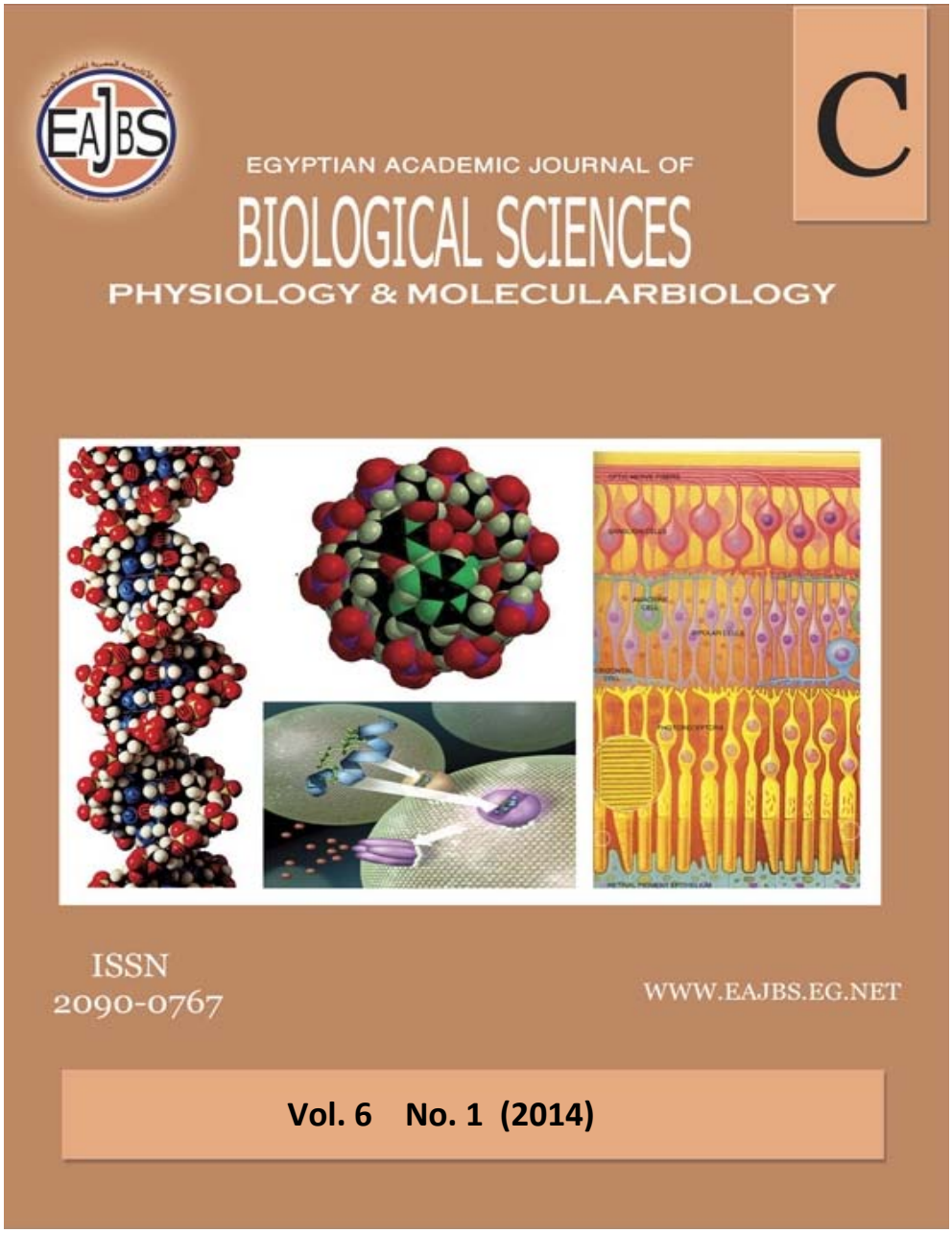

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences ,Department of Entomology ,Faculty of Sciences Ain Shams University .

Physiology & molecular biology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers that elucidate important biological, chemical, or physical mechanisms of broad physiological significance.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 6(1): 141 - 149 (2014)

Egyptian Academic Journal Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767

www.eajbs.eg.net

Association of Angiotensinogen M235T Gene variants, and Plasma Renin Activity with Preeclampsia

Ghada A Elfadil [1,2]; Dalia I. Wagea Alla [3]; Abdelgadir A Elmugadam [1,2]

1- College of Medical Laboratory Science, Sudan University of Science & Technology
2- College of Applied Medical Sciences, Taibah University, KSA
3- Medical Laboratory Science, Sharq-Elneel University College, Sudan

ARTICLE INFO

Article History
Received: 17/3/2014
Accepted: 10/5/2014

Keywords:
AGT gene
Mean arterial pressure
Preeclampsia
Renin
Sudanese

ABSTRACT

Preeclampsia is considered to be a multifactorial and multisystemic disorder with a genetic predisposition. Alterations in the renin–angiotensin system are considered to play a significant role in the pathogenesis of the disease. In order to investigate the possible association of the most common polymorphisms of the renin–angiotensin system genes with preeclampsia we have examined 50 women with preeclampsia and 50 normotensive pregnant women. DNA samples were genotyped for the M235T polymorphism of the angiotensinogen gene (AGT), plasma renin was determined by enzyme linked immunosorbent assay (ELISA).

Plasma renin was significantly decreased in patients with preeclampsia compared with normotensive pregnant women. The TT genotype of the M235T polymorphism was significantly increased in women who developed preeclampsia. In preeclampsia group the frequency of AGT gene, M235T misssense mutation was 92% (18% were heterozygous and 82%were homozygous), compared with 56% in control group (34% heterozygous, 66% homozygous). In preeclampsia group the frequency of M allele; 17% (n=13) versus 61% (n=39) for the control group, while the frequency of T allele in preeclampsia group; 83% (n=47) versus 39% (n=28) for the control group. Furthermore, there was a positive risk of developing preeclampsia when having TT genotype, and the results were highly statistically significant (odd ratio=2.597, X^2= 16.39, P =0.00) for TT genotype compared to MM genotype. There was significant positive correlation between plasma renin activity and mean arterial pressure (MAP) (r= 0.9, p= 0.000). There was no statistical significance between plasma renin activity and age in preeclampsia group (r= 0.044, p= 0.759).

In conclusion, an increased risk for preeclampsia in women carrying the TT genotype of the AGT gene was observed. Plasma renin activity was significantly decreased in patients with preeclampsia.

INTRODUCTION

Preeclampsia (PE) affects 5–7% of all pregnancies and is one of the leading causes of maternal mortality (Sibai B *et al*. 2005).

According to the 2005 World Health Organization report, PE occurs in approximately 3–5% of pregnancies worldwide (WHO. 2005).

Preeclampsia leads to 10–15% of direct maternal deaths, and the incidence is higher in developing countries than in developed countries (Duley L. 2009). The etiology of gestational hypertension and preeclampsia is not precisely known. However, it is acknowledged that these are not monocausal disorders but the result of a complex interaction between maternal constitutional factors, fetal factors, placental factors and pregnancy specific changes (Roberts JM *et al*.2002. Ohkuchi A *et al.* 2007).

Angiotensinogen (AGT) is the precursor of angiotensin II, that plays crucial roles in the regulation of blood pressure. The AGT gene, located on chromosome 1, has been reported to be related to the development of PE and essential hypertension (Ni SH *et al.* 2012). A common polymorphism AGT M235T (the substitution of threonine [Thr] for methionine [Met] at codon 235) was first identified in 1992 (Jeunemaitre X *et al*. 1992), and the Thr235 variant has been reported to be associated with higher AGT levels and abnormal remodeling of the uterine spiral arteries, which is an early cause of PE (Jeunemaitre X *et al*. 1992. Aggarwal S *et al*. 2011).

In the present study we have attempted to evaluate the association of AGT M235T polymorphisms, and serum renin activity with PE in a Sudanese population.

PATIENTS AND METHODS:

This was a cross sectional, case-control study that recruited patients from the antenatal clinic and labor room of Omdurman Maternity Hospital, Sudan, in the period from March 2012 to January 2014.

Fifty women with PE and 50 age-matched controls were included in the study. PE was diagnosed from the presence of blood pressure 140/ 90 mm Hg and proteinuria 0.3 g in a 24-hour urine specimen or urine dipstick 1+ or more that occurred after 20 weeks of gestation.

Patients with hypertension diagnosed before 20 weeks and pre-existing chronic hypertension were excluded. Women with hypertension detected for the first time in pregnancy after 20 weeks or after delivery, but with no previous records, were also excluded. Women with gestational hypertension were also excluded as this entity may be of varied etiological origin. Controls included normotensive women with normal pregnancy outcome. Chronic hypertension and concomitant diabetes or renal and cardiovascular diseases were regarded as exclusion criteria for cases and controls. A written, informed consent was taken from all subjects, and ethical clearance was obtained from the ethical clearance committee of the institute.

Five milliliters of venous blood were collected into two separate K2EDTA containers (2.5ml in each container) from each subject for plasma renin and DNA extraction for genotyping. Plasma was obtained by centrifugation at 3000 rpm for 5 minutes, then stored at -20c till used.

DNA was extracted using salting out method. Polymerase chain reaction (PCR) was used to amplify a 303 base pair fragment in AGT gene (exon 2) using synthetic oligonucleotide primers complementary to the DNA sequence of these regions. The sense primer strand, 5′-GAT GCG CAC AAG GTC CTG TC-3′, and-antisense-strand 5′-CAG GGT GCT GTC CAC ACT GGA CCC C-3, synthesized by the Trilink Biotechnology (San Diego California, USA). dNTPs were obtained from Intron Biotechnology (South Korea), PCR amplifications were conducted by a thermocycler Alpha laboratories (UK).

Each reaction 25 µL PCR reaction contained 2.5mM dNTPs, 2.5 U Taq DNA polymerase, 1µL genomic DNA template (200ng), 1µL of each primer (10pmol/µL), and 17µL nuclease free

water. PCR was carried out under the following conditions: denaturing at 94ºC for 2min, followed by 30 cycles at 94ºC for 20 s, annealing at 57for 30 s, extension at 72ºC for 30 s, and a final extension at 72º C for 5 min.

Ten µl of the 303-bp polymerase chain reaction product was exposed to 1 µl (4U/10 µL PCR product) restriction enzyme TthIII I for 1 hour at 65C° and electrophoresed on a 2% agarose gel with ethidium bromide staining. Gel was observed under UV and the size of RFLP product was compared with the molecular marker and photographed.

Plasma rennin activity was measured using DRG Renin ELISA Kit. The plasma renin activity expressed as pg/mL.

Statistical analysis

SPSS software (version 16) was used for the analysis of clinical variables. Descriptive statistics were used to analyze the demographic characteristics. Variables were compared between preeclampsia and control group by Student's *t* test. Linear regression analysis was used to assess correlation. Genotype and allele frequencies in control and preeclampsia groups were compared by Chi-square ($\chi 2$) analysis. Statistical significance was accepted at $p<0.05$. Odds ratio was used for the measurement of association.

RESULTS

The demographic and clinical data of the patients included in this study are shown in Table 1.

Table 1: Demographic and clinical data of study group

Characteristic	Normotensive pregnant	Preeclampsia pregnant	P
Maternal Age/(years)	25.26 ± 6.127	28.50 ±6.382	0.509
Parity Primigraved Multiparity	19(38.0%) 31(62.0%)	19(38.0%) (31)62.0%	
Proteinuria (±)	Negative(100% -)	+ve(72% ++, 28%+++	
DBP (mmHg)	74.86±2	100.62±17.01	0.000*
SBP (mmHg)	115.2±2.48	141.48±20.721	0.000*
MAP (mmHg)	88.27±2.178	114.35±16.87	0.000*
Renin (pg/ml)	77.9140±13.96	40.3560±9.92	0.000*

DBP= Diastolic Blood pressure, **SBP**= Systolic Blood pressure, **MAP**= mean arterial blood pressure
The table shows the mean ± SD and percent (%)
t-test was used for comparison.
* P value ≤ 0.05 is considered significant

Figure 1 shows the results of the Tth111I digestion on PCR products. AGT +704 T→C missense mutation (lead to amino acid substitution of AGT M235T) created a new restriction site with the sequence recognition: GACN NNGT↓C for Tth111I. Tth111I digested the fragment into 2 parts, the longer fragment; 279 bp and the shorter 24 bp. However, the 2% agarose gel was unable to retain the shorter fragment and it was suspected to have migrated out of the gel. Therefore, a band at 303 bp indicates homozygous wild-type (MM), a band at 279 bp indicates homozygous mutated (MT), and two bands at 303 bp and 279 bp indicates heterozygous mutation (TT).

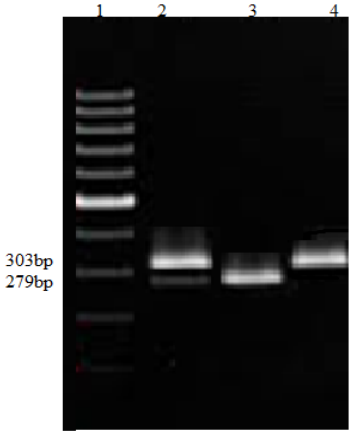

Fig 1: Agarose gel electrophoresis. Lane 1 is a 100 bp linear DNA ladder. Lanes 2 ,3 and 4 correspond to RFLP pattern of heterozygous(MT), homozygous mutant (TT), and homozygous wild-type (MM), respectively.

In preeclampsia group the frequency AGT M235T misssense mutation was 92% (18% were heterozygous and 82% were homozygous), compared with 56% in control group (34% heterozygous, 66%homozygous). In preeclampsia group the frequency of M allele; 17% (n=13) versus 61% (n=39) for the control group, while the frequency of T allele in preeclampsia group; 83% (n=47) versus 39% (n=28) for the control group as shown in Table 2. Furthermore, there was a positive risk of developing preeclampsia when having TT genotype, and the results were highly statistically significant (odd ratio=2.597, $\chi 2$= 16.39, P=0.00) for TT genotype compared to MM genotype

Table2: Distribution of Genotypes and allele of AGT gene M235T polymorphism in study group.

	Genotypes			Alleles	
	MM	MT	TT	M	T
Control	44% (n=22)	34% (n=17)	22% (n=11)	61% (n=39)	39% (n=28)
Cases	8% (n=4)	18% (n=9)	74% (n=37)	17% (n=13)	92% (n=46)
Total	26%	26%	48%		
$\chi 2$= 16.39.	odd ratio =2.597		df=1	P value= 0.000 *	

There were statistical significance in the mean of serum renin according to AGT genotypes as in Table 3, in preeclampsia; a maximum renin activity is associated with TT genotype, also there was significant positive correlation between plasma renin activity and MAP (r = 0.9, p = 0.000), while there was no statistical significance between plasma renin activity and age in preeclampsia group (r = 0.044, p = 0.759).

Table 3: means of plasma renin activity levels according to genotype of AGT gene in study group.

Genotype		Number	Means ±SD (pg/ml)	P value
MM	case	22	30.37±4.205	0.00*
	control	4	62.92±2.300	
MT	case	17	45.99±1.779	0.00*
	control	9	67.78±0.972	
TT	case	37	51.63±5.214	0.00*
	control	11	82.00±14.022	

The table shows the mean ± SD and probability (P).
t-test was used for comparison.
* P value ≤ 0.05 is considered significant

DISCUSSION

Preeclampsia is a multifactorial disorder with significant genetic contribution. It contributes to 16% of maternal and 23% of prenatal deaths worldwide (Khan KS *et al*. 2006. Khan KS *et al*. 2006).

PE is a significant obstetric problem in Sudan, however, the contribution of genetic polymorphisms to PE have not been well studied in this country. The present study showed that preeclampsia occurs at maternal age range from 15 to 38 years with mean (28.50±6.382 years), maternal age was considered to be risk factor for preeclampsia especially in young teenagers and women over 35 years (WHO. 2008). Nationwide US data suggest that the risk of preeclampsia increases by 30% for every additional year of age past 34 (Duckitt K *et al*. 2005). Our study showed 62% preeclampsia patients had multiparity. Epidemiological and family based studies in several geographically and ethnically distinct populations indicate that preeclampsia is a multifactorial disorder with a familial tendency and it is influenced by race, parity, health status of placenta, diet and body size. First degree relatives of women with preeclampsia have a fivefold increased risk while second degree relatives have twofold increased risk of having the disease compared with women who have no family history (Bouba I *et al*. 2003).

In the current study the mean of plasma renin activity (PRA) value among preeclampsia was significantly lower than that of control group; in addition there was a positive significant correlation between PRA and MAP. This finding has also been detected in previous studies (Bouba I *et al*. 2003, August P *et al*. 1990). The absence of stimulation of the renin angiotensinogen aldosterone system (RAAS) in women developing hypertension during pregnancy or in women with preeclampsia has been confirmed by many studies (Bouba I *et al*. 2003, August P *et al*. 1990).

Association studies of renin angiotensinogen system polymorphisms with PE from various other population groups have shown inconclusive results (Kim YJ *et al*. 2004, Medica I *et al*. 2007, Serrano NC *et al*. 2006, Kaur R *et al*. 2005).

In our study maximum renin activity in the preeclampsia was noted in carriers of TT genotype of AGT gene M235T polymorphism as compared to other genotypes. Despite the fact that plasma renin activity decreased in preeclampsia, an increase of its gene expression in the placental tissue and reciprocal inhibition of circulating RAAS activity were noted (Herse, R. *et al*. 2007, L. Anton and K. B. Brosnihan. 2008). The development of preeclampsia in TT genotype carriers was associated with maximum activity of plasma renin. Heterozygous genotype of the AGT gene M235T polymorphism in pregnant women with hypertension was associated with a 1.13-fold decrease in renin activity, and MM genotype with a 1.7-fold decrease (P=0.019) as compared to the TT genotype, these findings are agreed with study done by Radkov *et al*. 2013. This specific feature distinguishes preeclampsia from arterial hypertension in non-pregnant individuals, when TT genotype is associated with maximum angiotensinogen concentration and minimum plasma renin activity (Radkov *et al*. 2013).

In conclusion, an increased risk for preeclampsia in women carrying the TT genotype of the AGT gene was observed. Plasma renin activity was significantly decreased in preeclampsia.

REFERENCES

Aggarwal S, Dimri N, Tandon I and Agarwal S. (2011). Preeclampsia in

North Indian women: the contribution of genetic polymorphisms. J Obstet Gynaecol Res, 37: 1335–1341.

August P, Lenz T, Ales KL/ (1990). Longitudinal study of the renin–angiotensin–aldosterone system in hypertensive pregnant women: deviations related to the development of superimposed preeclampsia.Am J Obstet Gynaecol, 63:1612–21.

Bouba I, Makrydimasa G, Kalaitzidisb R, Dimitrios E. (2003). Interaction between the polymorphisms of the renin–angiotensin system in preeclampsia. European Journal of Obstetrics & Gynecology and Reproductive Biology., 110: 8–11

Duckitt K, Harrington D. (2005). Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. BMJ, 330:565.

Duley L. (2009). The global impact of pre-eclampsia and eclampsia. Semin Perinatol; 33: 130–137.

Herse, R. Dechend, N. K. Harsen, (2007). Hypertension, 49(5):604-611.

Jeunemaitre, X. Soubrier F, Kotelevtsev YV. (1992). Molecular basis of human hypertension: role of angiotensinogen. Cell, 71:169-180.

Kaur R, Jain V, Khuller M, Gupta I, Sherawat BS. (2005). Association of angiotensin-converting enzyme gene polymorphism with pregnancy-induced hypertension. Acta Obstet Gynecol Scand, 84: 929–933.

Khan KS, Wojdyla D, Say L. (2006). WHO analysis of causes of maternal death: A systematic review. Lancet; 367: 1066.

Kim YJ, Park MH, Park HS, Lee KS, Ha EH, Pang MG. (2004). Associations of polymorphisms of the angiotensinogen M235 polymorphism and angiotensin-converting-enzyme intron16 insertion/ deletion polymorphism with preeclampsia in Korean women. Eur J Obstet Gynecol Reprod Biol., 116: 48–53

L Anton and K. B. Brosnihan (2008). Ther. Adv. Cardiovasc. Dis., 2(5): 349-362

Medica I, Kastrin A, Peterlin B. (2007). Genetic polymorphisms in vasoactive genes and preeclampsia:A meta-analysis. Eur J Obstet Gynecol Reprod Biol; 131: 115–126.

Ngoc NT, Merialdi M, Abdel-Aleem H. (2006). Causes of stillbirths and early neonatal deaths: Data from 7993 pregnancies in six developing countries. Bull World Health Organ, 84: 699–705.

Ni SH, Zhang Y, Deng Y, Gong Y, Huang J, Bai Yand Zhou R. (2012). AGT M235T polymorphism contributes to risk of preeclampsia: evidence from a meta-analysis. Journal of the Renin-Angiotensin- Aldosterone System Aug 28.

Ohkuchi, A.; Hirashima, C. and Matsubara, S. (2007). Alterations in placental growth factor levels before and after the onset of preeclampsia are more pronounced in women with early onset severe preeclampsia. Hypertens Res., 30:151–159.

Radkov OV, Kalinkin M. N., and Zavarin V. V. (2013). Genophenotypic Analysis of Angiotensinogen Gene M235T Polymorphism and Preeclampsia. Bulletin of Experimental Biology and Medicine, 154: No. 3.

Roberts JM, Lain KY. (2002). Recent Insights into the pathogenesis of pre-eclampsia. Placenta, 23: 359–372.

Serrano NC, Díaz LA, Páez MC. (2006). Angiotensin-converting enzyme I/D polymorphism and preeclampsia risk: Evidence of small-study bias. PLoS Med; 3: 520.

Sibai B, Dekker G, KupfermincM. (2006). Pre-eclampsia. Lancet 2005; 365: 785–799.

WHO. (2008). managing eclampsia, Geneva.World health organization.

WHO. (2005). World health report: make every mother and child count. Geneva: WHO, p.63.

ARABIC SUMMARY

العلاقة بين المتغيرات الجينية لجين الأنجيوتنسين M235T و رينين البلازما عند النساء المصابات بتسمم الحمل

غادة الفاضل[1و2] – داليا وقيع الله[3] – عبدالقادر المقدم[1و2]

1- كلية علوم المختبرات الطبية – جامعة السودان للعلوم والتكنلوجيا

2- كلية العلوم الطبية التطبيقية – جامعة طيبة – السعودية

3- المختبرات الطبية – كلية شرق النيل الجامعية

يعتبر تسمم الحمل اضطراب متعدد العوامل مع وجود الاستعداد الوراثي للمرض . التغيرات التى تحدث في نظام الرينين أنجيوتنسين تلعب دورا هاما في حدوث المرض. من أجل التحقق من وجود ارتباط وراثى محتمل في الطفرات الجنيه فى نظام الرينين قمنا بفحص 50 امرأة مصابه بتسمم الحمل و 50 امراه سليمه كعينه ضابطه. تم مرمزة الحمض النووي لتعدد الأشكال M235T من الجين مولد الأنجيوتنسين (AGT)، وقياس تركيز الرينين فى بلازما الدم بواسطة انزيم مرتبط المناعي فحص (ELISA).

اظهرت النتائج انخفاض مستوى رينين البلازما بشكل ملحوظ في المرضى الذين يعانون من تسمم الحمل مقارنة بالنساء الحوامل فى العينه الضابطه . لوحظ ان هنالك زيادة فى النمط الجيني TT من تعدد الأشكال M235Tبشكل كبير في النساء المصابات بتسمم الحمل.

الذين لديهم الطفرة M235T فى الجين AGT 92 ٪ من المرضى (18 ٪ كان متخالف و 82 ٪ كانت متماثلة اللواقح) ، مقارنة مع 56 ٪ في المجموعة الضابطة (34 ٪ متخالف ، 66 ٪ متماثل) . تواتر الأليل M ؛ 17 ٪ (عدد = 13) مقابل 61 ٪ (عدد = 39) للمجموعة الضابطة ، في حين أن تواتر الأليل T في مجموعة تسمم الحمل ؛ 83 ٪ (عدد = 47) مقابل 39 ٪ (عدد = 28) للمجموعة الضابطة . علاوة على ذلك، كان هناك خطر إيجابية لحدوث تسمم الحمل عند وجود TT الوراثي ، وكانت النتائج ذات دلالة إحصائية عالية (نسبة = 2.597 ، $X^2 = 16.39$ ، ($P = 0.00$) ل TT الوراثي مقارنة MM الوراثي . كان هناك علاقة إيجابية هامة بين رينين البلازما و الضغط الشرياني. ولم تكن هناك أي دلالة إحصائية بين رينين البلازما والعمر في مجموعة المرضى $P = 0.759$