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Changes in Red Blood Cell Distribution Width in Patients with Multiple Sclerosis Treated with Rituximab: A Retrospective Cohort Study

Zabihollah Hashemzahi[1], Susan Sarhadi[2], Amirhossein Keykhaee[3], Saeedeh Sarhadi[4*]

[1]Neurology Department, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.

[2]Department of Pharmaceutics, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran.

[3]School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.

[4]Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

Abstract

Background: Recently, Anti-CD20 monoclonal antibodies have been proposed as second-line treatment options for patients with MS. However, there is still no strong laboratory evidence to establish standard prognostic criteria for treatment. Based on previous studies, the red blood cell distribution width (RDW) could be associated with autoimmune diseases. This study aimed to assess changes in RDW in MS patients treated with rituximab.

Methods: This retrospective cohort study included 50 MS patients treated with rituximab at Aliebn-Abitaleb Hospital. Patient records were used to collect information. Data collection included demographic data such as age (years) and gender (male/female), RDW, and the Extended Disability Status Score (EDSS) (before treatment and at 6 and 12 months after treatment). Finally, these findings were analyzed using SPSS software.

Results: The findings of the present study showed that EDSS scores decreased significantly during treatment ($P < 0.001$). This finding indicated an improvement in the disability status of patients with MS. RDW levels were also considerably reduced at 6 months and 1 year after rituximab treatment ($P=0.012$). However, the correlation between RDW and EDSS scores was not statistically significant ($P>0.05$).

Conclusion: RDW and EDSS score changed significantly over time and were influenced by rituximab, although there was no significant correlation between these two variables. RDW values may correlate with treatment response in patients with multiple sclerosis.

Keywords: Multiple Sclerosis; red blood cell distribution width; Extended Disability Status Score.

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Introduction

Multiple sclerosis (MS) is an inflammatory autoimmune chronic central nervous system (CNS) disease that can occur because of axon demyelination, which can create inflammatory lesions. As a result, nerve impulse conduction is disturbed. Epidemiologic data show that MS has a prevalence in children and adults around the world. MS patients suffer from numerous disorders, including visual impairment, double vision, blurred vision, balance disorders, cognitive impairment, fatigue, painful muscle spasms, and depression [1-10].

The number of MS patients was estimated at 2.8 million worldwide in 2020, an increase of 30% from 2013. The prevalence of patients in the U.S. and Europe is 117 and 142 per 100,000 population, respectively, which is significantly higher than in African, Southeast Asian, and Western Pacific countries. The disease typically develops between 20 and 50 years, with a peak in the 30s, although it can also occur in children and the elderly [11].

MS pathology involves multiple brain lesions that result from inflammatory immune system reactions. These plaques are mainly found in white matter surrounding the lateral ventricles and optic nerve. Studies have shown a positive correlation between the number of lesions and disease severity [12]. MS histopathological features indicate that T CD4+ cells predominantly react against myelin. However, some clinical reports indicate that CD8+ T cells act synergistically with myeloid cells [13]. Based on this

***Correspondence to:** Saeedeh Sarhadi, Email: dr.sarhadi93@gmail.com

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information, autoimmune processes in the CNS are induced by an imbalance between T helper-1 (Th1), Th17 cells, and regulatory T cells (Treg). Therefore, T cells are commonly considered the main drivers of inflammatory activity in MS, but other evidence also supports B cells' role in the disease's pathogenesis [14].

Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown effectiveness in reducing B cell activity. In 2017, this drug was approved for treating adult patients with MS. Rituximab has been indicated to significantly reduce the annualized relapse rate (ARR), slow disability progression, and prevent new MRI lesion formation [15]. Although the treatment approach is based on several factors, including the disease situation, the patient, the safety-risk balance, and disease progression[16].

Red blood cell distribution width (RDW) is an index that measures variation in red blood cell size and can be easily and cost-effectively assessed. It is calculated by using the standard deviation (SD) of red blood cell (RBC) volume and mean corpuscular volume (MCV): (RDW-SD)/(MCV)×100. RDW is frequently used to diagnose anemia but has also been associated with a variety of inflammatory conditions, including autoimmune disorders, cancer, and cardiovascular diseases[17]. In MS, where inflammation plays a key role, RDW changes may reflect alterations in erythrocyte morphology due to immune-mediated processes [18]This study aimed to explore the association between RDW changes and rituximab treatment in MS patients at Aliebn-Abitaleb Hospital in Zahedan, Iran, from 2018 to 2021.

Materials and Methods

This retrospective cohort study was conducted at Aliebn-Abitaleb Hospital, the referral hospital in southeastern Iran in Sistan and Baluchistan Province, from 2018 to 2021. This study was approved by the Ethics Committee of Zahedan University of Medical Sciences under number IR.ZAUMS.REC.1402.249.

Inclusion Criteria: The study included patients with complete records who were undergoing only second-line treatment for Secondary Progressive MS (SPMS). These patients had secondary progressive MS and were followed for 1 year. All patients were seen at one hospital every 6 months, evaluated using one piece of equipment, and checked by one neurologist.

Exclusion Criteria: Individuals with conditions that may affect the measurement of RDW and CBC parameters, including hemoglobinopathy, diabetes, hypertension, cardiovascular diseases, liver and kidney dysfunction, cancer, thyroid disease, and autoimmune and inflammatory diseases, were excluded based on a comprehensive history and laboratory tests.

Of 53 initially enrolled patients, 3 (5.7%) were lost to follow-up due to geographical constraints preventing clinic attendance. The final analysis included 50 patients who completed all study procedures.50 patients were assigned to treatment. Rituximab (1g every 6 months) (AryoGen Pharmed Pharmaceutical Company, Tehran, Iran) was prescribed in a hospital setting for MS patients and administered to CBC patients at each hospitalization.

This study's data collection method involved reviewing information forms. For this reason, the data from this periodic assessment were available in the hospital records. Data collection tools included demographic data such as age (years) and gender (male-female), red blood cell distribution width RDW-SD), which was obtained directly from the automated hematology analyzer and expressed in femtoliters (fL), with a reference range of 40–53 fL, and the Extended Disability Status Scale (EDSS score), which is a scale used to assess disease progression and is scored on a 10-point scale. EDSS score was evaluated by one neurologist, who also performed a comprehensive neurological examination.

Clinical and laboratory assessments were performed by the treating healthcare professionals as part of routine clinical care, and the results were documented in patients' hospital medical records. EDSS scores were assigned to each of the seven functional systems (Pyramidal, Cerebellar, Brainstem, Sensory, Bowel/Bladder, Visual, Cerebral) and evaluated the patient's walking ability, including walking speed and distance. Based on the FS scores and ambulation assessment, the neurologist determined the final EDSS score. These data were measured at three time points (before treatment, 6 months after treatment, and 1 year after treatment).

Based on Peng et al. (19), the mean RDW before and after intervention was 13.6 ± 0.83 and 12.5 ± 0.42, respectively. Assuming a conservative correlation of 0.10 between the data points, the effect size is approximately 1.2, which represents a large treatment effect. For this study, a conservative approach was used, and a moderate effect size of d = 0.50 was considered. With 90% power and a significance level of 0.05, the required sample size was estimated to be 44 participants. Accounting for potential attrition during follow-up, the final sample size was set at 50 subjects. The dataset was analyzed using IBM SPSS Statistics, version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. Friedman test was conducted to compare parameters over time. The Friedman test was used to compare median EDSS scores across the three time points because the EDSS scores were not normally distributed (Shapiro-Wilk test, $p < 0.05$).

The effect size in Table 1 was calculated as $W = X2/N(K-1)$, where W is Kendall's W value, X2 is the Friedman test statistic, and N is the sample size. k is the number of measurements per subject. Additionally, the Spearman correlation coefficient was computed to examine the association between the measured parameters and the EDSS scores. Statistical significance was defined as a p-value of < 0.05.

Results

This study retrospectively analyzed 50 medical records of multiple sclerosis (MS) patients treated with rituximab at

Table 1. Comparison of parameters at three time points: before, 6 months, and 12 months of rituximab treatment.

Variable	Before treatment				6 months later treatment				12 months after treatment				Effect sizes†	P-Value*
	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR		
WBC (10^9 cells/L)	7.5	2.7	7	3	6.7	1.9	6.4	2.4	6.88	1.8	6.8	2.7	0.03	**0.15**
Lym (%)	29.2	10.5	60	6.2	27.7	10.3	28.3	12.2	25.1	8.9	25.0	13.4	0.05	**0.74**
Hb (g/dL)	12.7	1.3	12.7	1.5	12.6	1.6	12.5	1.7	12.5	1.6	12.6	2.4	0.01	**0.94**
MCV, FL	80.4	6.4	81.1	9.1	81.1	6.3	81.1	8.9	81.7	6.8	82.2	9.2	0.10	**0.01**
MCH, FL	26.7	3.2	27.5	4.6	26.8	3.0	27.3	4.5	27.1	2.9	27.7	4.15	0.01	**0.18**
MCHC (g/dL)	33.1	1.7	33.3	2.3	32.7	1.7	33.1	3.1	33.1	1.5	33.1	2.0	0.01	**0.24**
PLT (10^9 cells/L)	263.4	70.6	262	112.5	258.2	70.8	251	101.2	250.9	67.4	253.0	108	0.02	**0.38**
RDW-SD (fL)	43.5	2.8	43.2	3.4	42.9	2.9	42.7	3.6	42.4	3.1	42.0	4.0	0.08	**0.012**
EDSS	2.9	1.43	2.50	2.0	1.47	1.50	1.0	2.13	1.22	1.43	1.0	2.00	0.65	**<0.001**

WBC: White blood cell; Lym.: Lymphocytes; Hb: Hemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelets; RDW: red cell distribution width; IQR: Interquartile range; SD: standard deviation; EDSS: Expanded Disability Status Scale; * Friedman test; †Kendall's W value

Table 2. Correlation between EDSS Scores and RDW in Multiple Sclerosis Patients Treated with Rituximab.

Variables	Spearman coefficient	95% confidence interval (CI)		Sig. (2-tailed)
		lower	upper	
EDSS Score -RDW (baseline)	-0.05	-0.33	0.23	0.68
EDSS Score -RDW (6th month)	-0.13	-0.40	0.15	0.35
EDSS Score -RDW (12th month)	-0.05	-0.33	0.23	0.70

EDSS: Expanded Disability Status Scale, RDW: red cell distribution width

Aliebn-Abitaleb Hospital in Zahedan, Iran, from 2018 to 2021. The primary objective was to evaluate and compare alterations in hematological parameters among MS patients receiving rituximab therapy. The mean ± standard deviation (SD) of the age of the study participants was 36.5 ± 10.2 years. The mean ± SD age at MS diagnosis among patients treated with rituximab was also 30.6 ± 9.60 years. The mean ± SD of Disease duration was 5.70 ± 4.0 years. The gender distribution of the study population included 38 females (76%) and 12 males (24%).

MCV and RDW levels changed significantly during rituximab treatment, as shown by the Friedman test results. Moreover, MCV increased significantly (p=0.014), while RDW decreased (p=0.012). However, no statistically significant differences were observed in other CBC parameters ($p > 0.05$). The median EDSS scores at baseline, Month 6, and Month 12 were 2.5 (IQR: 2), 1.0 (IQR: 2.13), and 1.0 (IQR: 2.0), respectively. The Friedman test showed a statistically significant difference in EDSS scores over time ($p < 0.001$) (Table 1).

The association between EDSS scores and RDW values in rituximab-treated multiple sclerosis patients was evaluated using Spearman's correlation analysis. The lack of statistical significance (P>0.05) points indicate no statistically significant correlation between EDSS and RDW during the study period. There is consistently no significant correlation between EDSS and RDW in MS patients treated with rituximab, according to our combined correlation analysis (Table 2) and scatter plots (Figure 1). Notably, neither the scatter plot's R2 (0.010) nor the initially observed inverse correlation at baseline (Spearman rho=-0.05, p=0.68) was statistically significant. Null relationships were confirmed by both methods at 6 and 12 months (all p=0.35, $R^2 < 0.07$).

Discussion

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) mediated by CD4+ T cells reacting against myelin antigens[13]. In recent years, anti-CD20 monoclonal antibodies have been suggested for the treatment of patients with MS (20). Despite these advancements, valid criteria are needed to assess patient prognosis, particularly in clinical practice.

Currently, changes in red cell distribution width (RDW) have been noted in various pathological conditions, including blood transfusion, chronic liver disease, autoimmune disorders, cancer, and cardiovascular diseases (17). Given its broad association with systemic inflammation and immune dysregulation, RDW may also be associated with MS. The present study found that RDW levels were significantly reduced at 6 months and 1 year after rituximab treatment.

Some studies have highlighted RDW changes in autoimmune neurological diseases. Rocha et al. examined RDW as a correlated marker of disability in relapsing-remitting multiple sclerosis (RRMS). In this retrospective study, they evaluated the association of baseline RDW and RDW 5 years after diagnosis with disability. The results showed that higher RDW at baseline was associated with greater disability at 5 years post-diagnosis. Also, RDW levels equal to or higher than 13.5% may be useful in predicting disability risk [21]. At the same time, no statistically

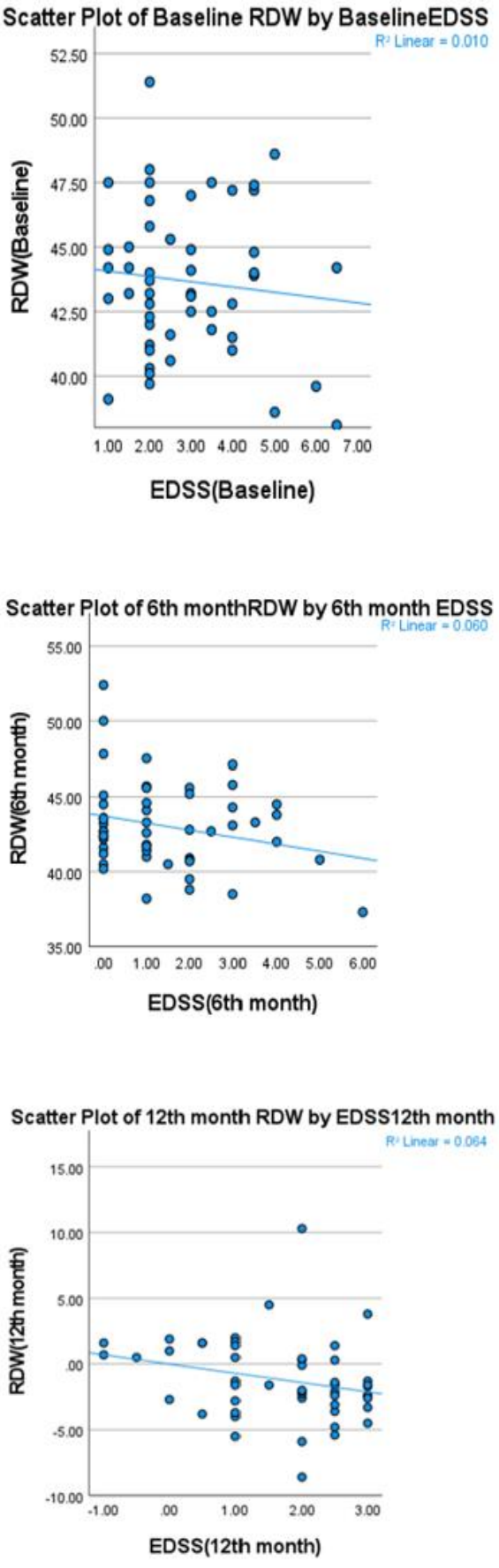

Figure 1. Scatter plot of RDW and EDSS score (baseline,6 th month,12thmonth).

significant correlation was observed in the present study between RDW and EDSS score. In a similar study, the relationship between RDW and multiple sclerosis was evaluated. 109 MS patients treated with interferon β-1a and 130 healthy subjects were enrolled. CBC and EDSS were assessed in MS patients before and after treatment with IFN-ß1a. A significant positive correlation was observed between RDW score and disability scale in MS patients (R=0.789, P=0.001). There was a significant difference in RDW and EDSS scores between untreated and treated patients. They showed that RDW values were significantly reduced in responders (P = 0.025). Their results reported that high RDW values were associated with EDSS scores in MS patients (19). Our study also showed that both RDW levels and EDSS scores decreased following treatment. Inflammatory factors in autoimmune and inflammatory diseases like MS affect RBC production, leading to RBCs of varying sizes and increased RDW levels. After treatment with anti-inflammatory drugs, reduced inflammation allows RBC production and maturation to normalize, resulting in a decrease in RDW.

A retrospective cohort study conducted across MS centers in Switzerland and the Netherlands examined the long-term effects of rituximab in 88 patients with secondary progressive MS (SPMS) between 2004 and 2017. The findings of this study also showed that treatment with rituximab for up to 10 years can significantly reduce EDSS score, consistent with our own observations (22). These data indicate that anti-B cell therapy (both low-dose and standard-dose) can improve prognosis and slow disease progression.

Another study was conducted in India, a developing country, to investigate the efficacy and safety of rituximab in multiple sclerosis. In this cohort study, 118 patients with multiple sclerosis treated with rituximab were evaluated from 2015 to 2019 at three tertiary care centers. Rituximab was administered at doses of 500 mg every 6–12 months, or 1–2 g every 6 months for progressive symptoms. The findings of this study showed that among the 80 patients who received rituximab, the EDSS score improved (ranging from 0.5 to 2 points) in 68 patients (85%), and most patients (91%) tolerated the rituximab injection well, with no reports of injury or opportunistic neoplasms. Therefore, these results reinforce the efficacy and safety profile of rituximab across MS patients [23].

Similarly, a study of different doses of Rituximab was reported in China. In this retrospective control study, 9 patients with relapsing-remitting MS (RRMS) were studied, and rituximab 100 mg per week for 3 weeks was used as induction therapy, followed by rituximab 100 mg every 6 months during the first year and then every 6 to 12 months as maintenance therapy. These findings showed that repeated low-dose rituximab treatment significantly reduced median ARR. This study showed that repeated low-dose rituximab treatment significantly reduced median ARR, mean EDSS score, and the number of T2 lesions on MRI. Thus, they concluded that repeated low-dose rituximab treatment for RRMS patients is cost-effective and has a

good safety profile [24]. Although our study used a different dosing regimen (1 g every 6 months), these findings suggest that both standard- and low-dose rituximab could effectively improve clinical and radiological outcomes in MS.

This study has several limitations that should be considered when interpreting the results. First, as a single-center investigation conducted within the framework of a medical doctoral thesis, our research was subject to the inherent financial and time constraints typical of academic training projects. While the single-center design may limit generalizability, our institution serves as the exclusive tertiary referral center for multiple sclerosis in southeastern Iran, which enhances the regional representativeness of our patient cohort. Additional limitations include the relatively modest sample size, which was adequate for a thesis project but may affect statistical power, and the absence of a Control Arm. Furthermore, we lacked systematic longitudinal MRI data to assess treatment-related changes in CNS activity. Despite these constraints, our findings regarding the association between RDW and EDSS scores provide clinically relevant insights that warrant further investigation in larger, multicenter studies with more extensive biomarker and neuroimaging assessments.

Conclusion

In this study, we evaluated rituximab as a treatment protocol. RDW can demonstrate an association with the inflammatory state of patients with multiple sclerosis (MS). However, future studies with larger patient cohorts and comparative analyses of other immunomodulatory and immunosuppressive therapies are warranted to better define the role of RDW across different treatment modalities. RDW significantly changed during 12 months of rituximab treatment in MS patients; however, the clinical significance of this finding and its relationship with disability remain uncertain.

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Ethical consideration

Not applicable.

Competing Interests

The authors declare no conflict of interest.

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