Evaluation of Homocysteine, hsCRP and Microalbuminuria in Hypertensives without and with end Organ Damage-A Case Control Study

Authors
Hermes R.S1*, Dr Santhi Silambanan2, Dr Emmanuel Bhaskar3, Dr Kalaiselvi V.S.4
1Research Scholar, 2Professor & Head, Dept of Biochemistry Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai, India
3Professor, Dept of General Medicine, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai, India
4Professor Dept of Biochemistry, Sri Balaji Medical College and Hospital, Chennai, India
1*Corresponding Author

Abstract

Background: Homocysteine considered to be a marker of endothelial dysfunction is formed from a sulfur-containing amino acid, methionine. The total plasma homocysteine levels are between 5-15 µmol / L in healthy individuals. As reported by various prevalence studies, the rate is found to be comparatively higher in Indians with a mean level of 19.5-23.2 µmol / L. Hyperhomocysteinemia is the most common risk factor for stroke and cardiovascular diseases by causing vascular dysfunction. Elevated levels of homocysteine could also lead to alterations in mediators of endothelial vasodilatation.

Methods: A total of 496 individuals between the age group of 20-55 years of age, from both sexes who were attending the Hypertensive clinic and Master health check up programme in Sri Ramachandra Medical College & Research Institute were enrolled for the study. They were grouped as three-Group I being controls, group II being hypertensives without complications and group III being hypertensives with end organ damage. After overnight fasting, blood and spot urine samples were collected. All the biochemical parameters were estimated by standard methodologies. The plasma homocysteine was determined by ELISA method (Axis Shield, UK), urinary microalbumin and hsCRP was determined by Immunoturbidimetric method. Albumin Creatinine Ratio (ACR) was also calculated.

Results: The results are expressed as Mean ± SD. The mean values of SBP, DBP and BMI showed a statistically highly significant difference between the 3 groups (p<0.001). Regarding the biochemical parameters LDL c, Chol /HDL ratio , the renal parameters microalbumin, ACR and the markers plasma homocysteine and the hsCRP levels showed a statistically highly significant A strong positive correlation was detected between hsCRP and DBP between the group 2 & 3.

Conclusion: The inflammatory marker hsCRP was found to show a strong positive association with DBP among hypertensives with and without end organ damage indicating that the process of inflammation starts early in the disease. Homocysteine correlates with urinary microalbumin and albumin /creatinine ratio indicating that renal dysfunction could be due to hyperhomocysteinemia.
A significant increase in microalbumin, hsCRP and homocysteine in essential hypertensives suggests that monitoring the levels of these parameters would be of great value in assessing the onset of end organ damage in essential hypertensives. Hyperhomocysteinemia plays a role in the setting of endothelial morphology and function.

**Keywords:** Cardiovascular risk, Endothelial dysfunction, Essential hypertension, Homocysteine, hsCRP, Microalbuminuria.

**Introduction**

Cardiovascular disease is a major contributor of mortality worldwide; the precipitating factor being essential hypertension. It is usually asymptomatic and manifests as severe organ damage. Homocysteine plays a prominent role in the pathogenesis of vascular diseases presenting as increase in blood pressure. Hyperhomocysteinemia (HHcy) is found to be a causative factor in the pathogenesis of vascular lesions in patients with hypertension especially in Indians with a different genetic variations compared to other populations. Microalbuminuria is a marker of renal injury that can often be detected earlier than any tangible decline in glomerular filtration rate. Microalbuminuria is now widely accepted as an independent risk factor for cardiovascular morbidity and mortality \[^1\]. Homocysteine causes endothelial dysfunction by increasing oxidation of endothelium, altering balance of vasomotor dilatation and constriction, and also increasing the coagulability of blood \[^2\]. Studies regarding homocysteine causing renal damage in hypertensives is lacking. The aim of this study was to assess the influence of Hcy on microalbuminuria in essential hypertensives with and without end organ damage.

Many risk factors have been identified leading to an increased blood pressure. Thus emphasis has been given to the early diagnosis at prehypertension stage itself. There is lot of research indicating the role of vascular inflammation in the setting and progression of hypertension\[^3\]. Tumor Necrosis Factor-a (TNF-a), Interleukin-6 (IL6) and C-Reactive Protein (CRP) are markedly elevated in people with hypertension \[^4\].

**Materials & Methods**

**Type of study:** Case-Control Study.

Group I consists of healthy individuals with normal blood pressure without any history of any illness or disease.

Group II consists of newly diagnosed hypertensives as well as known hypertensives under control without any evidence of end organ damage viz renal, cardiac, retinal changes and serum creatinine level < 1.2 mg/dl.

Group III consists of hypertensives with clinical or laboratory evidence of end organ damage viz renal cardiac, retinal changes and serum creatinine level > 1.2 mg/dl.

People taking steroids, oral contraceptive pills and fibric acid derivatives and subjects having diabetes mellitus, smokers and chronic alcoholics were excluded from the study.

This study was conducted in hypertensive clinic of General Medicine Department in Sri Ramachandra Medical College. The study was been approved by the Institutional Ethics Committee. Out of 296 samples, 173 belonged to Group I, 161 to Group II and 162 to Group III. The fasting blood and spot urine samples were collected from the subjects after informed consent. The anthropometric measurements were recorded. The plasma homocysteine was determined by ELISA method (Axis Shield, UK), urinary microalbumin and hsCRP was determined by Immuno turbidimetric method. Albumin Creatinine Ratio (ACR) was also calculated as urinary albumin concentration (mg)/ mmol of creatinine.

The obtained data were analysed by ANOVA and post hoc test. SPSS Software Version 19.0 was used. P value <0.05 was considered significant.
Results
The values are expressed as Mean ± SD
A statistically significant difference was observed in waist hip ratio (p value <0.05) . A highly statistical difference was observed in SBP, DBP and BMI ( p value <0.001).( Table 1)
There is no statistical significant difference in plasma glucose values between the groups. The total cholesterol, triglyceride, HDL cholesterol showed a significant difference (p value <0.05) between the groups. There is statistically highly significant difference (P value <0.001) between the groups with respect to other biochemical parameters. (Table 2)
The plasma homocysteine levels showed a highly statistical difference indicating that homocysteine levels show a marked increase as disease progresses. The mean hsCRP levels are higher in hypertensives with end organ damage.
There is statistically highly significant difference (p value <0.001) between the groups in SBP and DBP. Homocysteine and hsCRP. There is significant difference in Total cholesterol , HDL c, LDL c, Chol /HDL ratio, urinary microalbumin and ACR between groups 2 & 3 between 1 & 3, except HDL c which shows significant difference between 1 & 2. Regarding the anthropometric measurements, Waist /hip ratio shows a statistical difference between group 1 & 2 and a highly statistical difference between group 1 & 3. (Table 3)

Correlation analysis of homocysteine with other variables of the 3 different groups
When homocysteine was compared against routine biochemical parameters, microalbuminuria and albumin /creatinine ratio in group 2 showed correlation with p value <0.05. Other biochemical parameters in group 1,2 as well as 3 did not show significant difference in correlation with homocysteine.(Fig 1 & 2)

Correlation analysis of hsCRP with DBP in Group 2 & 3
In the same way , when hsCRP was compared against routine biochemical parameters , Diastolic blood pressure in group 2&3 showed correlation with p value <0.05. Other biochemical parameters in groups 1,2 as well as 3 did not show significant correlation with hsCRP (Fig 3 & 4).

Table 1 : Showing anthropometric data and blood pressure measurements

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Group-1 (n=173)</th>
<th>Group-2 (n=161)</th>
<th>Group-3 (n=162)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age (years)</td>
<td>40 ± 10</td>
<td>49 ± 8</td>
<td>48 ± 7</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>SBP(mmHg)</td>
<td>114 ± 9</td>
<td>136 ± 15</td>
<td>141 ± 16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3.</td>
<td>DBP(mmHg)</td>
<td>77 ± 6</td>
<td>88 ± 11</td>
<td>90 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4.</td>
<td>BMI(kg/m²)</td>
<td>23.6 ± 3.4</td>
<td>24.1 ± 3.38</td>
<td>25 ± 3.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5.</td>
<td>Waist / Hip Ratio</td>
<td>0.87 ± 0.08</td>
<td>0.89 ± 0.08</td>
<td>0.89 ± 0.05</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Table 2 : Biochemical parameters of the 3 groups

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Group-1 (n=173)</th>
<th>Group-2 (n=161)</th>
<th>Group-3 (n=162)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Plasma glucose (F)(mg/dl)</td>
<td>84.9 ± 19.11</td>
<td>88 ± 12.4</td>
<td>86 ± 15.0</td>
<td>0.197</td>
</tr>
<tr>
<td>2.</td>
<td>Plasma glucose ( PP)(mg/dl)</td>
<td>103.21 ± 31.0</td>
<td>106 ± 19.0</td>
<td>107 ± 29.2</td>
<td>0.411</td>
</tr>
<tr>
<td>3.</td>
<td>Total chol (mg/dl)</td>
<td>156.86 ± 26.15</td>
<td>157.03 ± 39.0</td>
<td>166.2 ± 31.0</td>
<td>0.012</td>
</tr>
<tr>
<td>4.</td>
<td>Triglyceride (mg/dl)</td>
<td>111.01 ± 58.43</td>
<td>119.34 ± 45.04</td>
<td>128 ± 48.0</td>
<td>0.010</td>
</tr>
<tr>
<td>5.</td>
<td>HDL-C (mg/dl)</td>
<td>44.63 ± 8.31</td>
<td>47 ± 8.0</td>
<td>44.5 ± 8.1</td>
<td>0.008</td>
</tr>
<tr>
<td>6.</td>
<td>LDL c (mg/dl)</td>
<td>108.78 ± 25.11</td>
<td>113.09 ± 26.30</td>
<td>125.2 ± 31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7.</td>
<td>Chol /HDL ratio</td>
<td>3.59 ± 0.83</td>
<td>3.37 ± 0.96</td>
<td>3.8 ± 0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8.</td>
<td>Urinary microalbumin mg /L</td>
<td>10.71 ± 8.28</td>
<td>16 ± 20</td>
<td>53.1 ± 93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>9.</td>
<td>ACR(mg/ mmol)</td>
<td>1.2 ± 1.44</td>
<td>1.75 ± 1.84</td>
<td>4.6 ± 10.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10.</td>
<td>Homocysteine (µmol/L)</td>
<td>20.28 ± 12.8</td>
<td>28.63 ± 13.51</td>
<td>43.8 ± 14.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>11.</td>
<td>hsCRP(mg/dl)</td>
<td>0.97 ± 0.75</td>
<td>2.01 ± 1.46</td>
<td>3.93 ± 1.29</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*P value <0.05 is considered statistically significant.
**P value <0.001 is considered statistically highly significant
Table 3: Multiple comparative study between the 3 groups

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Group 1 &amp; 2 p value</th>
<th>Group 2 &amp; 3 p value</th>
<th>Group 1 &amp; 3 p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>SBP (mm Hg)</td>
<td>&lt; 0.001 **</td>
<td>0.035 *</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>2.</td>
<td>DBP (mm Hg)</td>
<td>&lt; 0.001 **</td>
<td>0.057</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>3.</td>
<td>BMI (kg/m²)</td>
<td>0.307</td>
<td>0.482</td>
<td>0.022 *</td>
</tr>
<tr>
<td>4.</td>
<td>Waist / Hip ratio</td>
<td>0.039 *</td>
<td>0.846</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>5.</td>
<td>Total chol (mg/dl)</td>
<td>0.899</td>
<td>0.028 *</td>
<td>0.021 *</td>
</tr>
<tr>
<td>6.</td>
<td>Triglyceride (mg/dl)</td>
<td>0.296</td>
<td>0.253</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>7.</td>
<td>HDL-Chol (mg/dl)</td>
<td>&lt;0.001 **</td>
<td>&lt;0.001 **</td>
<td>0.899</td>
</tr>
<tr>
<td>8.</td>
<td>LDL c (mg/dl)</td>
<td>0.324</td>
<td>&lt;0.001 **</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>9.</td>
<td>Chol /HDL ratio</td>
<td>0.081</td>
<td>&lt;0.001 **</td>
<td>0.030 *</td>
</tr>
<tr>
<td>10.</td>
<td>Urinary Microalbumin mg/L</td>
<td>0.652</td>
<td>&lt;0.001 **</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>11.</td>
<td>A/C Ratio (mg)/ mmol</td>
<td>0.661</td>
<td>&lt;0.001 **</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>12.</td>
<td>Homocysteine (µmol/L)</td>
<td>&lt;0.001 **</td>
<td>&lt;0.001 **</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>13.</td>
<td>hsCRP (mg/dl)</td>
<td>&lt;0.001 **</td>
<td>&lt;0.001 **</td>
<td>&lt;0.001 **</td>
</tr>
</tbody>
</table>

*P value <0.05 is considered statistically significant.

**P value <0.001 is considered statistically highly significant.

Fig 1: Correlation of Homocysteine with urinary microalbumin in Group 2 (n=161)

\[ y = 0.272x + 8.067 \]
\[ R^2 = 0.034 \]
\[ p \text{ value} = 0.018 \]

Fig 2: Correlation of Homocysteine with ACR in Group 2 (n=161)

\[ y = 0.023x + 1.068 \]
\[ R^2 = 0.030 \]
\[ p \text{ value} = 0.026 \]
Discussion
It has already been known that Hyperhomocysteinemia (Hhcy) has a major role in hypertension. Patients with slightly higher blood levels of homocysteine compared to normal are found to be predisposed to endothelial diseases, in the absence of other risk factors of endothelial dysfunction. Hyperhomocysteinemia causes impaired functioning and endothelial and smooth muscle cells of vessels [5,6].

It is found that Hhcy contributes to elevation in the blood pressure by diminishing vasodilatation by specific vasodilators like nitric oxide, causing the increased release of reactive oxygen species and leads to alterations in the elasticity of the vascular wall [7].

Studies by [8,9] have shown that impaired vasodilatation correlates with high levels of Hcy in essential hypertension. The mechanism involves a possible interaction between Hcy and endothelium by increasing thrombogenesis thus precipitating angina or myocardial infarction. Recent studies have demonstrated the deleterious effects of increased plasma Hcy levels on the
vital organs. Diet induced chronic Hhcy also leads to alterations in cardiac and renal function [10,11]. Leakage of small amounts of proteins in urine has been considered since 1980s a crucial sign of early kidney disease, especially in hypertensive patients. In hypertension, microalbuminuria was shown to associated with altered metabolism [12,13,14] Essential hypertension causes renal ischemia and nephrosclerosis, leading to renal impairment [15].

Correlation analysis showed that the levels of plasma Hcy had positive relationship with microalbuminuria (r=0.946, P<0.01) [16]. In our study also levels of plasma Hcy showed a positive relationship with microalbuminuria and ACR in group 2 (r=0.187, P=0.01) and r=0.1752, p=0.026) respectively. Various studies [17,18,19,20], have shown that Hhcy leads to endothelial damage in all the organs in hypertension patients which is manifested in the kidneys as microalbuminuria.

Hcy is shown to induce oxidative stress in endoplasmic reticulum of meningeal cells through the activation of MAP kinase pathway [21]. Hhcy alters the cell signaling pathway mediated by ceramide leading to an increased expression of monocyte chemo attractant protein-1 [22,23]. Thus kidneys can be affected at pre-hypertension state indicating the involvement of Hcy leading to renal dysfunction [24,25].

Homocysteine acts as a prooxidant resulting in renal cell injury [26]. This leads defective functioning of glomerular basement membrane and raised intraglomerular pressure. Decreased nutritional status in the form of inadequate blood levels of pyridoxine, cobalamin and folate can lead to hyperhomocysteinemia. But studies have shown that prophylactic supplementation of the above mentioned vitamins does not bring about good outcome in all cases. Various studies [27,28,29] found linkage between homocysteinemia and renal failure. Homocysteine is degraded almost completely in kidney tissue [30]. In patients with hyperhomocysteinemia there is decreased renal clearance of homocysteine resulting in increased levels in blood [31,32,33].

It is a well established fact that CRP being an acute phase protein gets increased to varying high levels during acute or chronic inflammation. Many studies have proven that CRP levels are increased in hypertensive people [34,35]. According to our study, a significant association exists between DBP and hs CRP in hypertensives (groups II & III) (r= 0.2003, p=0.010 for group II; r=0.1681, p=0.0324 for Group III) but not in control group(r=0.0287, p=0.707).

Many studies [36,37,38] do find the same kind of result as ours. Sesso et al [39] found that individuals with prehypertension especially with increased DBP have increased hsCRP. Inflammation also contributes to the development of hypertension. CRP increases blood pressure by various pathways –decreased vasodilation by interfering with production of nitric oxide, increased leukocyte adhesion, platelet activation, oxidation, thrombosis and upregulation of angiotensin type-1 receptor [40,41]. Recently many circulating inflammatory markers are found to be associated with indices of arterial stiffening suggesting the possible role of inflammation [42,43,44]. According to Ki Chul Sung, studies showed elevated hsCRP in hypertensives [45]. Several mechanism have been elucidated for the possible role of hsCRP in the setting and progression of hypertension; salient ones being oxidative stress, increased level of adhesion molecules, plasminogen activator inhibitor-1 and oxidized LDL [46].

Studies suggest that the levels of hsCRP increases with the increase in the blood pressure [47]. more so with the duration of hypertension. However [48,49], did not find any association between hsCRP and the duration of hypertension [50,51] has reported that hsCRP levels in pre-hypertension leads to adverse cardiovascular events in addition to hypertension. Inflammatory processes lead to increased collagen deposition and fibrosis resulting in decreased myocardial function [52,53,54,55,56].
In patients with diastolic cardiac decompensation, collagen in the extracellular matrix is found to be anatomically and physiologically defective \[57,58\]. Inflammatory marker CRP alters the collagen/elastin ratio of the blood vessels \[59,60\].

**Conclusion**

Hyperhomocysteinemia in essential hypertension increases the risk of complications of hypertension resulting in damage to organs such as kidney, retina, heart etc. Early screening in hypertensive patients for microalbuminuria, hyperhomocysteinemia and increased hsCRP levels may reduce risk of further complications like chronic renal and cardiac diseases.

**Acknowledgement**

This work was supported by Founder-Chancellor Research Fellowship, Sri Ramachandra University, Porur, Chennai -India,

**Conflicts of Interests:** None

**References**


32. Brattström L, Lindgren A, Israelsson B, Andersson A, Hultberg B. Homocysteine...


Abbreviations

ACR = Albumin Creatinine Ratio
ANOVA = Analysis of Variance
BMI = Body Mass Index
DBP = Diastolic Blood Pressure
ELISA = Enzyme Linked Immunosorbent Assay
HDL chol = High Density Lipoprotein cholesterol
Hhcy = Hyperhomocysteinemia
hsCRP = High sensitivity C-reactive protein
LDL c = Low Density Lipoprotein cholesterol
MAP kinase = mitogen-activated protein kinase
mg / dl = Milligram per deciliter
mg / L = Milligram per liter
mm Hg = Millimeter of Mercury
mg / mmol = Milligram per millimole
µmol /L = Micromole per liter
SBP = Systolic Blood Pressure
SD = Standard deviation