

Neutrophil-to-Lymphocyte Ratio in Adult Patients with Anemia: A Hospital-Based Cross-Sectional Study

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ABSTRACT

Background: Neutrophil-to-lymphocyte ratio, a well-defined marker of immunity, is raised in many conditions which are characterized by abnormal inflammatory and immunological responses. Anemia of chronic disease, the most prevalent type in hospitalized patients, is a dominant clinical picture in protracted stimulation of immunity. This study aimed to assess the neutrophil-to-lymphocyte ratio in adult participants with anemia and to compare of the ratio with non-anemic participants.

Methods: In a cross-sectional study conducted in the Department of Clinical Physiology of Karnali Academy of Health Sciences, Teaching Hospital, Jumla, Karnali Province, Nepal, in the months of August, 2023, data on clinico-demographic variables were retrieved from the laboratory information system of hospital records of February–March, 2023. From the hematological parameters retrieved, the status of anemia and the neutrophil-to-lymphocyte ratio were calculated.

Results: After scrutiny of the laboratory records of 1421 adult patients, with 69.7% (N=990) female, the prevalence of anemia was 12.4% (N=176) and the median neutrophil-to-lymphocyte ratio was 2.21 (IQR: 1.41–3.75). The median neutrophil-to-lymphocyte ratio was significantly greater in the anemic participants among the overall ($p < 0.001$) participants and across the subgroups of gender ($p < 0.001$) and age ($p < 0.05$).

Conclusion: The neutrophil-to-lymphocyte ratio was significantly greater in the adult anemic than in the non-anemic participants. To this end, further studies assessing the association of this ratio with the various clinical aspects of anemia, such as subtypes, severity, course, prognosis and treatment responses are needed.

Key Words: anemia; hemoglobin; lymphocyte; neutrophil; neutrophil-to-lymphocyte ratio

INTRODUCTION

Neutrophil-to-lymphocyte ratio (NLR) is a hematological marker that takes into account two domains of the immunity, i.e., innate and adaptive.¹ Raised NLR serves as a key indicant of pathological conditions including infections,²⁻⁴ stroke,⁵ myocardial infarction,⁶ atherosclerosis,⁷ trauma,⁸ cancer,⁹ complexities associated with surgery¹⁰ and systemic inflammatory response syndrome (SIRS) due to inflammation induced by tissue damage.¹ Anemia, a condition with reduced hemoglobin or hematocrit or red blood cells counts, manifests in association with nutritional inadequacy, infections, inflammation, chronic diseases, and heritable defects of the red blood corpuscles.^{11,12} Anemia of chronic disease, the most prevalent type in hospitalized patients, is a dominant clinical picture in protracted activation of the immune system.¹³

In the face of a multitude of research studies reporting the associations of both NLR and anemia of chronic disease with various pathological conditions with infectious/inflammatory etiologies, the obviously plausible association between the ratio and anemia clearly justifies some investigation. In one of the very few studies conducted along this line, Hegde and Puranik¹⁴ assessed the alteration of NLR in patients with iron deficiency anemia, with the findings pointing specifically to the link between iron and immunity. Notwithstanding, in that study, the overall association between anemia in general and NLR was clearly lacking in the generalized anemic patients. In this backdrop, this study was designed to evaluate the NLR in adult anemic patients and compare the ratio between the anemic and non-anemic participants, overall and across the subgroups of gender and age.

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MATERIALS AND METHODS

It was a quantitative study with an observational, cross-sectional design (retrospective chart review) wherein, the research data was acquired from the laboratory information system (LIS) of Central Clinical Laboratory at Karnali Academy of Health Sciences (KAHS), Teaching Hospital, Jumla, Karnali Province, Nepal. Central Clinical Laboratory of KAHS Teaching Hospital is the clinical laboratory where biological specimens (blood in the present study) of the patients visiting KAHS Teaching Hospital are analyzed. KAHS Teaching Hospital is a tertiary healthcare center that caters to the healthcare needs of a significant proportion of population in the Karnali Province, especially those residing in the upper (hills and mountains) Karnali regions.

The study population comprised of the patients visiting KAHS Teaching Hospital, with the sampling unit being the study population who had their blood investigated at Central Clinical Laboratory of KAHS Teaching Hospital for various hematological parameters. Using the census or complete enumeration sampling strategy, the total number of patients who had their blood investigated for hematological parameters in any one month period (randomly selected as February–March 2023 AD or Falgun 2079 BS from the 12 previous months, ie., September 2022 to August 2023) were selected. From these, the hematological records considered for analysis were chosen based on the following criteria: adult participants (age 18 years or above)¹⁵ of both gender were included, while those with incomplete/dubious records were excluded from the final analysis. Likewise, only one set of values of the records of individuals (with more than one record in the LIS) was considered eligible for the analysis. In cases of multiple entries of the same lab parameter, only the parameter that was analyzed first was included, excluding all the other subsequent values.

After obtaining ethical clearance from the Institutional Review Committee of KAHS (Ref: 080/081/19), data were collected from the Laboratory Information System (LIS) of the hospital records of KAHS Teaching Hospital. Using a pre-designed proforma, the principal investigator recorded values of all the variables of interest. Based on these, the calculated variables were: anemia status (no anemia/

anemia), neutrophil count (per microliter), lymphocyte count (per microliter) and neutrophil-to-lymphocyte ratio. Anemia was defined in accordance with the WHO criteria as hemoglobin levels less than 12 g/dL in adult females and less than 13 g/dL in adult males.¹⁶ Likewise, neutrophil-to-lymphocyte ratio was calculated from the differential counts of the neutrophils and lymphocytes. The principal investigator, in alliance with the co-investigators, were involved in the supervision and monitoring throughout all the steps of the research.

Statistical Analysis: The collected data was initially entered in LibreOffice Calc (LibreOffice, The Document Foundation). After preliminary data management (data cleaning, re-coding, etc), data were entered and analyzed in Statistical Package for Social Science (SPSS), version 16.0 software. Categorical variables were described using frequency and percentage. Regarding the continuous variables, normality was tested using Kolmogorov Smirnov test. As their distributions were found to significantly deviate from the Gaussian distribution, median values with interquartile intervals were used to describe the data. As for the inferential statistics, comparison of the median NLR between the comparison groups was performed using non-parametric tests. Mann-Whitney U test was employed for comparing the hematological parameters in different categories. Finally, the median NLR amongst the study participants of the age-quartiles was compared using the Kruskal Wallis test. Statistical significance was defined as p-value less than 0.05 at 95% confidence level.

RESULTS

In a hospital-record-based cross-sectional study, the laboratory records of a total of 1421 adult patients were scrutinized. Of these participants, 990 (69.7%) were female. The median age of the overall participants was 36 years (IQR: 25–40 years), with the range being 67.0 years, ie., 18–85 years. As per the WHO criteria, the prevalence of anemia in the study participants was 12.4% (n=176). Likewise, the median neutrophil-to-lymphocyte ratio (NLR) was 2.21 (IQR 1.41–3.75) with the range being 0.16–47.00. (Table 1)

Table 2 depicts the comparison of the median values of different hematological parameters between the par-

Table 1: Baseline clinico-demographic attributes of the participants

Variables		Mean±SD	Median (Q1–Q3)	Range (Min–Max)
Age (years)		40.39±16.98	36.00 (25.00–54.00)	67.00 (18.00–85.00)
Gender	Female	N=990 (69.7%)		
	Male	N=431 (30.3%)		
RBC Count (x 10 ⁶ /μL)		4.85±0.82	4.80 (4.40–5.30)	7.50 (1.10–8.60)
Hemoglobin (g/dL)		14.67±2.59	14.60 (13.30–16.00)	24.60 (4.20–28.80)
Anemia Status	No Anemia	N=1245 (87.6%)		
	Anemia	N=176 (12.4%)		
TLC (x10 ³ /μL)		7.94±4.06	7.10 (5.50–9.30)	51.00 (1.00–52.00)
Neutrophil Count (x10 ³ /μL)		5.34±3.97	4.29 (3.02–6.55)	47.60 (0.24–47.84)
Lymphocyte Count (x10 ³ /μL)		1.99±0.84	1.95 (1.43–2.49)	9.31 (0.28–9.59)
Neutrophil/Lymphocyte Ratio (NLR)		3.42±3.84	2.21 (1.41–3.75)	46.84 (0.16–47.00)

Table 2: Median values of hematological parameters according to the anemia status

Variables	No Anemia (N=1245)	Anemia (N=176)	Test of Significance†	
			Z-value	p-value
RBC Count (x 106/ μ L)	4.90 (4.50–5.40)	3.70 (3.40–4.10)	-17.78	<0.001*
Hemoglobin (g/dL)	15.00 (13.80–16.20)	11.00 (9.50–11.70)	-21.36	<0.001*
TLC (x103/ μ L)	7.10 (5.50–9.00)	7.70 (5.60–11.20)	-2.38	0.017*
Neutrophil Count (x103/ μ L)	4.19 (3.01–6.28)	5.58 (3.59–8.23)	-4.87	<0.001*
Lymphocyte Count (x103/ μ L)	2.00 (1.50–2.52)	1.51 (0.98–2.15)	-6.43	<0.001*
NLR	2.10 (1.36–3.40)	3.76 (2.34–7.41)	-8.22	<0.001*

†: Mann-Whitney U Test

*: Statistically significant at 95% Confidence Intervals

Table 3: Comparison of NLR between the anemic and non-anemic participants across the subgroups and gender and age.

Variables	No Anemia (N=1245)		Anemia (N=176)		Test of Significance†	
					Z value	P value
Gender						
Female (n = 990)	2.03 (1.33–3.29)	Z = -2.45†	3.57 (2.29–7.35)	Z = -1.05	-7.38	<0.001*
Male (n = 431)	2.19(1.45–3.75)	P = 0.01*	4.25 (2.77–8.27)	P = 0.29	-3.99	<0.001*
Age-Quartiles						
First (18– 25 yrs)	2.74 (1.80–4.28)	χ² = 67.2‡	3.41 (2.39–6.30)	χ² = 1.71	-2.73	0.006*
Second (25–36 yrs)	1.94 (1.30–2.92)	P<0.001*	3.96 (2.00–7.74)	P=0.64	-4.93	<0.001*
Third (36–53 yrs)	1.71 (1.20–2.57)		3.04 (2.29–5.27)		-4.16	<0.001*
Fourth (54–85 yrs)	2.14 (1.42–3.62)		4.23 (2.35–8.30)		-4.01	<0.001*

†: Mann-Whitney U Test

‡: Kruskal Wallis Test

*: Statistically significant at 95% Confidence Intervals

†: Mann-Whitney U Test

‡: Kruskal Wallis Test

*: Statistically significant at 95% Confidence Intervals

participants with and without anemia. As shown, the median total leucocyte count (TLC) was significantly greater in the participants with anemia ($p=0.017$). Likewise, the median neutrophil counts were significantly greater and the median lymphocyte counts were significantly lower in these participants ($p<0.001$). However, the median NLR was significantly greater in the participants with anemia than in the non-anemic participants ($p<0.001$).

Table 3 illustrates the comparison of median values of neutrophil-to-lymphocyte ratios of different categories of gender and age between participants with and without anemia. As depicted the differences were statistically significant in all the subcategories of gender (male and female) and age (age-quartiles) ($p<0.05$). Likewise, the values were significantly different amongst the gender and age categories only in participants without anemia ($p<0.05$). In participants with anemia, the median NLR was greater in males than females but was not significant ($p=0.29$). Similarly, amongst the various age-quartiles, median NLR was the highest in the fourth age-quartile and lowest in the third age-quartile, with the overall difference in the ratio being statistically non-significant ($p=0.64$).

DISCUSSION

The study was prompted by a multitude of research studies reporting associations of neutrophil-to-lymphocyte ratio (NLR) and anemia of chronic disease independently with various pathological conditions with infectious/inflammatory components. It was designed with an aim of assessing the neutrophil-to-lymphocyte ratio in adult patients with anemia and comparing the ratio between the

participants with and without anemia, in the overall participants and across the subgroups of gender and age. In this study, out of the total of 1421 adult patients with the age ranging from 18 to 85 years, 69.7% (N=990) were female. The median age of the overall participants was 36 years (IQR: 29 years, ie., 25–54 years).

The proportion of participants with anemia was 12.4% (N=176/1421). As per the report from the analytical outcomes of Global Burden of Disease Study 2021,¹⁷ 24.3% of the population worldwide suffered from anemia. Further, as summarized by Weiss et al,¹³ anemia with inflammatory component was present in up to 2/5th of the cases of anemia, globally. Notably, the low proportion of anemic patients in the current study as compared to the global trend points to two major possibilities. First, because the cutoff at hemoglobin concentration was used for the diagnosis of anemia, the hemoglobin concentration in the overall participants can be expected to have increased with high altitude¹⁸ in a place like Jumla, confounding the diagnosis. Secondly, as the study participants only included the adults (18–85 years), the exclusion of children with possibly higher prevalence of anemia could have decreased the overall burden of the condition in the study.

In this study, median neutrophil-to-lymphocyte ratio (NLR) was 2.21 (IQR: 1.41–3.75) with the range being 0.16–47.00. The median NLR in the participants without anemia was 2.10 (IQR: 1.36–3.40). In an analysis of five contemporaneous randomized clinical trials, Adamstein et al⁷ reported the baseline median NLR to vary from 2.10 to 2.36. Forget et al,¹⁹ in their study on healthy adults (excluding the geriatric population), reported the NLR to range between 0.78–3.53, with the mean of 1.65. As reported in the Rotterdam study,²⁰ the baseline mean NLR was 1.76 (95% CI: 0.83–3.92).

Although the variation of NLR in anemia has been described in a very studies, it has been logically deduced in studies that the increased NLR can be due to alterations in total leucocyte count, and counts of differential neutrophil and lymphocyte, with counts of total leucocyte and neutrophil in the direction of the altered ratio and lymphocyte count, in the opposite direction.¹ As determined in this study, the median total leucocyte and differential neutrophil counts were significantly greater in the anemic participants ($p=0.017$) while the median lymphocyte counts were significantly lower in these participants ($p<0.001$). Hegde and Puranik reported that total leukocyte count, differential lymphocyte and neutrophil counts were significantly different between the control subjects and those with moderate and severe iron deficiency anemia.¹⁴ Additionally, they also reported that the total leucocyte and neutrophil counts were significantly greater and differential lymphocyte counts, significantly smaller, in the subjects with iron deficiency anemia. These findings, although specific for iron deficiency anemia, are analogous to the present study. In this study, the median NLR was significantly greater in the anemic than in the non-anemic participants ($p<0.001$) a finding similar to the study by Hegde and Puranik¹⁴ who reported a significantly increased NLR in the moderate and severe iron deficiency anemia patients. Moreover, the same authors also assessed the relationship between of NLR with various hematological parameters and obtained a statistically significant association of the ratio with Red Blood Cell count, Hemoglobin and other parameters.. In another comparable study, Fisher et al²¹ cited anemia, along with other commodities, as an autonomous determiner of an increased NLR during admission.

Comparison of the median values of the neutrophil-to-lymphocyte ratios (in participants under different subcategories of gender and age) between participants with and without anemia showed that the differences were statistically significant across all subcategories of gender (male and female) and age (age-quartiles) ($p<0.05$). Likewise, the ratio was significantly different amongst the gender and age quartiles only in participants without anemia ($p<0.05$). In the anemic patients, although the median NLR was greater in the male than in the female gender group, the difference was not statistically significant ($p=0.29$). In contrast, the Rotterdam study²⁰ reported the mean NLR to be significantly higher in the male subjects. ($p<0.05$). Risch et al²² also reported the similar finding. Similarly, amongst the various age-quartiles, median NLR was the highest in the fourth age-quartile, with the lowest being in the third age-quartile, with the overall difference in the ratio being statistically non-significant ($p=0.64$). This finding can be complemented by Risch et al,²² who, in their study, reported the NLR to have significantly increased with age.

The limitations of the present study can be envisioned from many standpoints including study design, exclusion of many necessary variables potentially affecting the independent and dependent variables, and limited ability to properly address the bias stemming from the design. Foremost, a cross-sectional study, such as this, fails to clearly elucidate the causal relationship between NLR and anemia. Moreover, the failure of distinction of the types of anemia (the records from the LIS providing no such diagnostic information) might further befuddle its association with the NLR. To add, despite the NLR being an established

parameter of inflammation, we couldn't include other parameters like CRP because of the technical issues like non-availability of hsCRP analysis at our laboratory setting and possibly limited sample size, if the CRP status (positive/negative) was included. Further, apart from the age and gender, no further information about necessary extraneous variables was available. This limited the scope of statistical analysis using these variables could have significantly affected the relationship between the independent (anemic status) and dependent (NLR) variables. To address these, a longitudinal study could be planned to assess the relationship between the NLR and the anemia considering the pathological course of anemia, use of medications, the various subtypes of this condition and other variables potentially affecting the relationship. Regarding the bias, one important bias in the study was the measurement/information bias resulting in potential errors in data due to inability of supervision/quality assessment during the laboratory investigations. Lastly, despite the supposedly sufficient sample size, the short duration of data collection clearly subverted the likely seasonal fluctuation of the obtained association between the addressed variables.

Despite the limitations outlined above, the present study has paved a clear way as to the relationship between anemia and NLR, an approach not undertaken by many studies in the region. Moreover, the findings have reiterated the predominance of anemia of chronic disease in the milieu of hospital visiting patients.

CONCLUSION

In this study, the neutrophil-to-lymphocyte ratio was significantly greater in anemic patients, overall and across different subcategories of gender and age. In the face of NLR being an established marker (especially for predicting the outcomes) of many diseases with inflammatory and immunological basis, evaluation of NLR in anemia definitely sets the foundation for evaluation of patients in the clinical setting and not to mention, for planning further studies, wherein the patients may be followed up and their prognosis can be evaluated in real time. Further studies with apt design and consideration of more variables could be planned to further elucidate the relationship.

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Authors' Contribution: All the authors conceptualized the research; NM and KuB designed the research; All the authors performed literature search; NM collected data; KuB performed data analysis; All the authors interpreted data; NM and KuB collaborated in penning the manuscript; All the authors reviewed the manuscript for essential intellectual content; NM performed the final approval of the version ready for submission. All the authors agreed to be responsible for all domains of the work; NM corresponded to journal.

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