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Original Article

Study of Hematological Changes and Neutrophil to Lymphocytes Ratio in Type 2 Lepra Reaction Cases

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Type 2 Lepra reaction (T2R) is an immune mediated systemic inflammatory response in leprosy patients with high bacterial load. Various cytokines e.g., TNF-α, INF-γ, IL-1β, IL-17A, IL-6 play important role in pathogenesis of ENL. Cytokines response affects the bone marrow derived cells lines and reflects as qualitative and quantitative changes in peripheral blood cells, which in T2R may serve as inflammatory marker for diagnosis and grading of reaction. The aim of study was to assess hematologic changes e.g., Hb, Total WBC count, neutrophils (%), lymphocytes (%), Platelet count, mean platelets volume (MPV), red cell distribution width (RDW) (%), neutrophils (%) to lymphocytes (%) ratio (NLR) in type 2 lepra reaction (T2R). A retrospective analysis was carried out at the tertiary care center. Data were collected with inclusion of all case record of leprosy & T2R from January 2018 to December 2021. Data of Hb, RDW, TC, Neutrophils (%), Lymphocytes (%), Platelets, MPV) were obtained for non-reaction leprosy (LL & BL) as well as during each episode of T2R patients. It was observed that there was significant low Hb while raised TC, RDW, NLR were found in T2R, which were more prominent in severe reaction. For diagnosis of T2R, AUC of NLR was 0.930 (0.885-0.975) with cut off value 6.08 (sensitivity 78.4%, specificity 92.2%, accuracy 90.2%). For severity of T2R, AUC was 0.833 (0.713, 0.953) with cut off value 9.1 (sensitivity 77.3%, specificity 82.8%, accuracy 77.3%). The limitation of study was small sample size and no objective scoring like EESS (ENLIST ENL Severity Scale). T2R patients with multiple episodes were already taking systemic corticosteroids and other infective conditions may also have effect on leucocyte count. There is significantly low Hb with raised TC, RDW, NLR in T2R. Hematologic changes are more prominent in severe reactions. NLR has value in grading severity of T2R.

Keywords : Leprosy, Type 2 Lepra Reaction, ENL, NLR, RDW, MPV

Introduction

Type 2 lepra reaction or Erythema nodosum leprosum (ENL) is an immune mediated systemic inflammatory response seen mostly in lepromatous leprosy (LL) & borderline lepromatous (BL) leprosy and less commonly noted in other types of leprosy e.g., BB (Borderline borderline) leprosy (Pocaterra et al 2006, Saunderson et al 2006). Higher bacillary index increases the risk of T2R/ ENL (Saunderson et al 2006, Polycarpou et al 2017). Clinical manifestations of T2R/ENL range from mild to severe constitutional symptoms and self-limiting skin lesions to severe persistent, recurrent skin lesions of varied morphology (Voorend & Post 2013).

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Neutrophils in tissue biopsy are an essential feature of ENL lesions and thought to be immune complex mediated drive. Various studies on ENL (Sher et al 1978, Polycarpou et al 2017, Schmitz et al 2019) have highlighted the presence of neutrophils in ENL lesions with increased neutrophils activation. Schmitz et al (2016) have earlier reported increased expression of CD64 on circulating neutrophils which corelate with ENL severity and is suggested as early marker for ENL diagnosis and severity measurement. Tavares et al (2021) demonstrated increased activated phenotype low density Neutrophils (LDNs) in peripheral blood in ENL, and also in-vitro generation of LDNs in whole blood by *M. leprae* in dose dependent manner. Neutrophils Extracellular Traps (NET) formation is unique characteristic of neutrophils, which is composed of nuclear DNA, histones and granular proteins and play vital role in elimination of extracellular micro-organisms. da Silva et al (2019) have demonstrated formation of Neutrophils Extracellular Traps (NET) in ENL lesions as well in serum of T2R cases and highlighted role of NET in pathology of T2R. Evidence suggested the role of various cytokines and inflammatory cells play role in pathogenesis of ENL. Cytokines such as TNF-α, INF-γ, IL-1β, IL-17A, IL-6 significantly rise in ENL (Pandhi & Chhabra 2013, Polycarpou et al 2017). Systemic cytokines response affects the bone marrow derived cells lines and that reflects as qualitative and quantitative changes in peripheral blood cells. Quantitative changes of peripheral blood cells are primarily used for assessing the systemic inflammatory response in various infectious and non-infectious conditions (Tefferi et al 2005, Adewoyin et al 2014). In T2R, inflammatory response reflected as quantitative changes in peripheral blood cells may help in assessing severity of episode. Easy availability of hematological investigations led us to explore the hematological changes and neutrophils to lymphocytes ratio (NLR) as inflammatory marker in type 2 lepra reaction.

Materials and Methods

This retrospective observational study was conducted after approval of institutional ethics committee (IEC-SMIMER Reference No 38; 15/10/2022) at our tertiary care teaching hospital in south Gujarat. All leprosy case records were maintained under specialty clinic from January 2018 to December 2021 with clinical diagnosis of type 2 lepra reaction/ ENL, lepromatous leprosy, and borderline leprosy (BL) were included for data collection and analysis. Inclusion criteria for T2R cases were case record with smear positive leprosy and clinical diagnosis of T2R/ENL, and for comparison group, smear positive borderline leprosy (BL) & lepromatous leprosy (LL) without any type of lepra reaction. Case records with missing data required for study, smear negative borderline leprosy cases with or without features of T2R and other leprosy cases e.g. Tuberculoid, indeterminate & pure neural leprosy with or without T2R were not enrolled in the study. Diagnosis was made according to the Indian Association of Leprologists classification (IAL 1982). T2R was categorized as mild and severe and was treated according to national leprosy eradication program guidelines (NLEP 2009). Mild ENL included intermittent crops of few ENL, mild fever (< 100^0F), no organ involvement and no neuritis. Severe ENL/T2R included multiple/ innumerable ENL in crops, neuritis, High fever (> 100^0F), Ulcerated ENL, involvement of eye, joints, lymph node, testes, kidney, liver, bone marrow, endocardium and recurrent ENL with more than 4 episodes in a year, mild reaction not responding to NSAIDs within 2-4 weeks. Clinical and laboratory data of each episode of T2R/ ENL cases and data of leprosy (LL & BL) without reactions of each enrolled case were obtained from records. Data of age, sex, clinical type of leprosy, type of ENL as per WHO grading of T2R

(mild/severe), Hemoglobin (Hb gm/dL), Total count (TC 10^3 /µL), differential lymphocyte and neutrophils count, platelet count, mean platelet volume (MPV fL), red cell distribution width (RDW %), were collected and entered in Microsoft excel sheet for analysis. Double data entry was done to reduce entry errors. The NLR was calculated by dividing the differential neutrophil count by the differential lymphocyte count. Patient's demographics and T2R characteristics were summarized descriptively. Numerical laboratory variables were expressed as mean ± standard deviation (SD). Normality of distribution was analyzed by Kolmogorov- Smirnov test. The statistical significance of laboratory variables among the groups were calculated using T-Test, and Microsoft excel sheet. As NLR ratio among different group was not normally distributed as per Kolmogorov- Smirnov test, statistical analysis of NLR among group was analyzed using Mann-Whitney U test with help of online statistical calculator form https://www.socscistatistics.com. ROC and cut off value of variables were generated using a web-tool for ROC curve analysis (ver. 1.3.1) available free from http://www.biosoft.hacettepe.edu.tr/easyROC/. (Goksuluk et al 2016; Last access 28th December 2022).

Results

Retrospective data analysis of age, sex and type of leprosy among case records of T2R /ENL and non-reaction comparison group is summarized in Table 1. Total 25 patients' records were found with T2R, 20 were male and 5 were female. There were 22 severe and 29 mild category reaction episodes (Total 51 episodes) among 25 patients. Out of 25 patients, 19 (76%) patients were having LL and 6 (24%) were having BL leprosy as clinical diagnosis.

Out of 25 T2R cases, 16 (64%) had mild reaction episodes, while 4 (16%) had severe and 5 (20%) had both mild and severe T2R episodes. Out of 6 BL cases, one case had multiple episodes and all episodes were of mild category. Out of 19 LL

Table 1 : Demographic characteristics of cases with and without type 2 reaction.

Parameters	Mild T2R (n=16)	Severe T2R (n=4)	Mild & Severe T2R (n=5)	Total T2R (n=25)	BL-LL without reaction	Cases with episodes of ENL	
						Multiple	Single
Male	12	4	4	20	39	7	**13**
Female	4	0	1	5	12	3	2
Total	16	4	5	25	51	10	15
BL	6	0	0	6	14	1	5
LL	10	4	5	19	37	9	10
Total	16	4	5	25	51		
Age							
Mean	38.7	34.5	38.8	38.14	34.87	38.43	37.88
SD	12.69	9.88	15.9	12.86	13	12.19	13.88
Median	37	35	35	35	35	38	35
Episodes	29	22	-	51		36	15

Abbreviations: BL – Borderline Leprosy; LL- Lepromatous Leprosy; T2R – Type 2 lepra reaction

Table 2 : Hemoglobin (Hb), Red cell distribution width (RDW), Total white cell counts (TC), Neutrophils, Lymphocytes, Platelets counts, Mean Platelet volume (MPV) among T2R & Non-reaction cases.

Parameters	Mild Episodes (n= 29)	Severe Episodes (n= 22)	All T2R Episodes (n= 51)	Non-reaction LL-BL (n =51)	ENL Episodes (n=51)		P value (T-test)		
					Multiple (n= 36)	Single (n= 15)	Mild Vs Severe	Reaction Vs non-Reaction	Multiple Vs Single
Hb (gm/dL) (Mean ± SD)	11.59 ± 2.43	9.06 ± 2.43	10.5 ± 2.72	12.57 ± 1.48	9.94 ±2.55	11.83 ± 2.73	P = 0.000630	P < 0.00001	P = 0.02256
RDW (%) (Mean ± SD)	16.57 ± 1.67	18.13 ± 2.48	17.24 ± 2.18	15.41 ± 2.45	17.31 ±1.83	17.07 ± 2.91	P = 0.0152	P = 0.000124	P = 0.723
TC × 10 3/µL (Mean ± SD)	12.73 ± 3.73	15.77 ± 5.78	14.04 ± 4.91	8.74 ±2.89	13.42 ±4.80	17.07 ± 2.91	P = 0.03862	P < 0.00001	P = 0.162
Neutrophils (%) (Mean ± SD)	80.27 ± 5.45	86.43 ± 6.07	82.93 ± 6.45	66.96 ± 8.45	83.02 ±6.66	82.72 ± 6.15	P = 0.0003935	P < 0.00001	P = 0.883
Lymphocytes (%) (Mean ± SD)	12.32 ± 3.84	7.1 ± 4.04	10.07 ± 4.68	20.89 ± 7.27	10.04 ±4.94	10.13 ± 4.14	P = 0.00001	P < 0.00001	P = 0.951
Platelets × 10 3/ µL (Mean ± SD)	286.72 ± 89.57	300.45 ± 135.78	292.65 ± 110.83	264.67 ± 88.42	299.03 ±115.40	277.33 ± 101.08	P = 0.683434	P = 0.16181	P = 0.530
MPV (Mean ± SD)	8.9 ± 1.39	8.36 ± 1.7	8.67 ± 1.54	8.55 ± 1.14	8.60 ±1.60	8.83 ± 1.44	P = 0.238123	P = 0.65087	P = 0.623
NLR									
Mean ± SD	7.56 ± 3.92	17.18 ± 11.22	11.71 ± 9.2	3.68 ± 1.52	**11.80 ± 8.96**	11.48 ± 10.08	P = 0.0007375 (T-test)	P = 0.00001 (T-test)	P = 0.912 (T-test)
Median	6.72	13.47	7.55	3.37	**7.56**	7.55			
1st Q	4.97	9.29	6.28	2.59	**6.42**	6.28	P < 0.00001 (Mann-Whitney U Z= 4.03194)	P < 0.00001 (Mann-Whitney U Z = 7.48245)	P = 0.84148 (Mann-Whitney U Z = 0.19639.)
3rd Q	7.59	20.19	13.47	4.84	**15.48**	10.68			
IQR	2.61	10.90	7.19	2.25	**9.05**	4.40			
Max - Min	20.44 – 3.58	41.3 – 4.2	41.3 - 3.58	6.97 – 0.96	41.30 – 3.58	38.16 – 4.57			

*Quartile, **Inter Quartile range

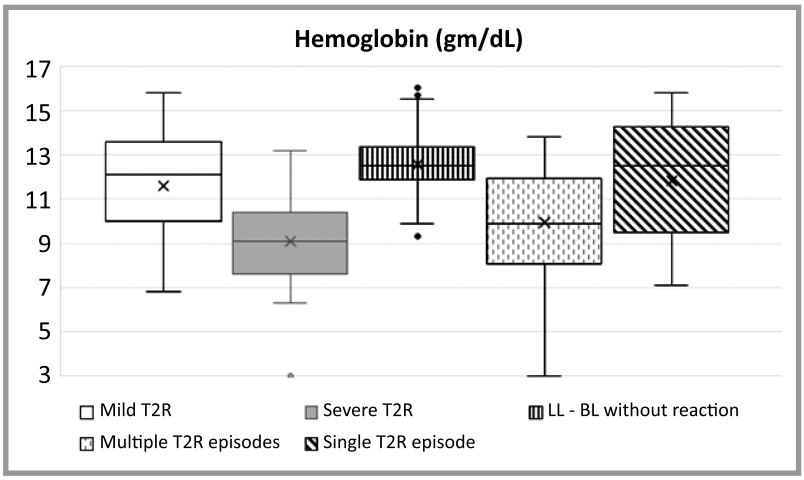

Fig. 1 : Box Plot chart comparison of Hemoglobin (Hb) in cases having ENL episodes with non-reaction (BL & LL) leprosy patients.

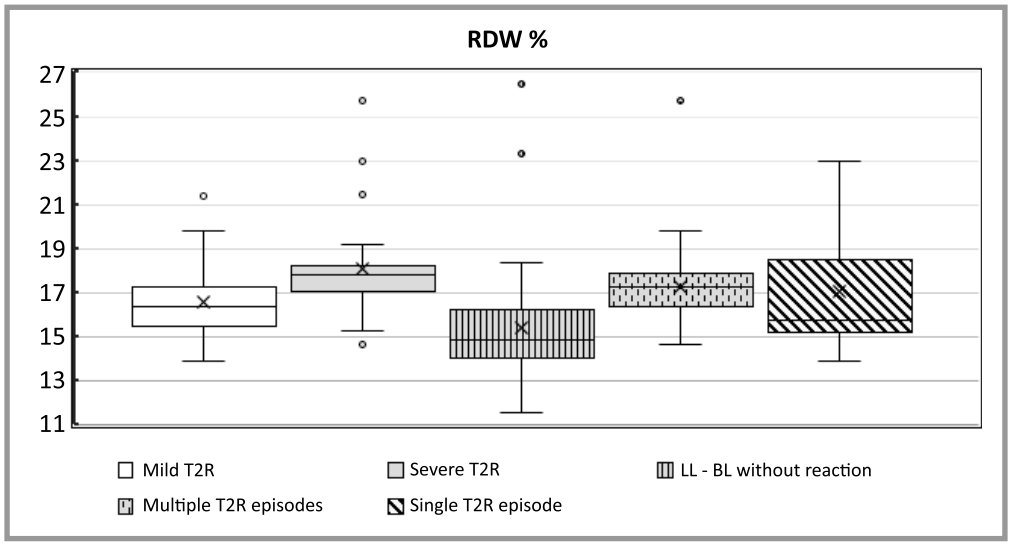

Fig 2 : Box Plot chart comparison of RDW of cases with ENL episodes with non-reaction (BL & LL) leprosy patients.

cases, 9 (47.37%; n=19) were associated with multiple episodes. Severe T2R was noted only in LL cases.

Out of 20 male patients, 13 had single episode (total 13 episodes) and 7 had multiple episodes of ENL (total 28 episodes). Out of 7 patients with multiple episodes, 1 had multiple severe episodes (total 5 episodes), 2 had multiple mild episodes (total 5 episodes), while 4 had multiple mild (total 7 episodes) and severe (total 11 episodes).

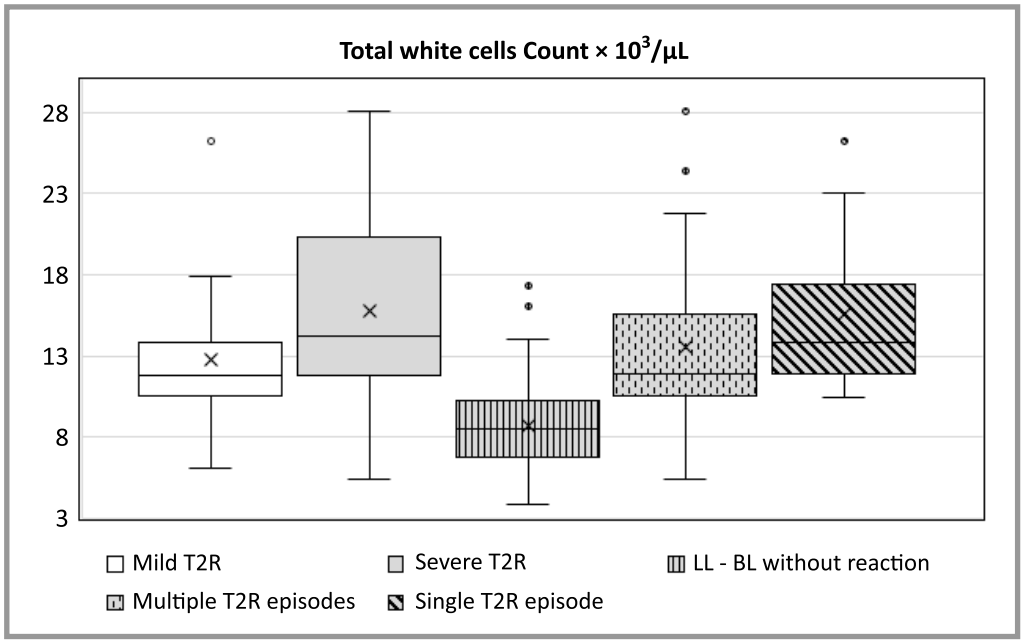

Fig. 3 : Box Plot chart comparison of WBC in cases having ENL episodes with non-reaction (BL & LL) leprosy patients.

Out of 13 patients with single ENL episode, 3 had severe ENL and the rest 10 had mild ENL episode.

Out of 5 females, 1 patient had one mild and three severe episodes (total 4 episodes), 2 patients had 2 mild episodes (total 4 episodes), while 2 had single mild episode. The mean age of patients with only mild, only severe and both types of ENL were 38.7 (Median 37), 34.5 (Median 35) and 38.8 (Median 35) years respectively.

Details of Hemoglobin (gm/dl), total white cell count (TC 10^3 /µL) and differential neutrophils, lymphocytes count, platelets count, mean platelet volume (MPV fL), red cell distribution width (RDW %), neutrophil lymphocyte ratio (NLR) of mild, severe, and non-reaction cases are summarized in Table 2**.** Box plot comparison of Hb, RDW, TC & NLR of mild, severe, multiple & single T2R episode with non-reaction leprosy cases are shown in Figs. 1, 2, 3, 4 respectively and ROC for NLR is shown in Fig. 5.

Platelets count & Mean Platelet Volume (**MPV)**:

There were marginally higher mean platelets count among T2R cases (292.65±110.83 ×10^3/µL) compared to non-reaction leprosy (264.67 ± 88.42 × 10^3/µL) cases and difference was not statistically significant (P > 0.05). Mean MPV remained within normal range among reaction cases and there was no statistical difference among non-reaction cases (P=0.65; T-test) as well severe Vs mild T2R case (P=0.24; T-test).

Haemoglobin (Hb):

There was tendency towards low Hb (g/dL) among T2R with significantly (p < 0.0001 T-test) lower mean Hb (10.5 ± 2.72g/dL) compared to non-reaction (12.57 ± 1.48g/dL) cases (Fig.1).

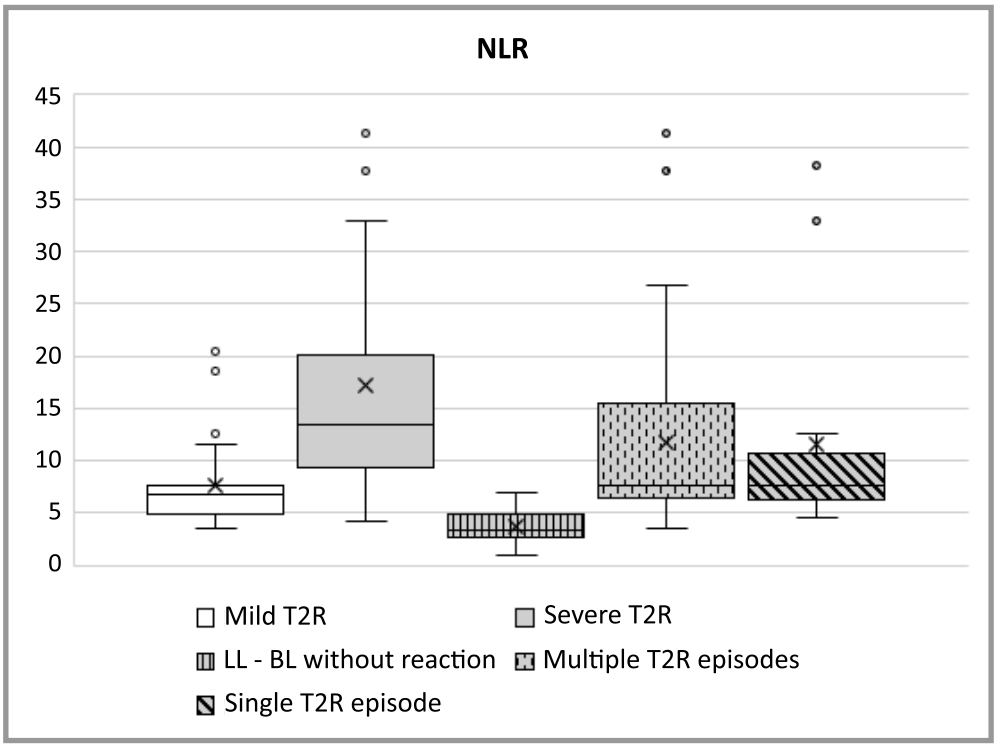

Fig. 4 : Box Plot chart comparison of NLR in patients having ENL episodes with non-reaction (BL & LL) leprosy patients.

Significant low Hb was also noted among severe T2R (P= 0.00063; T-test) and cases with multiple reaction episodes (P = 0.0225; T-test).

Red cell distribution width (RDW):

There was rise of RDW (%) among T2R. Raised mean RDW was also significantly higher compared to non-reaction cases and in severe cases compared to mild cases (P = 0.0152; T- test), but there was no statistical difference of RDW among multiple Vs single episode cases (Fig. 2).

Total & differential count:

In T2R cases & non-reaction leprosy cases mean TC (10^3 /µL) was 14.04 × 10^3 /µL (SD ± 4.91) & 8.74 × 10^3 /µL (SD ± 2.89), mean neutrophil (%) count was 82.93% (SD ± 6.45) & 66.96% (SD ± 8.45), & mean lymphocyte (%) count was 10.07% (SD ± 4.68) & 20.89% (SD ± 7.27) respectively. There was significant rise of total count with neutrophilia (Fig.3) and lymphopenia among T2R cases (P < 0.0001). Neutrophilia and lymphopenia resulted in raised NLR. NLR value was significantly high in T2R as compared to non-reaction leprosy cases (P < 0.0001) and also significant among mild Vs severe T2R (P < 0.0001), but not significant in comparison of single and multiple episodes (P > 0.05). Box plot chart (Fig. 4) analysis showing non-normal distribution of NLR data with comparison of mean, median and quartile range for mild, severe, multiple, single episodes of T2R and LL-BL cases without reaction. Centre lines showing the medians, box limits indicate the 25th and 75th percentiles. Fig. 5 shows receiver operating characteristic curve (ROC curve), sensitivity and

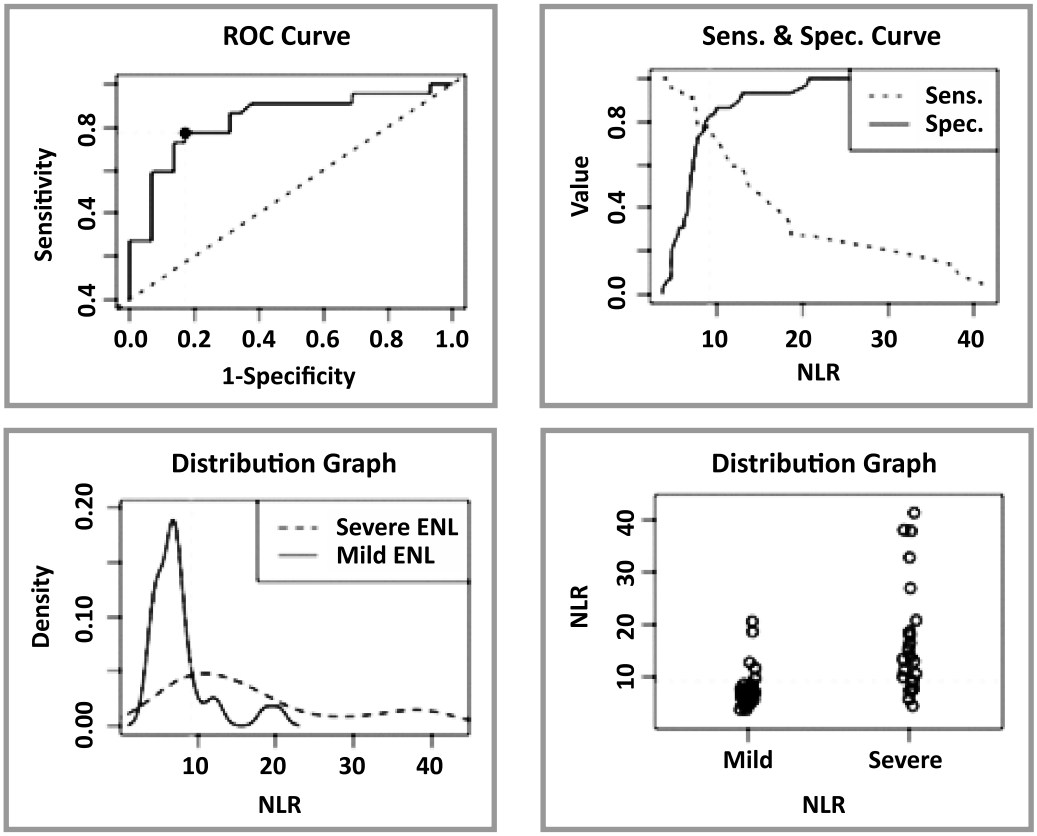

Fig. 5 : ROC curve, sensitivity & specificity curve and distribution graph of NLR in mild and severe T2R.

specificity curve and distribution graph of NLR comparing mild and severe T2R episodes. Area under curve (AUC) of NLR was 0.930 (0.885-0.975) with cut off value 6.08 for diagnosis of T2R (sensitivity 78.4%, specificity 92.2%, accuracy 90.2%). AUC was 0.833 (0.713, 0.953) with cut off value of 9.1 for severe T2R (sensitivity 77.3%, specificity 82.8%, accuracy 77.3%).

Discussion

Retrograde data analysis of T2R cases showed male predominance (80%) with mean age of 38.14 (SD ± 12.86) years and median 35 years. Recent published studies by Padhi et al (2019) & Singla et al (2021) reported similar findings in their study with mean age < 40, & common in male Systematic review published by Voorend et al (2013) did not find gender & age as risk factors for T2R but noted that most of the hospital-based study in India have found male predominance.

The majority of cases (n=19; 76%) were lepromatous leprosy & having clinically mild and severe type of reaction, while BL cases (n=6; 24%) were having clinically mild episodes only. Severe T2R were noted only in LL cases, thus LL appears to be the risk factor for severe T2R episodes. Singla et al (2021) reported 50 (37.3%) BL & 84 (62.7%) LL with T2R and noted 61.2 % cases with

mild to moderate episodes and rest 38.8 % with severe ENL episodes. Padhi et al (2019) reported 72 (74.22%) LL cases with T2R. Systematic review also (Voorend & Post 2013) highlighted higher proportion of ENL reactions in LL cases compared to BL cases.

Out of 25 T2R cases, 15 patients (60%) had only one-episode (15 episodes) during the study period, while 10 patients (40%) had multiple (total 36) episodes in study period. Out of 10 patients with multiple episodes, 9 (47.36%) had LL and only 1 had BL leprosy. Padhi et al (2019) reported only 26.8 % (26 out of 97) patients having single episodes. Systematic review (Voorend & Post 2013) noted multiple episodes range from 39% to 77.3% of ENL patients in hospital-based studies & 44 to 63% in field study of all ENL cases. This review also noted 30 – 50% moderate to severe ENL cases.

In the present study statistically significant low Hb (gm/dL) were found in T2R cases (10.5 ± 2.72 gm/dL) compared to non-reaction (12.57 ± 1.48 gm/dL) leprosy cases ($p < 0.0001$; T-test) and have relation with severity ($P= 0.00063$; T-test) and multiple episodes ($P = 0.0225$; T-test) of T2R. Freitas & Fleury et al (1996) have also reported significantly low Hb among moderate to severe ENL cases with mean value in moderate to severe and mild cases were 7.36 gm/dL (±1.42), 11.60 gm/dL (±1.09) respectively. Low Hb was related to multiple factors e.g., chronic inflammation in leprosy with acute reaction episode & associated rise of various cytokines that have direct or indirect effect on hematopoiesis. Other factors like hemolysis and nutritional deficiency also contributed to low Hb.

There was statistically significant ($p < 0.001$) rise of RDW (%) in T2R cases (17.24 ± 2.18 %) compared to non-reaction leprosy (15.41 ± 2.45 %) cases. Difference was also significant ($p < 0.05$) among mild (16.57 ± 1.67 %) and severe (18.13 ± 2.48 %) T2R, but not significant on comparing single and multiple T2R episodes. Ambalia et al (2021) reported significant rise of RDW (%) in both T1R & T2R lepra reaction cases and changes were independent of change in Hb. RDW (%) rise associated with or without low Hb and reflected the changes in average size of red blood cells. Raised RDW without anemia is thought to be related to inflammation and oxidative changes in RBCs and premature release of red cells from marrow.

In present study mean platelet counts increased with T2R compared to non-reaction leprosy cases ($P > 0.05$). Severe T2R had slightly higher mean platelet count compared to mild cases ($P > 0.05$). Rise of platelet counts may be related to cytokines effect on marrow and increase synthesis and release in peripheral blood (Norol et al 1998). Freitas & Fleury et al (1996) in their study reported significantly ($P < 0.05$) higher mean platelet count in moderate to severe ENL cases compare to mild cases. Mean platelet volume (MPV) remained within range in reaction and non-reaction cases. There was no significant change in MPV with increasing TC count in reaction compared to non-reaction cases ($P > 0.05$). Mean MPV was lower in severe T2R cases compared to mild T2R and non-reaction cases ($P > 0.05$).

Comparing the platelet count and MPV, there was negative correlation between MPV and platelet count ($p <0.05$, $r= -0.4732$ (mild), -0.5895 (severe), -0.5392 All T2R). Overall, there was a rise of platelets count and decreased MPV with rise of TC. Increased platelet count and decreased MPV suggest there is newly formed platelets released from marrow that have comparatively lower size and contribute to decreased MPV. Peripheral consumption of larger size activated platelets also contributes to low MPV. While decreased platelets count and raised MPV suggest increased platelet activation (as activated platelets are larger in size) and platelet consumption.

Interaction with PMN in ongoing acute systemic inflammation with activation & consumption in intravascular coagulation and Neutrophil extracellular traps (NET) formation may limit the platelet counts in peripheral blood (Afsar et al 2017, Zucoloto & Jenne 2019, Pogorzelska et al 2020). Our study only measured quantitative platelets changes in peripheral blood, but there is much more complex interaction between platelets and other immune cells thus platelet function assessment may throw more light inside in pathogenesis of T2R.

Data of our study revealed significant rise of mean total WBC count with significant neutrophilia and lymphopenia among T2R cases. Mean value was also significantly high among severe T2R episodes compare to mild episodes, but not significant among multiple episodes compare to single episodes. Recently published study by Tanojo et al (2022), reported significant rise of total WBC count in ENL compared to non-reaction leprosy.

Neutrophilia with lymphopenia results in higher neutrophils to lymphocytes ratio (NLR). NLR was significantly higher in severe T2R ($P < 0.0001$) compared to mild reaction. NLR is considered as useful indicator of morbidity and mortality in infection associated conditions and has been suggested as prognostic maker in sepsis and septic shock and serve as predictor of adverse outcome (Huang et al 2020). Neutrophilia is appeared to be associated with onset of T2R reaction but primary trigger that neutrophil expansion in peripheral blood is not known. Bacillary antigenic load and increased immunoglobulins level due to predominant humoral response results in immune complex formation and thought to precipitate T2R. It has been noted that occurrence of ENL is usually associated with dead and granular bacilli (Manandhar et al 1999, Fransisca et al 2021). There may be direct neutrophil activation by rising *M. leprae* antigen and immune complexes. Recent study highlighted role of Th 17 T-cells in leprosy that secrete IL-17A, IL-17F, and IL-22 upon activation. As neutrophils production is under control of G-CSF which is synthesized under the effect of IL-17A. Th-17 cells activation with rise of IL-17A may trigger neutrophilia with onset of T2R (Schmitz et al 2019).

Rise of peripheral WBC count with neutrophilia suggest systemic inflammatory response and results of proliferative effect of inflammatory mediators on bone marrow cells. T2R fulfils the clinical criteria of systemic inflammatory response syndrome (Dellinger et al 2008) e.g., fever and leukocytosis, but sepsis and multi-organ dysfunction syndrome have not been reported in T2R. Neutrophil activation in blood and resultant systemic inflammatory response from release of various pro-inflammatory cytokines, chemokines, various enzymes, further drive inflammatory process. Severity may depend on local as well systemic inflammatory response with neutrophils activation. Release of neutrophilic granular enzymes, other cytokines & NET formation (da Silva et al 2019) drive the tissue inflammation and tissue injury. On going tissue damage during T2R may further augment inflammation and inflammatory loop may be responsible for progress and severity of T2R.

Peripheral blood cells count depends on many physiological and pathological factors and thus peripheral blood cells ratio is not specific for diagnosis of specific disease condition. In our study median NLR was higher in severe (13.47) than mild (6.72) T2R, while median NLR of single (7.55) and multiple (7.56) T2R episode group were near equal. There was statistically significant difference ($p < 0.0001$) of NLR among T2R and non-reaction leprosy cases as well between mild and severe T2R cases. The diagnostic cut-off value of NLR for T2R was 6.08 with 78.4% sensitivity, 92.2% specificity and 90.2% accuracy. The diagnostic NLR cut-off for severity assessment was 9.1 (77.3% sensitivity, 82.8% specificity,

77.3% accuracy). Our ENL diagnostic cut-off results were higher than the result by Tanojo et al (2022) & Gomes et al (2020)**,** who reported the NLR cut-off value 4.99 (86.4%, sensitivity, 82.5% specificity, and 82.97% accuracy) and 2.95 (81.0%, sensitivity, 74.0% specificity, and 78.0% accuracy) respectively. Tanojo et al (2022) & Gomes et al (2020) described importance of NLR in T2R, while our study results also showed significant difference of NLR value in mild and severe ENL episodes.

Our study period also included Covid-19 pandemic time which might have had influence on haematological parameters. In patient data, we found 20 new LL-BL leprosy patients without any lepra reactions and 8 patients with T2R during January 2020 to December 2021. Out of 8 patients, 4 had single (mild only) episodes, while the other 4 had multiple episodes. Out of 4 patients with multiple episodes, 1st patient had two mild episodes, 2nd patient had 2 severe episodes, 3rd patient had one mild and one severe episode, and 4th patient had one mild episode. (4th patient had previous episode in 2019). 2nd and 3rd patients had one episode during Covid wave (2020), 2nd patient with severe and 3rd patient with mild episode, but both were RT-PCR negative for Covid-19 and improved with corticosteroid treatment.

Conclusion

T2R is systemic inflammatory response and neutrophils play major role in pathology of reaction which is evident by peripheral blood neutrophilia. There is significant low Hb while TC, RDW, NLR in T2R are raised and more prominent in severe reaction. Though NLR is not specific for diagnosis of T2R, it has value in grading severity of T2R. We have attempted to explore the value of NLR as a predictor of severity in T2R and there is significantly higher NLR value in severe T2R compared to mild T2R. This study has limitation as grading of T2R was available as mild and severe category without objective scoring like ENLIST ENL Severity Scale (EESS) which limit the analysis of co-relation between variables. Further T2R patients with multiple episodes were already taking systemic corticosteroids and other infective conditions may have effect on leucocyte count. These aspects need to be investigated in well-designed prospective studies.

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