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

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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 the M235T polymorphism was significantly increased in women who developed preeclampsia. In preeclampsia group the frequency of AGT gene, M235T missense 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, X²= 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 -20°C 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
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PCR was carried out under the following conditions: denaturing at 94°C for 2 min, followed by 30 cycles at 94°C for 20 s, annealing at 57°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°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 65°C 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 rennin 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 (χ2) 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>
<thead>
<tr>
<th>Characteristic</th>
<th>Normotensive pregnant</th>
<th>Preeclampsia pregnant</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age/(years)</td>
<td>25.26 ± 6.127</td>
<td>28.50 ±6.382</td>
<td>0.509</td>
</tr>
<tr>
<td>Parity Primigravida</td>
<td>19(38.0%)</td>
<td>19(38.0%)</td>
<td></td>
</tr>
<tr>
<td>Multiparity</td>
<td>31(62.0%)</td>
<td>(31)62.0%</td>
<td></td>
</tr>
<tr>
<td>Proteinuria (+)</td>
<td>Negative(100% -)</td>
<td>+ve(72% ++, 28%+++</td>
<td></td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74.86±2</td>
<td>100.62±17.01</td>
<td>0.000*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115.2±2.48</td>
<td>141.48±20.721</td>
<td>0.000*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>88.27±2.178</td>
<td>114.35±16.87</td>
<td>0.000*</td>
</tr>
<tr>
<td>Renin (pg/ml)</td>
<td>77.9140±13.96</td>
<td>40.3560±9.92</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

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 TthI111I 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↓ for TthI111I. TthI111I 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 missense 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.

Table 2: Distribution of Genotypes and allele of AGT gene M235T polymorphism in study group.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td>MT</td>
<td>TT</td>
</tr>
<tr>
<td>Control</td>
<td>44% (n=22)</td>
<td>34% (n=17)</td>
</tr>
<tr>
<td>Cases</td>
<td>8% (n=4)</td>
<td>18% (n=9)</td>
</tr>
<tr>
<td>Total</td>
<td>26%</td>
<td>26%</td>
</tr>
</tbody>
</table>

$\chi^2$ = 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.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Means ±SD (pg/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td>case</td>
<td>22</td>
<td>30.37±4.205</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>4</td>
<td>62.92±2.300</td>
</tr>
<tr>
<td>MT</td>
<td>case</td>
<td>17</td>
<td>45.99±1.779</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>9</td>
<td>67.78±0.972</td>
</tr>
<tr>
<td>TT</td>
<td>case</td>
<td>37</td>
<td>51.63±5.214</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>11</td>
<td>82.00±14.022</td>
</tr>
</tbody>
</table>

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


العلاقة بين المتغيرات الجينية لجين الانجيوتنسين M235T ونين البلازما عند النساء المصابات بتسمم الحمل

غادة الفاضل١ - داليا وقع الله١ - عبدالقادر المقدم١
1 - كلية علوم المختبرات الطبية - جامعة السودان للعلوم والتكنولوجيا
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 التي تحدث في نظام الرينين أنجيوتنسين تلعب دورا هاما في حدوث المرض. من أجل التحقق من وجود ارتباط وراثي محتمل في الطرقات الجينية في نظام الرينين نقوم بفحص 50 امرأة مصابة بتسمم الحمل و 50 امرأة سليمة (AGT)، كعينه ضابطة. تم مزج تفصاب المرض النووي لتعدد الأشكال M235T من الرينين مولد الانجيوتنسين (ELISA). وقياس تركيز الرينين في بلازما الدم بواسطة ترميز مرتبط المناعي فحص (ELISA).

ظهرت النتائج انخفاض مستوي رنين البلازما بشكل ملحوظ في المرضى الذين يعانون من تسمم الحمل مقترنة بالنساء الحوامل في العينة الضابطة. لوحظ ان هناك زيادة في النطاق الجيني TT من تعدد الأشكال في النساء المصابات بتسمم الحمل بشكل كبير في النساء المصابات بتسمم الحمل M235T الذين لديهم الوراثة AGT 92% من المرضى (18% كان متفاوت و82% كانت متماثلة اللوائح)، مقابلة مع 56% في المجموعة الضابطة (34% متفاوت، 66% متماثل). تأثر الأليل M متماثل اللوائح (34% متفاوت، 66% متماثل). تأثر الأليل T ؛ 17% (عدد = 13) مقابل 61% (عدد = 39) لمجموعة الضابطة، في حين أن تأثر الأليل T في مجموعة تسهم الحمل ؛ 83% (عدد = 47) مقابل 39% (عدد = 28) لمجموعة الضابطة. على ذلك، كان هناك خطر إيجابية لتحديد حمل الوراثة عند وجود الوراثي الفرعي، وكانت النتائج ذات دلاله إحصائية عالية (نسبة X² ل P = 0.00 ، X² = 16.39 ، V = 2.597 بين رنين البلازما و الضغط الشرياني. ولم تكن هناك أي دلاله إحصائية بين رنين البلازما والعمر في M235T مرضا 0.759.