

Variant (rs2576178) of *Renalase* Gene and Its Association with End Stage Renal Disease Patients in Hilla Province

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Abstract

Renalase, a newly discovered flavin-adenine dinucleotide-dependent amine oxidase released by the kidney, circulates in the blood and has an impact on heart function and systemic blood pressure. We conducted the research in Babylon from February to July of 2016. We collected 50 blood samples from patients with end stage renal disease admitted to Merjan Teaching Hospital in Babylon Province, Iraq. In addition to the control group (50 samples) of healthy people aged 11 to 80, all patients and control groups were from the same ethnic group (Arab). The current study's findings revealed the mean variation of renalase level (pg/ml) across patient and control groups. Renalase levels were much higher ($P < 0.05$) in the serum of people with end-stage renal disease (184.21 ± 4.97 pg/ml) than in the healthy group (44.06 ± 7.21 pg/ml). The findings showed that people with end-stage renal failure were more likely to have GG, GA, and AA in the *renalase* (*RNLS*) gene (18%, 20%, and 62%) than people in the control group (19.3%, 22%, and 58.7%). In the Iraqi population, the *Renalase* (rs2576178) G>A mutation was not related with vulnerability to end-stage renal failure.

Keywords: Renalase, rs2576178, Polymorphisms, End stage renal disease, Kidney

Introduction

One flavoprotein enzyme that helps in catecholamine metabolism is renalase, which is another name for monoaminoxidase-C (MAO-C) [1]. With the help of nicotinamide adenine dinucleotide (NADH), superoxide breaks down catecholamines and other chemicals that have similar structures [2]. The renalase enzyme protein exists in a minimum of four different forms. While two of these, h-renalase 1 and 2, retain their original amino acid domains, the third and fourth, h-renalase 2, have shorter domains [3].

Unlike other amine oxidases, such as flavin adenine dinucleotide (FAD), the kidneys primarily produce and then release renalase into the bloodstream. It plays a role in the metabolism of catecholamines in circulation [4]. Pargyline and

clorgyline, two well-known MAO inhibitors, have no effect on renalase activity [5].

Patients with chronic renal disease had decreased renalase concentrations, which may contribute to the onset of hypertension and its associated complications [2]. Researchers who looked at uremia in rats that had their kidneys removed confirmed that people with end-stage renal disease (ESRD) have a significant renalase deficiency [6]. This deficit increases the risk of cardiovascular disease by causing abnormal catecholamine breakdown and undue stress on the sympathetic nervous system [7].

Individuals with renal failure struggle to break down catecholamines, leading to elevated blood concentrations and an overactive sympathetic nervous system due to impaired

renalase function. Guo *et al.* [8]. found that cardiovascular disease risk increases with sympathetic nervous system activity levels that are high. According to Malyszko *et al.* [9], the *renalase* gene (RNLS) is located on chromosome 10 (q23.33), contains 342 amino acids, and has a molecular weight of 37.8 kDa. The gene consists of 311,000 base pairs (bp) and 13 exons. Researchers have found multiple single nucleotide polymorphisms (SNPs) in the *RNLS* gene, indicating its high degree of polymorphism [10]. Most recently, researchers found that three *RNLS* gene SNPs (rs2296545, rs2576178, and rs10887800) were associated with several diseases. The rs2296545 (C/G) mutation is in the second exon of *RNLS*. It changes the Asp37Glu amino-acid substitution, which in turn changes how this gene works [11]. Intron 6 contains the polymorphism rs10887800, while the 5-flanking region contains the polymorphism rs2576178 (G/A). These SNPs may affect the regulation and gene expression of *RNLS* [12].

The aim of this study was to investigate the potential relationship between two polymorphisms in the renalase gene and hypertension in patients with ESRD.

Methods

Sampling

Between February 2016 and July 2016, researchers from the University of Babylon's Biotechnology and Genetic Engineering Laboratory, Department of Biology, College of Science, conducted a case-control study. Blood samples from patients undergoing dialysis at Iraq's Merjan Teaching Hospital in Babylon Province was collected.

This experiment made use of one hundred blood samples. We studied people with ESRD and 50 healthy individuals. The Merjan Teaching Hospital Dialysis Center had patients hospitalized for periods spanning eleven years to eighty years. Conversely, they matched the chronic kidney disease (CKD) and ESRD groups. A licensed physician examined each subject. People with hepatitis were not included.

Renalase quantity assay by ELISA

Elascience, a Chinese company, supplied the specific kit (ELISA) that was used to measure the level of human renalase.

DNA extraction

Using the Favergen Extraction and Purification Kit (Taiwan), genomic DNA was extracted and purified from whole blood cells.

Genotypic identification using RFLP- PCR amplification

We amplified the following DNA regions with specific primers. We detected polymorphisms in the *renalase* gene

using one primer (rs2576178) from Bioneer, IDTDNA (USA). Both the forward and reverse primer sequences were 5-AGCAGAGAAGCTTAACCT-3 and 5-TTATTGCAAGTCGTAAC-3, respectively. For the polymerase chain reaction (PCR), we made the reaction volume with 20 microlitres of water, 1 microlitre of each of the reverse and forward primers, 12.5 microlitres of Green Master Mix, 3 microlitres of genomic DNA, and 2.5 microlitres of nuclease-free water. We used thermocycling to achieve amplification, which involved two minutes at 94°C, thirty-five cycles of five minutes at 94°C, one minute at 60°C, and one minute at 72°C, followed by a final expansion of five minutes. After electrophoresing the PCR products in a 1% agarose gel at 75 V for 1 hour, we observed them using ethidium bromide. We captured the photos using the Gel documentation framework. We performed the PCR-RFLP technique, following the Promega Company Protocol, after cutting the PCR product using *MSPI* restriction endonuclease. After *MSPI* digestion, we subjected the reaction to electrophoresis on 3% agarose gels at 75 V for 1 hour and set up a control at 75 V, 20 Am for 160 minutes. We subsequently stained the gels with ethidium bromide. We captured images using the gel documentation system (EBOXCX, UK).

Statistical analysis

All statistical analysis were done using the SPSS applied mathematics software system (25; SPSS Inc., Chicago , IL), and P- values of <0.05 were considered statistically significant.

Results

Serum renalase concentration in both genders of the study groups

Table 1 displays the renalase levels (ng/ml) for both the patient and control groups. In contrast to the healthy control group, whose serum renalase levels were 44.06 ± 7.21 ng/ml, the ESRD group's levels were significantly higher (184.21 ± 4.97 ng/ml) ($P < 0.05$).

Genetic polymorphisms of renalase gene associated with renal diseases

The genomic DNA (**Figure 1**) was extracted from the blood samples as a first step to amplify the target region of *RNAL* gene.

Figure 3 shows the results of cleaving the *RNLS* target sequence PCR products with the *MspI* (5' CCG 3') restriction enzyme, which allowed us to identify the rs2576178 SNP in the 5'-flanking region of the *RNLS* gene. **Table 2** shows the data of the ESRD patient group, *RNAL* polymorphism frequencies were 62% and 18%, according to the PCR-RFLP findings compared to the healthy control group that possessed all three polymorphic alleles at rates of 19.3%, 58.7%, and 22%, respectively.

Table 1. Serum renalase levels in both study groups.			
Groups	ESRD	Control	P=
Renalase Level (ng/ml)	184.21 ± 4.97	44.06 ± .21	0.001*
*P ≤ 0.05; SE: Standard Error; ESRD: End Stage Renal Disease			

Table 2. Genotype distribution and odd ratio of <i>RNAL</i> gene polymorphisms between the patients and healthy control.				
Genotype	ESRD No. (%)	Control No. (%)	Sig.	OR (95%) ESRD
AA ^a	30 (62)	27 (58.7%)	1.00	
AG	12 (20)	11 (22%)	0.58	1.01 (0.38-2.68)
GG	8 (18)	12 (19.3%)	0.23	1.66 (0.50-4.69)
Total number	50	50		
A allele	72	65	0.28	1.09(0.60-1.97)
G allele	28	35		
^a reference; *P ≤ 0.05; OR= (95%CI); CKD: Chronic kidney disease; ESRD: End- stage renal disease				



Figure 1. Genomic DNA electrophoresis pattern from renal patients and healthy control groups. The genomic DNA from blood samples (1-10 patients and 11-20 controls) is represented by lanes 1–20. The electrophoresis settings were 1% agarose, 75 V, 20 mA for 1 hour (10 µl in each well), and ethidium bromide was used for staining in the experiment.

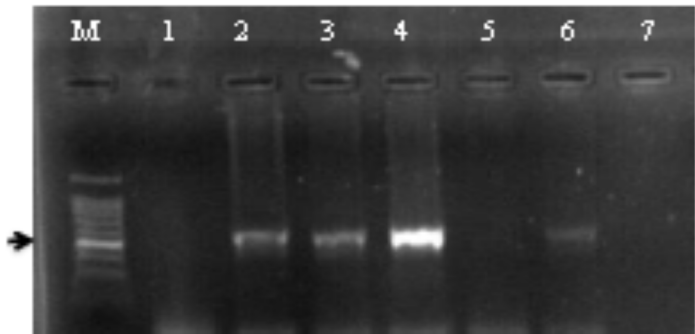


Figure 2. RNLS amplified product patterns of renal patients and healthy control groups as determined by agarose gel electrophoresis. The amplified products, which were a single band measuring 525 bp in size, are shown in Lanes 2-4 and 6, with M: standing for DNA size marker. The negative controls are located in Lanes 1 and 7. The conditions for electrophoresis and Ethidium bromide staining followed a 120-minute run at 75 V and 20 mA with a 1% agarose concentration.

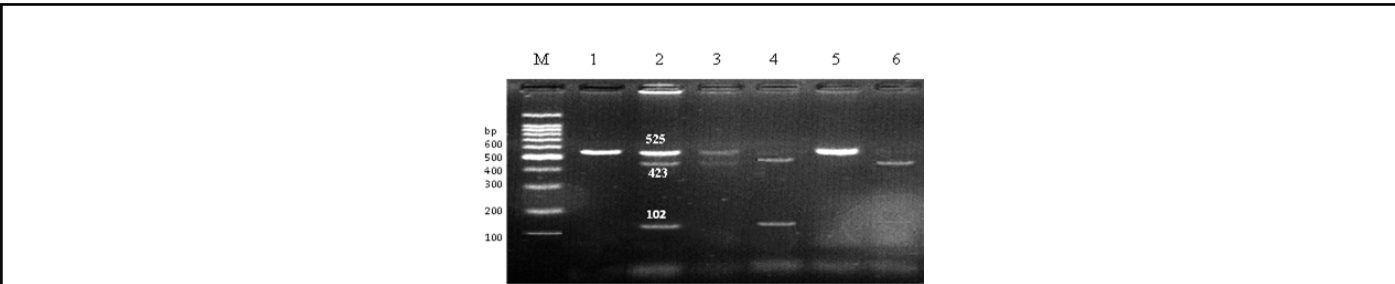


Figure 3. Electrophoresis patterns of allelotyping of the 5'-flanking region of the *RNLS* gene employing the *MspI* enzyme by PCR-RFLP technique in renal patients and healthy control groups. The DNA ladder ranges from 100 to 1100 base pairs. The first two lanes show a single band with a molecular size of 525 base pairs, while the second pair shows three bands with sizes of 525, 423, and 102 base pairs. The third, fourth, and sixth lanes show two bands with sizes of 423 and 102 base pairs, while the last pair represents a homozygous allele (GG).

Table 3. Mean differences in renalase levels associated with rs2576178 genotype frequencies.			
Variable	(Mean ± SD)	Genotype	LSD
ESRD	134.97 ± 16.81	GG	1.059
	100.88 ± 11.10	AA	
	84.06 ± 15.65	GA	
Control	27.40 ± 4.27	GG	2.76
	38.00 ± 6.59	AA	
	29.80 ± 3.08	GA	

ESRD: End Stage Renal Disease; SD: Standard Deviation; *P* ≤ 0.05.

The digested products migrated into 3% agarose, 75 V, 20 mA for 120 min; 10 µl in each well; stained with ethidium bromide.

Discussion

The importance of renalase in regulating blood pressure calls for further investigation into the causes and potential treatments for hypertension, which is increasingly common among hemodialysis patients [13]. There is an SNP called rs2576178 that is located in the 5'-flanking regions of the *RNLS* gene. It replaces a single nucleotide with another, potentially influencing the regulation and expression of the *RNLS* gene [12].

It is already known that changes in the *renalase* gene (rs2576178 SNP) are connected to high blood pressure, heart disease, stroke, ESRD, coronary cardiopathy, and naturally occurring high blood pressure [14]. Patients with end-stage renal illness had a frequency of 0.32 for the rs2576178 SNP minor, G allele, while the healthy control group had a frequency of 0.34. These investigations' findings have been inconclusive for the patient and control groups. The frequency of the minor G allele was 0.34 in the dbSNP database. In 2011, Gambaro *et al.* [15] discovered that 0.35 of Egyptians with a parathyroid gland had the minor G allele frequency for the rs2576178 SNP. This was a little different from what we found. Li *et al.*

[16] discovered that systolic and diastolic blood pressure values differed significantly depending on genotype, and that individuals with parathyroid glands had a significantly higher GG genotype of the rs2576178 SNP [17,18].

When comparing ESRD patients to healthy controls, the present study did not find a statistically significant correlation (*P* 0.05) between blood renalase levels and rs2576178 genotype frequencies. This could mean that the GG genotype (rs2576178 SNP) in the 5'-flanking region of the *RNLS* gene, which was found to have a big effect on gene expression before, either doesn't have much of an effect on renalase expression or doesn't have any effect at all. Other single-nucleotide polymorphisms (SNPs) in the *RNLS* gene, namely rs10887800 and rs2296545, are usually linked to high blood pressure and may help explain why patients with kidney disease have higher serum renalase levels. While we did test serum renalase levels, we did not measure enzyme activity in our experiment. According to our results, high levels of renalase in the blood of people with renal failure—most of whom also had high blood pressure—may be because the enzyme cannot break down the catecholamines (epinephrine and noradrenaline) that it needs as substrates. This, in turn, raises blood pressure. Thus, it is necessary to assess the level and activity of serum renalase in patients with renal insufficiency and hypertension.

When the rs2576178 SNP in the 5'-flanking region of the *RNLS* gene was tested on people with end-stage renal failure who were on dialysis, there were no big differences at the GG genotype. Consistent with earlier studies, this study did not detect a difference in the frequency of the G allele or the GG genotype between HD patients and contributors [19]. A highly Swedish population review also failed to find a correlation between upset and the SNP rs2576178 [20]. Additionally, Ahlawat *et al.* [21] found no link between the *RNLS* gene (rs2576178) polymorphism and the bothersome, long-lasting renal failure illness, based on genotype delivery and gene frequencies at SNP rs2576178.

Lastly, renal disorders are complex diseases involving multiple factors. These factors include inflammation, nutrition, environmental factors, and a large number of genes that are involved in renal functions or have risk variants that could worsen the disease.

Conflicts of Interest

The author states that they have no conflicts of interest.

Data Availability

None.

Funding Source

None.

Ethics Approval

Ethical clearance was granted for the study by the Babylon Health Directorate in Babil, Iraq, with reference number 246. Research conducted in accordance with the Declaration of Helsinki standards.

Authors' Contributions

Zahraa Isam Jameel and her colleagues came up with the concept and wrote, reviewed, and edited the final version.

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