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Intron 6 Allelic Variant Of Renalase Gene And Its Association With Preeclampsia.

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ABSTRACT

Hypertensive disorders of pregnancy greatly increase the maternal and neonatal morbidity and mortality. Renalase, a flavoprotein, effectively metabolizes catecholamines and helps in regulating arterial blood pressure. Renalase deficiency, could be a missing link in the aetiology and the pathophysiology of preeclampsia. Single Nucleotide Polymorphisms of renalase gene (RNLS) have also been strongly associated with preeclampsia. The variant of renalase gene studied is the A/G transition at rs10887800 located at intron 6. The case control study included 30 women with pre-eclampsia and 30 normotensive age and gestational weeks matched women. DNA was extracted from whole blood samples. The PCR products for A/G transition at rs10887800 at intron 6 of polymorphism were separated electrophoretically using 2 % agarose gel**.** On genotyping studies, frequencies were noted to be AA-5(16.66%); GG-18(60%); AG-7(23.33%) among cases and AA-8(26.66%); GG-14(46.66%); AG-8(26.665%) among controls. Using Kruskal Wallis test, p value was found to be non-significant (p value-0.532). The findings of our study did not show association of SNP of Renalase gene at rs10887800 with occurrence of preeclampsia. Further investigations on a larger sample size is necessary to show the effect of renalase gene polymorphisms on the pathophysiology of Preeclampsia.

Keywords: Preeclampsia, Renalase, Single Nucleotide Polymorphism, rs10887800, alleles, genotype.

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INTRODUCTION

Hypertensive disorders of pregnancy greatly increase the maternal and neonatal morbidity and mortality. These disorders affect 10% of all pregnancies worldwide, of which preeclampsia (PE) complicates about 3-5%. The incidence of preeclampsia in India is between 8-10%[1]. This is a syndrome of decreased organ perfusion, which is specific to pregnancy. It is one of the leading causes of maternal and perinatal mortality and morbidity and also carries the risk of the women developing cardiovascular and metabolic diseases in later years [2]. Though recent advances have been made in identification of patients and prevention of major complications of preeclampsia, its exact aetiology is still in the dark.

Renalase, a novel protein, is a Flavin adenine dinucleotide-dependent amine oxidase (MAO-C). It is an enzyme with 342 amino acids with a molecular weight of ~38 kDa. It is expressed strongly in human kidney and heart [3]. The main function of Renalase is to metabolize catecholamines and thus help in regulating arterial blood pressure. This is done by a mechanism different from that of classical monoamine oxidases(MAO-A&B). MAO-A & MAO-B, are intracellular since they are bound to the outer mitochondrial membrane through the carboxyl terminus. Whereas, renalase is secreted into the blood and it is the only aminooxidase which is active extracellularly [4]. It is found to be secreted through the basolateral membrane in the proximal tubules, thus entering the circulation to breakdown catecholamines [5]. Human and animal studies have revealed strong evidence to substantiate the role of renalase in blood pressure control [6].

The gene (RNLS) encoding renalase is on chromosome 10 at q23.33 which has ten exons and 311000 base pairs. Single Nucleotide Polymorphisms of RNLS gene has been associated with diseases like hypertension, chronic kidney disease, coronary disease, Type I diabetes, etc [3]. Studies linking preeclampsia and SNPs of renalase gene have also been strongly associated with these SNPs [7, 8]. Renalase deficiency, could be a missing link in the aetiology and the pathophysiology of preeclampsia. It is being tried as a therapeutic agent in diseases like hypertension and kidney diseases. Animal studies have shown promising results that renalase could be a potent therapeutic agent.

Pre-eclampsia represents a complex genetic disorder. Variants at various loci, taken together could eventually contribute to its susceptibility [9]. Variations in genes responsible for fluid balance and response of vasculature to sympathetic system are under study [10].

This study was one such pursuit to link a SNP of renalase gene with preeclampsia. The variant of renalase gene to be studied is the A/G transition at rs10887800 which is located at intron 6 (near the intron/exon border).

If this association is to be established, then renalase could be a potent therapeutic target in future. Thus, it could go a long way in reducing maternal and neonatal mortality and morbidity.

Aim Of The Study

To analyse the potential association of rs10887800 polymorphism of the renalase gene in patients with pre-eclampsia.

Objectives

- To study the presence of the allelic variant, rs 10887800, in the south Indian population.
- To identify the association of this polymorphism with preeclampsia.

MATERIALS AND METHODS

This case-control study was done over a period of 6 months (from June 2018- November 2018) after obtaining Institutional Ethical Committee clearance. The study population comprised of the antenatal mothers attending either the out-patient or admitted in the antenatal / labour wards of Kilpauk Medical College, Chennai, Tamilnadu. The control subjects were age and gestational weeks matched normal pregnancy mothers with no evidence of preeclampsia.

Inclusion Criteria

The study group consisted of 30 antenatal women in the age group of 20-40 years with the gestational age of 24-36 weeks, who were fulfilling the diagnostic criteria of preeclampsia by ACOG of: Blood Pressure ≥140/90mmHg measured on two occasions 4hours apart; Proteinuria ≥ 300mg/24hrs or 1+ in dipstick test or with; symptoms including visual disturbances, headaches, epigastric pain and the rapid development of oedema.

Exclusion criteria

Pregnancy complicated by renal disease, maternal thyroid disorders, isolated Proteinuria / elevated liver enzymes(AST/ALT) without rise in BP, maternal liver disorders. After obtaining the informed consent from the subjects after explaining about the study, baseline data including age, gender, occupation, detailed medical history, were recorded. Under strict aseptic precautions, 5ml of blood was taken from the antecubital vein in 2ml EDTA tubes and inverted appropriately for genetic analysis.

Genetic study

DNA extraction was done by Purefast Human Blood DNA Minispin prep kit (Helinibiomolecules, Chennai) as per the procedure. Extracted DNA was identified by 1% agarose gel electrophoresis and compared with a known molecular weight 1kb DNA (Lambda DNA) ladder. The extracted DNA was quantified spectrophotometrically. Polymerase chain reaction was done using Helini rs 10887800 human SNP genotyping PCR kit, using the Amplification Refractory Mutation System (ARMS). Amplification of the extracted DNA was carried out in Eppendorf Mastercycler nexus GX2.Amplified product – amplicons were identified by 2.5% agarose gel electrophoresis by comparison with a known 100bp DNA ladder. Negative control was included for each run. In order to visualize nucleic acid molecules in agarose gels, ethidium bromide was used. Illumination of the agarose gels with 300-nm UV light (e gel imager) from Life Technologies was subsequently used for visualizing the stained nucleic acid. The following were obtained on separation: A allele: PCR product size: 259bp; G allele: PCR product size: 400bp; Control PCR product size: 659bp.

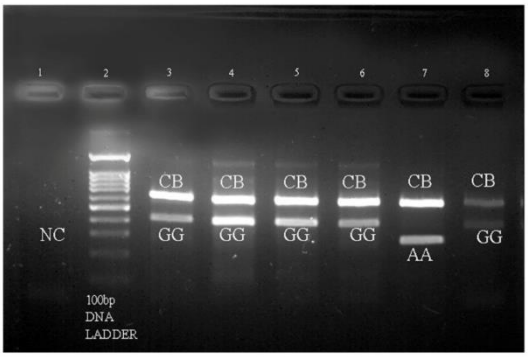

Image Parameters

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Band Detection Parameters

Background subtraction method	Rolling ball disk size :70
Band detection sensitivity	100
Molecular weight standard name	
Molecular weight regression type	CubicSpline
Mass regression type	Linear
Mass regression formula	N\A
Mass regression R square	N\A

Figure 1: PCR Products of Cases And Controls Under UV Illumination

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Statistical Analysis

SPSS version 21 package was used for statistical analysis. The groups were compared using Student t test. The genotypes were compared between cases and controls using Kruskal Wallis test. p value of <0.05 was considered significant. Genotype frequency distribution between cases and controls were compared with the x^2 test for 2×2 contingency table. Allele frequency by using the equation p + q = 1 where p and q are the frequencies of each allele at the particular locus. Hardy Weinberg equation equilibrium as shown by p2 + q2 + 2pq = 1.

RESULTS

Comparison of Demographic characteristics between cases and controls

The results of the demographic features are shown in Table.1 and the bar diagram depicted in Fig.14.The mean age group of cases was 27.03±4.58 and that of controls was 25.86±4.56 with a p value of 0.327(non-significant).The mean gestational age was found to be 33.31±2.36 in cases and 31.52±4.46 in controls, with a p value of 0.07. There was no significant difference in terms of height between cases and controls (p value- 0.5).But a significant increase in weight was noted in cases than in the controls, with a mean of 72.8±13.04 vs 64.23±9.82 and a p value of 0.006. Among the cases 37% had 1+ proteinuria (Table no.1).

Table 1: Demographic features of women with preeclampsia and controls

Variables	Cases	Controls	pvalue
Age(years)	27.03±4.58	25.86±4.56	0.327
Gestational age (weeks)	33.31±2.36	31.52±4.46	0.070
Height(cm)	153.93±4.65	152.96±6.38	0.505
Weight(kg)	72.8±13.04	64.23±9.82	0.006
Systolic BP(mmHg)	159±15.03	103.33±10.61	0.001
Diastolic BP(mmHg)	102±8.05	69±7.11	0.001

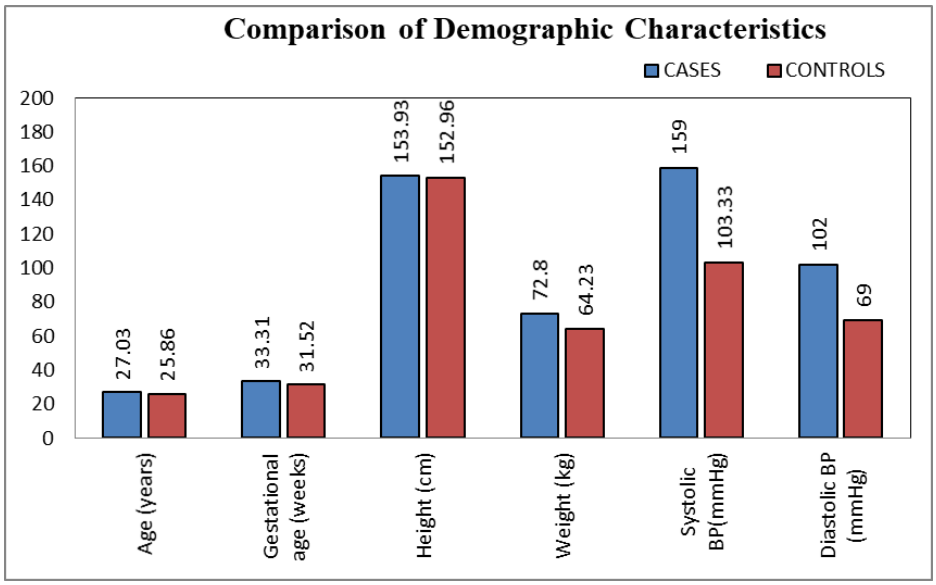

Figure 2

Genotype Distribution In Study Population

On genotyping studies, frequencies were noted to be AA-5(16.66%); GG-18(60%); AG-7(23.33%) among cases and AA-8(26.66%); GG-14(46.66%); AG-8(26.665%) among controls. Using Kruskal Wallis test, p value was found to be non-significant (p value-0.532).

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Table 2: Genotype distribution in study population

Genotype	Cases	Controls
AA	5(16.66%)	8(26.66%)
GG	18(60%)	14(46.66%)
AG	7(23.33%)	8(26.665%)
pValue-0.532		
Non-Significant		
Kruskal Wallis Test		

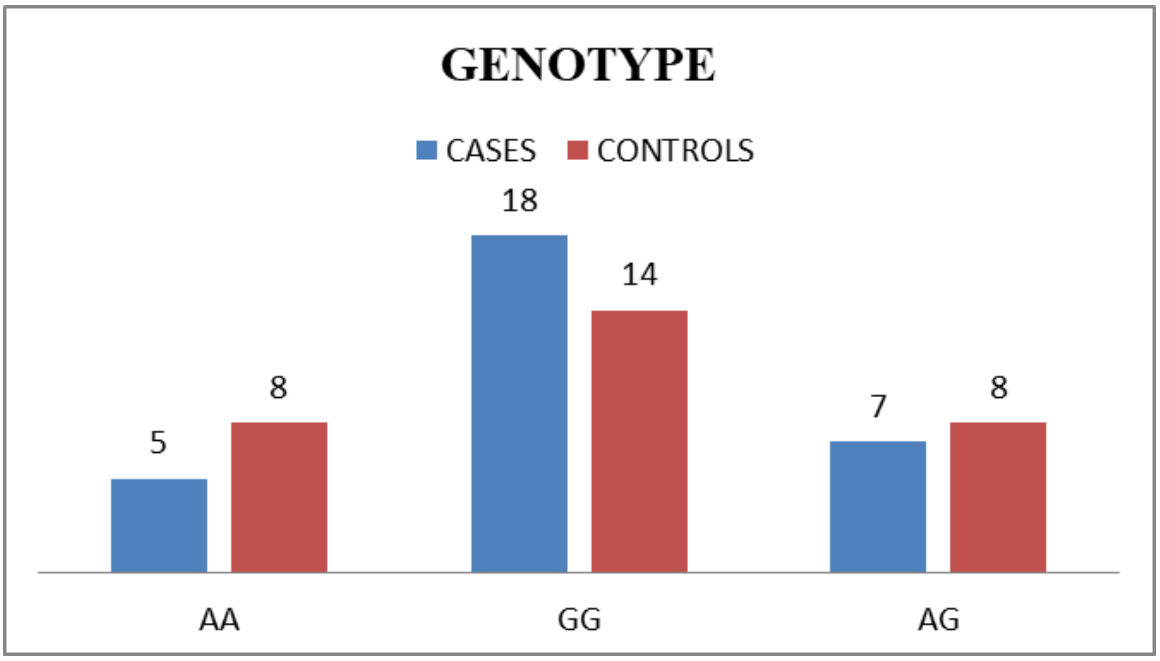

Figure 3

Allele Frequency

The allele frequencies were the following: A allele frequency in the Cases was 29.3% and in the Controls was 40%. G allele frequency in the cases and controls was 71% and 60% respectively. The allele frequency was found to satisfy Hardy Weinberg equilibrium. The chi-square statistic is 1.8154. The p-value is 0.177(>0.05); Odds Ratio:1.68 (Table:3)

Table 3: Allele frequency

Allele	Cases	Controls	p Value	Odds ratio
A	17	24	0.177	1.68
G	43	36		
	Among cases	Among controls		
A Allele Frequency	29.30%	40%		
G Allele Frequency	70.70%	60%		

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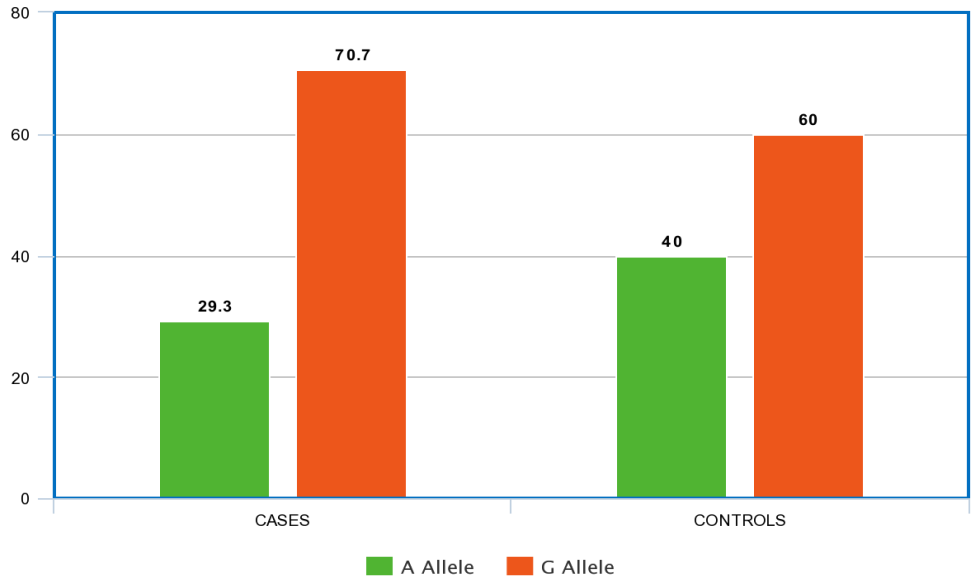

Figure 4: Allele frequency

DISCUSSION

In the recent days, much of the lime-light is on renalase, which is said to play a major role in controlling blood pressure, by regulating the metabolism of catecholamines. Renalase was found to be synthesized by kidney. It has also been shown to be expressed in cardiac, skeletal muscles, small intestine, etc. Renalase had weak similarities with amino acids of MAO-A&B and comes under flavin-dependent aminooxidase [11]. Recent studies have identified it as an intracellular and extracellular protein. The concentration in blood is approximately 5 µg/ml. Its functions are both that of a flavoenzyme and of a cytokine [12].

Unlike MAO-A & MAO-B, which are intracellular, renalase is secreted into the blood and it is the only aminooxidase which is active extracellularly, entering the circulation through the basolateral membrane of proximal tubules to breakdown catecholamines [13, 14]. Functionally also, it differs from these amine-oxidases. However, many theories were put forward regarding the mechanism of action [15].

Oxidation of catecholamines including NAD(P)H as co-substrate and two electrons was the most proposed theory [16]. Order by which the vasopressors are oxidized are epinephrine>>L-dihydroxyphenylalanine (L-DOPA)>dopamine/norepinephrine (NEp) [17, 18]. The end products of renalase reaction are different from those of MAOs and COMT. As O2-radical is involved, aminochromes are the products of renalase action on catecholamines. Epinephrine is broken down to adrenochrome; L-DOPA to dopachrome, NEp to noradrenochrome [19]. Besides enzymatic properties, renalase also acts like a cytokine by providing cytoprotective actions. This action is mediated by its interaction with PMCA4b, a plasma membrane receptor which in turn activates many cellular pathways [20]. This is achieved via activation of a signalling cascade and then protein kinase B, mitogen activated kinase, etc [21]. Animal studies show that Renalase protects against acute kidney injury (ischemic and toxic) owing to this cytoprotective function. Renalase gene -*RNLS* rs2576178 and rs2296545 polymorphisms and its association with hypertension were first studied. SNPs of rs2576178 and rs10887800 were studied in PIH patients in an Egyptian cohort. They showed significant correlation between the genetic polymorphisms and the blood pressure of preeclamptics [22]. Bagci et al., studied Caucasians of Turkish origin and obtained a significant correlation of rs10887800 polymorphism and PE , with the G allele frequency was 0.46 in cases as against 0.34 in controls.. However, there was no difference between cases and controls with regard to rs2576178 genotype [23].

Ours was the first study done in the Indian population, to analyze the renalase gene polymorphism with PE. In this cohort of south Indian population, no association could be obtained between the genetic polymorphism at rs10887800 of renalase gene and the preeclamptic status, with respect to the blood pressure.

Two previous studies relating renalase gene polymorphism and PE showed strong association of SNP at rs10887800 with blood pressure. Khaled Elsetohy A, et al. did the first study relating the polymorphisms of rs2576178 and rs10887800 with blood pressure of eclamptics in an Egyptian cohort [22]. They showed significant correlation of AG genotype with PIH and suggested that besides being associated with aetiology it can also be used as a predictor of PIH. The G allele frequency was 0.66 in cases as against 0.27 in controls. The A allele frequency of controls was 0.73 and of cases 0.33.

Cristiano Fava, et al., studied in a Swedish urban population (including more than 5000 subjects), the effect of two common RNLS SNPs (RNLS rs2576178 A > G and rs2296545 C > G) and demonstrated no association with hypertension and cardiovascular diseases [24]. Pawlik et al included 250 renal allograft patients and studied the association between renalase gene rs2296545 polymorphism and serum creatinine concentrations and blood pressure after transplantation. No significant association was found in this group [25].

Zhao et al. revealed that in Chinese population, rs2576178 GG and rs2296545 CC genotypes had significant association with an increased risk of hypertension.[26] Zhang et al found that SNP at rs10887800 had association in hypertensive patients with stroke [27].

There are studies assessing the serum renalase level with hypertension and other conditions. Yang Wang et al., showed no significant association of serum renalase levels with systolic, diastolic and mean arterial pressure and with brachial ankle pulse wave velocity among a cohort of Chinese adults [28]. The study of Schlaich et al showed serum renalase to be higher in normotensives than in the cases with resistant hypertension [29]. Maciorkowska et al. contradicted the finding and showed higher renalase levels was seen in patients with hypertension than in the normal controls [30]. Also, Koc-Zorawska et al.could not detect any significance in the renalase levels in the hemodialysed patients with or without hypertension [31]. Studies in patients with PE by Yilmaz et al. showed decreased renalase levels [32].

Our present study,is at variance with the previous ones. We found no association between the renalase gene polymorphism at the intronic rs10887800 with preeclampsia related clinical traits in the sample selected from the population attending our tertiary care hospital.

CONCLUSION

In an attempt to elucidate the causes of preeclampsia, single nucleotide polymorphisms of Renalase gene were studied. Renalase modulates BP through its action on circulating catecholamines and that could represent a novel therapeutic target for the treatment of hypertension. However, our study did not show association of SNP of Renalase gene at rs 10887800 with occurrence of preeclampsia. This polymorphism was found to be of negligible importance in our South Indian study population which included patients attending the tertiary care centre, Government Kilpauk Medical College and Hospital, Chennai.

Limitation

This discrepancy of our study with previous reports could be due to the difference in design, criteria of selection and genetic background.

Further investigations on a larger sample size is necessary to show the effect of renalase gene polymorphisms on the pathophysiology of PE.

The end product was not measured to establish the difference between the cases and controls.

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