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The Renalase (rs2296545) Polymorphism is Associated with End-Stage Renal Disease

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Abstract**—***Renalase is a recently identified enzyme that is dependent on flavin-adenine dinucleotide and acts as an amine oxidase. It is secreted by the kidney. It travels through the bloodstream, where it impacts the functioning of the heart and the overall blood pressure in the body. The study was conducted in Babylon between February and July 2023. (50) Blood samples were obtained from patients who were admitted to the Merjan Teaching Hospital in Babylon Province, Iraq, and were diagnosed with severe renal illness. The control group comprised 50 samples of healthy individuals spanning an age range from 11 to 80 years. The present investigation observed that the average fluctuation in renalase level (expressed in picograms per millilitre) was similar in both the patient and control groups. The serum of end-stage renal disease groups exhibited significantly elevated levels of renalase (P<0.05) compared to the healthy participant group (170.21 ± 4.97 pg/ml vs. 40.06 ± 7.21 pg/ml). The findings revealed that the frequencies of CC, CG, and GG genotypes of the renalase (RNLS) gene in individuals with end-stage renal failure were 19.6%, 39.3%, and 41%, respectively, whereas in the control group, the frequencies were 53%, 25.6%, and 21.4%. Conclusion: The presence of the Renalase mutation (rs2296545) C>G mutation in the Iraqi population was found to be linked to an increased susceptibility to end-stage renal failure.*

Keywords**—** *Renalase, rs2576178, polymorphisms, ESRD, Kidney*.

I. INTRODUCTION

Renalase, sometimes referred to as monoamine oxidase C (MAO-C), is a flavoprotein enzyme that plays a role in the breakdown of catecholamines [1-3]. The study conducted by Desir et al. [4] describes a technique that involves the breakdown of catecholamines and similar chemicals using nicotinamide adenine dinucleotide (NADH) as a cofactor. The degradation mechanism is dependent on the presence of superoxide. There exist a minimum of four isoforms of the renalase enzyme protein. Among these, two (h-renalase 1 and 2) possess amino acid domains that are conserved, but the other two have truncated domains (h-renalase 2). [5].

Meanwhile, renalase is rathe related with the kidneys than the other amine oxidases that use FAD. Thus, it is directly secreted into the bloodstream instead of the urine. It leads to the impairment of circulating catecholamine [(catecholamines) that are released after exercise] pool. [6]. One of the merits of renalaase is that it is insensitive to of the generally known MAO inhibitors such as pargyline and clorgyline [7].

Desir et al. in their study [6] found ir that the patients suffering f om chronic renal diseases have badly incorpo raed the red neckase. This implies that renalase (which Q may mean) might be a factor that contributes to the causes of hypertension and furthermore the impact that hypertension has on the health of a person. In a group of rats with partial or total (nephrectomized) kidney removals, the administration of creatinine, and addition of other chemicals can induce kidney disease with all-time low renal activity [7]. Therefore, this defect arises from disturbed enzymatic uncoupling of cathecholamines, which overstimulates the peripheral sympathetic system in a non-physiological manner. This exhaustive activation correlates with a more risk to life-threatening diseases [8].

The deficiency of kidney in persons with kidney failure leads to the decrease in catecholamines breakdown contributing to the increase of these substances in blood and ultimately triggering constant excitation of the sympathetic nervous system. And so, it gives rise to this stress situation. Enhancement of the sympathetic nervous system activity is associated to the heightened vulnerability to the cardiovascular diseases. Sincerely, help develop the given sentence.[9-11]

Renalase gene (RNLS) is a comprised of 311,000 bp of DNA sequence within chromosome 10 q23.33 and contains 13 exons. This protein has a sequence of 342 amino acids, a molecular weight of 37.8 kilodallatons based on the data presented by Malyszko et al. in [12]. The RNLS gene is quite variable because of the different single nucleotide polymorphisms (SNPs) that have been found out other than those along the same kind of gene that has already been investigated ([13]). A number of recent studies have shown a relationship between particular single nucleotide polymorphisms (SNP) carrying alleles in the RNLS gene, for example rs2296545, rs2576178, and rs10887800, and a collection of different diseases. The rs2296545 variant, a C to G conversion with a G allele percentage of 87%, is an example of a single-nucleotide change found in the second exon of the RNLS gene. As a result of the amino acid substitution of aspartate (Asp37) and glutamate (Glu) the normal function of the gene is affected leading to translation of abnormal proteins [14].

The identity, rs2576178, is located in the 5'-flanking region, whereas the identity, rs10887800, is in intron 6. This genetic diversity can influence the binding of RNLS [15] to their targets and therefore their regulation and expression.

Zahraa A. Al-Ajeeli and Hameed H. Hameed, "The Renalase (rs2296545) polymorphism is associated with End-Stage Renal Disease," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 7, Issue 4, pp. 16-20, 2024.

We set out to evaluate if the prerenalase variants affecting the renalase gene museum might impact blood pressure in ESRD patients.

II. METHODS

Sampling

A retrospective case-control study was conducted from February 2023 to July 2023. Furthermore, blood samples were collected from patients at the Dialysis Centre located at Merjan Teaching Hospital in Babylon Province, Iraq.

A total of 100 blood samples were utilised in this investigation. Patients diagnosed with end-stage renal disease (ESRD) and a control group of 50 individuals underwent blood sample collection. Individuals ranging in age from 11 to 80 years were hospitalised to the Merjan Teaching Hospital Dialysis Centre. Each participant got a comprehensive assessment conducted by a qualified medical professional. Exclusion criteria encompassed patients diagnosed with hepatitis.

Renalase Quantity Assay by ELISA

The quantity of human renalase was determined using a particular kit (ELISA) provided by Elascience, a Chinese business.

DNA extraction

Genomic DNA was extracted and purified from whole blood cells using the Favergen Extraction and Purification Kit (Taiwan).

Genotypic Identification Using RFLP- PCR Amplification

Specific primers were used to amplify the specified DNA regions: The author designed a primer (rs2296545) for the purpose of detecting genetic variations in the Renaelse gene. The primer was obtained from Bioneer, IDTDNA (USA). The forward primer sequence was 5'-CAGTCCTCTTTTCCCAGGTTTTG-3', and the reverse primer sequence was 5'-CCCGCCGCAGAGACTCA-3'. The PCR procedure was conducted using a reaction volume of 20 microliters, comprising 1 microliter of both reverse and forward primers, 12.5 microliters of Green Master Mix, and 3 microliters of genomic DNA. In order to increase the total volume of the reaction to 20 litres, 2.5 microliters of water that is free from nuclease were introduced. The amplification procedure was conducted in a thermo cycler for a length of two minutes at a temperature of 94 °C. Subsequently, a series of 30 cycles, each lasting 5 minutes, were conducted with the following temperature conditions: an instantaneous temperature of 94 °C, an instantaneous temperature of 62 °C, and a temperature of 72 °C for one minute. Lastly, there was a concluding extension phase that ran for a duration of five minutes. The PCR findings underwent electrophoresis on a 1% agarose gel at a voltage of 75 volts for a duration of 1 hour. The products were visualised using ethidium bromide. The photos were acquired with the gel documentation system.

Statistical analysis

The SPSS applied mathematics software system (version 25; SPSS Inc., Chicago, IL) was utilised for all mathematical analyses. Statistical significance was determined by considering P values less than 0.05.

III. RESULTS

Serum renalase concentration in both study groups

The renalase concentration, quantified in ng/ml, was presented in Table (1) for both the patient and control cohorts. The renalase concentration in the blood serum of the group with end-stage renal disease (ESRD) was substantially higher ($P < 0.05$) compared to the healthy control group. The blood samples from persons with end-stage renal disease (ESRD) had a renalase concentration of 170.21 ± 4.97 ng/ml, while the control group of healthy individuals had a renalase value of 40.06 ± 7.21 ng/ml.

TABLE 1. displays the serum renalase levels for both genders in the study groups.

Group	Mean± SE		
	ESRD	Control	P=value
Renalase Level (ng/ml)	170.21 ± 4.97	40.06 ± .21	0.001*

*P ≤ 0.05; SE: Standard error; ESRD: End- stage renal disease

Genetic polymorphisms of the renalase gene associated with renal diseases

The genomic DNA (Fig.1) was obtained from the blood samples as an initial step in amplifying the specific area of the RNAL gene.

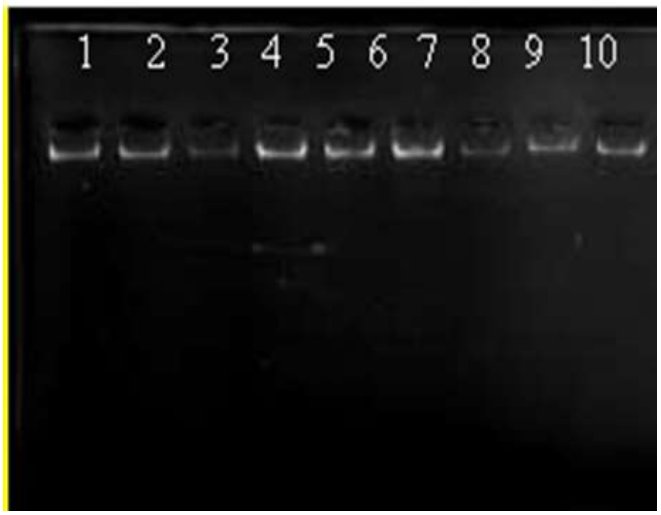

Fig. 1. displays the electrophoresis pattern of genomic DNA obtained from blood samples of renal patients and healthy control groups.

Lane 1 to Lane 10 represent genomic DNA extracted from blood samples of 10 patients and a control group. The electrophoresis was performed using 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.

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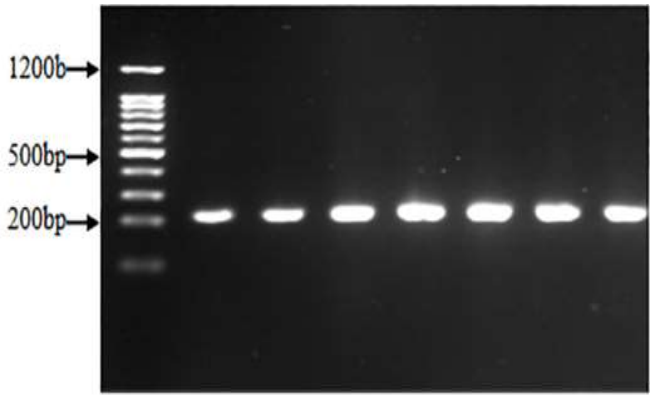

Fig. 2. Agarose gel electrophoresis of amplified *RNLS* amplified product patterns from renal patients and healthy control groups

The amplified products were a single band of 200 bp in size; Electrophoresis conditions: 1% agarose concentration, 75 V, 20 mA for 120 min, stained with ethidium bromide

Among patients with end-stage renal disease (ESRD), the occurrence of homozygous alleles CC and CG for the renalase polymorphism was 19.6% and 39.3%, respectively. The GG variant had a frequency of 41%. In comparison to the control group of individuals without health issues, the frequencies of the polymorphic alleles CC, GG, and CG were 53%, 21.4%, and 25.6% respectively, as indicated in Table (2).

Genotype	ESRD No. (%)	Control No.(%)	*P*pvsalue	OR (95%)
CC [a]	10 (19.6%)	27 (53%)		
CG	22 (39.3%)	11 (25.6%)	0.001	5.40 (1.93-15.04)
GG	18 (41%)	12 (21.4%)	0.004	4.41 (1.55-12.54)
Total	50	50		
C allele	42	65		
G allele	58	35	0.001	2.56 (1.44-4.54)

*P<0.05: significant

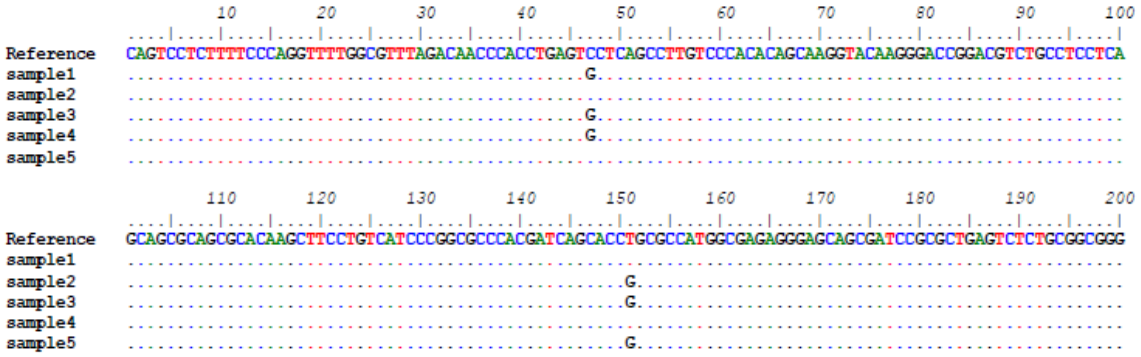

Fig. (3): Sequences alignment ID: NC_000010.11 results for Homo sapiens, *renalase* gene polymorphisms by the Bio Edit program version 7.2.5.

TABLE 3: Mean differences in Renalase levels and associated with *rs2296545* genotype frequencies

Variable	(Mean± SD)	Genotype	LSD
ESRD	134.97±16.81	CC	1.059
	100.88±11.10	CG	
	84.06±15.65	GG	
Control	27.40±4.27	CC	2.76
	38.00±6.59	CG	
	29.80±3.08	GG	

ESRD: End- stage renal disease; SD: Standard, deviation; *P* <0.05.

IV. DISCUSSION

The role of renalase in keeping the systolic pressure low to normal is substantial. A notable portion of hemodialysis patients suffer from hypertension, and therefore, it is undoubtedly necessary to establish what causes it and seek for the ways to solve this issue. The SNP rs2296545 is in the IVNL gene and a C to A single nucleotide polymorphism (SNP) is substituted. This variant (SNP) of the combination of ATC codon of the RNLS gene has the ability to change the nature of the expresion of this gene, [17].

Genetic variations or polymorphisms in human genes, especially the rs2296545 single-nucleotide polymorphism (SNP), turn out to be significant in such health conditions as hypertension, stroke, ESRD and coronary artery disease (CAD). On the other hand, the subsequent research showed that polymorphisms of such genes are involved in a course of various genetic diseases including hypertension [18].

So, the exploration showed the frequency of the lesser letter G of the rs2576178 is 42 in patients with ESRD and 65 in healthy populations. A multitude of studies have shown that there is no significant difference between the two-group patients and controls, in this case depending on their weight loss strategy. The information in the dbSNP database shows the frequency of a G minor allele, this being 0.34, as indicated by Gambaro et al. [19]. Followed by that, sequencing data has shown even more similarity but also comparatively less to the database of single nucleotide polymorphisms. What is interesting here is the particular SNP rs2296545 which has a minor G allele frequency of 0.35 among Egyptian patients with parathyroid gland. Presenting impartial accounts, an investigation disclosed that the prevalence of the GG genotype by SNP rs2296545 increased in a group of people suffering from the parathyroid problem. In addition, a significant difference was observed in the systolic and diastolic blood pressure from the person to person basis and more identified with the genotype. The citation if from the article by Li et al is. [20].

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There was demonstrated this fact that a statistically significant correlation (R = 0.95) between renalase serum levels and rs2296545 genotype frequencies was present in the patients with ESRD when their healthy counterparts have been compared. This result implies that the genotype GG represents the risk factor (rs2296545 SNP) that could significantly affect renalase expression and therefore renalasa gene expression would be influenced. Blood renalase concentrations in individuals having renal disease could be the results of ongoing mutations in RNLS gene, together include rs10887800 and rs2576178 SNPS, which are normally associated with hypertension. The experiments were done already but the data was obtained for the serum levels of renalase and now this data will be clearly compared with the serum levels of other enzyme activities. The abuse of serum renalase in chronic renal failure patients whose primary pathogenic agent is high blood pressure has been found to be extremely high as a result of the failure to carry out its pre defined functions. During the malfunctioning stage, it may cause energy sustenance through reductions of catalytic activity, or inactivity in processing and the breaking down of its substrates such as catecholamines. Examples are adrenaline (epinephrin) and noradrenaline (norepinephrin) in the bloodstream. Therefore, it can be concluded that the pressure of the heart will be higher than usual. Therefore, among the patients with kidney failure and hypertension, as good management strategies, is the measurement of serum renalase level and activity. [21-26].

Renal disorders are complex diseases caused by multiple variables, including various genes that affect kidney function. Genetic risk variants can worsen the disease, along with other factors like inflammation, nutrition, and environmental conditions [27-29].

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