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Nanocurcumin improves regulatory T-cell frequency and function in patients with multiple sclerosis

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ABSTRACT

Background: Multiple sclerosis is a chronic incapacitating disease of the central nervous system, it has been reported that the disturbance in the development and function of Treg subpopulations is associated with the disability status in the RRMS. Accordingly, in the current study, the objective was to specify nanocurcumin effects on Treg cells frequency, and function in patients with RRMS.

Methods and materials: 50 patients with RRMS were enrolled in this study in which 25 were treated for at least six months with nanocurcumin capsules while the other half received placebo capsules as the control group. The blood sample was collected prior to the administration of nanocurcumin and placebo capsules and following six months. At baseline and after a six-month treatment, the frequency of Treg lymphocytes, the expression of transcription factor related to these cells and the secretion levels of cytokines were assessed by flowcytometry, real-time PCR and ELISA, respectively.

Results: A significant reduction was observed in the proportion of peripheral Treg cell frequency, and the levels of TGF-β, IL-10 and FoxP3 expression in patients with RRMS. Our data revealed that the frequency of Treg cells ($p = .0027$), the expression of FoxP3 ($p = .0005$), TGF-β ($p = .0005$), and IL-10 ($p = .0002$) and the secretion levels of the TGF-β ($p = .033$), and IL-10 ($p = .029$) in cultured PBMCs are increased in nanocurcumin-treated group compared to placebo group.

Conclusion: The results of the current work indicated that nanocurcumin is capable of restoring the frequency and function of Treg cells in MS patients.

1. Introduction

Multiple sclerosis (MS) is a demyelinating disorder of the central nervous system (CNS) principally mediated by T lymphocytes with specificity to neuronal antigens in hereditarily disposed individuals (Buc, 2013; Danikowski et al., 2017). The autoreactive T cells transfer through the blood–brain barrier (BBB) and mediate impairment against the central neurons and their myelin sheaths (Dolati et al., 2017). Immunologically, MS is associated with Treg dysfunction, enhanced Th1 and Th17 responses, and autoreactive B cell over activity (Danikowski, Jayaraman, 2017).

Such immune imbalance could be initiated by the failure in the Treg suppression of effector T cells, destroying myelin and leading to neuronal damage and neuroinflammation (Bjerg et al., 2012; Dolati et al., 2018a). **Regulatory T cells** are a subset of $CD4^+$ lymphocytes playing a crucial role in the self-tolerance maintenance and the modulation of

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overall immune responses against infections and tumor cells (Fujio et al., 2010). Treg cells secrete TGF-β and IL-10 and in order to differentiate them, a particular cytokine TGF-β and the transcription factor forkhead box P3 (FoxP3) are needed (Sakaguchi et al., 2006). Treg cells can suppress inflammation and immune responses via several mechanisms including the secretion of IL-10 or TGF-β, which is essential to prevent immune activation (Wan and Flavell, 2008).

TGF-β induces FoxP3 expression in T cell receptor (TCR) stimulated naive $CD4^+$ cells, hence, it is considered as a vital factor for prompting FoxP3(Buckner, 2010). In the absence of considerable inflammation, TGF-β stimulates Treg differentiation, thereby, sustaining immune tolerance. MS is resulted from the self-tolerance failure leading to autoreactivity development of lymphocytes and this failure in MS self-tolerance might be associated with Tregs numerical, functional, and/or migratory deficits (Kleinewietfeld and Hafler, 2014).

Curcumin is the hydrophobic bioactive component isolated from the rhizome of the herb *Curcuma longa L* which has been well-documented for its wide range of biological and pharmacological activity (Shome et al., 2016; Xie et al., 2011). Currently, a large number of clinical trials are in progress, each offering a promising therapeutic prospective of curcumin in the near future (Mohajeri et al., 2015; Naksuriya et al., 2014). Several studies have illustrated that curcumin exhibits several biological activities, is fairly safe and well-tolerated and is also non-toxic even at doses up to 8 g per day (Ghosh et al., 2015). However, it should be noted that curcumin is less effective compared to a host of clinically used chemotherapeutic drugs. The limited in vivo bioavailability of curcumin results from its insolubility in body fluids, poor intrinsic activity, and quick clearance from the body. Nonetheless, encapsulation within nanoparticles has proved to increase the loading, release, and bioavailability of curcumin (Yallapu et al., 2015).

Nanocurcumin derived from curcumin is a better-quality form of the compound with diminished particle size, enhanced delivery to the diseased tissue, better pharmacokinetic properties, proper internalization and decreased systemic removal (Trivedi et al., 2017).

Nanocurcumin is a curcumin product (SinaCurcumin®) for oral use which is industrialized in Nanotechnology Research Center of Mashhad University of Medical Sciences, Mashhad, Iran and marketed by Exir Nano Sina Company in Tehran-Iran (IRC:1228225765). Each soft gel of Nano-curcumin has 80 mg of curcumin encapsulated in nano-micelle (Rahimi et al., 2016).

In the present study, we examined the effect of Nano micelle curcumin on Treg cells balance in MS patients compared with the placebo group as a controls.

2. Materials and methods

2.1. Study population

Fifty patients with relapsing–remitting MS referred to Imam Reza Hospital of Tabriz University of Medical Sciences were enrolled according to MacDonald criteria during June 2016 to January 2017. The evaluation of patients was directed in accordance with the local ethics committee. Written informed consent was taken from each patient and control subjects prior to participation, and the study protocol was accepted by the Ethics Committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.1177). This study was conducted in a randomized, double-blind, placebo-controlled way. Randomization was done according to a computer-generated list while the randomization code and allocation group were made blinded to the participants and hospital staff. MS patients were divided into 2 subgroups with a block randomization, 25 out of 50 received a daily dose of 80 mg oral nanocurcumin and 25 patients received placebo as control group for a period of 6 months. The patients were 28–51-year-old. The body mass index (BMI) of the patients was between19–30. At the beginning of the study, the EDSS of patients ranged 0–5.5 and no relapses had happened for at least 4 months before the study.

All patients who entered in present study received weekly interferon beta-1a (Actovex) (Gemabiotech S A, Argentina) injections for at least 3 months before the intervention and also they were weekly under IFN-beta treatment during supplementation. All patients underwent medical history evaluation, physical and neurological examination, screening laboratory tests, and brain magnetic resonance imaging (MRI). Treatment with anti-inflammatory drugs and/or immunosuppressive agents or a history of diabetes and other chronic or autoimmune diseases were considered as exclusion criteria. Patients were divided into two groups. Twenty-five patients were treated with nanocurcumin capsules (SinaCurcumin®, Mashhad, Iran) for at least six months and the remaining received placebos over the same period (control group). Additionally, we enrolled 35 age- and sex-matched healthy persons with a mean age of 35.6 ± 13.4 years (SD) as healthy control group. Each soft gel of Nano-curcumin contained 80 mg of curcumin encapsulated in nano-micelle. Patients were donated two types of blood samples, the first collected instantly prior to the administration of nanocurcumin and placebo capsules and the second taken following six months. Nine patients left the study, three patients did not sign the informed consent, five lived fairly far from the place where follow-up examinations were done, and one developed cancer. The current trial was registered on Iranian Registry of Clinical Trials number IRCT2016042227520N1. The comprehensive demographic and clinical data of patients are listed in Table 1 (Dolati et al., 2018b).

2.2. Blood sampling and cell culture

10–15 ml whole blood samples were collected from all patients prior to the administration of the capsules and 6 months post-treatment. Sampling was conducted on the morning following admission, while patients were in a fasted state. PBMCs were isolated from heparinized blood samples by adding 1.077 g/ml Ficoll (lymphosep) (Biosera, UK) and centrifugation at 450 ×*g* for 25 min followed by twice washing with Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma,

Table 1
Clinical characteristics of Placebo and Nanocurcumin treated RRMS subjects and Clinical endpoints after treatment.

	Before treatment		P value	After treatment		P value
	Treatment group	Placebo group		Treatment group	Placebo group	
Number	25	25	NS	20	21	NS
Age	35.2 ± 4.2 (28–51)	34.6 ± 5.3 (28–51)	NS	34.6 ± 5.2 (28–51)	34.5 ± 4.8 (28–51)	NS
Gender (Female/ Male)	16/9	15/10	NS	14/6	13/8	NS
BMI	24.6 ± 4.1	23.5 ± 4.8	NS	23.8 ± 4.6	23.4 ± 5.2	NS
EDSS	1.77 ± 0.33	1.96 ± 1.12	NS	0.98 ± 0.29	1.72 ± 1.06	0.041
Disease duration	4.8 ± 1.2 (2–6 yrs)	4.5 ± 1.6 (2–6 yrs)	NS	4.3 ± 1.5 (2–6 yrs)	4.2 ± 1.8 (2–6 yrs)	NS
Mean relapse rate 12 months before study period	1.08 ± 0.12	1.11 ± 0.34	NS			
Mean relapse rate during the study period (6 months)	0.72 ± 0.06	0.85 ± 0.7	NS			

Data are presented as mean ± SD or frequencies.
BMI: Body Mass Index; EDSS: Expanded Disability Status Scale.

Table 2
Primer sequences for real-time-PCR.

Gene	F-Primer (5′-3′)	R-Primer (5′-3′)
Foxp3	TCATCCGCTGGGCCATCCTG	**GTGGAAACCTCACTTCTTGGTC**
TGF-β	CGACTACTACGCCAAGGA	**GAGAGCAACACGGGTTCA**
IL-10	CATCGATTTCTTCCCTGTGAA	**TCTTGGAGCTTATTAAAGGCATTC**
β-actin	AGAGCTACGAGCTGCCTGAC	**AGCACTGTGTTGGCGTACAG**

FoxP3: Forkhead Box P3; IL-10: Interleukin 10; TGF-β: Transforming Growth Factor Beta.

Germany). The harvested cells were cultured for 48 h in a medium containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 200 mM L-glutamine and phytohemagglutinin (PHA). Consequently, the cultured cells were subjected for RNA extraction and the supernatant was exploited for cytokine assay using enzyme linked immuno-sorbent assay (ELISA).

2.3. Cell separation and Flow cytometry

For analysis frequency of Treg cells, the cells were not stimulated; instead, such chemical stimulators as monensin (eBioscience, San Diego, CA, USA) were used to improve the staining of intracellular cytokines. Cells were assessed using FACS Calibour flow cytometer with FLowJo software (Becton Dickinson, Mountain View, CA, USA). Cells were washed and incubated with FITC-conjugated anti-CD4, PE-conjugated anti-CD25, and PE-cy7-conjugated anti-CD127 monoclonal antibodies (eBioscience, San Diego, CA, USA), or isotype-matched IgG controls before staining.

2.4. Real-time PCR

Total RNA was extracted from PBMCs using RNX-PLUS Solution (SinaClon, Tehran, Iran) and quantified through spectrophotometric measurement (Nano Drop; Agilent Technologies, USA). Next, complementary DNA (cDNA) was synthesized using Revert Aid Reverse Transcriptase kit (Thermo Fisher, Waltham, MA, USA). In order to

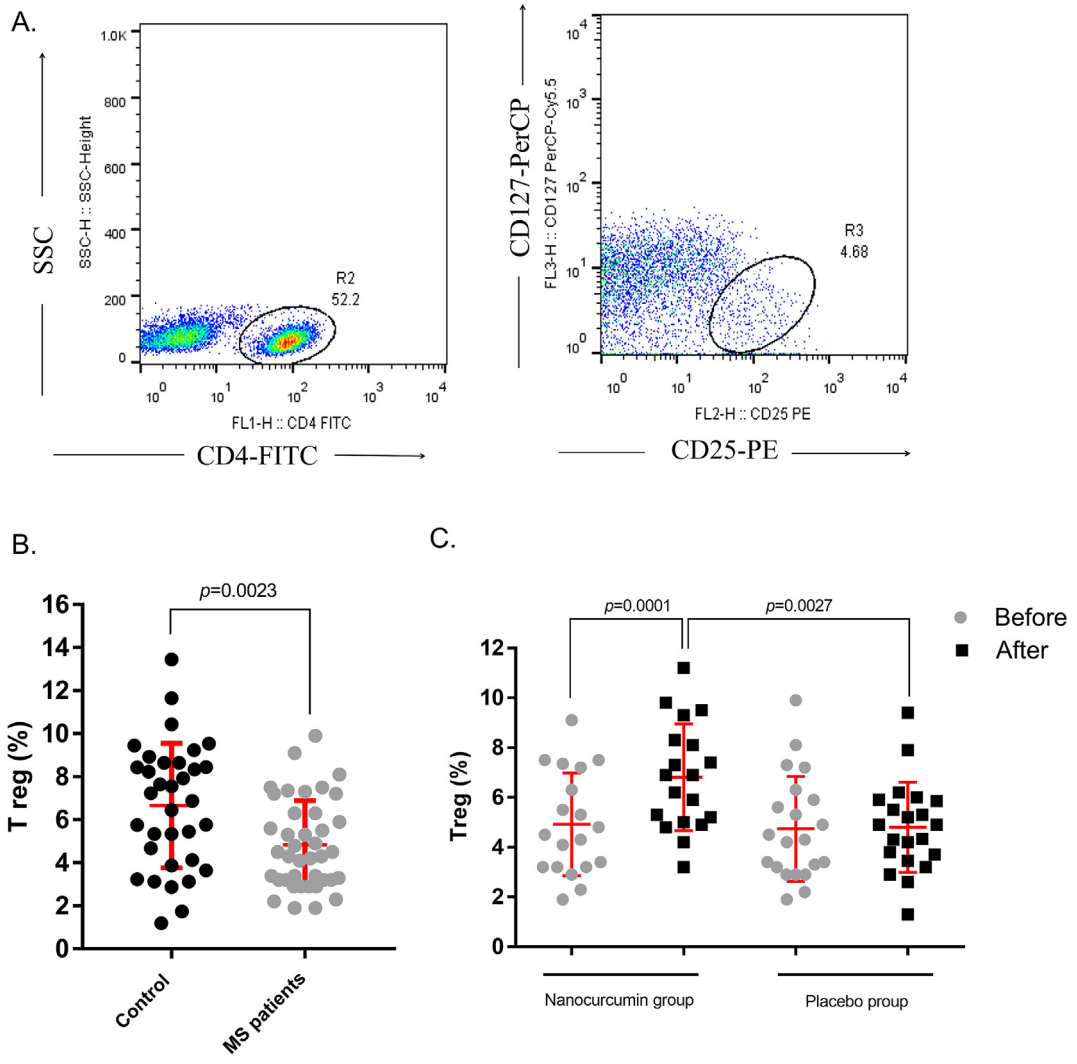

Fig. 1. The frequency of Treg cells in healthy control group, nanocurcumin and placebo treated RRMS patients. Representative dot plots demonstrate the analyzing method used for enumeration of Treg (A). The results pertaining to the flow cytometry analysis of the nanocurcumin treated and placebo treated patients with RRMS. (A) The percentage of $CD4^+$ $CD25^+$ $CD127^-$ (Treg) cells among the total $CD4^+$ T cell population is indicated. (B) The percentages of Treg cells in healthy control group is significantly higher in compared with MS patients ($p = 0.0023$).
The statistical analysis of Treg proportions in RRMS patients (C). There was an obvious increase in the frequency of Treg cells (p value $= 0.0001$) in nanocurcumin group in compare with before treatment and also in compare with placebo treated group (p value $= 0.0027$). Data are presented as, mean ± SD (Healthy control group n = 35, Nanocurcumin group n = 20, Placebo group n = 21). $p < 0.05$ was considered as statistically significant.

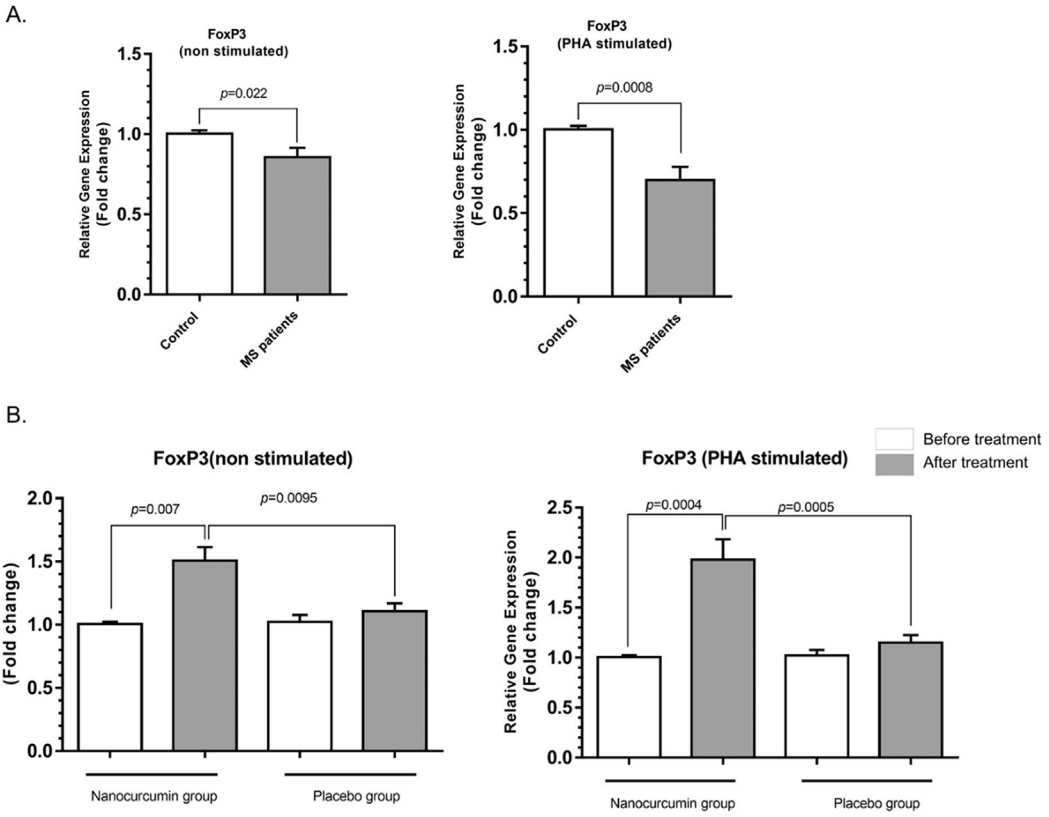

Fig. 2. The mRNA expression levels of transcription factor in PHA-stimulated cells and non-stimulated Tregs from healthy controls, nanocurcumin and placebo group at baseline and 6 months after treatment. (A).The mRNA expression levels of FoxP3 in PHA-stimulated cells and non-stimulated cells was in low levels in MS patients and have significantly difference with healthy control group, (p = .0008) and (p = .02), respectively. (B). The mRNA expression levels of FoxP3 in PHA-stimulated cells recognizably increased following the use of nanocurcumin compared to the basal level, 1.98 ± 1.023 (p = .0004); and expression level in placebo group compared with nanocurcumin group was decreased to 1.150 ± 0.37 (p = .0005). The mRNA expression levels of FoxP3 in non-stimulated cells recognizably increased following the use of nanocurcumin compared to the basal level, (p = .0009); and expression level in placebo group compared with nanocurcumin group was decreased significantly (p = .0025). FoxP3 expression analysis was performed in PHA-stimulated cells and non-stimulated cells. Data are presented as, mean ± SD (Healthy control group n = 35, Nanocurcumin group n = 20, Placebo group n = 21). $p < .05$ was considered as statistically significant.

analyze FoxP3, TGF-β and IL-10 mRNA levels, SYBR Green method of polymerase chain reaction (PCR) was employed. Standard curves were plotted according to six standards made via 10-fold serial dilutions of a concentrated sample of the genes to calculate the PCR efficiency. The standard condition of SYBR Green method in the first step was 10 min at 95 °C which was repeated for 40 cycles of denaturation (10 s at 95 °C), followed by 58 °C (for FoxP3) and 60 °C (for TGF-β and IL-10) for 30 s for annealing and then 20 s at 72 °C for extension. In order to confirm the amplification, an electrophoresis analysis was performed on 2% agarose gel and DNA sequencing by Biosystems (SEQLAB, Germany). The PCR reactions were performed in a 25 µl reaction volume containing primer, and the SYBR Green kit reagents (TaKara). The $2^{-\Delta\Delta CT}$ method was used to calculate the expression relative to the β-actin housekeeping control to normalize the expression folds of target gene. The primer sequences are presented in Table 2. All of the gene expression has been carried out in PHA-stimulated cells with the exception of FoxP3; its expression has been evaluated in PHA-stimulated cells and also in non-stimulated cells to avoid the FoxP3 unspecific expression.

2.5. Measurements of cytokine levels

Secretion of TGF-β and IL-10 were evaluated in the serum and supernatant acquired from cultured PBMCs using ELISA (Mybiosource, San Diego, USA). The concentration gradients of the kit standards or positive controls render an estimated sensitivity of 4.69 and 18.75 pg/ml for IL-10 and TGF-β, respectively. In brief, a 96-well plate was coated overnight with 100 µl of coating antibody; the plate was subsequently washed with phosphate buffered saline (PBS) containing 0.05% Tween and incubated for 1 h with blocking buffer on a shaker. 100 µl of samples or standards were added to the wells for 1 h on a shaker. After washing, the wells were initially incubated with 100 µl of biotinylated antibody for 1 h and then with 100 µl of horseradish peroxidase (HRP)-conjugated streptavidin for 30 min. After washing, 100 µl of tetramethylbenzidine substrate solution was added to the wells. The reaction was terminated following 30 min and the absorbance values were measured at 450 nm by a Medgenix ELISA reader (BP-800, Biohit, USA). The concentration of the samples was calculated using appropriate standard calibration lines and the Softmax software of the reader. Cytokine secretion analysis was performed in PHA-stimulated cells.

2.6. Statistical analysis

Statistical analysis was performed using SPSS PC Statistics (version 19.0; SPSS Inc., Chicago, IL, USA). Scale variables were assessed for normal distribution using the Kolmogorov–Smirnov test. As appropriate Tests were performed with 95% confidence intervals (tow tailed). Paired t-test was applied to compare the statistical differences of immunologic factors before and after nanocurcumin and placebo treatment. For drawing the graphs, the GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com) was used p-value < 0.05 were reported to be statistically significant.

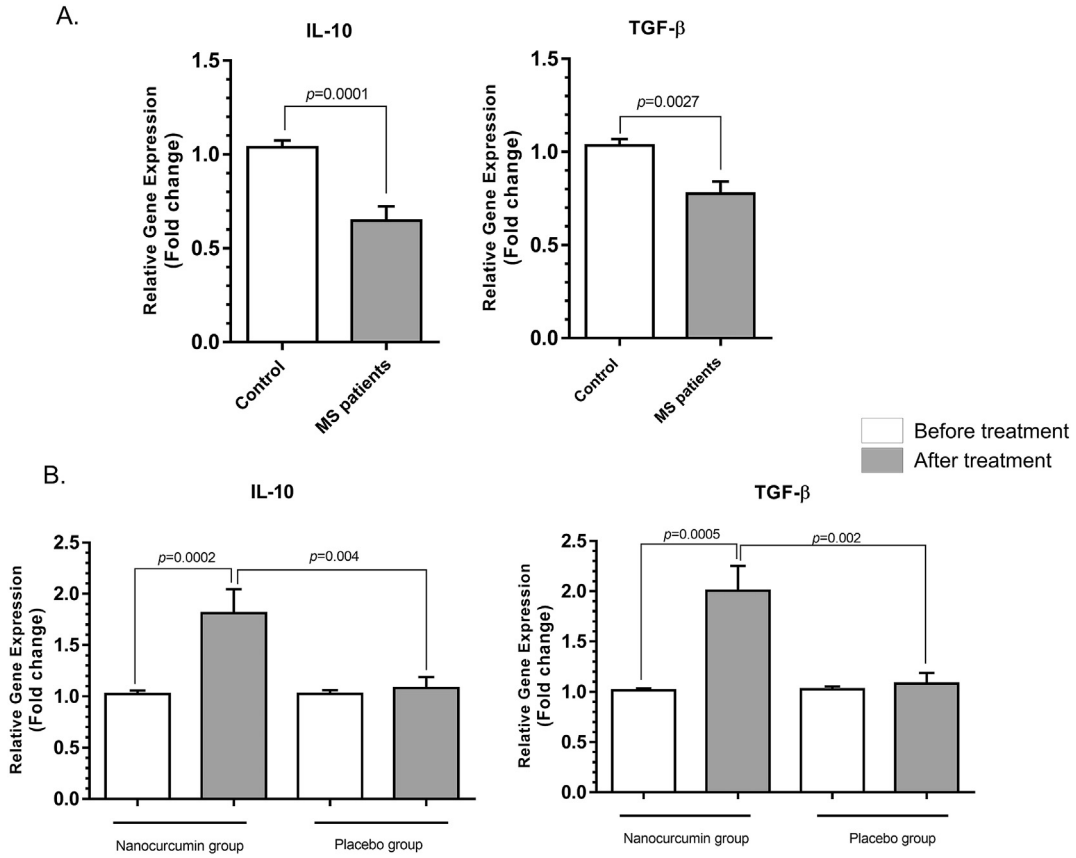

Fig. 3. mRNA expression level of TGF-β and IL-10 of healthy controls, nanocurcumin and placebo treated groups. (A). The mRNA expression levels of TGF-β and IL-10 were in low levels in MS patients and have significantly difference with healthy control group, (p = .0027) and (p = .0001), respectively. (B).The IL-10 and TGF-β mRNA expression levels indicated a significant increase in nanocurcumin treated group compared with before treatment (p value =.0002) and (p value =.0005), respectively and also in compare with placebo treated group (p value =.004) and (p value =0.002), respectively. Cytokine expression analysis was performed in PHA-stimulated cells. Data are presented as, mean ± SD (Healthy control group n = 35, Nanocurcumin group n = 20, Placebo group n = 21). $p < .05$ was considered as statistically significant.

3. Results

3.1. Nanocurcumin increases the frequency of circulating Tregs in MS patients

In a complementary set of experiments, flow cytometry analysis was employed for the enumeration of peripheral blood Treg cells in the nanocurcumin and placebo treated RRMS patients having immune cell abnormalities. The outcomes revealed that the proportion of $CD4^+$ $CD25^+$ $CD127^-$ Treg was considerably influenced by nanocurcumin in all the RRMS patients (Fig. 1 A).

The percentages of Treg cells in healthy control group was significantly higher compared to MS patients (p = .0023) (Fig. 1B).

In all treated patients, nanocurcumin significantly augmented the frequency of Treg cells from 4.92 ± 2.01% to 6.80 ± 2.09%. No differences were observed in frequency of Tregs in placebo group compared to basal levels. However, the proportion of Treg cells in nanocurcumin treated group significantly increased compared to basal level (before treatment) (p = .0001), (Fig. 1C). Additionally, the frequency of Treg cells in nanocurcumin treated group significantly increased compared to placebo group (p = .0027) (Fig. 1C).

3.2. Nanocurcumin increases the in vivo expression level of FoxP3 in PHA-stimulated and non-stimulated cells in MS patients

Low levels of FoxP3 mRNA expression in PHA-stimulated and non-stimulated cells was observed in MS patients and showed a significant difference with healthy control group, (p = .0008) and (p = .022), respectively (Fig. 2A). The mRNA expression levels of FoxP3 in PHA-stimulated cells recognizably increased following nanocurcumin use compared to the basal level, (1.98 ± 1.023, p = .0004). Furthermore, in PHA-stimulated cells the expression levels of FoxP3 in placebo group was in lower levels compared to nanocurcumin-treated group (1.150 ± 0.37, p = .0005) (Fig. 2B). Additionally, in non-stimulated cells the FoxP3 mRNA expression levels significantly increased following nanocurcumin use compared to the basal level, (p = .007). The expression level of FoxP3 in non-stimulated cells and in placebo group was also significantly lower than that of nanocurcumin group (p = .0095) (Fig. 2B).

3.3. Nanocurcumin increases the in vivo expression level of PBMCs isolated from RRMS patients

The mRNA expression levels of TGF-β (p = .0027) and IL-10 (p = .0001) were significantly lower in MS patients compared to healthy controls (Fig. 3A). Following nanocurcumin treatment, TGF-β and IL-10 mRNA levels were significantly increased (Fig. 3). Mean cytokine expression for TGF-β (2.004 ± 1.22, p = .0005) and IL-10 (1.83 ± 0.933, p = .0002) were significantly higher in nanocurcumin-treated group compared to placebo group. (Fig. 3 B).

3.4. Nanocurcumin increases the serum levels of TGF-β and IL-10 and in vivo levels of these cytokines in cultured PBMCs of MS patients

The serum levels of TGF-β (p = .043) and IL-10 (p = .015) in healthy control group were significantly higher compared to MS

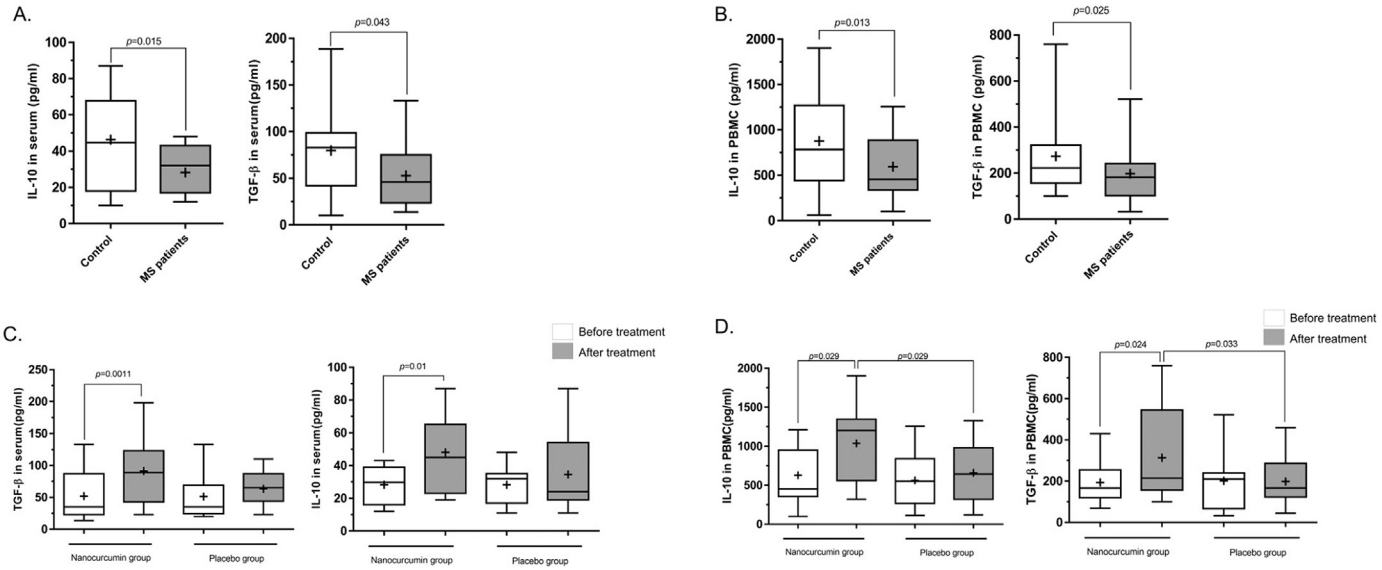

Fig. 4. Serum and Treg -associated cytokines secretion. (A). The serum levels of TGF-β and IL-10 secretion levels in healthy control group were in higher levels and in MS patients were in lower levels and is significantly different (p = .043) and (p = .015).
(B). The TGF-β and IL-10 secretion levels in healthy control group were in higher levels and in MS patients were in low amount and is significantly different (p = .025) and (p = .013).
(C). Serum levels of TGF-β and IL-10 were diminished in MS patients, yet increased after treatment with nanocurcumin; (p = .0011) for TGF-β, and (p = .01) about IL-10. (D).The secretion levels of IL-10 and TGF-β were significantly increased observed in the nanocurcumin group after treatment in compare with before treatment (p value =.029) and (p value =.024), respectively and also in compare with placebo treated group (p value =.029) and (p value =.033), respectively. Cytokine secretion analysis was performed in PHA-stimulated cells. Data are presented as, mean ± SD (Healthy control group n = 35, Nanocurcumin group n = 20, Placebo group n = 21). p < .05 was considered as statistically significant.

patients (Fig. 4 A). The TGF-β (p = .025) and IL-10 (p = .013) secretion levels were significantly higher in healthy control group compared to MS patients in cultured PBMCs (Fig. 4B). Nanocurcumin administration caused significant increase in TGF-β (p = .0011) and IL-10 (p = .01) serum levels in MS patients. However, in placebo group the levels of mentioned cytokines were still lower compared to nanocurcumin treated group. Additionally, in placebo group, no significant differences were observed in cytokines levels before and after intervention (Fig. 4 C).

After nanocurcumin treatment, TGF-β (p = .024) and IL-10 (p = .029) levels in PBMC were increased. However, these levels were significantly lower in placebo group (p = .033 and p = .029 for TGF-β and IL-10, respectively). In placebo treated group no significant differences were observed in cytokines levels compared to the baseline (Fig. 4D).

3.5. Nanocurcumin treatment significantly reduced the total EDSS in MS patients

After nanocurcumin treatment, the EDSS was significantly decreased (p = .039) compared to the baseline levels. Additionally, the EDSS were significantly different between nanocurcumin treated group and placebo controls (p = .041).

4. Discussion

Clonal deletion of self-reactive T cells in the thymus and the anergy induction do not alone describe the self-tolerance, because the pathogenic auto-reactive T cells are possibly existent in the periphery of healthy individuals (Viglietta et al., 2004). Therefore, extra regulatory mechanisms occur in order to prevent autoreactive T cells from immune disorders induction (Fritzsching et al., 2011; Hosseini et al., 2018).

The frequency of Tregs significantly reduced in PBMCs of MS patients. In this study, we also revealed that expression levels of Treg cytokines and transcription factor were diminished. Furthermore, TGF-β and IL-10 concentration levels decreased in MS patients. Our results confirmed important roles of Treg cells in autoimmune responses suppression.

A proper understanding of the MS essential mechanisms prove its reflection in numerous immunotherapeutic agents advancement, each affecting the enduring immunopathogenic processes in a different way, trying to restore a prior physiological state (Buc, 2013).

Interferon beta (IFN- β) influences the activity of Treg cells by increasing the number of ligands related to glucocorticoid-induced tumor necrosis factor receptor family–related protein (GITR) receptors in the membranes of dendritic cells. The contact between GITR in Treg-cells and GITR-ligands in dendritic cells stimulates the proliferation of Treg lymphocytes, followed by an augment in their amounts and active suppressive activities (Chen et al., 2012; de Andrés et al., 2007).

Glatiramer acetate (GA) is another traditional disease modifying drug for MS which increases the synthesis of immunosuppressive IL-10. It is believed that GA-activated T cells enter the CNS and enhance their anti-inflammatory and neuroprotective properties (Haas et al., 2009). GA also maintains the suppressive activities of Treg cells by upregulation of their co-inhibitory molecules, namely T cell Ig and ITIM domain (TIGIT), T cell immunoglobulin domain and mucin domain (TIM-3) (Hong et al., 2005).

In this study, we have examined curcumin effects on Tregs function in MS patients.

In the current study, we prepared curcumin with nano-micelles (nanocurcumin) and investigated its effect on the responsiveness of Treg-mediated suppression in MS patients. The nanocurcumin-treated MS patients had a better Treg-mediated suppression compared with the control group. Our results exhibited that frequency of $CD4^+$ $CD25^+$ $CD127^-$ Treg was significantly influenced by nanocurcumin in all the RRMS patients. Additionally, Proportion of Treg cells increased in nanocurcumin treated group as compared with basal levels before treatment and also versus placebo group.

Furthermore, patients exposed to the nanocurcumin showed higher levels of FoxP3 mRNA, as measured in total PBMC compared with

placebo group as controls.

In this clinical trial, we found that a six-month oral administration of nanocurcumin could increases TGF-β and IL-10 mRNA and secretion levels in serum and cultured PBMC in MS patients.

We also exhibited that, IL-10 concentration levels showed a higher increase than that of TGF-β concentration levels in nanocurcumin treated group. Mostly, EDSS score was considerably better-quality in the group supplemented by nanocurcumin compared with placebo group.

Furthermore, in patients with short disease duration, decrease in EDSS, suggests recovery from a relapse rather than "real improvement" of disability.

Based on above results, we established that, nanocurcumin as an immunomodulatory agent, could regulate immune system function and prevent autoreactivity through influence Treg cells frequency and function, consequently by affected peripheral tolerance.

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Conflict of interests

The authors declare no conflict of interests.

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