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Research Article

SNP Variety of UMOD Gene in Patients with Hypertension Disease in Hilla Province

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Abstract— A major risk factor for cardiovascular illnesses, hypertension—also known as high blood pressure—is shaped by both environmental and hereditary elements. Recent research have shown several genetic variations linked to hypertension, among which the rs13333226 variant found in the Uromodulin gene (UMOD). This paper explores the biological relevance of Uromodulin, consequences of the rs13333226 variation, and possible function in hypertension. Fifty blood samples in all came from diabetic patients visiting the Diabetes Center at Merjan Teaching Hospital in Babylon, Iraq. Five samples in all were also gathered to act as a control group. PCR magnificues DNA taken from blood samples. The SOD2 gene was genotyped using a polymerase chain reaction (PCR) method then applying the DNA single-stranded conformation polymorphism (SSCP) method. The single-stranded conformation polymorphism (SSCP) technique thus verified the genuineness of these DNA polymorphisms. The findings show several haplotypes seen in SOD2. The findings showed that in the sick group the DNA polymorphism distribution was 84% and 16%; in the control group it was 70% and 30%. Understanding hypertension depends much on the rs13333226 variation on the Uromodulin gene. Clarifying the link between this variation and blood pressure control would help scientists open the path for better risk evaluation and focused treatments in the control of hypertension. Complete knowledge of the consequences of this variation and its possible influence in cardiovascular health depends on ongoing research.

Keywords— Hypertension, Umod , Gene Polymorphisms, SSCP, PCR

1. Introduction

More than seven million people died in 2010 as a result of hypertension, according to the World Health Organization (WHO), accounting for around 12.8% of all deaths that year. Hypertension itself accounts for around 3.7% of all global deaths [1]. We can categorize hypertension based on its causes. A) Secondary hypertension: This form of the disease can develop in people who already have arteriosclerosis, kidney disease, dysfunction of the adrenal glands, or a thyroid issue. B) Primary or Essential Hypertension (EH): It comprises all cases of hypertension for which the cause or origin cannot be determined; this kind accounts for as much as 95% of all diagnosed cases [2].

A mucoprotein that has been found in urine in large quantities since 1950 has been dubbed the Tamm-Horsfall protein (THP) after its discoverers, Tamm and Horsfall [3]. Decher and Muchmore [4] studied and characterized Uromodulin (UMOD), a urine glycoprotein, decades after the discovery of THP. Only kidney cells express this protein, proving that UMOD and THP are the same protein .These days, people refer to the same protein as either THP or UMOD. New studies show that the UMOD protein can control the activity of the NKCC2 co-transporter and the reabsorption of NaCl in TAL cells. However, more research is needed to fully understand how uromodulin proteins affect blood pressure [5]. Furthermore, it is known that the UMOD protein can bind to a number of cytokines, including tumor necrosis factor-α (TNF-α), via its epidermal growth factor (EGF) elements. In an autocrine way, TAL makes TNF-α, which controls the expression of NKCC2A and lowers the reabsorption of NaCl at this site [6].

Prior to 2010, there was no established link between the UMOD gene and hypertension (aside from the nephropathic effect). A large genome-wide association study, on the other hand, helped a group of researchers from Glasgow University find the rs13333226 variant on the Uromodulin gene as a quantitative blood pressure locus. The minor G allele of rs13333226 is linked to a lower risk of high blood pressure, less uromodulin protein in the urine, and better kidney function [6]. Our second goal was to create and test a PCR-

SSCP genotyping method that can be used with this mutation so that researchers in the future can make an informed choice.

2. Related work

A complicated disease shaped by genetic, environmental, and lifestyle elements, hypertension with the urmodulin gene (UMOD) rising as a major contender, recent studies have concentrated on the part of particular genes in the onset of hypertension. Changes in kidney function and blood pressure control have been linked to UMOD gene variations, which makes it a vital field of research. The body of studies on urmodulin gene polymorphisms and hypertension emphasizes the need of genetic variables in the pathophysiology of this disease. Variants in the UMOD gene have been connected to kidney function as well as hypertension, implying that more research on this gene could provide useful knowledge for controlling and preventing hypertension. To completely clarify the mechanisms by which urmodulin affects blood pressure and to investigate possible therapeutic targets, more study is absolutely required [4-7].

3. Materials and methods

3.1 Hypertension patients and healthy control subjects

The present case-control study included 50 patients with ***Hypertension*** and 50 healthy control subjects. These individuals visited Marjan Hospital in Babylon, Iraq.

3.2 DNA Extraction

Using the extraction and purification kit provided by Favergen Company (Taiwan), the DNA comprising the genome was separated and polished from whole WBCs.

3.3 Measurement of Concentration and Purity of Extracted DNA

Measuring the absorbance at 260 and 280 nm correspondingly, a spectrophotometer (NanoDrop) analyzed the quantity and quality of DNA. Using the 260/280 ratio, purity was calculated from DNA concentration expressed in nanograms per millilititer (ng/ml). Regarding pure DNA solutions, the ratio runs between 1.2 and 2.[7-10].

3.4 PCR Amplification

Primers created especially for this use were used to preferentially amplify the specified DNA sections. SOD2 (rs13333226) was found with a single primer purchased from Bioneer, IDTDNA (USA). 5'- GTCCCTACAAAGGACATGAACTC -3' was the forward sequence, and 5'- CCAACACTACTCACCAGTTCTG -3' was the reverse. Mix 1.5 µl of forward and reverse primers, 12.5 µl of Green Master Mix, 3 µl of genomic DNA, and 1.5 µl of nuclease-free water to get the desired outcome. As a result, 20 µl will be the total reaction volume. Standard molecular markers were used in every electrophoresis session. Gels that were exposed to UV light were photographed. [11-14].

3.5 Statistical analysis

Statistics were examined using SPSS version 19. The Chi-square test was used to assess the Hardy-Weinberg equilibrium (HWE) of antioxidant genes in both subjects with Hypertension and in the healthy control group.

4. Results

Genomic DNA (Fig.1) was obtained from blood samples as an initial procedure to amplify the desired gene area.

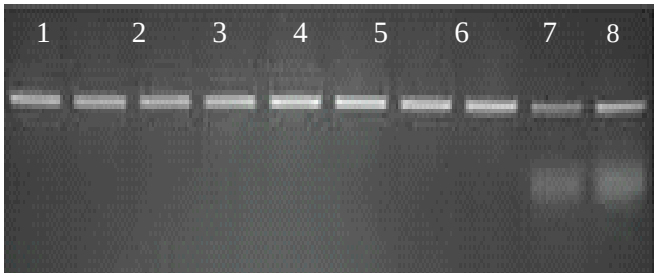

Figure 1 shows the electrophoresis pattern of genomic DNA obtained from blood samples from both the hypertension group and the healthy control group.

Lanes 1 to 7 represent genomic DNA extracted from blood samples. Electrophoresis was performed using a 1% agarose gel, with a voltage of 75 V and a current of 20 mA for 1 hour. Each well contained 10 µl of DNA sample, which was then stained with ethidium bromide.

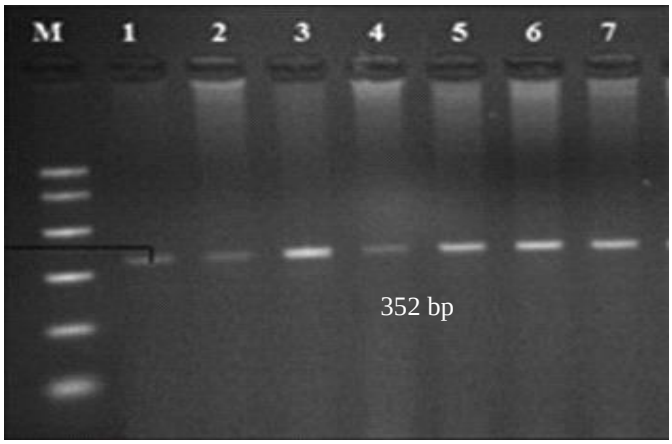

Figure 2: Agarose gel electrophoresis of amplified products of UMOD

M; refers to DNA size marker lane 1-7 lane refers to the patterns of amplified products of SOD2 (352 bp).

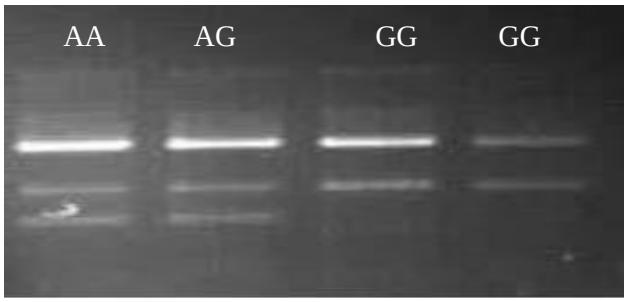

Figure 3: DNA polymorphisms of the UMOD gene of hypertension patients using the PCR-SSCP technique

Lanes 1-4 represent the PCR-SSCP pattern of SOD2. The term "C" denotes control, while the term "P" represents groups of patients. The SSCP gels obtained were compared to ascertain the number of haplotypes present. Two separate SSCP band patterns were detected in the gels.

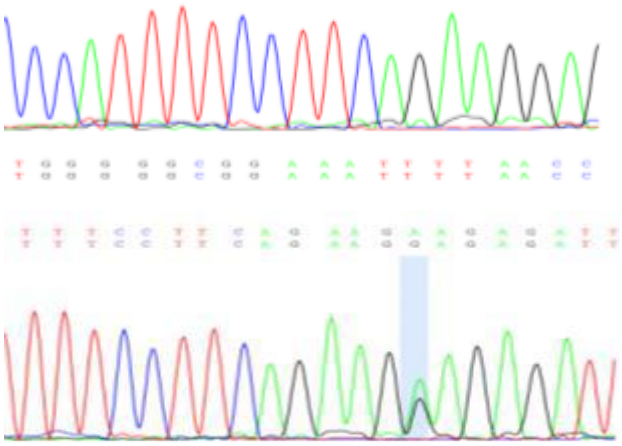

Figure 4: DNA polymorphisms of the UMOD gene of hypertension patients using the PCR-Sequencing technique

Table1-The determination of the genetic sequence of each amplified DNA and the criteria for SSCP-PCR depend on the diversity of single-stranded DNA (ssDNA) in SSCP gels.

Genotype	patients	Control	*p*-value	OR (CI-95%)
AA[a]	20	30		
AG	8	15	0.005*	3.85(1.46-10.17)
GG	22	5	*0.02	4.12(2.2-8.98)
Total	50	50		

P ≤ 0.05; OR= (95%CI); [a] reference;

5. Discussion

Previous animal studies have implicated uromodulin in the regulation of blood pressure and the development of hypertension. In 1950, Graham et al. discovered that mice lacking UMOD (UMOD−/−) weren't susceptible to changes in blood pressure caused by salt, and that their SBP was much lower than that of wild-type mice. The UMOD knockout mice exhibited a leftward shift in the pressure-natriuresis curve. In contrast, Trudu et al. (15) showed that when UMOD levels were high, BP went up in a way that depended on the dose. This was because of higher UMOD expression and excretion. Based on these findings, uromodulin may influence hypertension progression via TAL salt transport modification. A prior clinical investigation also linked the UMOD gene to hypertension.

A large genome-wide association study (GWAS) with European cohorts found that people with the minor G allele of rs13333226 had a lower risk of hypertension. In contrast, Algharably et al. (15) looked at a group of 1,218 white people and found that there were no significant links between rs12917707 and mean 24 h SBP, DBP, or any other BP phenotype.

A genetic variation called Rs13333226 (HGVS names 16:g.20354332A>G) in the UMOD gene promoter region was linked to lower levels of uromodulin protein expression in urine (16). A G allele of this variation was found in some individuals. The association of UMOD gene promoter polymorphism rs6497476 (-744) with EH has been the subject of a small number of studies prior to 2010. Iwai et al. (2006) looked at this variant in the Japanese population and found that the minor allele was linked to a lower risk for EH, but the link wasn't strong enough to be statistically significant. Another study says that a change in the UMOD gene is connected to a higher estimated glomerular filtration rate (eGFR) and a lower risk of getting chronic kidney disease (CKD). Additionally, studies [17] have linked this polymorphism to a decrease in urinary uromodulin protein excretion.

Dongdong Zeng et al. [18] did a large genome-wide association study and found a link between the rs13333226 minor G allele and a lower risk of high blood pressure, less uromodulin protein in the urine, and better kidney function. Our results are somewhat similar to these results. The study recruited 1,621 cases and 1,699 controls, and after that, 16,541 controls and 19,845 cases underwent follow-up analyses. Additionally, the same group of researchers measured the glomerular filtration rate (eGFR) of 13,446 people. They discovered that rs13333226 was independently associated with hypertension in both the non-adjusted and adjusted versions of the eGFR calculations. They also found that the G allele of rs13333226 was linked to less uromodulin being released in the urine. Finally, they found that these variants can change blood pressure by affecting sodium homeostasis. They suggested that the UMOD locus could be a possible target for antihypertensive drugs to lower the risk of hypertension in heart diseases.

As Finn Grey et al [20] found, the opposite is true. They looked at 1000 people from the Chime population and found that those with the rs13333226 G allele had higher diastolic blood pressure than those with the AA genotype ($p = 0.035$). Based on our findings, individuals of Arab descent from the Babylon province may be at a lower risk of developing essential hypertension if they carry the rs13333226 variant of the UMOD gene [19-26].

Commonly known, hypertension is a disease that seriously endangers health by causing kidney problems and heart disease. Recent studies have drawn particular attention to the uromodulin gene (UMOD) as one of several genetic variables influencing the onset of hypertension. This paper investigates the relationship between uromodulin gene polymorphisms and hypertension, so addressing the consequences of these genetic variations on blood pressure control and possible treatment options [27-30].

Finding people with particular UMOD gene polymorphisms could help to evaluate their likelihood of getting hypertension. In personalized medicine, genetic screening might be a useful tool since it would enable customised treatments depending on a person's genetic makeup. Moreover, focusing on uromodulin pathways could provide fresh treatment options for controlling high blood pressure [31-35].

6. Conclusions and future scope

The results show that, in comparison to control groups, DNA polymorphisms correlate in number of bands with patients. Conventional cross-sectional and retroactive case-control studies have some blame for the discrepancy in the link between genes and hypertension illness. These studies might have missed important changes in statistical analysis and have to consider selection bias. There are notable differences in the gene distribution among ethnic groups, which could help to explain the variations in study studies [36-40].

Investigation of the uromodulin gene polymorphisms will provide a valuable way to explore the pathogenesis of renal and cardiovascular diseases. Further study in this area is needed in order to formulate targeted interventions and improve patient's quality of life.

Wider Genetic Investigations: Further investigations in the field must carry out studies of great samples in terms of GWAS to find further uromodulin polymorphisms and their correlation to different diseases. This might contribute to our understanding of the genetic structure of kidney and cardiovascular diseases.

Functional Studies: Important are the biological mechanisms by which uromodulin polymorphisms alter protein function and disease susceptibility. Animal models and functional tests might shed light on the pathophysiological functions of these variations.

Translating genetic discoveries into clinical practice will require conducting longitudinal studies to evaluate how uromodulin polymorphisms affect treatment response and disease progression over time.

Future clinical trials could investigate the effectiveness of treatments aimed at uromodulin pathways in genetically varied populations. This individualized medicine strategy might maximize therapeutic results.

Combining uromodulin genetic data with other omics technologies—genomics, proteomics, metabolomics—could offer a thorough knowledge of its function in health and disease, therefore opening the path for creative therapeutic ideas [41-55].

Conflict of interest statement
The author declares that she has no conflict of interest in this work.

Data Availability
None.

Funding Source
None.

Authors' Contributions
S.M conceived the idea and wrote the original draft of the manuscript, and the author reviewed and edited the final version.

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