Evaluation of Neutrophil-Lymphocyte Ratio and Arterial Stiffness Index in Middle Aged Prehypertensive and Hypertensive Men

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Abstract

Background: To evaluate the neutrophil-lymphocyte ratio and arterial stiffness index in prehypertensive and hypertensive middle-aged men.

Methods: In this descriptive study a total of ninety male subjects aged 35-55 years were studied in three groups i.e. normotensive, prehypertensive and hypertensive. Each group comprised of thirty subjects. Blood pressure was measured with mercury sphygmomanometer according to the standard protocol. Arterial stiffness index was calculated from digital volume pulse recorded by photoplethysmography with iWorx-214 physiological interface system. The blood cell count and differential leukocyte count was done by automated hematology analyzer and confirmed by light microscopy, after staining the peripheral blood film with Leishman’s stain.

Results: The neutrophil-lymphocyte ratio was normal and comparable in the three groups, while arterial stiffness index was significantly different amongst the groups. The neutrophil-lymphocyte ratio was correlated with arterial stiffness index in normotensive subjects (r=0.432; p=0.01), whereas, it was moderately correlated with systolic and diastolic blood pressure (r=0.50; p=0.001) in the hypertensive subjects. The arterial stiffness index strongly correlated with systolic (r=808; p=0.01) and diastolic (r=739; p=0.001) blood pressures in subjects of all groups together.

Conclusions: The inflammation contributes in the process of stiffening of the vascular wall, which may precede the development of hypertension. Arterial stiffness index is associated with blood pressure irrespective of the neutrophil-lymphocyte ratio.

Key Words: Neutrophil-lymphocyte ratio; Arterial Stiffness Index, photoplethysmography, prehypertension, hypertension.

Introduction

Hypertension is a progressive increase in blood pressure. The prevalence of hypertension has been increasing all over the globe including Pakistan. In the United States, twenty-nine percent of the adults above nineteen years in age have hypertension and approximately thirty-seven percent are prehypertensives. In Pakistan, it has been estimated that one-third of population above forty-five years and twenty percent above fifteen years have hypertension.

Hypertension is a multifactorial disorder caused by various environmental, physiological and genetic factors. The endothelial dysfunction, vascular stiffness, abnormal sympathetic outflow, alterations in renin-angiotensin system and increased plasma aldosterone levels are the major culprits involved in the increased arterial pressure.

The measurement of arterial stiffness index (ASI) has been proposed to be a useful non invasive tool for the cardiovascular risk assessment because of its ability to identify early target organ damage. The stiffness of the arterial wall determined by analysis of digital volume pulse contour, is a simple, reproducible and noninvasive method of assessing arterial stiffness. There has been a strong evidence that the stiffness index score derived by this technique is comparable to the arterial stiffness determined by pulse-wave velocity, which is the gold standard marker. ASI may serve as a noninvasive tool for assessing the patients at risk of stroke, coronary artery disease and heart failure. It has been documented to be more sensitive tool in risk stratification for cardiovascular diseases (CVD) in comparison to plasma glucose, total cholesterol, and waist to hip ratio in apparently healthy population.

It has been documented that white blood cell count (WBC) and neutrophil-lymphocyte ratio (NLR) may predict the inflammatory status in various cardiovascular pathologies. The increased neutrophil count has been associated with ongoing inflammatory process in patients with type II diabetes mellitus and raised NLR has been associated with metabolic syndrome. The NLR above 2.5 has been linked with poor outcome after reperfusion procedures in acute coronary syndrome. In patients with coronary artery
disease undergoing angioplasty, higher NLR has been associated with decreased ejection fraction and high rate of mortality and morbidity.7

Patients and Methods
This case control study, carried out at Army Medical College in collaboration with Military Hospital Rawalpindi, from December 2013 to May 2014, included ninety male subjects aged between 35-55 years (n=90). Equal number of normotensive, prehypertensive and hypertensive subjects were selected from the medical OPD of Military Hospital Rawalpindi by non-probability, convenience sampling and placed in three groups (n=30). The grouping of subjects into normotensive, prehypertensive and hypertensive subjects was done in light of JNC-VII report.8 Subjects having allergic diseases, acute coronary syndrome, malignancy, chronic inflammatory disease, hypo or hyperthyroidism and any other prolonged illness were excluded. The study was started after approval from the Ethical Review Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College.

Arterial blood pressure was measured by auscultatory method with the mercury sphygmomanometer. First reading was taken at the time of filling the proforma, second during recording of pulse wave contour for measurement of the stiffness of the vessel wall and third before drawing the blood sample. The average systolic (SBP), diastolic (DBP), pulse pressure (PP=SBP-DBP) and mean arterial pressure (MAP = DBP + 1/3 (SBP - DBP) were calculated. The random blood sugar was checked by the glucometer in order to screen for diabetes mellitus and those having random blood sugar above 140 mg/dl were excluded from the study. Arterial Stiffness Index was measured by placing photoplethysmograph on the volar surface of the distal segment of middle finger and digital volume pulse (DVP) was recorded by Human/Animal Physiology interface system; iWorx-214. The recorded data was analyzed, using LabScribe software and reflection time was calculated by placing cursor on the two peaks of DVP. The arterial stiffness index was calculated as [ASI = Height (meters)/ Reflection time (seconds)]. The total and differential blood cell counts were measured by automated hematology analyzer. The differential leucocyte count (DLC) was verified by direct microscopy after staining the peripheral blood smear by Leishman stain under the supervision of hematologist. ESR was measured by Westergren’s method. Statistical analysis was performed by using the IBM-SPSS version 20. The study variables in the three groups were compared by one way ANOVA followed by Post-Hoc Tukey’s test. In order to determine the correlation between NLR, ASI and BP variables, Pearson’s correlation coefficient was calculated. The p-value <0.05 was considered statistically significant.

Results
The mean and standard deviation of the demographic variables have been compared by one way ANOVA (table-1).

### Table 1: Comparison of age, body mass index, blood pressure variables, neutrophil-lymphocyte ratio and arterial stiffness index (by one-way ANOVA)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>WBC (k/μL)</th>
<th>PP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>SBP (mm Hg)</th>
<th>ASI (m/s)</th>
<th>ESR (mm at end if first hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive n=30</td>
<td>7.85 ± 0.62</td>
<td>11.47 ± 5.27</td>
<td>164.03 ± 13.63</td>
<td>86.20 ± 2.25</td>
<td>129.93 ± 4.46</td>
<td>6.86 ± 1.45</td>
<td>13.27 ± 4.80</td>
</tr>
<tr>
<td>Prehypertensive n=30</td>
<td>7.57 ± 1.67</td>
<td>11.96 ± 5.27</td>
<td>164.03 ± 13.63</td>
<td>86.20 ± 2.25</td>
<td>129.93 ± 4.46</td>
<td>6.86 ± 1.45</td>
<td>13.27 ± 4.80</td>
</tr>
<tr>
<td>Hypertensive n=30</td>
<td>7.96 ± 1.67</td>
<td>11.96 ± 5.27</td>
<td>164.03 ± 13.63</td>
<td>86.20 ± 2.25</td>
<td>129.93 ± 4.46</td>
<td>6.86 ± 1.45</td>
<td>13.27 ± 4.80</td>
</tr>
</tbody>
</table>

All values have been expressed as Mean ± SD
(BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure; WBC: white blood cell count; NLR: neutrophil-lymphocyte ratio; ESR: erythrocyte sedimentation rate; k/μL: 1000/microlitre; ASI: arterial stiffness index)

### Table 2: Comparison between the two groups (By Post-Hoc Tukey’s Test)

<table>
<thead>
<tr>
<th>Age(years)</th>
<th>Normotensive vs Prehypertensive</th>
<th>Normotensive vs Hypertensive</th>
<th>Prehypertensive vs Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>DBP</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>MAP</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>PP</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>ASI (m/s)</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

(SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure; ASI: arterial stiffness index)

ASI was significantly different amongst the groups and strongly correlated (p=<0001) to the systolic, diastolic and mean arterial pressure (r=0.808; r=0.739 and r=0.781 respectively). No correlation was found between NLR and ASI in prehypertensive and hypertensive groups but moderate correlation was...
observed in normotensive group ($r=0.432$; $p=0.01$) (Table 2).

The neutrophil-lymphocyte ratio had a moderate positive correlation with systolic ($r=0.473$; $p=0.001$) and diastolic ($r=0.472$; $p=0.001$) blood pressure in hypertensive group, but no correlation was observed in normotensive and prehypertensive groups.

**Discussion**

Few studies compared the NLR and ASI in prehypertensive and hypertensive subjects. The results of our study showed that white blood cell counts and NLR were comparable in normotensive, prehypertensive and hypertensive subjects. These results were similar to the findings of the studies conducted by Acar *et al.*$^9$ and Julius *et al.*$^{10}$ but contrary to the findings of two other studies by Pusuroglu *et al.*$^{11}$ and Rajkumari *et al.*$^{12}$ These contrary findings may be due to the difference in the span of the hypertension in the subjects participating in these studies. The hypertension over long period of time has been associated with elastin fiber fatigue and fracture due to the continuous stretching of arterial tree which may lead to systemic inflammation.$^3$ Since there was no difference of NLR observed amongst the normotensive, prehypertensive and hypertensive groups therefore, NLR may not be a suitable inflammatory marker in hypertensive patients.

In our study, there was rise in NLR with increasing ASI but this relationship was not statistically significant. These results were similar to the findings of Blann *et al.*$^{13}$ in patients with stable coronary artery disease. In his study, ASI was strongly related with age and blood pressure but not with the markers of systemic inflammation. When the correlation between NLR and ASI was analyzed separately in each group, there was moderate correlation ($r=0.432$; $p=0.001$) in normotensive subjects but no correlation was observed in the prehypertensive and hypertensive subjects. Our results supported the findings by Malahfji *et al.*$^*$ that NLR was associated with ASI in asymptomatic individuals free of CVD. In a study, conducted by Park *et al.* NLR was independently associated with arterial stiffness irrespective of blood pressure variations, which is contrary to our findings.$^{15}$ This conflict in the findings of the two studies may be due to the difference in the study design. In their study, the grouping was based on NLR and it was studied as a qualitative or categorical variable. The NLR values had been divided into four quartiles and it was revealed that the mean blood pressure and arterial stiffness was higher in the highest quartile of NLR and blood pressure was associated with the increasing quartile of NLR. However, in our study, NLR was studied as a numerical or quantitative variable and its association with arterial stiffness index in equal number of normotensive, prehypertensive and hypertensive subjects was investigated. Statistically, the results of co-relational studies are more dependable when both the variables are quantitative while, the categorization of numerical data may yield misleading results.$^{16}$

There was a weak positive correlation of NLR with blood pressure in our study, which showed that the inflammatory processes may have a role in the pathophysiology of hypertension. Interestingly, when this relationship was evaluated separately in each group, the NLR was associated relatively strongly with blood pressure variables in hypertensive group but no such correlation was seen in normotensive and prehypertensive subjects. These results are consistent with the findings of Schillaci *et al.*$^{17}$ and Tian *et al.*$^{18}$

The initial stiffening in the vessel wall may involve inflammatory process and inflammation could precede the development of hypertension. This observation is supported by the findings of various studies, in which elevated WBC count and especially raised neutrophil count were associated with higher incidence of hypertension.$^{14}$

It may be deduced from the findings of the present study and observations of the published data that initiation of vascular stiffening could be due to inflammatory processes. However, continuous stretching of the vessel wall over a prolonged period due to sustained hypertension may lead to the mechanical injury and inflammation, which may be responsible for the strong association of inflammatory cells in hypertensive subjects. Therefore, a vicious cycle develops which leads to further stiffening of the vessel wall. These inflammatory processes may play a pivotal role in cardiovascular complications and end organ damage as a consequence of continuously high blood pressure. The raised NLR seen in acute coronary syndrome, stroke, peripheral arterial occlusive disease, extent of myocardial damage after acute ischemic event and post angioplasty arrhythmias may be due to the activation of complex cascade of events after an acute insult to injury prone arteriosclerosed vessel wall. It leads to the activation of platelets and polymorphonuclear cells leading to the release of autocrine and paracrine hormones resulting in the raised NLR. These inflammatory processes may further result in worsening of these diseases and poor prognosis.
Many studies have revealed that ASI may be used in risk stratification of CVD in preclinical stages but various intricate and costly techniques have been employed to measure the ASI in these studies.\textsuperscript{5,7} ASI appears to be a useful non invasive marker in predicting the extent of arterial stiffness, atherosclerosis and arteriosclerosis in a single clinic visit and may predict blood pressure pressure variations better than the mean blood pressure. The measurement of ASI may provide an opportunity to prevent target organ damage in preclinical stage and may serve as prognostic tool in hypertensive patients on medications, because arterial stiffness improved with physical activity and medications.\textsuperscript{5,20} The ASI measured via this technique is quite simple and can be done in office setting or at the bed-site. Therefore, the ASI may be employed in clinical practice for the risk stratification of CVD and may serve as an important diagnostic and prognostic tool.

Conclusions
1. The inflammation contributes in the process of stiffening of the vascular wall, which may precede the development of hypertension.
2. Arterial stiffness index is associated with blood pressure irrespective of neutrophil-lymphocyte ratio.
3. Inflammation precedes hypertension and not merely because of the mechanical injury resulting from raised blood pressure, though the mechanical stretching results in worsening of inflammation and the hypertension.

References