Renalase levels and genetic variants are associated with preeclampsia: a hospital based study in Chinese cohort

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Research article

Keywords: preeclampsia, renalase, gene polymorphism, Chinese

Posted Date: August 18th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-58899/v1>

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Abstract

Background

Pre-eclampsia (PE) is a major contributor of maternal and fetal morbidity and mortality. There are many host related biomolecules that regulate the pathophysiology PE. Critical analysis of these parameters is of crucial importance as some of these may be used as prognostic/diagnostic marker of this serious ailment and can be targeted for developing therapeutic measures against the disease. The aim of the current study is to examine the role of renase in the context PE in a Chinese cohort.

Methods

A hospital based case control study was designed to investigate role of renase in PE. 384 Chinese women consisting of subjects with normotensive pregnancy (n = 105), women with PE (n = 121) and healthy pregnancy (n = 158) were included in the study. Serum renase level was measured in recruited subjects by ELISA. Renase gene polymorphisms (rs10887800, rs2576178 and rs2296545) were genotyped by PCR-RFLP.

Results

A higher level of serum renase in healthy pregnant women compared to controls, whereas, subjects with PE demonstrated a reduced level of this enzyme. Renase level was negatively correlated with systolic as well as diastolic blood pressure, whereas, a positive association was observed with glomerular filtration rate. Prevalence of homozygous mutant (GG) and minor allele (G) of rs10887800 and rs2576178 polymorphisms were higher in PE patients compared to normotensive pregnant women and healthy controls. Furthermore, association of G-G-C haplotype with susceptibility to PE was observed.

Conclusions

Low level of renase may be associated with high risk of PE during pregnancy. Renase gene polymorphisms (rs10887800 and rs2576178) are correlated with with serum renase and associated with predisposition to development of PE in Chinese cohort.

Background

Pre-eclampsia (PE) is the most common complication during pregnancy that affects about 2–8% of pregnancies and contributes to the leading cause of pregnancy-related mortality in developed countries [1]. The syndrome poses a significant risk to both the mother and the fetus. Although the exact cause and pathogenesis of this ailment are not understood with reasonable clarity, it is assumed that the disease is driven by several factors released from trophoblast, induced by placental pressure and promotes an

overwhelming maternal inflammatory response [2]. In addition to morbidity and mortality in pregnant women, PE may cause prenatal death, preterm birth, as well as intrauterine growth restriction [3].

The association for American College of Obstetricians and Gynecologists (ACOG) defines PE as women with hypertension ($> 140/90$ mmHg), high proteinuria (> 0.3 g/24 hrs) and liver dysfunctions after 20 weeks of gestation [4]. Further, worldwide about 14% of maternal death accounted due to PE and its related hypertensive disorders [5]. There are ample instances of the occurrence of PE in China. In a retrospective study at three different hospitals of China, among 67,746 pregnant women included, the prevalence of mild or severe preeclampsia was 1.42 or 0.49%, respectively [6].

Renalase is a form of monoamine oxidase that directly degrades several catecholamines such as epinephrine, norepinephrine and dopamine. Renalase is synthesized and secreted within the peripheral nervous system, hypothalamus and the pituitary [7]. The major function of renalase enzyme is to reduce blood pressure levels by inhibiting cardiac contractility and maintain optimal heart rate [8, 9]. Since renalase is directly involved in maintenance of blood pressure, serum renalase is considered as potent biological marker for primary hypertensive disease [10].

The involvement of this enzyme in regulating reproductive health during pregnancy is well known. An augmented level of renalase has been reported in ovaries during gestation [11]. Alteration in serum renalase level has been found to be strongly correlated with pathophysiologic process in pregnant subjects with PE [12]. Additionally, renalase levels are also found to be associated with systolic and diastolic blood pressure levels, glomerular filtration rate and urinary protein excretion in pregnant women with PE [13]. However, pathophysiological role of renalase in Chinese pregnant women with PE is limited and this gap triggered us to examine the possible correlation of this biomolecule with disease pathogenesis during PE.

Renalase gene is located at the long arm of 10th chromosome (q23.33) having 16 exons and spans around 310 kb. Several single nucleotide polymorphisms have been reported in the renalase gene, but some polymorphisms with functional relevance have been investigated widely. A significant association of GG genotype and minor allele "G" of rs10887800 polymorphism has been associated with susceptibility to development of PE, in Turkish PE patients [14]. Similarly, combined homozygous mutations of rs10887800 and rs2576178 polymorphisms increased eight fold chances to develop PE clinical phenotype in pregnant women [15].

Based on importance on role of renalase in PE and limited study in Chinese population, we investigated distribution of renalase gene polymorphisms (rs10887800, rs2576178 and rs2296545) and serum renalase levels between healthy controls, pregnant women with and without PE.

Materials And Methods

Study population

The current study was undertaken between a period from July 2017 to September 2019 at Department of obstetrics, Daqing peoples's Hospital after approval of study protocol by Institutional Review Board of Daqing people's hospital, Daqing. In total, 384 women aged 25–35 years were recruited in the study. Essentially, three study populations were considered, namely, subjects with normotensive pregnancy with gestation weeks > 20 weeks (n = 105), women with newly diagnosed PE (n = 121), and healthy non-pregnant women (n = 158). Before the investigation, written informed consent was taken from each study participant.

The major inclusion criteria for patients with PE involved; third-trimester pregnancy complicated with PE, blood pressure > 140/90 with proteinuria > 300 mg in 24 hours (According to ACOG) [4]. In the group with normotensive pregnancy, women with a record of PE in previous pregnancies, hypertension, cardiovascular and renal diseases were excluded. A healthy control group included age-matched normotensive women with no pregnancy and no previous record of PE or hypertension or cardiovascular/renal diseases. Data for clinical characteristics, such as body mass index (BMI), fetal birth weight, blood pressure (systolic as well as diastolic), serum creatinine, glomerular filtration rate, WBC count, serum uric acid and urea were collected from hospital records.

Collection of serum

About 2.5 ml of blood (without anticoagulant) was collected intravenously from all participants (pregnant women, non-pregnant controls, and PE patients) at the time of enrollment. 500 ul blood samples were dissolved with anticoagulant, and 2 ml of blood samples were used for isolation of serum. Serum was separated by centrifuging the blood at 2500 rpm for 5 minutes and was stored at -20 °C until further use.

Enzyme-Linked Immunosorbent Assay

Serum level renalase was determined in the subjects from three study categories by ELISA using human renalase ELISA kit (R&D Systems, Inc, USA) following the manufacturer's instructions. All serum samples were measured in duplicate, and the average absorbance value was recorded for a study subject.

Isolation of genomic DNA

From the 500 ul of blood samples collected with anticoagulant, 200 ul of whole blood was used for isolation of genomic DNA by QIAamp DNA Blood Mini kit (Qiagen, Germany). The instructions provided by the manufacturer were followed for isolation of the genomic DNA. The purity of the isolated DNA was accessed by a Nanodrop spectrophotometer.

Genotyping of renalase (rs10887800, rs2576178 and rs2296545) polymorphisms

Genotyping of renalase polymorphisms (rs10887800, rs2576178 and rs2296545) were performed by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) as described earlier by various groups [16, 17]. Different primers pairs were used for amplification of genetic fragment flanking respective mutant positions- rs2576178: F-5'- CAGGAAAGAAAGAGTTGACAT-3', R-5'-

AAGTTGTTCCAGCTACTGT-3'; rs10887800: F-5'-AGCAGAGAAGCAGCTTAACCT-3', R-5'-TTATCTGCAAGTCAGCGTAAC-3'; rs2296545: F-5'-GGAAGTCCCC GATCACGTGAC-3', R-5'-TGCTGTGTGGGACAAGGCTGA-3'. A total volume of 25 ul PCR reaction was prepared with a master mix, respective primers pairs, and 10–40 ng of genomic DNA. The thermal cycling condition was initial denaturation of 95°C for 7 minutes, followed by 35 cycles of denaturation at 95°C for the 30 s, annealing at 60°C for 30 s, and extension at 72°C for 45 s. The final extension was for 7 minutes at 72°C. The PCR reactions yielded an amplicon size of 554 bp for rs10887800, 525 bp for rs2576178, and rs2296545 for 209 bp. The amplicons were digested with PstI, MspI, and Eco81I, respectively. Based on differential digestion products by specific restriction enzyme as visualization under UV illuminator, genotypes of subjects were determined as follows- for rs10887800: 554 bp (AA), 415 + 139 bp (GG) and 554 + 415 + 139 bp (AG); rs2576178: 525 bp (AA), 423 + 102 bp (GG) and 525 + 423 + 102 bp (AG) and banding pattern for genotyping of rs2296546 were CC (139 + 70 bp), GG (209 bp) and for CG (209 + 139 + 70 bp).

Statistical analysis

All statistical analysis was performed by using GraphPad Prism version 7.0 (GraphPad Software, Inc, La Jolla, CA, USA). For comparison of various parameters among different groups, unpaired Student's t-test or one-way ANOVA were used as appropriate. Further, correlation analysis between the variables was performed by Spearman's correlation test. Data were expressed as mean $\pm$ standard error (SE). Allele and genotype frequency was determined by direct counting. Fisher's exact test compared the distribution of genotypes and alleles among different groups. P-value, odds ratio, and 95% confidence interval were calculated. Case-controls haplotype analysis and linkage disequilibrium were accessed by SNPalyze software. The distribution of various genotypes in healthy controls was tested for Hardy-Weinberg equilibrium. For all statistical analyses, a P value of less than 0.01 was considered statistically significant.

Results

Baseline characteristics of participants

The baseline clinical features of the different study groups are demonstrated in Table-1. The results showed a significant difference in parameters such as duration of gestation, body mass index (BMI) at the time of blood draw, frequency of standard delivery, percentage of caesarian section, fetal birth weight, systolic/diastolic blood pressure (mmHg), white blood cell count ($\times 10^9/L$), levels of urea and uric acid (mg/dL) and glomerular filtration rate (GFR) among three study populations. However, serum creatinine levels were reasonably similar in three study populations.

Serum renalase levels in study subjects

Serum renalase levels were measured in three clinical categories, and observations are shown in Figure-1. The mean $\pm$ SE renalase levels in PE patients, normotensive pregnant and normotensive non-pregnant women were found to be 170.7 ± 2.38 ug/ml, 279.1 ± 3.65 ug/ml and 199.6 ± 1.08 ug/ml, respectively. The

results indicate a significant difference in the level of this enzyme among the groups (One way ANOVA, $P < 0.0001$). Normotensive pregnant subjects had significantly higher renalase levels as compared to non-pregnant controls ($P < 0.0001$). On the other hand, subjects with PE displayed considerably lower levels of renalase when compared normotensive pregnant subjects ($P < 0.0001$).

Association of serum renalase level with blood pressure and glomerular filtration rate

Previously, the role of renalase on the regulation of blood pressure has been well defined. Thus in the present study, we tested the possible relationship of this enzyme with systolic as well as diastolic blood pressure. A significant inverse association was observed between the serum renalase level and systolic blood pressure ($r = -0.5949$, $P < 0.0001$) as well as diastolic blood pressure ($r = -0.6243$, $P < 0.0001$).

As the renalase productions and circulation in the plasma are regulated by kidney function, we examined the association of these enzyme levels with glomerular filtration rate. Correlation analysis suggested a direct association of renalase with glomerular filtration rate in the study subjects ($r = 0.0567$, $P < 0.0001$).

Distribution of renalase gene polymorphism in healthy Chinese women

A total of 158 healthy non-pregnant women were enrolled in the present study, and the PCR-RFLP method was employed for genotyping renalase polymorphisms (rs10887800, rs2576178, and rs2296545). as shown in Table-2, heterozygous mutants (rs10887800: AG = 51%, rs2576178: AG = 48% and rs2296545: CG = 48%) were more frequent in comparison to wild type (rs10887800: AA = 22%, rs2576178: AA = 20% and rs2296545: CC = 27%) and homozygous mutants (rs10887800: GG = 24%, rs2576178: GG = 32% and rs2296545: GG = 25%). Distribution of genotypes were in accordance with Hardy-Weinberg equilibrium (rs10887800: $\chi^2 = 0.11$, $P = 0.73$; rs2576178: $\chi^2 = 0.10$, $P = 0.75$; and rs2296545: $\chi^2 = 0.22$, $P = 0.63$).

Association of renalase polymorphisms with susceptibility to PE

Prevalence of renalase polymorphisms (rs10887800, rs2576178, and rs2296545) was compared among different clinical categories of subjects enrolled in the present investigation by Fisher exact test. As shown in Table-2, the homozygous mutants and minor alleles of rs10887800 and rs2576178 polymorphisms were significantly more frequent in PE patients compared to NNP [rs10887800 (GG: $P = 0.008$, OR = 2.66; G: $P = 0.007$, OR = 1.60), rs2576178 (GG: $P = 0.04$, OR = 2.42; G: $P = 0.02$, OR = 1.53)] and NP categories [rs10887800 (GG: $P = 0.03$, OR = 2.10; G: $P = 0.01$, OR = 1.55), rs2576178 (GG: $P = 0.02$, OR = 2.49; G: $P = 0.009$, OR = 1.67)], indicating an important role of renalase variants with susceptibility to development of PE in Chinese cohort. In contrast, the other renalase polymorphism (rs2296545) failed to demonstrate such an association.

Renalase haplotype distribution among different clinical categories of enrolled subjects

Linkage disequilibrium analysis revealed a higher degree of linkage between all pair of renase gene polymorphisms [rs10887800-rs2576178: $D'=0.867$, $r^2 = 0.678$, Akaike's information criterion (AIC) = 601.84; rs10887800-rs2296545: $D'=-0.738$, $r^2 = 0.413$, AIC = 347.99; rs2576178-rs2296545: $D'=-0.772$, $r^2 = 0.410$, AIC = 344.52]. Case-controls haplotype analysis was performed by SNPAllyze software, and results are shown in Table-3. Prevalence of G-G-C haplotype (rs10887800-rs2576178-rs2296545) of the renase gene was significantly high in PE patients when compared to NNP ($P = 1 \times 10^{-3}$) and NP ($P = 0$) cases. Also, haplotype A-G-G was more frequent in PE patients compared to NP ($P = 0$), indicating an important role of renase haplotype on predisposition to the development of PE pathogenesis.

Association of renase polymorphisms with serum renase levels, blood pressure, and glomerular filtration rate

We observed significant differences in serum renase, blood pressure, and glomerular filtration rate among different groups of enrolled subjects. Based on these observations, we hypothesized that variations in the renase gene (rs10887800, rs2576178, and rs2296545) would be associated with those parameters. As shown in figure-3A, homozygous mutants (GG) of renase rs10887800 polymorphism displayed lower serum renase compared to heterozygous (AG) and wild type (AA). A similar observation was also noticed for rs2576178, AA genotype had higher serum renase levels than heterozygous (AG) and homozygous mutant (GG) subjects (Figure-3B). However, the other renase polymorphism (rs2296545) failed to demonstrate a genotype-phenotype relationship (Figure-3C). Also, no significant association of renase polymorphisms with blood pressure and glomerular filtration rate was noticed (data not shown).

Discussion

PE is a clinical phenotype in pregnant women associated with unrestricted activation of the inflammatory response. Prior shreds of evidence have suggested a substantial role of many inflammatory mediators (including cytokines) in promoting the pathogenesis of PE. However, other biomolecules in pregnant women with distinct physiological functions during pregnancy should also be simultaneously looked at to understand the pathophysiology of PE in detail. Among other biologically relevant molecules, catecholamine levels have been found to be enhanced in pre-eclamptic women [18]. Catecholamines are degraded by different monoamine oxidases, and renase is therefore presumed to play a role in regulating the pathogenesis of PE.

Before examining the role of renase during PE, we analyzed the baseline characteristics in three categories of study populations. Table-1 clearly showed a significant reduction in the duration of gestation, the percentage of vaginal delivery, and fetal birth weight in subjects with PE as compared to healthy pregnant women. On the other hand, pre-eclamptic women demonstrated an increased body mass index (BMI), percentage of caesarian section, systolic and diastolic blood pressure, WBC count,

serum urea, and uric acid levels as compared to healthy controls. All these data are in accordance with the previous report [19].

Importantly, the glomerular filtration rate (GFR) was found to be significantly lower in subjects with PE as compared to healthy pregnant women. Many earlier reports are supporting our results [12], [20]. It is assumed that the decreased GFR in pre-eclamptic subjects may be due to reduced renal blood flow that results due to high renal vascular resistance. An earlier study demonstrated that the decreased GFR in PE subjects is due to a fall in renal blood flow and a reduction in the ultrafiltration coefficient in glomerular capillary [21].

A significant elevation of serum renalase was noticed in pregnant women with healthy gestations as compared to those without pregnancy. Additionally, the level of the enzyme was significantly diminished in subjects with PE. The overall results of the present investigation were in line with previous observations [12, 20]. However, our results are in contrary to a report in Pakistani PE patients [22], that demonstrated comparable renalase levels among subjects with PE, healthy pregnant women, and non-pregnant controls. Discrepancies among studies can be attributed to a lower number of subjects enrolled in the reports from the Pakistan population (non-pregnant controls: $n = 30$, healthy pregnant women: $n = 45$, and subjects with PE: $n = 20$). Compared to the earlier report [22], in the present study, we enrolled sufficiently large numbers of samples, further strengthening our observations.

Renalase is secreted into the peripheral circulation, and its levels are regulated by renal function and catecholamine concentrations [23]. Prior pieces of evidence support the occurrence of systemic and renal hemodynamic alterations during pregnancy [24]. During pregnancy, systemic renal vasodilation is associated with a 30–40% rise in renal blood flow and GFR [24]. Therefore, a higher level of renalase in pregnant subjects in our study may be due to increased GFR.

A notable reduction in renalase level in preeclamptic women was observed in the present study when compared to the healthy pregnancies. Previously, it was shown that the manipulation of gonadotropin-releasing hormone (GnRH) might alter the expression of renalase and GnRH antagonists downregulate the renalase secretion [25]. As the placenta has a central role in PE [26], ischemic changes observed during PE possibly accompany low GnRH level and diminish GnRH would facilitate a low level of renalase in pre-eclamptic women.

Prior evidence from both animal and human suggested a regulatory role of renalase on blood pressure [13]. Further, renalase deficiency was found to be associated with hypertension and increased sympathetic activity [27]. Notably, in patients showing resistance to hypertension, the plasma levels of renalase are inversely associated with systolic blood pressure [28]. Our study indicates an inverse association of renalase level with systolic as well as diastolic blood pressure in study subjects. Collectively, all this evidence strongly demonstrated the regulatory importance of renalase with blood pressure.

We found a direct correlation of serum renalase level with GFR. Prior studies have indicated a direct correlation of GFR with renal mass [20]. Additionally, it is also known that the release of renalase into peripheral blood can be influenced by renal function performance. Since GFR is known as the best index for examining renal function, we speculate the direct relationship of GFR with renalase level. Overall, the results of our investigation and earlier reports highlighted an important pathophysiological relevance of serum renalase in the development of PE.

Genetic association studies on the association of common renalase variants with susceptibility to PE is minimal. To date, two independent studies in Turkish [14] and the Iranian population [15] have demonstrated the importance of renalase mutants on predisposition to PE development. In the present report, we observed a significant association of homozygous mutant (GG) and minor allele (G) of rs10887800 and rs2576178 polymorphisms with risk for development of PE in the Chinese population. These observations are following earlier reports, including Iranian [15] and Turkish patients [14]. However, Bagci et al. failed to demonstrate the association of rs2576178 polymorphism with PE. Furthermore, G-G haplotype (rs10887800-rs2576178) has been associated with susceptibility to PE risk in Iranian patients. Similarly, in the current report rs10887800-G rs2576178-G rs2296545-C haplotype was linked with predisposition to PE when compared to women with or without pregnancy. Our present study is more advantages over the previous report: i) we have included three SNPs of the renalase gene, and ii) normotensive non-pregnant and normotensive pregnant women were enrolled as controls. Collectively results of the present report and earlier studies further strengthn association of renalase polymorphisms with susceptibility to PE.

As we observed significant associations of renalase gene polymorphisms with susceptibility to PE development, further, we analyze the functional relevance of those studied SNPs. Elevated serum renalase was observed in homozygous genotype compared to wild type and heterozygous mutant for rs10887800 and rs2576178 polymorphisms. In contrast, an earlier report highlighted the association of elevated renalase levels in the GG genotype of rs10887800 compared to AA genotype and nonexistence of the relationship between rs2576178 polymorphism and plasma renalase levels in patients undergoing hemodialysis [29]. Also, an earlier report showed an association of high SBP and DBP with GG genotype of rs10887800 polymorphism and for GG genotype of rs2576178 only with SBP. However, in the present study, we did not observe any association of renalase polymorphisms with SBP, DBP, and GFR levels in patients and controls.

Conclusions

Renalase levels diminished in PE patients and negatively associated with SBP and DBP. Lower levels of renalase in PE patients are possibly due to a higher prevalence of GG genotype of rs10887800 and rs2576178 polymorphisms in PE patients. Combinely, the present study revealed a significant role of renalase with susceptibility and pathophysiology of PE in the Chinese population. However, further studies in other communities are required to validate our findings.

Declarations

- **Ethics approval and consent to participate:** The study protocol was approved by the institutional Human Ethical Committee and informed consent was obtained from all participants.
- **Consent for publication:** Not Applicable
- **Availability of data and materials:** Data and materials will be available upon request to corresponding author.
- **Competing interests:** authors declare no conflict of interest.
- **Funding:** No specific fund has received for this report.
- **Authors' contributions:** XL, QH and JX contributed to design, perform experiments, write the manuscript and finalize the manuscript
- **Acknowledgements:** authors would like to thank all patients and healthy controls for their voluntary participation in the present report.

References

1. Duley L: **The global impact of pre-eclampsia and eclampsia.** *Semin Perinatol* 2009, **33**(3):130-137.
2. Tjoa ML, Levine RJ, Karumanchi SA: **Angiogenic factors and preeclampsia.** *Front Biosci* 2007, **12**:2395-2402.
3. English FA, Kenny LC, McCarthy FP: **Risk factors and effective management of preeclampsia.** *Integr Blood Press Control* 2015, **8**:7-12.
4. Bulletins–Obstetrics ACoP: **ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002.** *Obstet Gynecol* 2002, **99**(1):159-167.
5. Say L, Chou D, Gemmill A, Tuncalp O, Moller AB, Daniels J, Gulmezoglu AM, Temmerman M, Alkema L: **Global causes of maternal death: a WHO systematic analysis.** *Lancet Glob Health* 2014, **2**(6):e323-333.
6. Xiao J, Shen F, Xue Q, Chen G, Zeng K, Stone P, Zhao M, Chen Q: **Is ethnicity a risk factor for developing preeclampsia? An analysis of the prevalence of preeclampsia in China.** *J Hum Hypertens* 2014, **28**(11):694-698.
7. Li G, Xu J, Wang P, Velazquez H, Li Y, Wu Y, Desir GV: **Catecholamines regulate the activity, secretion, and synthesis of renalase.** *Circulation* 2008, **117**(10):1277-1282.
8. Xu J, Li G, Wang P, Velazquez H, Yao X, Li Y, Wu Y, Peixoto A, Crowley S, Desir GV: **Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure.** *J Clin Invest* 2005, **115**(5):1275-1280.
9. McCowan L, Stewart AW, Francis A, Gardosi J: **A customised birthweight centile calculator developed for a New Zealand population.** *Aust N Z J Obstet Gynaecol* 2004, **44**(5):428-431.
10. Li X, Huang R, Xie Z, Lin M, Liang Z, Yang Y, Jiang W: **Renalase, a new secretory enzyme: Its role in hypertensive-ischemic cardiovascular diseases.** *Med Sci Monit* 2014, **20**:688-692.

11. Gu R, Lu W, Xie J, Bai J, Xu B: **Renalase deficiency in heart failure model of rats—a potential mechanism underlying circulating norepinephrine accumulation.** *PLoS One* 2011, **6**(1):e14633.
12. Yilmaz ZV, Akkas E, Yildirim T, Yilmaz R, Erdem Y: **A novel marker in pregnant with preeclampsia: renalase.** *J Matern Fetal Neonatal Med* 2017, **30**(7):808-813.
13. Desir GV, Wang L, Peixoto AJ: **Human renalase: a review of its biology, function, and implications for hypertension.** *J Am Soc Hypertens* 2012, **6**(6):417-426.
14. Bagci B, Karakus S, Bagci G, Sancakdar E: **Renalase gene polymorphism is associated with increased blood pressure in preeclampsia.** *Pregnancy Hypertens* 2016, **6**(2):115-120.
15. Teimoori B, Moradi-Shahrehabak M, Rezaei M, Mohammadpour-Gharehbagh A, Salimi S: **Renalase rs10887800 polymorphism is associated with severe pre-eclampsia in southeast Iranian women.** *J Cell Biochem* 2019, **120**(3):3277-3285.
16. Kandil NS, Sharkawy RME, Desouky LMI, Kandil LS, Masoud IM, Amin NG: **Renalase gene polymorphisms (rs2576178 and rs10887800) in Egyptian hypertensive end stage renal disease patients.** *Egyptian Journal of Medical Human Genetics* 2018, **19**(4):379-383.
17. Rezk NA, Zidan HE, Elnaggar YA, Ghorab A: **Renalase gene polymorphism and epinephrine level in chronic kidney disease.** *Appl Biochem Biotechnol* 2015, **175**(4):2309-2317.
18. Kjeldsen SE, Eide I, Aakesson I, Oian P, Maltau JM, Lande K, Gjesdal K: **Increased arterial adrenaline is highly correlated to blood pressure and in vivo platelet function in pre-eclampsia.** *J Hypertens Suppl* 1985, **3**(3):S93-95.
19. Darmochwal-Kolarz D, Michalak M, Kolarz B, Przegalinska-Kalamucka M, Bojarska-Junak A, Sliwa D, Oleszczuk J: **The Role of Interleukin-17, Interleukin-23, and Transforming Growth Factor-beta in Pregnancy Complicated by Placental Insufficiency.** *Biomed Res Int* 2017, **2017**:6904325.
20. Allam HA, Abdelaal DE: **Renalase as a Biomarker in Gestations with Preeclampsia.** *Journal of Gynecology and Women's Health* 2018, **11**(4).
21. Moran P, Baylis PH, Lindheimer MD, Davison JM: **Glomerular ultrafiltration in normal and preeclamptic pregnancy.** *J Am Soc Nephrol* 2003, **14**(3):648-652.
22. Jamil Z, Shahid S, Baig E, Ahmad R, Subhani F, Fatima SS: **Serum anti mullerian hormone and renalase levels in predicting the risk of preeclampsia.** *Taiwan J Obstet Gynecol* 2019, **58**(2):188-191.
23. Xu J, Li G, Wang P, Velazquez H, Yao X, Li Y, Wu Y, Peixoto A, Crowley S, Desir GV: **Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure.** *J Clin Invest* 2005, **115**(5):1275-1280.
24. Tkachenko O, Shchekochikhin D, Schrier RW: **Hormones and hemodynamics in pregnancy.** *Int J Endocrinol Metab* 2014, **12**(2):e14098.
25. Zhou M, Liang T, Wang Y, Jin D, Wang J, Jia L, Zhang S: **Expression and tissue localization of renalase, a novel soluble FAD-dependent protein, in reproductive/steroidogenic systems.** *Mol Biol Rep* 2013, **40**(6):3987-3994.

26. Salafia CM, Pezzullo JC, Ghidini A, Lopez-Zeno JA, Whittington SS: **Clinical correlations of patterns of placental pathology in preterm pre-eclampsia.** *Placenta* 1998, **19**(1):67-72.

27. Zhao Q, Fan Z, He J, Chen S, Li H, Zhang P, Wang L, Hu D, Huang J, Qiang B *et al.* **Renalase gene is a novel susceptibility gene for essential hypertension: a two-stage association study in northern Han Chinese population.** *J Mol Med (Berl)* 2007, **85**(8):877-885.

28. Symplicity HTN1, Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Bohm M: **Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial.** *Lancet* 2010, **376**(9756):1903-1909.

29. Stec A: **Rs10887800 renalase gene polymorphism influences the level of circulating renalase in patients undergoing hemodialysis but not in healthy controls.** *BMC Nephrol* 2017, **18**(1):118.

Tables

Table-1 Baseline characteristics of study subjects

Parameters	Normotensive non-pregnant (n = 158)	Normotensive pregnant (n = 105)	Pregnant with PE (n = 121)
Age (years)	31.6 ± 5.2	33.7 ± 6.6	29.2 ± 4.5
Primiparas (%)	NA	58.9	61.6
Duration of gestation (days)	NA	269.5 ± 19.6	249.5 ± 14.2*
BMI at blood draw (Kg/m ²)	21.1 ± 3.8	26.2 ± 4.1#	30.1 ± 5.2*#
Vaginal delivery (%)	NA	57.4	14.6*
Caesarian section (%)	NA	44.3	86.8*
Fetal birth weight (grams)	NA	3126	2561*
Systolic blood pressure (mmHg)	106.3 ± 0.7	95.3 ± 0.9	142.6 ± 1.3*#
Diastolic blood pressure (mmHg)	79.6 ± 0.5	69.6 ± 0.3	87.8 ± 0.6*#
Serum creatinine (mg/dL)	0.6 ± 0.4	0.5 ± 0.3	0.7 ± 0.3
Glomerular filtration rate (mL/min)	127.7 ± 0.3	133.3 ± 0.5	107.1 ± 1.1*#
White blood cell (x 10 ⁹ /L)	8.9 ± 3.1	9.4 ± 3.1#	10.4 ± 3.7*#
Uric acid (mg/dL)	3.7 ± 2.1	3.9 ± 1.7	6.2 ± 1.9*#
Urea (mg/dL)	17.7 ± 4.7	18.5 ± 4.1	23.09 ± 13.7*#
Data are presented as either means or mean ± SE. NA: Not applicable. *: P < 0.05 - Subjects with PE versus healthy pregnant women; #: P < 0.05 - Subjects with PE versus healthy non pregnant women.			

Table-2 Distribution of reninase gene polymorphisms (rs10887800, rs2576178 and rs2296545) in healthy controls, pregnant woman and preeclampsia patients

Genotype/Allele	NNP (n = 158)	NP (n = 105)	PE (n = 121)	NNP vs NP (P value, OR, 95% CI)	NNP vs PE (P value, OR, 95% CI)	NP vs PE (P value, OR, 95% CI)
rs10887800 (A > G)						
AA	35 (22)	22 (21)	15 (12)	Ref, 1	Ref, 1	Ref, 1
AG	81 (51)	54 (51)	58 (48)	0.87, 1.06, 0.57 to 2.05	0.17, 1.67, 0.82 to 3.32	0.25, 1.57, 0.76 to 3.31
GG	42 (24)	29 (25)	48 (40)	0.85, 1.09, 0.53 to 2.22	0.008, 2.66, 1.28 to 5.72	0.04, 2.42, 1.07 to 5.35
A	151 (48)	98 (47)	88 (36)	Ref, 1	Ref, 1	Ref, 1
G	165 (52)	112 (53)	154 (64)	0.85, 1.04, 0.73 to 1.48	0.007, 1.60, 1.13 to 2.25	0.02, 1.53, 1.05 to 2.22
rs2576178 (A > G)						
AA	32 (20)	25 (24)	17 (14)	Ref, 1	Ref, 1	Ref, 1
AG	76 (48)	47 (45)	48 (40)	0.51, 0.79, 0.42 to 1.51	0.72, 1.18, 0.61 to 2.30	0.35, 1.50, 0.70 to 3.14
GG	50 (32)	33 (31)	56 (46)	0.72, 0.84, 0.43 to 1.66	0.03, 2.10, 1.07 to 4.16	0.02, 2.49, 1.19 to 5.41
A	140 (44)	97 (46)	82 (34)	Ref, 1	Ref, 1	Ref, 1
G	176 (56)	113 (54)	160 (66)	0.72, 0.92, 0.65 to 1.31	0.01, 1.55, 1.10 to 2.20	0.009, 1.67, 1.15 to 2.44
rs2296545 (C > G)						
CC	43 (27)	26 (25)	33 (27)	Ref, 1	Ref, 1	Ref, 1
CG	76 (48)	49 (47)	61 (51)	0.87, 1.06, 0.58 to 1.92	0.88, 1.04, 0.58to 1.81	1, 0.98, 0.51 to 1.84
GG	39 (25)	30 (28)	27 (22)	0.60, 1.27, 0.65 to 2.49	0.86, 0.90, 0.47 to 1.73	0.45, 0.70, 0.35 to 1.51

Note: data are presented in number (%). NNP: Normotensive non-pregnant, NP: normotensive pregnant woman, PE: preeclampsia patients, OR: odds ratio, CI: confidence interval.

Genotype/Allele	NNP (n = 158)	NP (n = 105)	PE (n = 121)	NNP vs NP (P value, OR, 95% CI)	NNP vs PE (P value, OR, 95% CI)	NP vs PE (P value, OR, 95% CI)
C	162 (51)	101 (48)	140 (58)	Ref, 1	Ref, 1	Ref, 1
G	154 (49)	109 (52)	102 (42)	0.53, 1.13, 0.80 to 1.61	0.12, 0.76, 0.54 to 1.07	0.05, 0.67, 0.46 to 0.98
Note: data are presented in number (%). NNP: Normotensive non-pregnant, NP: normotensive pregnant woman, PE: preeclampsia patients, OR: odds ratio, CI: confidence interval.						

Table-3 Haplotype analysis of renalase polymorphisms in

Haplotype	Overall	NTNP	PE	P value
G-G-G	0.4173	0.414	0.4221	0.852
A-A-C	0.3336	0.3642	0.2932	0.071
G-G-C	0.1239	0.0776	0.1836	1.E-3
A-G-C	0.0449	0.0654	0.0174	0.013
A-A-G	0.0338	0.0483	0.0151	0.062
A-G-G	0.016	9.21E-9	0.038	0
G-A-C	0.0154	5.481E-3	0.0306	0.059
G-A-G	0.015	0.0251	6.905E-12	0.033
Haplotype	Overall	NTP	PE	P-value
G-G-G	0.4299	0.4392	0.4221	0.711
A-A-C	0.3383	0.392	0.2932	0.035
G-G-C	0.1262	0.0595	0.1836	0
A-G-C	0.0101	3.609E-7	0.0174	0.19
A-A-G	0.0253	0.0353	0.0151	0.184
A-G-G	0.0378	0.0394	0.038	0.945
G-A-C	0.0298	0.0295	0.0306	0.85
G-A-G	2.611E-3	5.101E-3	6.905E-12	0.44
Note: data are presented in percentage, distribution of haplotype among case and and cotrols were comapred by SNPAlzye software. NNP: Normotensive non-pregnant, NP: normotensive pregnant woman, PE: preeclampsia patients, OR: odds ratio, CI: confidence interval.				

Figures

Figure 1

Serum renalase levels in different clinical categories of enrolled subject. ELISA was employed for quantification of renalase levels in PE (n=121), normotensive pregnant women (n=105) and normotensive non pregnant controls (n=158). Data represented Mean $\mu\text{g/ml} \pm \text{SE}$ and were analyzed with one way ANOVA. $P < 0.01$ was considered statistically significant.

Figure 2

Correlation between serum renalase with blood pressure and glomerular filtration rate. Serum renalase, systolic, and diastolic blood pressure and glomerular filtration rate were measured in all participants and correlation was analyzed by the Spearman rank correlation coefficient. A negative correlation of renalase was observed with diastolic (A), and systolic blood pressure (B) and a positive relationship were noticed with glomerular filtration rate (C). A P-value < 0.01 was considered as significant.

Figure 3

Association of renalase polymorphisms (rs10887800, rs2576178, and rs2296545) with different serum renalase levels in patients and controls. Serum renalase in all participants were quantified by ELISA and their distribution was compared among different genotypes of rs10887800 (A), rs2576178 (B) and rs2296545 (C). ANOVA was used for comparison of differential levels of serum renalase among different genotypes. A P value <0.01 was considered as significant.