Current Neuropharmacology, 2022, *20,* 1600-1612

SYSTEMATIC REVIEW ARTICLE

Interferon-Beta Injection in Multiple Sclerosis Patients Related to the Induction of Headache and Flu-Like Pain Symptoms: A Systematic Review and Meta-Analysis of Randomised Controlled Trials

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ARTICLE HISTORY

Received: September 02, 2021
Revised: October 06, 2021
Accepted: October 28, 2021

DOI:
10.2174/1570159X19666211101142115

Abstract: Multiple sclerosis (MS) is a chronic neurodegenerative, inflammatory, and autoimmune disease characterised by the demyelination of the central nervous system. One of the main approaches for treating MS is the use of disease-modifying therapies (DMTs). Among the DMTs are interferons (IFNs), which are cytokines responsible for controlling the activity of the immune system while exerting immunomodulatory, antiviral, and antiproliferative activities. IFN-beta (IFN-β) is the first-choice drug used to treat relapsing-remitting MS. However, the administration of IFN-β causes numerous painful adverse effects, resulting in lower adherence to the treatment. Therefore, this study aimed to investigate the headache and flu-like pain symptoms observed after IFNβ injection in MS patients using a systematic review and meta-analysis of randomised controlled trials. A total of 2370 articles were identified through research databases. Nine articles were included (three involving IFNβ-1b and six involving IFNβ-1a). All studies included in the meta-analysis had a low risk of bias. The odds ratio of headache and flu-like pain symptoms increased in MS patients treated with IFN-β. Thus, the adverse effects of headache and flu-like pain symptoms appear to be linked to IFN-β treatment in MS. The protocol of the study was registered in the Prospective International Registry of Systematic Reviews (registration number CRD42021227593).

Keywords: Adverse effects, disability, disease-modifying therapies, immunomodulatory, neuroinflammation, treatment.

1. INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune-mediated neurodegenerative disease of the central nervous system, characterised by inflammatory demyelination [1, 2]. Thus, it can lead to physical disability, cognitive impairment, and decreased quality of life in patients [3]. Additionally, most MS patients suffer from chronic neuropathic pain [4]. Another pain symptom frequently found in MS is headache [5].

MS can be classified into primary progressive MS (PPMS), secondary progressive MS (SPMS), and relapsing-remitting MS (RRMS) [6].

The progressive MS forms are characterised by intense neurodegeneration without symptom recovery [7]. Most of the patients presented with RRMS clinical form, which is characterised by reversible episodes of neurological dysfunction in the initial stages of the disease [8]. The RRMS incidence is three females to each male, whereas, in the progressive MS forms, the incidence is equal in male and female patients [9]. Furthermore, MS typically presents in young female adults (mean age of onset, 32 years) [10] but has also been reported in younger and older people [11].

Thus, as the MS clinical forms have different pathophysiological characteristics, different treatment approaches may be used [12]. Three primary treatment modalities are found in MS management: intervention for disease exacerbations, disease-modifying therapies (DMTs), and symptomatic approach [13]. The latter include the administration of interferon-beta (IFN-β), monoclonal antibodies, fingolimod, dimethyl fumarate, teriflunomide, and glatiramer acetate [14]. Monoclonal antibodies, fingolimod, dimethyl fumarate, teriflunomide, and glatiramer acetate administration have better efficacy for progressive MS forms and RRMS [15]. However, IFN-β injection is the treatment most indicated for RRMS treatment [12].

In that sense, IFN-β can reduce relapse rates in MS, and is considered an effective option for RRMS treatment [16, 17]. However, despite aiding in treatment, it induces many adverse effects that may reduce the MS patient's quality of life, which is the main reason for discontinuing treatment [17]. The main adverse symptoms of IFN-β treatment are flu-like pain symptoms (painful symptoms related to flu-like syndrome, such as myalgia, arthralgia and headache) [18] and headaches [5]. Although prophylactic analgesics are used to treat pain associated with IFN-β injection, there is no

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1875-6190/22 $65.00+.00

standard therapy indicated for these adverse effects [19]. Moreover, the mechanisms involved in IFN-β associated pain induction are not well explored [20].

Nevertheless, there is still a lack of recent systematic reviews describing the types of pain observed after IFN-β treatment. Consequently, the investigation of flu-like pain symptoms and headache frequencies is needed to guide new advances in treatment approaches. Therefore, this study aimed to investigate the headache and flu-like pain symptoms observed after IFN-β injection in MS patients using a systematic review and meta-analysis.

2. METHODS

This systematic review followed the protocoled reporting items preferred for systematic reviews and meta-analyses (PRISMA) 2020 [21]. Our study was registered in the Prospective International Registry of Systematic Reviews (PROSPERO) (registration number CRD42021227593).

2.1. Research Strategy

The research was carried out using the Pubmed, Excerpta Medica Database (Embase), and Sci Verse Scopus (Scopus) scientific databases, using the publication period from 2005 to 2021. We utilised the following keywords combined for IFN, MS, and pain, based on the Medical Subject Heading (MeSH) words; the entire search string can be found in Supplementary **1**. The search was conducted by two independent researchers (L.P. and P.R.).

The search for the articles was carried out in January and March 2021. First, the duplicate records, review articles, and conference papers were automatically removed using the EndNote X9® software before the screening. Subsequently, the articles were screened in three phases. In the first phase, title analysis was performed, followed by, in the second phase, abstract analysis, and finally, in the third phase, full-text analysis. The articles selected underwent review by two researchers (F.V. and J.F., and L.P. and P.R.); if there was disagreement, a fifth researcher was consulted (G.T.). Subsequently, a sixth researcher (L.G.) searched the selected articles' references and other related reviews manually.

2.2. Exclusion and Inclusion Criteria

Randomised clinical trials (RCT), written in English, that address the subjects' pain (headache and flu-like pain symptoms) in MS patients treated with IFNs are included. Exclusion criteria were studies that included children, pre-clinical studies, articles that only assessed injection site pain, follow-up studies, case-report articles, review articles, pre-existing headache, unspecified type of pain, and the absence of a placebo group in the article. Two pairs of independent reviewers (F.V. and J.F., and L.P. and P.R.) analysed the data exclusion or inclusion, and discrepancies were evaluated and resolved by the third researcher (G.T.).

2.3. Data Extraction and Quality Assessment

Two pairs of independent reviewers (F.V. and J.F., or L.P. and P.R.) performed the data extraction by reading the full-text articles, and the results were recorded in two tables. The relevant outcomes included the number of patients in the experimental groups (IFN and control), age, sex, MS diagnostic criteria, clinical aspects, and risk of bias. The references cited in the methodology or clinical trials registration database were examined, and the authors of the article were consulted if the clinical aspects of the patients were not described in the article (Table **1**). When the type of study in the article was not given, it was classified by the authors [22]. The type of IFN, administration route, dose, time, pain assessment, outcomes, and risk of bias, were extracted and are described in Table **2**. Moreover, the quality of evidence for the pain level in MS patients after IFN treatment was specified as high, medium, low, or relatively low depending on the circumstances described by GRADE [23]. The quality of evidence was evaluated using GRADEprofiler (GRADEpro) 3.6.1 software for randomised clinical trials.

2.4. Risk of Bias

Individual studies were assessed for the source of bias by two pairs of independent reviewers (F.V. and J.F., and L.P. and P.R.), including the fifth reviewer (G.T.) when necessary. For Table **1**, the sources of bias include clinical characteristics of patients being undefined, the inclusion of progressive MS patients, patient use of alcohol or other substances, and unconventional MS diagnostic methods. In Table **2**, painful adverse effects of INFβ-1a and INFβ-1b treatments and sources of bias (use of analgesic drugs, self-referral pain assessment, re-randomisation, and placebo data not shown) are described.

This data was classified into high, unclear, or low subcategories and expressed using the Cochrane Risk of Bias tool [24]. Bias was rated high when progressive MS patients were included, analgesic drugs were used in allocation concealment, re-randomisation was used in random sequence generation, or placebo data was not shown in incomplete outcome data. Bias was classified as unclear when the use of alcohol or other substances, or clinical characteristics of patients were not defined in allocation concealment. Additionally, publication bias was evaluated using Egger's test [25], Begg's test [26], and funnel plots.

2.5. Statistical Analysis

Considering the risk of bias outcomes, whenever the study showed bias over two standard deviations (SD) above the total percentage of high bias (25%), it was excluded from the study [27]. The meta-analysis was extended using the number of patients who experienced painful adverse effects (flu-like pain symptoms and headache) and the total number of individuals extracted from the placebo group. The studies are shown as forest plots. Data were pooled using the random-effects model using weighted averages relative to the sample size of the single studies [24]. The heterogeneity was analysed using the I^2 statistics, classifying < 25% as no heterogeneity; 25-50% as mild heterogeneity; 50-75% as moderate heterogeneity; and > 75% as high heterogeneity [28]. Statistical analyses were performed using RStudio software or RevMan software version 5.4, the Cochrane 2020 collaboration [24], with a minimum significance level of $p < 0.05$.

3. RESULTS

3.1. Article Selection and Characteristics

Our research yielded 2370 studies that were obtained through the Pubmed, Scopus, and EMBASE databases. The

Table 1. Data extraction from clinical aspects of MS patients treated with IFNβ.

Experimental Groups, Age, and Sex Distribution	MS Diagnosis/ Disability Assessment	Clinical Aspects	Risk of Bias	References
PegIFNβ-1a 2 qw (N = 512, 36.9 years, 361W, 139M). PegIFNβ-1a 4 qw (N = 500, 36.4 years, 352W, 148M). Placebo (N = 500, 36.3 years, 358W, 142M).	Diagnostic confirmation: McDonald criteria of Polman and colleagues [45]. Disability evaluation: EDSS.	Eligibility criteria: patients aged 18-65 years with RRMS, an EDSS score of 0.0-5, and no less than two clinical relapses in the three years preceding study entry, with one or more in the 12 months before. Progressive MS, and preceding treatment with IFN for MS for at least four weeks or discontinuation during six months or less before baseline were excluded. Use of other medications used for MS therapy during the study was excluded. Patients characteristics: disease duration mean PegIFNβ-1a 2 qw: 4 years; PegIFNβ-1a 4 qw: 3.4 years; Placebo: 3.5 years. EDSS score: PegIFNβ-1a 2 qw: 2.47; PegIFNβ-1a 4 qw 2.48; Placebo 2.44. Treatment lasted for 0.92 years.	Bias was not detected.	Calabresi (2014) [32].
IFNβ-1a, 44 tiw (N = 171, 30.6 years, 114W, 57M). IFNβ-1a, 44 qw (N = 173, 30.7 years, 106W, 59M). Placebo (N = 171, 30.9 years, 112W, 59M).	Diagnostic confirmation: McDonald criteria of Polman and colleagues [45]. Disability evaluation: EDSS.	Eligibility criteria: patients aged 18-50 years, and EDSS score of 0.0-5.0, the occurrence of only one event suggestive of MS in the 60 days preceding study entry, and evidence of two or more silent T2-weighted lesions on brain MRI scan of at least 3 mm, that should be either ovoid, periventricular, or infratentorial. There was no exposure to other immunomodulatory or immunosuppressive therapies. Treatment for MS relapses with corticosteroids in short courses was allowed following the trial protocol according to the treating physician. Patients characteristics, EDSS score, mean INFβ-1a three times a week 1.50 (0-4.0), INFβ-1a once a week 1.50 (0–3.5), Placebo 1.50 (0-3.5). Treatment lasted for 2 years.	Inclusion of progressive MS patients	Comi (2012) [36].
IFNβ-1a (N = 120). Placebo (N = 60).	Diagnostic confirmation: McDonald criteria of Polman and colleagues [45]. Disability evaluation: EDSS.	Eligibility criteria: patients aged 18-60 years, with RRMS, and an EDSS score of 0.0-5.5 with at least two or more relapses in the last three years and active disease (at least one clinical event) within 6 months before study entry. Treatment lasted for 0.76 years.	Patient use of alcohol or other substances. Clinical characteristics of patients being undefined.	De Stefano (2012) [37].
MS patients (N = 293, 33.5 years, 241W, 52M). IFNβ-1a, 22 μg (N = 95). IFNβ-1a, 44 μg (N = 98). Placebo 22 μg (N = 49). Placebo 44 μg (N = 51).	Diagnostic confirmation: Poser and colleagues [47]. Disability evaluation: EDSS.	Eligibility criteria: patients with RRMS and an EDSS score of 0.0-5.0 with at least one relapse in the last two years. Patients' characteristics: mean EDSS score of 2.5. Treatment lasted duration: 0.92 years.	Patient use of alcohol or other substances. Clinical characteristics of patients being undefined.	Freedman (2005) [33].
IFN β-1a, 22 μg (N = 189, 34.5 years, 127W, 62M). IFN β-1a, 44 μg (N = 184, 34.7 years, 122W, 62M). Placebo (N = 187, 34.6 years, 141W, 46M).	Diagnostic confirmation: Poser and colleagues [47]. Disability evaluation: EDSS.	Eligibility criteria: patients aged 18-50 years, with RRMS and EDSS score of 0.0-5.0 with at least one relapse in the last two years. Any preceding systemic treatment with interferons, cyclophosphamide, or lymphoid irradiation was excluded. There was no exposure to immunomodulatory or immunosuppressive treatments in the last year (3% of patients went through immunosuppressive therapy before that period of time). Patients' characteristics - disease duration: Placebo 4.3 years, IFNβ-1a 22 μg 5.4 years, IFNβ-1a 44 μg 6.4 years. EDSS score mean: Placebo 2.4, IFNβ-1a 22 μg 2.5, IFNβ-1a 44 μg 2.5. Treatment lasted for 2 years.	Patient use of alcohol or other substances.	Gold (2005) [34].

(Table 1) contd….

Experimental Groups, Age, and Sex Distribution	MS Diagnosis/ Disability Assessment	Clinical Aspects	Risk of Bias	References
IFNβ-1a (N = 54, 38 years, 32W, 22M). Placebo (N = 54, 38.5 years, 36W, 18M).	Diagnostic confirmation: McDonald criteria of Polman and colleagues [45]. Disability evaluation: EDSS.	Eligibility criteria: patients, aged 18-55 years, with RRMS and an EDSS score of 1.0-6.0 with at least two relapses within the three years preceding study entry and at least one relapse one year before baseline; and no less than six T2 lesions on MRI scan or two relapses during the year before the screening. Progressive MS, patients with more than 15 years of disease and with EDSS score of 2 or less, past or current diagnosis of another neurological or systemic autoimmune diseases, use of rituximab, lymphocyte-depleting therapy, or lymphocyte trafficking blockers in the last 24 weeks, treatments utilizing INFβ-, glatiramer acetate, intravenous immunoglobulin, plasmapheresis, and immunosuppressives in the last 12 weeks, use of systemic glucocorticoids in the last four weeks, and past exposure to IFNβ-1a were excluded. Patients' characteristics disease duration: Placebo 2.7 years, IFNβ-1a 3.3 years, EDSS score mean: Placebo 3.0 (1.0-6.0) IFNβ-1a 2.8 (1.0-6.0). Treatment lasted for 2 years.	Bias was not detected.	Kappos (2011) [35].
IFNβ-1b (N = 292, 30.7 years, 207W, 85M). Placebo (N = 176, 30.5 years, 124W, 52M).	Diagnostic confirmation: McDonald criteria of Polman and colleagues [45]. Disability evaluation: EDSS.	Eligibility criteria: patients aged 18-45 years, with MS and an EDSS score of 0.0/5.0 that had a first neurologic suggestive of MS with at least 24 hours of duration and no less than two no visible lesions of at least 3 mm on T2-weighted brain MRI scan, one or more of which should be ovoid, periventricular, or infratentorial. Disorders other than MS that could explain to patients' signs and symptoms, previous situations that could be caused by an acute demyelinating event, prior therapy with immunosuppressive, and complete transverse myelitis or bilateral optic neuritis were excluded. In order to exclude other diseases with similar symptoms, a complete diagnostic evaluation was executed in the screening period in which vasculitis/collagenosis, borreliosis, vitamin B12 deficiency, and neurosarcoidosis tests were performed. Patients characteristics EDSS score mean: IFNβ-1b 1.5 (0-4.0), placebo 1.5 (0-4.0). Treatment lasted for 2 years.	Patient use of alcohol or other substances. Inclusion of progressive MS patients.	Kappos (2006) [29].
IFNβ-1b (N = 36, 45 years, 15W, 21M). Placebo (N = 37, 44 years, 22W, 15M).	Diagnostic confirmation: Schumacher and colleagues [46]. Disability evaluation: EDSS.	Eligibility criteria: patients aged 18-65 years, with progressive MS (with slow or accelerated clinical progression in which signs and symptoms should last at least one year) or transitional MS (with a single relapse, before or during progression) with at least one year of duration and EDSS score of 3.0-7.0. Other MS forms, probable diseases to cause signs and symptoms not correctly excluded by spinal cord MRI, exposure to immunomodulatory or immunosuppressive treatments, alcohol, or other substances used within 90 days before the examination, and suicide attempts were excluded. Patients' characteristics: disease duration mean - placebo 11.4 years, IFN β-1b 11.3 years. EDSS score mean: placebo: 5.2 and IFNβ-1b: 5.3. Treatment lasted for 2 years.	Inclusion of progressive MS patients.	Montalban (2009) [30].
IFNβ-1b (N = 65, 37.5 years, 48W, 17M). Rapid-titration (N = 31) IFNβ-1b. Slow-titration (N = 34) IFNβ-1b Placebo (N = 33, 35 years, 24W, 9M).	Diagnostic confirmation: Poser and colleagues [47]. Disability evaluation: EDSS.	Eligibility criteria: patients aged 18-55 years, clinically or laboratory-supported diagnoses of RRMS for one or more years and with EDSS score of 0.0-5.5. Women were requested to present negative results for pregnancy and utilize contraception methods correctly. Patients disabling condition should be only RRMS. There was no exposure to total lymphoid irradiation, murine or T-cell antibodies, IFNs or other recombinant DNA cytokines previously in the study, or immunosuppressant therapy six months before study baseline, or adrenocorticotrophic hormone in the month preceding study entry, medication intolerance, and alcohol or drug abuse, depression, or suicide attempts history were excluded. Patients' characteristics: EDSS score mean: placebo: 2.92 and IFNβ-1b: 3.09. Treatment lasted for 90 days.	Bias was not detected	Wroe (2005) [31].

Abbreviations: EDSS (Expanded Disability Status Scale); IFNβ (Interferon beta); Peg-IFN (Pegylated interferon); M (Male); MS (Multiple sclerosis); MSFC (Multiple sclerosis functional composite); MRI (Magnetic resonance imaging); N (Number of subjects); QW (once a week); RRMS (Relapsing-remitting multiple sclerosis); TIW (three times a week); W (Woman).

Table 2. Painful effects caused by INFβ-1a and INFβ-1b treatments in multiple sclerosis patients.

IFN Type	Route, Dose, Time	Pain Assessment	Outcomes	Risk of Bias	References
Peg-IFNβ-1a	Peg-IFNβ-1a (125 µg, sc, every 2 or 4 weeks). Placebo (sc).	Self-referral.	Flu-like pain symptoms: Peg-IFNβ-1a treated group 47% of patients (2 and 4 qw) and 13% of patients for the placebo group. Headache: Peg-IFNβ-1a injection 44% for 2 qw and 41% for 4 qw, or 33% for the placebo group. This study did not assess the difference between the IFN and placebo groups.	Use of analgesic drugs. Self-referral pain assessment.	Calabresi (2014) [32].
IFNβ-1a	IFNβ-1a (44 µg, sc, tiw; or 44 µg, sc, qw). Placebo (sc, tiw).	Medical Dictionary for Regulatory Activities.	Flu-like pain symptoms: IFNβ-1a treated group 54% (tiw) or 71% (qw) of patients and 13% of patients for the placebo group. This study did not assess the difference between the IFN and placebo groups.	Use of analgesic drugs.	Comi (2012) [36].
IFNβ-1a	IFNβ-1a (44 µg, sc, tiw). Placebo (sc, tiw).	Medical Dictionary for Regulatory Activities.	Flu-like pain symptoms: IFNβ-1a treated group 55% of patients and 16.7% of patients for the placebo group. Headache: IFNβ-1a injection 31.7% or 16.7% for the placebo group. This study did not assess the difference between the IFN and placebo groups.	Use of analgesic drugs.	De Stefano (2012) [37].
IFNβ-1a	IFNβ-1a (22 µg, sc, qw; or 44 µg, sc, qw). Placebo (sc, qw for IFNβ-1a 22 and 44 µg groups).	Self-referral.	Flu-like pain symptoms were frequent in IFNβ-1a (54% for 22 µg qw versus 67% for 44 µg qw, P = 0.06). Placebo data for flu-like pain symptoms was not found. This study only compared the IFNβ-1a doses used (22 µg and 44 µg qw) for flu-like pain symptoms.	Use of analgesic drugs. Self-referral pain assessment. Re-randomisation. Placebo data not shown.	Freedman (2005) [33].
IFNβ-1a	IFNβ-1a (22 µg, sc, tiw; or 44 µg sc, tiw). Placebo (sc, tiw).	Self-referral.	Flu-like pain symptoms: IFNβ-1a treated group 56% (22 µg) or 59% (44 µg) of patients and 51% of patients for the placebo group. Headache: IFNβ-1a injection 65% (22 µg) or 70% (44 µg) or 63% for the placebo group. This study did not assess the difference between the IFN and placebo groups.	Use of analgesic drugs. Self-referral pain assessment.	Gold (2005) [34].
IFNβ-1a	IFNβ-1a (30 µg, im, qw). Placebo (iv, qw).	Self-referral.	Flu-like pain symptoms: IFNβ-1a treated group 19% of patients and 0% of patients for the placebo group. Headache: IFNβ-1a injection 9% or 6% for the placebo group. This study did not assess the difference between the IFN and placebo groups.	Self-referral pain assessment. Re-randomisation.	Kappos (2011) [35].
IFNβ-1b	IFNβ-1b (250 µg, sc, QOD). Placebo (sc, QOD)	EuroQoL-5 Dimensional Questionnaire.	Flu-like pain symptoms: IFNβ-1b treated group 42.2% of patients and 18.2% of patients for the placebo group. Headache: IFNβ-1a injection 26.7% or 17% for the placebo group. This study did not assess the difference between the IFN and placebo groups.	Use of analgesic drugs. Self-referral pain assessment.	Kappos (2006) [29].

(Table 2) contd….

IFN Type	Route, Dose, Time	Pain Assessment	Outcomes	Risk of Bias	References
IFNβ-1b	Day 1: 125 μg for two weeks (14 days, sc). Day 15: the dose was increased to 250 μg (sc) for the rest of the treatment (24months). Placebo (sc).	Self-referral.	Flu-like pain symptoms were more frequent in IFNβ-1b (41.7%) treated groups when compared with placebo (5.4%) (P < 0.001). Headache was more frequent in IFNβ-1b (38.9%) group when compared with placebo (10.8%) (P = 0.005).	Use of analgesic drugs. Self-referral pain assessment.	Montalban (2009) [30].
IFNβ-1b	Rapid-titration: IFNβ-1b 125 μg QOD for 2 weeks (sc, 7 doses). Day 15 (sc, dose 8): the dose was increased to 250 μg (sc). Slow-titration: IFNβ-1b 62.5 μg QOD for 9 days (sc, 5 doses) and in day 11, the dosage was increased to 125 μg (sc) for five doses, with further increments on day 21 (to 187.5 μg for five doses, sc) and day 31 (to 250 μg for 30 doses, sc). Placebo (sc).	Self-referral.	Flu-like pain symptoms were more frequent in IFNβ-1b (36,9%) treated group when compared with placebo (15%) (P < 0.001). Headache was more frequent in IFNβ-1b (50.8%) treated group when compared with placebo (30.3%) (P > 0.05).	Use of analgesic drugs. Self-referral pain assessment.	Wroe (2005) [31].

Abbreviations: IFNβ (Interferon beta); Peg-IFN (Pegylated interferon); IM (Intramuscular); IV (Intravenous); MS (Multiple sclerosis); QOD (Every other day); QW (Once time a week); SC (Subcutaneous); TIW (Three times a week).

selection of studies is shown in the flowchart (Fig. **1**). In the classification phase, 369 duplicated articles were excluded, and after the title and abstract revision, 2260 articles were excluded due to failure to meet the inclusion criteria. Next, the full text of 110 articles was analysed in order to confirm studies' eligibility, resulting in the final inclusion of seven items. Furthermore, 69 articles were identified through manual searching, from which we included two further articles after full-text analysis.

Finally, we included nine prospective randomised studies in this review, including 3806 patients, of which 2489 were treated with IFNβ and 1317 were part of the placebo group. In three studies using IFNβ-1b [29-31], 393 MS patients were treated with IFN, and 266 patients were in the placebo group. IFNβ-1a was the most frequent type used for MS treatment. We selected six studies using INFβ-1a [32-37], with a total of 2086 patients treated with IFNβ-1a and 1057 patients in the placebo group. A description of individual study characteristics is given in Table **1**.

Self-referral was the most frequent form of pain assessment, used in six articles [30-35], the Medical Dictionary for Regulatory Activities was used in two articles [36, 37], and one study used questionnaires [29] (Table **2**). The most frequent doses used for IFNβ-1a were 22 μg and 44 μg, and for IFNβ-1b were 125 μg and 250 μg. The most commonly used route of administration for both IFNβ-1b and IFNβ-1a was subcutaneous (Table **2**).

Table **2** describes the flu-like pain and headache symptoms observed in nine articles. In this review, one of the most frequent painful symptoms after IFN treatment was flu-like pain symptoms. However, six articles did not assess the difference between the IFN and placebo groups [29, 32, 34-37]. Additionally, the study of Freedman and colleagues [33] did not report the data for the placebo group and only compared the frequency of flu-like pain symptoms between the doses used (22 or 44 μg of IFNβ-1a, P = 0.06), but no difference was detected between the groups. For flu-like pain symptoms, only two studies compared the groups treated with IFNβ-1b or placebo [30, 31], and both found a significant difference, where IFNβ-1b induced more flu-like pain symptoms compared to placebo (Table **2**). In seven articles, headache symptoms were assessed [29-32, 34, 35, 37]. However, only two studies compared the groups treated with IFNβ-1b or placebo [30, 31], and only Montalban and colleagues observed that IFNβ-1b induced headache more frequently when compared to placebo [30] (Table **2**).

3.2. Risk of Bias

Most of the studies (88%) have a high risk of bias due to allocation concealment [29, 31-34, 36, 37] (Fig. **2A**). One article (11%) had a high risk of bias due to incomplete outcome data [33]. In terms of random sequence generation, only one study (11%) had a high risk of bias [33].

The other six articles (66%) were classified as having unclear bias [29-34] in our selective reporting. In summary, in the blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), and other bias, we assessed a low risk of bias (Fig. **2B**).

Furthermore, to examine the possibility of publication bias, four funnel plots were produced for the nine studies, plotting the standard error of the study estimate against the symptom mean difference. The funnel chart revealed asymmetries for all meta-analyses (Fig. **3**). Egger's and Beggs's tests for meta-analyses with IFNβ-1b were not performed because there were fewer than four articles. Moreover, the

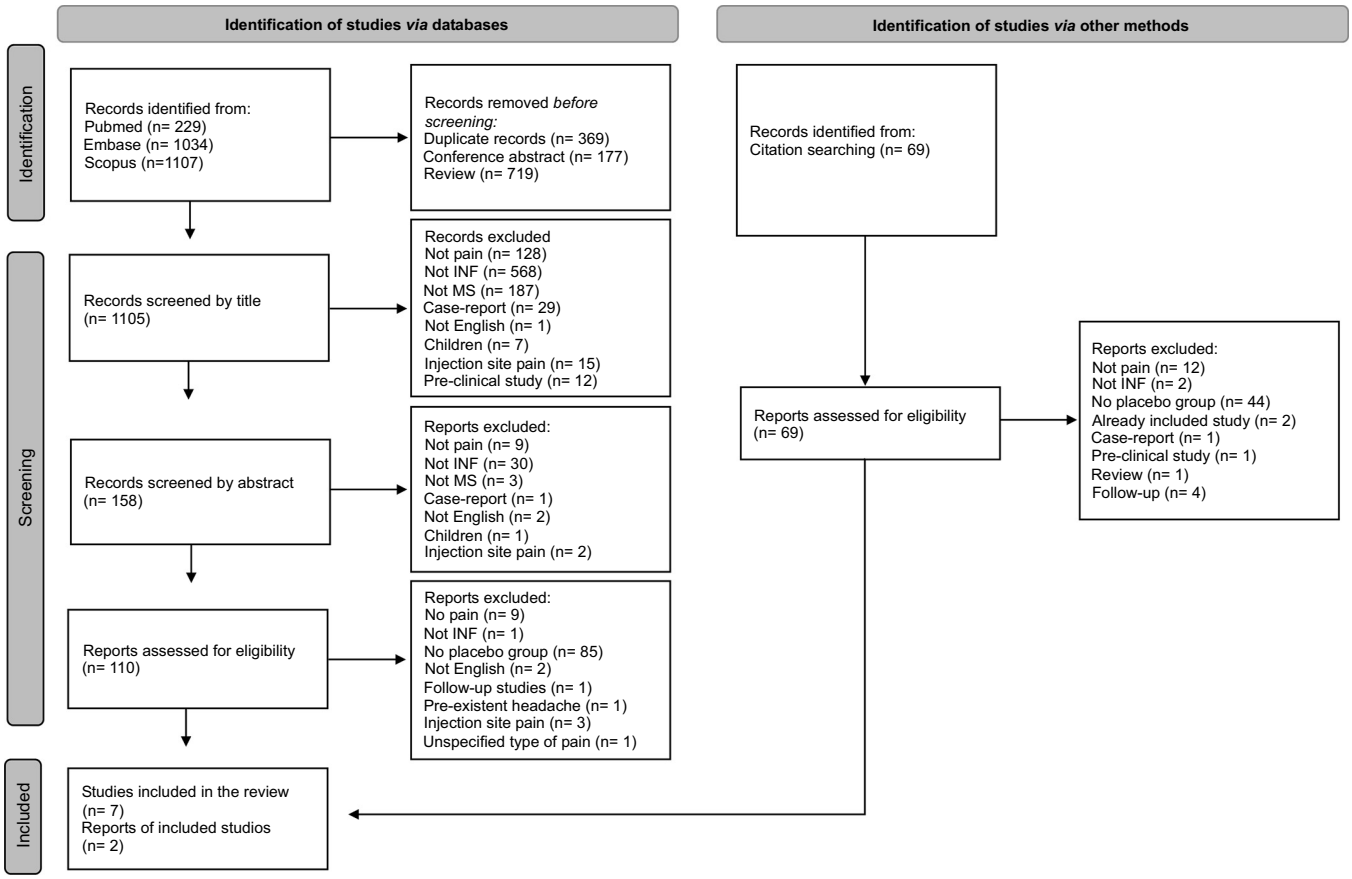

Fig. (1). Flow diagram of studies identification.

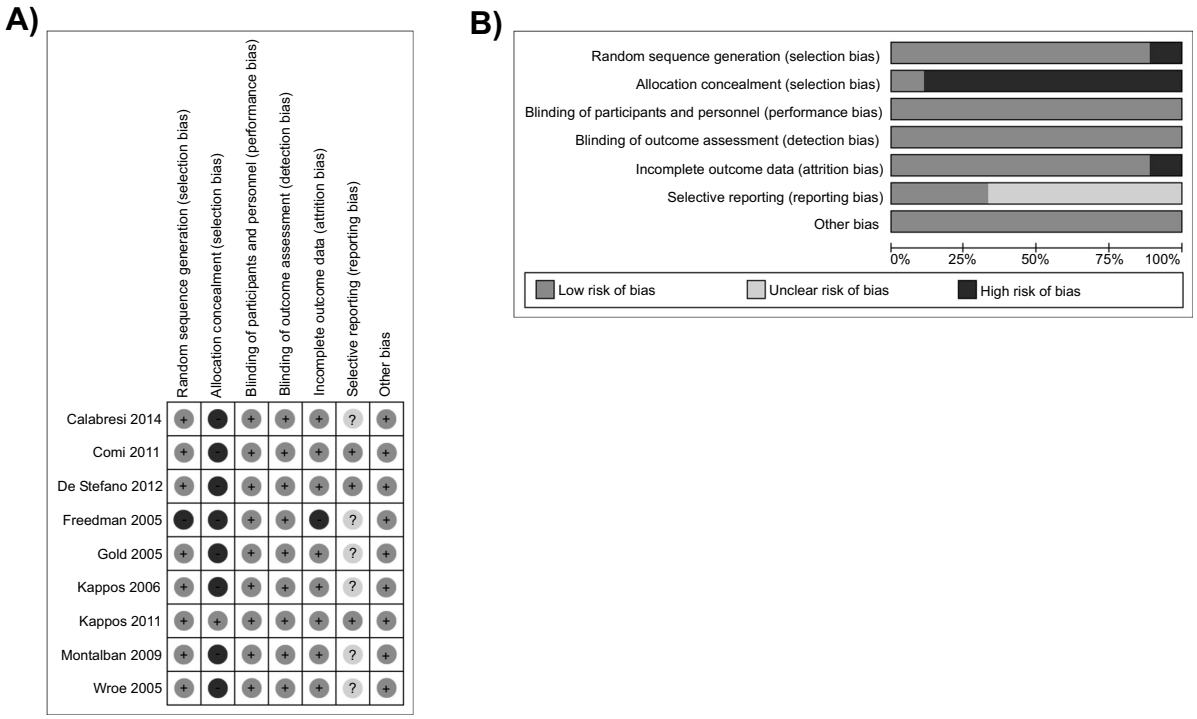

Fig. (2). Risk of bias of the articles that evaluated the pain symptoms from INF-treatment in multiple sclerosis patients versus placebo. **a**) Risk of bias for each study included and **b**) calculated percentage for each indicator evaluated. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

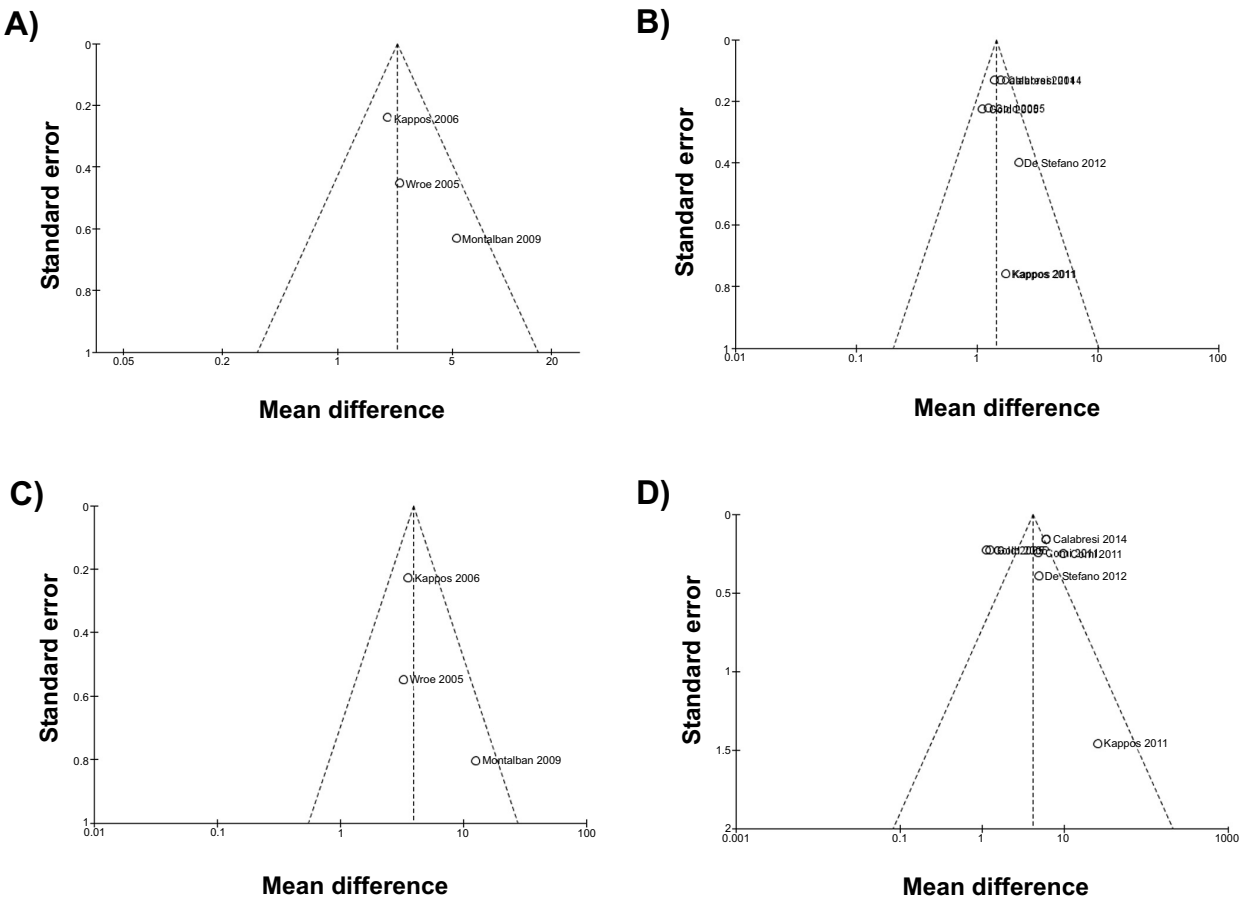

Fig. (3). Funnel plot of pain symptoms from INF-treatment in multiple sclerosis patients versus placebo. **a**-**b**) headache; **c**-**d**) flu-like pain symptoms. The Egger's test and Begg's test were used to assessing the evidence of publication bias. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

Egger's test for headache in IFNβ-1a meta-analysis results in p = 0.6581 and for flu-like pain symptoms IFNβ-1a meta-analysis results in p = 0.9871, suggesting no evidence of publication bias in these studies. Similarly, the Beggs's test for headache in the IFNβ-1a treatment meta-analysis gave a p-value of 0.8510, and for flu-like pain symptoms, the IFNβ-1a meta-analysis resulted in a p-value of 0.4579, suggesting no evidence of publication bias in these studies.

3.3. Meta-analysis Results and Quality Assessment

The study by Freedman and colleagues [33] was excluded from the meta-analysis due to re-randomisation and the lack of the placebo group data before the study was re-randomised. However, we include the study by Gold and colleagues [34] and Kappos and colleagues [35] since we used the data from before the re-randomisation.

The forest plots and quality of evidence showed the frequencies of painful headache after treatment with IFNβ-1a or IFNβ-1b in MS patients (Fig. **4**). In the first meta-analysis, three studies [29-31] were included in order to assess the frequency of headache in MS patients undergoing IFNβ-1b treatment. The analysis demonstrated that the odds ratio increased for MS patients receiving IFNβ-1b treatment experienced headache compared to placebo group (Z = 3.09, OR = 2.25, 95% CI = 1.35–3.78), P = 0.002), with low heterogeneity (Tau² = 0.06 Chi² = 2.68, df = 2, P = 0.26; I² = 25%) (Fig. **4a**). In the meta-analysis of headache in MS patients treated with IFNβ-1a, four studies were examined [32, 34, 35, 37]. The analysis shows that the odds ratio increased for patients receiving IFNβ-1a treatment experienced headache compared patients in the placebo group (Z = 4.57, OR = 1.43, 95% CI = 1.23–1.66, P < 0.00001), with low heterogeneity (Tau² = 0.00, Chi² = 3.63, df = 5, P = 0.60, I² = 0%) (Fig. **4b**).

The forest plots and quality of evidence showed the frequencies of flu-like pain symptoms during treatment with IFNβ-1a or IFNβ-1b in MS patients (Fig. **5**). In the meta-analysis of flu-like pain symptoms, three studies described MS patients receiving IFNβ-1b treatment [29-31]. The analysis shows that the odds ratio increased for MS patients who received IFNβ-1b treatment to experience flu-like pain symptoms compared to placebo group (Z = 5.28, OR = 3.97, 95% CI = 2.38 to 6.62, P < 0.00001), with a low level of heterogeneity (Tau² = 0.04, Chi² = 2.37, df = 2, P = 0.31, I² = 15%) (Fig. **5a**).

In the meta-analysis of flu-like pain symptoms in MS patients receiving IFNβ-1a treatment, six studies were used [4, 7, 11, 22, 29]. The analysis shows that the odds ratio increased for MS patients who received IFNβ-1a treatment to experience flu-like pain symptoms compared to placebo group (Z = 4.61, OR = 4.08, 95% CI = 2.24 to 7.40,

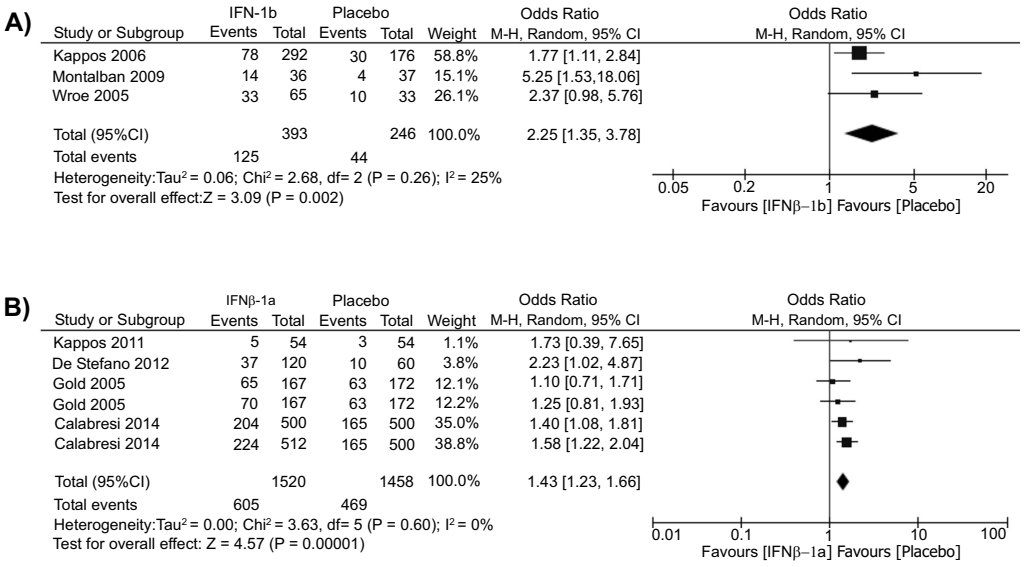

Fig. (4). Meta-analysis of headache expressed as forests plots. **a**) the influence of INFβ-1a treatment in multiple sclerosis patients versus placebo; **b**) the influence of INF-1b treatment in multiple sclerosis patients versus placebo. The forest plots presented the number of INFβ-treatment patients with and without headache, and the total number of patients from placebo with and without headache using the random-effects model. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

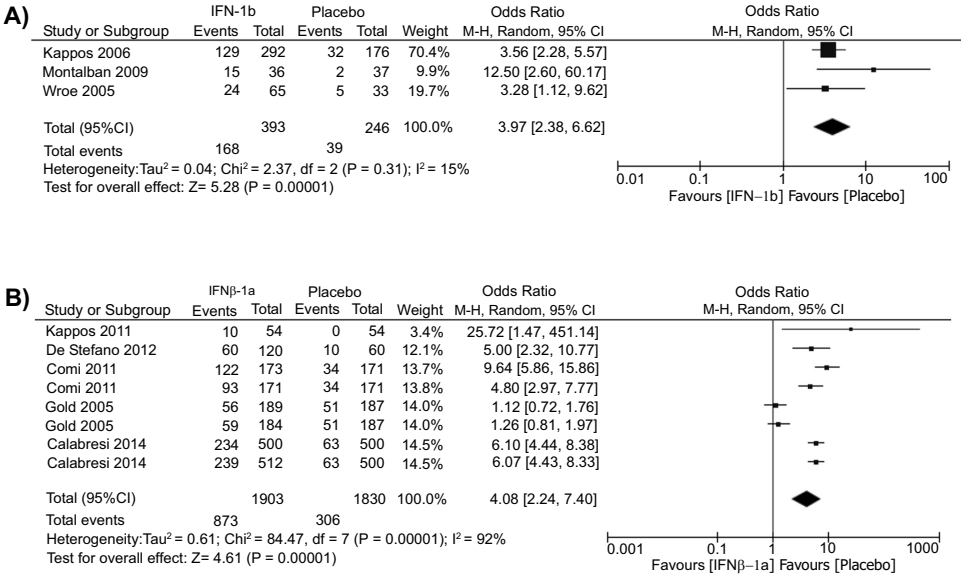

Fig. (5). Meta-analysis of flu-like pain symptoms expressed as forests plots. **a**) the influence of INFβ-1a treatment in multiple sclerosis patients versus placebo; **b**) the influence of INFβ-1b treatment in multiple sclerosis patients versus placebo. The forest plots presented the number of INFβ-treatment patients with and without flu-like pain symptoms, and the total number of patients from placebo with and without flu-like pain symptoms using the random-effects model. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

$P < 0.00001$), with a high level of heterogeneity ($Tau^2 = 0.61$, $Chi^2 = 84.47$, $df = 7$, $P < 0.00001$, $I^2 = 92\%$) (Fig. **5b**). All meta-analyses showed high-quality final evidence (Supplementary **2**).

4. DISCUSSION

Treatment with IFN-βs in MS patients is known to cause diverse adverse effects, including pain [38, 39]. Although some prophylactic analgesics are used in the treatment of the side effects of IFN-β injection, the efficacy of these drugs still needs to be investigated [19]. Moreover, the mechanism of pain induction by INF-βs is not well known [20]. Therefore, this research aimed to investigate the headache and flu-like pain symptoms caused by IFN-βs injection in MS patients. This systematic review and meta-analysis is the first to propose an association between pain induction and treatment with IFN-βs in MS patients. In this setting, we observed that treatment with IFN-βs in MS patients generates headache and flu-like pain symptoms.

Our systematic review and meta-analysis selected only prospective randomised controlled trials (RCT). No study is likely to demonstrate causality on its own, but the randomisation aids in balancing participant characteristics between the groups, allowing attribution of any differences in outcome to the study intervention. Thus, the RCT study design is unique, reduces bias, and provides an excellent tool for examining causal relationships between an intervention and outcome [40].

In some selected studies, progressive MS patients were included, but IFN-β is more often indicated as a first-line treatment for RRMS [12, 41]. The disease course is different in the different clinical forms of MS (RRMS and PMS) [6]. RRMS is characterized by episodes of neurodegeneration and recovery, while PMS progresses without remission. DMTs aim to treat neuroinflammation that can lead to neurodegeneration, and therefore may have an indirect effect on this process [12]. However, we have not included this criterion as an exclusion point in our study because we have few selected studies, and some types of IFN-βs could be used to treat progressive MS as discussed below.

Moreover, RRMS incidence is three females to each male [9], suggesting a sex difference in MS development [9]. Two studies [37, 41] did not show the sex distribution of the MS patients in the groups; the lack of this information can undermine the reliability of these studies. In four studies [29, 33, 34, 37], the use of alcohol and illicit drugs was not evaluated. However, alcohol consumption and illicit drugs could alter the perception of pain due to their analgesic effects [12, 42, 43]. Therefore, lack of availability of these factors was considered to lead to a high risk of bias in the allocation concealment topic (selection bias). Additionally, the MS diagnostic criteria have been refined over the years [44], with Mcdonald's method coming into use after 2005 and being improved over the years [45]. Until 2005, the diagnosis of MS was based on Schumacher and colleagues [46] and Poser and colleagues [47]. Therefore, the included articles use varying diagnostic criteria. Thus, some articles may include patients with imprecise or even mistaken diagnoses.

Our study included three articles that used IFNβ-1b and six that used IFNβ-1a, which are proteins with different amino acid sequences. IFNβ-1a has the same amino acid sequence as human IFNβ, whereas IFNβ-1b does not. Also, unlike IFNβ-1b, IFNβ-1a is glycosylated [48]. Both IFNβ types are used in the treatment of RRMS [14]. Currently, Avonex® (IFNβ-1a) and Extavia® (IFNβ-1b) are indicated for patients undergoing the first demyelinating event of RRMS, whereas Plegridy® (pegINFβ-1a) is indicated for RRMS in general. Rebif® (IFNβ-1a) and Betaseron® (IFNβ-1b) are indicated for RRMS and SPMS [15, 19, 49]. Most studies used the subcutaneous route to administer both types of IFNβ (Table **2**). The subcutaneous route is the most commonly used, since only Avonex® is administered intramuscularly [38]. In addition, the doses used in the selected studies follow the current guidelines for the safe prescription of these medications (Avonex® 30 μg, Betaseron® 0.25 mg, Rebif® 22 or 44 μg, and Plegridy® 125 μg) [19].

Moreover, the pain measurement protocols used in the selected studies were not consistent. Only one study used a questionnaire (EuroQoL-5 Dimensional Questionnaire) [29], and two studies [36, 37] used a medical dictionary. All of the other studies used self-referral for pain determination. A possible solution to the self-referral problem is the use of specific questionnaires for reporting adverse effects that can distinguish between them in a specific way. In addition, studies could add a questionnaire that included the visual analogue scale (VAS) to assess the pain resulting from the treatment [50]. However, none of the included studies aimed to assess only the pain induced by IFN-βs. Therefore, it is essential to develop further randomised double-blind, placebo-controlled trials that assess IFN-β-induced pain using a standard measure of pain. Some studies included in our review recommend prophylactic treatment with analgesics. However, even with this recommendation, it was not possible to avoid the painful side effects.

We detected in the meta-analyses that both IFNβ-1a and IFNβ-1b induced headache and flu-like pain symptoms. Additionally, the meta-analysis of flu-like pain symptoms showed that they were induced at a significant level by IFNβ-1a ($P < 0.00001$), despite presenting a high level of heterogeneity. A number of factors, such as differences in clinical aspects, the low number of studies [51], different routes of administration, the inclusion of progressive MS, analgesic use, and different doses, may explain the high level of heterogeneity.

A standardised method for diagnosing side effects may reduce heterogeneity. Thus, our results indicate the lack of studies investigating the adverse effects caused by IFN-β in a standardised way, where both groups were treated with analgesics and only RRMS patients were used. Also, the low number of articles may explain the asymmetry of the funnel graph, as this analysis is recommended to be carried out with more than ten articles, according to Crochrein guidelines [24].

Consequently, the results of our meta-analyses must be assessed in light of these limitations, as the effectiveness depends on the quality of the articles. First, the low number of articles makes it impossible to analyse the funnel plot properly. Furthermore, the self-referral diagnostic methodology increased the likelihood of errors. In addition, the use of analgesics without ensuring that all patients follow the guidelines equally may introduce bias. These facts may explain the small increase in headache and flu-like pain symptoms in patients treated with IFNβ-1a, as well as the small increase in flu-like pain symptoms and headache. Therefore, the frequency of headache and flu-like pain symptoms should be measured with a more accurate assessment system and a detailed prescription for the use of analgesics in order to more accurately measure the increase in these adverse effects.

Our results show that the frequency of flu-like pain symptoms and headache increased in MS patients treated with IFN-βs. In this view, IFN-β injection in MS is accompanied by pain induction. Moreover, we believe that this study suggests that this painful symptom should be better monitored in the clinic to indicate its treatment, improving patients' quality of life. In addition, it will be necessary to study which analgesic drugs should be used as the standard therapy for this type of pain caused by IFN-βs and its mechanisms. Effective prophylactic treatment can reduce IFN-β

treatment dropout, as one of the most frequent reasons for MS therapy withdrawal is the painful adverse effects [52].

CONCLUSION

In conclusion, our study was the first systematic review and meta-analysis of randomised controlled trials that evaluated the pain symptoms of IFN-β treatment in MS patients. The results show that IFN-β injection in MS patients is related to headache and flu-like pain symptoms. However, more randomised double-blind, placebo-controlled trials are needed to investigate pain symptoms in IFN-β-treated patients. Additionally, standard pain measurement tools must be used to evaluate the pain intensity.

CONSENT FOR PUBLICATION

Not applicable.

STANDARDS OF REPORTING

PRISMA guidelines & methodologies were followed.

FUNDING

Fellowships from the Conselho Nacional de Desenvolvimento Científico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) are also acknowledged. L.G.P is recipient of a fellowship CAPES [#88887.570091/2020-00]. G.T is recipient of a fellowship from CNPq [process #303531/2020-7] and funding of Prêmio Capes de Teses - Ciências Biológicas II, CAPES, 2014 [processo #23038.006930/2014/59].

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material and PRISMA checklist are available on the publisher's website along with the published article.

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