Association of Hemoglobin with Ambulatory Arterial Stiffness Index in Untreated Essential Hypertensive Patients Without Anemia

Huimin Chen, Qi Hua and Haixia Hou

Abstract

Objective Increased hemoglobin (Hb) levels are known to be associated with increased cardiovascular events and mortality in hypertensive patients, but the underlying mechanism remains unclear. However, an increased Ambulatory Arterial Stiffness Index (AASI), the surrogate maker of arterial stiffness, has been proven to be an independent predictor of cardiovascular disease. This pilot study evaluated the association between Hb and AASI in untreated essential hypertensive patients without anemia.

Methods A total of 566 untreated essential hypertensive patients without anemia were divided into Normal-Hb and High-Hb groups according to their Hb levels. The AASI and its symmetric calculation (Sym_AASI) were derived from 24h-Ambulatory Blood Pressure Monitoring (24h-ABPM). A multivariable linear regression analysis was performed to determine the relationship between Hb and AASI, Sym_AASI.

Results High-Hb group (n=127) showed higher AASI and Sym_AASI (0.51±0.11 vs 0.43±0.12, p<0.001; 0.33±0.10 vs 0.27±0.08, p<0.001) compared to Normal-Hb group (n=439). Univariate correlation analysis showed that Hb levels were positively related to AASI and Sym_AASI values (r=0.459, p<0.001; r=0.353, p<0.001). After adjustment for age, sex, BMI, current smoker, eGFR, uric acid, total cholesterol, high-density lipoprotein, 24h-SBP, 24h-PP and dipper status, Hb persisted as an independent determinant of AASI and Sym_AASI (β=0.402, p<0.001 and β=0.298, p<0.001, respectively).

Conclusion High hemoglobin seems to be associated with increased AASI in untreated essential hypertensive patients without anemia.

Key words: hemoglobin, ambulatory arterial stiffness index, hypertension, arterial stiffness


Introduction

Previous epidemiologic studies have shown an association between elevated hemoglobin (Hb) and cardiovascular (CV) event. High hemoglobin levels have been related to increased CV risk and may induce left ventricular hypertrophy (LVH) in patients with hypertension (1, 2). Recent studies have also indicated that relatively high baseline Hb levels are associated with increased risk of all-cause mortality and the composite endpoint in hypertensive patients (3, 4).

However, the underlying mechanisms by which high hemoglobin levels lead to LVH and increased CV events remain unclear. Increased arterial stiffness is associated with hypertension and is recognized as an important determinant of cardiovascular risk (5, 6). It is thus reasonable to hypothesize that there might be a close association between Hb and arterial stiffness.

In the present study, we employed the Ambulatory Arterial Stiffness Index (AASI), taken as the surrogate marker of arterial stiffness (7), which is associated either with preclinical target organ damage in hypertension or with an increased risk of cardiovascular mortality in hypertensive patients and stroke in the general population (8-11).

The purpose of this study was to investigate the relationship between Hb levels and AASI in untreated essential hy-
pertensive patients without anemia.

Materials and Methods

Patients

In this cross-sectional study, we recruited consecutive hypertensive patients in the Outpatient Department of Xuanwu Hospital from February 2009 to February 2011. Inclusion criteria were age ≥ 18 years and a diagnosis of untreated essential hypertension according to the world health organization (WHO) criteria (12), as determined by average office systolic blood pressure (SBP) ≥ 140 mm Hg and/or office diastolic blood pressure (DBP) ≥ 90 mmHg on at least three occasions, and corroborated by 24-h ambulatory blood-pressure monitoring (ABPM), and required a daytime BP mean ≥ 135 and/or ≥ 85 mmHg for SBP and DBP at the time of recruitment. Thus, subjects with white-coat hypertension were not included in the study.

The exclusion criteria were: 1) secondary hypertension, 2) presence of anemia (Hb < 130 g/L for males and Hb < 120 g/L for females according to the WHO criteria for anemia) (13), 3) pregnant women, 4) presence of cardiovascular and cerebrovascular diseases, except for essential hypertension, 5) major surgery in the previous six months, 6) chronic renal disease (except for essential hypertension), 5) smoking status was defined as a nocturnal systolic BP reduction of >2 hours, if data were obtained while patients had an irregular rest-activity schedule, or if the nighttime sleep period was <6 hours or >12 hours during ABPM. Dipper status was defined as a nocturnal systolic BP reduction above 10%.

Other measurements

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Glomerular filtration rate (eGFR) was estimated from serum creatinine using the abbreviated Modification of Diet in Renal Disease equations for each sex (16). Diabetes mellitus (DM) was defined as a fasting blood glucose ≥ 7.0 mmol/L or use of antidiabetic medication. Smoking status was defined as current smoking or having a history of smoke in the previous 3 months.

Statistical analysis

Data management and analysis were performed using SPSS 13.0 (SPSS, Chicago, IL) software. Data are presented as mean ± SD for continuous variables with normal distribution and as proportions for categorical variables. Pearson’s and Spearman’s correlations were used to test the relationships between Hb and other variables to AASI and Sym_AASI as appropriate. Unpaired student’s t test was used to determine the differences between the two groups. Chi-square test was used for categorical comparison of data. Multivariable stepwise linear regression analysis was performed to determine the relationship between Hb and AASI, Sym_AASI to control the potential contribution of influential factors. Two-tailed p<0.05 indicated statistical significance in all analyses.

Results

Demographic characteristics of the study population

A total of 556 untreated essential hypertensive patients without anemia were enrolled. Demographic and clinical characteristics of the patients in the study were reported in Table 1.

According to the Hb levels, 439 (78%) patients and 127 (22%) patients have normal and high Hb levels respectively.
There were no significant differences between the two groups in age, presence of DM, eGFR, triglyceride (TG), low-density lipoprotein (LDL-C), hypereosensitivity C-reaction protein (hs-CRP). Patients with high Hb levels were more likely to be male or to smoke, with higher BMI, higher office-SBP, higher office-DBP, higher uric acid (UA), higher cholesterol (TC), lower high-density lipoprotein (HDL-C) in comparison to Normal-Hb patients (Table 1).

On the other hand, no significant difference was observed from the 24h-pulse pressure (PP), 24h-heart rate (HR), and dipper status of 24h-ABPM profiles between the two groups. But the High-Hb group was more likely to have higher 24h-systolic BP (SBP), 24h-diastolic BP (DBP) and 24h-mean arterial pressure (MAP), compared to the Normal-Hb group. Meanwhile, High-Hb group showed higher AASI values (0.33±0.10 vs. 0.27±0.08, p<0.001) compared with the Normal-Hb group (Table 2). Fig. 1 shows the distribution diagram of 24h-BP between Normal-Hb and High-Hb groups of patients (r=0.505, p<0.001; r=0.635 p<0.001, respectively).

**Univariate correlation analysis in study patients**

Univariate correlation analysis between AASI, Sym_AASI and other possible affecting factors are shown in Table 3. Both AASI and Sym_AASI were positively correlated with age (r=0.367, p<0.001; r=0.263, p<0.001), sex (r=0.247, p=0.002; r=0.258, p=0.001), BMI (r=0.232, p=0.003; r=0.197, p=0.030), current smoker (r=0.238, p=0.002; r=0.246, p=0.001), Hb (r=0.459, p<0.001; r=0.353, p<0.001), UA (r=0.302, p=0.001; r=0.253, p=0.001), 24h-SBP (r=0.294, p<0.001; r=0.243, p=0.001) and 24h-PP (r=0.421, p<0.001; r=0.387, p<0.001).
Figure 1. Distribution diagram of 24h-BP between Normal-Hb group (A) and High-Hb group (B) of patients.

Multivariable regression analysis in study patients

The multivariable stepwise linear regression analysis showed that the positive relation between AASI, Sym_AASI and Hb persisted ($\beta$=0.402, $p<0.001$ and $\beta$=0.298, $p<0.001$, respectively) even after adjustment for age, sex (female=1, male=0), BMI, current smoker (yes=1, no=0), eGFR, UA, TC, HDL-C, 24h-SBP, 24h-PP, dipper status (yes=1, no=0). The two models are shown in Table 4.

Discussion

To our knowledge, this is the first study to examine the relations between Hb and arterial stiffness in hypertensive patients. The results of our study showed that in untreated essential hypertensive patients without anemia, hemoglobin levels were independently associated with arterial stiffness, assessed by AASI and Sym_AASI.

The AASI is a recently proposed index derived from ABPM for the evaluation of arterial stiffness (7). Previous studies have shown that AASI is a predictor of cardiovascular outcome in the general population and in patients with hypertension (8-11). However, the correlation with the Pulse Wave Velocity, which is considered the gold standard for arterial stiffness measurement, has been subsequently reported to be weak, since the regression slope of diastolic on systolic BP in significantly influenced by several factors other than arterial stiffness, such as systolic and diastolic BP data scattering and nocturnal dipping (17). For this reason, AASI has been rather proposed as an integrated measure which reflects the combined effects of components of arterial stiffness and the reflection of the arterial pulse wave. On the other hand, an “adjusted” way to calculate AASI based on a symmetrical regression (Sym_AASI) has been recently introduced, for which no such artificial relationships are found (14, 18). Therefore we employed both AASI and Sym_AASI as makers of arterial stiffness in this study.

In our study, there were systematic disparities in CV risk factors between the two groups defined by levels of Hb. We performed the distribution diagram of the 24h-SBP and 24h-DBP between the two groups of patients (Fig. 1). In this diagram, the High-Hb patients had significantly higher 24h-SBP and 24h-DBP compared with the normal patients. And more patients of the High-Hb level group had more severe hypertension than those of Normal-Hb level group. Meanwhile, the left ventricle might be markedly thickened in these patients with severe hypertension untreated for years. The results shown here were in line with previous studies (2, 4). Also, High-Hb patients were more likely to have higher BMI, higher TC, lower HDL-C and higher uric acid. And those risk factors might be considered to contribute to the increased arterial stiffness in these patients. After adjustment for the high proportions of men, current smoker and those CV risk factors. Hb was associated with AASI and Sym_AASI.

The close association between elevated Hb levels and increased AASI, the marker of increased arterial stiffness, might be explained by the following mechanisms. First, there was evidence that elevated Hb level was associated with increased blood pressure in the general population (19-21). Hb affects peripheral vascular resistance in two ways according to the Poiseuille-Hagen Equation: primarily by influencing the viscosity of blood (22), and secondarily by affecting the caliber of peripheral arterioles (23). The present study also showed that High-Hb patients had significantly higher 24h-SBP and 24h-DBP, compared with normal-Hb patients. It is known that arterial stiffness increases transiently as blood pressure rises. Arterial stiffening also increases as the structure of the artery changes. Persistently elevated blood pressure accelerates atherosclerosis, arterial smooth muscle hyperplasia and hypertrophy, and collagen synthesis, thereby increasing arterial stiffness. Secondly, it has been reported that cell-free Hb infusion does cause hypertension because of its intrinsically high rate of nitric oxide (NO) scavenging (24, 25). NO has been proven as an...
endocrine vasoregulator that could reduce the arterial stiffness by increasing vasodilation and improving endothelial function (26, 27). Thirdly, heme oxygenase-1 (HO-1) was found to play an important beneficial role in attenuating oxidative stress and inflammation by regulating vascular endothelial growth factor in hypertension (28-31). It is the inducible rate-limiting enzyme in the degradation of hemoglobin, generating free iron, biliverdin, and carbon monoxide (CO) (32). And CO has vasodilating properties, which are widely accepted. Based on literature and previous research, HO-1 deficiency might be involved in hypertension and increased arterial stiffness in high-Hb patients. However, confirmation is needed for further study. These explanations are good support to our hypothesis.

In addition, most hypertensive patients with anemia might be the result of severe hypertensive target-organ damage such as renal insufficiency (33), myocardial infarction (34), stroke (35), or even from antiplatelet or anticoagulant drugs for routine treatment. Therefore we eliminated anemia patients (Hb<130 g/L for male, Hb<120 g/L for female) in the present study, as their poorer CV situations generally meet the exclusion criteria. In the future we plan to work with this group of patients with anemia. Also, we found that 24h-PP was almost the same between High-Hb and Normal-Hb groups despite the higher value of AASI and Sym_AASI in high-Hb patients. The 24h-PP was positively related to

### Table 3. Correlation Analysis between AASI, Sym_AASI and Other Possible Affecting Factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>AASI Correlation coefficient</th>
<th>p value</th>
<th>Sym_AASI Correlation coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.367</td>
<td>&lt;0.001</td>
<td>0.263</td>
<td>0.001</td>
</tr>
<tr>
<td>Sex(female=1,male=0)</td>
<td>0.247</td>
<td>0.002</td>
<td>0.258</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.232</td>
<td>0.003</td>
<td>0.197</td>
<td>0.030</td>
</tr>
<tr>
<td>Current smoker (yes=1,no=0)</td>
<td>0.238</td>
<td>0.002</td>
<td>0.246</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus (yes=1,no=0)</td>
<td>0.062</td>
<td>NS</td>
<td>0.071</td>
<td>NS</td>
</tr>
<tr>
<td>Hb</td>
<td>0.459</td>
<td>&lt;0.001</td>
<td>0.353</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.194</td>
<td>0.033</td>
<td>-0.223</td>
<td>0.012</td>
</tr>
<tr>
<td>UA</td>
<td>0.302</td>
<td>0.001</td>
<td>0.253</td>
<td>0.001</td>
</tr>
<tr>
<td>TG</td>
<td>0.116</td>
<td>NS</td>
<td>0.093</td>
<td>NS</td>
</tr>
<tr>
<td>TC</td>
<td>0.168</td>
<td>0.026</td>
<td>0.202</td>
<td>0.010</td>
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<td>HDL-C</td>
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<td>0.017</td>
<td>-0.208</td>
<td>0.022</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.052</td>
<td>NS</td>
<td>0.037</td>
<td>NS</td>
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<tr>
<td>Hs-CRP</td>
<td>0.090</td>
<td>NS</td>
<td>0.059</td>
<td>NS</td>
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<tr>
<td>24h-SBP</td>
<td>0.294</td>
<td>&lt;0.001</td>
<td>0.243</td>
<td>0.001</td>
</tr>
<tr>
<td>24h-DBP</td>
<td>0.017</td>
<td>NS</td>
<td>0.058</td>
<td>NS</td>
</tr>
<tr>
<td>24h-MAP</td>
<td>0.101</td>
<td>NS</td>
<td>0.033</td>
<td>NS</td>
</tr>
<tr>
<td>24h-PP</td>
<td>0.421</td>
<td>&lt;0.001</td>
<td>0.379</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24h-HR</td>
<td>-0.097</td>
<td>NS</td>
<td>-0.066</td>
<td>NS</td>
</tr>
<tr>
<td>Dipper (yes=1, no=0)</td>
<td>-0.192</td>
<td>0.035</td>
<td>-0.181</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 2.

### Table 4. Multiple Stepwise Linear Regression Analysis for Assessing the Determinants of AASI and Sym_AASI among the Study Patients

<table>
<thead>
<tr>
<th>Dependent variable: AASI*</th>
<th>Beta</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>0.402</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24h-PP</td>
<td>0.367</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.285</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>UA</td>
<td>0.189</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent variable: Sym_AASI*</th>
<th>Beta</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h-PP</td>
<td>0.434</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb</td>
<td>0.298</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.227</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 2.

*Adjusted for age, sex, BMI, current smoker, eGFR, UA, TC, HDL-C, 24h-SBP, 24h-PP, dipper status.
AASI and Sym_AASI in our study, though the degree of correlation was not very powerful (r=0.421; r=0.379, respectively). We then performed the univariate linear regression analysis between 24h-PP and AASI, Sym_AASI. The results showed that every 0.01 increase of AASI and Sym_AASI may cause the value of 24h-PP to increase by 0.29 mmHg and 0.25 mmHg, respectively (data not shown). Thus, AASI might have little affect on 24h-PP levels. On the other hand, because of the comparatively smaller sample size and low prevalence (about 22%) of High-Hb patients in our study, the baseline characteristics of both groups were not well matched. Thus, some potential confounding factors may affect the PP level as well. We hypothesize that if a study has a larger sample size and is well designed, a significant difference in the PP levels between the two groups will be observed. We therefore involved the 24h-PP in the multivariable stepwise linear regression mode in our study. It turned out that Hb persisted as an independent determinant of AASI and Sym_AASI.

There were still some limitations in this study. First of all, the cross-sectional design limited our ability to infer a causal relationship between Hb and AASI, Sym_AASI. Indeed, a totally opposite explanation might also reasonable. As a result, the relationship between Hb and arterial stiffness should be verified in future prospective studies. Second, although a number of potential confounding factors such as age, sex, BMI, current smokers, eGFR, UA, lipid profile and characteristic of ambulatory blood pressure monitor were controlled in multivariable regression analysis, the existence of other unrecognized confounding variables was always possible. Third, though the gender was controlled, the high-Hb patients in this study were male dominated, which might influence the results in some ways. Fourth, because of within-subject variation, the single-measurement of hemoglobin and several risk factors will reflect long-term averages less precisely than repeated measurement. This misclassification is likely to be random and thus will lead to an underestimation of the association.

In conclusion, this study performed in hypertensive patients without anemia showed that Hb was independently associated with AASI and Sym_AASI, the direct relationship between Hb and AASI, Sym_AASI suggested that increased arterial stiffness might be the link between high Hb levels and increased cardiovascular events and mortality in hypertensive patients. Hypertensive patients with higher Hb levels usually come with worse CV outcomes, further study is required to provide the information on the sensitivity and specificity of Hb value in the diagnosis of arterial stiffness in hypertensive patients.

The authors state that they have no Conflict of Interest (COI).

References