Renalase gene polymorphisms in end-stage renal disease patients: An Egyptian study

El-Sayed Abdallah[1], Dina Sabry[2]

Departments of [1]Internal Medicine, and [2]Medical Biochemistry, Cairo University
drsayed@gmail.com

Abstract: Background: Renalase is a novel protein produced by the kidneys, participating in the metabolism of circulating catecholamines. Patients with end-stage renal disease have a significant renalase deficiency, which could be a pathophysiologic mechanism partially explaining high prevalence of hypertension in patients with end-stage renal disease. The aim of the current study is to assess possible relationship of renalase gene polymorphism with hypertension in patients with end-stage renal disease. **Patients and methods**: 139 patients on chronic hemodialysis were recruited in this study. RS2576178 and RS10887800 was genotyped using polymerase chain reaction. Patients were subgrouped into hypertensive and non-hypertensive patients. Allele and genotype frequencies were compared in both groups. In addition fifty healthy subjects were enrolled as a control group and subjected to the same genotyping.**Results**: Analyzing genotype and allele frequency of RS2576178 renalase polymorphism revealed that the difference between both patient groups was not statistically significant. The slightly higher prevalence of G allele in hypertensive group was also not statistically significant.Analyzing genotype and allele frequency of R10887800 renalase polymorphism revealed no difference in frequency of GG genotype. The prevalence of G allele was similar in both groups. Analyzing allele frequency of RS2576178 showed a higher frequency of G allele in ESRD group compared to healthy control. Carrier state of G allele was associated with 7.2 times higher risk of developing ESRD (OR=7.188; 95% CI: 3.5-14.7). Analyzing allele frequency of RS10887800 showed a higher prevalence of G allele in ESRD group compared to healthy control. Carrier state of G allele was associated with 12.3 times higher risk of developing ESRD (OR=12.3; 95% CI: 5.6-27.1).**Conclusion**: Unlike previous reports of association of RS2576178 and RS10887800 polymorphisms with developing hypertension in patients with ESRD, the present study does not support a significant association in our population. We rather suggest an association of these polymorphisms with end stage renal disease.

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1. Introduction

Renalase is a novel soluble monoamine oxidase that regulates cardiac function and blood pressure, first described by Xu *et al.,* in 2005 [1]. It consists of a secretory signal peptide, a flavin adenine dinucleotide (FAD) binding region and an amine oxindase domain and is present in at least 4 isoforms (h-renalase 1 to h-renalase 4) [2]. Renalase specifically degrades circulating catecholamines. Excess catecholamines have been shown to facilitate the conversion of prorenalase (an inactive form) to renalase. Excess catecholamines also promote the synthesis and secretion of prorenalase [3]. Xu *et al.,* also have shown that renalase was virtually undetectable in blood of patients with end stage renal disease [1].

The human renalase gene resides on chromosome 10, contains 9 exons and spans about 311 Kb [4]. Many polymorphisms of this gene has been reported in association with different diseases related to dysfunction of renalase and hence catecholamine metabolism disturbance. Farzaneh-Far *et al.,* reported association of functional polymorphism in renalase (Glu37Asp) with cardiac hypertrophy, dysfunction and ischemia [5]. Buraczynska *et al.,* reported association of renalase gene polymorphism with hypertension in type 2 diabetes patients. They also reported a strong association of rs10887800 polymorphism with stroke in patients with and without diabetes [6]. Insufficiency of renalase has been reported in patients with end stage renal disease and might be involved in developing hypertension among these patients. An association between two renalase polymorphisms and hypertension in patients with end stage renal disease has recently been suggested [7]. The aim of this study is to investigate the role of two renalase polymorphisms in development of hypertension in patients with end stage renal disease.

2.Patients and methods:

In this study we included 139 patients attending hemodialysis unit of Cairo University hospitals. They had end stage renal disease and were on regular hemodialysis. After approval of the research ethics committee and obtaining ann informed consent, the patients were subjected to full medical history and clinical examination. Hypertension was defined as having systolic blood pressure > 140, diastolic blood pressure > 90 according to current diagnostic criteria

[8] or being known to be hypertensive and taking regular antihypertensive therapy. Based on this patients were divided into two groups, group I hypertensive patients and group II non-hypertensive patients. In addition to the patients' groups 50 volunteer healthy subjects who were normotensive were included as a control group.

In both patients groups as well as the control group renalase gene polymorphism was determined by polymerase chain reaction. Genomic DNA was extracted from peripheral blood leucocytes. Template DNA was then amplified by using two pairs of oligonucleotide primers (Table 1) for detection of renalase gene polymorphism, 10 pmol of each primer. The PCR mixture contained 20 ng of genomic DNA, 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.4, 5% dimethyl-sulphoxide (DMSO), each of 0,5 mM deoxyribonucleoside triphosphate (dNTPs) and 1 unit of Taq polymerase (Pharmacia, Uppsala, Sweden) in a final volume of 50 µL. Amplification was performed by denaturation denaturation at 94^0C for 1 min, annealing (nucleotide primer attachment) at 58^0C for 1 min, and extension (DNA elongation) at 72^0C for 2 min as many as 30 cycles followed by a final extension at 72^0C for 4 min.

The instrument utilized in the study was PCR Thermal (Biometra, Germany). Results of PCR were separated by electrophoresis on a 2% agarosa gel, which had been enriched with ethidium bromide (0.1%), then visualized by ultraviolet light and were documented by using the gel doc (Biometra, Germany). This was followed by restriction fragment length polymorphism, PCR products were digested with the appropriate restrictive endonuclease at a temperature of 37°C for 6–10 hrs. The reaction products were separated by electrophoresis in 1.5–2.5% agarose gel.

Statistical analysis:

Data are represented as mean ± standard deviation. Genotype distribution and allele frequency were analyzed using chi-square test of independence. The risk for developing hypertension in presence of an allele was assessed by calculating the odds ratio with 95% confidence interval. Statistical significance was considered if p value < 0.05.

Table (1): Primers for detection of polymerase

Renalase polymorphism	Sequence of primers	Type of restriction	AA genotype	GG genotype
rs2576178	sense: 5'-AGCAGAGAAGCAGCTTAACCT-3'	Msp I	525 bp	423+102 bp
	antisense:5'-TATCTGCAAGTCAGCGTAAC-3'			
rs10887800	sense: 5'-CAGGAAAGAAAGAGTTGACAT-3'	Pst I	554 bp	415+139 bp
	antisense: 5'-AAGTTGTTCCAGCTACTGT-3'			

3.Results

The study included 139 patients with end stage renal disease, 99 of them were hypertensive (group I) and 40 were non hypertensive (group II). Group I included 50 males and 49 females, while group II included 17 males and 23 females (p-value 0.39).

Serum creatinine was 3.8±0.84 in all patients, 3.8±0.76 in group I and 3.8±0.88 in group II.

Genotype and allele distribution of rs2576178 renalase gene polymorphism is summarized in Table 2.

Table 2: genotype distribution and allele frequency of rs2576178

Genotype	GG	GA	AA	Total		Allele	G	A	Total
Hypertensive	55	30	14	99		Hypertensive	140	58	198
Normotensive	24	6	10	40		Normotensive	54	26	80
Total	79	36	24	139		Total	194	84	278

Comparison of rs2576178 polymorphism between hypertesive and normotensive patients with end stage renal disease (ESRD) revealed no statistically significant difference in the GG genotype distribution between hypertensive and normotensive groups (p-value 0.63). The prevalence of G allele was slightly higher in the hypertensive group (0.71 vs 0.68) yet this was statistically insignificant (p-value 0.29). Carrier state of G allele was associated with 2.02 times higher risk of developing hypertension, yet this was statistically insignificant (OR=2.02; 95% CI: 0.81-5.04).

Genotype and allele distribution of rs10887800 renalase gene polymorphism is summarized in table 3.

Table 3: genotype distribution and allele frequency of rs10887800

Genotype	GG	GA	AA	Total		Allele	G	A	total
Hypertensive	28	60	11	99		Hypertensive	116	82	198
Normotensive	8	29	3	40		Normotensive	45	35	80
Total	36	89	14	139		Total	161	117	278

Comparison of rs10887800 polymorphism between hypertensive and normotensive patients with ESRD revealed no statistically significant difference in the GG genotype distribution between both groups (*p*-value 0.31). The prevalence of G allele was not different between both patient groups (0.59 in hypertensive group vs 0.56 in normotensive group; *p*-value 0.36). Carrier state of G allele was associated with 0.65 times higher risk of developing hypertension, again this was statistically insignificant (OR=0.65; 95% CI: 0.17-2.46).

Distribution of renalase gene polymorphisms in the control group is summarized in table 4.

Table 4: Renalase gene polymorphism in control group

Genotype	GG	GA	AA
rs2576178	8	12	30
rs10887800	6	15	29

Comparison of rs2576178 polymorphism between patients with ESRD and healthy control group revealed a statistically significant higher frequency of GG genotype in the ESRD patients group (56% vs 16% in control group; *p*-value < 0.01). The prevalence of G allele was significantly higher in ESRD group compared to healthy control (0.69 vs 0.28; *p*-value < 0.01). Carrier state of G allele was associated with 7.2 times higher risk of developing ESRD, which was statistically significant (OR=7.188; 95% CI: 3.5-14.7).

Comparison of rs10887800 polymorphism between patients with ESRD and healthy control group revealed a statistically significant higher frequency of GG genotype in the ESRD patients group (26% vs 12% in control group; *p*-value 0.04). The prevalence of G allele was significantly higher in ESRD group compared to healthy control (0.58 vs 0.27; *p*-value < 0.01). Carrier state of G allele was associated with 12.3 times higher risk of developing ESRD, which was statistically significant (OR=12.3; 95% CI: 5.6-27.1).

4.Discussion

Renalase has been the focus of many studies identifying susceptibility to diseases. In 2007 Zhao *et al.,* [9]assessed correlations of single nucleotide polymorphisms (SNPs) of the renalase gene with primary hypertension. His study revealed association of essential hypertension with rs2576178 and rs2296545 renalase gene polymorphisms. In 2011 Stec *et al.,*[7] studied two renalse gene polymorphisms in ESRD patients affected by hypertension. They established an increased risk of hypertension in ESRD patients who were carriers of G allele in both rs2576178 and rs10887800 renalase gene polymorphism. In the study presented here we found no statistically significant difference in G allele frequency between hypertensive and normotensive ESRD patients, nor did we find a significant increase in risk of developing hypertension in G allele carriers for both rs2576178 and rs10887800 renalase gene polymorphism. Interpopulation genetic differences might acount for the different results in the present study from the aforementioned studies (one was conducted in Asian population and the other one in Polish population). Similarly Fava *et al.,* [10] found no association between rs2576178 renalase gene polymorphism and hypertension in a Swedish urban-based cohort.

In the study conducted by Stec *et al.,*[7], no healthy control group was examined. In the present study we enrolled fifty healthy subjects as a control group. We found a significant difference in distribution of G allele between healthy controls and patients with ESRD for both rs2576178 and rs10887800 renalse gene polymorphisms. We found that the carrier state of G allele in rs2576178 and rs10887800 renalase gene polymorphisms is associated with 7.2 and 12.3 times higher risk of developing end stage renal disease.

In conclusion according to our results and unlike previous reports of association of RS2576178 and RS10887800 polymorphisms with developing hypertension in patients with ESRD, the present study does not support a significant association in our population. We do suggest an association between these renalase gene polymorphisms and end stage renal disease. This finding needs to be confirmed by further studies involving larger number of patients.

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