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Highlights

- There are more than 2 million individuals affected by multiple sclerosis (MS) worldwide, and most patients are faced with a poor prognosis after several remission and relapse cycles. Vitamin D deficiency is widely considered to be an environmental risk factor for MS. And some studies have reported significantly lower vitamin D levels in MS patients than in healthy controls. Therefore, some studies used vitamin D as a therapy of MS. Prompted by the lack of meta-analyses investigating the association between MS and vitamin D, the purpose of this study was to evaluate the effectiveness of vitamin D in MS patients.

- In this analysis, our findings suggest that vitamin D appeared to have no therapeutic effect on Expanded Disability Status Scale (EDSS) scores and annual relapse rate (ARR) in the patients with MS.

The efficacy of vitamin D in multiple sclerosis: a meta-analysis

Chao Zheng [1#], Liang He [2#], Lingling Liu[1], Jie Zhu[1,3], Tao Jin[1*]

[1]Department of Neurology and Neuroscience Center, the First Hospital of Jilin University, Changchun, China.

[2]Department of Urinary Surgery, the First Hospital of Jilin University, Changchun, China.

[3]Department of Neurobiology, Care Sciences and Society, Karolinska Institute, Karolinska University hospital Huddinge, Stockholm, Sweden.

# These authors contributed equally to this work.

***Corresponding author:**

Dr. Tao Jin, Department of Neurology and Neuroscience Center,

The First Hospital of Jilin University,

Xinmin Street 71#, 130021, Changchun, China

Tel: +86 431 88782356;

Fax: +86 431 88782378;

E-mail: drtao.jin@hotmail.com

Abstract

Background: Multiple sclerosis (MS) is a chronic inflammatory demyelination disorder in the central nervous system (CNS) leading to a high level of neurological disability. The pathogenesis of MS remains largely unknown, which explains the lack of significant efficacy of therapy in MS. Vitamin D deficiency is widely considered to be an environmental risk factor for MS. Many studies investigating the therapeutic effects of vitamin D on MS have been applied. The objective of this systematic review and meta-analysis was to evaluate the effectiveness of vitamin D in MS patients.

Methods: To obtain a more comprehensive estimate of the efficacy of vitamin D on MS patients, we conducted a meta-analysis to determine the role of vitamin D in MS. The PubMed, EMBASE and Cochrane databases were searched in October 2017. Randomized, double-blind, placebo-controlled clinical trials recorded within the three main databases were considered. The analysis was conducted for two specific outcomes: Expanded Disability Status Scale (EDSS) score and annual relapse rate (ARR).

Results: Vitamin D_3 as add-on treatment had no significant therapeutic effect on MS according to EDSS score (mean difference -0.01 [95% CI -0.34 to 0.33]). The ARR was higher in the vitamin D group than in the placebo group (mean difference 0.05 [95% CI 0.01 to 0.1]).

Conclusion: Our findings suggest that vitamin D appeared to have no therapeutic effect on EDSS score or ARR in the patients with MS.

Keywords: Multiple sclerosis, Meta-analysis, Vitamin D, Randomized clinical trials

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelination disorder in the central nervous system (CNS) characterized by multifocal areas of myelin sheath destruction, oligodendrocyte, axonal and neuronal damage (Pierrot-Deseilligny and Souberbielle 2017). There are more than 2 million individuals affected by MS worldwide. The disease results in a series of clinical symptoms including paresthesia, ataxia, cognitive impairment, as well as loss of vision and mobility (Lassmann and Bradl 2017). Most MS patients are faced with a poor prognosis after several remissions and relapse cycles. The majority of patients affected by MS are young adults. The effect of MS at the peak of patients' active lives brings enormous burden to their families and society in general (Vargas and Tyor 2017).

Despite decades of research, the etiology of MS remains unclear. It is generally assumed that MS is caused by several factors including gender, inherited and environmental factors. It has been observed that the geographical latitude gradient is strongly related to MS morbidity. The higher the latitude is, the greater the prevalence of MS is (Mokry, Ross et al. 2015). Moreover, the connection between MS and latitude may be due to low ultraviolet (UV) radiation exposure and low vitamin D status in high-latitude areas (Simon, Munger et al. 2012, Mokry, Ross et al. 2015). Consistent with this viewpoint, some studies have reported significantly lower vitamin D levels in MS patients than in healthy controls. Consequently, vitamin D deficiency is widely considered to be an environmental risk factor for MS (Gelfand, Cree et al. 2011).

Vitamin D is a type of fat-soluble secosteroid with pleiotropic effects on the body. For most humans, the main source of vitamin D is skin exposure to UV-B radiation from the sun. Food intake is also source of vitamin D for humans (Simon, Munger et al. 2012).

Several studies have reported the immunomodulatory effects of vitamin D on the immune response (Joshi, Pantalena et al. 2011, Gatenby, Lucas et al. 2013). Because of the deficiency of vitamin D found in some MS patients, many studies have applied vitamin D or its active form as a therapeutic compound in experimental autoimmune encephalomyelitis (EAE), the animal model for MS, and obtained positive results. Vitamin D administered either orally or intraperitoneally has been shown to alleviate clinical symptoms in EAE (Chang, Cha et al. 2010, Chiuso-Minicucci, Ishikawa et al. 2015, Zhen, Feng et al. 2015). In a study to explore the mechanism behind Vitamin D with therapeutic effect, Chang et al. reported that 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) reduced the differentiation and migration of T-helper 17 cells in EAE (Chang, Cha et al. 2010). Moreover, a significant decrease in the number of mature splenic dendritic cells was observed after administration of $1,25(OH)_2D_3$ (Chiuso-Minicucci, Ishikawa et al. 2015). Furthermore, $1,25(OH)_2D_3$ could also inhibit the onset of EAE by influencing the expression of apoptotic proteins (Zhen, Feng et al. 2015).

Based on these preclinical studies, other clinical studies investigating the therapeutic effect of vitamin D on MS have been performed. To our knowledge, however, there have been no meta-analyses investigating the association between MS and vitamin D. To obtain a more comprehensive estimate of the association, we conducted a meta-analysis to evaluate the effectiveness of vitamin D in MS patients.

2. Materials and Methods

2.1 Search strategy

The PubMed, EMBASE and Cochrane databases were searched in October 2017 for clinical trials assessing the relationship between vitamin D and MS. The search was restricted to articles published in English. The search used different combinations of the search terms “vitamin D” with “multiple sclerosis” or “MS”. In addition, all reference lists of relevant articles were manually reviewed for further relevant studies.

2.2 Study selection

Two reviewers screened the articles independently by appraising titles and abstracts. Articles not meeting the eligibility criteria and duplicate articles were excluded. The full text of the remaining articles was read to assess for inclusion. Any uncertainties were discussed between the reviewers to reach consensus.

2.3 Inclusion and exclusion criteria

Inclusion criteria for the present meta-analysis were as follows: randomized, double-blind, placebo-controlled trials involving humans; the patients diagnosed with MS; the patients receiving vitamin D, active form of vitamin D (such as cholecalciferol and calcitriol), or placebo as treatments; the articles focused on treatment effect on clinical aspects, such as Expanded Disability Status Scale (EDSS) score, annualized relapse rate (ARR), multiple sclerosis functional composite (MSFC) and quality of life (QoL). Studies that focused only on laboratory parameters or not published in English were excluded.

2.4 Quality assessment

The Jadad scoring system was used to evaluate the quality of included studies. Each study was evaluated and scored in the following terms: randomization (0 = inappropriate randomization method; 1 = study only described as randomized; 2 =

study described appropriate randomization method); double-blinding (0 = inappropriate method of blinding; 1 = study only described as double blinded; 2 = study described appropriate method of blinding); and withdrawal or dropout (0 = study did not describe withdrawal or dropout patient number and reason; 1 = study described withdrawal or dropout patient number and reason). The maximum Jadad score is 5, and studies scoring ≥3 are considered to be high-quality; studies scoring ≤2 are classified as low quality.

2.5 Data extraction

To ensure correctness in data, the two reviewers assessed all of the included studies independently. The following data were extracted from the studies: first author, publication year, sample size, study design, inclusion and exclusion criteria, details of treatment and main study results. Disagreements between the two reviewers were resolved by consultation with a third reviewer.

2.6 Statistical analysis

All statistical analyses were performed using RevMan 5.3 (The Cochrane Community, London, United Kingdom). Heterogeneity was evaluated using the Q and I-square (I^2) test. According to the Cochrane Handbook for Systemic Review of Interventions, value of $pQ > 0.1$ was considered to be milder heterogeneity and $pQ < 0.1$ as substantial heterogeneity. An $I^2 < 50\%$ was considered to be mild heterogeneity, I^2 between 50% and 75% as substantial heterogeneity, and $I^2 > 75\%$ as considerable heterogeneity. In the present study, if I^2 was > 50% and/or , the data would be pooled using a random-effect model. In contrast, if I^2 was < 50%, the data would be pooled using a fixed-effect model (Sivaramakrishnan and Sridharan 2017). Subgroup analysis was performed by disease subtype classification. Funnel plot was not done due to the small number of included studies. Sensitivity analysis was conducted by excluding studies one at a time to investigate the effect of the individual study on the pooled data.

3. Results

3.1 Search results

The search strategy initially produced 107, 2025, and 196 studies from the PubMed, EMBASE, and Cochrane databases, respectively. Twenty-two studies remained for further screening after removal of duplicates, and a review of titles and abstracts by the two reviewers. Among these studies, 6 were excluded due to lack of full texts, 8 due to concentration on laboratory parameters, and 2 due to different results presentation. Finally, 6 randomized control trials (RCTs) (Burton, Kimball et al. 2010, Mosayebi, Ghazavi et al. 2011, Kampman, Steffensen et al. 2012, Shaygannejad, Janghorbani et al. 2012, Soilu-Hanninen, Aivo et al. 2012, Golan, Halhal et al. 2013) were included in the review (Fig. 1).

3.2 Study characteristics

Of the 6 included studies, two were from Iran (Mosayebi, Ghazavi et al. 2011, Shaygannejad, Janghorbani et al. 2012), one from Norway (Kampman, Steffensen et al. 2012), one from Canada (Burton, Kimball et al. 2010), one from Israel (Golan, Halhal et al. 2013), and one from Finland (Soilu-Hanninen, Aivo et al. 2012). At baseline, the studies included 337 patients (169 patients received vitamin D and 168 patients received placebo as treatment). Among these included studies, 247 patients were female and 90 were male. Table 1 summarizes the baseline characteristics of the patients in the included studies.

3.3 Efficacy

3.3.1 Effects of vitamin D on EDSS

Data from a total of 6 studies were analyzed, representing 318 patients (158 patients received vitamin D and 160 received placebo as treatment). In the meta-analysis, the therapeutic effect of vitamin D on the EDSS score of MS patients was investigated using a random-effects model due to substantial heterogeneity (I^2 = 52%; P = 0.06).

The pooled results indicated that using vitamin D_3 as add-on treatment had no significant therapeutic effect on MS according to EDSS score (mean difference = -0.01 [95% CI-0.34 to 0.33]) (Fig 2).

Subgroup analysis for different MS subtype were conducted. The therapeutic effect of vitamin D on the EDSS score of relapsing remitting MS (RRMS) patients and all types of MS patients were investigated respectively. In RRMS patients (Shaygannejad, Janghorbani et al. 2012, Soilu-Hanninen, Aivo et al. 2012, Golan, Halhal et al. 2013), pooled results indicated that using vitamin D_3 as add-on treatment had no significant therapeutic effect on MS according to EDSS score (mean difference = -0.03 [95% CI-0.39 to 0.33]). The heterogeneity of this subgroup was mild (I^2 = 19%; P = 0.29) (Fig 3). When pooled the data of studies including all types of MS patients (Burton, Kimball et al. 2010, Mosayebi, Ghazavi et al. 2011, Kampman, Steffensen et al. 2012), the result was the same as RRMS subgroup (mean difference = -0.00 [95% CI-0.55 to 0.55]). The heterogeneity of this subgroup, however, was substantial (I^2 = 65%; P = 0.06) (Fig 3).

3.3.2 Effects of vitamin D on ARR

Data from 5 studies (Burton, Kimball et al. 2010, Kampman, Steffensen et al. 2012, Shaygannejad, Janghorbani et al. 2012, Soilu-Hanninen, Aivo et al. 2012, Golan, Halhal et al. 2013) were analyzed, representing 259 patients (132 patients received vitamin D and 127 received placebo as treatment). The therapeutic effect of vitamin D on ARR of MS patients was investigated using a fixed-effects model because of low heterogeneity (I^2 = 5%; P = 0.38). The ARR was higher in the vitamin D group than in the placebo group (mean difference 0.05 [95% CI 0.01 to 0.1]). The pooled results demonstrated that using vitamin D significantly increased the ARR of MS patients (Fig 4).

3.4 Sensitivity analysis

There was no significant variation in the pooled data by excluding any of the studies, supporting the robustness of our results.

4. Discussion

In 1974, Goldberg hypothesized that 25(OH)D_3 deficiency may be an environmental factor that increases the risk for MS development. Since then, this hypothesis has been verified by a large number of studies (Kubicka and Pierzchala 2013). Some studies revealed that 25(OH)D_3 levels in MS patients were negatively correlated with MS activity (Fitzgerald, Munger et al. 2015). Based on these studies, several studies investigating the therapeutic effect of vitamin D supplementation on MS patients have been performed (Stein, Liu et al. 2011, Kampman, Steffensen et al. 2012, Shaygannejad, Janghorbani et al. 2012, Soilu-Hanninen, Aivo et al. 2012, Achiron, Givon et al. 2015, Ashtari, Toghianifar et al. 2016). However, the evaluation indexes and study results are conflict. To obtain a more comprehensive estimate of the efficacy of vitamin D on MS patients, we conducted a meta-analysis to determine the role of vitamin D in MS.

In a previous systematic review, Pozuelo-Moyano et al. summarized five clinical trials to investigate the efficacy of vitamin D on MS (21). Among these 5 trials, 4 demonstrated no effect of vitamin D on any outcome (Mosayebi, Ghazavi et al. 2011, Stein, Liu et al. 2011, Kampman, Steffensen et al. 2012, Shaygannejad, Janghorbani et al. 2012), while only one showed a significant effect on the number of lesions in the CNS of MS (Soilu-Hanninen, Aivo et al. 2012). Although this systematic review did not pool data, we still obtain a negative result from it. Similar to that systematic review, our meta-analysis displayed that vitamin D supplementary therapy had no significant effect on the EDSS score of MS patients ($P = 0.9$). Even worse, supplementary vitamin D therapy significantly increased the ARR of MS patients ($P = 0.01$).

Aside from the EDSS score and ARR, the studies included in our review article also measured other indexes. Soilu-Hanninen et al. found that the patients with oral

vitamin D treatment had fewer new T2 lesions and T1-enhancing lesions compared with control (Soilu-Hanninen, Aivo et al. 2012). Kampman et al. compared the MS functional composite (MSFC) components and Krupp's fatigue severity scale (FSS) scores between vitamin D treatment group and the controls. They found there was no significant difference between groups in these two metrics after being treated with vitamin D for 96 weeks (Kampman, Steffensen et al. 2012). Golan et al. reported that the health related QoL (HRQoL) between the two groups demonstrated no significant differences (Golan, Halhal et al. 2013). Because these results were only reported in individual studies, our review did not include them in the pooled analysis.

Regarding the adverse effects of vitamin D therapy, high doses of vitamin D may have a direct toxic effect. Long-term oral therapeutic doses of vitamin D can cause severe side effects, including hypercalcemia, nephrolithiasis, and metastatic vascular calcifications (Rajakumar, Reis et al. 2012, Ramos, de Santana et al. 2014). In these 5 studies, however, vitamin D treatment was tolerated well and its adverse effects were infrequent. Gastrointestinal discomfort, such as severe constipation, diarrhea and dyspepsia, were the most common side effects. In addition, fever, headache, and fatigue were also reported. Among these trials, the longest study period was 12 months; we speculate that the short length of study period may be the reason for the absence of severe side effects of vitamin D.

Here some studies investigated the efficacy of vitamin D on MS were excluded by "inclusion and exclusion criteria", some excluded studies due to the focusing on laboratory parameters (Mahon, Gordon et al. 2003, Kimball, Vieth et al. 2011, Ashtari, Toghianifar et al. 2015). In these studies, vitamin D increased significantly the effect on lymphocyte proliferation, functions, and subsets, as well as cytokine levels. Kimball et al. reported MS patients treated with cholecalciferol plus calcium for 12 months and found that that this combination significantly suppressed the proliferation responses of peripheral blood mononuclear cells (PBMC) from MS patients to

disease-associated antigen challenge (Kimball, Vieth et al. 2011). In terms of cytokines, Mahon et al. reported that applying vitamin D as adjuvant therapy for 6 months significantly increased serum transforming growth factor (TGF)-h1 levels in MS patients. TGF-h1 is considered to be an important anti-inflammatory cytokine in MS and EAE (Mahon, Gordon et al. 2003). Additionally, Ashtari et al displayed that serum interleukin-10 (IL-10) levels in relapsing-remitting MS patients increased significantly after taking high-dose vitamin D_3 for 3 months. IL-10 is a regulatory cytokine that has the ability to promote an anti-inflammatory response in the pathogenesis of MS (Ashtari, Toghianifar et al. 2015).

However, in these excluded studies, the clinical indexes were not evaluated. Therefore, we cannot infer whether vitamin D had a therapeutic effect on the clinical symptoms of MS patients from these studies (Mahon, Gordon et al. 2003, Kimball, Vieth et al. 2011, Steffensen, Jorgensen et al. 2011, Ashtari, Toghianifar et al. 2015). It is known that a quantitative change occurs before a qualitative change. Therefore, we infer that the supplementary vitamin D therapy only affects the progress of MS at biological change levels such as cytokines and lymphocytes. Effective therapy may be need incremental dose of vitamin D or an extension of the treatment period to achieve a significant effect on clinical symptoms in MS.

There were some limitations to our meta-analysis that should be taken into consideration. First, our pooled results regarding the EDSS score need to be interpreted with caution due to study heterogeneity even though a subgroup analysis was conducted. Second, subgroup analysis for different dose of vitamin D, study duration, lifestyle habits (including smoking, alcohol and coffee consumption), age and sex differences, as well as ethnicity were not conducted due to the lack of detailed information in the included articles. Third, the sample size of this study was not large.

5. Conclusion

Available randomized trial data suggest that vitamin D probably has no significant effect on MS patients, at least in terms of EDSS score. The ARR increased after treatment with vitamin D. However, due to the limited sample size in our study and the non-definitive results of preclinical studies, large multicenter RCTs are needed to confirm our findings.

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Conflict of interest

The authors have no conflicts of interest.

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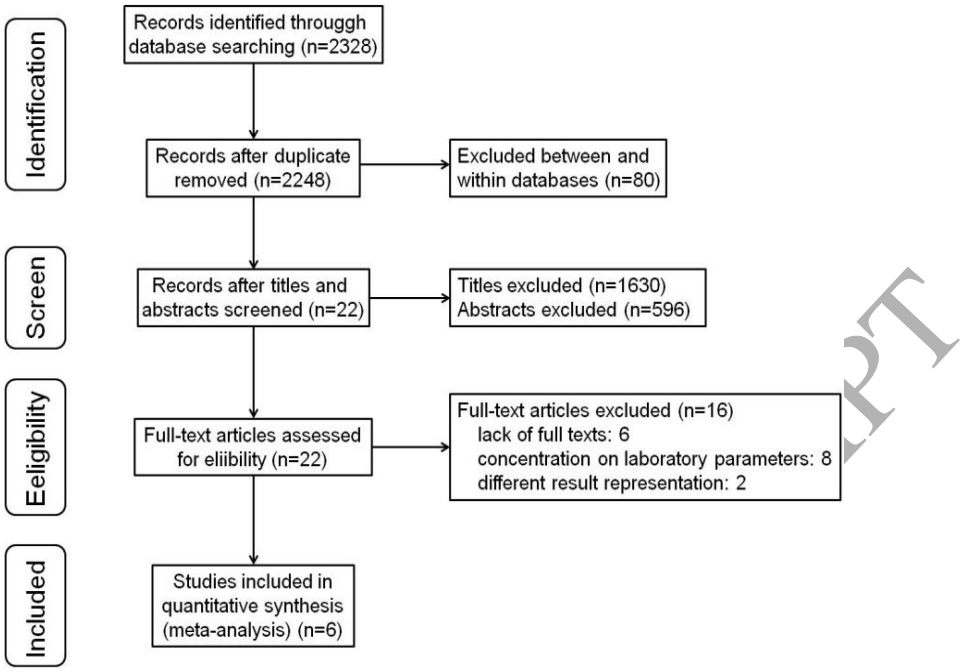

Fig 1. Flow diagram summarizing the selection of eligible studies.

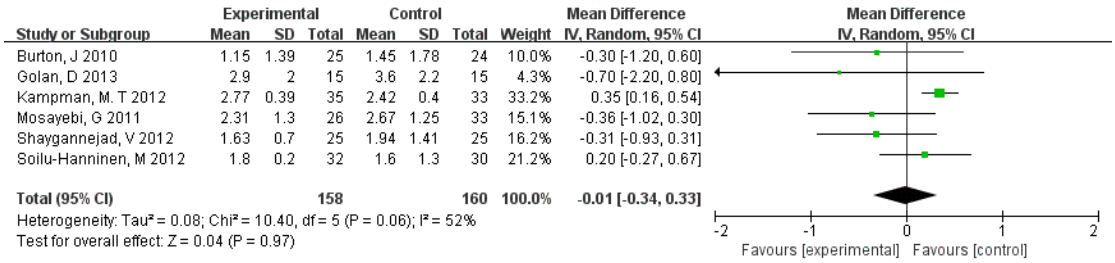

Fig 2. Meta-analysis and Forest plot. Effect of vitamin D on the Expanded Disability Status Scale (EDSS) score of multiple sclerosis patients (158 received vitamin D and 160 received placebo as treatment). SD = standard error; IV = inverse variance; CI = confidence interval.

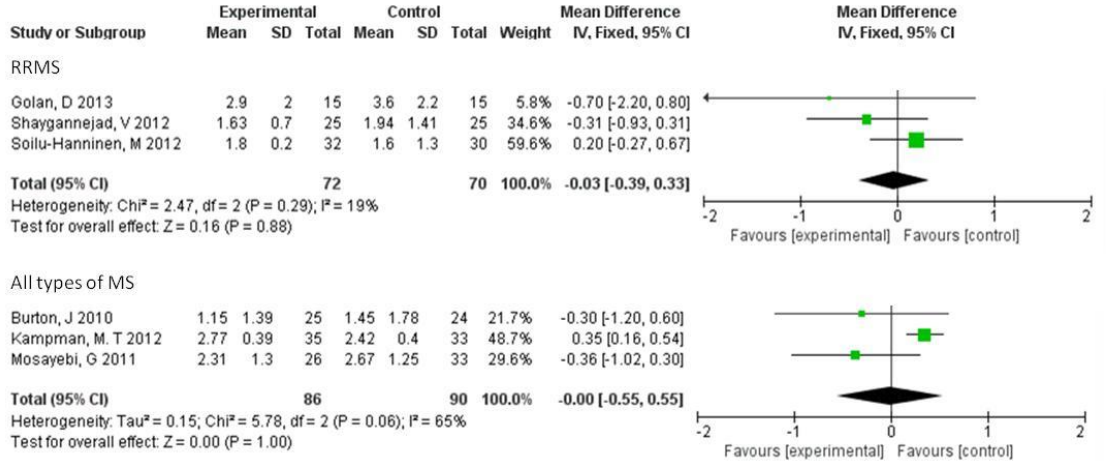

Fig 3. Meta-analysis and Forest plot. Respective pooled estimate of SD and 95% CI of EDSS score in RRMS patients and all types of MS patients.

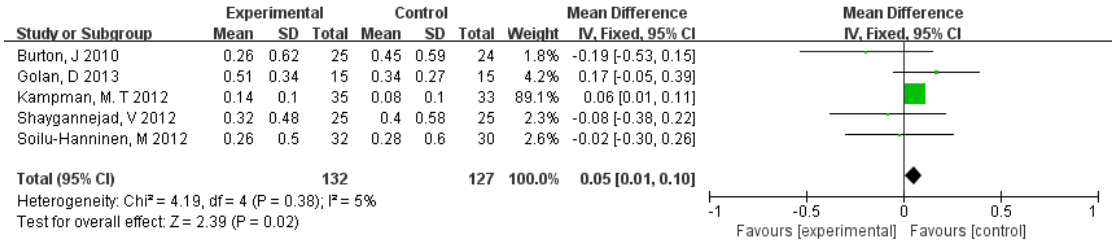

Fig 4. Meta-analysis and Forest plot. Effect of vitamin D on the annualized relapse rate of multiple sclerosis patients (132 received vitamin D and 127 received placebo as treatment).

Authors, year	Jadad score	Number of patients at base line (treatment group/controls)	Research period	Intervention		Sex (male/female)		EDSS at base line (mean [SD])		ARR at base line (mean [SD])	
				Treatment group	Control	Treatment group	Controls	Treatment group	Controls	Treatment group	Controls
Burton et al, 2010	3	25/24	52 weeks	40000 IU/day （for 28 weeks）+10000 IU/day (for 12 weeks) +0 IU/day to the end +1200 mg calcium/day	Less than 4000 IU/day	4/21	5/19	1.46 (1.55）	1.23 (1.63）	0.44(0.77)	0.54(0.72)
Golan et al, 2013	4	24/21	12 months	4370IU/day	800IU/day	5/19	8/13	2.9 (2.0)	3.6 (2.2)	0.28 (0.23)	0.38 (0.26)
Kampman et al, 2012	5	35/33	96 weeks	20000 IU/week+500 mg calcium/day	500 mg calcium/day	11/24	9/24	2.61 (0.37)	2.27 (0.38)	0.11 (0.09)	0.15 (0.09)
Mosayebi et al, 2011	3	26/33	6 months	300000 IU/month	Placebo	9/17	8/25	/	/	/	/
Shaygannejad et al, 2012	5	25/25	12 months	0.25 g (for 2 weeks)→0.5 μg/day	Placebo	3/22	3/22	1.6 (0.7)	1.7 (1.2)	1.04 (0.20)	1.04 (0.2)
Soilu-Hanninen et al, 2012	5	34/32	12 months	20 mg/week	Placebo	13/21	12/20	2.0 (0.2)	1.5 (1.2)	0.49 (0.5)	0.51 (0.5)

Table 1. The characteristics of the patients included in in the studies. SD = standard error.