Role of Gender in the Associations of Microalbuminuria with Inflammatory Markers in Hypertensive Subjects: A Cross-Sectional Study

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Key Words
C-reactive protein  •  Essential hypertension  •  Cytokines  •  Atherosclerosis

Abstract
Background: Though the association between microalbuminuria (MA) and inflammatory markers has been studied, the possible gender differences in these associations have not yet been analyzed. Our study aims to analyze the role of gender in the associations of MA and inflammatory markers.

Methods: 1,060 hypertensive patients were assessed for MA (albumin-creatinine ratio), plasma levels of HsCRP (high-sensitivity C-reactive protein), IL-18, and sCD40L (soluble CD40 ligand). Patients with diabetes mellitus, metabolic syndrome and overt nephropathy were excluded.

Results: Mean age was 46 ± 9.6 years, with 560 males and 500 females. The prevalence of MA was 35.6% (n = 378). MA was associated with HsCRP (OR: 2.13, CI: 1.155–3.168, p = 0.001) and sCD40L (OR: 2.35, CI: 1.014–3.912, p = 0.013) in the premenopausal females, whereas in males (OR: 1.83, CI: 1.037–3.920, p = 0.023) and postmenopausal females (OR: 2.31, CI: 1.688–3.274, p = 0.031) MA was associated only with HsCRP and not with sCD40L or IL-18.

Conclusions: Association between MA and HsCRP is consistent in all hypertensive patients. However, MA is associated with sCD40L only in premenopausal females and not in males and postmenopausal females.

Background
Microalbuminuria (MA) in patients with hypertension is an important marker of significant end-organ damage and is also an independent risk factor for morbidity and mortality in these patients [1, 2]. MA has been consistently shown to be a risk for cardiovascular disease and mortality due to its established association with proatherogenic factors, endothelial dysfunction and chronic inflammation [3–7].

Various molecules have been implicated to play a part in the inflammatory cascade associated with atherosclerotic cardiovascular disease. Well established among this group is HsCRP (high-sensitivity C-reactive protein) [8]. More recently, a variety of others, especially soluble CD40 ligand (sCD40L) and IL-18, have also been implicated [6, 9–16].

Data are consistent with respect to the association between MA and HsCRP [6, 17]. However, data are rather scanty with respect to the association between MA and the other inflammatory mediators, namely sCD40L and IL-18. The possible gender differences in this association have not yet been analyzed. If such a gender difference is identified, the possible role of hormones in this gender difference can be studied which may throw further light on our understanding of the process of atherosclerosis. In these lines, our present study is an effort to analyze the
possible association between microalbuminuria (MA) and the inflammatory mediators namely HsCRP, sCD40L and IL-18 in hypertensive patients and to analyze any gender variations in this association.

Materials and Methods

The study period spanned over two and a half years (August 2006 to January 2009). From a total of 1,450 consecutive patients who attended the outpatient hypertension clinic of our tertiary care center in South India during the study period, 350 were excluded as they had one of the following exclusion criteria: diabetes mellitus and prediabetes as defined by the American Diabetes Association [18], metabolic syndrome as defined by NCEP ATP III guidelines [19], patients already known to have hypertension and were receiving treatment, presence of overt nephropathy (creatinine >1.5 mg/dl), presence of overt proteinuria (ACR >300 mg/g), BMI >30, smokers, active infection as observed by the treating physician, and secondary forms of hypertension as per standard diagnostic guidelines [20]. 40 patients were further excluded as they refused to give informed consent for the study. Consequently, 1,060 consecutive, newly diagnosed, previously untreated, adult patients (aged >18 years, range 28–69 years, median 48 years) with essential hypertension were enrolled in the study and they formed the final study cohort. All study patients were from a low socioeconomic status. Informed consent was obtained from all patients. The study was approved by the hospital’s ethics committee.

Method of Patient Evaluation

Patients were questioned about their age by the principal investigator. Two office blood pressure recordings were taken, 30 min apart, as per standard methods [20]. Blood pressure severity was classified according to JNC VII classification [20]. Venous blood samples were obtained at 8 a.m. after an overnight fast. Lipid levels, fasting glucose, plasma HsCRP, IL-18, sCD40L were measured. MA was measured as an average of two non consecutive overnight spot urine samples for ACR using a quantitative assay (DCA 2001, Bayer Diagnostics Europe, Dublin, Ireland) with a coefficient of variation of 2.8% [21–23]. Microalbuminuria was diagnosed if ACR ranges between 17–250 mg/g in males and between 25 and 355 mg/g in females [24]. Plasma levels of HsCRP were assessed using validated high sensitivity assay (Dade Behring N high sensitivity CRP assay, Marburg, Germany), with a coefficient of variation of 3.6%. Plasma HsCRP level <2 mg/l was considered normal. sCD40L levels were estimated by the ELISA method (Bender Medsystems, Austria) with a coefficient of variation of 4%. sCD40L levels <2.5 ng/ml were considered normal. IL-18 was measured by the ELISA method (Biosource International, Nivelles, Belgium), with a coefficient of variation of 6%. IL-18 levels <275 pg/ml were considered normal. A standard 12-lead electrocardiogram (ECG) was taken for all patients and Romhilt and Estees criteria were used to classify left-ventricular hypertrophy (LVH) [25]. Body mass index (BMI) was calculated for all the study patients using standard methods [26].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normo-albuminuric patients</th>
<th>Micro-albuminuric patients</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>43 ± 5.8</td>
<td>47 ± 8.2</td>
<td>0.312</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>376</td>
<td>184</td>
<td>0.41</td>
</tr>
<tr>
<td>Females</td>
<td>324</td>
<td>176</td>
<td>0.067</td>
</tr>
<tr>
<td>BMI</td>
<td>23 ± 3.21</td>
<td>24 ± 4.1</td>
<td>0.216</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>139 ± 14.21</td>
<td>153 ± 18.7</td>
<td>0.041</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>84 ± 9.21</td>
<td>92 ± 11.43</td>
<td>0.021</td>
</tr>
<tr>
<td>LVH</td>
<td>107</td>
<td>112</td>
<td>0.073</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>239 ± 15.33</td>
<td>231 ± 11.56</td>
<td>0.245</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>132 ± 16.82</td>
<td>129 ± 15.91</td>
<td>0.124</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>41 ± 7.21</td>
<td>40 ± 4.51</td>
<td>0.061</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>131 ± 21.4</td>
<td>142 ± 18.9</td>
<td>0.37</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>83 ± 9.65</td>
<td>88 ± 6.34</td>
<td>0.11</td>
</tr>
<tr>
<td>HsCRP, mg/l</td>
<td>1.27 ± 0.71</td>
<td>2.96 ± 1.58</td>
<td>0.036</td>
</tr>
<tr>
<td>IL-18, pg/ml</td>
<td>246 ± 51.7</td>
<td>281 ± 39.25</td>
<td>0.026</td>
</tr>
<tr>
<td>sCD40L, ng/ml</td>
<td>2.43 ± 0.91</td>
<td>2.97 ± 0.48</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Statistical Analysis

Baseline characteristics of the study patients were expressed in mean ± SD and percentage. Student’s t test was used to analyze differences in baseline characteristics between patients with or without MA. The χ² test was used to compare categorical variables between the two groups. Linear regression analysis was applied to identify the association of MA with demographic and laboratory variables. Multiple logistic regression analysis was used to identify the associations between demographic and laboratory variables (age, BMI, systolic BP, diastolic BP, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, LVH, HsCRP, IL-18, sCD40L), and MA individually in four different groups namely (1) all study patients, (2) male patients, (3) premenopausal female patients, and (4) postmenopausal female patients. HsCRP, IL-18 and sCD40L were analyzed as a categorical variable (normal or elevated). p < 0.05 was considered statistically significant. All values presented fall within the 95% CI. Statistical analysis was performed using SPSS windows version 15.0 software (SPSS Inc., Chicago, Ill., USA).

Results

The final study cohort comprised of 1,060 patients. Mean age of the study population was 46 ± 9.6 years, with males (n = 560) marginally outnumbering the female patients (n = 500). Among the 500 female patients 263 were in the premenopausal age group and 237 were in the postmenopausal age group. 20% (n = 212) were overweight (BMI between 25 and 30), and 20.7% (n = 219)
had LVH in ECG. 275 patients (25.9%) had pre-hypertension according to JNC-VII classification. 636 patients (60%) had class I and 149 (14.1%) had class II hypertension according to JNC VII. The prevalence of MA in the study cohort was 35.6% (n = 378). 325 patients (30.7%) had an elevated HsCRP levels. 309 patients (29.2%) had an elevated IL-18 levels and 374 (35.3%) had an elevated sCD40L levels. Analysis of baseline differences between microalbuminuric and normoalbuminuric patients using ‘t’ test showed no difference in age, gender, BMI, LVH, lipid levels and fasting glucose levels (table 1). However microalbuminuric group had significantly, higher systolic BP, diastolic BP, higher HsCRP, IL-18, and sCD40L levels compared to normoalbuminuric group. Linear regression analysis identified positive correlation between MA and age (r = 0.472, p = 0.026), systolic BP (r = 0.713, p = 0.035), HsCRP (r = 0.529, p = 0.012), IL-18 (r = 0.611, p = 0.014), sCD40L (r = 0.723, p = 0.001). However, the other variables did not have a statistically significant association with MA. Multiple logistic regression identified age (OR: 1.31, CI: 1.287–2.047, p = 0.031), HsCRP (OR: 3.12, CI: 1.329–5.301, p = 0.001), IL-18 (OR: 2.45, CI: 1.144–4.571, p = 0.014) and sCD40L (OR: 1.93, CI: 1.104–3.783, p = 0.021) to be associated with MA when analyzed for the entire study cohort (table 2). In male (OR: 1.83, CI: 1.037–3.920, p = 0.023) and post-menopausal female patients (OR: 1.688, CI: 1.688–3.274, p = 0.031) MA was associated only with HsCRP and not with sCD40L or IL-18 (tables 2, 3). Whereas in pre-menopausal female patients MA was associated with HsCRP (OR: 2.131, CI: 1.155–3.168, p = 0.001) and sCD40L (OR: 2.355, CI: 1.014–3.912, p = 0.013) but not with IL-18 (table 3).

Discussion

Our study has shown a high prevalence of MA (35.6%) in a cohort of newly diagnosed hypertensive patients free from other co-morbid illness. MA is relatively common in hypertensive patients with a prevalence ranging from 6.7 to 40% [17, 27–31]. The MAGIC study [31] has reported a very low prevalence of MA (6.7%) among untreated patients (males 49 ± 0.6 years, females 52 ± 0.6 years) with mild-to-moderate hypertension. The methodology of their study was similar to our study where patients with comorbid conditions were excluded. Even then, we observed a higher prevalence of MA. Similar to us, a recent study from South India with a similar methodology involving elderly hypertensive patients (age >65 years) also observed a high prevalence of MA (39.4%) [17]. Hence, it is possible that South Indian hypertensive patients may have a higher prevalence of MA probably due to the influence of ethnicity on MA [32].

In our study, we observed the association between MA and age of the patient. Existing evidence also favors this association [6, 33]. The possible explanation that could be offered is that the longer a patient lives, the longer will the kidneys be exposed to the damaging effects of hyperten-

![Table 2. Associations of microalbuminuria in the total study cohort and in male patients](image-url)
sion and consequently the higher will be the chances of developing MA.

In our study, a raised HsCRP was significantly associated with MA in males, females and in the study population as a whole (tables 2, 3). Our observation is consistent with the global data with regard to this association [6, 17, 33–35]. It is possible that chronic exposure to low-grade inflammation in the form of raised HsCRP alters glomerular functions resulting in MA.

Our study observed that IL-18 and sCD40L were associated with MA when analyzed for the entire study cohort. Similar observations have been reported in the literature [36–38]. However, our study findings are contradictory to that observed by Tsioufis et al. [6]. In their cohort of male essential hypertensive subjects, MA had no association with sCD40L and IL-18. The important difference between their study and ours was that their study involved male essential hypertensive patients aged between 30 and 65 years. Female patients were not included in their study. In contrast, we included both male and female patients. Interestingly, further analysis of our data observed that in male patients and in postmenopausal females, MA was not associated with IL-18 and sCD40L, whereas in premenopausal female patients MA was associated with sCD40L but not with IL-18. When comparing these results with that of Tsioufis et al. [6], our male subgroup and postmenopausal female subgroup had similar findings to their cohort of male hypertensive subjects. Summarizing these results, it is possible to hypothesize that an association between MA and sCD40L occurs only in premenopausal female hypertensive patients and not in males and postmenopausal females. The possible mediator that might play a role in this observation may be estrogen, as estrogen is known to alter serum levels of sCD40L [39, 40]. Women that are not pregnant have been observed to have significantly higher sCD40L levels when compared to pregnant women [39]. Pregnancy is a progesterone-predominant and estrogen-deficient state. Also, women with polycystic ovarian syndrome have higher sCD40L levels compared to healthy women [40]. Polycystic ovarian syndrome is a state of estrogen excess. Hence, higher estrogen levels may be associated with a higher serum levels of sCD40L, which may explain why our premenopausal female microalbuminuric patients had significant association with sCD40L whereas our male patients and our postmenopausal female patients did not. The lack of statistically significant correlation between MA and IL-18 in subcohorts (i.e. men, pre- and postmenopausal women) is probably due to lack of statistical power; as this association was significant when analyzed for the entire study cohort.

Limitations

A single measurement of HsCRP, sCD40L, HsCRP and IL-18, a cross-sectional study design with lack of follow-up to see if these observations remain valid over time and with treatment, the exclusion of significant numbers of patients with other cardiovascular risk factors like

<table>
<thead>
<tr>
<th>Variables</th>
<th>Premenopausal women (n = 263)</th>
<th>Postmenopausal women (n = 237)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p value</td>
</tr>
<tr>
<td>Age</td>
<td>0.21 (0.122–2.168)</td>
<td>0.258</td>
</tr>
<tr>
<td>BMI</td>
<td>0.57 (0.265–1.118)</td>
<td>0.31</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.37 (0.127–1.315)</td>
<td>0.14</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.16 (0.021–1.537)</td>
<td>0.37</td>
</tr>
<tr>
<td>TC</td>
<td>0.61 (0.366–1.49)</td>
<td>0.44</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.44 (1.123–1.944)</td>
<td>0.43</td>
</tr>
<tr>
<td>HDL</td>
<td>0.63 (0.379–1.020)</td>
<td>0.171</td>
</tr>
<tr>
<td>LDL</td>
<td>0.73 (0.205–1.115)</td>
<td>0.291</td>
</tr>
<tr>
<td>LVH</td>
<td>0.62 (0.134–2.509)</td>
<td>0.305</td>
</tr>
<tr>
<td>HsCRP</td>
<td>2.13 (1.155–3.168)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-18</td>
<td>1.04 (0.199–1.144)</td>
<td>0.275</td>
</tr>
<tr>
<td>sCD40L</td>
<td>2.35 (1.014–3.912)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Figures within the table that are printed in bold indicate significant associations.
smoking, obesity and diabetes which are known to affect MA and inflammation limit the generalization of the study findings.

Conclusions

The prevalence of MA was high in our hypertensive cohort. Increase in age, HsCRP, sCD40L, and IL-18 were significantly associated with MA in this population. Association between MA and HsCRP was consistent in the entire study cohort. Association between sCD40L and MA was significant in premenopausal female patients. However, a similar association was not observed in males and postmenopausal females. From these observations, it is possible to hypothesize that estrogen may play a role in this variability in the association of MA and sCD40L. This hypothesis requires validation in future studies.

References

Microalbuminuria and Inflammation in Hypertensive Patients


