Cyclophosphamide Versus Rituximab in Progressive Forms of Multiple Sclerosis

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Abstract- This study aimed to compare the efficacy of rituximab versus Cyclophosphamide on active secondary progressive multiple sclerosis (SPMS). The randomized clinical trial was performed from 2015 to 2017 in multiple sclerosis (MS) clinics affiliated to Isfahan MS society (IMSS). Patients were randomized to two groups, and one of them received Rituximab that was repeated every six months in case of medical indication. The other one received a monthly pulse of methylprednisolone plus cyclophosphamide (Endoxan, Baxter, UK) until two years. Expanded disabilities status scale (EDSS), clinical, and MRI findings were assessed every six months. Statistical analysis was performed using SPSS software. 39 patients in the Rituximab group and 30 in the Cyclophosphamide group with similar age and gender distribution were entered for analysis. At baseline, the mean number of attacks in the Rituximab group was significantly more than the Cyclophosphamide group (P=0.0001). After 6, 12, and 18 months of treatment, the rate of attacks was similar between groups although it increased significantly in the Rituximab group (P=0.030) after 24 months of treatment. EDSS was increased in the Rituximab group more than the other group at the end of the study. Both drugs were well-tolerated by patients. The EDSS was increased in the Rituximab group but the disability score did not worsen in the Cyclophosphamide group. Both therapies were associated with a reduction in disease attacks and improvement in radiologic findings in a two-year period of follow-up.

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Introduction

Multiple Sclerosis (MS), the demyelinating devastating disease of the central nervous system (CNS), has been associated with a high prevalence of morbidity among young women (1). Beyond the classic classification (2) of the disease, which was regarding the disease progression, MS presentation has been introduced as either relapsing-remitting or progressive pattern in recent studies (3). Each subtype can be seen in an active or inactive form regarding clinical, imaging (presence of Gadolinium enhancing lesions) or CSF findings. Further, an article in 2017 suggested a new classification of the disease regarding active elements in the cerebral spinal fluid (CSF). Combined active progressive subtype was found to have higher CSF cell counts, IgG-index, MBP, NFL and CHI3L1 CXCL13 and MMP-9 versus the inactive form with only higher levels of IgG-index and MBP in CSF (4).

T cell-mediated autoimmune reactions were the assumed etiology of the disease by some studies, while humeral B cells played the most important role (5,6). The theory was supported by further achievements in treatment following targeting B cells (7). Depleting mature B cells by binding to CD20 molecule on the surface area and the consequent apoptosis was the main underlying mechanism of the two recent successful medications, Rituximab and Ocrelizumab (8,9). In the latest review article in 2018 (10), Rituximab shortened and prevented relapses both clinically and radiologically via modifying inflammatory activities in spite of contradictory results in improving the expanded

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disabilities status scale (EDSS). Thus, the role of Rituximab is still under inquiries. The effect of the drug on different stages of MS was investigated in prior studies (11-13) but it lacks sufficient pieces of evidence in patients with aggressive progressive forms of MS.

Lots of policies have been introduced for patients with aggressive or progressive forms of MS. B-cell depleting agents like Rituximab were associated with serious adverse events, especially infections that may require surgical interventions and consequents recurrent immunoglobulin monitoring in some cases (14-16). By our current knowledge, treatment options for aggressive forms of MS have lots of inconsistency (17) and need more considerations in both aspects of outcome and medication side effects.

Cyclophosphamide is one of the known drugs used for aggressive MS. The mechanism of action in MS comes from its ability to reduce pro-inflammatory T helper (Th) 1 cytokine interferon-g (IFNg) and interleukin (IL)-12 and increasing the secretion of the anti-inflammatory Th2 cytokines IL-4 and IL-10 in CSF and peripheral circulation (18). Cyclophosphamide has an immunomodulatory effect on T cells that can impact on higher expressions of CXCR3+ and CCR5+ IFNg producing T cells in relapsing attacks. Progressive MS was experimentally correlated with higher levels of peripheral IFNg and IL-12 (19,20) that could be modulated by Cyclophosphamide in patients with secondary progressive MS (20). These theories motivate clinical trials and more studies on human beings about the efficacy of this medication in aggressive forms of MS. The medication was proved to be tolerable by MS patients, although it lacked pieces of evidence in those suffering from aggressive forms.

The burden of the side effects of each approach versus the potential advantages is another issue that has to be compared between different medications (21). This study aimed to compare the efficacy of Rituximab and Cyclophosphamide on active secondary progressive multiple sclerosis (SPMS).

Materials and Methods

Ethics

The proposal of this study was enrolled in the ethical review board for clinical trials at Isfahan MS society (IMSS). The study was performed from October 2015 to April 2017 in MS clinics affiliated to IMSS. Helsinki declaration was highly respected during all steps of the study, and ethical aspects were the first regarded issue in our study considering the painful and distressing quiddity of the disease. Regarding the aim of the study and patients' interests, medications, side effects, advantages and design of the study were clarified for patients during multiple sessions. The written consent form was signed by those patients who agreed to participate in our study. During the study, routine follow-up was performed whether there were any complaints or not. All potential side effects and improvement were checked by the same neurologist and if there was any serious complication or deterioration of the disease, the patient was excluded from the study to receive appropriate medical supports regarding standard protocols for managing MS attacks or any potential medication side effects.

Sample size and patients

This study was a pragmatic and superiority randomized clinical trial. The total number of 80 was calculated regarding the last studies in the related field, considering the best estimation for rejecting the null hypothesis (alpha error of 0.05) and preserving the highest power of 80%. MS patients with active secondary progressive subtype were selected if there were two or more attacks during the last year, more than three gadolinium-enhanced lesions in brain magnetic resonance imaging (MRI) surveys or more than one score progression in EDSS within the last year. The enrollment was limited to patients under sixty years old with a known diagnosis of secondary progressive multiple sclerosis and an EDSS of less than six at the initiation of the study. Exclusion criteria were as follows: suffering from any other types of MS rather than SPMS, neuromyelitis optica, history of myelopathy or neurodegenerative disorders, history of other autoimmune disorders, recent or recurrent infections and presence of any hematologic, immunologic or metabolic laboratory abnormalities. Simple sampling continued until all calculated populations were recruited. In the case of MS deterioration or presentation of any symptoms in favor of medication side effects, the patient was excluded from the study and received the appropriate alternative treatment.

Randomization and allocation concealment

Classification of patients was performed regarding cluster randomization. Randomization was performed using random allocation software, and each patient was given a number in a concealed envelope. Odds and even numbers were considered to receive Rituximab and Cyclophosphamide, respectively. The envelope was opened by the first neurologist who was not blind about the drug and provided educational supports regarding the medication and appropriate dosage (refer to blinding

section).

Blinding

This trial could not be blinded on all sides. As patient safety was the first issue in our survey, a non-blinded neurologist prescribed the medication and educated possible side effects and alarm signs about the specific administered drug. So it was risky to be blinded about the medication and also the medical history of the patients. All medications were offered to patients in a covered pocket with only educational notifications. The second neurologist who evaluated patients during each visit was blinded to the consumed drug and recorded his examination assessment in a previously designed checklist. The second blinded neurologist was the same for all patients and checked possible medication side effects during each session regardless of the consumed drug. The act reduced possible observational biases. Statistical analysis was performed by a blinded individual to aim and design of the study who was not involved with medical aspects.

Intervention and follow-up

Demographic data including age, sex, the age of onset of MS, and the first presentation sign were all documented. All medications administered for MS treatment were discontinued by the beginning of the study except Natalizumab and Fingolimod which needed to be stopped three and two months prior to initiation of a new drug, respectively.

One group of patients received a single dose of Rituximab, 1000 milligrams intravenous (IV) infusion that was repeated after two weeks, and then every six months with the same dosage if there was an increase in CD19 and CD 20 level. The second group was administered a monthly pulse of 1 gram IV methylprednisolone plus 1 gram IV cyclophosphamide (Endoxan, Baxter, UK) every month until two years.

EDSS, symptoms, and signs of MS attack and gadolinium contrast assisted MRI findings were assessed by the same blinded neurologist at the study initiation and every six months for a period of two years. Complete blood count, CD19 level, CD20 level, liver, and kidney enzymes were measured every 6 months.

Data analysis

Statistical analysis was completed using SPSS software for Windows (SPSS, Inc., Chicago, IL, USA, version 22). Descriptive statistics are reported as mean±SD, median, or number (percent) as appropriate. Independent sample t-test and chi-square tests were used to compare prevalences and means between the two groups, respectively. Nonparametric alternatives, Mann-Whitney U test and Friedman test, were used if Kolmogorov Smirnov test results revealed significant P values. If it was needed to determine whether there was any significant difference between the means of more than two independent groups, an analysis of variances (ANOVA) was applied. Repeated measurement ANOVA was applied to evaluate the trend of progression. All hypothesis testing was two-tailed and the level of significance was considered to be less than 0.05 in all tests.

Results

Figure 1 shows the flowchart of the study (Figure 1).

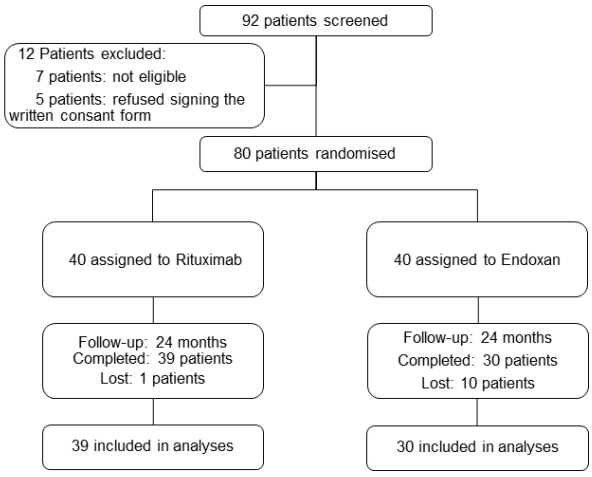

Figure 1. The flowchart of the study

To select eligible patients, 92 MS patients were screened. Of them, seven patients were not eligible, and five did not agree to enter our study. Eligible patients randomly assigned to two Rituximab or Cyclophosphamide groups. During the follow-up period in the Rituximab group, one patient and in the Cyclophosphamide group, 10 patients withdrew treatments and were excluded from the analyses. Finally, 39 in the Rituximab group and 30 in Cyclophosphamide group were included in the final analysis. The most common first symptom in patients who received Rituximab was paraparesis and blurred vision (28.2%), and in Cyclophosphamide group was paraparesis (33.3%).

Table 1 shows the characteristics of studied patients (Table 1).

Table 1. Baseline characteristics of the studied patients by groups

		Rituximab group (n=39)	Endoxan group (n=30)	*P*
Age (year)		31.9 ± 7.7	37.9 ± 7.5	0.002*
Age of onset (year)		24.3 ± 5.8	26.5 ± 7.3	0.187*
Sex	**Male**	4 (10)	8 (90)	0.075ᵗ
	Female	35 (27)	22 (73)	
First Symptom	**Paraparesis**	11(28.2)	10 (33.3)	0.661ᵗ
	Blurred vision	11 (28.2)	8 (26.7)	
	Sensory symptoms	8 (20.5)	4 (13.3)	
	Upper limb weakness	5 (12.8)	4 (13.3)	
	Diplopia	2 (5.1)	4 (13.3)	
	Ataxia	2 (5.1)	0	
	Adverse effects	13 (33.3)	7 (23.3)	0.364ᵗ
	Anemia	0	3 (42.9)	0.033ᵗ
	Pneumonia	3 (23.1)	3 (42.9)	
	Infusion-related reactions	3 (23.1)	0	
	Headache	5 (38.5)	0	
	Urinary tract infection	2 (15.4)	1 (14.3)	

Data are mean ± SD and number (%)
P calculated by *Independent Sample t-test or ᵗChi square test

Age of onset and gender were both normally distributed in each group ($P>0.05$). Patients in the Cyclophosphamide group were significantly older than patients in the Rituximab group ($P=0.002$). The prevalence of each first presenting symptom was not different between the two groups ($P=0.661$), Adverse effects were reported in 20 of patients in both groups (33.3% in the Rituximab group versus 23.3% in Cyclophosphamide group, $P=0.364$). Types of reported adverse effects were significantly different between groups ($P=0.033$). The most commonly reported adverse effect in the Rituximab group was headache (38.5%), but in the Cyclophosphamide group were pneumonia and anemia (42.9%).

The comparison of the studied endpoints between Rituximab and Cyclophosphamide groups are presented in table 2 (Table 2).

At baseline, the mean of number of attacks in the Rituximab group was significantly more than Cyclophosphamide group ($P=0.0001$), but 6, 12, and 18 months after treatment, the rate of attacks was similar between groups while within 24 months after treatment, the attacks were significantly higher in the Rituximab group again ($P=0.030$). The trend of attack number was significant changes over time in both groups ($P=0.0001$).

The mean EDSS was similar between two groups at the initiation of the study ($P>0.05$), while the trend of EDSS change was significant in the patients treated with Rituximab in worsening pattern ($P=0.001$). The number of new lesions in T2 weighted imaging in both groups during the study was similar ($P>0.05$), but the trend of changes in MRI lesions during the study period decreased significantly in the Cyclophosphamide group ($P=0.040$). Also, the number of gadolinium-enhanced lesions

between the studied groups was not significantly different (P>0.05), whereas the trend of changes in the number of gadolinium-enhanced lesions during the study period was statistically significant in both groups (P=0.0001).

A comparison of the trend of changes in endpoints during the follow-up period between groups is presented in figure 2 (Figure 2).

The decrease in the number of attacks from baseline was significantly different between groups (Figure 2A, P=0.002).

Table 2. Comparison of endpoints between studied groups

		Rituximab group (n=39)	Endoxan group (n=30)	P^1
Number of Attacks	**One year before initiation of the drug**	1.97 ± 0.43	1.53 ± 0.51	0.0001
	6 Months	0 ± 0	0 ± 0	1
	12 Months	0.05 ± 0.22	0 ± 0	0.211
	18 Months	0.23 ± 0.48	0.13 ± 0.34	0.418
	24 Months	0.41 ± 0.5	0.17 ± 0.34	0.030
	P^2	0.0001	0.0001	
Expanded Disability Status Scale	**Baseline**	3.8 ± 0.58	3.7 ± 0.54	0.846
	6 Months	3.8 ± 0.59	3.7 ± 0.51	0.522
	12 Months	3.8 ± 0.63	3.6 ± 0.51	0.127
	18 Months	4.0 ± 0.79	3.7 ± 0.57	0.143
	24 Months	4.0 ± 0.95	3.7 ± 0.64	0.248
	P^2	0.001	0.202	
New T2 lesion in MRI	**Baseline**			
	6 Months	2.1 ± 1.7	2.1 ± 1.8	0.985
	12 Months	1.2 ± 1.6	0.87 ± 1.0	0.744
	18 Months	1.4 ± 1.8	1.1 ± 1.5	0.645
	24 Months	1.2 ± 1.7	1.0 ± 2.0	0.482
	P^2	0.096	0.040	
Gadolinium-enhanced lesions	**Baseline**	5.8 ± 2.7	4.8 ± 3.5	0.037
	6 Months	0 ± 0	0 ± 0	1.000
	12 Months	0.28 ± 0.60	0.03 ± 0.18	0.035
	18 Months	0.36 ± 0.54	0.53 ± 0.51	0.125
	24 Months	0.34 ± 0.48	0.40 ± 0.50	0.626
	P^2	0.0001	0.0001	

P^1, comparison of variables between two groups in each time and calculated by Mann-Whitney test
P^2, the comparison of the trend of variables within groups during the study and calculated by Friedman test

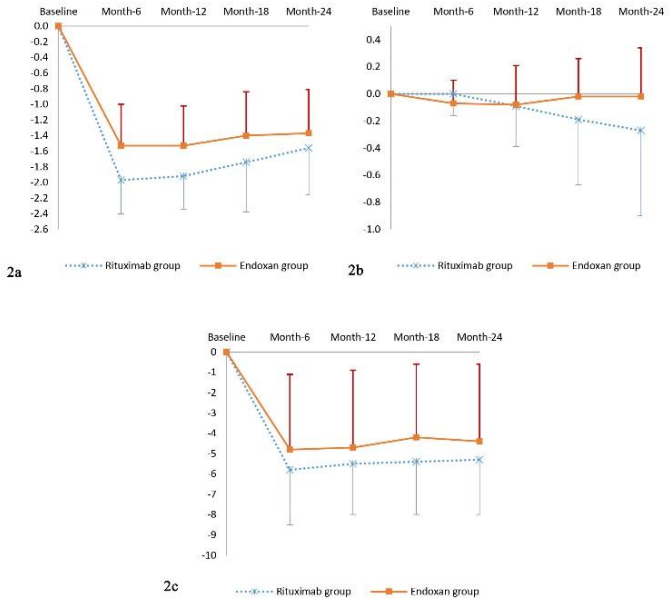

Figure 2. The trend of changes in endpoints during the follow-up period between groups using repeated measurements of ANOVA. A Attacks number, (P=0.002); B, Expanded Disability Status Scale, (P=0.023); C, Gadolinium-enhanced lesions, (P=0.247)

Discussion

Secondary-progressive MS is a type of MS that develops following relapsing-remitting MS (RRMs) in some patients, with a constant worsening of neurological disabilities (22). The neurodestructive quiddity of the disease, especially in severe progressive forms, raises serious consideration in treatment policies.

The current study was designed to appraise the efficacy of Rituximab and Cyclophosphamide in the most severe, devastating forms of MS. Both drugs have been used for MS treatment via modulating B-cell activities, although cyclophosphamide contributes to more mechanisms of actions (8,18,23). The findings of our study showed that attack numbers and gadolinium-enhanced lesions decreased significantly using each drug. Also, EDSS stabilization occurred in the Cyclophosphamide group, while the disability progression in the Rituximab group was significant (Figure 2). The trend (speed) of new lesion formation in brain T2 MRI was significantly decreased in patients who received Cyclophosphamide therapy. Both drugs could positively influence on reducing the number of attacks during our survey, after two years. Significant more attacks in patients who received Rituximab during different intervals of drug initiation could be attributed to the significantly higher initial number of attacks in this group.

The number of studies regarding the use of Cyclophosphamide in the treatment of SPMS is limited (12).

In a study by Zipoli *et al*., the effectiveness of Mitoxantrone versus Cyclophosphamide was assessed among patients with SPMS. In their study, they found that cyclophosphamide was accompanied by a 63%decrease in brain MRI lesions. Another similar study by Perini *et al*., showed that disease progression, disability progression, and EDSS score reduced significantly by Cyclophosphamide administration during a two-year period with a tolerable safety profile (24).

Studies about Rituximab are more available but mostly in patients with primary progressive MS. In a study by Rommer *et al*., Rituximab caused acceptable remission and stabilized EDSS in patients with active SPMS. The effect of Rituximab is still under investigation. There are some articles with conflicting results in 2018. Alcala *et al.*, (25) surveyed the effect of Rituximab on progressive and relapsing forms of MS. They reported a significant decrease in annualized relapse rate and EDSS during the first year of treatment although EDSS did not change during the second year. In our study, after a period of two-year follow-up, both groups showed a significant decrease in the number of attacks although EDSS was significantly increased in those who received Rituximab. As it was seen, Rituximab showed varied results in different populations. Imaging findings by T2 and Gadolinium-enhanced MRI were both significantly improved after two years of Cyclophosphamide usage while the significant change in T2 MRI findings was not achieved in those who received Rituximab. Cyclophosphamide plus methylprednisolone may not cause a statistically significant improvement in the disability score by referring to our findings, but its considerable impact on reducing the number of attacks and imaging findings is not ignorable. As both imaging and clinical improvement have been considered for severity definition and classifying subtypes of the disease by the most recent studies (26), it seems that Cyclophosphamide deserves to be paid more attention in parallel to other focused medications like Rituximab. Alcala-Vicente *et al*., in 2017 (25), scrutinized imaging findings and relapse rates within a year of follow-up after administration of fingolimod. The significant improvement in findings in different subgroups after about 30 months was regardless of EDSS or the interval between drug administration and the onset of the disease. Although the consumed medication and its mechanism was not the same as our study, it showed that improvement in imaging and annualized attacks could be a discrete purpose in treating patients with MS. Accordingly, failure in achieving a significant decrease in EDSS can not necessarily raise doubts about the efficacy of Cyclophosphamide in aggressive forms. However, despite no significant improvement in EDSS in patients who received Cyclophosphamide in our study, the disability score was not increased. This unpleasant event happened in the Rituximab group and EDSS increased after two years. It seems that the B-call depleting agent, Rituximab, is not certainly (15,27) associated with EDSS improvement in patients with active SPMS. Also, high infection rates among patients given Rituximab [Scotti] and the possible appearance of tolerance to treatment over time propose the necessity of introducing more acceptable alternatives like Cyclophosphamide.

Infusion reactions have been reported as one of the most popular side events of Rituximab (25). Regarding the higher prevalence of the mentioned effect and also headache among patients who received Rituximab in our study, Cyclophosphamide appeared more tolerable especially in those young patients without anemia or

other chronic diseases. The mentioned medication was previously introduced as a safe drug in MS by applying the usual dosage. Potential side effects, including alopecia, gastrointestinal symptoms, and cystitis, were not that much severe or permanent. Amenorrhea prevalence was relatively higher but occurred among women older than 40 (24,27). None of these phenomena occurred in our study. However, three patients experienced anemia that was transient and mild.

The main limitation of our study included the loss of cerebrospinal fluid analyses in regard to inflammatory markers (above mentioned in the introduction) as a severity predictor of MS.

The current study compared the effectiveness of Cyclophosphamide versus Rituximab, one of the most popular recent drugs in MS. The main strength of our survey was assessing the potency of the drugs on severe aggressive forms of MS rather than other types that were investigated a lot in previous studies.

Our findings showed that both therapies were associated with a reduction in disease attacks and improvement in radiologic findings in a two-year period of treatment with either Rituximab or Cyclophosphamide.

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