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A study on Association between the Neutrophil-to-Lymphocyte Ratio and Prehypertension in Kashmiri Patients

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Abstract

Background: Managing prehypertension is essential to halting the development of hypertension because it is a precursor to possible cardiovascular problems. According to recent research, inflammatory indicators may contribute to prehypertension. One promising inflammatory marker that has developed is the neutrophil-to-lymphocyte ratio (NLR).

Objectives: The study's objective is to investigate the relationship between NLR and prehypertension, investigating the possibility that this ratio could operate as a precursor to or contributing factor to prehypertension.

Materials and methods: A total of 103 participants were chosen, comprising 50 normotensive control people and 53 recently diagnosed PHT patients. A systolic blood pressure (BP) of 120–139 mm Hg and/or a diastolic BP of 80–89 mm Hg were considered prehypertensive.

Results: Based on NLR values, patients were categorised into three tertiles: 1.18 (0.9–1.42) in tertile 1, 1.55 (1.43–1.78) in group 2, and 2.41 (1.82–4.5) in tertile 3. The frequency of the prehypertensive state was significantly higher among patients in the upper NLR tertile compared to the middle and lower tertiles (41 (77.3 %), 7 (13.2 %), and 5 (9.4 %), respectively; $p < 0.001$). There was no significant difference between the middle and lower NLR tertile groups in terms of pre hypertensive state ($p = 0.558$). Systolic BP and diastolic BP were significantly higher among patients in the upper NLR tertile (systolic: 128 ± 8 mm Hg, $p < 0.001$; diastolic: 84 ± 4 mm Hg) than among those in the middle (systolic: 114 ± 12 mm Hg, $p < 0.001$; diastolic: 74 ± 8 mm Hg, $p < 0.001$) and lower (systolic: 110 ± 11 mm Hg, $p < 0.001$; diastolic: 71 ± 9 mm Hg, $p = 0.001$) NLR tertiles.

Conclusion: Our research revealed a correlation between PHT and NLR. The monocyte count and NLR assessment can both be used to identify a higher risk of prehypertension.

Keywords: Prehypertension, SMHS, Kashmir, Neutrophil-to-lymphocyte ratio, Hematology.

Introduction

Hypertension is a major risk factor and a leading contributor to premature mortality and morbidity due to CVDs, cerebrovascular events, retinopathy, and chronic renal diseases [1,2]. Globally, an estimated 10.7 million all-cause fatalities in 2015 were related to systolic BP ≥110–115 mmHg, and 7.8 million deaths were related to systolic BP ≥140 mmHg. India—the most populous country with a growing economy [3], an aging population, and increasing urbanization—is also witnessing an increasing prevalence of HT over the years[4] similar to any other Lower Middle-Income Countries

(LMIC). The United Nations Population Fund projects that the elderly population in India is set to nearly double, reaching a staggering 192 million by the year 2030[5].

Prehypertension is a condition characterized by blood pressure readings that are higher than normal but not yet high enough to be classified as hypertension. Clinically, Prehypertension (PHT) has been defined as a systolic blood pressure (BP) of 120–139 mm Hg and/or a diastolic blood pressure of 80–89 mmHg[6]. It is an important stage in the continuum of blood pressure abnormalities and a significant risk factor for the development of hypertension and cardiovascular diseases. Identifying markers that predict prehypertension could facilitate early intervention and prevention. The Prehypertensive term is a predictor method to identify susceptible individuals at higher risk for developing hypertension, in comparison to those with ideal blood pressure (< 120/80 mm Hg)[7,8]. Prehypertensive individuals have a higher risk of coronary vascular disease, such as coronary heart disease and myocardial infarction, according to a number of prospective and cross-sectional studies conducted in the last few years [9-13]. Therefore, it is critical to comprehend the pathophysiology of PHT, since in certain instance PHT may serve as the initial indicator of cardiovascular disease (CVD). Most studies done have reported inflammation plays a crucial role in development of cardiovascular diseases in different ethnic populations[14,15]. Increased incidence of cardiovascular illnesses, including atrial fibrillation, HT, and coronary artery disease, has been linked to elevated levels of systemic immune markers[16]. Increased cardiovascular risk factors are associated with a higher white blood cell (WBC) count and its subtypes[17]. NLR reflects inflammation and physiologic stress PLR reflects platelet and clotting system activation, local vessel wall inflammation and endothelial dysfunction[17].

The neutrophil-to-lymphocyte ratio (NLR) is a simple, cost-effective marker derived from complete blood counts. It reflects the balance between neutrophil and lymphocyte counts and has been linked with various inflammatory conditions and cardiovascular diseases[18]. Despite the importance of PHT on cardiovascular outcomes, insufficient data exists on the potential association between the NLR and PHT in different ethnic populations. Therefore, the aim of this study was to explore the NLR in patients with PHT in Kashmiri ethnic population. Hence, objective was to investigate the potential association between NLR and prehypertension, examining whether an elevated NLR could be an early indicator of prehypertension in Kashmir.

Materials and methods

Study Design and Population

This study was conducted in Post Graduate Department of Physiology Government Medical College (GMC) Srinagar in collaboration with the Department of Immunohematology, SMHS, Hospital Srinagar. G Power analysis suggested that a sample size of 45 was needed with an alpha of 0.05 for a power of 0.80 A total of 103 cases and controls age and sex matched were enrolled for the study, after clearance from intuitional ethical committee. This study utilized a cross-sectional design involving 103 voluntary female participants from Government Medical College Srinagar and Associated SMHS Hospital. The cohort included individuals aged 25-49 years, excluding those with a history of hypertension, chronic inflammatory diseases, or other serious health conditions. Blood pressure measurements were classified according to the American College of Cardiology (ACC) guidelines, with participants categorized into normotensive, prehypertensive, and hypertensive groups.

Prehypertension was defined as a systolic BP of 120–139 mmHg and/or a diastolic BP of 80–89 mmHg. BP measurements were performed on the right arm using a sphygmomanometer (Erka, Erlangen, Germany) after 10 minutes of rest in a seated position. The average of two blood pressure measurements obtained at least three minutes apart was recorded for each patient. The nurse specialist determined the mean BP. Ambulatory BP monitoring was not performed. Patients who had PHT but were otherwise healthy were included. Exclusion criteria included drug use, morbid obesity (body mass index ≥ 35 kg/m2), diabetes mellitus, metabolic syndrome, dyslipidemia, renal dysfunction (serum creatinine > 1.5 mg/dl, blood urea nitrogen > 40 mg/dl), heart failure, valvular diseases, asthma, chronic obstructive pulmonary disease, peripheral and cerebral vascular disease,

hematological disorders, acute or chronic infection, cancer, inflammatory disease, and hepatic dysfunction

Data Collection
Complete blood counts were performed to determine neutrophil and lymphocyte levels. The NLR was calculated by dividing the neutrophil count by the lymphocyte count. Blood pressure measurements were taken using standardized equipment and protocols.

Sample Collection
Under universal sterile precautions 5 ml of venous blood sample was collected for estimation of complete blood count (CBC) and biochemical parameters (Lipid Profile: Triglycerides, Total Cholesterol, HDL, LDL). 3ml sample was transferred into Green Top tubes for Lipid Profile estimation and 2ml whole blood was transferred into purple top EDTA Vials for CBC analysis. Serum sample was centrifuged within 30 minutes of collection at 4000 rpm for 60 secs to separate the serum. Separation of serum sample was done in Biochemistry Laboratory at SMHS hospital. The serum samples were analyzed for lipid profile by chemiluminescent microparticle immunoassay (CMIA) method on Allinity1 Abbott (USA) analyzer and CBC was done on Sysmex hematology analyzer (Germany) in the clinical Pathology Department at SMHS Hospital.

Calculations
The Body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m^2) and the NLR was calculated by dividing the neutrophil count by the lymphocyte count.

Table 1: Method and sources of kits used for estimation of various biochemical parameters

Kits	Method	Source
Cholesterol	CHOD-PAP	ABBOTT; USA
HDL	New clearance method	ABBOTT; USA
LDL	New clearance Method	ABBOTT; USA
TG	GPO-PAP	ABBOTT; USA

Statistical Analysis
Data were analyzed using the SPSS software version 21.0 for Windows (SPSS Inc., Chicago, Illinois). Descriptive statistics was used to summarize the demographic and clinical characteristics of the participants. Chi-square tests assessed differences in NLR between the blood pressure categories. ANOVA was conducted to evaluate the association between NLR and prehypertension while controlling for potential confounders such as age, sex, body mass index (BMI). Pearson analysis was used to evaluate correlations. Statistical significance was defined as $p < 0.05$.

Results
Demographic and Clinical Characteristics.

Table 2: Comparison of age, height, weight and BMI between Prehypertensive and control groups.

Parameters	Study Group N=53	Control Group N=50	P-Value
Age	49.88±8.8	47.88±6.0	>0.05
Height	155.1±7.7	153.1±6.0	>0.05
Weight	68.88±11.8	67.88±10.0	>0.05
BMI	28.88±2.8	47.88±3.0	>0.05

*BMI: Basal Metabolic Rate

Table 2 shows the comparison of age, height, weight, BMI between two groups weren't statistically significant (p>0.05). Both groups were comparable to each other

Table 3: Comparison of Baseline Clinical and hematological characteristics of Prehypertensive and control subjects.

Parameters	Prehypertensive N=53	Control Group N=50	P-Value
Mean SBP, mm Hg	128±5	106±4	**<0.001**
Mean DBP, mm Hg	84±4	71±5	**<0.001**
Hemoglobin, g/dl	13.5±1.5	14.0±1.0	**>0.05**
Triglycerides, mg/dl	114±33	110±25	**0.65**
Total Cholesterol, mg/dl	187±32.2	180±30.5	**<0.05**
HDL, mg/dl	45±20	46±22	**0.12**
LDL, mg/dl	109±19	128±25	**0.08**
Red-cell distribution width , %	15.8±1.1	15.1±0.8	**0.20**
Platelet count, /mm3	259±61	261±65	**0.85**
Mean Platelet Volume, Fl	8.5±1.1	8.0±1.0	**0.12**
White Blood Count, $x10^3/\mu L$	7.5±1.7	7.4±1.3	**0.85**
Neutrophil Count, $x10^3/\mu L$	4.3±1.3	3.7±0.9	**0.02**
Lymphocyte Count, $x10^3/\mu L$	2.1±0.4	2.8±0.6	**0.001**
Neutrophil/Lymphocyte ratio (NLR)	2.08±0.75	1.42±0.85	**<0.001**

*HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; *SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.

Table 2 demonstrates the characteristics of the prehypertensive patients compared to the normotensive controls. Biochemical and hematological parameters were comparable between groups except neutrophil and lymphocyte counts. In the PHT group, neutrophil count was significantly higher (4.3 ± 1.3 versus 3.7 ± 0.9; p = 0.02) and lymphocyte count was significantly lower (2.1 ± 0.4 versus 2.8 ± 0.6; p = 0.001) than in normotensive subjects. As a result, patients with PHT had significantly higher NLR values compared to the control group (2.08 ± 0.75 versus 1.42 ± 0.85; p < 0.001). Pearson analysis revealed a positive correlation between NLR and both systolic BP (r = 0.423; p < 0.001) and diastolic BP (r = 0.482; p < 0.001).(Fig:1).

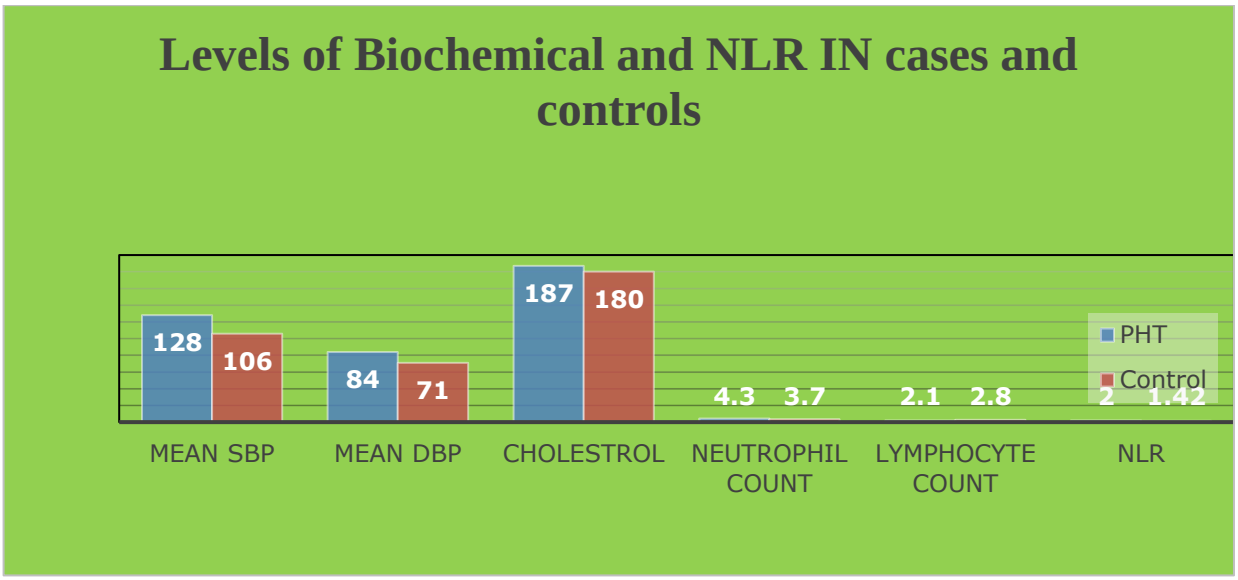

Figure 1: Histogram depicting levels of biochemical and NLR in prehypertensive and controls.

Table 4: Clinical and hematological characteristics of Patients by neutrophil-lymphocyte-ratio tertiles.

Parameters	Tertile 1 (1.18±0.18) N=53	Tertile 2 (1.55±0.08) N=53	Tertile 3 (2.41±0.64) N=53	P-Value
Age, years	48±7	49±11	44±13	**0.74**
Prehypertensive, n (%)	5(9.4)	7(13.2)	41(77.3)	**<0.001**
Body Mass Index , kg/m^2	26.9±3.5	28.2±3.5	27.2±3.8	**0.54**
Mean SBP, mm Hg	110±11	114±12	128±8	**<0.001**
Mean DBP, mm Hg	71±9	74±8	84±4	**0.001**
Hemoglobin, g/dl	14.5±1.5	13.9±1.0	14.0±1.8	**0.96**
Triglycerides, mg/dl	123±33	113±25	103±40	**0.25**
Total Cholesterol, mg/dl	187±32.2	180±30.5	195±35.0	**0.45**
HDL, mg/dl	48±20	50±15	40±10	**0.30**
LDL, mg/dl	121±27	109±31	110±18	**0.40**
Red-cell distribution width , %	15.3±1.1	15.1±0.8	15.0±1.3	**0.92**
Platelet count, /mm3	259±66	251±55	261±67	**0.84**
Mean Platelet Volume, fL	8.4±1.1	8.1±1.0	8.2±1.2	**0.91**
White Blood Count, x10^3/µL	6.8±1.1	7.4±1.3	7.9±1.9	**0.14**
Neutrophil Count, x10^3/µL	3.1±0.4	4.0±0.8	4.8±1.2	**<0.001**
Lymphocyte Count, x10^3/µL	2.7±0.4	2.6±0.5	2.0±0.5	**<0.001**

HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; *SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.

Table 4 summarizes NLR values in tertiles. Patients were divided into tertiles based on NLR values: 1.18 (0.90–1.42) in tertile 1; 1.55 (1.43–1.78) in tertile 2; and 2.41 (1.82–4.50) in tertile 3. Baseline demographic, hemodynamic, and biochemical and hematological characteristics of the study population tertiles are shown in Table 1. There were no statistically significant differences between the groups with respect to age, body mass index, or hemoglobin. The frequency of the prehypertensive

state was significantly higher among patients in the upper NLR tertile compared to the middle and lower tertiles (41 (77.3 %), 7 (13.2 %), and 5 (9.4 %), respectively; $p < 0.001$). There was no significant difference between the middle and lower NLR tertile groups in terms of pre hypertensive state ($p = 0.558$). Systolic BP and diastolic BP were significantly higher among patients in the upper NLR tertile (systolic: 128 ± 8 mm Hg, $p < 0.001$; diastolic: 84 ± 4 mm Hg) than among those in the middle (systolic: 114 ± 12 mm Hg, $p < 0.001$; diastolic: 74 ± 8 mm Hg, $p < 0.001$) and lower (systolic: 110 ± 11 mm Hg, $p < 0.001$; diastolic: 71 ± 9 mm Hg, $p = 0.001$) NLR tertiles.

Discussion

This is a first kind of study done in ethnic population of Kashmir. In the present study, a higher NLR, a reliable marker of inflammation, was found to be statistically associated with the presence of PHT. In addition, the NLR was significantly correlated with both systolic and diastolic BP. Numerous prospective cohort studies have shown a correlation between blood pressure and the risk of cardiovascular disease (CVD), stroke, and early mortality[7,18]. At systolic blood pressure as low as 115 mm Hg[19], there is an increased risk of CVD. The Framingham Heart Study's longitudinal data unequivocally demonstrate that people with blood pressure readings between 120 and 139/80 and 89 mmHg are more likely than those with readings under 120/80 mmHg[8] to experience full-blown hypertension and cardiovascular disease (CVD) later in life. Increased WBC count and BP[20–22] have been linked in studies conducted in the US and other populations. It is commonly known that leukocytes raise the risk of cardiovascular disease. Numerous CVDs have an inflammatory component to their aetiology[23,24]. Increased WBC count is linked to cardiovascular mortality, myocardial infarction, congestive heart failure, stroke, and the severity of coronary heart disease in addition to cardiovascular mortality and morbidity combined[25–30].

The ability of the total WBC count and its subgroups to predict the risk of myocardial infarction and death was investigated by Horne et al. They discovered that a higher frequency of cardiovascular events is independently correlated with high neutrophil and monocyte counts, low lymphocyte counts, and high NLR[31]. Moreover, NLR and leukocyte subtype numbers are markers of systemic inflammation [32]. Numerous cardiovascular disorders have been shown to benefit from the predictive and prognostic significance of the NLR, according to recent research[33–34]. WBC counts were observed to be greater in syndrome X patients compared to control persons by Atmaca *et al*[34]. A significant positive connection has been observed by Demirkol *et al* between the readings of plasma NLR[34] and carotid intima-media thickness. In CVD, these indicators are important prognostic factors. Numerous recent investigations have bolstered the theory that immune cells have a role in HT. Tian *et al* recently examined the relationship between certain circulating leukocyte types and BP and discovered that there is a relationship between BP and lymphocytes as well as neutrophils[35]. Kawada *et al* demonstrated a separate correlation between HT[36] and neutrophil count. Our findings agreed with the findings of this investigation.

To our knowledge, this is the first study to show an association between PHT and NLR. Further studies are needed to clarify the role of NLR in PHT patients, especially in relation to biochemical and clinical parameters, before we conclude that NLR may be used as a follow-up marker. This study was limited by a relatively small sample size. The findings suggest a significant association between the neutrophil-to-lymphocyte ratio and prehypertension. Prehypertensive people with increased NLR may have an underlying inflammatory condition that has a role in the development of elevated blood pressure. The idea that inflammation contributes to the development of hypertension in its early stages is supported by this connection. This association could be explained by several ways. Pro-inflammatory cytokines that neutrophils are known to emit may exacerbate endothelial dysfunction, a major contributing factor to the development of hypertension[37-38]. On the other hand, T-cells in particular are anti-inflammatory lymphocytes, and their decreased number may indicate a diminished ability to counteract inflammation[37-40.] Neutrophils were believed to be mediators engaged in the release of reactive oxygen species (ROS) and regulating complicated interactions involved in

inflammatory processes. Nitric oxide (NO) levels may be impacted by inflammation, potentially leading to vascular endothelium damage[41] and hypertension[42,43]. Furthermore, NO is an indicator of oxidative stress. ROS can lead to vascular diseases by impairing NO function, which is a component of NO-based cell signalling[44]. In an animal model, Ghosh *et al* also showed that oxidative stress was a factor in the common hypertension[45]. Furthermore, atherogenesis was linked to endothelial dysfunction and oxidative stress[46,47]. T cells, sometimes referred to as lymphocyte subsets, take role in the process of hypertension. One of the main theories linking antigen-driven autoimmune regulation to blood pressure control was that T-cell receptor ligation and a costimulatory interaction between CD28 (present on T lymphocytes) and B7 ligation (present on antigen-presenting cells) activated T lymphocytes. The hypertensive state was lessened by restricting or blocking these two routes[48,49]. Moreover, T cells' p47phox NADPH subunit was crucial to the angiotensin II-mediated hypertensive response. T cells also invaded other organs, such as the heart, kidney, and arteries, which could worsen hypertension by causing endothelial dysfunction and aortic stiffness[50]. T cells were discovered by Crowley *et al* to have invaded the renal interstitium, particularly in the vicinity of the renal vasculature[51]. Youn *et al* in Korea showed that human hypertension was associated with elevated CXC chemokine receptor type 3 and increased CD8+ T lymphocytes[52]. However, further research is needed to fully understand the connection between T cells and prehypertension in people. To the best of our knowledge, this work is the first to use the NLR as an inflammatory marker and explore the relationship between prehypertension in Kashmir, stratified by age and BMI, and the NLR. More studies are warranted with large sample size on prehypertension and NLR in ethnic population of Kashmir to elucidate the its role in early prognosis and diagnosis.

Conclusion

Our data findings suggests that the there is a connection between PHT and NLR. The monocyte count and NLR assessment can both be used to identify a higher risk of prehypertension. One potentially useful measure for identifying people at risk of prehypertension is the neutrophil-to-lymphocyte ratio. It is a desirable alternative for risk assessment and early screening because to its affordability and ease of use. To validate these results and investigate the underlying mechanisms that connect NLR to prehypertension, more long-term investigations are required.

Contribution by authors

Author contributions were as follows: Lab work, patient selection, and data extraction were performed by Anam Shameem Hakak , Samia Mearaj , Humairah Shafi , Dr Shabir ud din Lone . Anam Shameem Hakak wrote the initial draft of the manuscript, while Dr Shabir ud din Lone reviewed it. Successive drafts were critically revised and approved in their final form by all authors. All authors have read and approved the final manuscript.

Author Disclosure Statement

The authors assert that there exist no commercial or financial associations that could be interpreted as a possible source of conflict of interest.

Competing interests

The authors assert that there were no conflicting interests.

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Search Strategy

In this study, publicly available data from published sources were utilized. A comprehensive search was conducted on the PubMed, EMBASE, and MEDLINE (via PubMed) databases to retrieve all pertinent literature on the topic. We employed a blend of relevant keywords to conduct our search. Furthermore, a thorough examination of the reference list encompassing all pertinent papers and reviews was conducted to ascertain supplementary information. This study encompassed all observational research endeavors that documented the correlation between Neutrophil-to-Lymphocyte Ratio and Prehypertension in Kashmiri Patients

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Ethical statement
Ethical approval was sought before the start of the study.

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