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Original Article

Assessment of Neutrophil-lymphocyte Ratio and Platelet-lymphocyte Ratio in Iron Deficiency Anemia

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ABSTRACT

Background and Aim: The present study is undertaken to investigate and establish the alteration in the neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in iron deficiency anemia patients and to elucidate the link between iron with immunity and hemostasis. **Methods:** Complete hemogram along with differential leucocyte count and platelet count was done in 100 untreated iron deficiency anemia patients, who are visiting/admitted to KIMS, OPD, Hubballi, using Automated analyzer (HP 120). **Results:** The results of the present study showed clearly that there is a significant increase ($P<0.05$) in neutrophil-lymphocyte ratio as well as platelet-lymphocyte ratio among moderate and severe anaemic patients compared to normal individuals. Moreover, this increase in the ratio is more evident as the severity of anemia is increased. **Conclusion:** The alteration in neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in these patients may be due to low levels of iron as iron has a vital role in proliferation of different blood cells, especially lymphocytes. Thus, the outcome of the present study may be suggestive of a probable link between iron and immunity.

Key words: Iron deficiency anemia, Neutrophil, Lymphocyte, Platelets, Immunity, Hemostasis.

INTRODUCTION

Iron Deficiency Anemia (IDA) is one of the most prevalent forms of malnutrition. Globally 50% of anemia is attributable to iron deficiency and accounts for approximately nearly a million deaths per year worldwide.[1] Anemia is a major health problem in India. IDA can result from lack of iron supplementation in the diet, impaired absorption, as found in spruce, other causes of fat metabolism, chronic diarrhea, gastrectomy, increased absorption as found in growing infants, children, adolescents, as well as premenopausal women, particularly during pregnancy and chronic blood loss.[2]

Neutrophil-lymphocyte ratio (NLR) calculated from complete blood count with differential count, is readily available and inexpensive marker of inflammation in ischemic heart disease,[3] as early marker of diabetic neuropathy,[4] as a predictable marker for preeclampsia and a marker for colorectal cancer.[5] Similarly, platelet-lymphocyte ratio (PLR) is a cost effective and inexpensive inflammatory biomarker with independent prognostic value in solid tumors.[6] Many studies have shown that iron is necessary for proliferation and maturation of immune cells, particularly lymphocytes which is associated with the specific response to infection. Furthermore, the immune system uses iron as intermediary in the production of bacteriostatic cells.[7]

To the best of our knowledge, so far, there is no comprehensive study published on the role as well as predictive value of NLR and PLR in iron deficiency anemia. Thus, the present study is undertaken to investigate and establish the alteration in the ratio of these blood cells in IDA patients and to elucidate the role of iron in immunity and hemostasis.

MATERIALS AND METHODS

Setting

The present study was conducted in iron deficiency anemic patients who are visiting/ admitted to 'Karnataka Institute of Medical Sciences Out Patient Department, Hubballi. Informed consent from the patients/ subjects for the collection of blood was obtained in the vernacular language. The study protocol was approved by Institutional Ethical Committee (IEC) before starting the work of this study.

(Approval No: IEC. No -PGS/SYN/423/2018-19 dated 10-10-2018)

Study Population

Hundred iron deficiency anemic patients were recruited for the present study after scrutiny based on inclusion and exclusion criteria. They were grouped as moderate anemic (10-13 gm % of Hb) and severe

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anemic (7-8 gm % of Hb) with 50 in each group. Also, 50 healthy, age and sex matched normal individuals were recruited into control group.

Inclusion Criteria

Control subjects/ patients of both gender in the age group of 20-40 years were recruited for this study.

Exclusion Criteria

Patients with history of diabetic mellitus, hypertension, thyroid dysfunction, cardiovascular, neurological or any other chronic diseases were excluded. And patients who had HIV or any other immunodeficiency diseases and recent infections were not included in this study.

Procedure of Data Collection

Blood sample (5 ml) was taken in a sterilized syringe and maintained in anticoagulant used tubes. Then, the sample was analyzed using "Automated analyzer" (HP 120). Complete hemogram including differential leucocyte count and platelet count were performed and NLR and PLR were calculated.

For grouping the subjects into control group, moderately anemic and severely anemic groups, RBC count, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and hematocrit (PCV) were taken into account.

Statistical Analysis of Data

The statistical analysis was done by using 'SPSS 21' software. The results were expressed in frequency, percentages, mean and standard deviation. Student's *t* test and ANOVA were used as tests of significance. NLR and PLR were calculated using absolute lymphocyte and absolute neutrophil count. Correlation of NLR and PLR with all parameters was done using Pearson's correlation. P value<0.05 is considered statistically significant.

RESULTS

Table 1 shows the comparison of RBC count, hemoglobin, PCV, MCV, MCH and MCHC between the iron deficiency and control subjects. There was a significant reduction (P<0.05) in RBC count, hemoglobin, PCV, MCV, MCH and MCHC iron deficiency anemia patients compared to control subjects.

WBC (white blood cell) count and Platelet count were significantly increased (P<0.05) in moderate anemia. However, platelet count was decreased in severe iron deficiency anemia patients compared to control subjects**.** Differential neutrophil count was increased whereas differential lymphocyte count was decreased in both the moderate and severe anemic patients (Table 2).

NLR was increased significantly in both moderate and severe anaemic patients (Table 3). This increase in this ratio was more evident as the severity of anaemia increases. PLR was been increased significantly in both moderate and severe anaemic patients (Table 3). However, this

Table 1: Comparison of RBC count, hemoglobin content, PCV, MCV, MCH and MCHC between iron deficiency anemia patients and control subjects.

Groups	RBC count (million/mm³)	Hb (g/dl)	PCV (%)	MCV (femtolitre/cell)	MCH (picogram/cell)	MCHC (g/dl)
Control (n = 50)	4.866±0.206	14.57±0.5	42.80±2.3	88.06±5.24	29.99±1.49	34.12±1.86
Moderate Anemic (n = 50)	3.657±0.207	8.09±0.34	26.90±0.8	73.74±3.65	22.20±1.47	30.11±1.47
Severe Anemic (n = 50)	2.843±0.274	5.39±0.62	19.60±1.3	69.35±5.40	19±1.68	27.49±2.50

Data expressed as mean ± SD. RBC: Red blood cell; Hb: Hemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration.

Table 2: Comparison of WBC count, platelet count, differential neutrophil count and differential lymphocytes count between iron deficiency anemia patients and control subjects.

Groups	WBC count (thousand/ mm³)	Platelet count (lakhs/ mm³)	Differential neutrophil count (%)	Differential lymphocytes Count (%)
Control (n = 50)	7.04±0.55	3.05±0.41	50.33±4.6	38.56±1.8
Moderate Anemic (n = 50)	7.21±0.48	4.12±0.44	67.58±4.84	24.33±2.32
Severe Anemic (n = 50)	7.53±1.10	2.28±0.4	74.84±3.12	21.61±1.89

Data expressed as mean ± SD. WBC: White blood cell.

increase in ratio is more evident in moderate anaemic, compared to severe anaemic patients.

Correlation of NLR and PLR with all other blood parameters was shown in Table 4. Similarly correlation between NLR and Haemoglobin (Figure 1) and between PLR and Haemoglobin (Figure 2) are shown. The NLR was negatively correlated with haemoglobin and statistically significant (P<0.001). The PLR was negatively correlated with haemoglobin and statistically significant (P<0.001).

DISCUSSION

The present study was undertaken to demonstrate and establish the link between iron and immunity, by examining the Neutrophil-lymphocyte ratio in Iron deficiency anaemia. In this study we found that NLR has been increased in moderate and severe anaemic patients. This increase in the ratio is more evident as severity of anaemia is increased. As per individual white blood cell is concerned, the present data showed an increase in neutrophil count as well as decrease in lymphocyte count, which result in an increase in the ratio of these cells.

Few studies showed that the association between the neutrophil to lymphocyte ratio is anew marker of systemic inflammation.[8] The prognostic value of neutrophil to lymphocyte ratio in patients, presenting with ST segment elevation due to myocardial infarction undergoing the 'primary per cutaneous coronary intervention' also mentioned the similar conclusion.[9]

A small peptide called "hepcidin" has a crucial role in the control of iron availability to the tissues. Hepcidin expression is up regulated by inflammation. IDA is frequently associated with anaemia of chronic diseases, including chronic heart failure, cancer, chronic kidney disease, rheumatoid arthritis, obesity and inflammatory bowel diseases.[10] It has also been shown that interleukin-6 (IL-6) enhances the synthesis of hepcidin in the liver, which regulates iron recycling, resulting in anaemia due to Hypoferremia.[11,12] As anaemia is associated with hypoxia and ischemia, it may affect WBCs via increasing vascular reactivity to catecholamine through glucocorticoids.

Platelets are known to be active players in the induction of inflammatory response, in addition to their participation in haemostasis. Contrast to acute infections with viruses or bacteria, chronic inflammation is often related with increased megakaryopoiesis and reactive thrombocytosis.[9,13] In the present study, we have observed that platelet-lymphocyte ratio has been increased significantly in both moderate and severe anaemic patients. However, this increase in ratio is more evident in moderate anaemic, compared to severe anaemic patients.

Iron deficiency is a recognized cause of reactive thrombocytosis even outside the setting of inflammation.[14,15] Moreover, platelets produced in iron deficient animals displayed a higher mean platelet volume and increased aggregation.[16] IL-6 plays a key role in the systemic Inflammatory response and thrombocytosis by several mechanisms including; increasing the synthesis of acute phase proteins such as C-reactive protein (CRP), serum amyloid A protein and/or fibrinogen, decreasing albumin biosynthesis in the liver,[11] stimulating human megakaryocytic proliferation and differentiation synergistically with IL-3,[17] and stimulating thrombopoietin production.[11]

Figure 1: Scatter plot showing correlation between NLR and hemoglobin. NLR: Neutrophil-lymphocyte ratio.

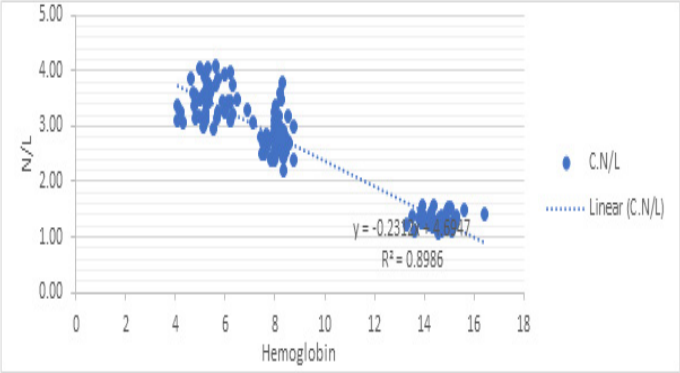

Figure 2: Scatter plot showing correlation between PLR and Hemoglobin. PLR: Platelet-lymphocyte ratio.

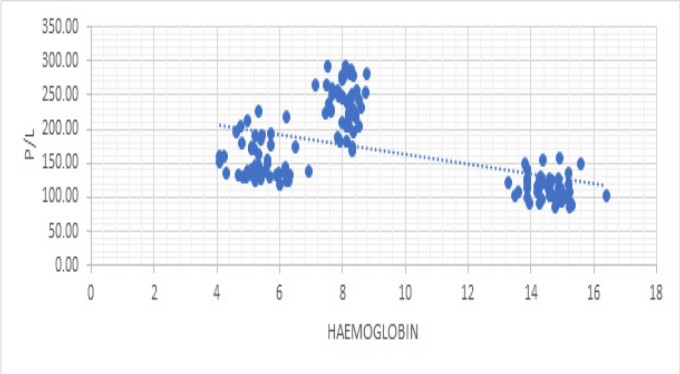

Table 3: Comparison of absolute neutrophil count, absolute lymphocyte count, NLR and PLR between iron deficiency anemia patients and control subjects.

Groups	Absolute Neutrophil count (cells/ mm³)	Absolute Lymphocyte count (cells/ mm³)	NLR	PLR
Control (n = 50)	3546.90± 459.2	2716.55± 261.1	1.30± 0.12	113.19± 17.43
Moderate Anemic (n = 50)	4880.27±512.6	1755.76±201.1	2.80±0.33	237.30±32.11
Severe Anemic (n = 50)	5634.63±830.4	1627.03±261.1	3.48±0.31	144±33.45

Data expressed as mean ± SD. NLR: Neutrophil-lymphocyte ratio; PLR: Platelet-lymphocyte ratio

Table 4: Correlation between neutrophil-lymphocyte ratio and platelets-lymphocytes ratio with all other blood parameters.

Blood parameters		NLR	PLR
Hemoglobin	*r*	-.948	-.486
	P	0.000	0.000
RBC Count	*r*	-.916	-.384
	P	0.000	0.000
PCV	*r*	-.944	-.460
	P	0.000	0.000
MCV	*r*	-.825	-.440
	P	0.000	0.000
MCH	*r*	-.908	-.467
	P	0.000	0.000
MCHC	*r*	-.767	-.352
	P	0.000	0.000
Lymphocyte	*r*	-.966	-.611
	P	0.000	0.000
Neutrophil	*r*	.939	.427
	P	0.000	0.000
WBC Count	*r*	.240	-.224
	P	0.003	0.006
Platelet Count	*r*	-0.148	.709
	P	0.071	0.000
Absolute Lymphocyte Count	*r*	-.892	-.687
	P	0.000	0.000
Absolute Neutrophil Count	*r*	-.942	-.542
	P	0.000	0.000

Correlation was done by Pearson correlation analysis. The *r* represents correlation coefficient and P represents level of significance. P<0.05 was considered statistically significant. RBC: Red blood cell; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cell. NLR: Neutrophil-lymphocyte ratio; PLR: Platelet-lymphocyte ratio

However, the exact mechanism of thrombocytosis in IDA is not yet clear. Megakaryocytic cell lines grown in iron-depleted conditions exhibited reduced proliferation but increased ploidy and cell size. One of the data suggests that iron deficiency increases megakaryopoietic differentiation and alters platelet phenotype without changes in megakaryocyte growth factors, specifically thrombopoietin. Iron deficiency-induced thrombocytosis may have evolved to maintain or increase the coagulation capacity in conditions with chronic bleeding.

The results of the present study indicated clearly that there is a significant increase in Neutrophil-lymphocyte ratio as well as platelet-lymphocyte ratio among moderate and severe anemic patients compared to normal individuals. Moreover, this increase in the ratio is more evident as the severity of anaemia is increased. This alteration in neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in these patients may be due to low levels of Iron as Iron has a vital role in proliferation of different blood cells, especially lymphocytes. Thus, the outcome of the present study may be suggestive of a probable link between iron with immunity.

Limitations of the Study

Other inflammatory markers were not measured and compared. Hematopoietic growth factors and hepcidin was not measured.

CONCLUSION

This study was conducted to establish and demonstrate the link between Iron with immunity and hemostasis, by examining the neutrophil-lymphocyte ratio and the platelet-lymphocyte ratio in Iron deficiency anemia patients. The results of the present study indicated clearly that there is a significant increase in neutrophil-lymphocyte ratio as well as platelet-lymphocyte ratio among moderate and severe anemic patients compared to normal individuals. Moreover, this increase in the ratio is more evident as the severity of anaemia is increased.This alteration in NLR and PLR in these patients may be due to low levels of iron as iron has a vital role in proliferation of different blood cells, especially lymphocytes. Thus, the outcome of the present study may be suggestive of a probable link between iron with immunity.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of Interest.

ABBREVIATIONS

NLR: Neutrophil to Lymphocyte Ratio; **PLR:** Platelet to Lymphocyte Ratio; **RBC:** Red Blood Cell; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **PCV:** Packed Cell Volume; **WBC:** White Blood Cell; **IL-6:** Interleukin-6.

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