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THE PREDICTIVE EFFECT OF NEUTROPHIL LYMPHOCYTE RATIO (NLR) AND PLATELET TO LYMPHOCYTE RATIO (PLR) IN CULTURE NEGATIVE FEVER OF UNKNOWN ORIGIN– A COMPARATIVE STUDY

ANALYSIS OF NLR AND PLR IN FEVER OF UNKNOWN ORIGIN

Dr. Madhumitha. M [1], Dr. Prathish Kumar [2], Dr. M. Nishanthy [3] and Dr. M. P. Brundha [4]*

[1] Graduate, Madha Medical College, and Research Institute, Kovur, Chennai, Tamilnadu, India. Email: mmadhumithaa@gmail.com
[2] Associate Professor, Department OF General Medicine, Madha Medical College and Research Institute, Kovur, Chennai.
[3] Assistant Professor, Department of Microbiology, Madha Medical College and Research Institute, Kovur, Chennai.
[4] Professor, Department of Pathology, Madha Medical College, and Research Institute, Kovur, Chennai, Tamilnadu, India.
*Corresponding Author Email: generalpathology2015@gmail.com

Abstract

Background: FUO refers to prolonged febrile illnesses with no established etiology, despite extensive evaluations and diagnostics. It is possible to predict severity and mortality among patients with Fever of unknown origin using several inflammatory markers like NLR and PLR. A neutrophil lymphocyte ratio (NLR) is the ratio between the absolute neutrophil count and the absolute lymphocyte count in the body. The platelet lymphocyte ratio (PLR) is a numerical value that represents the ratio of platelet count to lymphocyte count in a blood sample. This study discusses the importance of NLR and PLR in culture negative Fever of unknown origin. Material and Methods: The study was conducted in a tertiary care hospital in South India. Reports of CBC (completed blood count) tests of Culture negative FUO patients hospitalized between October 2022 and March 2023 were retrospectively collected. Total White Blood Cell Count, Absolute Neutrophil Count, Absolute Lymphocyte Count, Platelet Count, and ratio of neutrophil lymphocytes to platelets lymphocytes, were collected and calculated and analyzed. Results: Fever of unknown origin was associated with higher total WBC, absolute neutrophil count, and lymphocyte count. There is a decrease in platelet count compared to the control group. In terms of NLR and PLR, the group with FUO exhibited a mild reduction in NLR and a significant reduction in PLR compared to the control group. Conclusion: Decreased NLR and PLR are the most important predictive markers of severity in Culture Negative Fever of Unknown Origin.

Keywords: NLR, PLR, Fever, FUO, CBC.

INTRODUCTION

Fever of Unknown Origin

Fever can be caused by a variety of organisms. Any febrile illness without a clearly defined etiology is commonly referred to as Fever of unknown origin (FUO) by clinicians[1]. A diagnosis is usually made only after a febrile illness has resolved or developed distinguishing characteristics. FUO refers to prolonged febrile illnesses with no established etiology, despite extensive evaluations and diagnostics[2]. In 1961, Petersdorf and Beeson defined FUO as an illness lasting at least three weeks with fevers above 38.3°C (101°F) twice and uncertain diagnosis despite one week of inpatient care[3]. Patients with FUO are typically hospitalized only when their clinical condition needs it, not for diagnostic purposes alone; it has, therefore, been decided that the requirement for in-hospital evaluation should no longer be included in the definition. The following are the criteria for the diagnosis of FUO[4]: 1. Fever >38.3'C

(101'F) on at least two occasions; 2. Illness duration of >3 weeks; 3.No known immunocompromised state; 4. Diagnosis that remains uncertain after a history taking, physical examination and following obligatory investigations like determination of Erythrocyte Sedimentation Rate and C Reactive Protein, Platelet Count, Leukocyte Count and Differential Count, measurement of levels of Hemoglobin, Electrolyte, Creatine Kinase, Ferritin, Antinuclear Antibodies, Rheumatic Fever, Protein Electrophoresis, Urine Analysis, Blood Culture, Urine Culture, Chest X Ray, Abdominal Ultrasonography and Tuberculin Skin Test or Interferon Gamma Release Assay[5, 6]. FUO has evolved since its first definition due to changing diseases, widespread antibiotic use, and most importantly, new diagnostic techniques. CT and ultrasound have made it easier to detect intra-abdominal abscesses and tumors, for example. Moreover, blood culture and echocardiography have improved, making infective endocarditis less common[7].

FUO has a wide range of differential diagnoses. An atypical presentation of a rather common disease is more likely to cause FUO than a very rare disease. Endocarditis, Diverticulitis, Vertebral Osteomyelitis, and Extrapulmonary Tuberculosis have atypical presentations[8, 9]. Through comprehensive and repeated history-taking, physical examination, and the necessary investigations, potentially diagnostic clues (PDCs) are used to diagnose them. All localizing signs, symptoms, and abnormalities that might lead to a diagnosis are considered PDCs[10]. A concise list of probable diagnoses can only be made with the help of PDCs, even though they are often misleading. Information about the fever pattern and duration should be included in the history, as well as earlier medical history, drug use in the past and present, as well as family and sexual history, country of origin, recent and remote travel, unusual environmental exposures associated with travel or hobbies, and animal contact[11].

It is possible to predict severity and mortality among patients with Fever of unknown origin using several inflammatory markers[12]. There has been research on platelet count, mean platelet volume, platelet distribution width and platelet lymphocyte ratio as markers of severity and mortality in patients with Fever of unknown origin. This study discusses the importance of NLR and PLR in Fever of unknown origin.

Neutrophil to Lymphocyte Ratio

A neutrophil lymphocyte ratio is the ratio between the absolute neutrophil count and the absolute lymphocyte count in the body[13]. Neutrophils are a type of white blood cell (leukocytes) that act as the immune system’s first line of defence. There are three types of white blood cells: granulocytes, lymphocytes, and monocytes. A neutrophil, along with an eosinophil and a basophil, is a subset of a granulocyte. Together, white blood cells protect the body from infection and injury. An absolute neutrophil count may be used to check for infection, inflammation, leukemia, and other conditions. Neutrophil counts are inversely related to infection risks, the lower the count, the greater the risk[14]. As part of the innate immune response, lymphocytes play an essential role in the regulation of antimicrobial phagocytic and cytotoxic activity. Absolute lymphocyte count calculates as the total white blood cell count (WBC) multiplied by the percentage of lymphocytes in the peripheral blood[15]. The neutrophil-lymphocyte ratio (NLR) is an easy way to assess an individual's inflammatory status. Studies have shown it to be useful in predicting the prognosis of inflammatory or infectious pathologies and in some cancers, postsurgical complications. NLR in normal male and female are 1.65± 1.47 and 1.69± 1.37[16].

Symptom severity is associated with increased NLR. For example, in a case of Pneumonia with Symptoms like fever, fatigue, coughing, are associated with a higher Neutrophil lymphocyte ratio. In contrast, a lower neutrophil to lymphocyte ratio says improvement in clinical conditions such as decreased cough, improved fever, and supported lung disease in such patients. When a reduction in NLR in case of fever and ITP, says chronicity and good prognosis[17].

Platelet Lymphocyte Ratio

The platelet lymphocyte ratio (PLR) is a numerical value that represents the ratio of platelet count to lymphocyte count in a blood sample. It is a calculated value that can provide information about the balance between the body's inflammatory response and the ability to form blood clots. Platelet lymphocyte ratio originally served as a systemic inflammatory biomarker to predict the prediction of neoplastic diseases. Normal PLR are 117.05±47.73 for both the gender[18]. Changes in the PLR may indicate certain conditions or diseases, although it is important to note that the PLR alone is not diagnostic of any specific condition and should be interpreted in the context of other clinical findings. In general, an increased PLR suggests an increased platelet count and/or a decreased lymphocyte count. This can be seen in various inflammatory conditions, such as infections, autoimmune diseases, and certain types of cancer[19, 20]. It may also be associated with an increased risk of cardiovascular disease and poor outcomes in some cancers. On the other hand, a decreased PLR suggests a decreased platelet count and/or an increased lymphocyte count. This can occur in certain viral infections, such as HIV, as well as in some Hematological disorders, like lymphocytic leukemia. It's worth noting that the PLR is not routinely used as a standalone diagnostic tool, but rather as an adjunctive marker in conjunction with other clinical and laboratory assessments. The interpretation of PLR values should be done by a qualified healthcare professional who can consider the individual's medical history, symptoms, and other relevant factors[21].

Although there have been extensive investigations on neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) in many diseases, their roles in Fever of unknown origin still are unclear. The purpose of the present study is to evaluate NLR and PLR in patients with Fever of unknown origin and explore their clinical significance. Our present study of the predictive effect of PLR and NLR on patients with FUO is to provide help for relaxed clinical diagnosis and prognosis.

MATERIAL AND METHODS

Our research initiative was a retrospective investigation carried out at a tertiary care hospital in South India, with ethical approval obtained from the Institutional Ethical Committee under the reference IHEC number MMCRI/IEC/2023/015. This study focused on patients with Fever of Unknown Origin (FUO) and aimed to comprehensively analyze their clinical profiles and hematological parameters. The scope of our study encompassed the retrospective collection of Complete Blood Count (CBC) test reports from patients who were hospitalized with FUO during the period between October 2022 and March 2023. The inclusion criteria were stringent, targeting patients aged between 21-50 years, who had been hospitalized for more than a week due to a fever exceeding 38.3°C, and whose blood cultures tested negative for organisms. Both male and female patients were incorporated into our study to ensure a comprehensive representation of the FUO population. To maintain precision in our

investigation, exclusion criteria were defined. Patients with fevers of less than a week's duration, those not hospitalized specifically for fever, and pediatric cases were deliberately excluded from the study. This strategic selection process ensured that our study population was homogenous and specifically tailored to the unique characteristics of adult individuals experiencing prolonged fever of unknown origin.

Information pertaining to the study population was meticulously collected, including demographic details such as age and gender, as well as key clinical parameters like the duration of fever, Total White Blood Cell Count, Absolute Neutrophil Count, Absolute Lymphocyte Count, and Platelet Count. These parameters were chosen to provide a comprehensive overview of the hematological status of the individuals under investigation. From the amassed data, we calculated essential ratios, specifically the ratios of neutrophil to lymphocyte and platelet to lymphocyte. These ratios, derived from the absolute counts, served as valuable indicators of the immune response and hematological balance in the context of FUO. Our data analysis was conducted with rigor, utilizing statistical tools such as SPSS version 26. The independent t-test, Chi Square analysis, and Pearson correlation study were employed to scrutinize the collected data and derive meaningful insights. These statistical methodologies allowed us to explore potential differences between groups, assess the significance of various parameters, and examine correlations, particularly with respect to the duration of fever.

RESULTS

A total of 130 records were collected for this study. 65 patients of fever of unknown origin and 65 control samples who were normal for routine health check-up were taken. Results were obtained and analysed using SPSS software version 26.

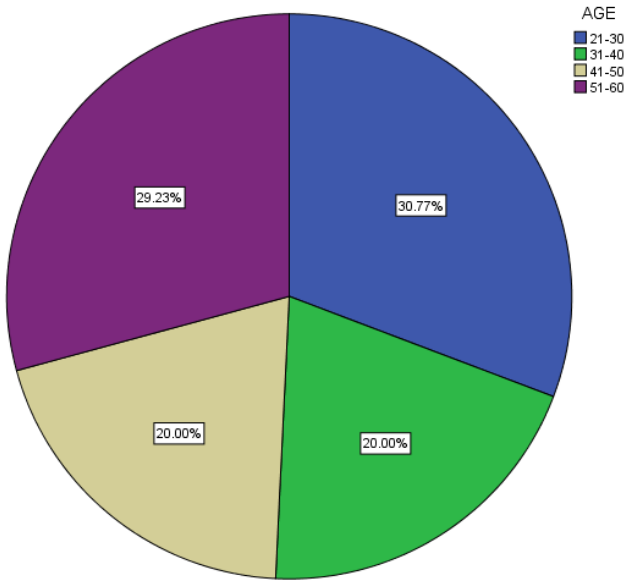

Figure 1: Age Distribution of Study Population

Figure 1 shows the age distribution of patients with FUO. Although the Fever of Unknown Origin is highly prevalent among the study population of 21-30 years (30. 8%), followed by 51-60 years (29.2%), 31-40 years (20%), and 41-50 years (20%), but there is no significant difference between these age groups statistically ($p>0.05$).

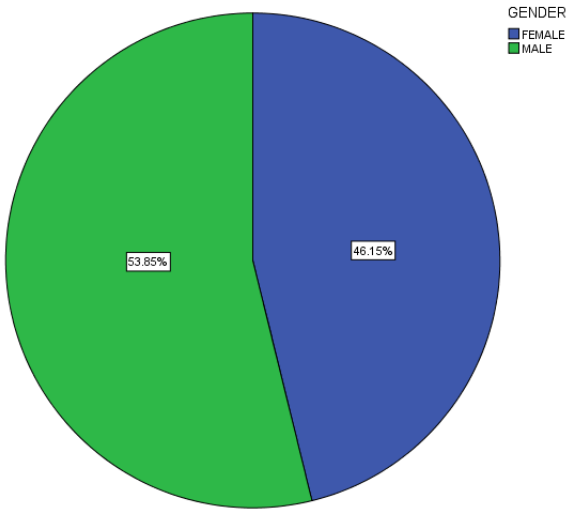

Figure 2: Gender Distribution of Study Population with Fever of Unknown Origin

Figure 2 shows that the prevalence of Fever of Unknown Origin is higher in males (54%), compared to females (46%). However, there is no statistically significant difference (p>0.05).

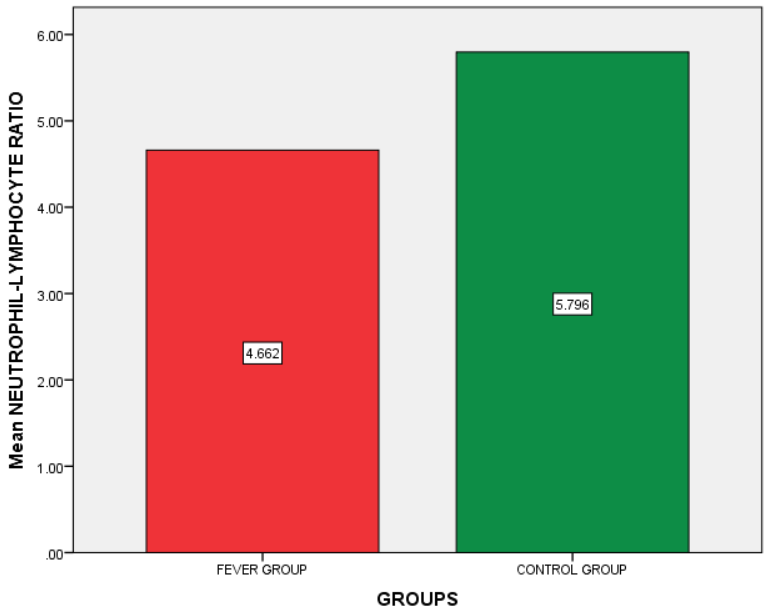

Figure 3: Comparison of Neutrophil Lymphocyte Ratio

Figure 3 shows the mean Neutrophil Lymphocyte Ratio among the study population. Patients with fever of unknown origin have a lower Neutrophil Lymphocyte Ratio (4.66) than the control group (5.79). Statistically, there is no significant difference between the two groups (p>0.05).

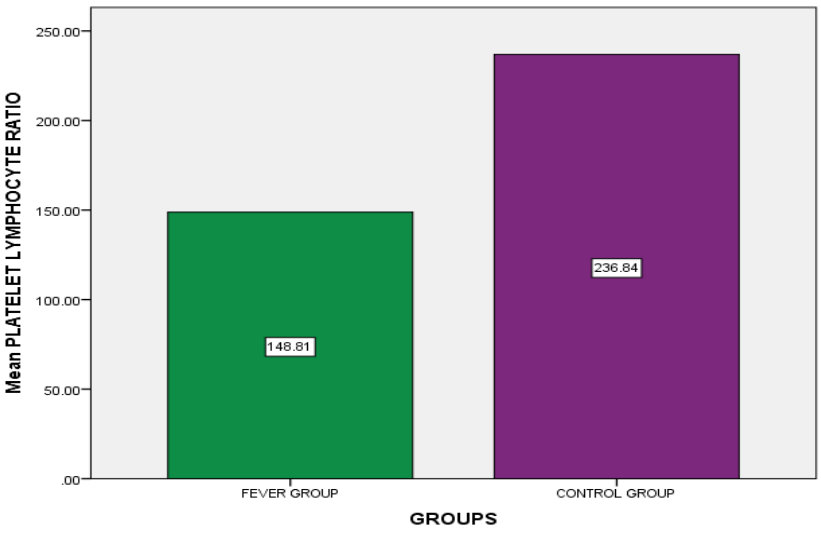

Figure 4: Comparison of Platelet Lymphocyte Ratio

Figure 4 shows the mean Platelet Lymphocyte Ratio among the study population. Patients with fever of unknown origin have a lower Platelet Lymphocyte Ratio (4.66) than the control group (5.79). Statistically, there is a significant difference between the two groups P=0.001(<0.05)

Table 1: Statistical Analysis

	Groups	N	Mean	Std. Deviation	Sig.
Total WBC Count	Fever Group	65	10258.4615	5761.42594	
	Control Group	65	7425.5385	1766.92355	0.000
Absolute Neutrophil Count	Fever Group	65	7138.6462	4874.20659	
	Control Group	65	5399.2615	1692.06559	0.000
Absolute Lymphocyte Count	Fever Group	65	2490.7385	2045.14947	
	Control Group	65	1704.3692	900.07317	0.001
Platelet Count	Fever Group	65	244892.3077	131396.00487	
	Control Group	65	252400.0000	80280.91305	0.001
Neutrophil-Lymphocyte Ratio	Fever Group	65	4.6615	6.41716	
	Control Group	65	5.7960	7.76075	0.298
Platelet-Lymphocyte Ratio	Fever Group	65	148.8138	144.59670	
	Control Group	65	236.8435	241.18676	0.007

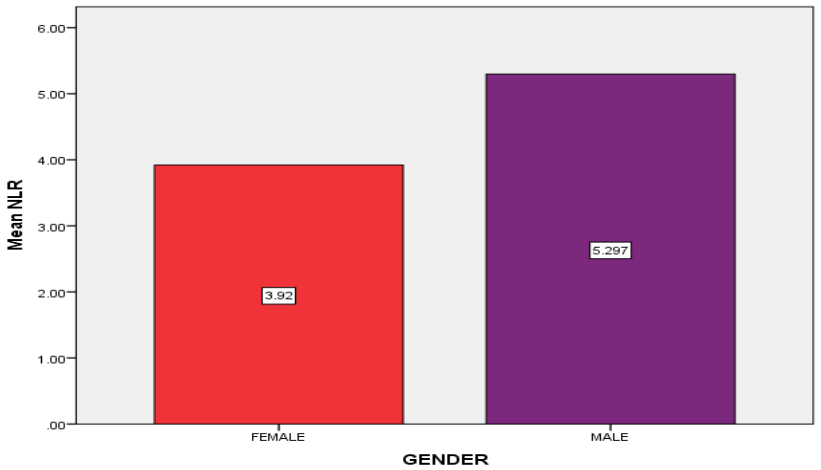

Figure 5: NLR with Gender Association

Figure 5 showed gender association analysis for NLR among the study population. Females have low NLR (3.9) than males (5.2). However, there is no difference between the gender in relation to Neutrophil-Lymphocyte Ratio statistically (Chi square p>0.05).

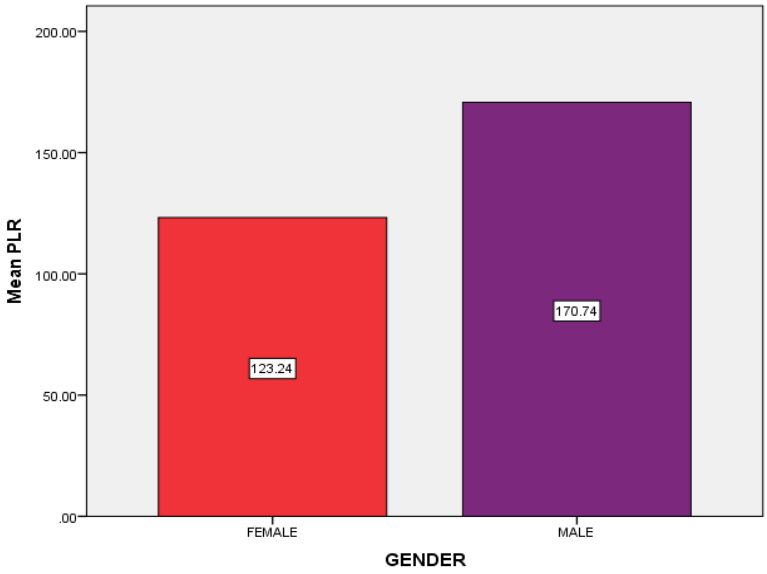

Figure 6: PLR with Gender Association

Figure 6 showed gender association analysis for PLR among the study population. Females have low PLR (123.2) than males (170.7). However, there is no difference between the gender in relation to Platelet-Lymphocyte Ratio statistically (Chi square p>0.05).

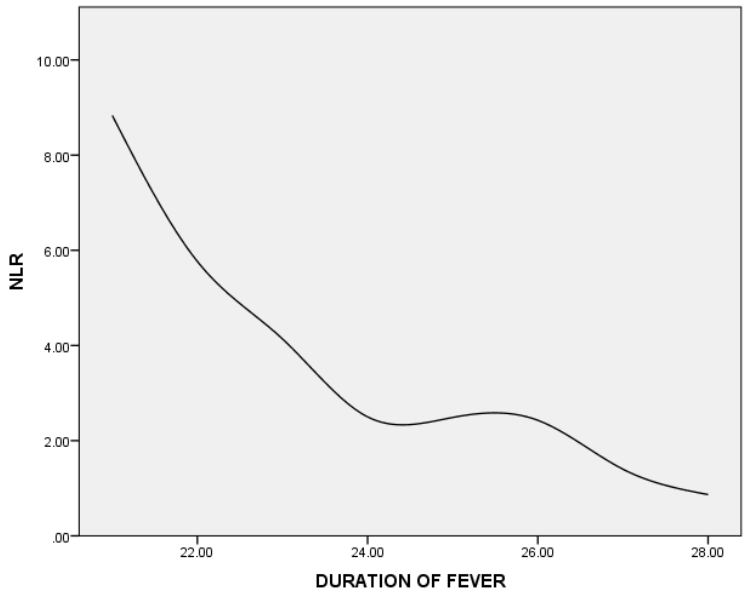

Figure 7: Correlation Analysis of NLR with Fever Duration Indicated that the Ratio of NLR Decreased as the Fever Duration Increased.

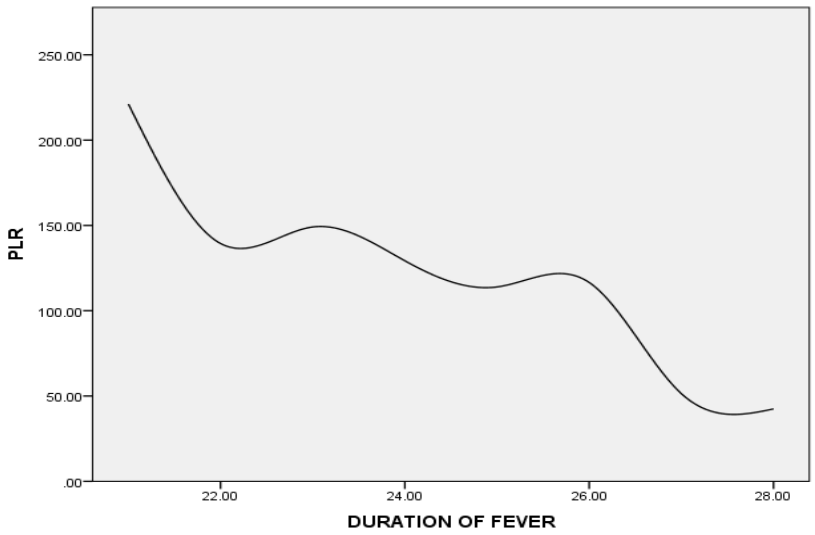

Figure 8: Correlation Analysis of PLR with Fever Duration Indicated that the Ratio of NLR Decreased as the Fever Duration Increased.

DISCUSSION

Fever of Unknown Origin (FUO) poses a diagnostic challenge, necessitating a thorough investigation into its hematological manifestations. This research delves into the intricate interplay of hematological parameters, shedding light on potential indicators for understanding and monitoring FUO. The study not only explores demographic details but meticulously analyzes Complete Blood Count (CBC) reports, contributing valuable insights into the complex landscape of FUO. Figure 1 depicts the age distribution within the study cohort, revealing intriguing patterns. A subtle inclination towards higher incidence in the 21-30 age group, resonate with seminal work by Kadri et al. in 2000, highlighting a preponderance of FUO in the younger age group [22]. Gender distribution, as illustrated in Figure 2, unveils that males constitute 54% of the study population, with females at 46%. Noteworthy is the heightened incidence of FUO in males. However, meticulous statistical analysis reveals no significant gender-based differences ($p > 0.05$). This trend aligns with findings from a 2007 study, emphasizing a higher prevalence of FUO in males [23].

The investigation into hematological parameters unfolds a complex narrative, providing critical insights into the host's immune response during FUO. The mean Total White Blood Cell (WBC) count in the FUO group is significantly elevated compared to the control group, affirming existing literature associating FUO with heightened Total WBC count [24,25]. This finding underscores the potential diagnostic value of WBC count in identifying and monitoring FUO. The analysis of Absolute Neutrophil Count (ANC) reveals a stark contrast between the FUO and control groups, with a mean value of 7,138±4874 cells/mm3 and 5,399±1692 cells/mm3, respectively. This significant statistical divergence aligns seamlessly with a contemporaneous study conducted in 2022 [26].

The exploration of Absolute Lymphocyte Count (ALC) further fortifies the findings, exposing a substantial and statistically significant elevation in ALC among those with FUO. These revelations harmonize with the observations of Haidar et al., who posited a concomitant increase in ALC with Total WBC count in FUO cases [27]. Turning our attention to platelet count, the FUO group exhibits a notably lower count compared to

the control group, demonstrating a statistically significant difference. This corroborates findings from a study conducted on the North Indian population, which similarly identified a reduction in platelet count among individuals experiencing FUO.

The exploration extends to the calculation of Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) from the absolute counts. As in figure 3 and table 1, the mean NLR in the FUO group is 4.66±6, while in the control group, it is 5.79±7. Despite the reduction in NLR in FUO cases, the statistical analysis does not yield significant differences (p=0.298). The decrease in NLR implies a dynamic shift in the immune response during FUO. Neutrophils and lymphocytes play crucial roles in different phases of the immune response – neutrophils are rapid responders to acute infections, while lymphocytes contribute to adaptive immunity. A reduction in NLR may suggest alterations in the balance between these components, reflecting a nuanced modulation of the immune system during FUO[22]. Moreover, NLR is recognized as a marker of systemic inflammation, with lower values often associated with a less inflammatory environment.

The reduction in NLR in FUO cases suggests a potential modulation of the inflammatory response. This could be attributed to various factors, including the nature of the underlying infection or inflammatory condition, as well as the stage of the immune response [23]. The decrease in NLR is, in part, driven by changes in lymphocyte counts. A deeper investigation into lymphocyte subsets could provide insights into the specific populations involved. Understanding whether the decrease is driven by a reduction in specific lymphocyte subsets, such as T cells or B cells, could offer valuable information regarding the nature of the immune response in FUO. The clinical significance of a decreased NLR in FUO warrants careful consideration. While NLR has been proposed as a prognostic marker in various medical conditions, its specific role in the context of FUO remains an area of active investigation. Monitoring NLR dynamics alongside other clinical parameters may provide insights into the severity and progression of FUO, aiding in diagnostic and prognostic assessments.

In contrast, PLR in the FUO group (148±144) is markedly lower than in the control group (236±241), demonstrating a significant difference (p=0.007)(Figure 4 and Table 1). This suggests the potential utility of PLR as an indicator for monitoring FUO patients, considering its role in gauging the level of cytokine storm. Inflammation in FUO leads to a decrease in platelet counts, inducing the production of thrombopoietin, which in turn accelerates platelet production. The observed decrease in Platelet-to-Lymphocyte Ratio (PLR) in the context of Fever of Unknown Origin (FUO) reflects a noteworthy hematological alteration that merits comprehensive exploration. PLR, a calculated parameter derived from absolute platelet and lymphocyte counts, serves as a valuable indicator of the intricate interplay between platelets and the immune response.

Understanding the following implications of a decreased PLR in FUO requires delving into the underlying mechanisms and potential clinical significance. A reduced PLR in FUO suggests a dynamic modulation in the inflammatory response. Platelets and lymphocytes, both integral components of the immune system, play key roles in the body's defense mechanisms. A decrease in PLR may signify alterations in the balance between pro-inflammatory and anti-inflammatory processes during FUO [24]. The observed decrease in platelet counts could be influenced by heightened levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) in FUO. This, in turn, may stimulate

thrombopoietin production, impacting platelet dynamics. The regulatory mechanisms governing thrombopoiesis and their response to inflammatory stimuli could be pivotal contributors to the altered PLR [25]. The clinical significance of a decreased PLR in FUO warrants careful consideration. While PLR has been implicated as a prognostic marker in various inflammatory conditions, its role in the context of FUO remains an area of active investigation. Monitoring PLR dynamics alongside other clinical parameters may offer insights into the severity and progression of FUO, aiding in diagnostic and prognostic assessments. Platelets, traditionally viewed as hemostatic cells, actively participate in immune responses, interacting with leukocytes and modulating inflammatory processes .

Exploration of potential correlations between gender and NLR/PLR, although showing a slight skew towards males (figure 5 and 6), does not reveal significant gender-based disparities. This concords with a 2020 study involving fevers suspected to be caused by Corona infections. Studies have suggested that hormonal variations, particularly the influence of sex hormones like testosterone and estrogen, contribute to divergent immune profiles between males and females [26]. These hormonal disparities may manifest in variations in neutrophil and lymphocyte counts, thus impacting NLR and PLR. Testosterone, known for its immunosuppressive effects, may influence neutrophil counts, potentially contributing to a higher NLR in males. On the other hand, estrogen, with its immunoenhancing properties, could be associated with lower NLR and PLR in females. While these hormonal influences are complex and multifaceted, they underscore the need to consider gender as a critical factor when interpreting hematological parameters. Intriguingly, the study investigates the temporal dimension by examining the duration of fever concerning NLR and PLR (Figure 7 & 8). Remarkably, a significant reduction in both NLR and PLR is observed as the fever duration increases, unveiling a positive correlation between the duration of fever and these hematological parameters.

This dynamic observation suggests a potential temporal modulation in the immune response during prolonged febrile episodes. The observed reduction in NLR and PLR with prolonged fever duration suggests a nuanced temporal modulation of the immune response. Fever, as a hallmark of inflammatory and infectious processes, triggers a cascade of immune events. The decrease in NLR and PLR over time may signify a shifting balance between neutrophils, lymphocytes, and platelets, reflecting the evolving nature of the host's response to the underlying cause of fever [27]. While the study contributes significantly to understanding hematological alterations in FUO, acknowledging inherent limitations is essential. The absence of culture-positive samples and limited CBC reports upon discharge restrict the depth of analysis. Additionally, the control group, comprising non-fever cases, lacks a detailed exploration of the systemic illnesses affecting these patients. Future research initiatives should aim to rectify these gaps, thereby advancing our understanding of FUO and its hematological manifestations more comprehensively.

CONCLUSION

Within the constraints of our study, we draw the conclusion that Fever of Unknown Origin (FUO) is linked to elevated total White Blood Cell (WBC) counts, increased Absolute Neutrophil Count, and heightened Lymphocyte Count. A substantial decrease in platelet count was observed when compared to the control group. While the Neutrophil-Lymphocyte Ratio (NLR) did not exhibit statistical significance, the

group with FUO demonstrated a slightly lower NLR compared to the non-fever control group. Notably, Platelet-Lymphocyte Ratio (PLR) displayed a significant reduction in FUO compared to the control group. This emphasizes the potential of PLR as a more pivotal inflammatory marker in FUO when contrasted with NLR.

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