Association of renalase genotypes with their serum levels in Iraqi hypertensive patients

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Abstract

Due to its worldwide prevalence, hypertension poses a significant threat to public health. Renalase helps regulate blood pressure and heart function by breaking down catecholamines in the bloodstream. As its effects are linked to alterations in multiple organs. This study aims to investigate the association of polymorphism of *renalase* gene rs (2296545) to their serum levels among Iraqi hypertensive patients A total of 70 volunteers (50 verified hypertension patients and 20 healthy individuals who served as controls) were selected using a practical selection technique. Genetic polymorphism of the *renalase* gene (rs2296545) was carried out using RFLP-PCR. Serum levels of renalase were performed using the enzyme-linked immunosorbent assay technique (ELISA) using a Human- renalase kit . This study demonstrates that renalase serum levels were markedly higher in hypertensive patients although there was an insignificant difference (72.2±12.6), controls (65.40±8.91), P= 0.071. The results of this study also demonstrate a marked increase in the levels of renalase among patients with hypertension who have the genotype CC 104.02±9.10 compared to controls43.58±4.04, P> 0.05 while the hypertensive patients with GG genotype had the lowest level of renalase70.68±22.16 compared to controls 28.233± 0.0, P> 0.05.

Keywords— hypertension, renalase, ,genotype, sera.

1. Introduction

Due to its worldwide prevalence, hypertension constitutes a significant public health issue[1]. It is anticipated that by 2025 there will be 1.56 billion adults with hypertension[2]. Renalase is a kidney-secreted flavin adenine dinucleotide-dependent amine oxidase. Renalase is responsible for regulating blood pressure and heart function. It does this by breaking down catecholamines that are circulating in the body [3; 4]. 10q23.33 is the location of the human renalase gene [5] and is highly expressed in the kidney and heart [6; 7]. This study examines the relationship between renalase gene polymorphism and hypertension. Moreover, the single nucleotide polymorphisms (SNP) analyzed in earlier studies are commonly located within the predicted functional areas. There are indications that the SNP rs2296545 is related to hypertension [8] or hypertension and concomitant diabetes [9]. Genetic polymorphisms may contribute to the differences in disease risks amongst different individuals. Different forms of genetic variants are found in the human genome. Single-nucleotide polymorphisms (SNPs) account for more than 90% of genomic variants and are the major form of genetic polymorphisms [10].

There are presently no studies of Iraqi population groups that have explored the polymorphism of renalase rs2296545 in hypertension patients or measured its levels in their sera. This study aims to assess the relationship between the renalase gene rs(2296545) polymorphism and Iraqi hypertensive patients, as well as to test renalase levels in these patients and controls.

2. Materials and Methods

This study included70 participants 50 confirmed hypertensive patients whose their age range between 40–73 years and 20 apparently healthy individuals (controls) subjects (12males and 13 females) and their age range between 40–69 years were selected using a convenient sampling methods.

Five milliliters of blood were collected from all participants and placed in a tube without anticoagulant and placed in a centrifuge at a speed of 2000 rpm for 10 minutes. After that, the serum was withdrawn into an eppend or ff tube 2ml and preserved after being labeled with deep freezing until further processed.

Genetic polymorphism of renalase gene (rs2296545) was carried out using RFLP-PCR restriction enzyme ***Bsu 36I*** **(BioLabs/ USA)**. Two primers were used to detect the polymorphism of SNP of rs2296545. The sequences of these primers are Forward 5′GGAAGTCCCCGATCACGTGAC-3.Reverse 5′TGCTGTGTGGGACAAGGCTGA -3′. the optimal conditions of ***renalase*** gene detection

by PCR . Initial denaturation 94°C, 5 min. 1Cycle. Denaturation 9 4°C,40 sec. Annealing 60,°C40 sec. Extension-1,72°C, 40 sec. 45 cycle . Extension -2 ,72°C,10 min1 cycle .

3. Results and Discussion

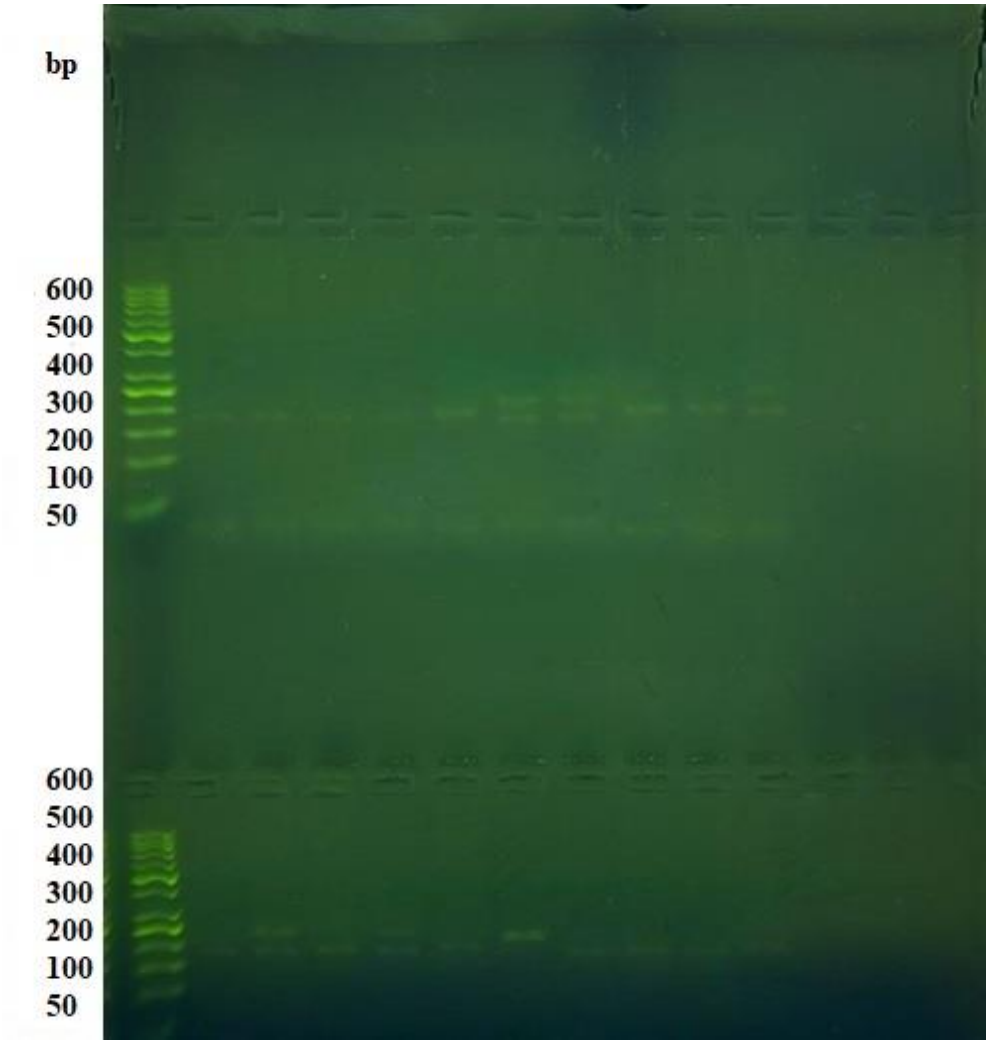

Figure (3-1): Electrophoresis pattern of PCR product digested with *Bsu 36I* restriction enzyme (2.5% agarose gel). : DNA molecular marker 50 plus bp size .by Red stain stained bands in the gel.

3.1 Serum levels of renalase according to *renalase* genotypes

Serum levels of renalase according to *renalase* genotypes are shown in Table (3-11). Hypertensive patients with CC genotypes showed higher levels of renalase than that with

CG, GG genotype CC (104.02±9.10, GC76.80±19.03 and GG 70.68±22.16, P ≤ 0.05. There is a clear increase in the levels of renalase among patients with hypertension who have the genotype CC, and this result confirms what we have found in the relationship of the genotype with the predisposition to disease, as this genotype increases the possibility of developing hypertension. On the other hand, the patients with the GG genotype showed the lowest level of this renalase among hypertension patients, and this supports the previous results, as this genotype does not affect the degree of predisposition to the disease.

Table 3.1 Serum levels of renalase according to renalase genotypes

Parameters / Groups	Pg/ml Mean+SD		
	GG	**GC**	**CC**
Controls	**28.233± 0.0**	**46.88±2.76**	**43.58±4.04**
Hypertensive Patients	**70.68±22.16**	**76.80±19.03**	**104.02±9.10**
P-value	**0.682**	**0.104**	**0.385**
LSD	**0.342**	**0.233**	**0.121**
Statistical significance	**NS**	**NS**	**NS**

Pg:Picogram

***P ≤ 0.05**

NS :Non-significant P> 0.05

SD: Standard deviation

4.Discussion

The renalase gene is a viable candidate for participation in blood pressure regulation. In case-control studies, gene polymorphism analysis indicated a connection between the renalase gene and a variety of human diseases. The Renalase gene is involved in the regulation of blood pressure [11].

Compared to controls, the proportion of hypertension patients with GG genotype was significantly higher, whereas the number of hypertensive patients with CC genotype was remarkable. The CC genotype was the most prevalent among both sick and healthy individuals. The C allele was predominant among both hypertension patients and healthy controls. The frequency of the allele C of rs2296545 is closely associated with hypertension. Moreover, there is evidence [9; 12]. Provides evidence that the allele C frequency of

renalase gene rs2296545 may be related to diabetes. The present study's findings are consistent with those of Adel Abou [13], who discovered that the rs2296545 CC genotype was significantly elevated in hypertension patients compared to healthy controls. In addition, the polymorphism of renalase rs2296545 in this study is consistent with [9; 14]. Analysis of renalase gene (rs2296545) gene polymorphism and hypertension susceptibility revealed a significant risk correlation between the GG genotype and hypertensive patients of both sexes. These findings imply that the rs 2296545 GG genotype is a risk factor for hypertension, but the rs2296545 CG and CC genotypes are protective. Following Zhao et al., [8]who studied the association of single nucleotide polymorphisms of the renalase gene with primary hypertension in the northern Han Chinese population, in a group of 2586 individuals (1,317 patients with essential hypertension and 1,269 healthy controls), rs2296545 was found to be associated with essential hypertension. In contrast, Fava *et al.*[15]observed no association between the rs2296545 gene polymorphism and cardiovascular events, such as hypertension, in a Swedish urban-based cohort of over 5000 individuals. Lv *et al.* [16]demonstrated in a meta-analysis that the rs2296545 polymorphism in the renalase gene is strongly related to an elevated risk of hypertension. Using renalase polymorphisms, renalase (amine oxidase) functions as a blood pressure regulator and can predict many disorders, including preeclampsia and cardiovascular diseases, particularly in patients with hypertension. This study indicates that renalase serum levels were significantly elevated in hypertension patients, while the difference was not statistically significant. Renalase is a 2005-discovered new amine oxidase implicated in the pathophysiology of hypertension[17]. Experimental and clinical research have confirmed the correlation between serum renalase and blood pressure; nevertheless, the results are still inconclusive. In a study including 2,586 Chinese adults, Zhao *et al.* [8]discovered two single-nucleotide polymorphisms (SNPs; rs2576178 GG genotype and rs2296545 CC) within the renalase gene were associated with essential hypertension (defined as $BP \geq 160/100$ mmHg) [8]. In a study conducted by Maciorkowska *et al.* (2015)[4] in adult patients, the median serum renalase was statistically significantly higher in patients with SBP and $DBP > 140/90$ mmHg compared to those with readings of $< 140/90$ mmHg, measured by ambulatory blood pressure monitoring ($p = 0.018$). A recent study reveals that serum renalase levels and BPs showed a linear correlation. In addition, serum renalase was significantly associated with the risk of hypertension [*OR* = 1.018 (1.006–1.030)] [18]. [19] verified these findings in 88 teenagers. In 34 hemodialyzed patients [20] and 50 patients following surgical repair of aortic coarctation, hypertensive participants exhibited significantly lower levels of serum renalase than their normotensive counterparts. Schlaich *et al.* (2010)[21] determined that the serum renalase levels of 22 patients with resistant hypertension were lower than those of 4 normotensive controls. Diverse study demographics, sample sizes, and racial variances may also

contribute to the divergent findings. Despite the clear increase in renalase levels in hypertension patients compared to healthy people, the insignificance of the study's results is primarily attributable to changes in the sample size.

The results of this study also demonstrate a marked increase in the levels of renalase among patients with hypertension who have the genotype CC. While the hypertensive patients with GG genotype had the lowest level of renalase and they may be a genetic risk factor for hypertension. These results were in concordance with the results of susceptibility association that GG genotypes increased the likelihood of contracting the disease. The small sample size and hospital setting constituted a limitation of the current investigation. The discrepancies with other research are typically attributable to the diverse genetic makeup and ethnic origins of these people, and resolving the issue would require additional large-scale studies.

5. Conclusions

Identifying individuals with renalase polymorphism which is an important factor in the development of hypertension.

6.References

[1] Erem, C., Hacihasanoglu, A., Kocak, M., Deger, O., and Topbas, M. (2009). Prevalence of prehypertension and hypertension and associated risk factors among Turkish adults: Trabzon Hypertension Study. *Journal of public health*-47 ,(1)31 , .58
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[2] Tabrizi, J. S., Sadeghi-Bazargani, H., Farahbakhsh, M., Nikniaz, L., and Nikniaz, Z. (2016). Prevalence and associated factors of prehypertension and hypertension in Iranian population: the lifestyle promotion project (LPP). *PLoS One*, *11*(10), e016 .5264.

[3] Baroni, S., Milani, M., Pandini, V., Pavesi, G., Horner, D., and Aliverti, A. (2013). Is renalase a novel player in catecholaminergic signaling? The mystery of the catalytic activity of an intriguing new flavoenzyme. *Current pharmaceutical design*, *19*(14), 2540-2551 .

[4] Maciorkowska, D., Zbroch, E., & Malyszko, J. (2015). Circulating renalase, catecholamines, and vascular adhesion protein 1 in hypertensive patients. *Journal of the American society of hypertension*, *9*(11), 855-864.

[5] Xu, J., Li, G., Wang, P., Velazquez, H., Yao, X., Li, Y., ... & Desir, G. V. (2005). Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure. *The Journal of clinical investigation*, *115*(5), 1275-1280.

[6] Fedchenko, V., Globa, A., Buneeva, O., & Medvedev, A. (2013). Renalase mRNA levels in the brain, heart, and kidneys of spontaneously hypertensive rats with moderate and high hypertension. *Medical science monitor basic research*, *19*, 267.

[7] Hennebry, S. C., Eikelis, N., Socratous, F., Desir, G., Lambert, G., & Schlaich, M. (2010). Renalase, a novel soluble FAD-dependent protein, is synthesized in the brain and peripheral nerves. *Molecular psychiatry*, *15*(3), 234-236.

[8] Zhao, Q., Fan, Z., He, J., Chen, S., Li, H., Zhang, P., ... & Gu, D. (2007). Renalase gene is a novel susceptibility gene for essential hypertension: a two-stage association study in northern Han Chinese population. *Journal of Molecular Medicine*, *85*(8), 877-885.

[9] Buraczynska, M., Zukowski, P., Buraczynska, K., Mozul, S., & Ksiazek, A. (2011). Renalase gene polymorphisms in patients with type 2 diabetes, hypertension and stroke. *Neuromolecular medicine*, *13*(4), 321-327.

[10] Collins, F. S., Brooks, L. D., & Chakravarti, A. (1998). A DNA polymorphism discovery resource for research on human genetic variation. *Genome research*, *8*(12), 1229-1231.

[11] Desir, G. V., Wang, L., and Peixoto, A. J. (2012). Human renalase: a review of its biology, function, and implications for hypertension. *Journal of the American society of hypertension*, *6* .426-417 ,(6)

[12] Stec A, Semczuk A, Furmaga J, Ksiazek A, Buraczynska M: Polymorphism of the renalase gene in end-stage renal disease patients affected by hypertension. Nephrol Dial Transplant 2012;27:4162-4166.

[13] Abou Zaghla, H. M. A., El-Masry, T. M., Salem, R., Zahra, M. A., & Hassan, M. S. (2020). Polymorphisms of renalase gene in Egyptian prevalent hemodialysis patients with and without hypertension. *Journal of Renal Endocrinology*, *6*(1), e04-e04.

[14] Li, X., Jiang, W., Li, L., Huang, R., Yang, Q., Yang, Y., ... & Tang, X. (2014). Renalase gene polymorphism in patients with hypertension and concomitant coronary heart disease. *Kidney and Blood Pressure Research*, *39*(1), 9-16.

[15] Fava, C., Montagnana, M., Danese, E., Sjögren, M., Almgren, P., Engström, G., ... & Melander, O. (2012). The Renalase Asp37Glu polymorphism is not associated with hypertension and cardiovascular events in an urban-based prospective cohort: the Malmö Diet and cancer study. *BMC medical genetics*, *13*(1) , 1-10.

[16] Lv, Y. B., Wang, Y., Ma, W. G., Yan, D. Y., Zheng, W. L., Chu, C., ... & Mu, J. J. (2016). Association of renalase SNPs rs2296545 and rs2576178 with the risk of hypertension: a meta-analysis. *PLoS One*, *11*(7), e0158880.

[17] Zbroch, E., Małyszko, J., Małyszko, J., Zórawski, M. J., & Myśliwiec, M. (2012). Kidney and hypertension: is there a place for renalase. *Pol Arch Med Wewn*, *122*(4), 174-9.

[18] Wang, Y., Chen, C., Hu, G. L., Chu, C., Zhang, X. Y., Du, M. F., ... & Mu, J. J. (2022). Associations of Renalase With Blood Pressure and Hypertension in Chinese Adults. *Frontiers in cardiovascular medicine*, *9*

[19] Lemiesz, M., Tenderenda-Banasiuk, E., Sosnowska, D., Taranta-Janusz, K., & Wasilewska, A. (2018). Serum renalase levels in adolescents with primary hypertension. *Pediatric cardiology*, *39*(6), 1258-1264.

[20] Wybraniec, M. T., Mizia-Stec, K., Trojnarska, O., Chudek, J., Czerwieńska, B., Wikarek, M., & Więcek, A. (2014). Low plasma renalase concentration in hypertensive patients after surgical repair of coarctation of aorta. *Journal of the American Society of Hypertension*, *8*(7), 464-474.

[21] Schlaich, M., Socratous, F., Eikelis, N., Chopra, R., Lambert, G., & Hennebry, S. (2010). Renalase plasma levels are associated with systolic blood pressure in patients with resistant hypertension: 9C. 01. *Journal of Hypertension*, *28*, e437.