ABSTRACT

Objective: Hypertension is a common but incompletely understood disease. Emerging evidence supports a novel view of hypertension as a disease of inadequate or aberrant responses to angiogenic factors. In the present study we aimed to investigate the serum levels of vascular endothelial growth factor (VEGF), an angiogenic factor and soluble angiopoetin receptor Tie-2 (sTie-2) in patient with essential hypertension.

Methods: In the present study 90 individuals (mean age 48 ± 7 years, 56 males and 34 females) have been divided in to three groups as 30 patients with hypertension, 30 healthy individuals (with a family history of hypertension) and 30 healthy individuals (no family history of hypertension). All individuals have been evaluated in terms of blood pressure and biochemical parameters. The levels of VEGF and Tie-2 receptor have been evaluated by using The enzyme-linked immunosorbent assay (ELISA) method.

Results: The findings suggested that the serum VEGF, soluble Tie-2 receptor, LDL and triglyceride levels in the hypertensive patients were significantly higher than in the controls (p < 0.05). However, the level of HDL-cholesterol in the patients was significantly lower than in the controls (p < 0.05). In correlation analysis, a positive correlation was found statistically significant between the values of VEGF and sTie-2 (r = 0.405, p = 0.026).

Conclusion: As a result of this study, our data indicate that serum levels of VEGF and Tie-2 receptor may be related to the primer hypertension. This study could inspire to further studies to explore the roles of VEGF and Tie-2 receptor in essential hypertension.

Keywords: Angiopoetin receptor, endotelial disfunction, hypertension, Tie-2 receptor, VEGF

From: 1Departments of Physiology and 2Department of Cardiology, Cumhuriyet University School of Medicine, 58140 Sivas, Turkey.

Correspondence: Dr E Ozdemir, Department of Physiology, Cumhuriyet University School of Medicine, 58140 Sivas, Turkey. E-mail: ercan_ozdemir@hotmail.com . Tel: +90 346 219 1010, Fax: +90 346 219 1602

West Indian Med J;  DOI: 10.7727/wimj.2015.357
INTRODUCTION

Hypertension is an important medical problem because constant high blood pressure has harmful effects on target organs such as the heart and kidney, bringing about severe cardiovascular disease and renal failure (1). However, the pathophysiological mechanisms of essential hypertension are not exactly elucidated. Essential hypertension is associated with altered function and structure of vessels, as well as altered platelet function and insulin resistance and imbalance in angiogenesis (2, 3). Angiogenesis, the formation of new blood vessels from pre-existing vessels, is the physiological process that occurs in response to tissue cell hypoxia and other stimuli (4). Enhanced nitric oxide (NO) in several processes and cardiovascular disorders increases angiogenesis and reduced NO biosynthesis disrupts angiogenesis in several tissues (5). Angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) induce NO biosynthesis. Several studies indicate that angiogenesis is impaired in arterial hypertension (6, 7).

Evidence encourages a novel view of hypertension as a disease of insufficient or abnormal responses to angiogenic growth factors such as VEGF. Hypertensive patients have decreased microvascular density, with some evidence supporting a significant role for rarefaction in hypertension (8). Changes in different angiogenic markers have been shown in essential hypertension. For instance, serum levels of VEGF are increased in hypertension, and correlate with cardiovascular risk and normalizes with treatment (9) demonstrating a relation between proangiogenic factors and arterial blood pressure.

Angiopoietins are proangiogenic factors indispensable for vascular development and maturation in angiogenesis (10, 11). Angiopoietin-1 (Ang-1) and its antagonist angiopoietin-2 (Ang-2) bind to the tyrosine kinase receptor (Tie-2) which is particularly expressed on vascular endothelial cells (12, 13). Angiopoietin-1 is released by vascular smooth muscle cells...
and pericytes in blood vessel and stabilizes the development of newly formed blood vessels by recruiting vascular smooth muscle cells and pericytes. In addition, this angiogenic factor supports structural integrity of mature vessels (14). Ang-1 has robust vascular protective effects which is suppressing plasma leakage, preventing vascular inflammation and endothelial cell death. Whereas, this molecule has also been implicated in induction of angiogenesis and pulmonary hypertension (10). Vascular endothelial growth factor and angiopoietins/Tie-2 receptor system are important regulators of angiogenesis and stimulated by a lot of factors including markers of hypoxia and inflammation (15–17). It is likely that in arterial hypertension, which is related with rarefaction of the small vessels, these factors are increased as a compensative mechanism for the hypoxia that this rarefied small vessels may cause (6). Accordingly, it has been suggested that Ang-2 and its receptor Tie-2 are increased in the presence of relative hypoxia (18, 19).

In the presence of VEGF, Ang-2 may play a important role in stabilizing vessels, sprouting and regression for pathophysiological angiogenesis (20). Chen et al (21) suggested that the angiopoietins/Tie-2 system is essential for the protection of vascular system integrity, vessel remodeling and induction of angiogenesis. The role of Ang-1 and Ang-2 in the reorganization of angiogenesis is dependent on other growth factors (VEGF, fibroblast growth factor and platelet derivated growth factor).

Otherwise, it is known that the angiopoietin/Tie-2 system influences vascular smooth muscle hyperplasia in primer pulmonary hypertension (22). However, the pathophysiological role of VEGF and the angiopoietin/Tie-2 signalling pathway in essential hypertension has not been studied in humans. In light of this information, our aim of the study was to investigate the serum levels of vascular endothelial growth factor and soluble angiopoetin receptor Tie-2 (sTie-2) in patient with essential hypertension.
METHODS

Study population and design

Thirty patients previously diagnosed with essential hypertension, 30 healthy individuals (control group 1) and 30 healthy individuals (control group 2, with a family history of hypertension) admitted to the Cardiology Department of Cumhuriyet University Education and Research Hospital between March 2012 and April 2013 were included in the study. The volunteer subjects were informed about the procedures. The study protocol was approved by the Cumhuriyet University School of Medicine Ethics Committee (Ethic No: 2011/061) and written informed consent was obtained from all participants in accordance with the Helsinki Declaration.

The participant with a history of antihypertensive drug use, peripheral artery disease, cancer, diabetes mellitus, atherosclerotic heart disease and heart failure is not included in the study. The demographic characteristics (age, gender, body weight and height) of volunteers were recorded and measurements of body mass index (BMI) were carried out separately. Fasting blood glucose, serum electrolytes and lipids were measured and renal function tests were performed.

Measurement of Blood Pressure

Participants rested for about 10 minutes before arterial blood pressure was measured. The systolic (SBP) and diastolic (DBP) blood pressures were measured at the same time every day for seven days to identify hypertensive patients. Hypertension stages were determined by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7). The blood pressure defined four categories in the JNC-7 guidelines (23): normal blood pressure (SBP < 120 mm Hg and DBP < 80 mm Hg), prehypertension (SBP 120–139 mm Hg or DBP 80–89 mm Hg), Stage I hypertension (SBP 140–159 mm Hg
or DBP 90–99 mm Hg) and Stage II hypertension (SBP > 160 mm Hg or DBP > 100 mm Hg).

**Biochemical Parameters**

Fasting blood glucose by enzymatic hexokinase method, creatinine, high density lipoprotein, low density lipoprotein and triglyceride by enzymatic colorimetric method and blood urea nitrogen by kinetic test were measured.

**Measurement of VEGF and soluble Tie-2 (sTie-2) receptor**

Venous blood samples (3 mL) were collected from each hypertensive patients and health individuals. All blood samples were drawn during routine blood tests on the same day and processed within one hour. Serum was separated by centrifugation at 4000 g for five min at 4 °C and sample aliquots were immediately stored at -70 °C until assayed. Serum concentrations of VEGF (VEGF_{165}) and sTie-2 were measured in duplicate with a quantitative enzyme-linked immunosorbent assay (ELISA) technique (Boster Biological Technology Co. Ltd. Fremont, CA 94538 USA) according to the manufacturer’s guidelines.

**Statistical Analysis**

Statistical analysis was performed using SPSS for Windows version 17.0 (SPSS, Chicago, IL, USA). Results are expressed as mean with standard deviation (SD) or as median with inter-quartile range for the normally distributed data and skewed data respectively. Baseline characteristics of groups were compared using Chi-squared test test. Non-parametric tests (Kruskall-Wallis test for three independent samples and Bonferroni corrected Mann-Whitney test for statistical difference between the two groups) were used to compare the three groups. Spearman's correlation test was used for correlation analysis. $p < 0.05$ was considered statistically significant.
RESULTS

The demographic and clinical characteristics of the study population

Demographic characteristics of 90 subjects (n = 30 for all groups) are summarized in Table 1.

Table 1: Baseline demographic and clinical characteristics of all subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient (n = 30)</th>
<th>Control 1 (n = 30)</th>
<th>Control 2 (n = 30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>19/11</td>
<td>21/9</td>
<td>16/14</td>
<td>0.182</td>
</tr>
<tr>
<td>(％)</td>
<td>(63/37)</td>
<td>(70/30)</td>
<td>(53/47)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.13 ± 6.32</td>
<td>48.86±7.03</td>
<td>47.33±8.14</td>
<td>0.512</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>78.40 ± 19.32</td>
<td>77.28±21.06</td>
<td>73.50±18.30</td>
<td>0.634</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68±0.07</td>
<td>1.67±0.08</td>
<td>1.68±0.10</td>
<td>0.781</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.65±3.90</td>
<td>27.60±3.67</td>
<td>26.06±2.63</td>
<td>0.247</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>148.50±17.42</td>
<td>109.83±5.16</td>
<td>106.50±7.08</td>
<td>0.020*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>95.33±11.05</td>
<td>66.00±6.07</td>
<td>59.50±8.02</td>
<td>0.003**</td>
</tr>
</tbody>
</table>

Control 1, participants with a family no history of hypertension; Control 2, participants with a family history of hypertension. Data were expressed as mean ± SD. *p < 0.05 and **p < 0.01, the differences between the patient group and the control groups.

There was no statistically significant difference between mean age of the patient (50.13 ± 6.32 years), control 1 (48.86 ± 7.03 years), and control 2 (47.33 ± 8.14 years) groups. Body weight (kg) and height (m) showed no difference between all study groups. All groups compared to body mass index (BMI), the difference was not significant (p > 0.05).

Comparison of the biochemical parameters of hypertensive subject with controls

Serum levels of creatinine, fasting blood glucose, sodium, potassium, calcium and BUN in patient group was not statistically significant compared with the controls (Table 2). HDL-cholesterol (mg/dL) level of the patient group (37.80 ± 8.93) was significantly lower than the control 1 (43.03 ± 7.58) and control 2 [42.93 ± 4.38] (p < 0.05). However, the patient’s serum
LDL-cholesterol [mg/dL] (93.37 ± 27.30) and TG (139.20 ± 80.30) levels were significantly higher in the control 1 (69.80 ± 14.72; 83.06 ± 16.32, respectively) and control 2 [71.30 ± 10.94; 79.26 ± 18.96, respectively] \((p < 0.01)\).

Table 2: Biochemical parameters of patients and the control group.

<table>
<thead>
<tr>
<th></th>
<th>Patient ((n = 30))</th>
<th>Control 1 ((n = 30))</th>
<th>Control 2 ((n = 30))</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Ca}^{2+}) (µmol/L)</td>
<td>8.81 ± 0.50</td>
<td>8.85 ± 0.48</td>
<td>8.78 ± 0.44</td>
<td>0.827</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>85.0 ± 10.50</td>
<td>87.9 ± 10.70</td>
<td>86.4 ± 10.30</td>
<td>0.216</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>13.2 ± 2.70</td>
<td>12.8 ± 3.10</td>
<td>14.5 ± 4.40</td>
<td>0.105</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>0.82 ± 0.20</td>
<td>0.80 ± 0.20</td>
<td>0.84 ± 0.20</td>
<td>0.911</td>
</tr>
<tr>
<td>Na(^+) (mmol/L)</td>
<td>140.2 ± 2.30</td>
<td>139.5 ± 3.60</td>
<td>141.2 ± 0.40</td>
<td>0.780</td>
</tr>
<tr>
<td>K(^+) (mmol/L)</td>
<td>4.5 ± 0.40</td>
<td>4.5 ± 0.30</td>
<td>4.5 ± 0.40</td>
<td>0.683</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>37.80 ± 8.93</td>
<td>43.03 ± 7.58</td>
<td>42.93 ± 4.38</td>
<td>0.023*</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>93.37 ± 27.30</td>
<td>69.80 ± 14.72</td>
<td>71.30 ± 10.94</td>
<td>0.009**</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>139.20 ± 80.30</td>
<td>83.06 ± 16.32</td>
<td>79.26 ± 18.96</td>
<td>0.004**</td>
</tr>
</tbody>
</table>

FBG, fasting blood glucose; BUN; blood urea nitrogen, Cr, creatinine; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride. *\(p < 0.05\), compared to control 1 and 2; **\(p < 0.01\), compared to patient.

**Serum levels of VEGF and sTie-2 receptor in patient and control groups**

The serum levels of VEGF in patients (51.91 ± 7.83) were significantly higher than the control 1 (35.03 ± 3.71) and control 2 (27.19 ± 3.21) \([p < 0.01; \text{Fig. 1}]\).

![Fig. 1](image-url)  

**Fig. 1.** Serum levels of VEGF in patient and control groups. Data are given as mean ± SD. VEGF, vascular endothelial growth factor. *\(p < 0.01\) compared with control 1 and control 2 subjects.
VEGF and Tie-2 Receptor Levels in Hypertension

Similarly, sTie-2 receptor levels of patients (193.13 ± 41.38) were statistically significant higher compared to the control 1 (116.11 ± 9.02) and control 2 (104.30 ± 16.60) \([p < 0.05; \text{Fig. 2}].\)

![sTie-2](image)

**Fig. 2.** Seum levels of sTie-2 receptor in patient and control groups. Data are given as mean ± SD. sTie-2, soluble Tie-2 receptor. *\(p < 0.05\) compared with control 1 and control 2 subjects.

Serum VEGF and sTie-2 levels according to the stage of hypertension in patients

VEGF values were evaluated according to the stages of hypertension. The serum levels of VEGF in stage 2 group (53.20 ± 12.17) were higher than in prehypertension (39.71 ± 6.78) and stage 1 group (45.68 ± 10.23). However, the difference between groups was not statistically significant (Fig. 3, \(p > 0.05\)).

![VEGF](image)

**Fig. 3.** Seum levels of VEGF in patients. Data are given as mean ± SD. \(n = 8\), prehypertension; \(n = 11\), stage 1 and \(n = 11\), stage 2.
Compared the concentration of sTie-2 receptor according to the stage of hypertension, the levels of sTie-2 in stage 2 group (251.55 ± 39.94) were significantly higher than in prehypertension (78.02 ± 13.26) and stage 1 group (115.89 ± 26.69) [Fig. 4; $p = 0.001$, $p = 0.032$, respectively].

**Fig. 4.** Serum levels of sTie-2 in patients. Data are given as mean ± SD. *$p < 0.05$ compared with prehypertension and stage 2 subjects ($n = 8$, prehypertension; $n = 11$, stage 1 and $n = 11$, stage 2).

**Correlation analysis levels of serum VEGF and sTie-2 receptor in patients**

A positive correlation was found statistically significant between the values of VEGF and sTie-2 [$r = 0.405$, $p = 0.026$, Spearman’s test] (Fig. 5).

**Fig. 5.** The correlation between VEGF and sTie-2 parameters in patients with hypertension. ($n = 30$). $r = 0.405$, $p < 0.05$ (Spearman’s test).
DISCUSSION

This is the first comprehensive study on circulating VEGF and sTie-2 receptor in essential hypertension. The decisive results are: (i) compared with healthy or disease controls, hypertensive patients are characterized by an excess of circulating sTie-2 and VEGF; (ii) the concentrations of sTie-2 receptor in stage 2 group were significantly increased; (iii) the levels of VEGF were correlated with the sTie-2 receptor in patients; (iv) in accordance with hypertension, HDL-cholesterol levels in patients was found to be extremely low. These findings have implications for the role of angiopoetins in the pathogenesis of arterial hypertension.

Hypertension is a very widespread but poorly understood disease. There are many explanations for pathophysiological mechanisms in essential hypertension, including increased adrenergic activity, impaired renin-angiotensin-aldosteron system, constitutional and environmental factors (24). Obtained evidences support a novel view of hypertension, as a disease of insufficient or abnormal responses to angiogenic factors and its associated vascular rarefaction and remodeling (8, 25).

At the present time, the best way to prevent or decrease cardiovascular complications in hypertension is lowering the blood pressure. For all that, several studies have demonstrated that controlling blood pressure does not fully prevent vascular or renal complication in essential hypertension (26). For this reason, we need more understanding regarding the vascular changes related to blood pressure, endothelial dysfunction or vascular remodeling about the angiogenic factors in the pathophysiological mechanism in hypertension (6, 26). Kim et al. suggested that Ang-1, as an angiogenic factor, has the miscellaneous vascular effects on microvascular rarefaction and target organ damage in hypertension (27). Ang-1 is involved in angiogenesis and increases endothelial stabilization by boosting vascular integrity. In addition, the specific characteristic of Ang-1 is the formation of tight vascular network,
compared to VEGF (28, 29). Angiopoietin and its receptors (Tie-1 and Tie-2) have important roles in the late stages of vascular development, where they control stabilization and formation of the vessels. Therefore, Ang-1 is required for excellent organization and maturation of newly formed vessels, and supports the stability and structural integrity of vasculature (10). Ang-2 demonstrates very different features on angiogenesis. Ang-1 stimulates Tie-2 receptor and Ang-2 can inhibit this receptor. Whereas, Ang-2 demonstrates angiogenic activity only in the presence of VEGF (27).

Tie-2 is an angiopoietin receptor and is extensively expressed on the endothelial cells. This receptor also plays a significant role in angiogenesis (30). The Tie-2 receptor seems to be important for vascular stabilization and angiogenic remodelling that occur after the effects of VEGF (31, 32). Several reports indicate that the Ang-1/Tie-2 signalling pathway is involved in endothelial cell-matrix interactions (33). Therefore, this signalling pathway also helps stabilize new vessel formation and the alterations of all these three in hypertensives reflects the abnormal angiogenesis that is seen here. The soluble form of the Tie-2 receptor in plasma has been identified both in diseased and in healthy humans (32). However, the exact role of soluble form of this receptor in essential hypertension is not known completely. Plasma levels of soluble Tie-2 are increased in malignant tumors (34) and congestive cardiac failure (35). In the present analysis, we have demonstrated that soluble Tie-2 levels elevated in patients with hypertension. On the other hand, Lee et al (28) suggested that Ang-1 prevents hypertension and target organ damage through its interaction with vascular endothelial Tie-2 receptor. Ang-1 was found to be effective in preventing hypertension and reducing target organ damage in hypertensive rats. In addition, Ang-1 was shown to quite increase the plasma level of nitric oxide (NO), through the endothelial specific Tie-2/endothelial NO synthase (eNOS) signalling pathway (29). Circulating NO plays a significant role in controlling arterial blood pressure by regulating vasodilation and it is also important for sustaining endothelial homeostasis.
Consistent with our findings, an analysis of 248 patients with hypertension demonstrated a positive correlation between more severe hypertension and higher VEGF levels (2). Bevacizumab, a recombinant human monoclonal antibody to VEGF, has been attempted as anti-angiogenic treatment of various cancers including renal cell and colorectal carcinoma (36, 37). However, it has been indicated that bevacizumab treatment induces hypertension in patients with cancer (38).

In conclusion, our findings suggested that the changes serum levels of VEGF leads to hypertension and increased sTie-2 receptor level may be associated with essential hypertension. In the light of these findings, it may be concluded that serum VEGF and angiopoietin/Tie-2 signalling system play an important role in essential hypertension.

ACKNOWLEDGEMENTS

This research was supported by Cumhuriyet University Scientific Research Project (T-514, CUBAP, Sivas, Turkey).
REFERENCES


