

*Original Article*

## Effects of low dose methotrexate on relapsing-remitting multiple sclerosis in comparison to Interferon $\beta$ -1 $\alpha$ : A randomized controlled trial

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### Abstract

**BACKGROUND:** Methotrexate, a toxic antimetabolite that limits cellular reproduction by acting as an antagonist to folic acid, has been used to control autoimmune disease with different results. The aim of this study was to evaluate the effectiveness of low dose Methotrexate in the relapsing-remitting multiple sclerosis (RRMS).

**METHODS:** Eighty patients with definite RRMS aged 15 to 55 years were randomly allocated to receive a 12-month treatment course of either oral Methotrexate (7.5 mg/week) or intramuscular Interferon  $\beta$ -1 $\alpha$  (30  $\mu$ g/week). Response to treatment was assessed at 12 months after start of therapy.

**RESULTS:** The results of the study demonstrated significant reduction in relapse rate in both groups ( $p < 0.01$ ). In 40 patients treated by Methotrexate, the mean value (SD) of relapse rate decreased from 1.75 (0.74) to 0.97 (0.83) ( $p < 0.01$ ). Correspondingly, the mean value (SD) of relapse rate in patients treated by Interferon  $\beta$ -1 $\alpha$  decreased from 1.52 (0.59) to 0.57 (0.78) ( $p < 0.01$ ). Decrease of relapse rate in Interferon  $\beta$ -1 $\alpha$  group was more than that in the other group ( $p = 0.06$ ).

**CONCLUSIONS:** This study suggests that although treatment with Methotrexate may significantly reduce relapse rate and slow progression of disease in patients with RRMS, its efficacy is less than Interferon  $\beta$ -1 $\alpha$  and it may be better used as add-on therapy.

**KEYWORDS:** Multiple Sclerosis, Methotrexate, Interferon Beta.

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Multiple sclerosis (MS), the most prevalent chronic neurologic disease, is an inflammatory, demyelinating, immune-mediated disease affecting young adults and often associated with significant disability and impaired quality of life.<sup>1-3</sup> Disease modifying therapies only slow the progression of the disease, therefore, symptom management must have a long-term consideration.<sup>4</sup>

At present, there is no cure for MS and prime task of treatment is to prevent the relapses and to slow down the neurological deterioration. Despite extensive research to develop effective pharmacological treatments to al-

leviate exacerbation and chronic neurological damage in MS, current available drugs have limited efficacy and considerable adverse effects.<sup>5,6</sup>

At present, three Interferon beta (IFN $\beta$ ) formulations, and glatiramer acetate are used as the first step of treatment with only partially effect on reducing disease activity in relapsing-remitting MS (RRMS). Tysabri and Mitoxantrone are used as the second line in patients with worsening RRMS.<sup>7-9</sup> These drugs are expensive with only modest benefit and show no or little efficacy or are not well tolerated in many patients.<sup>5,6,10,11</sup>

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Because of these limitations, most of patients initially or after some duration of therapy request for more potent, less expensive and friendly useable substitution. Recently, frequent studies are performed on oral drugs that most of them are immunosuppressive or immunomodulative (such as Daclizumab, Fingolimod, Laquinimod, Alemtuzumab, Rituximab and ...).<sup>7,12-14</sup>

Immunosuppressive drugs were used in treatment of MS patients according to hypothesis of central nervous system inflammation in MS pathophysiology.<sup>15</sup> Recently, oral Methotrexate (MTX) was used in treatment of progressive MS without significant side effects.<sup>10,16,17</sup> This was used as a potent immunosuppressive drug from 30 years ago in the treatment of MS and about 10 percent of MS patients received this drug for treatment.<sup>8,15,18</sup> Methotrexate with effect on immune system prevents inflammation and by this way affects the MS disease course.<sup>19</sup>

Multiple studies were performed to evaluate efficacy and safety of Methotrexate in treatment of MS patients with inconsistent results.<sup>17,20,21</sup> In some studies, Methotrexate was recommended as second line in treatment of MS as combination therapy,<sup>22</sup> and in some studies the effect of drug is questionable;<sup>23,24</sup> however, there is limited evidence of its efficacy in MS patients, so this study was designed to evaluate efficacy of low dose Methotrexate in prevention of MS progression in Iranian RRMS patients compared to IFN $\beta$ -1 $\alpha$ .

## Methods

This randomized single-blind clinical trial was done to compare the effect of low dose Methotrexate and weekly injection of Interferon  $\beta$ -1 $\alpha$  (CinnoVex) on the prevention of MS progression.

### Patients

There were 80 MS patients recruited from neurology outpatient clinics of Isfahan University of Medical Sciences in 2007. Entry criteria included men and women 15-55 years with a clinical definite RRMS according to McDonald criteria:<sup>25</sup> with  $\geq 2$  relapses within the 2-years

prior to study, stable neurological functioning for at least one month prior to study entry and have an expanded disability status scale (EDSS)<sup>26</sup> score  $\leq 4$  and a willingness to continue current medications for the duration of the study. Exclusion criteria included evidence of substantial psychiatric, cardiac, endocrinological, hematologic, hepatic, renal, or metabolic disease and pregnancy or lactation as determined by history, physical examination, and screening blood tests. Women of child-bearing potential had to practice a clinically accepted method of contraception.

Tenets of current version of the Declaration of Helsinki were followed, institutional ethical committee approval was granted, and the nature of the trial was explained to the patient. After a detailed discussion with the neurologist, patients made a final decision and each patient signed an informed consent.

### Randomization Scheme

A total of 97 patients were eligible for study. 17 patients were excluded because they refused entry or discontinuing treatment. Ultimately, eighty patients, 25 men (31.2%) and 55 women (68.8%) completed their treatments without interruption and were assigned randomly and equally to one of the two self-administered treatment groups. Patients were randomized according to a preexisting list produced by a computer program that differed from a random number generator only in that it assigned equal numbers of patients into each treatment group. The first treatment group received 7.5 mg of oral Methotrexate weekly (with 1 mg daily folic acid supplementation) for 12-months. The second group received 30  $\mu$ g weekly intramuscular Interferon  $\beta$ -1 $\alpha$  (CinnoVex) for 12-months. Patients were allowed access to their routine symptomatic therapy. Compliance with study treatment was established by asking the patients about missed doses. In the month preceding the trial, all patients had a pre-treatment evaluation that consisted of obtaining demographic data, complete neurologic and medical history, physical and neurologic examination, and previous treatment. Every 3 months, laboratory tests

including CBC, LFT, BUN, creatinine, and albumin were checked in all patients

#### Patient Evaluation

The trial was single-blinded and physician (MRS) who assessed the outcome was not aware of type of treatment of every patient. Patients were evaluated at baseline and 12-months after the start of the therapy by a neurologist to evaluate the development of side effects of the medications, compliance of the patients, and efficacy parameters. Assessment of the course of the disease was done by change in EDSS and number of relapses.

#### Statistical Analysis

Statistical analysis was based on an intention-to-treat principle. Comparison between groups receiving Methotrexate and Interferon  $\beta$ -1 $\alpha$  was made using t-test for independent samples; comparisons between before and 12-months after treatment within each group were done by paired student's t-test. Comparisons between proportions were done by chi-square or Fisher's exact test. Results are expressed as mean (SD) and  $P < 0.05$  was considered statistically significant. All statistical tests were two-sided. The analyses were done on a personal computer using SPSS for Windows (SPSS Inc., Chicago, IL).

#### Results

Ultimately 80 patients who met the entry criteria were enrolled for the study and completed 12 months treatment. The two treatment groups were generally well matched at baseline with regard to age, gender, duration of

disease, EDSS, and relapses in previous year before study. The patients had a mean (SD) age of 34.25 (8.34) years in MTX group and 30.75(8.75) in INF group without significant differences between the two groups ( $p < 0.05$ ).

Mean (SD) EDSS at the start of treatment with first and second groups was 2.32 (0.96) and 2.02 (0.65), respectively. There was no statistically significant difference between them (Table 1). Treatment was well tolerated. Changes of mean EDSS and number of relapses before and after receiving Methotrexate or Interferon  $\beta$ -1 $\alpha$  are shown in Table 2.

In both groups, the average EDSS score did not significantly change before and after treatment ( $p = 0.82$ ). The average EDSS score increased from baseline by 0.05 point (95% CI; 0.18, 0.38) in Methotrexate, compared to a decrease of 0.04 point (95% CI; 0.09, 0.37) in Interferon  $\beta$ -1 $\alpha$  group. After 12-month treatment in the Methotrexate group, the mean (SD) relapse rate decreased from 1.75 (0.74) at baseline to 0.97 (0.83) at the end of study period ( $p < 0.01$ ).

Correspondingly, in the Interferon  $\beta$ -1 $\alpha$  group, the mean (SD) relapse rate decreased from 1.52 (0.59) at baseline to 0.57 (0.78) at the end of study ( $p < 0.01$ ).

The overall cross-tab analysis revealed significant decrease of relapses in both groups without any statistically differences between them (Table 2), and although EDSS increased slightly in Methotrexate group compared with Interferon  $\beta$ -1 $\alpha$  group, there was no significant difference in disability changes before and after treatment.

**Table 1.** Characteristics of patients by treatment group at baseline

Characteristics	Treatment group at baseline		P value
	Methotrexate (n = 40) Mean (SD)	Interferon $\beta$ -1 $\alpha$ (n = 40) Mean (SD)	
Age (year)	34.25 (8.34)	30.75 (8.75)	0.10
Duration of MS* (year)	6.6 (3.6)	5.7 (2.9)	0.09
Expanded Disability Status Scale	2.32 (0.96)	2.02 (0.65)	0.10
Relapses in previous year	1.75 (0.74)	1.52 (0.59)	0.14
Gender	Men 13 (32.5)	Men 12 (30.0)	0.11
	Women 27 (67.5)	Women 28 (70.0)	

\* MS: Multiple sclerosis

**Table 2.** Comparison of Expanded Disability Status Scale (EDSS) and relapse rate before and after treatment with Methotrexate and Interferon  $\beta$ -1 $\alpha$ 

	Treatment group		<i>P</i> value (95% CI)
	Methotrexate (N= 40) Mean (SD)	Interferon $\beta$ -1 $\alpha$ (N = 40) Mean (SD)	
EDSS at Baseline	2.32 (0.96)	2.02 (0.65)	0.10 (-0.67, 0.66)
EDSS after 12 months	2.37 (0.99)	1.98 (0.83)	0.06 (-0.02, 0.79)
<b>P value (95% CI)</b>	0.82 (0.18, 0.38)	0.824 (0.09, 0.37)	
Relapses at baseline	1.75 (0.74)	1.52 (0.59)	0.14 (-0.07, 0.52)
relapses at 12-months	0.97 (0.83)	0.57 (0.78)	0.06 (0.04, 0.75)
<b>P value (95% CI)</b>	0.006 (0.42, 1.12)	< 0.001 (0.35, 1.02)	

## Discussion

In current study, although oral administration of low dose Methotrexate over 12-months significantly decreased the relapse rate in RRMS patients, this efficacy was less than that in interferon  $\beta$ -1 $\alpha$  group.

No unusual or unexpected safety risks were found with our patients and the spectrum of most frequent adverse events was similar to previous studies such as the study of Goodkin that was reported no major side effect during 36 months treatment by Methotrexate.<sup>27</sup> In our study, gastrointestinal symptoms were the most common side effects and no major side effect was reported such as those in previous studies.<sup>28-30</sup> Headache and fever were the most common side effects of Interferon  $\beta$ -1 $\alpha$  group after injection. There was not any discontinuation of drugs among patients of the two groups according to the side effects.

The results of this study were similar to the previous study that showed a trend towards fewer relapse in the RRMS.<sup>31</sup> An open-label study was performed to evaluate the safety and efficacy of combination therapy with weekly oral Methotrexate (20 mg) and Interferon  $\beta$ -1 $\alpha$  (IFN $\beta$ -1 $\alpha$ ) in patients with MS;<sup>32</sup> 44% reduction in the number of Gadolinium-enhanced lesions on MRI scan was observed during combination therapy ( $p = 0.02$ ). There was a trend towards fewer exacerbations too and the results of study suggested this combination therapy may be safe and well tolerated. According to a clinical trial of Methotrexate for progressive MS,<sup>27</sup> there were significant differences between Methotrexate and placebo treated groups.

In another study, Methotrexate was effective as add-on treatment in preventing progression in patients with poor responses to Interferon- $\beta$  treatment alone.<sup>17</sup>

The mechanism of action of Methotrexate on MS has not been fully elucidated yet. MS have been identified as Th1-mediated autoimmune diseases.<sup>33</sup> In contrast, Th2-mediated responses have beneficial effects on the severity and progression of the disease,<sup>34,35</sup> and are one of the major mechanisms underlying Ag-specific immune tolerance induction.<sup>36-39</sup> Methotrexate competitively and reversibly inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis.

Lower doses of Methotrexate have been shown to be effective for the management of some disease such as rheumatoid arthritis, crohn's disease, and MS. In these cases, inhibition of DHFR is not thought to be the main mechanism, but rather the inhibition of enzymes involved in purine metabolism, leading to accumulation of adenosine, or the inhibition of T cell activation and suppression of intercellular adhesion molecule expression by T cells to inhibit acute autoimmune attack on the central nervous system. Therefore, previously mentioned side effects with high dose of Methotrexate are not expected to occur with low dose administration for MS treatment.

This trial showed effectiveness of Methotrexate in RRMS but less than that of Interferon  $\beta$ -1 $\alpha$ . There was limitation in keeping patients blind because of the different administration rout of drugs (intramuscular injection vs. oral administration) So, we tried to do randomiza-

tion carefully as Schultz and co-workers reported that careful randomization is more important than a double-blind design.<sup>36</sup>

### Conclusion

This comparative trial suggested that although Methotrexate may reduce the relapses of RRMS and slow down the neurological deterioration, its efficacy is less than Interferon  $\beta$ -1 $\alpha$ .

So, it can be used as an add-on therapy in RRMS. Further studies, with longer follow-ups are needed to assess this finding.

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### Conflict of Interests

Authors have no conflict of interests.

### Authors' Contributions

Both authors have carried out the study, participated in the design of the study and acquisition of data performed the statistical analysis and wrote the manuscript. Both authors read and approved the final manuscript.

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