Indian J Lepr 2021, 93 : 341-347

http://www.ijl.org.in

Original Article

Study of Red Blood Cell Distribution Width (RDW) as Hematological Inflammatory Marker in Leprosy and Lepra Reactions

DG Ambalia[1], JB Vaishnani[2], VA Desai[3]

Received : 10.09.2020 Accepted : 09.08.2021

Red blood cell distribution width (RDW) is routinely reported in complete blood count and reflects variability in the size of circulating red blood cells. The degree of anisocytosis in a blood sample is known as the red cell distribution width (RDW). RDW has emerged as a prognostic marker in various disorders, including acute or chronic conditions and is positively correlated with inflammatory markers. This study aimed to assess RDW value in leprosy and lepra reaction patients. An observational study was conducted at the tertiary care centre. Details of complete blood count and CRP were collected for 77 newly diagnosed leprosy patients and those on treatment experiencing lepra reaction attending leprosy clinic between July 2018 & December 2019. Seventy-seven patients presented with various other dermatological problems, e.g. chronic eczema, prurigo, keloid scars etc., were enrolled for comparisons. A total of 154 patients were enrolled. Mean RDW was lowest in TT leprosy (13.44 ± 1.14) and highest in type 2 lepra reaction (17.68 ± 2.23). RDW was significantly higher in lepra reactions (17.09 ± 2.09) cases (T1R & T2R) compare to leprosy cases without Lepra reaction ($X2 = 31.1332$ $p << 0.001$) & comparison group ($X2 = 17.47$; $p << 0.001$). Raised RDW was associated with raised CRP (86.67%) in T2R and (30%) in T1R cases. Reactive CRP was statistically significant at $p < 0.05$ ($X2 = 6.2266$; $p = 0.0125$) in lepra reaction cases compare to non-reaction cases. A statistically significant rise of RDW in lepra reaction cases (Type 2 & Type 1 reactions) compared to leprosy cases without lepra reaction and a non-leprosy comparison group was observed which is also associated with raised CRP. RDW along with CRP appear to be potentially useful markers to study the progress of reaction cases in leprosy.

Keywords : Leprosy, Lepra reactions, Red Blood Cell Distribution - RDW, CRP, MCV

Introduction

Leprosy is a chronic mycobacterial disease with varied clinical presentations ranging from no visible inflammatory signs (e.g. hypopigmented lesions) to marked inflammatory signs (e.g. erythematous & infiltrated lesions) across the spectrum of leprosy and lepra reactions.

Red blood cell distribution width (RDW) is routinely reported in complete blood count & reflects variability in the size of circulating red

[1] Dr DG Ambalia, MBBS, PG Resident
[2] Dr JB Vaishnani, MD (Dermatology), Professor & Head
[3] Dr VA Desai, MBBS, PG Resident
Department of Dermatology Venereology & Leprology, Surat Municipal Institute of Medical Education & Research (SMIMER); Near Sahara Gate, Umarwada, Surat-395010, Gujarat, India.
Corresponding Author: Dr Jignesh B Vaishnani, **Email**: jigsmimer@yahoo.co.in

blood cells known as anisocytosis. Higher the variability in the size of cells higher the RDW. Thus, a high RDW means that cells are of very different sizes, whereas a low RDW indicates that cells are more or less of the same size. The RDW reference range in one Indian population study was 12.2 to 15.5 (Sehgal et al 2013). The MCV is the average volume of a red blood cell and reflects the size of cells with a normal reference value of 80fL – 95fL. MCV and RDW values will give an idea about changes in the size of RBCs. A high MCV indicates a large red blood cell, and a low MCV indicates a small red blood cell. High RDWs with Low MCV are usually indicative of Iron Deficiency Anemia; high RDW with high MCV indicates Vitamin B12 and Folate Deficiency; High RDW and normal MCV indicate iron/Vit B12/ folic acid deficiency and Hemorrhage (Salvagno et al 2015).

RDW has emerged as a prognostic marker in a variety of disorders such as cardiovascular disease, cancer, diabetes, chronic obstructive pulmonary disease, kidney failure, as well as in other acute or chronic inflammatory conditions and positively correlated with inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) (Lippi et al 2009, Lappé et al 2011, Zou et al 2016). CRP is an acute-phase reactant and rises with acute increase of systemic inflammatory response. The rise of RDW with the rise of CRP may indicate the change of RDW value during acute inflammatory process and thus can be used as acute inflammatory marker.

Chronic inflammatory state in leprosy and enhanced immune response during lepra reactions with increased inflammatory cytokines may have widespread collateral effects. RBCs homeostasis is affected by systemic inflammatory changes, and affects the size, shape and maturity of red cells and reflects as a change in RDW value. The status of RDW in leprosy & lepra reaction patients was not evaluated in the past, and the present study was aimed to evaluate the status of RDW and its relation with different types of leprosy & lepra reaction.

Materials & Methods

This prospective observational study was conducted after approval of the institutional ethics committee at the tertiary care centre of south Gujarat. Based on clinical and laboratory investigations, all newly diagnosed leprosy patients and those already on treatment and presented with lepra reactions attending leprosy clinic from July 2018 to December 2019 were enrolled. Based on Indian Association of Leprologists (IAL 1982) classification cases were clinically grouped as Tuberculoid (TT), Pure Neuritic (PN, Borderline (BL) without reaction, Lepromatous (LL) without reaction, Type 1 reaction (T1R), and type 2 reaction (T2R). Cases with the presence of hemoglobinopathies or other conditions such as sickle cell anaemia, thalassemia were excluded. Data including age, sex, clinical characteristics of leprosy & lepra reaction, complete blood counts with RDW, MCV & CRP were recorded. Those patients who presented with various other dermatological problems, e.g. chronic eczema, prurigo, keloid scars etc., were enrolled for comparisons. CRP is an acute-phase reactant and rises with an acute increase of systemic inflammatory response and tissue damage. Rise of RDW with the rise of CRP may indicate the change of RDW value during acute inflammation. As RDW is a part of RBCs indices, its value needs to interpret in relation to other red cells indices for diagnosis of anaemia & hemoglobinopathies. Complete blood count (CBC) along with RDW & MCV were done on semi-automatic haematology analyser (Beckman Coulter). Serum CRP was done by latex semi-quantitative method (CRP latex method – Beacon), and results were reported as qualitative (reactive/non-reactive) and quantitative (mg/dl

after multiplying highest dilution of reactive serum with 0.6). The reference range of RDW in our laboratory was 12.1(low) to 16.2 (High), and RDW ≥ 16.2 was considered abnormal. MCV < 80 fl was considered low.

Data of RDW, Hemoglobin (Hb), Mean Corpuscular Volume (MCV), C-reactive protein (CRP) were entered in MS Excel sheet and excel sheet formula, and Epi-info-7 statistical calculator was used for analysis. Quantitative data were expressed as frequency, mean, while unpaired t-test & Chi-square tests were used to calculate the statistical significance of RDW value in leprosy cases with and without reaction and with the comparison group. P<0.05 was considered statistically significant.

Results

A total 154 patients were included in the study. 77 patients had leprosy, while the other 77 patients having other dermatological diseases were enrolled for comparison. The clinical characteristics of study participants are summarised in Table 1. Out of 77 leprosy cases, 52 cases were without lepra reactions, and 25 had lepra reactions. Out of 52 non reaction leprosy cases, 10 (12.99%) were TT, 6 (7.79%) were PN, 26 (33.77%) were BL and 10 (12.99%) were LL cases. Out of 25 lepra reaction cases, 10 (12.99%) were T1R, and 15 (19.48%) were T2R cases. There was no significant difference in the mean age of study group leprosy cases (31.53 ± 12.83) and comparison non-leprosy dermatological disease cases (31.57 ± 12.91).

Red blood cell distribution width (**RDW)**: Out of the total of 77 leprosy cases, 22 (28.57%) cases had RDW ≥ 16.2, out of which 4 were non-reaction leprosy cases, and 18 were reaction (T1R & T2R) cases. Out of these 4 non- reactions cases, 2 were PN cases & 2 were BL cases without lepra reactions. Out of 18 reactions cases, 05 (50%) cases were T1R, and 13 (86.66%) cases were T2R. All TT & LL without lepra reaction cases had RDW values within normal range. Mean RDW values in TT, PN, BL without reaction and LL without reaction cases were 13.44 (SD ± 1.14), 14.82 (SD ± 1.91), 14.72 (SD ± 1.41) & 14.24 (SD ± 0.88), respectively. Mean RDW value in lepra reaction cases was 16.16 (SD ± 1.09) in T1R & 17.68 (SD ± 2.23) in T2R reaction cases. In the comparison group, the mean RDW was 13.98 ± 0.87 and 2 (2.59%) cases had RDW ≥ 16.2.

Mean Corpuscular Volume (MCV): Out of 77 cases, 35 (45.45%) cases were having MCV <80 fL and seen in 01 (16.67%) PN, 13 (50 %) BL without reaction, 06 (60 %) LL without reaction, 06 (60 %) T1R & 09 (60%) T2R cases. Mean MCV values in TT, PN, BL without reaction, LL without reaction were 89.38 (SD ± 5.097), 85.92 (SD ± 8.73), 81.64 (SD ± 9.67), 76.41 (SD ± 8.08), respectively. Mean MCV value in T1R & T2R were 77.18 (SD ± 8.87) & 78.58 (SD ± 8.67) respectively. In comparison group mean MCV was 85.98 ± 6.44 and 10 (12.99%) cases having MCV < 80.

Hemoglobin (Hb): Out of 77 leprosy cases Hb < 10 gm/dl was seen in 11 (14.29%). Out of 11 cases, 7 (63.63%) were T2R cases, 2 (18.18%) BL without reaction cases, 1 (9.09%) each TT & PN Leprosy. These 4 non-lepra reaction cases with Hb < 10 gm/dl were not associated with raised RDW (≥ 16.2). While out of 7 T2R cases, 6 (7.79%) were associated with raised RDW (≥ 16.2). So out of 11 cases with Hb < 10 gm/dl, only 6 (7.79%) cases were associated with RDW ≥ 16.2. One (1.30%) case in comparison group was having Hb < 10 gm/dl. In comparison, 2 (2.59%) cases were having RDW ≥ 16.2, and out of these, 1 was associated with Hb < 10 gm/dl.

C-reactive Protein (CRP): Reactive CRP (> 1.2 mg/dl) was found in 21 (27.27%) cases, out of which 5 were without lepra reactions, and 16 were with lepra reactions. Out of 5 (9.62%) non reaction cases, CRP > 1.2 mg/dl was seen in 2 (2.6%) LL

Table 1 : Characteristics according to RDW, Hb, MCV and CRP status among leprosy cases

	Frequency (%)	Age (Mean ± SD)	RDW % (Mean ± SD)	Hb (gm/dl) (Mean ± SD)	MCV (fl) (Mean ± SD)	MCV < 80 fl	RDW % ≥ 16.2	Hb ≤ 10 gm/dl	↑RDW with ↓Hb < 10gm/dl	CRP > 1.2 mg/dl	↑RDW with CRP > 1.2 mg/dl
TT Leprosy	10 (12.99%)	28.3 ± 6.53	13.44 ± 1.14	13.85 ± 1.80	89.38 ± 5.09	00 (0%)	00	01 (10%)	0 (10%)	1	0
PN Leprosy	6 (7.79%)	31.7 ± 12.6	14.82 ± 1.91	12.55 ± 1.79	85.92 ± 8.73	01 (16.67%)	02 (33.33%)	01 (16.67%)	0	2 (33.33%)	1
BL Leprosy (Without reaction)	26 (33.77%)	30 ±15	14.72 ± 1.41	12.93 ± 1.58	81.64 ± 9.67	13 (50 %)	02 (7.69%)	02 (7.69%)	0	0	0
LL Leprosy (Without reaction)	10 (12.99%)	35 ±15	14.24 ± 0.88	12.41 ± 0.63	76.41 ± 8.08	06 (60 %)	00	00	0	2 (20%)	0
T1R	10 (12.99%)	32 ±9.9	16.16 ± 1.09	12.21 ± 1.76	77.18 ± 8.87	06 (60 %)	05 (50%)	00	0	3 (30%)	3
T2R	15 (19.48%)	34 ±12	17.68 ± 2.23	10.6 ± 1.81	78.58 ± 8.67	09 (60 %)	13 (86.66%)	07 (46.67%)	6 (7.79%)	13 (86.67%)	13
Total (Leprosy Cases)	77 (100%)	31.53 ± 12.83	15.52 ± 2.20	12.41 ± 1.86	81.12 ± 9.32	35 (45.45%)	22 (28.57%)	11 (14.29%)	6 (7.79%)	21 (27.27%)	17
Comparison Group	77 (100%)	31.57 ± 12.91	13.98 ± 0.87	13.42 ± 1.39	85.98 ± 6.44	10 (12.99%)	02 (2.59%)	01 (1.30%)	1 (1.3%)	01 (1.30%)	0

TT: Tuberculoid, BL: Borderline, LL: Lepromatous; PN: Pure neural; T1R-type 1 lepra reaction; T2R - type 2 lepra reaction; PB - paucibacillary, MB multi-bacillary; Hb - Hemoglobin; CRP: C-reactive protein; RDW: Red cell distribution width, MCV: mean corpuscular volume.

without reaction, 2 (2.6%) PN, and 1 (1.30%) TT leprosy. Out of 16 lepra reactions cases, CRP > 1.2 mg/dl was seen in 3 (3.90%) T1R and 13 (16.88%) T2R cases. Out of 21 (27.27%) cases with CRP > 1.2mg/dl, 17 cases were associated with raised RDW $\geq$ 16.2 and included 1 non-reactions case (PN), and 16 reactions cases (3 T1R & 13 T2R). 2 BL without reactions and 2 BL cases with T1R were having RDW $\geq$ 16.2 but not associated with raised CRP > 1.2 mg/dl. All 13 T2R cases had raised RDW ($\geq$ 16.2) with raised CRP (>1.2 mg/dl). In comparison, group 1 (1.30%) case having reactive CRP > 1.2 mg/dl but was not associated with raised RDW.

Discussion

In the present study, we assessed RDW in leprosy cases with and without lepra reaction and compared it with RDW values of non-leprosy patients. We found that there was no statistically significant difference (P = 0.9623) of RDW among the comparison group (13.98 ± 0.87) and leprosy cases without lepra reactions (14.40 ± 1.40). RDW was significantly higher in lepra reactions (17.09 ± 2.09) cases (T1R & T2R) compare to leprosy cases without lepra reaction (X^2 = 31.1332 p << 0.001) & comparison group (X^2 = 17.47; p << 0.001). Raised RDW was associated with raised CRP (86.67%) in T2R and (30%) in T1R cases. Reactive CRP was statistically significant at p < 0.05 (X^2 = 6.2266; p = 0.0125) in lepra reaction cases compare to non-reaction cases. Mean MCV was within the reference range in TT, PN & BL without reaction cases, but 50% of BL without reaction had MCV below the reference range. Mean MCV was low (< 80 fL) in LL without reaction, T1R & T2R. MCV value was not statistically different among reaction and non reaction cases (X^2 = 2.35; p = 0.1252845), while anaemia was statistically higher in reaction cases compared with non reaction cases (X^2 = 4.148; p = 0.0416684).

An abnormally high RDW means that cells are of a variety of different sizes and are generally expected to occur in situations of deficiencies (Iron, Vitamin B12, folic acid – disease-related), a condition that leads to ineffective erythropoiesis (abnormal production), or an increase premature release of large red blood cells into the peripheral circulation, or decrease survival of circulating erythrocytes. Various factors like inflammation, oxidative stress, directly and indirectly, affect the RBCs homeostasis has been implicated in higher RDW. Inflammation contributes to increasing RDW levels by affecting iron metabolism, inhibiting the erythropoietin and premature release of erythrocytes, or shortening red blood cell survival resulting from oxidative damage and indirectly phagocytosis or lysis of RBCs (Ganz 2019).

Raised inflammatory cytokines in leprosy and lepra reactions, e.g. IFN-γ, TNF-α, IL-6, have been implicated in the development of anaemia and altered iron homeostasis in the form of decreased iron availability low MCV & abnormal RDW value (Freitas & Fleury 1996). In the present study, anaemia was seen in 11 (14.29%) cases, out of which 7 (63.63%) were having T2R lepra reactions. Rea (2001) reported that a fall in mean Hb is statistically significant after the onset of symptoms of T2R in polar or subpolar lepromatous leprosy. In the present study, out of 15 T2R cases, 13 cases had raised RDW. Out of 13 cases with raised RDW, Anaemia was seen in 06 (7.79%) cases, and the rest 7 cases did not have anaemia despite raised RDW value. Similarly, out of 10 T1R cases, 5 (50%) cases had raised RDW value, and anaemia was not seen in any of these cases. In such cases, this raised RDW value may be attributed to inflammatory response during the reaction and independent of factors related to anaemia.

Cytokines such as IL-1 beta, IFN-gamma, TNF-alpha and IL-6 inhibit erythropoiesis via direct suppression of erythroid precursors and also promote apoptosis of precursor cells (Agarwal 2012). TNF-α cause down-regulation of erythropoietin (EPO) receptors and blunt the erythropoietic effect of erythropoietin directly (Zamai et al 2000). IFN-γ may affects the RDW value by the effect on iron metabolism, inhibition of erythtopoiesis & red cell survival by increase phagocytosis of RBCs and apoptosis of precursor cells, thus contributes to anaemia and different sized RBCs (Ganz 2019). Increased hepicidin expression in leprosy also contribute to the inhibition of erythropoiesis by inhibiting iron release (Souza et al 2012). There is a significant rise in IL-6, IL-6 mRNA, IFN-γ, TNF-α in ENL/T2R, with increased expression of hepcidin, cathelicidin, anti-microbial peptides C1q & defensins. (Polycarpou et al 2017). Also, in T1R there is a significant rise of IFN-γ, TNF-alpha, IL-1, IL-2 compared to non-reaction cases (Fonseca et al 2017, Belgaumkar et al 2007). As mentioned earlier, the rise of cytokines and hepcidin creates an iron deficiency state and affects erythropoiesis and is reflected as low MCV and anaemia and may contribute to a rise in RDW value. Low MCV and normal RDW in leprosy cases without lepra reactions may results from chronically decreased iron availability for erythropoiesis due to raised cytokines in chronic inflammation. In reactions cases, low MCV may result from a chronic inflammatory state, and high RDW may result from mixed acute and chronic effects of inflammation.

Inflammatory state associated with raised oxidative stress and in leprosy oxidative stress is found to be high in multibacillary & lepra reaction cases and implicated in the pathogenesis of leprosy (Jyothi et al 2008, Swathi & Tagore 2015). Oxidative stress associated with increased phagocytosis & hemolysis of RBCs, and release of juvenile erythrocytes. Oxidative stress also causes changes in shape and size of RBC by membrane glycoprotein changes and may contribute to the RDW value. Oxidative stress has been found to trigger eryptosis by activation of calcium-dependent K+ channels, which leads to loss of KCL from erythrocytes and osmotic loss of water results in shrinkage of RBCs. Shrinkage of RBCs, changes in the size of the cell directly contributes to rising of RDW under the effect of raised systemic oxidative stress (Repsold & Joubert 2018). The increase in oxidative stress may have direct & acute contributory effects on RDW, and this could be independent of anaemia.

Reactive CRP (>1.2mg/dl) was found in 21 (27.27%) cases, and most of the cases 16 (76.19%), were having lepra reaction (T1R & T2R). Reactive CRP (>1.2mg/dl) among lepra reactions and non-lepra reaction cases was statistically significant at 0.05 (X^2=6.2266; p=0.0125 & Fischer Exact value 0.0075). All 13 (876.66%) T2R cases with raised RDW had reactive CRP $\geq$ 1.2 mg/dl. In T1R, out of 5 (50%) cases with raised RDW, only 3 (30%) cases had raised reactive CRP > 1.2 mg/dl. There is a positive correlation between CRP and RDW, and the correlation coefficient calculated for quantitative CRP and RDW among all cases were 0.418901. CRP is an acute-phase reactant and rises with the acute increase of systemic inflammatory response under the effect of cytokines, e.g. IL-6 and tissue damage. CRP also augments the local inflammation and phagocytosis of antigen. The rise of RDW with the rise of CRP may indicate changes may be of value in monitoring acute inflammation.

Conclusion

In summary, this study highlights a significant rise of RDW value along with increase of CRP in lepra reaction cases. As the sample size may be small in the present study, further study with larger sample size is required to confirm the present findings. Further, as RDW value depends

on many factors that affect RBC homeostasis, RBC indices and comparison of value during reaction state with pre-reaction baseline value will provide more accurate status of RDW among leprosy and lepra reactions.

References

1. Agarwal S (2012). Red cell distribution width, inflammatory markers and cardiorespiratory fitness: results from the National Health and Nutrition Examination Survey. *Indian Heart J.* **64(4):** 380-387.
2. Belgaumkar VA, Gokhale NR, Mahajan PM et al (2007). Circulating cytokine profiles in leprosy patients. *Lepr Rev.* **78(3):** 223-230.
3. Fonseca AB, Simon MD, Cazzaniga RA et al (2017). The influence of innate and adaptative immune responses on the differential clinical outcomes of leprosy. *Infect Dis Poverty.* **6(1): 1-8.**
4. Freitas TC, Fleury RN (1996). Hematologic Profile of Leprosy Patients in Reactional Episode of Erythema Nodosum Leprosum. *Hansen Int.* **21:** 59-66.
5. Ganz T (2019). Anaemia of inflammation. *New Engl J Med*. **381:**1148-1157doi: 10.1056/NEJMra 1804281. PMID: 31532961.
6. Indian Association of Leprologists (1982). Clinical, histopathological and immunological features of the five type classification approved by Indian Association of Leprologists. *Lepr India*. **54** : 22-25.
6. Jyothi P, Riyaz N, Nandakumar G et al (2008). A study of oxidative stress in paucibacillary and multibacillary leprosy. *Indian J Dermatol Venereol Leprol.* **74(1)**: 7-10.
7. Lappé JM, Horne BD, Shah SH et al (2011). Red cell distribution width, C-reactive protein, the complete blood count, and mortality in patients with coronary disease and a normal comparison population. *Clinica Chimica Acta; Int J Clin Chem.* **412(23-24):** 2094–2099.
8. Lippi G, Targher G, Montagnana M et al (2009). Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med.* **133:** 628-632.
9. Polycarpou A, Walker SL, Lockwood DNJ (2017). A systematic review of immunological studies of erythema nodosum leprosum. *Front. Immunol.* **8:233.** doi: 10.3389/fimmu.2017.00233
10. Rea TH (2001). Decreases in mean hemoglobin and serum albumin values in erythema nodosum leprosum and lepromatous leprosy. *Int J Lepr Other Mycobact Dis.* **69(4):** 318-327.
11. Repsold L, Joubert AM (2018). Eryptosis: An erythrocyte's suicidal type of cell death. *Biomed Res Int.* 2018:9405617. https://doi.org/10.1155 /2018/9405617
12. Salvagno GL, Sanchis-Gomar F, Picanza A et al (2015). Red blood cell distribution width: A simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci*. **52(2):** 86-105.
13. Sehgal KK, Tina D, Choksey U et al (2013). Reference range evaluation of complete blood count parameters with emphasis on newer research parameters on the complete blood count analyzer Sysmex XE-2100. *Indian J Pathol Microbiol*. **56**: 120-124.
14. Souza VN, Malaspina TS, Campanelli AP et al (2012). Increased hepcidin expression in multi-bacillary leprosy. *Mem do Inst Oswaldo Cruz.* **107(Suppl. 1).** 183-189.
15. Swathi M, Tagore R (2015). Study of oxidative stress in different forms of leprosy. *Indian J Dermatol.* **60:** 321. https://www.e-ijd.org/ text.asp?2015/60/3/321/156426
16. Zamai L, Secchiero P, Pierpaoli Set al (2000). TNF-related apoptosis-inducing ligand (TRAIL) as a negative regulator of normal human erythro-poiesis. *Blood.* **95(12):** 3716-3724.
17. Zou XL, Lin XJ, Ni X et al (2016). Baseline red blood cell distribution width correlates with disease activity and therapeutic outcomes in patients with systemic lupus erythematosus, irrespective of anemia status. *Clin Lab.* **62(10):** 1841-1850.

How to cite this article : Ambalia DG, Vaishnani JB, Desai VA (2021). Study of Red Blood Cell Distribution Width (RDW) as Hematological Inflammatory Marker in Leprosy and Lepra Reactions. *Indian J Lepr.* **93**: 341-347.