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Research Article

Evaluation of NLR and PLR in Immune Thrombocytopenic Purpura; Is it Worth Doing?

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Abstract

Background: Immune thrombocytopenia (ITP) is an autoimmune disorder. The clinical biomarkers like Neutrophils to lymphocytes ratio (NLR) and platelet to lymphocytes ratio (PLR) can be used as differential diagnostic tool in ITP. The current study was planned to evaluate utility of NLR and PLR in ITP diagnosis and their association with disease prognosis and response to treatment.

Methods: A case control study (1:1) was conducted from January 2015 to December 2017 with 111 ITP patients and 111 healthy controls. Peripheral blood was collected and CBC were recorded using Sysmex XN-1000.The calculation of NLR and PLR was done using absolute value of neutrophils, lymphocytes and platelets counts. The significant difference ($p = <0.05$) between ITP patients and healthy control groups was determined by Kruskal wallis test, Dunn's test and spearman's correlation test was done to evaluate platelet count correlation with IPF using SPSS ver.23.

Results: Low hemoglobin and platelet counts with high total leucocyte count (TLC) and IPF were detected in ITP patients as compared to healthy individuals ($p = <0.05$).Among all groups of ITP patients, very low platelet count with median(IQR) of 2(3.8)x109/l was observed in ND-ITP group. The NLR was high with prognosis of disease as higher levels were observed in P-ITP. The PLR was significantly low in ND-ITP, P-ITP, C-ITP, R-ITP and compared to controls with $p = <0.001$.

Conclusion: The simple, reliable and calculated NLR and PLR ratios can be used in predicting prognosis and response to treatment in ITP and to some extend the severity of disease.

Keywords: Immune Thrombocytopenic Purpura (ITP); Neutrophils to Lymphocytes Ratio (NLR); Platelet to Lymphocytes Ratio (PLR); Immature Platelet Fraction (IPF)

Introduction

Thrombocytes or platelets are non-nucleated and membrane disc like cells which activates the coagulation factors by activating phospholipids in membrane during blood clotting due to blood vessels impairment [1]. Decreased platelets counts or thrombocytopenia (i.e. <1,50000/mm^3) is commonly observed with autoimmune diseases or microbial infections. Thrombocytopnia can be divided based on degree which is divided into mild (<100000/mm^3), moderate (20000-50000/mm^3) and severe (<20000/mm^3). Thrombocytopenia usually occurs may due to i) elevated destruction of platelets in diseases such as immune thrombocytopenia (ITP), disseminated intravascular coagulation (DIC), and thrombotic thrombocytopenic purpura (TTP) or ii) reduced platelet production as hypo-production thrombocytopenia are related with other bone marrow diseases [2].

The ITP is a hematologic disorder without any apparent clinical cause and isolated thrombocytopenia [3]. To date the exact etiology of ITP is still unknown. It has been proposed that various factors including excessive platelets destruction due platelet autoantibodies production, T-cell mediated or oxidative stress dependent platelets destruction and cessation of megakaryopoiesis etc. may contribute in the pathogenesis of ITP. Bleeding is the commonest clinical manifestation which occurs with or without bruises and epistaxis in ITP and bleeding correlates generally with severity of the thrombocytopenia [4,5]. With the advancement of technology the new generations of automated hemo-analyzers have incorporated new complete blood counts (CBC) parameters including extended platelets indices such as immature platelets fraction (IPF). The IPF represents a population of newly formed platelets or reticulated platelets (RP) with high concentration of residual RNA due to excessive peripheral platelets destruction [6]. In ITP, improvement in risk-stratification algorithms is needed by incorporating sensitive markers such as neutrophils to lymphocytes ratio and platelets to lymphocytes ratio. In many benign and malignant diseases a significant role of inflammation has been observed. For indirect evaluation of inflammation can be made by easy, inexpensive and easily calculated parameter; Neutrophil lymphocyte ratio (NLR) has been used [7,8]. The elevated NLR predicts disease course, prognosis and response to treatment in autoimmune disease like ITP [9,10]. In addition to NLR, another inflammatory biomarker is platelet to lymphocytes ratio(PLR) which predicts the prognosis in female reproductive system and gastrointestinal tumors [11,12]. The PLR can also be used as prognostic biomarker in many diseases [13,14]. The role of PLR has been associated with clinical characteristics and outcomes in ITP [15-17]. With growing evidence, the data regarding the association between ITP and inflammatory markers is not enough. The current study was planned to observed NLR and PLR in ITP patients and compared with healthy controls.

Materials and Methods

A case control time bound study was conducted at National Institute of Blood Diseases & Bone Marrow Transplantation hospital in Karachi; from January 2015 to July 2017.A total of 111 patients and equal number of healthy control individuals with ratio of 1:1 were included. The study was approved by NIBD - Research Ethics Committee and informed consent was obtained by all study participants following guidelines of world medical association of Helsinki. All healthy study participants of either age or gender with no past history of illness and medication voluntarily participated in the study. Considering clinical and laboratory investigations of ITP patients were separated into four groups: newly diagnosed ITP (ND-ITP), persistent ITP (P-ITP), chronic ITP (C-ITP) and refractory ITP (R-ITP) as per International working group guidelines [4]. The clinical response to treatment was calculated based on number of platelet counts. The venous whole blood samples (3cc) were collected using sterile disposable plastic syringes after cleaning the vein puncture area with 70% ethanol. The Sysmex XN-1000 analyzer (Sysmex Corporation, Kobe, Japan) was used to evaluate all blood cell parameters along with extended parameters of RBCs, WBCs and platelets to predict response of bone marrow with respect to peripheral counts and Leishman's stain was used for microscopic examination of blood smear from EDTA tube. Further the ratios of neutrophils to lymphocyte counts and platelets to lymphocytes counts were estimated by following formulas.

$$N{:}L = \frac{\text{Absolute count of neutrophils}}{\text{Absolute count of lymphocytes}}$$

$$PLR = \frac{\text{Number of platelet counts}}{\text{Absolute number of lymphocyte counts}}$$

Statistical analysis

The median and Interquartile ranges were used to describe parameters. Kruskal wallis test and Dunn's test was used to evaluate significant difference between ITP patients and healthy control groups with ($p = <0.05$) and spearman's correlation test was done to evaluate platelet count correlation with IPF and N:L ratio using SPSS ver.23.

Results

One hundred and eleven (111) ITP patients along with healthy control individuals (111) with same gender were included in the present study. The median age of the patients and healthy control population was 23 ± 17.1 and 29 ± 8.5 yrs respectively. A total of 38(34%) Chronic ITP, 32(29%) newly diagnosed ITP, 31(28%) persistent ITP and 10(09%) refractory ITP patients along with similar total of 111 healthy individuals were included. Out of 111 ITP patients, 101 received treatment as 10 was refractory ITP. Thirty eight(34.2%) received oral prednisolone followed by Methlyprednisolone (IV) 23(20.7%), Immuran 30(27%), IVIg and Revolade 4(3.6%) and dexamethasone 2(1.8%). Out of 111 pa-

tients, 70(76.2%) were females and 41(36.9%) were males. Mean hemoglobin, TLC and platelet counts were evaluated and significant difference was observed (Table 1). Platelet parameters such as IPF are important for diagnosis of ITP and can predict bone marrow response towards platelet destruction. In the present study significantly high IPF was observed in ITP patients (p = <0.001) as compared to healthy controls suggesting increased reticulated platelets. The correlation of platelet counts and IPF in all studies groups, the spearman correlation r values was (r = -0.013** p = 0.000), (r = -0.063 p = 0.734), (r = -0.386* p = 0.017), (r = -0.794** p = 0.006) and (r = -0.226* p = 0.017) in ND-ITP, P-ITP, C-ITP, R-ITP and healthy control individuals respectively (Figure 1). The neutrophils to lymphocytes ratio was higher P-ITP 2.64(3.95), C-ITP 2.53(3.41), ND-ITP 2.46(2.36), R-ITP 2.02(4.39)as compared to controls 1.77(0.84) with p = <0.001 revealing high ratio with prolonged disease (Figure 2). In contrast to it, the assessment of inflammatory marker of platelet to lymphocytes ratio revealed significant low levels in ND-ITP 0.52(1.43), 10.52(19.07) P-ITP, 13.25(20.76) C-ITP, 18.95(10.12) R-ITP and compared to controls 114(95) with p = <0.001 as platelet were less in number in all ITP patients (Figure 3). According to platelet counts, 53(47.7%) ITP patients was in active phase, 17(15.3%) partial remission, 11(9.9%) complete remission and 10(9.0%) patients were unresponsive to all given treatment. The NLR ratio was high in active phase of disease with median of 2.58(2.47) indicating active patients were more prone to infection and PLR was raised in partial remission and patients with median of 38.4(36.9) suggesting inflammation with disease progression (Table 2).

ITP phases	N(%)	Platelet count M(IQR)	NLR M(IQR)	PLR M(IQR)
Active phase	53(47.7)	16(35)	2.58(2.47)	5.22(16.3)
Partial remission	17(15.3%)	108(22)	1.81(2.53)	38.4(36.9)
Complete remission	11(9.9)	26(10)	2.22(2.98)	10.5(9.0)
Non responders	10(9)	43.5(41.5)	2.02(3.2)	18.9(10)

Table 2: Phases of ITP patients according to platelet counts.

N (%): Number of patients (percentage), M (IQR): Median (Interquartile ranges).

Figure 1: Correlation of platelets count and IPF in (a) newly diagnosed ITP patients, (b) persistent ITP, (c) chronic ITP patients, (d) refractory ITP and (e) healthy control population.

Figure 2: Evaluation of N: L ratio in studied groups.

Figure 3: Variation of PLR in studied groups

Discussion

Immune thrombocytopenia is an autoimmune disorder which causes thrombocytopenia. In ITP, peripheral destruction of platelets has been observed but the pathogenesis remains complex and elusive. ITP can arise at any age. However the yearly incidence of ITP is between 2.6, 9.5 and 1.6 in Europe, USA and UK [18-20].

In ITP, besides clinical parameters the laboratory parameters were also important to make final diagnosis by evaluating complete blood picture (CBC) which predicts the bone marrow function by depicting values of three cell lines including red cells, white cells and platelets and several other research parameters.

Parameters	ND-ITP	P- ITP	C- ITP	R-ITP	Controls	P-value
	M(IQR)R	M(IQR)R	M(IQR)R	M(IQR)R	M(IQR)R	
Hb(g/dl)	9.9(2.9) 5.5-15.0	12.0(4.0) 8.0-15.5	11.6(3.4) 4.8-15.6	11.8(12.7) 6.3-15.4	14.3(1.6) 11.8-17.2	p1 = <0.05 p2 = <0.05 p3 = <0.05 p4 = <0.05
TLC(x10^9/l)	9.72(7.71) 3.24-24.6	10.64(4.73) 4.76-38.5	8.67(5.72) 2.50-25.8	8.07(10.18) 1.18-24.82	7.15(2.33) 4.65-13.0	p1 = <0.05 p2 = <0.05 p3 = <0.05 p4 = <0.05
Platelet(x10^9/l)	2(3.8) 0-53	26.0(36) 2-129	25(46.5) 2-143	43.5(46.3) 3-79	274(72) 154-445	p1 = <0.05 p2 = <0.05 p3 = <0.05 p4 = <0.05
IPF(%)	25.4(19.8) 0-87.3	17(22.6) 5.8-51.4	12.9(13.9) 2.9-42	16.5(13) 9.9-38.4	3.1(1.9) 1-5.9	p1 = <0.05 p2 = <0.05 p3 = <0.05 p4 = <0.05
ANC(x10^9/l)	6.04(5.03) 1.28-22	6.93(4.99) 2.18-37.8	5.16(8.46) 0.52-23.3	5.36(7.07) 0.51-20.65	4.15(1.58) 2.38-6.83	p1 = <0.05 p2 = <0.05 p3 = <0.05 p4 = <0.05
ALC(x10^9/l)	2.79(2.82) 0.66-11.4	2.69(2.47) 0.57-7.2	1.99(1.89) 0.63-7.82	2.35(1.42) 0.62-4.64	2.38(0.96) 2.38-6.83	p1 = >0.05 p2 = >0.05 p3 = >0.05 p4 = >0.05

Table 1: Hematological characteristics of ITP patients and controls.

M(IQR)R = Median (Interquartile Range) Ranges, p1 = Difference Between ND-ITP and Controls, p2 = Difference Between P-ITP and Controls, p3 = Difference Between C-ITP and Controls, p4 = Difference Between R-ITP and Controls. † Hb: Hemoglobin, TLC: Total Leucocytes Counts, IPF: Immature Platelet Fraction, ANC: Absolute Neutrophils Counts, ALC: Absolute Lymphocyte Counts.

In the present study, among the red cell parameters hemoglobin content depicts anemia and significant low levels of hemoglobin 9.9(2.9) was observed in ND-ITP which was consistent with study of Fahim and Monir [21] as low levels of hemoglobin were observed in ITP patients. The second cell lineage, white blood cells primarily provide defense against microorganisms. In this study cohort of P-ITP patients exhibited raised TLC with p = <0.001. The ITP is diagnosis of exclusion and generally was diagnosed on the basis of decreases platelet counts with certain clinical symptoms [22]. Recently several platelets parameters offered by automated hemo-analyzers have gained attraction in presumptive diagnosis of ITP. The reduction in platelets number was the main observation in ITP patients and found significant reduction of platelet counts in all ITP patients groups including 2(3.8) in ND-ITP followed by C-ITP, P-ITP, R-ITP as compared to normal number of platelets in healthy individuals which was consistent with studies of El-Rashedi FH., *et al*. and Talaat RM., *et al*. [23,24] as thrombocytopenia was observed in acute ITP. For platelet, the other advance parameter which is immature platelet fraction (IPF) is a supportive parameter which evaluates the reticulated platelets in blood giving clue of bone marrow response to thrombocytopenia (Table 1). The IPF or reticulated platelets with high RNA content expressed as percentage (%) or absolute IPF by Sysmex XN-1000 is a simple, reliable and novel parameter used to enumerate reticulated platelets. The platelets RNA content or increased IPF directly correlates with megakaryocytic activity specially observed under conditions of thrombocytopenia as reported in previous studies [25,26]. The IPF with platelet counts were correlated and observed significant inverse relationship with all ITP patients groups with <0.05 which is consistent with findings of Lindsey., *et al*. [27] reported inverse correlation of platelet counts and IPF with acute bleeding scores in ITP patients (Figure 1).

In the past, the neutrophils to lymphocytes ratio(PLR) was used as marker to predict systemic inflammation [7]. In the present study, the N:L ratio was high with ND-ITP and P-ITP as compared with study of Eren R., *et al*. reported the median of 2.51 ranging (1.15-11.35) suggesting it as a biomarker to be used with response to steroids in ITP patients [28] (Figure 2). The inflammation in disease state might be predicted by the PLR which is used as an inflammatory biomarker in tumors of GI and female reproductive systems and also predict prognosis of several other diseases [11,29]. The low PLR ratio observed in the present cohort of ITP patients is comparable to a Chinese study (Figure 3). In that study re-occurrence of ITP was studied over a period of four years. About 13% decreased risk of relapsed was observed in NDITP patient with low PLR ranging between 0.86-9.7 [30]. In another study, the elevated levels of NLR and PLR were observed in autoimmune disease; Systemic lupus erythmatosus suggesting it to be used as inflammatory biomarkers [31]. In a Turkish study, significant correlation was found between raised NLR and recurrence rate in ITP [32]. Thus, the NLR and PLR can be used in predicting infections, inflammation and recurrence in ITP. The study was conducted with limited number of sample size. Further extensive studies with larger number of sample size with long term follow-up are needed to observe these biomarkers to be used in response to treatment.

Conclusion

ITP is diagnosis of exclusion. The clinical utility of simple parameter like IPF to predict bone marrow response to low platelet counts. The NLR and PLR can be used to predict inflammation in patients which is simple, easy to calculated and inexpensive method of predicting prognosis and response of treatment in ITP.

Contributions

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. AA did methodology, data collection, analyzed visualized and validate results.SM had the basic concept, reviewed, edited and finalized the study. TS supervised and finalized the study.

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