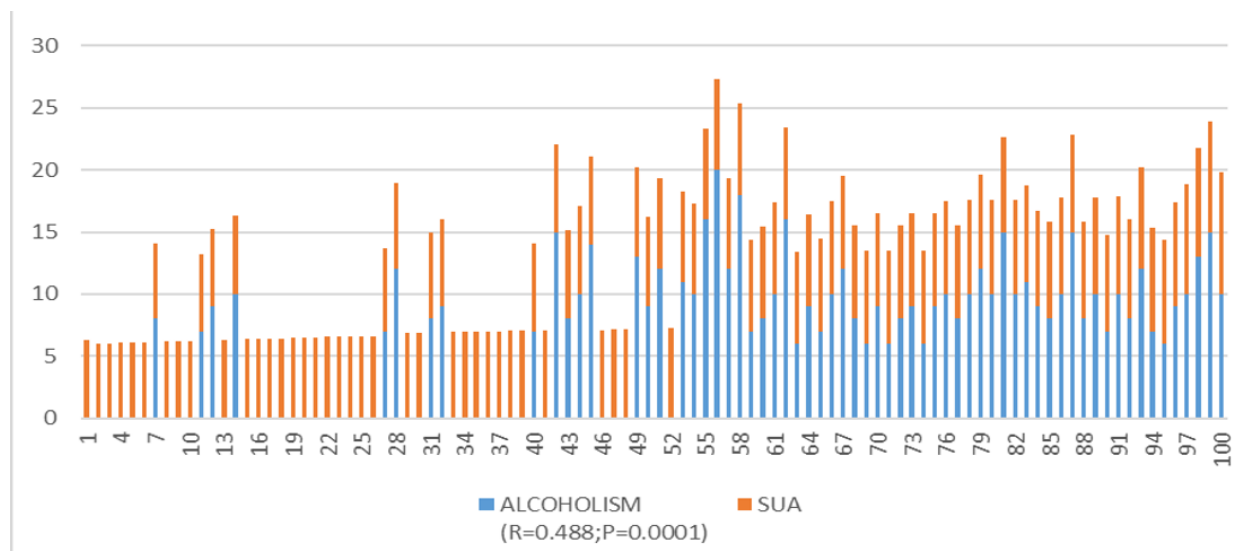


Figure 2: The correlation between Non Alcoholic and Alcoholic Hyperuricemia

upstream and downstream ATCAGAGTCATTT-TATCCACACA and rs2231137 were upstream TTGCAATCTCATTATCTGGAC and downstream CAAGGTAGAAAGCCACTCTTCAG. About 25 μ l reaction mixture was included with 1x assay buffer, 150ng genomic DNA, dNTP 0.2mM, 1 pM of primers, 1.5mM MgCl₂ reaction mixture was included with 1x assay buffer, 150ng genomic DNA, dNTP 0.2mM, 1pM of primers, 1.5mM MgCl₂ and 1unit TaqDNA polymerase. The PCR program involves denaturation (95^oC: 3min), cycle denaturation (95^oC - 30s), annealing (60^oC - 30s); extension (72^oC:1min) and final extension 72^oC for 5min and no. of cycles were 25. The obtained PCR product was analyzed on 2% agarose gel. The 150 base pairs amplicon was subjected to restriction digestion with 10 units of XcmI (New England Biolabs, USA) at 37^oC for 2 hours and analyzed on 2% agarose gel by using ethidium bromide staining.

RESULTS AND DISCUSSION

Clinical characteristics of the participants

Among the included participants of 100, the mean age of the patients was found to be 56 years. All the patients were suffering from either type 2 diabetes or hypertension or both.

The mean values in the studied population are as follows, fasting sugar 242.15 \pm 25.45 mg/dl, urea 26.52 \pm 8.58mg/dl, creatinine 1.35 \pm 0.31 mg/dl and uric acid 6.68 \pm 2.5 mg/dl (Table 1). An average of 3 readings of BP was taken. Mean systolic blood pressure is 220 \pm 13.97, while the diastolic blood pressure is 94.15 \pm 9.78 mm of Hg (Table 2).

Association of hyperuricemia with characteristics proved by univariate and multivariate logistic regression analysis

tic regression analysis

In the univariate regression analysis, hyperuricemia is positively associated with the male gender in terms of high BMI, alcoholism, elevated triglycerides, fasting glucose increased DBP. Multi colinearity was observed between variables and from the multivariate regression analysis, hyperuricemia was positively associated with age, male gender and alcoholism.

Statistical analysis

Statistical analysis was performed by SPSS 20 version software. Hardy-Weinberg equilibrium was applied for the results. The Chi-square test was performed to compare ABCG2 rs2231142 gene at intron G/T that showed Hardy Weinberg value 0.65 (Table 3) genotypic and allelic distributions among the studied population.

Genotyping ABCG2 polymorphism

Band patterns of ABCG2 genotype were identified as the cut 90 bp and 60 bp and the uncut 150 bp. All the samples were selected for result confirmation. Up to 30% of the results were concordant.

Genome-wide association of hyperuricemia in the studied population

From the analysis of the present study, SNP (rs 2231142) exhibited the strongest association with hyperuricemia of the ABCG2 gene and showed a genome-wide significance. Carriers of the rs2231142 allele had significantly higher UA levels ($p < 0.001$).

While obesity and high alcohol intake are the known factors to influence uric acid levels in the blood, hyperuricemia is known to be one of the causal

agents for developing hypertension and type 2 diabetes (Shah and Keenan, 2010; Kolz et al., 2009). According to a worldwide study, the number of hypertensive and diabetic adults would increase upto 300 million and 1.56 billion, respectively, by the year 2025 (Choi et al., 2014; Kodama et al., 2009). Hence, there is a greater need to find for the causative agents of such disease conditions and their underlying mechanisms.

Among the various factors that lead to hyperuricemia induced hypertension and metabolic syndrome, genetic inheritance is one of the significant factors that change the direction of the utilization and elimination of uric acid.

In the present study, we have selected the known cases of type 2 diabetes and hypertension and are diagnosed with hyperuricemia for the first time. We aimed to see a possible link between obesity, alcoholic status, BMI, triglycerides and total cholesterol levels with that of the fasting sugar and uric acid levels in the serum sample of all the participant patients.

Our results showed a strong positive correlation between serum uric acid and fasting blood sugar ($R=0.943; P<0.00001$), which are in line with the previous studies (King et al., 1981). Insulin resistance may be the link between elevated Glucose and Uric Acid levels (Kearney et al., 2005). Our study also demonstrated an increase in serum uric acid level in type II diabetes subjects, which corresponds to the studies on higher risk for developing impaired glucose tolerance and type II diabetes in hyperuricemia (Modan et al., 1987).

Serum Creatinine serves as a sensitive indicator in the early diagnosis of kidney failure, so we tried estimating the serum creatinine in these patients, which showed a positive correlation with serum uric acid. It is said that uric acid mediates the relation between hypertension and renal disease via renal vasoconstriction and systemic hypertension. A common hypothesis also exists that the common association of hyperuricemia with CKD was solely attributed to the retention of serum uric acid that is known to occur as the glomerular filtration rate falls (Meisinger, 2012). The link between uric acid and arterial HTN was first noted in the 1960s. As observed in the existing research, there are two mechanisms involved, one being mediated by direct entry of uric acid into both endothelial and vascular smooth muscle cells, resulting in local inhibition of endothelial nitric oxide levels, stimulation of vasoactive and inflammatory mediators (Johnson et al., 2005).

As regards to uric acid, total cholesterol and

triglycerides, the existing studies show that altered lipoprotein metabolism is linked to high serum uric acid (Richard et al., 2013). Our data provide strong evidence for these studies that there is a positive correlation between elevated uric acid and the total cholesterol ($R=0.98; P<0.0001$) and triglyceride content ($R=0.95; P<0.00001$) of the study population.

Uric acid and BMI were positively correlated in our study ($R=0.96; P<0.00001$). The individuals who were suffering from both hypertension and diabetes showed a strong correlation than the hypertensive and patients suffering only diabetes. The prevalence of hyperuricemia in our study was 2 times more in obese patients when compared to normal-weight diabetics (Figure 1). This shows that obesity may serve as a clinical indicator for hyperuricemia. Though the mechanism pertaining to the association between elevated BMI and elevated serum uric acid is not well established, the possible reason has been observed as the abnormal fat distribution in the body (Matsuura, 1998).

Our data also suggests that there is a possible link between alcoholic status and elevated uric acid levels in patients who are suffering from type 2 diabetes and hypertension. For Uric acid and consumption of alcohol, we studied a combination of the patient population, which included alcoholic and non-alcoholic subjects. When we estimated serum uric acid in both hypertensives and diabetics, subjects who were alcoholics for more than 10 years with continuous intake of alcohol showed a 2 fold rise in their serum uric acid value than the normal range (Figure 2). The possible reason behind this elevation might be due to the overproduction of lactate due to alcoholic intake (Lieber, 1965).

Preliminary investigations also suggest that there is an indication of hyperuricemia, is indeed a component of Metabolic Syndrome and could also be used as a simple marker of insulin resistance. This link was explained by two mechanisms 1) reduction of NO bioavailability and endothelial supply, which inhibits the delivery of glucose to the skeletal muscle (Zhou et al., 2014) and 2) activation of NADPH oxidase, generating oxidized lipids and inflammatory mediators in adipocytes (Vuorinen-Markkola and Yki-Järvinen, 1994).

Further, we tried to investigate these criteria at a genetic level, taking into account of the previous genetic studies with respect to hyperuricemia. Studies have proved that genetic polymorphism on the Q141K variant of the ABCG2 gene is responsible for the abnormal uric acid levels (Sautin et al., 2007). So, we have selected the same SNP (rs 2231142) to establish this theory of hyperuricemia variation

with ethnicity. When adjusted with the covariates, serum uric acid levels showed a significant positive correlation in these subjects with BMI, alcoholism, fasting blood glucose, total cholesterol and serum triglycerides. Upon pooling the data, we could observe a strong association of rs 2231142 related hyperuricemia in alcoholic obese men with a significant ($R=0.488;P=0.0001$).

CONCLUSIONS

It is concluded from the above findings of the present study that Hyperuricemia is a risk factor in patients suffering from hypertension, type 2 diabetes, alcoholism and obese individuals and genetic polymorphism on SNP rs2231142 in ABCG2 is found more associated with hyperuricemia among Srikakulam population.

This study is one of the first genome-wide studies in the north AP region in connection with hyperuricemia. Hence, the findings may be of great significance in the early intervention of Hyperuricemia being a causal agent of metabolic syndrome induced by hypertension and type 2 diabetes complications.

As our study falls short with low sample size, future studies are required in this area of genome-wide association of hyperuricemia related Hypertension and Type 2 diabetes to delink other possible mechanisms involved.

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