

Interferon- β 1a Modulates Expression of RAGE but Not S100A12 and Nuclear Factor- κ B in Multiple Sclerosis Patients

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Keywords

IFN- β 1a · Multiple sclerosis · S100A12 · RAGE · Nuclear factor- κ B

Abstract

Objectives: Interferon- β 1a (IFN- β 1a) is a common strategy therapy for multiple sclerosis (MS) with unknown mechanisms. S100A12 (S100 calcium-binding protein A12) is a damage-associated molecular pattern molecule which binds to its receptor, RAGE (receptor for advanced glycation end products), and activates nuclear factor- κ B (NF- κ B). NF- κ B is transcribed from proinflammatory molecules, which may participate in the pathogenesis of MS. Therefore, the aims of this study were to compare mRNA levels of S100A12, RAGE, and NF- κ B in newly diagnosed MS patients with healthy controls and determine whether IFN- β 1a therapy

affects the expression of the molecules. **Methods:** S100A12, RAGE, and NF- κ B mRNA levels in 30 new cases of untreated MS patients and 35 healthy controls were evaluated using the real-time PCR technique. The mRNA levels were also evaluated in the MS patients after 6 months of IFN- β 1a therapy. **Results:** S100A12, RAGE, and NF- κ B mRNA levels were significantly decreased in the new cases of untreated MS patients in comparison to healthy controls. IFN- β 1a therapy results in upregulation of RAGE in MS patients, but not S100A12 and NF- κ B. **Conclusions:** It appears that S100A12 participates in the pathogenesis of MS, and it seems that IFN- β 1a modulates immune responses in an S100A12-inde-

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pendent manner. Based on the reported anti-inflammatory effects of RAGE, it seems that RAGE may be considered as a mechanism by IFN- β 1a to modulate immune responses. NF- κ B is produced permanently in the human cells and is inactive in the cytoplasm; therefore, the effects of IFN- β 1a may be related to its functions rather than expressions.

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Introduction

Multiple sclerosis (MS) is an autoimmune/proinflammatory disease which is associated with attack of the immune system to the central nervous system [1, 2]. Epidemiological information demonstrated that the rates of MS are growing in developed and developing countries [3, 4]. Iran, as a developing country, is associated with increased incidence of the disease; in Kerman Province, MS is found in 57.3/100,000 of its population [5]. It has been documented that MS patients suffer from increased production of proinflammatory molecules including proinflammatory cytokines [6]. Previous investigations revealed that nuclear factor- κ B (NF- κ B) is an important molecule which is responsible for transcription from proinflammatory molecules [7]. While NF- κ B is in an inactive form in the cytoplasm immune cells, in binding with I κ k, and after I κ k phosphorylation and degradation, NF- κ B is activated and translocated to the nucleus and transcripts from the target genes [8]. Several intracellular pathways result in activation of NF- κ B, including the S100A12 (S100 calcium-binding protein A12)/RAGE (receptor for advanced glycation end products) pathway [9]. It has been reported that S100A12 is a damage-associated molecular pattern (DAMP) that is produced and released during several pathologic conditions [10]. S100A12 attaches to its cell membrane receptor, RAGE, which leads to activation of intracellular pathways and consequently activation of NF- κ B [10]. Thus, it may be hypothesized that S100A12, its corresponding receptor, and NF- κ B may participate in the pathogenesis of MS. Therefore, a main aim of this study was to evaluate the expression levels of S100A12, RAGE, and NF- κ B in new cases of untreated MS patients in comparison to healthy controls. Moreover, it has been demonstrated that interferon- β 1a (IFN- β 1a) can modulate immune responses in MS patients via unknown mechanisms [11, 12]. Based on the plausible roles of S100A12, RAGE, and NF- κ B in the pathogenesis of MS, another aim of this investigation was to evaluate the effects of IFN- β 1a therapy on the expression of the molecules.

Material and Methods

Subjects

This experimental investigation was carried out on 22 female and 8 male relapse-remitting MS patients and 25 female and 10 male healthy controls. Expression levels of S100A12, RAGE, and NF- κ B were evaluated in the MS patients before they received any medication and were compared to healthy controls. The patients were treated with CinnoVex (IFN- β 1a, pharmaceutical compositions of the CinnaGen Company) for 6 months, and after that the expression of S100A12, RAGE, and NF- κ B were examined again and compared to that before treatment with IFN- β 1a. MS was diagnosed based on radiology, laboratory, and clinical outcomes, as well as neurologist observations, according to the revised criteria of McDonald et al. [13]. The patients with the following criteria were excluded from the study: other autoimmunity/proinflammatory diseases such as type 1 and 2 diabetes, type 1–4 hypersensitivities, heart/kidney/liver failure, and patients receiving anti-inflammatory medications.

All participants sign an informed consent form prior to entering the project. Ethical research permission was obtained from the Ethics Committee of Kerman University of Medical Sciences (Code: k/92/587). IFN- β 1a (CinnoVex) was purchased from CinnaGen (Tehran, Iran).

Real-Time PCR Condition

Expression levels of S100A12, RAGE, and NF- κ B were evaluated using the real-time PCR technique. Accordingly, total mRNA was extracted from peripheral blood immune cells using RNX solution (CinnaGen, Tehran, Iran), cDNA was synthesized using a commercial kit from Parstoos Company (Mashhad, Iran), and real-time PCR was performed in a Rotorgen thermocycler (Qiagen, USA). The protocols of the mentioned experiments were described in our previous study [2, 14]. Table 1 lists the primer sequences for S100A12, RAGE, NF- κ B, and β -actin (as the housekeeping gene).

The $2^{-\Delta\Delta CT}$ formula was used for calculation of the relative expression of S100A12, RAGE, and NF- κ B between either untreated MS patients versus healthy controls or untreated versus MS patients treated with IFN- β 1a for 6 months.

Data Analysis

SPSS-18 software was used for analyzing the raw data. The differences between new cases of untreated MS patients versus healthy controls and also expression of the molecules in male and female within the groups were computed using a Student *t* test, and the differences between untreated versus MS patients treated for 6 months and also sex-dependent expression of the molecules were computed using a paired-samples *t* test. The results are reported as means $\pm$ SEM and *p* < 0.05 was considered to be significant.

Results

Real-time PCR analysis revealed that mRNA levels of S100A12 (*p* = 0.005), RAGE (*p* < 0.001), and NF- κ B (*p* < 0.001) were significantly decreased in the new cases untreated patients when compared to healthy controls (Fig. 1). The results revealed that IFN- β 1a therapy had

Table 1. Primer sequences of S100A12, RAGE, and NF-κB

S100A12	F	GGAGGGAATTGTCAATATC
	R	ATCTTGATTAGCATCCAGG
RAGE	F	GACCCTGGAAGGAAGCAG
	R	CCCCTTACACTTCAGCACC
NF-κB	F	TCTCCCTGGTCACCAAGGAC
	R	TCATAGAAGCCATCCCGGC
β-Actin	F	GGCACCCAGCACAATGAAG
	R	CCGATCCACACGGAGTACTTG

no effects on the expression of S100A12 in total ($p = 0.626$), male ($p = 0.091$), and female ($p = 0.690$) MS patients (Fig. 2).

The results also showed that expression levels of RAGE in total, male, and female untreated new MS patients were 0.46 ± 0.19 , 1.13 ± 0.34 , and 0.17 ± 0.05 , respectively, while the data after 6 months of treatment with IFN-β 1a were 3.26 ± 1.01 , 0.06 ± 0.02 , and 4.27 ± 1.23 , respectively. The statistical analysis revealed that the differences before and after treatment in total ($p = 0.018$), male ($p = 0.024$), and female ($p = 0.005$) MS patients were significant (Fig. 3).

Expression levels of NF-κB were not altered after 6 months of treatment with IFN-β 1a in total ($p = 0.728$), male ($p = 0.298$), and female ($p = 0.615$) MS patients (Fig. 4).

Discussion

S100A12 is a DAMP which induces inflammation via binding to its corresponding receptor [10]. It has been documented that DAMPs are produced and released during damage, such as infections and proinflammatory diseases [15]. The results revealed that expression levels of S100A12 were decreased in untreated MS patients in comparison to controls. Thus, based on the results, it seems that S100A12 can be considered as a DAMP for MS and participate in the pathogenesis of the disease. In contrast with our results, Glasnović et al. [16] reported that mRNA levels of S100A12 were not different between new MS patients and healthy controls. Therefore, it seems that S100A12 production by MS patients may be various in different ethnic population and more investigations are needed to shed light on our understanding of the mechanisms played by S100A12 in the pathogenesis of MS. Interestingly, IFN-β 1a therapy had no significant effects on the expression of the molecule. Thus, it seems that IFN-β

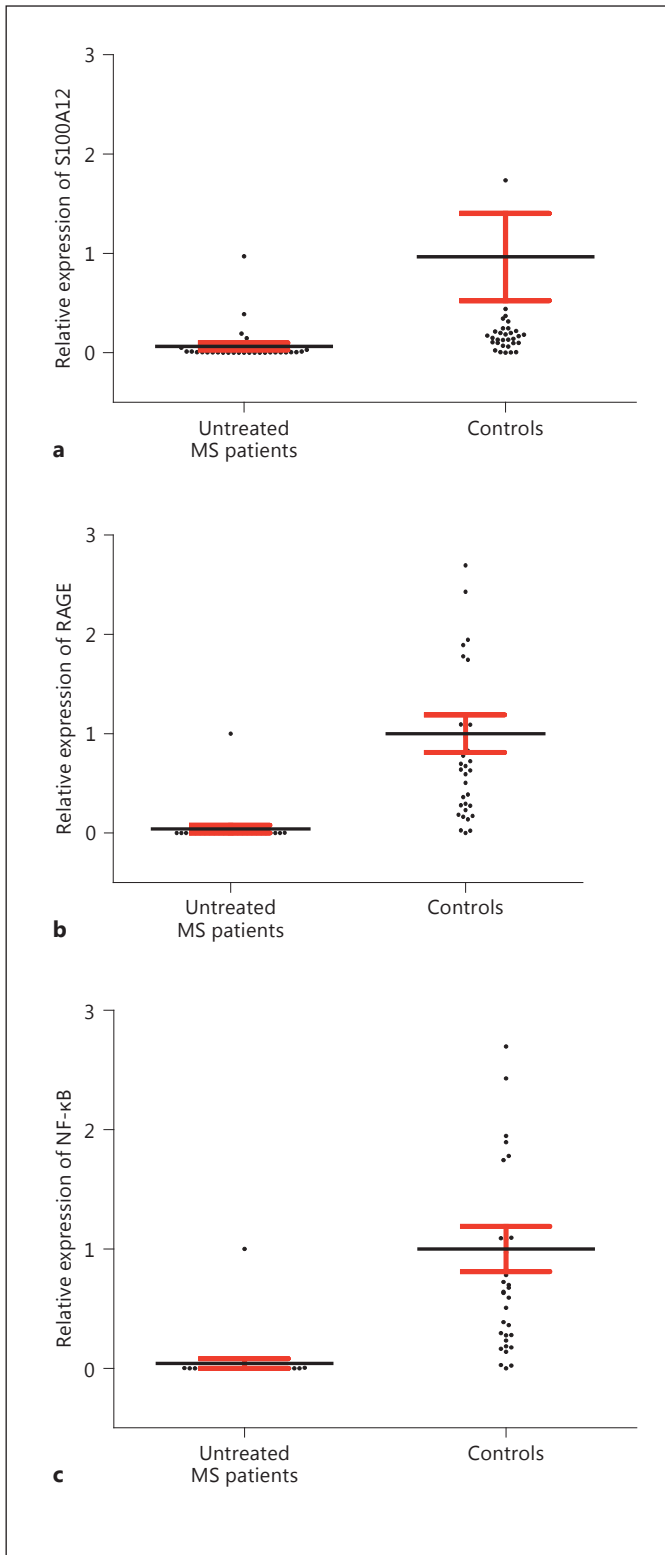


Fig. 1. Expression levels of S100A12 (a), RAGE (b), and NF-κB (c) in untreated MS patients compared to healthy controls. The figure shows mRNA levels of S100A12, RAGE, and NF-κB were significantly lower in MS patients than healthy controls.

1a modulates immune responses in an S100A12-independent manner.

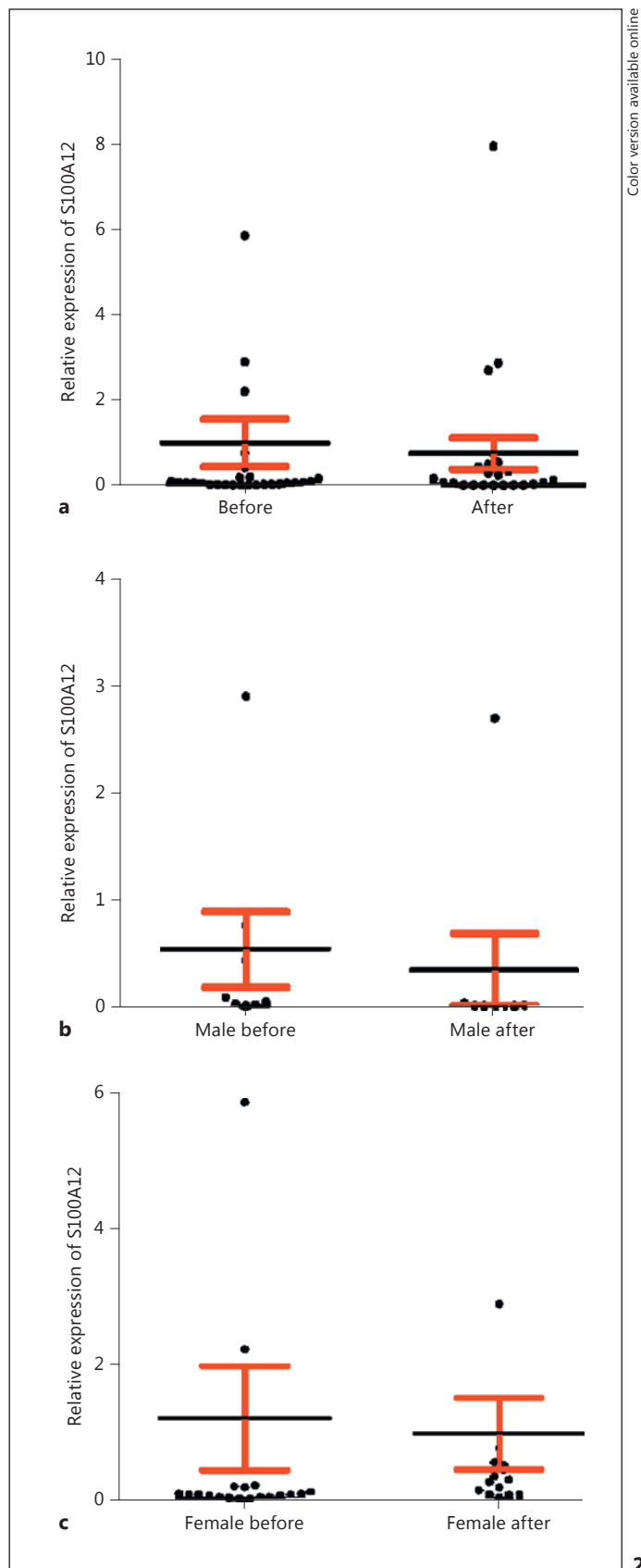
The results also showed that mRNA levels of RAGE, as the receptor of S100A12, were also decreased in MS patients in comparison to controls. The results also were confirmed by Glasnović et al. [16], who demonstrated that soluble RAGE was significantly decreased in the CSF of MS patients when compared to healthy controls. RAGE not only is a receptor for S100A12, but also binds to other molecules to induce intracellular signaling [17]. Additionally, its soluble form has anti-inflammatory effects [17], perhaps via trapping the proinflammatory ligands. Furthermore, results revealed that IFN- β 1a therapy leads to upregulation of RAGE in the MS patients. Therefore, it seems that RAGE may have anti-inflammatory effects and may be considered as a new MS treatment strategy. The results also demonstrated that IFN- β 1a therapy increased expression of RAGE in the female MS patients, but decreased the molecule in the male MS patients. Thus, it may be hypothesized that expression of RAGE is sex dependent and future treatment strategies need to take the gender of the patients into consideration. Parallel to these results, Tanaka et al. [18] reported that 17 β -estradiol and tumor necrosis factor- α (TNF- α) may cause increased levels of soluble RAGE in females. Furthermore, Nguyen et al. [19] showed that proinflammatory cytokines, such as TNF- α , are increased in female MS patients in comparison to male MS patients. Therefore, these differences may explain gender differences findings in the level of RAGE in our study. However, based on the fact that the number of males in the current investigation is lower than females, it appears that more investigations need to be performed to shed light on our understanding of the mechanisms played by IFN- β 1a to modulate expression of RAGE.

Fig. 2. Expression levels of S100A12 before and after treatment with IFN- β 1a in the total (a), male (b), and female (c) groups. S100A12 mRNA levels did not change 6 months after therapy with IFN- β 1a in total, male, and female MS groups. The data are reported as means $\pm$ SEM.

Fig. 3. Expression levels of RAGE before and after treatment with IFN- β 1a in the total (a), male (b), and female (c) groups. RAGE mRNA levels significantly increased in the total and female groups, while they decreased in the male group 6 months after therapy with IFN- β 1a. The data are reported as means $\pm$ SEM.

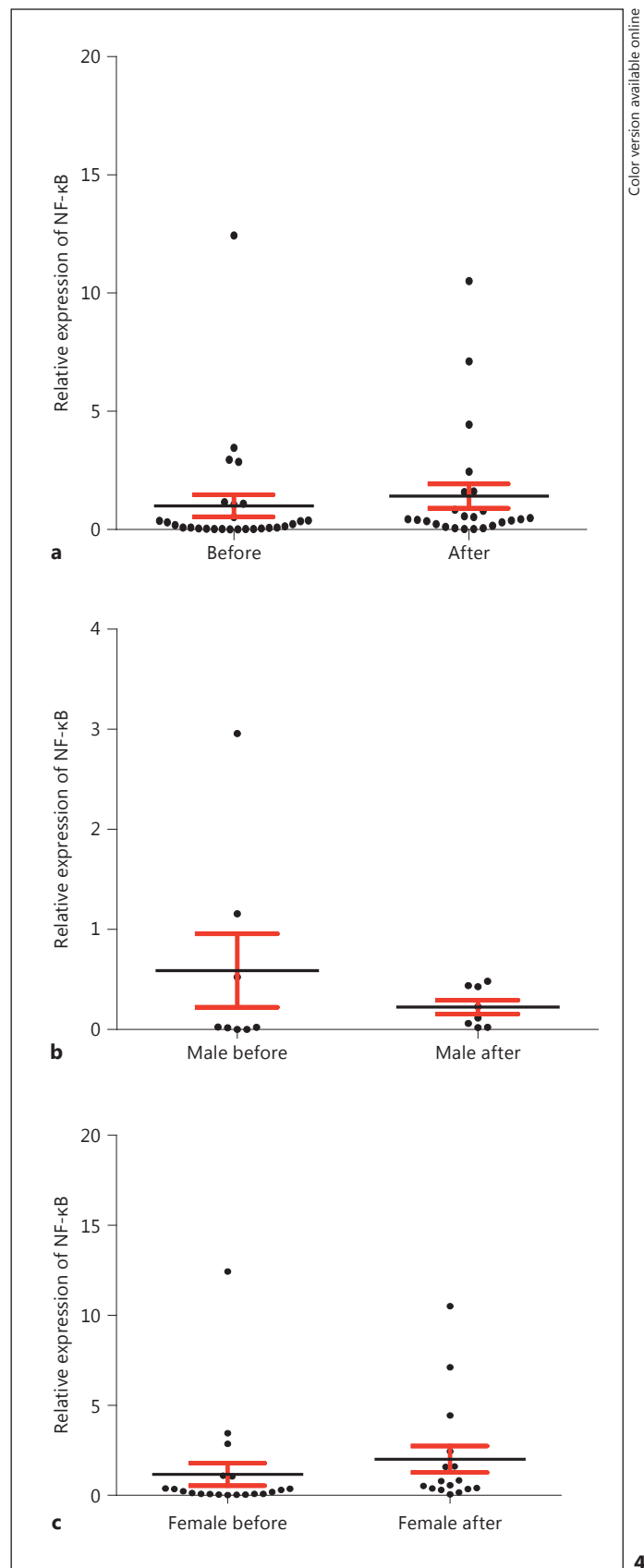
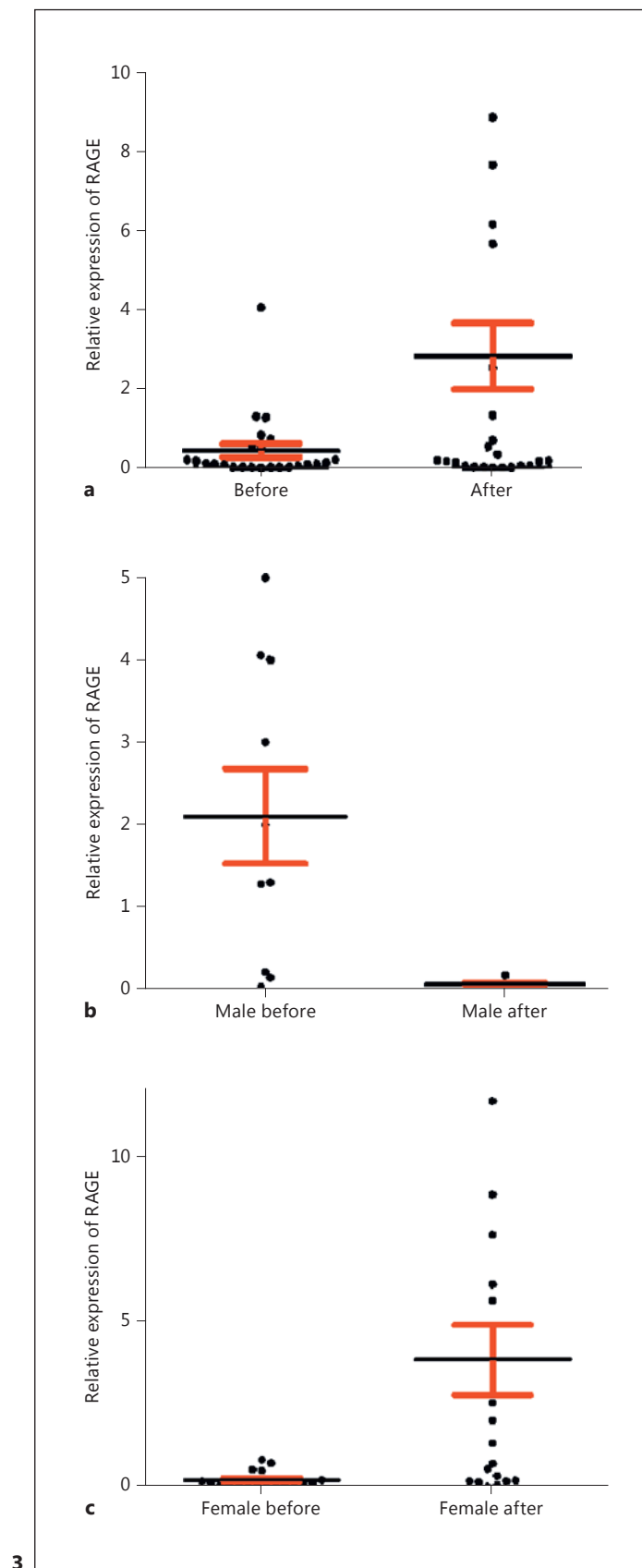
Fig. 4. Expression levels of NF- κ B before and after treatment with IFN- β 1a in the total (a), male (b), and female (c) groups. The figure demonstrates that 6 months of IFN- β 1a therapy had no effects on the expression of NF- κ B on total, male, and female MS patients. The data are reported as means $\pm$ SEM.

(For figures 3 and 4 see next page.)



Color version available online

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Data analysis showed that expressions of NF- κ B were significantly decreased in our MS population compared to controls. Based on the fact that NF- κ B is a target for several intracellular pathways [20, 21] and also according to the fact that the molecule has several strains [22], the investigations regarding expression levels of NF- κ B in MS patients are controversial [23–25]. Additionally, based on the fact that NF- κ B is produced and saved in its inactive form in the cytoplasm, the effects of exogenous factors on the functions of the molecule are associated with activation of NF- κ B (degradation of I κ B), rather than alteration in its expression. Thus, it may be hypothesized that the regulatory effects of IFN- β 1a on NF- κ B may be related to its functions rather than expression. In parallel with the conclusion, Eggert et al. [26] and Housley et al. [27] reported the increased functions of NF- κ B in MS patients. Interestingly, the Eggert et al. study approved the roles of anti-inflammatory drugs to decrease NF- κ B function and improve MS symptoms [26]. Collectively, it may be hypothesized that IFN- β 1a may decrease functions of NF- κ B rather than its expression in MS patients. Additionally, our previous investigations demonstrated that IFN- β 1a therapy leads to decrease expression of IL-1 β , as a target of NF- κ B, in MS patients [2]. Accordingly, it seems that although expression of NF- κ B was not changed after

IFN- β 1a therapy, its functions were decreased, which leads to downregulation of IL-1 β .

Taken together, it appears that although S100A12 participates in the pathogenesis of MS, it is not a target for IFN- β 1a therapy (in contrast to RAGE). The effects of IFN- β 1a on the functions of NF- κ B need to be evaluated in further investigations.

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Ethics Statement

This review article has been prepared according to the ethical roles of The Ethical Committee of Kerman University of Medical Sciences (Code: k/92 /587).

Disclosure Statement

The authors have no conflict of interest to declare.

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