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Review Article

Sex Hormone Therapy in Multiple Sclerosis: A Systematic Review of Clinical Trials

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ABSTRACT

In spite of the observed immunomodulatory properties of different sex hormones on Multiple Sclerosis (MS) in different investigations, to date, there has been no study to systematically review the documents to add more powerful data to the field. Therefore, in this paper we aim to systematically review clinical and Randomized Controlled Trials (RCT) assessing the effect of sex hormone therapies on individuals with MS. A comprehensive search of electronic databases including PubMed, EMBASE, and Scopus was conducted. Clinical trials and RCTs that assessed the impact of sex hormones on individuals with MS were selected and included in the systematic review. In the final phase of the search strategy, 9 papers reached the criteria for entering in the systematic review. Two independent reviewers extracted the relevant data from each article according to the standardized data extraction form. Two reviewers also assessed the quality of each study independently using PEDro scale. We categorized three different classifications of outcomes including clinical, MRI, and immune system findings and put each measured outcome in the category which matched best. In conclusion, the existed investigations on the effect of sex hormones on inflammatory and neurodegenerative components of MS are promising particularly in Relapsing-Remitting MS (RRMS).

Keywords: Multiple sclerosis; Sex hormones; Inflammatory; Neurodegenerative

INTRODUCTION

Multiple Sclerosis (MS) is a demyelinating inflammatory disorder causing a wide range of potential symptoms including impairments in vision, motor ability, sensation and balance. MS has a significant impact on the affected individual, families and society due to presentation of the symptoms usually at a highly productive stage of the person's life. According to estimates, the prevalence of MS has 30% increases in 2020 in comparison to 2013, affecting 2.8 million people worldwide [1]. The exact etiology of MS remains incompletely recognized and subsequently a definite cure is still lacking [1].

It has been described that the pathogenesis of MS has both inflammatory and neurodegenerative components [2]. The exact pathological mechanism of inflammatory component is not fully understood but peripheral activation of auto-reactive CD4+ cells which cross Blood Brain Barrier (BBB) and attack neuronal myelin sheath in the Central Nervous System (CNS) has been theorized as one of the key mechanisms in this component [3]. Upon recognizing myelin antigens, a chronic inflammatory cascade initiate, resulting in demyelination of axons, formation of demyelinated white matter plaques and astrocytic scars [4]. In addition, diffuse axonal damage, long-term disease progression, and permanent disability is the hallmark of neurodegenerative component [2,5]. However, whether neurodegeneration component is directly associated with inflammatory component is not clear yet. Axonal damage may occur separately from white matter inflammatory lesions and intense immunosuppressive medications are not usually sufficient to stop neurodegeneration [6,7].

On the other side of story, over the last two decades, neuroimaging

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and neuropathological studies have indicated another pathological mechanism which can be attributed to neurodegeneration in MS [8-10]. Several studies have represented gray matter atrophy to have stronger correlation with clinical disability and neurodegeneration than white matter lesions and inflammation [5,11,12]. There are studies suggesting gray matter atrophy as a surrogate marker for neurodegeneration and progression of disability in patients with MS [13,14]. Fisher et al., [14] in a neuroimaging study have demonstrated that gray matter tissue lesions govern the pathological process as MS progresses underling neurological disability [14]. However, they represented that the mechanisms differ in Relapsing-Remitting MS (RRMS) and Secondary Progressive MS (SPMS). It is worth noting that while effective anti-inflammatory medications have the potential to improve white matter lesions; gray matter atrophy may benefit less form such medications [15,16]. Given together, the need for shedding more light on novel treatment options combining anti-inflammatory and neuroprotective effects seems necessary.

Throughout the last two decades, sex hormones such as testosterone and estrogen (estriol or 17β-estradiol) have been administered as neuroprotective therapies to effectively target gray matter atrophy and prevent permanent disability based on two well-established clinical observations [2]. First, MS has a higher prevalence in females than males and second, during pregnancy particularly in the third trimester a decrease in disease activity occur [2]. Sicotte et al., [17] in a clinical trial study treated non-pregnant women with MS with oral estriol (8 mg/day). Their results showed that the pregnancy hormone estriol significantly decreased delayed type hypersensitivity responses to tetanus, interferon-gamma levels in peripheral blood mononuclear cells, and gadolinium enhancing lesion numbers and volumes based on monthly cerebral imaging in RRMS patients [17]. Furthermore, Gold et al., [18] treated ten men with MS with 10 g of gel containing 100 mg of testosterone in a cross over design [18]. They showed that testosterone has the potential to significantly reduce DTH recall responses inducing a shift in peripheral lymphocyte composition through decreasing CD4+ T cells and increasing NK cells. In addition, testosterone administration produced BDNF and PDGF-BB supporting the potential neuroprotective and immunomodulatory effects of this sex hormone for central nervous system [18].

Although the immunomodulatory properties of testosterone, progesterone and estrogen (estriol or 17_{β}-estradiol) have been well explored in the literature, to date, there has been no study to systematically review the existed data in order to add more powerful results to the field. Therefore, in this paper we aim to systematically review clinical and Randomized Control Trials (RCT) assessing the effect of sex hormone therapies on MS. Undoubtedly, our results could better establish the strength of evidence and formulate suggestions for future researches in the field of interventional strategies for patients with MS.

MATERIALS AND METHODS

Data source and searches

The search strategy, study selection, data extraction and analysis were conducted and reported according to standards of the Preferred Reporting Items for Systematic review and Meta-Analysis guidelines (PRISMA) in this study [19]. A comprehensive search of electronic databases including PubMed, EMBASE, and Scopus was conducted. Administrating the search strategy of population and intervention approach, the key terms and words were as follows: "Multiple Sclerosis" or "Autoimmune Diseases of the Nervous System" or "Demyelinating Autoimmune Diseases" or "Chronic Progressive, Multiple Sclerosis" or "Relapsing-Remitting, Multiple Sclerosis" or "Primary Progressive, Multiple Sclerosis" or "Secondary Progressive, Demyelinating Diseases" and "Gonadal Hormones" or "Sex hormones" or "Corpus Luteum Hormones" or "Gonadal Steroid Hormones" or "Estradiol Congeners" or "Progesterone Congeners" or "Testosterone Congeners" or Estr* or Testosterone or Progesterone and therap* or intervention or effect and "clinical trial" or "randomized control trial" or "open label study" or "open label clinical trial" or "open label trial" or "clinical study". A further manual search of references in review articles was conducted to find relevant investigations.

Eligibility criteria and study selection

Clinical trials and RCTs that assessed the impact of sex hormones on individuals with MS were selected and included in the systematic review. The inclusion criteria were to (1) be a clinical trial or RCT study (2) have MS as their exposure of interest (3) apply any type of sex hormones as their intervention of interest (4) be published between 2000-2022 and (5) be published in the English language. Animal studies, case reports, observational studies, letters, reviews, presentations or any reports without sufficient data were excluded from the study.

In the primary search and after removing the duplicates, 11503 studies were entered to the library. According to title and abstract checking 11450 papers were excluded and 53 papers were selected to be screened on their full text. In the final phase, 9 papers reached the criteria for entering in the systematic review. Papers were excluded for: using a sample not comprised individuals with MS (11467), not assessing the impact of sex hormones (547), being of a non-empirical nature (5), being a review study (4478) and assessing animals as their exposure of interests (534). Due to the fact that numerous articles had the combination of such categories, the number of each excluded group is approximate. Any inconsistencies or disagreements between two independent screeners who searched and selected the articles were resolved by discussion.

Data extraction

Two independent reviewers extracted the following data from each article according to the standardized data extraction form. Publication details (including the first author's name, publication's year, and the country in which the study conducted), study details (including study design, number of participants, gender of participants, age of participants, disease stage, disease type, and control group characteristics), intervention details (including type of sex hormone, duration of therapy, follow-ups, control interventions, and the reported outcomes), and moderator variables (including participant retention and dropouts, participant adherence, and adverse effects associated with sex hormones) were extracted from each selected study. A Non-Available (NA) or Not-Reported (NR) statement was assigned in cases in which supplementary methodological information was not provided either from the article or corresponding author's contact. Disagreements between the reviewers on the extracted data were resolved by a third reviewer or consensus-based discussion.

Quality assessment

We administered PEDro scale in order to rate independently each article for quality by two reviewers [20]. The PEDro scale which is developed to evaluate the methodological quality of intervention studies is an 11 points scale evaluating the following aspects: specific eligibility criteria, randomization allocation, concealed allocation, baseline demographic similarities, participant blinding, therapist blinding, outcome assessor blinding, whether more than 85% of participants completed follow-up for at least 1 primary outcome, intention to treat analysis, between group statistical comparisons, and point estimates and variability for at least one of the primary outcome measures. Five of the included studies were RCT studies and the remaining four studies were clinical trials. The quality of all selected studies was assessed by two independent reviewers. Discrepancy between two reviewers' opinion were resolved by discussion.

RESULTS

Study selection and characteristics

According to our primary search and after removing duplicates, 11503 articles were found. Title and abstract checking resulted in removal of 11450 papers and therefore 53 papers were entered to the phase of full text checking. Finally, nine articles meet the inclusion criteria for being systematically reviewed. Of the nine final studies, seven studies were clinical trials and the remaining three studies were RCTs. The main reasons for removing the articles were as follows: using a sample not comprised individuals with MS (11467), not assessing the impact of sex hormones (547), being of a non-empirical nature (5), being a review study (4478) and assessing animals as their exposure of interests (534). The articles were published in three different countries including USA (6 studies), France (2 studies), and Italy (1 studies) between 2002 and 2021. More than half of the articles (6 studies) evaluated the effect of sex hormones on women with MS while the remaining (3 studies) assessed men with MS (no study evaluated both). In total 482 individuals with MS were included in the studies with a mean sample size of 43.81 (Standard Deviation (SD): 57.87 range: 4-150). In addition, the age range of individuals with MS was almost defined in all selected studies (M: 41.3, SD: 6.03) except in one study [21]. Six studies examined the effect of sex hormones on females with MS and the remaining three studies assessed on males. No study investigated both genders. Tables 1-3 represents details and characteristics of the selected studies.

Quality assessment

Table 4 shows the results of quality assessment of clinical trial and RCT studies. The mean of scores for quality assessment was 8.24 (SD: 1.67) for all selected studies. Both reviewers agreed on the rating of all selected studies and therefore a high interrater reliability was achieved.

Table 1: Basic and demographic characteristics of selected clinical trials and RCTs.

S. No	Author/year	Country	Number of participants (exposed or cases)	Gender	Study design	Age (M)	Disease type and stage	Control group	Adjusted or controlled for
1	Sicotte et. al. 2002	USA	10	Female	Clinical trial; Cross over	44	RR (n=6) and SP (n=4)	Healthy female (n=6)	Pregnancy Taking oral contraceptives Receiving hormone replacement therapy
2	Soladn et al. 2003	USA	10	Female	Clinical trial; Cross over	44	RR (n=6) and SP (n=4)	NR	Pregnancy Taking oral contraceptives Receiving hormone replacement therapy Not receiving steroid treatment for at least 3 month/ IFN beta/glatiramer acetate for 6 months before participating in the study Receiving hormone replacement therapy
3	Sicotte et. al 2007	USA	11	Male	Clinical trial	46	RR	NR	Not receiving disease-modifying treatment Having an expanded disability status scale score of 0-5.0 Having at least 1 clinical relapse Having at least 1 greater than 3-mmgadolinium-enhancing lesion on brain MRI during the preceding 2 years

4	Gold et al. 2008	USA	10	Male	Clinical trial; Cross over	46	RR	NR	Not receiving disease-modifying treatment Having an expanded disability status scale score of 2
5	Vukusic et. al 2009	France	150	Female	RCT (computer-generated block randomization schedule)	NR	RR, SP	MS patients (n=150)	Age <18 years Having clinical isolated syndrome not fulfilling Mac Donald's criteria for MS, Primary progressive MS, Possible MS or no MS according to Mc Donald's criteria Having on-going or previous myocardial infarction, stroke or venous thromboembolism Having on-going or previous breast cancer, or cancer of the uterus, Severe liver disorder, Undiagnosed genital bleeding Having hypersensitivity to one of the study treatment Desire for lactation, desire for a MS disease-modifying treatment in the 24 weeks after delivery
6	Kurth et al. 2014	USA	10	Male	Open label clinical trial; cross over design	46	RR	subjects served as their own controls	Not receiving disease-modifying treatment
7	Voskuhl 2016	USA	83	Female	Clinical trial; randomized double-blind, placebo-controlled phase 2	37	RR	RRMS patients (n=81)	Not having progressive MS Taking glatiramer acetate for more than 2 months before randomization Smoking Taking other concurrent disease-modifying or hormonal treatments
8	De Giglio et al. 2017	Italy	48	Female	RCT	30	RR	RRMS patients (n=46)	Not having relapses or steroid intake in the previous 60 days Not having pathologies of the reproductive system/pregnancy/interruption of pregnancy in the previous 12 months prior immunosuppressive therapy Taking glatiramer acetate, IFN beta or any experimental drugs before entry into the study Taking estroprogestins in the previous 3 months Severe psychiatric illnesses, including severe depression, alcohol or substance abuse
9	Vukusic et al. 2021	France	150	Female	RCT	32	RR or SP	RR/SP MS patients (n=150)	Having a Clinically Isolated Syndrome not fulfilling MacDonald's criteria, primary progressive MS, Having ongoing or previous myocardial infarction, stroke, venous thrombo-embolism, breast or uterine cancer, severe liver disorder, undiagnosed genital bleeding. Hypersensitivity to one of the study treatments Willing to breastfeed, start a DMT in the 24 weeks after delivery Refusing a non-hormonal contraception in the 12 weeks following delivery

Table 2: Therapeutic features and characteristics of selected clinical trials and RCTs.

S. No	Author/year	Type of sex hormone and dosage	Measures	Duration of therapy/follow-ups	Adverse effects	Tapering off
1	Sicotte et. al. 2002	Estriol (8 mg/ day) and progesterone (100 mg/day)	Immune response: Delayed type hypersensitivity (DTH) responses to tetanus, candida, and levels of IFN-. Imaging: The number and volume of new and total gadolinium-enhancing lesions clinical and cognitive measures: EDSS, Nine-Hole Peg Test, and PASAT cognitive testing scores	6 months/6 months	NR	Each week for 2 weeks the dose was decreased by half before entering the post-treatment period
2	Soladn et al. 2003	Estriol (8 mg/ day) and progesterone (100 mg/day)	Immune response: Cytokine profiles of stimulated PBMCs by means of intracellular cytokine staining (IL-5, IL-10, IL-12 p40, TNF alpha, and IFN), cytometric bead array (IL-2, IL-4, IL-5, IL-10, TNF, and IFN. Imaging: The number and volume of new lesions	6 months/ 6 months	NR	over 2 week after the treatment period, the oral estriol dose was decreased
3	Sicotte et. al 2007	Daily treatment: 10 g of the gel containing 100 mg of testosterone	Clinical measures: disability and cognition (the Multiple Sclerosis Functional Composite and the7/24 Spatial Recall Test) Imaging: Enhancing lesion activity and whole brain volumes	12 months/ 1-12 months after initiation of treatment	NR	NR
4	Gold et al. 2008	Daily treatment: 10 g of the gel containing 100 mg of testosterone	Immune response: 1. lymphocyte subpopulation composition by means of flow cytometry and *ex vivo* protein production of cytokines (IL-2, IFN gamma, TNF alpha, IL-17, IL-10, IL-12p40, TGFbeta1), as well as growth factors (BDNF, PDGF-BB, NGF, and CNTF)	12 months/1-12 months after initiation of treatment	NR	NR
			2. In vivo functional immune measure: DTH skin recall tests Cognitive function: PASAT			
5	Vukusic et. al. 2009	Progestin, Estradiol (10 mg per day)	Clinical assessment: 1. Relapses (date of onset, symptoms, level of certainty, level of severity, corticosteroid treatment, hospitalization).	12 weeks/12 weeks	NR	NR
			2. Disability (EDSS scale). Imaging: MRI measures Biological assessment: hormonal levels, cytokine array and gene array			
6	Kurth et al. 2014	Testosterone (100 mg per day; topically	Imaging: Focal gray matter loss as a marker for neurodegeneration by means of voxel-based morphometry	12 months/ NR	NR	NR
7	Voskuhl et al. 2016	Daily oral estriol (8 mg), injectable glatiramer acetate 20 mg daily	Clinical assessment: 1. annualized confirmed relapse rate at 24 months, time to first confirmed relapse, annualized relapse event rate, as well as time to first relapse event. 2. Questionnaires: EDSS, Modified Fatigue Impact Scale (MFIS) score, Beck Depression Inventory (BDI) score, Multiple Sclerosis Quality of Life (MS QoL) score, Multiple Sclerosis Functional Composite (MSFC) PASAT score. Imaging: enhancing lesions, T2 lesions, brain volume	24 months/NR	The number of patients with severe adverse effects did not differ considerably between the estriol group and the placebo group	A 4-week tapering plan began after 24 months of treatment

8	De Giglio et al. 2017	Group 1: IFN beta only Group 2: IFN beta, 20 mcg ethinylestradiol, 150 mcg desogestrel Group 3: IFN beta, 40 mg ethinylestradiol and 125 mg desogestrel	Clinical assessment: Rao's Brief Repeatable Battery for assessing cognitive function. Imaging: enhancing lesion activity	NR/12 and 24 months	Group 3 showed significantly getting worse on the sexual function subscale of the 54-item MS quality of life questionnaire at month 24 post-treatment	
9	Vukusic et al. 2021	NOMAc, first 2weeks post-partum: one tablet of 5 mg daily. The next 10 weeks: 10 mg tablets in combination with weekly transdermal 17-beta-estradiol, 75 µg since day 15	Clinical assessment: Annualized relapse rate in the first 12 weeks after delivery Imaging: MRI measures	pregnancy/0-24 months post-partum	Four serious adverse events on placebo and six in the sex steroid group were reported. Six adverse effects were related to pregnancy and delivery, and two were related to MS. Table S2 in(31)	NR

Note: PBMC: Peripheral Blood Mono-Nuclear Cells.

Table 3: Outcomes of selected clinical trials and RCTs based on three categories of immune system function, Imaging/MRI findings and clinical assessments.

S. No	Author/year	Immune response	Imaging/MRI findings	Clinical measures
1	Sicotte et. al. 2002	After 6 months of treatment DTH responses to tetanus were considerably decreased. The reduction in the candida response did not meet significance. IFN /actin was reduced considerably at month 12 during treatment in the RR group, but not the SP group	The total volume and number of enhancing lesions for all 10 MS participants (6 RR, 4 SP) reduced (p=0.008)	Relapses showed no significant alterations during the study. EDSS and Nine-Hole Peg Test scores represented no major changes. PASAT cognitive testing scores were considerably enhanced in the RR group but not in the SP group.
2	Soladn et al. 2003	Levels of IL-5 and IL-10 significantly increased during estriol treatment TNF decreased considerable during estriol treatment. Changes were mostly robust in the RRMS group in comparison to SPMS group	Enhancing lesion volume and number were significantly decreased (months 7–12) compared with pretreatment baseline (months 1–6) in the RRMS, but not the SPMS group	NR

3	Sicotte et. al 2007	NR	Brain atrophy slowed to an annualized rate of -0.26% 9 months post-treatment representing a 67% reduction in the rate of brain volume loss in comparison to the pretreatment period. Gadolinium-enhancing lesion numbers or volume did not differ significantly	PASAT scores increased after 9 months and were considerably upgraded by month 12 of treatment. Spatial memory was improved in immediate and delayed recall subtests of the 7/24 Spatial Recall Test at treatment month 12. No alterations were measured in the EDSS score, the 9-HolePegTest, or the 25-ft timed walk.
4	Gold et al. 2008	DTH recall responses were significantly reduced A shift in peripheral lymphocyte composition was induced by decreasing CD4+ T cell percentage and increasing NK cells. PBMC production of IL-2 was expressively decreased. TGFbeta1 production was enhanced. PBMCs attained during the treatment period produced considerably more BDNF and PDGF-BB.	NR	Cognitive function as measured by PASAT was improved significantly.
5	Vukusic et. al 2009	NR (study is on-going)	NR (study is on-going)	NR (study is on-going)
6	Kurth et al. 2014	NR	Gray matter significantly increased in the right frontal cortex. A reduction in enhancing lesions was not obtained due to the fact that the level of enhancement at baseline in the cohort was low	NR
7	Voskuhl et al. 2016	NR	Pre-specified endpoints related to enhancing or T2 lesions or whole brain volume did not differ between groups. A difference between groups at 12 months for cortical grey matter and white matter was observed by Post-hoc analyses. Estriol group had larger cortical grey matter volume than placebo group for patients without enhancing lesions at baseline.	The annualized confirmed relapse rate was significantly reduced in the estriol group compared with placebo group. Time to confirmed relapse decreased significantly in the estriol group than in the placebo Time to relapse event was not decreased. Irregular menses were higher in the estriol group than in the placebo group Vaginal infections were lower in the estriol group than in the placebo group Fatigue was decreased significantly with estriol compared with placebo group after 24 months. Cognitive testing (PASAT scores) represented no changes at 24 months. PASAT scores increased significantly in the estriol group than in the placebo group at 12 months
8	De Giglio et al. 2017	NR	NR	Group 3 had a lower number of patients with cognitive impairment than Group 1 at month 24). Cognitive impairment was lesser in Group 3 over 24 months. Mood and fatigue scores were equivalent across the groups at both time points.
9	Vukusic et al. 2021	NR	No differences were observed in terms of secondary clinical and MRI outcomes.	Post-partum relapses did not change

Table 4: Quality score of selected clinical trial and RCT studies.

Author/year	Study type	Specific eligibility criteria	Randomization allocation	Concealed allocation	Baseline demographic similarities	Participant blinding	Therapist blinding	Outcome assessor blinding	85% of participants completed follow-up for at least 1 primary outcome	Intention to treat analysis	Between group statistical comparisons	Point estimates and variability for at least one of the primary outcome measures	Total score
Sicotte et. al. 2002	Clinical trial	1	1	0	1	0	0	1	1	1	1	1	8
Soladn et al. 2003	Clinical trial	1	0	0	1	0	0	1	1	1	1	1	7
Sicotte et. al 2007	Clinical trial	1	0	0	1	0	0	1	1	1	1	1	7
Gold et al. 2008	Clinical trial	1	0	0	1	0	0	1	1	1	1	1	7
Vukusic et. al 2009	RCT	1	1	1	1	1	1	1	1	1	1	1	11
Kurth et al. 2014	Clinical trial	1	0	0	1	0	0	1	1	1	0	1	6
Voskuhl et al. 2016	Clinical trial	1	1	1	1	1	1	0	1	1	1	1	10
De Giglio et al. 2017	RCT	1	1	1	1	1	0	1	1	1	1	1	10
Vukusic et al. 2021	RCT	1	1	1	1	1	1	0	1	1	1	1	10

Dosage and type of sex hormones across selected studies

Three RCTs and three clinical trials used different forms of estrogen including estriol, estradiol, ethinylestradiol, and transdermal 17-beta-estradiol. In addition, two RCTs and two clinical trials administered synthetic forms of progesterone such as progestin, desogestrel, and NOMAc. The remaining three clinical trials used testosterone in order to investigate the effect of sex hormone on individuals with MS. Five out of nine studies administered the combination of two different sex hormones and the remaining only used a single hormone. Estriol was administered 8 mg-day in all three studies which assessed the impact of this hormone in individuals with MS. In contrast a wider spectrum of dosage was used across three studies using forms of estradiol including 75 micrograms weekly, 8 mg daily, and 10 mg daily. Moreover, synthetic forms of progesterone were also used in different dosages of 10 mg/daily, 100 mg daily, and 125 mg daily. However, the dosage for testosterone was fixed across three studies administrating the effect of testosterone on individuals with MS with 100 mg/day.

Outcome measures

Table 3 shows details on the main findings of selected studies in the three categories. Different outcomes were measured following sex-hormone therapy across nine selected studies. Generally, we categorized three different classifications of outcomes including clinical, MRI, and immune system findings and put each measured outcome in the category which matched best. In one study, Vukusic et al., [21] reported no results in none of the categories since it is an on-going RCT project on the effect of progestin and estradiol in post-partum relapses [21]. Two studies measured outcomes of all three categories, one study assessed only one category and the remaining assessed a combination of two categories. Clinical assessments commonly consisted of batteries of cognitive function examination, disability (eg. EDSS scores), date and onset of relapses, symptoms, level of severity, fatigue, depression, quality of life, and functions of daily living. In the category of MRI finding, outcomes of the number and volume of new and total gadolinium-enhancing lesions, T2 lesions, enhancing lesion activity, whole brain volumes, and focal gray matter loss. In the final category,

measures of immune system modulation followed by sex-hormone therapy consisted of DTH responses to tetanus and candida as well as levels of IFN-alpha, Cytokine profiles of stimulated PBMCs determined by intracellular cytokine staining (IL-5, IL-10, IL-12 p40, TNF , and IFN), cytometric bead array (IL-2, IL-4, IL-5, IL-10, TNF, and IFN, lymphocyte subpopulation composition by flow cytometry, *ex vivo* protein production of cytokines (IL-2, IFN gamma, TNF alpha, IL-17, IL-10, IL-12p40, TGFbeta1) and growth factors (BDNF, PDGF-BB, NGF, and CNTF).

Clinical assessments: Six out of nine studies (four clinical trials two RCTs) evaluated outcomes relevant to clinical assessment category. In regard to frequency and duration of relapses, two studies (one RCT and one clinical trial) showed no significant changes, while one study showed that annualized confirmed relapse rate was significantly reduced in the estriol group in comparison to placebo group [22]. In addition, regarding cognitive function five studies reported a significant improvement in scores of different cognitive assessments. In this vein, five studies showed positive impact of sex hormone therapy on PASAT scores (one study showed positive impact only in RRMS and not in SPMS), one study on spatial memory test while two studies showed no effect for nine-hole peg test. Moreover, two studies showed a significant improvement in symptoms of fatigue and mood imbalance following sex hormone therapy.

MRI findings: Six out of nine studies (five clinical trials and one RCT) evaluated outcomes relevant to MRI findings category. In regard to number and volume of new enhancing GAD lesions, two studies showed improvements (one study showed improvement only in RRMS group and not in SPMS group) while two other studies showed no significant difference before and after sex-hormone therapy. One study showed an improvement in brain volume loss while study showed no difference in whole brain volume before and after sex-hormone therapy. Moreover, two studies showed improvements in gray matter volume following sex-hormone therapy.

Immune system: Three out of nine studies (clinical trials) examined outcomes relevant to immune system category. Two studies showed decreased DTH responses to tetanus following sex hormone therapy. One study showed interferon-alpha levels in peripheral blood mononuclear cell in RRMS not in the SPMS group. Another study also showed an increase in levels of IL-5 and IL-10 as well as a decrease in TNF-alpha following sex hormone therapy which were more prominent in RRMS than SPMS group.

DISCUSSION

The effect of sex hormone therapy in individuals with MS has been explored in numerous investigations. However, to our knowledge this is the first study systematically reviewed the outcomes of clinical trials and RCTs in MS. Although single investigations reported such a possible association, a comprehensive and systematically conducted study can add more valuable insight to the field. We summarized findings with regards to clinical assessments, MRI findings, and immune system outcomes.

All identified studies in the current systematic review, examined RRMS while four out of nine selected studies investigated the effect of a particular type of sex hormones on both RRMS & SPMS females. Interestingly in such studies which assessed both SPMS and RRMS individuals, the outcomes were more robust in females with RRMS than SPMS. Among these four studies, two studies [23,24] showed the positive impact of estriol on MRI findings and immune system outcomes only in RRMS group. Moreover, one out of these two studies showed no significant effect of estriol on EDSS scores and relapses. Vukusic et al., [21] in their recent investigation with a larger sample size (n=150) also failed to show the efficacy of progestin and transdermal 17-beta estradiol on both RRMS and SPMS groups in terms of preventing post-partum relapses in MS. Other studies recruiting only RRMS individuals showed improvements in different aspects of brain structure such as a decrease in brain atrophy, immune system function such as decrease in DTH, and cognitive function such as improvements in PASAT scores and fatigue symptoms. In regard to the impact of a particular type of sex hormone, we classified the results attributed to each sex hormone in two categories of testosterone and steroids in the following.

Testosterone

Previous estimates showed that approximately 40 percent of men with MS have low levels of testosterone which is correlated with their physical and cognitive disability as well as worse clinical outcomes [25]. In the current systematic review, three out of nine studies assessed only males [26-28]. The sex hormone which was evaluated in these three studies was testosterone and the type of MS was RRMS. Among these three studies, one study evaluated both MRI findings and clinical assessments, another study evaluated only immune function and the last study examined only MRI findings, 12 months after intervention had been started. Regarding MRI findings, their cumulative results showed that while testosterone could significantly improve brain atrophy, brain volume loss, and gray matter volume, no effect was observed in GAD lesions. Moreover, in terms of immune function outcomes, Gold et al., [18] showed prominent improvements in DTH response, peripheral lymphocytes, CD4 positive T cells, and increase in Natural Killer (NK) cells, TGF beta, and Brain Derived Neurotrophic Factor (BDNF). In addition, Sicotte et al., [17] represented positive effect of testosterone on PASAT scores, and spatial memory tests, while there was no significant improvement regarding EDSS and Hole-peg test scores on males with RRMS. Therefore, one can hypothesize a potential neuroprotective effect based on the increase in BDNF and platelet-derived growth factors following testosterone intervention in men with MS.

Steroid combinations

Five out of nine studies investigated the effect of steroid combinations (i.e., estrogen and progesterone) on females with both SPMS and RRMS. There was no study assessing the effect of steroids on men with MS. The cumulative results of these studies showed the positive impact of steroid combinations on immune system outcomes such as a decrease in DTH response, IFN/actin, and TNF as well as an increase in IL-5 and IL-10. In addition, two of these studies showed that steroid combinations could exert positive effect on reducing the number and volume of new enhancing brain lesions. It is worthy of note that the aforementioned findings were more robust in females with RRMS than SPMS. However, among the studies in this group, Vukusic et al., [27] failed to show any prominent difference before and after steroid therapy in females with both RRMS and SPMS. In addition, in terms of clinical outcomes, studies in this group showed improvements in mood and fatigue symptoms, and PASAT scores which were also more

prominent in females with RRMS. However, in term of EDSS scores, nine-hole peg test and relapses, these studies failed to represent any positive effect of steroid combinations on females with both RRMS and SPMS.

One remaining study conducted by Voskuhl et al., [22] assessed the effect of estriol without any other sex hormone combinations on females with RRMS. The results showed that estriol has positive impact on improving cortical and white matter volume, decreasing relapses, vaginal infection, fatigue symptoms and enhancing PASAT scores on females with RRMS.

Of the possible explanations for such positive effects of estrogen and progesterone is their neuroprotective, pro-myelinating and immunosuppressive mechanisms [29]. In animal models of Experimental Autoimmune Encephalomyelitis (EAE) which has many pathologic features similar to MS [30], estrogen has been shown to activate macrophages and microglia, increasing the induction of B cells. It is assumed that such regulatory feedback is one of the components of neuroprotection [31]. In addition, reducing immune response in the brain and regulating local growth factors, oligodendrocyte, and astrocyte function is attributed to the effects of estradiol and progesterone [32].

Possible mechanisms

Sex steroids seems to implement positive impacts on both inflammatory and neurodegenerative components of MS. One of the early indicators of the inflammatory component of MS is axonal injury caused by microglial activation and inflammation [33]. Altered microglia activation contributed to demyelination due to altered production of Nitric Oxide (NO) and tumor necrosis factor-alpha which is toxic to CNS cells [34]. In addition, proinflammatory cytokines induces hypoxia which in turn reduces phagocytosis in BV-2 microglia cells. Evidence has indicated that such pathologic mechanism can be prevented by sex hormones by means of acting directly on the microglia and astroglial regulation [35]. Besides, there is a high-affinity of steroid receptors in neurons and glia and the primary source of steroidogenic enzymes is asctrocytes which are in the rim and center of demyelinating lesions [36].

Sex steroids also regulate the ability of macrophages in participating in immune responses [37] and lower concentrations of sex steroids have been shown to be associated with higher serum levels of proinflammatory cytokines such as tumor necrosis factor-alpha and interferon-gamma. Moreover, studies on EAE have suggested the protective role of estrogen in gut microbiota changes [38]. In other words, the communication between gut microbiota and sex steroids may affect positive immune-regulation which results in neuro-protection.

It is worthy of note that while FDA-approved medications and treatments for MS diminish relapse and inflammation rate, but have not strong effects on re-myelination, gray matter atrophy and disability [26,35]. However, interestingly based on several MRI findings in the current systematic review, sex steroids may achieve re-myelination, and reduce gray matter atrophy. Several mechanisms may underlie such effect of sex hormones such as sex hormones' interaction with astroglia, insulin-like growth factor-1 and recruiting oligodendrocytes [39]. In addition, sex steroids modulate chemokine expression and signaling which is associated with demyelinating diseases [40-47].

CONCLUSION

In spite of possible valuable effect of sex hormones on pathological mechanisms of MS, many mechanisms and effects remain undefined yet in different pathological components and different types of MS. As represented in the current systematic review, the existed clinical trials and RCTs have shown a more robust impact of sex hormones on RRMS than SPMS. However, the exact justification of such observation is not debated yet. Besides, there was no clinical trial or RCT investigating the effect of sex hormone on Primary Progressive MS (PPMS). In addition, gender-specific responses to different sex hormone therapies are required more investigations due to gender-specific differences in responses to inflammation within CNS. For example, brain lesions in females with MS, the activation of 3β-hydroxysteroid-dehydrogenase, a precursor of progesterone and the progesterone receptor has been observed.

Moreover, regarding the appropriate combination of sex hormones and their effect on MS, there is also still poor information and remains to be studied. For example, previous evidence has shown that a high estrogen to progesterone ratio could give rise to a significantly greater number of active MRI lesions than a low ratio. Nevertheless, there is a paucity of data on sex hormone combinations in demyelinating diseases such as MS. Moreover, there are three estrogens with different strength including Estrone (E1), Estradiol (E2), and Estriol (E3) and the optimal efficacy of each one is yet to be defined for MS. In particular, immune-regulatory effect of estriol may be much more than estradiol. Besides, there are few studies investigating the effect of Estrone on different types of MS.

Given together, the existed investigations on the effect of sex steroids on inflammatory and neurodegenerative components of MS are promising. Different types of MS require therapeutic agents targeting improvement of neurodegenerative component and re-myelination as much as inflammatory component. However, further and more exact investigations are warranted to study such possibility of sex hormones either alone or in combination with other to induce re-myelination and consequently.

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