An evaluation of the serum biomarkers of Endothelial dysfunction in hypertensive and hypercholesterolemic subjects: A case-control study

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Abstract
Objective: To evaluate the serum biomarkers of Endothelial dysfunction in hypertensive and hypercholesterolemic subjects.

Methods: This was a case-control study conducted in a tertiary care hospital. The patients with hypertension and hypercholesterolemic were diagnosed using 7th Joint National Committee criteria. The cases were with primary Hypertensive and hypercholesterolemic patients without any other complication. Controls were healthy subjects without any complication. A total of 60 cases and 30 controls were included in the study.

Results: More than one third of patients of both cases (41.7%) and controls (43.3%) were above 60 years of age. More than half of patients of both cases (60%) and controls (56.7%) were males. There was no significant difference (p>0.05) in age and gender between cases and controls showing comparability of the groups in terms of age and gender. A significant (p=0.0001) increased level of total cholesterol, LDL and triglycerides was observed among cases compared to controls. However, significant (p=0.004) decreased level of HDL was found among cases than controls. Serum uric acid, creatinine, nitric oxide, hs-CRP and IL-6 were significantly (p=0.0001) increased among the cases than controls.

Conclusion: Endothelial dysfunction is an important component of primary hypertension as well as that of hypercholesterolemic state, the severity being determined by nitric oxide, hs-CRP, LDL cholesterol and HDL cholesterol levels independently.

Keywords: Hypertension, Hypercholesterolemic, Endothelial dysfunction.

Introduction
Traditional risk factors such as arterial hypertension, hypercholesteremia, hyperglycemia, smoking, age and obesity may initiate endothelial damage (Jalali, 2011). Endothelial dysfunction is a condition comprising not only attenuated endothelium dependent vasodilatation but also activation of endothelial inflammation. Endothelial dysfunction is a well-accepted predictor of atherosclerosis development and future cardiovascular events, but its role in hypertension is less well understood. It would be
naïve to assume that decreased endothelial vasodilator release through an increase in peripheral resistance directly translates into hypertension. It is well established that not only metabolic and local nervous factors have a much stronger effect on local vascular tone but also the renal and central control of blood pressure overrule local vascular factors in their effect on blood pressure (Brandes, 2014).

Hypertension is a common disease that causes significant human morbidity and mortality worldwide. Across the globe, 26% of the world population suffers from hypertension and is estimated to rise to 29% by the year 2025. As per American Heart Association, one third of the adult population of U.S. is hypertensive but one third of these remain undiagnosed. Hypertension predisposes to heart failure, ventricular arrhythmia, blindness, renal failure and other serious diseases resulting in high mortality each year worldwide (Kearney et al, 2005). WHO has reported that in adults aged 40-55 years, Indian males rank highest among those of 20 other developing countries in Blood pressure levels. In fact according to recent studies the current prevalence of hypertension in India is around 30-35% (Gupta, 2004). Of these, only 42% urban and 25% rural Indians are aware of their hypertensive status. And on top of that only 38% of urban and 25% rural Indians are being treated for hypertension. One-fifth of urban and one-tenth of rural Indian hypertensive population have their BP under control (Anchala et al, 2014).

The present case-control study was designed to evaluate the serum biomarkers of Endothelial dysfunction in hypertensive and hypercholesterolemic subjects.

**Material and Methods**

This was a case-control study conducted in a tertiary care hospital. The study was approved by the Ethical Committee of the Institute. The consent was taken from each participant before enrolling in the study. The patients with hypertension and hypercholesterolemic were diagnosed using 7th Joint National Committee criteria. The cases were with primary Hypertensive and hypercholesterolemic patients without any other complication. Controls were healthy subjects without any complication. A total of 60 cases and 30 controls were included in the study. Patients with history of diabetes, renal impairment, hepatic dysfunction, any other cardiac disease, acute infectious state, critically ill, septicemic and moribund patients were excluded from the study.

**Methods**

The protocol used for the study was in accordance with guidelines of institutional ethical committee. Sixty patients included in the study with primary hypertension and hypercholesterolemic were diagnosed using 7th JNC criteria. Subjects were included in the study if their medications were stable for three months and their blood pressure was ≥140/90 mm Hg.

**Blood Pressure Measurement:** The subjects were asked to rest for at least five minutes. The blood pressure was measured in both the upper extremities in the supine, sitting and standing position. An average of two sitting readings on two different visits was taken before assigning to specific group of JNC VII classification.

**Collection of Blood and Isolation of Serum:** Venous blood (5ml) for estimation of IL-6, hs-CRP, NO, serum uric acid, serum creatinine and lipid profile content were drawn after informed written consent from all the study group subjects with a disposable syringe & needle, under all aseptic conditions. Serum was separated by centrifuging the blood at 300 rpm for 20 minutes. Samples were stored in aliquots at -20° C until assayed. Nitric Oxide (NO) in serum was determined indirectly, by the measurement of stable decomposition products nitrite and nitrate, employing the Griess reaction.

**Statistical analysis**

The results are presented in frequencies, percentages and mean±SD. The Chi-square test was used to compare the categorical variables. The Unpaired t-test was used to compare
biomarkers between cases and controls. The p-value < 0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Results
More than one third of patients of both cases (41.7%) and controls (43.3%) were above 60 years of age. More than half of patients of both cases (60%) and controls (56.7%) were males. There was no significant difference (p>0.05) in age and gender between cases and controls showing comparability of the groups in terms of age and gender (Table-1).

A significant (p=0.0001) increased level of total cholesterol, LDL and triglycerides was observed among cases compared to controls. However, significant (p=0.004) decreased level of HDL was found among cases than controls (Table-2).

Serum uric acid, creatinine, nitric oxide, hs-CRP and IL-6 were significantly (p=0.0001) increased among the cases than controls (Table-3).

Table-1: Age and gender distribution between cases and controls

<table>
<thead>
<tr>
<th>Age and gender</th>
<th>Cases (n=60)</th>
<th>Controls (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>14</td>
<td>23.3</td>
<td>7</td>
</tr>
<tr>
<td>40-60</td>
<td>21</td>
<td>35.0</td>
<td>10</td>
</tr>
<tr>
<td>&gt;60</td>
<td>25</td>
<td>41.7</td>
<td>13</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>60.0</td>
<td>17</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>40.0</td>
<td>13</td>
</tr>
</tbody>
</table>

Chi-square test

Table-2: Comparison of lipid profile between cases and controls

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Cases (n=30)</th>
<th>Controls (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>221.6±23.2</td>
<td>183±23.5</td>
<td>0.0001*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>32.3±6.1</td>
<td>42.7±6.4</td>
<td>0.004*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>157.4±24.6</td>
<td>105.38±22.9</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>211.6±67.3</td>
<td>173.5±72.1</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

Unpaired t-test, *Significant

Table-3: Comparison of other biomarkers between cases and controls

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Cases (n=30)</th>
<th>Controls (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum uric acid (µmol/l)</td>
<td>258.50±40.52</td>
<td>177.5±23.5</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>80.56±24.15</td>
<td>62.15±18.8</td>
<td>0.0004*</td>
</tr>
<tr>
<td>Serum nitric oxide (µM)</td>
<td>5.98±1.25</td>
<td>7.8±2.14</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Serum hs-CRP (mg/L)</td>
<td>3.91±0.46</td>
<td>1.14±0.25</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Serum IL-6 (pg/ml)</td>
<td>11.05±3.12</td>
<td>6.05±1.15</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

Unpaired t-test, *Significant

Discussion
Interleukin 6 is a cytokine that performs functions in both innate and adaptive immunoresponses. It is synthesized by monocytes, endothelial cells, fibroblasts and other types of cells in response to microorganisms, but can also be stimulated by other cytokines, primarily interleukin-1 (IL-1) and TNF-α (Souza et al, 2008).

C-reactive protein is an inflammatory marker synthesized in hepatocytes in response to primary stimulation from IL-6. Under acute inflammatory conditions, CRP levels increase during the first 6 to 8 hours and may reach levels as high as 300 mg/dl within 48 hours (Steffel and Luscher, 2009).
C-reactive protein can be considered a biomarker of the process of endothelial dysfunction (Souza et al, 2008) and, at supraphysiological concentrations, as a predictor of vascular disease. It also plays an important role in down regulation of eNOS and in transcription of endothelial cells, which causes destabilization of eNOS-RNA. This process resulted in reduced production of NO (Tonet et al, 2010).

In this study, serum uric acid, creatinine, nitric oxide, hs-CRP and IL-6 were significantly (p=0.0001) increased among the cases than controls. Endothelial dysfunction, commonly observed in cardiovascular and renal diseases, is attributed to oxidative stress, dyslipidemia, accumulation of endogenous inhibitors of NO synthase, genetic factors, and other causes (Brunner et al, 2005).

The experimental data directly implicate UA in endothelial dysfunction (Khosla et al, 2005), but few studies were conducted in humans and available data are controversial. In patients with heart failure, with type 2 diabetes, at increased cardiovascular risk, and with hypercholesterolemia but not in patients with essential hypertension, allopurinol, a xanthine oxidase inhibitor that lowers UA and interacts with anion superoxide generation, improves endothelial dysfunction (Li and Shah, 2004).

In the study of Mercuro et al (2004), the beneficial effect of allopurinol could have been a direct consequence of the reduced UA levels rather than of superoxide anions mediated by xanthine oxidase inhibition because of the close correlation found between the amount of that decrease and the improvement of endothelial function. It is interesting that recent findings suggest that fructose-induced hyperuricemia reduce endothelial NO levels and induce insulin resistance, components of the metabolic syndrome (Nakagawa et al, 2005).

In healthy humans, there is an inverse circadian relationship between serum UA levels and NO, supporting the hypothesis that UA impairs endothelial function, even if this relationship does not necessarily suggest a causal and unidirectional relationship between UA and NO bioavailability. In keeping with this, L-arginine reduces serum UA (Maxwell and Bruinsma, 2001), a phenomenon that supports the reverse hypothesis that UA is modified by increased NO availability. Short-term infusion of UA in normal individuals does not modify the forearm blood response to basal blood flow and responses to ACh, SNP, and L-NG-monomethylarginine (Waring et al, 2004).

**Conclusion**

Endothelial dysfunction is an important component of primary hypertension as well as that of hypercholesterolemic state, the severity being determined by nitric oxide, hs-CRP, LDL cholesterol and HDL cholesterol levels independently.

**References**

6. Souza JR, Oliveira RT, Blotta MH, Coelho OR. Níveis séricos de interleucina-6 (IL-6), interleucina-18 (IL-18) e proteína-C reativa (PCR) na síndrome coronariana


