Published online:31/01/2016 Published print:02/2016

ORIGINAL PAPER

doi: 10.5455/medarh.2016.70.31-34
Med Arch. 2016 Feb; 70(1): 31-34
Received: October 25th 2015 | Accepted: December 25th 2015

Renalase Gene rs2576178 Polymorphism in Hemodialysis Patients: Study in Bosnia and Herzegovina

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ABSTRACT

Introduction: Renalase is a protein secreted in kidneys and considered as a blood pressure modulator. High rates of hypertension and its regulation in patients on hemodialysis demands search for potential cause and treatment. The aim of this study was to determine the genotype and allele frequencies of renalase gene rs2576178 polymorphism in population from Bosnia and Herzegovina. Also, the objective of present study was to find the possible association between renalase gene rs2576178 polymorphism and hypertension in patients on hemodialysis. **Material and Methods:** The genotype of renalase gene rs2576178 polymorphism was determined in 137 participants (100 patients on hemodialysis and 37 controls), using polymerase chain reaction (PCR) and subsequent cleavage with *MspI* restriction endonuclease. Genotype and allele frequencies were assessed for Hardy-Weinberg equilibrium using a Chi-squared test. The value of $P<0.05$ was considered as statistically significant. **Results:** Comparison of genotype distribution and allele frequency in participants on hemodialysis with and without hypertension, and healthy control showed no statistical difference. **Conclusion:** The results of the study suggest that renalase gene rs2576178 polymorphism is not a factor that influences blood pressure in patients on hemodialysis.

Key words: renalase gene, hypertension.

1. INTRODUCTION

Renalase was discovered in 2005 by groups of scientists guided by Xu and Desir and proposed to be blood pressure and cardiac function regulators (1). It is believed that Renalase is a hormone, mainly synthesized by kidneys and excreted into the blood, but recently, there were different findings. First report about this flavin adenine dinucleotide (FAD)-dependent protein in different region in Central Nervous System and peripheral nerves was published 2010, revealing new insight in its role (2).

Renalase was classified as a flavoprotein that functions as a FAD/NADH oxidase and metabolizes circulating catecholamines (1). So, it may play the role in the regulation of sympathetic tone and modulates blood pressure and cardiac function. Possible, it regulates disposition of neurotransmitter in the brain.

Renalase gene is located on chromosome 10 (q23,33) and encodes 331 kbp long protein that belongs to flavin adenine dinucleotide dependent amine oxidase.

The gene has 10 exones. There are several isoforms of renalase but the major one contains 342 amino acids with signal peptide, FAD binding domain, and monoamine oxidase domain (3).

There is a single nucleotide polymorphism at the 5' flanking region rs2576178 GG that has been linked to hypertension (4).

Renalase polymorphism	Sequence of primers	Restriction enzymes	AA genotype	GG genotype
rs 2576178	Sense 5'AGTGGCCGTTCAACAGTTAG 3'	MspI	200 bp	160 + 40 bp
	antisense: 5'GTGGGCTATTGTTGGGAGAA3'			

Table 1. Primers sequence and base pairs for renalase polymorphism genotypes

In advanced stages of the chronic kidney disease–end stage renal disease (ESRD), hypertension is presented in more than 80% of the patients, reaching up to 90% in those treated with hemodialysis (5). Hypertension is considered as a major risk for various cardiovascular diseases that are responsible for high mortality rate in patients on hemodialysis. Sympathetic nervous system function is associated with chronic renal failure (6). Besides the reduced catecholamine clearance, increased sympathetic nerve activity may be one of the causes of the increased plasma catecholamine levels evident in ESRD patients.

There isn't clear association between blood pressure values and mortality in ESRD patients, but it is believed that regulation of hypertension should be beneficial to the ESRD patient. Concerning the fact that the etiology of high blood pressure is multifactorial, especially in patients on hemodialysis, we investigated possible role of the renalase gene rs2576178 polymorphism in pathophysiology of hypertension in patients on hemodialysis. Also, objective of study was to determine the genotype and allele frequencies of renalase gene polymorphism in Bosnia and Herzegovina population.

2. PARTICIPANTS AND METHODS

A total of 137 unrelated participants (62 women; 75 men) were enrolled in this cross-sectional study. The rs2576178 polymorphism was genotype in 100 patients on hemodialysis (48 normotensive and 52 hypertensive) and 37 controls (apparently healthy, normotensive individuals). Patients on hemodialysis were recruited from Clinic for Hemodialysis, Clinical Center, Sarajevo, but control participants were healthy volunteers selected mostly from medical staff. The study was approved by Ethic Committee Faculty of Medicine University of Sarajevo and participants gave informed consents. The patients and controls were originated from Bosnia and Herzegovina.

Hypertension was defined as having systolic blood pressure ≥140, diastolic blood pressure ≥90 according to diagnostic criteria (7) or being taking regular antihypertensive therapy.

To obtain genomic DNA for genetic testing we collected buccal cells with two swabs. Participant's mouth was vigorously rubbed on the both sides of the cheek at least six times and swabs were placed inside of envelope. Used cotton swabs and the envelope were sterile. Upon receipt, the buccal swabs were placed and kept on room temperature to dry prior to DNA extraction. Genomic DNA was extracted from buccal swabs using a standard salting out procedure (Miller) (8).

Genotype Determination

Detection of the genetic polymorphism rs 2576178 was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the *MspI* restriction enzyme. For determination of polymorphisms we used a pair of primers designed for the project: sense: 5'AGTGGCCGTTCAACAGTTAG3' and antisense: 5'GTGGGCTATTGTTGGGAGAA3' (Table 1).

The PCR reactions were performed in final volume of 30 µl using 50 ng of genomic DNA, 0.25 M of sense and antisense primer each, 1.5 mM of $MgCl_2$, and 200 µM of dNTP 2 U of Taq polymerase in 10X M PCR buffer, containing 100 mM Tris-HCl (pH 8.3) and 50 mM KCl (*Applied Biosystems, Life technologies,* Thermo Fisher Scientific Inc. 2015). Amplification of DNA was carried out in a Tpersonal Thermocycler (Biometra, Germany).

PCR conditions were as follow: an initial denaturation at 95°C for 1 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s. The PCR was followed by a final step of elongation at 72°C for 7 minutes. The final step of DNA chain elongation lasted 7 minutes at 72 °C.

Obtained PCR product by this procedure was 200 bp. Amplified products were digested with restriction enzyme *MspI* (TAKARA BIO INK, Japan) at temperature of 37°C for 2 hours. After digestion, wild-type AA genotype stays uncut, but fragments with mutant allele were cleaved and showed two bands of 160 and 40 bp (Figure 1). The genotypes were determined after electrophoresis separation on 2.5% agarose gels, staining with ethidium bromide and visualization by using UVItec Gel Documentation System (UVITEC Cambridge, UK).

Statistical analysis

Hardy–Weinberg equilibrium for alleles was tested using the chi-square test.

Genotype distribution and allele frequencies were assessed by a chi-square test of independence with 2 x 2 contingency tables and z-statistics. Statistical significance was defined as $P<0.05$. Statistical calculation was performed with SPSS for Windows (version 19.0. SPSS Chicago, IL).

3. RESULTS

The genotypes of renalase gene polymorphism rs2576178 (5' flanking region) was determined in 137 participants (100 patients on hemodialysis and 37 controls). The mean age of hypertensive and normotensive patients were 53 (ranging from 23 to 65) and 62 (ranging from 18 to 79) years; respectively. Mean age of control group participants was 42 years (ranging from 16 to 61 years).

PCR products digested with *MspI*, were separated and visualized by electrophoresis in 2,5% agarose gel, stained with ethidium bromide. Line 1: uncut PCR product; lanes 3 and 4: AA (homozygous wild-type genotype); lanes 2 and 6: AG heterozygous; lane 5: GG (homozygous mutated); lines M: 50 bp DNA ladder.

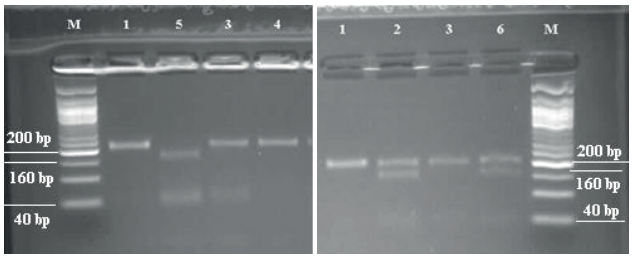

Figure 1. Allelic variants determination of renalase gene rs2576178 polymorphism done by RFLP-PCR.

For the rs2576178 polymorphism, the genotype distribution in all participants of study, was in Hardy-Weinberg Equilibrium (p=0.454). Also, distribution of studied genotypes in groups of normotensive patients on hemodialysis, hypertensive patients on hemodialysis treatment and control were in Hardy-Weinberg Equilibrium (p=0.712; P=0.457; p=0.855, respectively).

Genotyping results for the rs2576178 polymorphism are summarized in Table 2 and 3.

Genotypes rs2576178		Groups	
		N-HD n(%)	H-HD n(%)
AA		14(29)	16(32)
AG		25(52)	28(53)
GG		9(19)	8(15)
Total (n)		48	52
alleles	A	55.0	58.0
	G	45.0	42.0

Table 2. The distribution of genotypes and alleles frequencies of rs2576178 renalase polymorphism in normotensive and hypertensive hemodialysis patients

AA-participants with homozygous wild-type genotype; AG- participants with heterozygous genotype; GG- participants with homozygous; A-wild-type allele; G-mutated allele; N-HD- normotensive patients on hemodialysis treatment; H-HD–hypertensive patients on hemodialysis treatment; n-number of participants; (%)-percentage of genotype or allele in group of participants

Pearson chi-square test; genotype frequencies difference between N-HD and H-HD was at the level p=0.904 and allele frequencies difference was at the level p=0.669.

Genotypes rs2576178		Groups		
		HD n(%)	CG n(%)	Total
AA		30(30)	10(25)	(40)29.2
AG		53(53)	19(53)	(72)52.6
GG		17(17)	8(22)	(25)18.2
alleles	A	56	53	55.48
	G	44	47	44.52

Table 3. The distribution of genotypes and alleles frequencies of rs2576178 renalase polymorphism in patients on hemodialysis and healthy control

AA- participants with homozygous wild-type genotype; AG- participants with heterozygous genotype; GG- participants with homozygous; A-wild-type allele; G-mutated allele; HD- hemodialysis; CG-control group; n-number of participants; (%)-percentage of genotype or allele in group of participants

Genotype and allele frequencies between all groups of participants were not different (p>0.05). Pearson chi-square test; genotype frequencies difference between HD and CG was at the level p=0.814 and allele frequencies difference was at the level p=0.671.

4. DISCUSSION

Hemodialysis patients are at higher risk of developing the high blood pressure. There are many factors contributing hypertension in this population: hypervolemia, increased sympathetic activity, erythropoietin and among them recently studied genetic factors. In the last decade, renalase and its role in blood pressure regulation have been studied extensively.

In this study we analyzed genotype and allele frequencies of renalase gene rs2576178 polymorphism in patients on hemodialysis and control group of healthy individuals in order to find out the possible association between renalase gene rs2576178 polymorphism and blood pressure in HD patients.

Browse the published papers for results of investigating the link between rs2576178 polymorphism in renalase gene and hypertension, showed the opposite findings. In Zhao et al (9) study it has been reported the association of renalase gene rs2576178 polymorphism with hypertension, suggesting that genetic variants in this gene may influence susceptibility to essential hypertension in northern Han Chinese population.

In our study, renalase gene rs2576178 polymorphism between normotensive and hypertensive patients on hemodialysis revealed no significant difference in the GG genotype. Also, there was no difference in frequencies of GG genotype and G allele between HD patients and healthy participants. The frequencies of genotypes and alleles for rs2576178 were slightly different to those observed in Buraczynska et al (10). G allele frequency in our study was for hypertensive, normotensive patients on HD and controls 42%, 45% and 47%, respectively comparing to hypertensive, normotensive type 2 diabetes patients and controls 37%, 40% and 30%, respectively. Along with our results, Buraczynska et al (10) study revealed no association of renalase gene SNP with hypertension in patients with type 2 diabetes.

Also, study of Abdallah et al (11) that included 139 patients on chronic hemodialysis didn't support previous reports of the association of rs2576178 polymorphism and hypertension development in ESRD. Similarly, Fava et al (12) found no association between rs2576178 renalase gene polymorphism and hypertension in a Swedish cohort study. Case-control study of Ahlawat at al (13) revealed no significant difference among groups for genotype distribution and allele frequencies at SNP rs2576178 suggesting no association of renalase gene

rs2576178 polymorphism and hypertension, and chronic kidney diseases.

Different results have been published by Stec et al (14) who had been studied two renalase gene polymorphisms in ESRD patients affected by hypertension. They found higher risk of hypertension in ESRD patients who were carriers of G allele in both rs2576178 and rs10887800 renalase gene polymorphism. It has to be noted that healthy control was not examined in the Stec et al (14) study.

Limitations of this study were small sample size and subjects enrolled from one hemodialysis center. Genetic studies require large population.

5. CONCLUSION

Results couldn't confirm association of renalase rs2576178 gene polymorphism and hypertension in patients on hemodialysis. Also, we observed no association of this renalase gene polymorphisms and renal function in population of Bosnia and Herzegovina.

Probably other genetic factors than renalase rs2576178 gene polymorphism play role in the hypertension in patients with ESRD on replacement therapy by hemodialysis, nevertheless this hypothesis requires further investigations.

- **Acknowledgments:** The study was supported by Federal Ministry of Education, Bosnia and Herzegovina. Project number 05-14-4596-2/12. The authors wish to thank Prof. Lejla Kapur-Pojskic for valuable advice.
- **Author's contribution:** First author, Emina Kiseljakovic gave substantial contribution to conception and design but also for analysis and interpretation of data needed for this manuscript. She also critically revised the article for important intellectual content and performed genetic analysis. Co-author, Mirela Mackic-Djurovic, participated in making a concept, performing genetic analysis and the design of paper, but also did the final review of the paper before publishing. Sabaheta Hasic played role in critical revision of the article for the intellectual content and did the final review before publishing. Amela Beciragic collected the data. Halima Resic collected the data and critical evaluated the article. Amina Valjevac and Lejla Alic drafted the article.
- **Conflict of interest: none declared.**

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