Effects of atorvastatin on some inflammatory markers in patients with multiple sclerosis treated by interferon beta-1b

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ABSTRACT

Objective: To investigate the effect of Atorvastatin vs. placebo on some inflammatory markers in patients with multiple sclerosis treated by interferone beta-1b.To achieve the aim of this study, a randomized control comparative trial was adopted.

Patients and Methods: A total of 100 patients with multiple sclerosis were recruited and investigated for some inflammatory markers which included, interleukin-2, tumor necrosis factor-α, C-reactive protein, and erythrocyte sedimentation rate. The patients were divided into 2 groups, namely the atorvastatin group which consisted of 50 patients and the placebo group which consisted of 50 patients. The patient groups were followed- up for 12 weeks during which the above parameters were measured before starting therapies and at the end of the follow-up period using commercially available kits. The patient groups were compared with the control group consisted of 50 apparently healthy subjects.

Results: The IL-2,TNF-α,CRP and ESR at baseline in both patient groups were found significantly elevated as compared with the control group ($p< 0.001$) . The use of atorvastatinhas resulted in significant decrease on the above parameters with non-significant effects in the placebogroup. Atorvastatinappeared to be superior in compared with the placebo group.

Conclusion: The use of atorvastatinfor 12 weeks in patients withmultiple sclerosis treated by interferone-beta has beneficial effect on some inflammatory markers studied in this research(IL-2,TNF-α,CRP and ESR).

Keywords: Multiple Sclerosis, Atorvastatin,Interferone-beta-1b.

الخلاصة

أهداف الدراسة: لدراسة تأثير عقار الاتورفستاتين والدواء الكاذب على بعض دلالات الالتهاب لدى المرضى المصابين بتصلب الأعصاب المتعدد المعالجين بالانترفيرون-بيتا.لتحقيق أهداف هذه الدراسة، تم اعتمادتصميم محاولة عشوائية ضابطة.

طريقة العمل: تم إشراك مائة مريض مصاب بتصلب الأعصاب المتعدد لغرض إجراء الاختبارات على بعض دلالات الالتهابفي مصل الدم والتي شملت (IL-2,TNF-α,CRP and ESR) تم تقسيم المرضى إلى مجموعتين متساويتين: مجموعة الاتورفستاتين و مجموعة الدواء الكاذب ضمت كل واحدة على 50 مريضا. تمت متابعة المرضى لمدة 12 أسبوعا، أجريت خلالها اختبارات المعايير أعلاه قبل وبعد انتهاء فترة المتابعة بواسطة عدة عمل متوفرة في السوق المحلية. تم مقارنة مجموعة المرضى مع مجموعة ضابطة اشتملت على 50 شخصا سليما ظاهريا.

النتائج: وجدت دلالات الالتهاب قيد الدراسة(IL-2,TNF-α,CRP and ESR)مرتفعة معنويا في كلا مجموعتي المرضىمقارنة مع مجموعة الضبط.اظهر استعمال الاتورفستاتين تاثير مفيد على المعايير أعلاهمقارنة بالدواء الكاذب.

الاستنتاج: إن استعمالعقار الاتورفستاتين لمدة 12 أسبوعالدى المرضى المصابين بتصلب الأعصاب المتعدد المعالجين بالانترفيرون-بيتا لديه تأثير ايجابي على بعض اختبارات دلالات الالتهاب قيد الدراسة.

كلمات الدلالة: تصلب الأعصاب المتعدد، اتورفستاتين، انترفيرون-بيتا .

Multiple sclerosis (MS) is an inflammatory autoimmune disorder invading myelin sheath in central nervous system (CNS)[1]. It is the most common nontraumatic cause of disability in young adults, affecting approximately 2.5 million people worldwide; its prevalence is not uniform, with a latitudinal gradient of prevalence present in most studies[2].

The etiology of MS is unknown. It likely results from complex interactions between environmental and genetic factors, which lead to an aberrant immune response and damage to the myelin sheath, oligodendrocytes, axons, and neurons[3]. MS is considered to be an inflammatory autoimmune CD4 T-cell mediated disorder based on immune alterations in the blood and cerebrospinal fluid (CSF) as well as the pathological features in the brain[4]. Autoreactive activated T-cells invade the blood brain barrier and initiate an inflammatory response that leads to myelin destruction and significant neurological disability[5] .

Cytokines have crucial functions in the development, differentiation and regulation of immune cells. As a result, dysregulation of cytokine production or action is thought to have a central role in the development of autoimmunity and autoimmune diseases such as MS[6].An imbalance in cytokines exists in MS, with the proinflammatory T helper1 (Th1) cytokines predominating over the anti-inflammatory and regulatory (Th2) cytokines[7].This dysregulation in cytokine balance in individuals with MS is due to an increased Th1 immune response combined with a decreased Th2 response[8].This imbalance is characterized predominately by increased levels of interferon gamma (IFN- γ), interleukin (IL)- 2, interleukin (IL)- 12 and tumor necrosis factor alpha (TNF-α) and decreased levels of IL-4, IL-10, and transforming growth factor-beta (TGF-β)[9].

In addition to this imbalance in cytokines, MS is characterized by increased levels of inflammatory markers which include serum soluble vascular adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), neopterin, serum nitric oxide metabolites nitrate and nitrite and alpha 1-acid glycoprotein (AGP)[10].

Interferon-beta is an immune-modulatory drug that modulates T-cell activation and reduces inflammatory mediators and reduces the relapse rate in relapsing remitting MS[11].

Experimental and clinical studies have demonstrated that statins can downregulate both acute and chronic inflammatory processes, reduce circulating CRP and pro-inflammatory cytokines levels[12]. These data suggest the potential value of statins in the treatment of MS[13]. Thus, the present study aims to:

1) compare the concentrations of some cytokines and inflammatory

markers (IL-2, TNF-α, CRP, and ESR) between MS patients and healthy individuals.

2) evaluate the effect of 3 month use of statin (atorvastatin) versus placebo on the concentrations of the above parameters in MS patients treated by interferon beta-1b.

Patients and Methods

The study was conducted in Ibn-Siena Consultation Clinic of Neurology, Mosul, Iraq. The subjects included in this study were selected over the six-month period of 1st February to 1st June 2013. To achieve the aim of the present study, a randomized control trial (RCT), open-labeled design was adopted.

Out of one-hundred twenty patients recruited in this study, only one-hundred patients whose ages mean ±SD were 35.82±8.75years complaining from relapsing remitting multiple sclerosis (RRMS) in remission phase diagnosed according to McDonald criteria 2010 receiving subcutaneous interferon beta-1b 250 µg every other day were completed the three months follow-up period.

The patients who were eligible to the study divided randomly into 2 equal groups. The first group consisted of fifty patients started to receive atorvastatin 20 mg (Aditor) manufactured by Advanced Pharmaceutical Industries- Jordan twice daily in addition to their usual interferon beta-1btreatment. The second group consisted of fifty patients started to receive placebo capsules (glucose powder) twice daily in addition to their usual interferon beta-1b treatment.

The study was approved by the ethical committee of Nineveh Health Directorate and all patients signed a written informed consent form. Fifty apparently healthy volunteers, matched for age, BMI and gender with the patients, were considered as a control group.

About 10 mL of venous blood was withdrawn, using a disposable syringe at about 8.00 to 10.00 am from the two MS patient groups and control subjects prior to start taking any drug (atorvastatin or placebo) and after three months of the drug use. 1ml of the blood was added to EDTA tube to calculate ESR. The remaining blood (9ml) allowed clotting in a plain tube at room temperature and then the serum was separated by centrifugation at 3000 rpm for 10 minutes and kept frozen at -20˚C to be analyzed later on.

Determination of IL-2 and TNF-αconcentrations was done using ELISA technique by commercially available kit.Serum CRP was measured by slide agglutination,using CRP Latex Test Kit.The recommended method for ESR measurement was Westergren method.

Statistical methods: Data are presented as mean ± SD.Unpaired t-test was used to compare between age and sex and inflammatory parameters of the control and those of the patients. Paired t-test was used to compare between the studied inflammatory parameters before and after therapy. Mann whitney test was used to compare the effect of atorvastatin vs placebo. Results considered significant at *p* value equal or less than 0.05.

Results

The characteristics of the patients and controls were presented in table 1.

Table 2 shows that the serum levels of IL-2, TNF-α, CRP and ESR were significantly higher ($p<0.01$) in patients with MS allocated to interferon plus atorvastatin group before starting therapy as compared with the control group.By comparing the mean concentrations of IL-2, TNF-α,CRP and ESR in patients with MS before and after therapy, there was a

significantdecrease (p<0.01) in serum IL-2, TNF-α, CRP and ESR levels after three months use of interferon plus atorvastatin.

Table 3 shows that the serum IL-2,TNF-α, CRP and ESRlevels in patients with MS allocated to interferon plus placebo group were significantly higher (p<0.01) before starting therapy as compared with the control subjects.By comparing the mean concentrations of IL-2,TNF-α, ESR and CRPin patients with MS before and after therapy, there was no significant difference in the mean concentrations of IL-2,TNF-α, ESRand CRPafter three months use of interferon plus placebo.

Table 4 illustrates the comparative effect of interferon plus atorvastatin and interferon plus placeboafter three months. Interferon plus atorvastatin appeared to produce more significant decrease (p<0.01) for IL-2 , TNF-α, ESR and CRP with regard to its effects on cytokines and the markers of inflammation as compared to Interferon plus placebo therapy

Table 1. Characteristics of multiple sclerosis patients and control group

Parameters	Controls n=50	Interferon + atorvastatin n=50	Interferon + placebo n=50	P-value
Age(year)	35.82±8.75	35.30±8.62	37.56±7.50	NS
Gender Male No. (%) Female No. (%)	 16 (32.0%) 34 (68.0%)	 17 (34.0%) 33 (66.0%)	 10 (20.0%) 40 (80.0%)	 NS
BMI(kg/m^2)	25.44±3.63	25.45±3.72	26.23±3.93	NS

NS: Non significant difference from control group

Table 2. Comparison of IL-2 , TNF-α , CRP and ESR among control andmultiple sclerosis patients on interferon plus atorvastatin (before and after) therapy.

Parameters	Controls n=50	Interferon + atorvastatin before treatment n=50	Interferon + atorvastatin after treatment n=50
IL-2 (ng/l)	25.36±24.22	722.89±230.04	488.65±229.29
TNF-α (ng/l)	23.11±11.66	302.51±117.60	144.79±122.55
CRP (mg/l)	7.20±3.20	18.59±12.96	6.24±1.18
ESR (mm/H)	11.70±6.00	28.24±15.38	11.88±9.92

a) p<0.01 vs control
b) p<0.01 vsinterferon + atorvastatinafter treatment

Table 3. Comparison of IL-2,TNF-α, CRP and ESR betweencontrol &multiple sclerosis patients on interferon plus placebo (before and after) therapy.

Parameters	Controls n=50	Interferon + placebo before treatment n=50	Interferon + placebo after treatment n=50
IL-2 (ng/l)	25.36±24.22	725.02±201.11*	722.14±199.27
TNF-α (ng/l)	23.11±11.66	405.75±131.15*	402.63±134.33
CRP (mg/l)	7.20±3.20	12.84±15.38*	13.20±15.42
ESR (mm/H)	11.70±6.00	31.22±12.22*	31.32±11.68

* p<0.01 vs control

Table 4. Difference of percentage variation between multiple sclerosis patients on interferon plus atorvastatin and interferon plus placebo therapies.

Parameters	Interferon + atorvastatin n=50	Interferon + placebo n=50	P-value
IL-2 (ng/l)	-231.89±181.61	-2.87±21.88	<0.01*
TNF-α (ng/l)	-157.72±136.75	-3.12±14.77	<0.01*
CRP (mg/l)	-6.72±17.84	0.36±3.29	<0.01*
ESR (mm/H)	-16.36±10.98	0.10±4.46	<0.01*

*Significant differences using Mann Whitney test

Discussion

The current study was performed to assess the level of some Th1 cytokines and inflammatory markers in MS patients and to test whether atorvastatinuse can reduce these markers.The two MS groups and control group participate in this study were tested statistically for the absence of significant differences between the studied groups in concerning age, gender and body mass index (BMI) to exclude any effect of these parameters on the results of the study.

In this study, serum concentrations of IL-2 and TNF-αwere found significantly higher in patients with MS in both drug groups than the control subjects. This is not surprising as pro-inflammatory cytokines such as IL-1, TNF-α, IL-2, IFN-γ are believed to contribute to the tissue injury in MS, while anti-inflammatory cytokines produced by Th2 cells (IL-4, IL-10) have been shown to down regulate the immune response [14]; and the results of the current study were in agreement with a previous study published in 2010[15]. Despite clinically inactive disease and immunomodulator therapy, higher TNF-α and IL-2 of the patients suggested a continuous subclinical immune activity that could not be suppressed by treatment [16].

C-reactive protein is a generalized marker for inflammation, and elevated serum levels of CRP have been extensively studied in relation to cardio pathology. However, elevated levels of CRP have more recently been linked to CNS pathologies characterized by CNS inflammation including Alzheimer's disease and Parkinson's disease; this suggests that CRP may also play a role in modulating MS [17]. Actually many investigators have used CRP as an inflammatory marker in assessing inflammation in MS patients[18,19]. In this study, CRP was found higher than the control and this was in agreement with a previous study of 30 RRMS patients on interferon beta in whomCRP levels were higher than the control subjects[20].Conversely the current result disagreed with a study published in 2011 which did not report any difference in CRP level between MS and control group[21].

The current study found that ESR level in MS patients was higher than control subjects. One previous

study demonstrated that ESR may be moderately elevated in MS patients[22]; another study reported that ESR increased during relapses, and became significantly lower after intravenous glucocorticoid treatment[23]. While, and in contrast to our result, another study did not found any differencesin ESR between MS patients and healthy subjects[24].

Because of their immunomo-dulatory properties, statins are currently under investigation as treatment for MS. This concept was first tested in 1999 by Stanislaus *et al.,* who showed that lovastatin reduced mononuclear-cell infiltration into the brain and attenuated the clinical signs of experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis[25]. The attenuation of EAE by statins was attributed to the up-regulation of Th2 cytokines such as IL-4, IL-10 and transforming growth factor-b1 (TGF-b1)[26]. Statins improve proliferation and survival of oligodendrocyte precursors in vitro and improve myelination in vivo[27]. Oral atorvastatin was shown to prevent or reverse chronic or relapsing paralysis due to demyelination in a murine model. This was associated with a shift from Th-1type immune responsetowards Th-2 type responses in vivo. These results suggest a possible role of statins in inflammatory phase of MS and other Th-1 mediated autoimmune diseases including diabetes and rheumatoidarthritis[28].

Current study found that three months use of atorvastatin produced beneficial effect in MS patientsthrough significant decrease in IL-2, TNF-α, CRP and ESR.These results are in agreement with a previous study reported that simvastatin has shown to increase IFN- γ, IL-12 and IL-4 expression and to decrease TNF-α and IL-10 in T cells in vitro[29]. Another study found that mean levels of two Th2 cytokine (IL-4 and IL-10) increased after addition of atorvastatin to interferon-beta-1b; but failed to reach statistical significance[30].In contrast to this, aplacebo-controlled randomized trialfound non-significant difference between simvastatin and placebo group at 12 months duration and concluded that there is no beneficial effect of simvastatin 80 mg as add-on therapy to interferon beta-1a[31]. Another study also concluded that atorvastatin 40 mg/day added to interferon beta-1b did not have a beneficial effect on relapsing-remitting MS compared to interferon beta-1b monotherapy over a 12-month period[32].

In conclusion, the addition of atorvastatinfor three months to interferon beta-1b treatment has a beneficial effects on markers of disease activity such asIL-2,TNF-α, ESR and CRP in patients with MS.

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