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Polymorphism study of ATP1B1 gene in hypertension with CKD patients

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Abstract---The present study aims to examine the genetic variation of the *ATP1B1* gene concerning hypertension and chronic kidney disease CKD. (120) blood samples from participants were obtained and divided into three groups: a first group representing hypertensive patients (hyper), a second group representing Hypertensive CKD patients (hyper with CKD), and a third group representing a control group. DNA was extracted from all blood samples and then converted to cDNA and the ARMS-PCR technique was used to investigate the single nucleotide polymorphism (SNP) from the *ATP1B1*, genes. The result of this study investigated that SNP rs2901029 located in *ATP1B1* is significant association with hypertension and CKD.

Keywords---CKD, polymorphism, ATP1B1-gene, hypertension.

Introduction

Na-K-ATPase is a plasmalemmal ion transporter that can be found in all mammalian cells that have been identified. The Na⁺ gradient across the plasma membrane, which serves as the principal transporter, drives additional Na⁺-coupled transporters. Na-K-ATPase may therefore play a variety of roles in the anaemia of CKD (Pivovarov *et al.*, 2018).For each unit of ATP expended, the Na⁺ K⁺ ATPase pumps 3 Na⁺ out of the cell and 2K⁺ into the cell (Kopeck *et al.*, 2014). The ATP1B1 gene expression for the Na, K-1 ATPase's subunit. This plasma membrane-bound oligomeric enzyme catalyzes the exchange of three Na⁺ ions for two K⁺ ions across the cell membrane via a coupled active transport, maintaining the gradient's normal physiological state (Kaplan , 2002).

The gradients of concentration are resisted by sodium and potassium. The gradient between a higher quantity of sodium extracellularly and a higher level of potassium intracellularly is maintained by the Na⁺ K⁺-ATPase pump. The

persistent concentration gradient maintains the cell's resting membrane potential, controls cell volume, and facilitates cell signal transmission, all of which are essential for physiological functions in many organs (Pivovarov *et al.*, 2018).

It is essential for the maintenance of other physiological processes such as sperm motility, the generation of the neural action potential, and the filtration of waste materials in the nephrons (Clausen *et al.*, 2017). Blood pressure is affected by Na⁺/K⁺-ATPase activity in the cardiovascular system and renal tubular systems in different ways. When ouabain binds to the Na⁺/K⁺-ATPase subunit 1 in ventricular myocytes, it inhibits Na⁺/K⁺-ATPase activity and raises intracellular Ca²⁺ in a concentration-dependent way, raising blood pressure. In contrast, sodium is transported from the luminal to the interstitial side of renal tubular cells via the Na⁺/K⁺-ATPase. Therefore, suppressing sodium reabsorption and lowering blood pressure are both caused by the reduction of renal Na⁺/K⁺-ATPase activity (Peng *et al.*, 1996). So, this study aimed to the determination of genetic variation of the *ATP1B1* gene and its relation to CKD and hypertension, which may be reflected in the expression of Na⁺/K⁺-ATPase and then in the function of the kidney.

Material and Methods

Collection of samples

(1ml) of blood was collected from 120 individuals, divided into three groups, each group included forty individuals. The first group included Hypertensive patients (hyper), and the second group included Hypertensive with chronic kidney disease patients (hyper with CKD), while the last group included healthy individuals as a control group, then immediately blood Samples were drawn and placed into Dipotassium-EDTAVacutainer® Tubes for use in ARMS-PCR Technique.

DNA extraction and ARMS –PCR (primer amplification refractory mutation system–polymerase chain)

ARMS-PCR primers for *ATP1B1* rs2901029 gene polymorphism were designed in this study using the NCBI-SNP database and Primer1 ARMS-PCR primers were designed online. These primers were provided by (Scientific Researcher. Co. Ltd. Iraq) as following tables:

Table (1): The ARMS-PCR primers for ATP1B1 rs2901029 gene polymorphism with their sequence and amplicon size

| Primer | Sequence (5'-3') | Product size |
|-------------------------|-----------------------|--------------|
| Wildtype Forward Primer | TGCTAGCACCAAGCAAGGAAA | 501bp |
| Mutant Forward Primer | TGCTAGCACCAAGCAAGGAAG | |
| Common Reverse Primer | CTGCAGGTATTTGGGCTGCA | |

Statistical analysis

The data of genotype and allele frequencies of SNP2901029 barcode with SNP (*ATP1B1* rs2901029) gene was estimating of Odds ratios for genetic variants that

are associated with diseases and provided the 95% confidence interval (CI), corresponding to the effect of each specific SNP barcode on the occurrence of hypertension and CKD. SPSS for windows version (2010) was used for statistical analysis of the molecular study.

Result

Analysis of genotype and Allele

The results of genotype and allele frequencies of SNP2901029 located on the *ATP1B1* gene are shown in table (2) and figure 1 and 2. The homozygous genotype AA was more frequent in hyper patients 16(40%) and hyper with CKD 16(40%) in comparison with control group table (2) and (figure 1). In hyper group versus hyper with CKD, there were non-significant differences between them, [OR (CI) = 1(0.4088 to 2.4464) and P value = 1]. in hyper group versus control group, investigated significant associated with hyper in our study, [P value = 0.04 and OR (CI) = 2.6667(0.9808 to 7.2503)] means was found risk factor for disease take place.

In hyper with CKD group versus the control group, also showed a significant association with a high risk of hyper and CKD [P value = 0.04, and OR (CI) = 2.6667(0.9808 to 7.2503)] means was found risk factor for disease take place. On the other hand, the frequency of the heterozygous genotype AG was 12(30%), 18(45%) and 20(50%)] . In hyper, hyper with CKD patients and control groups, respectively, table (2) and (figure 1). In hyper group versus hyper with CKD ,the results showed non-significant differences between them ,P value = 0.16 ,and [OR (CI) = 0.5238(0.2089 to 1.3137)] .in hyper group versus control group , P value = 0.07 means was no found significant differences between them , and the value of OR (CI) = 0.4286(0.1713 to 1.0725)].P = 0.65 in hyper with CKD group versus control group means was no found significant differences between them , [OR (CI) = 0.8182(0.3398 to 1.9701)].The value of Odds ratio indicated that AG genotype samples indicated that resistant factor.

The homozygous genotype GG was more frequent in hyper group 12(30%) and control group 12(30%) Comparison with hyper with CKD group 6(15%) table (2) and (figure 1). in hyper group versus hyper with CKD, there was non-significant different between them, P value = 0.11, and [OR (CI) = 2.42(0.8082 to 7.2978] Also, in hyper group versus control group the result showed non- significant different between them (p=1) , and [OR (CI) = 1(0.3843 to 2.6023)]. In hyper group with CKD versus control group there was a non significant differences between them, P = 0.11 and [OR (CI) = 0.4118(0.1370 to 1.2373)]. The value of the Odds ratio indicated that AG genotype samples in all groups indicated that GG genotype risk factor for disease

Allele A was more frequent in hyper group 50(62.5%) and hyper with CKD group 44(55%) compared with a control group 36(45%) (table 2)and figure (2). in hyper group versus hyper with CKD comparison [P = 0.33, OR (CI) = 0.7333(0.3899 to 1.3791)], the results demonstrated that was a non-significant difference between them. also in hyper group versus group comparison noted non significant differences between them, [P = 0.20 ,OR (CI) = 1.4938(0.8012 to 2.7851)]. In the

hyper with CKD versus the control group, there were significant differences between them, [$P = 0.02$, OR (CI) = 2.03(1.0832 to 3.8308) that indicated A Allele risk factor for disease take place.

Allele G was more frequent in control 44(55%) compared with hyper group 36(45%) and hyper with CKD (table 2)and figure (2). $P = 0.33$ in hyper group versus hyper with CKD means were no found significant differences between them, OR (CI) = 1.3636(0.7251 to 2.5645) means that no found significant differences between them. $P = 0.20$ in the hyper group with a control group, there were non-significant differences between them, OR (CI) = 0.6694(0.3591 to 1.2481) means was found risk factor for disease. $P = 0.02$ in hyper with CKD versus control group means was found significant differences between them, OR (CI) = 0.4909(0.2610 to 0.9232) means was found risk factor for disease.

Table (2) Genotype and Allele Frequency for ATP1B1rs2901029

| Genotypes and allele | Groups | | | Hyper vs Hyper with CKD | | Hyper. vs Control | | Hyper with CKD vs Control | |
|----------------------|----------------------|------------------------------------|-----------------------|-------------------------|--------------------------|-------------------|--------------------------|---------------------------|--------------------------|
| | G1 Hyper. N=40 | G2 Hyper with CKD N=40 | G3 Control N=40 | P value | OR(CI) | P value | OR(CI) | P value | OR(CI) |
| Genotypes | | | | | | | | | |
| AA | 16(40) | 16(40) | 8(20) | 1 | 1(0.4088 to 2.4464) | 0.04 | 2.6667(0.9808 to 7.2503) | 0.04 | 2.6667(0.9808 to 7.2503) |
| AG | 12(30) | 18(45) | 20(50) | 0.16 | 0.5238(0.2089 to 1.3137) | 0.07 | 0.4286(0.1713 to 1.0725) | 0.65 | 0.8182(0.3398 to 1.9701) |
| GG | 12(30) | 6(15) | 12(30) | 0.11 | 2.42(0.8082 to 7.2978) | 1 | 1(0.3843 to 2.6023) | 0.11 | 0.4118(0.1370 to 1.2373) |
| Alleles | | | | | | | | | |
| A | 44(55) | 50(62.5) | 36(45) | 0.33 | 0.7333(0.3899 to 1.3791) | 0.20 | 1.4938(0.8012 to 2.7851) | 0.02* | 2.03(1.0832 to 3.8308) |
| G | 36(45) | 30(60) | 44(55) | 0.33 | 1.3636(0.7251 to 2.5645) | 0.20 | 0.6694(0.3591 to 1.2481) | 0.02* | 0.4909(0.2610 to 0.9232) |

* Significant difference at $P < 0.05$, OR: Odd ratio, CI: Confidence interval.

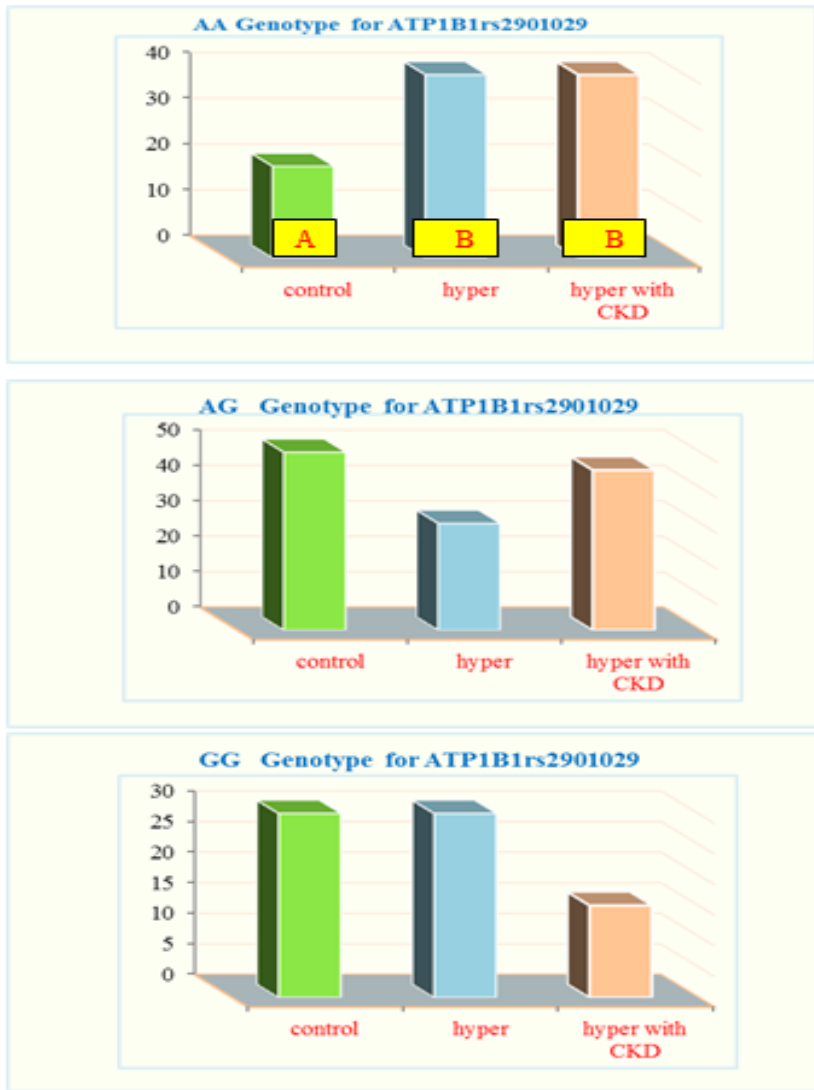


Figure (1) Ratio of Genotype for ATP1B1rs2901029 of hypertension and patients with CKD hypertension in comparison with control .

■ Control ■ hypertension ■ hypertension with CKD

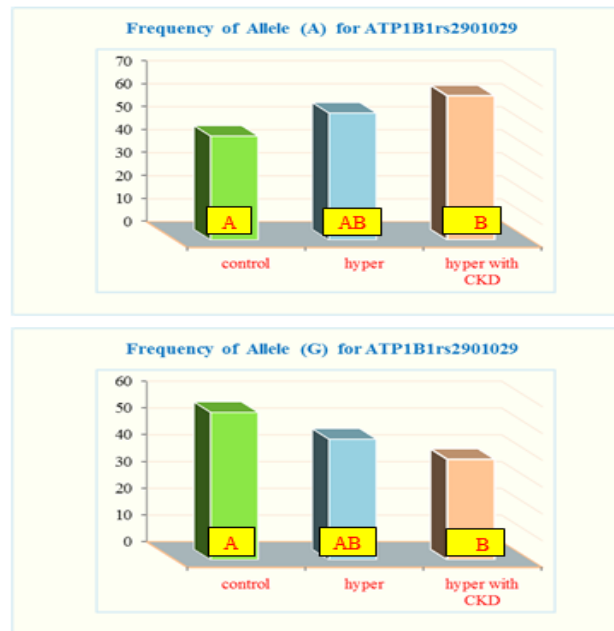


Figure (2). Ratio of Allele Frequency for ATP1B1rs2901029 of hypertension and patients with CKD hypertension in comparison with control .

■ Control ■ hypertension ■ hypertension with CKD

a different letters (A,B and C) represent significant different ($p < 0.05$) among groups

a similar letters represent no significant different ($p > 0.05$) among groups

Detection of ATP1B1 rs2901029 gene Polymorphisms

the distribution of ARMS-PCR for ATP1B1 gene polymorphisms (rs2901029) was detected by ARMS - PCR technique. At this locus, there are three genotypes; AG, GG and AA. (A A) homozygote genotype showed only A allele amplification at bp product size. the (G G) homozygote was shown in the G allele only, whereas the (G/A) heterozygote was shown in both G and A allele. The presence of the A or G allele was observed at bp 501 product size.

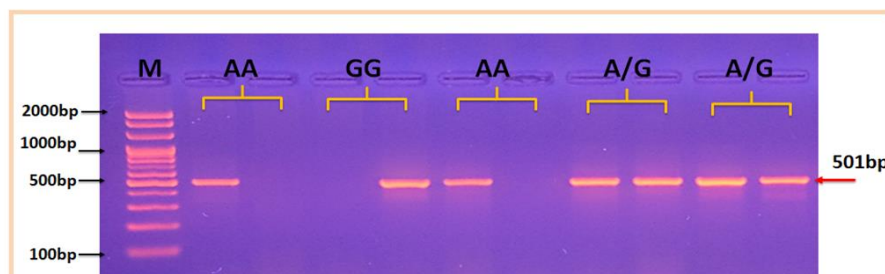


Figure (3): Agarose gel electrophoresis image that showed the ARMS-PCR product analysis of ATP1B1 rs2901029 gene polymorphism.

Discussion

Recently, genetic variants associated with hypertension- is being reported. In humans, Several genes in a chromosome 1q linkage region have been demonstrated to be related to hypertension. In our study, we examined variation in one SNP rs2901029 that is located on *ATP1B1* gene concerning hypertension. The findings of this study establish that genotypes AA was more frequent with Hypertension and Hypertension with CKD patients in comparison with control ,that suggest genotypes AA of *ATP1B1* rs2901029 in relation to hypertension and blood pressure. Which is consistent with Chang *et al.*, (2007) who proved that polymorphism in this genotype of *ATP1B1* associated with SBP in a cohort of African-Americans ; Xiao *et al.*, (2009). hypertension-susceptibility alleles *ATP1B1* is relatively common because the gene of *ATP1B1* consider one of the genes that responsible blood pressure regulation, *ATP1B1* encodes the ubiquitously expressed β subunit of Na,K-ATPase, an intrinsic oligomeric protein necessary for the maintenance of Na^+ and K^+ electrochemical gradients across the plasma membrane. This transporter is involved in multiple BP-regulating physiological processes: renal sodium reabsorption, vascular smooth-muscle-tone regulation, and cardiac muscle contraction.

In Hypertension patients Decreased Na^+ , K^+ -pump activity can result in a rise in intracellular Na^+ concentrations which in turn increase $\text{Na}^+/\text{Ca}^{2+}$ exchange, thereby raising intracellular Ca^{2+} levels. inhibition of the Na^+ , K^+ -pump can also reduce the driving force for renal tubular Na^+ reabsorption, elevating Na^+ excretion. By decreasing the membrane potential, thus allowing more efficient depolarization of nerve endings and by increasing intracellular Ca^{2+} , inhibition of the Na^+ , K^+ -pump can increase nervous tone (Haddy,1984) .also, Decreased Na,K-ATPase activity precedes the development of hypertension in animal models (Ferrandi et al.,1996). this study is also similar to the study of Xiao *et al.*, (2009) which confirmed the association of BP with *ATP1B1* genetic variants and haplotypes but did not include this SNP.

Conclusion

We conclude from the results of our study, and previous studies confirming the presence of several mutations in Na,k ATPase associated with high blood pressure and reabsorption and increase of Na^+ intracellular and inhibition of pump function in kidney nephrons leading to chronic kidney disease.

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