

## **Leukocyte Count a Fore warner of Hypertension**

<sup>1</sup>Raghav Prasad, Intern, Kempegowda Institute of Medical Sciences, Bangalore

<sup>2</sup>Manogna Sagiraju, Intern, Kempegowda Institute of Medical Sciences, Bangalore

<sup>3</sup>Dr Manjula CP, Associate Professor, Department of Pathology, Kempegowda Institute of Medical Sciences, Bangalore

**Corresponding Author:** Raghav Prasad, Intern, Kempegowda Institute of Medical Sciences Bangalore, Bangalore

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### **Abstract:**

**Background:** Hypertension is a major risk factor for coronary artery disease, stroke, renal failure, and various cardiac conditions. Leukocyte count has emerged as a potential marker for identifying individuals at risk of developing hypertension. This study aims to investigate the relationship between leukocyte count and hypertension in an Indian population and found a significant positive correlation between leukocyte count and systolic blood pressure in all blood pressure groups, indicating that as leukocyte count increased, systolic blood pressure also increased.

**Methods:** A total of 240 subjects between the ages of 18-60 years were enrolled in this observational study. The participants were divided into three groups: normotensive, pre-hypertensive, and hypertensive, based on their blood pressure measurements. Leukocyte counts were determined using a standardized method, and statistical analyses, including linear regression and Pearson correlation, were performed to assess the relationship between leukocyte count and systolic blood pressure.

**Results:** The analysis revealed a significant difference in mean leukocyte counts among the three blood pressure

groups. Normotensive individuals had a mean leukocyte count of  $7609 \pm 384$  cells/cu mm, while pre-hypertensive and hypertensive individuals had mean counts of  $8573 \pm 451$  and  $9578 \pm 519$  cells/cu mm, respectively. Linear regression analysis demonstrated a positive linear relationship between leukocyte count and systolic blood pressure ( $\hat{Y} = 100.6017 + 0.003535X$ ). Pearson correlation analysis confirmed a significant positive correlation between leukocyte count and systolic blood pressure in all blood pressure groups, indicating that as leukocyte count increased, systolic blood pressure also increased.

**Conclusion:** Elevated leukocyte count serves as an independent risk factor for hypertension in the Indian population. The findings suggest that leukocyte count could be utilized as an additional marker for risk assessment in the identification of individuals who may benefit from early interventions to prevent or manage hypertension and its associated complications. Integrating leukocyte count assessment into clinical practice may aid in risk stratification and facilitate personalized treatment approaches. Further research is necessary to evaluate the clinical utility of leukocyte count in guiding treatment decisions and its impact on long-term patient outcomes.

**Keywords:** Hypertension, Leukocyte count, Blood Pressure, Risk Factor, Cardiovascular Diseases, Indian Population, Inflammation, Predictive marker, Cardiovascular Risk.

### Introduction

Hypertension is one of the strongest risk factors for stroke, renal failure and almost all cardiovascular diseases including coronary artery disease, left ventricular hypertrophy, cardiac arrhythmias and heart failure [1].

- Globally it accounts for 9.4 million deaths each year due to complications arising from it [2].
- In India it is estimated that 10% of all deaths each year are attributable to hypertension [3].
- A meta-analysis on prevalence of hypertension in India, conducted between January 2000 and June 2012 revealed that urban population is more predisposed to hypertension (40.8%) as compared to rural population (17.9%) [4].

The modern epidemic of hypertension is a preventable disease. Studies have indicated that implementing targeted interventions to reduce blood pressure among individuals with hypertension can lead to a substantial decrease in the burden of cardiovascular diseases [5]. Therefore, it is crucial to prioritize effective screening strategies within the population to identify those at heightened risk of developing hypertension and its associated complications. By identifying individuals at an early stage, appropriate interventions can be implemented to mitigate the progression and impact of hypertension, ultimately improving public health outcomes.

It has been hypothesized that leukocytes play a role in pathogenesis of hypertension in multiple ways i.e., by causing a state of chronic inflammation [6], by platelet-leukocyte interactions [7] and adherence of leukocytes to the vascular endothelium [8]. Elevated leukocyte and

neutrophil counts have been shown to precede the development of hypertension in animal models [9,10].

Previous studies have shown a linear relationship between elevated leukocyte count and higher systolic blood pressure in a predominantly white population [11]. Two longitudinal studies investigating the association between white blood cell (WBC) count and incidence of hypertension have noted an increased incidence of hypertension in higher range of normal leukocyte count that is  $8.65-10.05 \times 10^9/L$  as compared to lower range of normal leukocyte counts [12,13].

Leukocyte Count estimation is a stable, well standardized, inexpensive and widely available test, with a high level of inter-assay precision [14]. The information obtained from this may be extrapolated to predict the risk of hypertension. If so, this simple routine test will have a major impact on the health of the general population. If we are able to expand on the inferences generated based on its findings.

The existing "normal" values utilized in clinical practice primarily stem from reference populations of European or European-American origin. Consequently, there exists a potential for misclassification of risk among other large population groups. This study aims to contribute to the expansion of knowledge and comprehension regarding the impact of leukocyte count on blood pressure specifically within the Indian population.

The study establishes a linear relationship between systolic blood pressure (dependent variable) and leukocyte count (independent variable). Additionally, it compares the mean leukocyte counts among different blood pressure groups, aiming to substantiate leukocyte count as a risk factor for hypertension. By conducting this investigation, the study seeks to provide valuable insights into the association between leukocyte count and hypertension in the Indian population.

## Review of Literature

The initial description of leukocytes was reported independently by Gabriel Andral, a French professor of medicine and William Addison, an English country practitioner, in the year 1843 [15,16]. As early as 1954, Cole et.al, made an observation that myocardial infarction (MI) patients having elevated leukocyte counts had a 4-fold higher risk of death as compared to patients with WBC counts in the normal range [17].

Many studies have suggested that elevated levels of inflammatory markers such as WBC count, C-Reactive Protein and IL-6 are associated with increased risk of hypertension [6,12,13,18,19,20] and cerebrovascular disease (CVD) [21-28]. Several epidemiologic studies have shown a positive association between neutrophil count and CVD [24,25].

The American Heart Association convened 'Prevention Conference V' to examine various strategies for the identification of patients at high risk of hypertension who need primary prevention. Among the strategies discussed was the measurement of markers of inflammation like leukocyte count [29]. The Conference concluded that "many of these markers are not yet considered applicable for routine risk assessment because of:

- Lack of measurement standardization,
- Lack of consistency in epidemiological findings from prospective studies with endpoints,
- Lack of evidence that the novel marker adds to risk prediction over and above that already achievable through the use of established risk factors."

The association between smoking and elevated white blood cell (WBC) count has been established, and smoking itself is recognized as an independent risk factor for hypertension [30,31]. Additionally, a study conducted on Japanese men observed that the risk ratios of hypertension associated with elevated leukocyte count

were more prominent among non-smokers compared to smokers [18]. These findings highlight the importance of considering smoking status as a potential confounding factor when examining the relationship between leukocyte count and hypertension.

It is hypothesized that leukocytes play a role in pathogenesis of hypertension in the following ways –

1. Elevated leukocyte counts cause chronic low-grade inflammation that alters endothelial cell function and affects production of nitric oxide and prostacyclins. Consequently, there is loss of vasodilator, antithrombotic and anti-atherogenic properties of the vascular endothelium [6].
2. Increased adherence of the stimulated leukocytes to the vascular endothelium, causing capillary leukocytosis and subsequent increased vascular resistance; a raised WBC count may therefore indicate increased catecholamine levels or enhanced sympathetic nervous system activity, thus causing an increase in blood pressure and eventually resulting in sustained hypertension [6,8].
3. Inflammation may play a key role in the initiation and development of hypertension via the proinflammatory actions of mediators such as adhesion molecules, chemokines, growth factors, heat shock proteins, endothelin-1 and angiotensin. Certainly, a persistent low-grade inflammatory state could result in high normal levels of inflammatory cytokines [11].
4. A significant relationship between blood pressure and reactive oxygen species (ROS) by neutrophils has been observed in apparently healthy subjects [32].

## Materials and Methods

Type of study: Observational Study (Clinical Investigation)

Subject area- Haematology

Study Site: Central Laboratory, Department of Pathology

Duration of Study: 2 months

Ethics: Ethical clearance has been taken from the Institutional Ethics Committee for the study. Informed consent form duly filled and signed by the patient has been taken before drawing blood samples.

### Patients

- Inclusion Criteria: All subjects between 18-60 years of age.
- Exclusion Criteria: Smokers, dyslipidemias, pregnancy, malignancy, diabetes mellitus, acute and chronic infections, inflammatory conditions, auto-immune disorders, immunodeficiency disorders.
- Sample Size: 240
- The study group will consist of all subjects with systolic blood pressure (SBP) >120mm of Hg and/or Diastolic Blood Pressure (DBP) >80mm of Hg<sup>33</sup>. They will then be divided into pre-hypertensives (SBP: 120-139 mm of Hg or DBP: 80-89 mm of Hg) and hypertensives (SBP ≥ 140 mm of Hg or DBP ≥ 90 mm of Hg).
- Control group consists of subjects with SBP<120mm of Hg and DBP<80mm of Hg.

### Method

1. The patient's informed consent is taken and information is recorded in the pro forma.
2. A tourniquet is applied above the puncture site and the site is disinfected with isopropyl alcohol for 30 seconds. 2 ml of venous blood is collected and stored in an EDTA vacutainer tube.
3. Leukocyte counts are estimated using Sysmex XT 1800i hematology auto analyzer.
4. Blood Pressure is recorded using mercury column sphygmomanometer. SBP and DBP were determined by Korotkoff phases I and V respectively.

5. The average of 3 readings is noted down.
6. The data will be tabulated based on JNC8 classification of hypertension<sup>33</sup> and the mean leukocyte count with 95% confidence interval for each class will be calculated.
7. A linear regression test will be done to check for the presence of a linear relationship between leukocyte count and blood pressure which will be followed by a Pearson Correlation.
8. A comparison of the mean of leukocyte counts of different blood pressure groups will be done.

### Results

Number of normotensives= 120

Number of pre-hypertensives = 60

Number of hypertensives = 60

A comparison of mean leukocyte counts of cohorts of different blood pressure groups.

Table 1: Comparison of Mean Leukocyte Counts

Blood Pressure Group	Mean Leukocyte Count (cells/cu mm)
Normotensive	7609 ± 384 (7230 to 7990)
Pre-Hypertensive	8573 ± 451 (8120 to 9020)
Hypertensive	9578 ± 519 (9060 to 10100)

There are a significant difference of mean leukocyte counts in each group.

### Linear Regression

A linear regression was made with leukocyte count as the independent factor and systolic blood pressure as the dependent factor (**Figure 1**).

The equation -  $\hat{Y} = 100.6017 + 0.003535X$

The residual plot (**Figure 2**) shows a random pattern which indicates that a linear model provides a decent fit to the data.

Figure 1: Linear Regression comparing Systolic Blood Pressure with Leukocyte Count

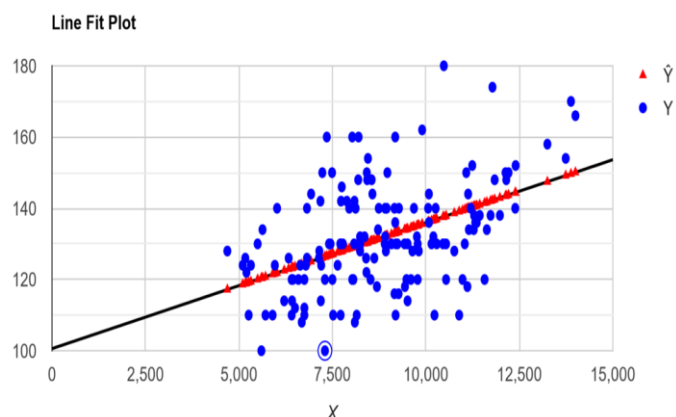
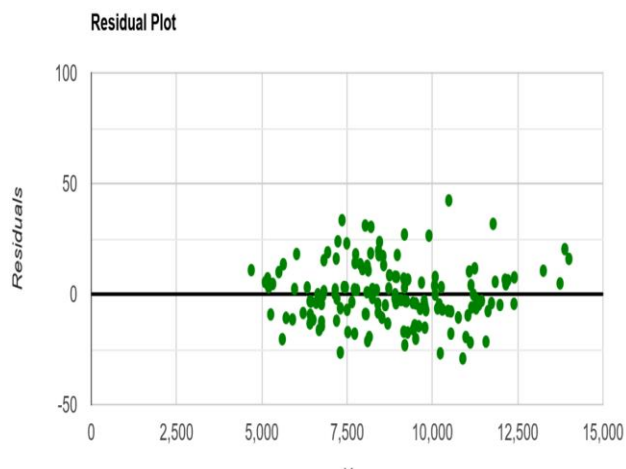


Figure 2



As a linear model fits the data set, a Pearson correlation is calculated to see the relationship between the variables.

Table 2

Blood Pressure Group	Pearson Correlation between SBP and LC
All	$r(238) = 0.467, p < 0.01$
Normotensive	$r(118) = 0.411, p < 0.05$
Pre-Hypertensive	$r(58) = 0.449, p < 0.05$
Hypertensive	$r(58) = 0.389, p < 0.05$

There is a positive correlation between the variables which is significant in all groups.

1. To rule out the effect of age on leukocyte count a Pearson correlation is calculated between age and leukocyte count.

$r(238) = 0.0814, p < 0.05$

A very weak correlation is shown which rules out age as a confounding factor.

2. To rule out the effect of sex on leukocyte count the mean count between males and females is compared.

Number of males= 135

Number of females= 105

Male =  $8829.910828 \pm 316.157439$

Female =  $8895.63158 \pm 303.741298$

There is no significant difference in the mean leukocyte count between males and females.

### Discussion

Our study attempts to establish leukocyte count as a viable risk factor for hypertension. The findings of our study demonstrate a statistically significant increase in leukocyte count among individuals with hypertension. This observation can be attributed to the elevated leukocyte levels inducing a state of chronic low-grade inflammation, which, in turn, disrupts endothelial function [6]. Moreover, the heightened leukocyte count promotes increased adherence of leukocytes to the vascular endothelium, leading to capillary leukocytosis and subsequently raising vascular resistance [8]. These mechanisms contribute to the development and progression of hypertension.

The present study also demonstrated that a direct correlation is evident between systolic blood pressure and leukocyte count across all blood pressure levels, as indicated by a Pearson Correlation coefficient of 0.467. Therefore, a statistically significant moderate positive correlation can be inferred to exist between these variables. This is similar to previous studies thus further cementing the hypothesis [11,12,13].

The normal leukocyte count is between 4000- 11000 cells/mm<sup>3</sup>, so any value within this range is mostly ignored by clinicians. The present study provides

evidence that elevated leukocyte counts within the high normal range can serve as a noteworthy risk factor for hypertension and subsequent cardiovascular diseases. However, there remains a lack of consensus among professional societies and governmental agencies regarding the appropriate utilization of leukocyte count assays in clinical practice. Consequently, it is imperative to conduct randomized clinical trials to ascertain whether risk stratification based on leukocyte count can effectively reduce the number of patients requiring treatment by identifying low-risk groups previously advised to undergo additional diagnostic testing or intensive interventions.

These studies should then clarify strategies, based on leukocyte levels, for additional treatment planning, further diagnostic testing and change in recommended targets for treatment of blood pressure. In patients with hypertension, leukocyte count measurement may be useful as an independent marker for assessing likelihood of complications like coronary artery disease, left ventricular hypertrophy, cardiac arrhythmias, heart failure, cerebral stroke and renal failure.

SBP is used as a dependent variable as it has a greater impact on morbidity and mortality of patients with hypertension [34,35,36], and even within the non-hypertensive range, imposes increased rates of cardiovascular disease [37].

To the best of our knowledge, this study represents the first investigation of this subject within the Indian population. Consequently, it provides valuable epidemiological data that can be juxtaposed with analogous studies conducted among European, American, and Japanese populations. Furthermore, the study findings indicate a minimal influence of sex or age on leukocyte count within this specific cohort.

The primary limitation of this study is the absence of C-reactive protein (CRP) levels, which is a specific inflammation marker known to be associated with hypertension and cardiovascular disease. Therefore, future investigations should focus on elucidating the independent roles of leukocyte count and CRP in predicting hypertension. Another limitation pertains to the use of a sphygmomanometer for blood pressure measurement, which can introduce observer or instrumentation errors. To mitigate this, an average of three readings was taken to minimize potential inaccuracies. Additionally, the phenomenon of white coat hypertension, wherein patients exhibit elevated blood pressure due to anxiety in a clinical setting, could potentially influence the results. Another area that warrants attention is the absence of comparative data concerning different types of leukocytes. Furthermore, the exclusion of patients with a history of smoking hinders the understanding of leukocyte count's impact on blood pressure among smokers. Future studies should be conducted to establish elevated leukocyte count as an independent risk factor in smokers. Additionally, it would be of interest to explore the utilization of pharmacological interventions to reduce leukocyte count in hypertensive patients and examine their effects on morbidity and mortality within this population. Finally, it is crucial to incorporate experimental study designs, in addition to observational approaches, to address and clarify these aforementioned limitations and issues.

### **Conclusion**

The study emphasizes that elevated leukocyte count can serve as an independent risk factor for hypertension. It reveals that increasing leukocyte counts are associated with higher blood pressure levels. Leukocyte count is a reliable, widely accessible, cost-effective, and standardized test, making it a valuable tool for

diagnosing high-risk individuals and informing treatment strategies. By incorporating leukocyte count in clinical practice, healthcare professionals can potentially enhance outcomes in hypertensive patients.

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