Increased plasma Angiotensinogen levels and its association with the M235T gene polymorphism and hypertension in Calabar and Uyo cities, Nigeria

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Abstract

Hypertension results from an interaction of many risk genes and environmental factors. The M235T allele of the angiotensinogen gene is thought to increase plasma levels of the angiotensinogen which is associated with hypertension. The angiotensinogen is an important substrate for renin in the RAAS that is finally converted into angiotensin II that plays a key role in the control of blood pressure. This study was designed to measure plasma angiotensinogen levels in an adult population in Calabar and Uyo (South-South), Nigeria in relation to the M235T allele and hypertension. Out of a large population of 1224 participants who had been genotyped for the M235T polymorphism, plasma was collected from a sample of 300 consisting of 150 patients and 150 controls. Protein A sandwich enzyme linked immunosorbent assay was carried out with the plasma samples to measure the angiotensinogen levels. Age, BMI, M235T allele, blood pressure and O.D values were compared between controls and patients using the independent t test. The absorbance values of plasma angiotensinogen were significantly higher in the patients (0.71) with M235T allele than in the controls (0.53). Further research still needs to be carried out to determine the actual concentration of the protein in the participants.

Keywords: angiotensinogen, M235T allele, hypertension, plasma.
1. INTRODUCTION

The M235T polymorphism of the angiotensinogen gene has been associated with a 10% - 30% increase in plasma AGT. Chronic increases in plasma AGT concentration is believed to slightly increase blood pressure thus facilitating hypertension (Corvol and Jeunemaitre, 1997).

The renin-angiotensin (R-A) system is a powerful pressure system which influences salt and water homeostasis. Angiotensinogen (AGT) is a key component of this system, it is cleaved by renin to yield angiotensinogen 1 (AGT 1), which is cleaved by angiotensinogen converting enzyme (ACE) to yield angiotensinogen II (AGT II), responsible for carrying out a range of functions that include i) prompting the constriction of blood vessels causing a rise in blood pressure, ii) ensuring the release of aldosterone by the adrenal cortex which acts on the tubules causing absorption of more water and salt from urine. Blood volume increases so does blood pressure. Potassium ions are excreted from the tubules in exchange for sodium iii) mediates the release of antidiuretic hormone from the pituitary that enhances the reabsorption of water, it also increases an individual’s appetite for salt and stimulates the sensation of thirst (Caulfield et al., 1994). The measurement of angiotensinogen concentration has proved to be a convenient method for monitoring the activity of R-A system in human populations since it circulates at relatively constant level. In Nigerians, Rotimi et al (1997) reported a significant association between the presence of the M235T allele and high mean AGT concentration which was also significantly related to hypertension status, though Cooper et al (1999) reported a high level of plasma angiotensinogen but low hypertension status in Igbo Ora, Nigeria. There are no documented data on the levels of angiotensinogen in populations in the southern parts of Nigeria.

This research was carried out to determine angiotensinogen levels in hypertensive patients and controls in a population in South- south Nigeria.

METHODOLOGY

A total of 300 individuals, 150 patients and 150 controls were selected from a adult population of 1224 who had been genotyped for the M235T angiotensinogen gene polymorphism by polymerase chain reaction(PCR) and restriction fragment polymorphism, these methods were described in detail (Kooffreh et al, 2012). Their plasma samples were screened using protein A sandwich ELISA for their angiotensinogen levels. The age, weight, height (was used to calculate Body mass index-BMI), Systolic and Diastolic blood pressure readings were also recorded.
control and hypertensive groups were compared by chi-square analysis. Continuous variables were compared between hypertensives and controls by independent t test. Means and standard deviation was used to describe results.

RESULTS
For the genotyping of the angiotensinogen polymorphism, The normal individual M235M gives an undigested 165bp, a mutant individual M235T gives two fragments of 141bp and 24bp. Recessive individual T235T gives a 141bp fragment. However agarose gel allows the visualization of a 165bp fragment for M235M, a 141bp fragment for T235T, a 165bp and 141bp for the M235T individuals respectively. Figs 1 and 2 (Procopciuc et al, 2002)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Patients</th>
</tr>
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<tbody>
<tr>
<td>TT</td>
<td>0.49 ± 0.20</td>
<td>0.66 ± 0.33</td>
</tr>
<tr>
<td>MT</td>
<td>0.53 ± 0.27</td>
<td>0.71 ± 0.33</td>
</tr>
<tr>
<td>MM</td>
<td>0.28</td>
<td>0.41</td>
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</tbody>
</table>

Table 1. Plasma angiotensinogen ELISA values and demographic characteristics among patients and controls

DISCUSSION
A sample population of individuals who had been genotyped for the M235T allele of the angiotensinogen gene were investigated using a protein A sandwich ELISA to measure plasma angiotensinogen levels in relation to the M235T allele. The T allele was associated with increased plasma AGT (Bloem et al, 1997, Rotimi et al, 1997). Elevation in plasma levels of angiotensinogen has been associated with hypertension. The protein A sandwich ELISA was sensitive enough to identify the presence of the angiotensinogen in the plasma of patients and controls, the mean O.D values for plasma angiotensinogen was significantly higher in the patients than the controls with the mutant T allele implying an association with hypertension which is in line with literature (Jeunemaitre et al, 1992 and Corvol et al, 1999). Corvol and Jeunemaitre (1997) reported that the M235T allele was associated with a 10-30% increase in plasma angiotensinogen which is able to increase blood pressure, thus facilitating hypertension. The elevation of O.D. values of angiotensinogen may suggest a role of the M235T genotype in hypertension however this needs further confirmation in larger sample size of this population.

CONCLUSION
The absorbance values of plasma angiotensinogen were significantly higher in the patients with M235T allele than in the controls. Further research still needs...
5. References


