# HIGHER SERUM URIC ACID LEVELS IN MULTIPLE SCLEROSIS PATIENTS AFTER LONG-TERM INTERFERON BETA TREATMENT

Gordana Toncev[1, 2], Katarina Vesic[2], Dejan Aleksic[2], Snežana Lazarevic[2]
[1]Clinic of neurology, Clinical Center Kragujevac, Serbia
[2]Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

# POVIŠEN NIVO MOKRAĆNE KISELINE KOD OBOLELIH OD MULTIPLE SKLEROZE NAKON DUGOTRAJOG LEČENJA INTERFERONOM BETA

Gordana Tončev[1,2], Katarina Vesić[2], Dejan Aleksić[2], Snežana Lazarević[2]
[1] Klinika za neurologiju, Klinički centar Kragujevac, Srbija
[2] Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija

Received / Primljen: 05.05.2016. Accepted / Prihvaćen: 05.07.2016.

## ABSTRACT

*Interferon beta is a safe and efficacious treatment for relapsing multiple sclerosis (MS). However, there is some evidence that uric acid, a scavenger of peroxynitrite, is involved in MS pathology and that increasing serum uric acid levels might have beneficial therapeutic effects. The aim of this study is to investigate serum uric acid levels in MS patients before and after long-term interferon beta treatment.*

*Blood samples from 101 MS patients (53 receiving interferon beta 1a treatment and 48 receiving interferon beta 1b treatment; 28 male and 73 female; mean age at treatment onset 32,4±7,3 years; mean duration of disease at treatment onset 5,1±3,2 years; mean EDSS 2±1,3) before and after interferon beta treatment (mean treatment duration 3±2 years) were analysed. Serum uric acid levels were measured using a quantitative enzymatic assay (Elitech Diagnostic, Sees, France). MS patients had significantly increased serum uric acid levels after treatment compared with those at the beginning of treatment (272,31±78,21 μmol/l vs. 210,17±53,65 μmol/l; p=0,019, Wilcoxon Mann-Whitney U-test). We did not find significant differences in serum uric acid levels between the interferon beta 1a and interferon beta 1b groups (p=0.98).*

*These results indicate that one of the beneficial effects of interferon beta in MS might be based on the elevation of serum uric acid levels as a natural scavenger of peroxynitrite.*

**Keywords:** *multiple sclerosis, interferon beta, uric acid*

## SAŽETAK

*Interferon beta je bezbedan i efikasan lek kod relapsno-remitentnog tipa multiple skleroze (MS). Međutim, postoje dokazi da je mokraćna kiselina, koja uklanja peroksinitrit, uključena u patologiju MS i da povišen nivo mokraćne kiseline može da ima korisne terapijske efekte. Cilj ovog istraživanja je da ispita nivo mokraćne kiseline kod obolelih od MS pre i posle dugotrajne terapije interferonom beta. Analizirani su krvni rezultati uzeti od 101 pacijenta (53 je primalo interferon beta-1a, a 48 je primalo interferon beta-1b; 28 muškaraca i 73 žene; prosečna starost na početku lečenja je 32,4 ± 7,3 godine, srednje trajanje bolesti na početku lečenja 5,1 ± 3,2 godine, srednji EDSS 2 ± 1,3) pre i posle lečenja interferonom beta (srednje trajanje lečenja 3 ± 2 godine). Nivo mokraćne kiseline u serumu je meren pomoću kvantitativnog enzimskog testa (Elitech Diagnostic, Sees, France). Oboleli od MS imaju značajno povišen nivo mokraćne kiseline nakon primene terapije u poređenju sa nivoima na početku bolesti (272,31 ± 78,21 μmol/l vs. 210,17 ± 53,65 μmol/l; p=0,019, Wilcoxon Mann-Whitney U-test). Nismo utvrdili značajnu razliku u nivoima mokraćne kiseline između grupa pacijenata sa terapijom interferon beta-1a pacijenata sa terapijom interferon beta-1b (p=0.98). Naši rezultati pokazuju da se jedan od korisnih efekata terapije interferonom beta kod MS može bazirati na povišenom nivou mokraćne kiseline koji prirodno uklanja peroksinitrit.*

**Ključne reči**: *multipla skleroza, interferon beta, mokraćna kiselina*

## ABBREVIATIONS

**MS** – multiple sclerosis,
**EDSS**– Expanded Disability Status Scale score,
**CNS** - central nervous system

UDK: 616.832-004.2-085.375; 615.375:577.85 / SER J EXP CLIN RES 2017; 18 (3): 227-230
DOI: 10.1515/SJECR-2016-0052

**Corresponding author:** Snežana Lazarević
0642707023; Email: simovicsnezana2@gmail.com

## INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) with unknown aetiology. In recent years, there have been advances in understanding the pathogenesis of MS. There is significant evidence that oxidative stress is involved in MS pathology (1, 2). The proximal agents of neuronal cell damage might be nitric oxide and peroxynitrate (forming in a reaction between nitric oxide and superoxide anion radicals), which can compromise antioxidant defences and cause oxidative damage to tissues (3, 4). Uric acid is an important physiological antioxidant in the total antioxidant capacity of blood plasma (5, 6, 7). There is some evidence that MS patients have lower serum uric acid levels (8, 9, 10, 11, 12, 13, 14) and that MS and gout (chronic hyperuricaemia) are mutually exclusive diagnoses (15, 16).

Interferon beta is an efficacious and safe first line treatment for relapsing MS. It is the first therapeutic intervention demonstrated to modify the natural history of MS (reduces the relapse rate, decreases radiological disease activity, and slows progression of MS). Interferon beta has a range of effects on the immune system, which could explain its positive effects on MS (17, 18, 19, 20).

The aim of this study is to estimate changes in serum uric acid levels before and after long-term interferon beta treatment.

## MATERIALS AND METHODS

Serum samples of 101 patients with definite relapsing remitting MS, according to the criteria set forth by McDonald, admitted to the Clinic of Neurology, Clinical Center Kragujevac, were analysed. Forty-eight patients with MS were treated with interferon beta-1b (Betaferon$^{R}$) in doses of 8 MIU s.c. every second day, while 55 MS patients were treated with interferon beta-1a (Rebif$^{R}$) in doses of 44 mg s.c. three times weekly, from January 2004 to January 2011.

The inclusion criteria for treatment included being over 18 years of age, having had at least two relapses in last two years, and having an EDSS score (Expanded Disability Status Scale score) (22) of less than or equal to 3.5 (criteria for including MS patients receiving interferon beta treatment in Serbia). During this seven-year period, 26 patients stopped the treatment (16 interferon beta 1a and 10 interferon beta 1b patients). We investigated the serum uric acid levels of the MS patients before initiating the treatment and at the end of the treatment (in cases of treatment discontinuation) or in January 2011 for the patients who continued the treatment.

All blood samples were taken as part of a routine laboratory screening for interferon beta treatment (looking for possible adverse events every six months or at the end of the treatment). Serum uric acid levels were measured using a quantitative enzymatic assay (Elitech Diagnostic, Sees, France) according to the manufacturer's protocol, and the results were standardized using a commercial uric acid standard solution. Blood samples were obtained from all subjects after overnight fasting. Because of the circadian fluctuation of uric acid, all blood samples were taken at the same time (at 8 am) (23). There were no patients with chronic renal disease or diabetes mellitus, nor were there any receiving acetylsalicylic acid and thiazide diuretics. In Clinical Center Kragujevac, the normal range of serum uric acid levels was 150 to 350 μmol/l for females and 210 to 420 μmol/l for males. All MS patients consented to be involved in the study. The local Ethical Committee approved this investigation.

**Table 1.**Demographic and clinical characteristics of MS patients

| | Total | Interferon beta 1a | Interferon beta 1b | p |
|---|---|---|---|---|
| Number of patients | 101 | 53 | 48 | ns |
| Male/Female | 73/28 | 41/12 | 32/16 | ns |
| Age at treatment onset: years (Mean±SD)<br>Range | 32.4±7.3<br>19-56 | 32.5±8.1<br>19-56 | 32.5±6.3<br>23-47 | ns |
| Disease duration at onset of treatment (Mean±SD)<br>Range | 5.1±3.2<br>1-15 | 4.6±2.5<br>1-11 | 5.6±3.7<br>1-15 | ns |
| EDSS score at treatment onset (Mean±SD)<br>Range | 2.1±1.3<br>0-3.5 | 2.3±1.2<br>0-3.5 | 1.9±1.5<br>0-3.5 | ns |
| Mean follow up period | 3.9±2.0 | 3.73±2.14 | 3.9±1.8 | ns |

EDSS- Expanded Disability Status Score; ns- not significant

The clinical and demographic characteristics of the MS patients are presented in Table 1.

The SYSTAT program was used for statistical analysis. Significant differences between groups was calculated by the Wilcoxon Mann-Whitney U test. Differences in mean values were calculated using t-test. Correlations were analysed using Spearman's rank correlation. The results are given as mean ± SD.

## RESULTS

Serum uric acid levels before and after treatments with interferon beta (i.e., the follow-up period) are pre-

**Table 2.**Uric acid levels in serum of MS patients before and after interferon beta treatment

| MS patients | Serum uric acid levels umol/l ± SD | p |
|---|---|---|
| Before onset of treatment | 210.17±53.65 | |
| After follow up period<br>Total<br>Interferon beta 1b<br>Interferon beta 1a | <br>272.31±78.21*<br>281.33±52.28*<br>269.89±93.71* | <br>0.019<br>0.015<br>0.022 |

* significant in comparison with serum uric acid levels before treatment

sented in Table 2. The MS patients were found to have significantly higher mean serum uric acid levels after a mean of 3,9 years of interferon beta treatment compared with pretreatment serum uric acid levels (272,31±78,21 μmol/l *vs.* 210,17±53,65 μmol/l; p=0,019, Wilcoxon Mann-Whitney U-test). On the other hand, there were no significant differences in serum uric acid levels in MS patients after treatment with interferon beta 1b and interferon beta 1a (281,33±52,28 μmol/l vs. 269,89±93,71 μmol/l, respectively; Wilcoxon Mann-Whitney U-test, p=0,298).

Pretreatment serum uric acid levels increased in 88 patients, decreased in five patients and were unchanged in eight patients after a mean 3,9 years of treatment. We also found a significant linear correlation between the duration of interferon beta treatment and changes (increasing) in serum uric acid levels (p=0,021).

## DISCUSSION

Interferon beta is a well-established and safe first line treatment for MS. To our knowledge, this is the first study to compare changes in serum uric acid levels before and after long-term interferon beta treatment. We previously reported higher uric acid levels after one year of interferon beta 1b treatment in a small preliminary sample of patients (24).

Several studies have demonstrated lower serum uric acid levels in MS patients and suggest that higher levels of uric acid may offer protection against the development of MS (5-16). There are some opinions that the lower urate levels among multiple sclerosis cases could be a consequence rather than a cause of the disease (25). Consequently, treatment attempts in human MS with a precursor of uric acid have been reported (26, 27). A few studies have demonstrated elevated serum uric acid levels after the administration of some drugs, such as glatiramer acetate (28) and high-dose methylprednisolone (29), indicating that some beneficial effects of these drugs might be due to the elevation of serum uric acid levels.

Interferon beta reduces the exacerbation rate in patients with relapsing remitting (RR) MS, decreases disease activity in the brain (measured as the identification of new or enlarging lesions in serial brain magnetic resonance imaging (MRI)), and slows the progression of MS. Interferon beta exerts a large range of effects on the immune system, which can explain its positive effects on MS, including antagonism of the proinflammatory cytokine interferon gamma (17, 18), inhibition of the production of chemokines and matrix metalloproteinases (19), and increased production of Interleukin 10 (20).

The results of this study indicate that one of the beneficial effects of interferon beta might be based on the elevation of serum levels of uric acid as a natural scavenger of peroxynitrite.

## REFERENCES


1. Rodriguez M. (1997) Multiple sclerosis:Insights into molecular pathogenesis and therapy. Mayo Clinic Proc 1997; 72:663-4.
2. Kemp K, Redondo J, Hares K, &Rice C, Scolding N, Wilkins A. (2016) Oxidative injury in multiple sclerosis cerebellar grey matter. Brain Res. 2016 Apr 14. pii: S0006-8993(16)30236-0. doi: 10.1016/j.brainres.2016.04.027. [Epub ahead of print]
3. Beckman JS, Koppenol WH. (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. Am J Physiol; 271:C1424-C1437.
4. Carvalho AN, Firuzi O, Gama MJ, van Horssen J, Saso L. (2016) Oxidative stress and antioxidants in neurological diseases: is there still hope? Curr Drug Targets. Vol: 17 DOI: 10.2174/1389450117666160401120514
5. Hooper DC, Bagasra O, Marini JC, Zborek A, Ohnishi ST, Kean R et al. (1997) Prevention of experimental allergic encephalo-myelitis by targeting nitric oxide and peroxynitrite: implications for the treatment of multiple sclerosis. Proc Natl Acad Sci U S A 1997; 94:2528-2533.
6. Zabaleta ME, Bianco NE, De Sanctis JB. (1998) Serum nitrotyrosine levels in patients with multiple sclerosis: Relationship with clinical activity. Medical Science Research 1998; 26:407-408.
7. Squadrito GL, Cueto R, Splenser AE, Valvanidis A, Zhang H, Uppu RM et al. (2000) Reaction of uric acid with peroxinitrite and implications for the mechanism of neuroprotection by uric acid. Arch Biochem Biophys 2000; 376 (2):333-337.
8. Moccia M, Lanzillo R, Costabile T, Russo C, Carotenuto A, Sasso G, Postiglione E, De Luca Picione C, Vastola M, Maniscalco GT, Palladino R, Brescia Morra V. (2015) Uric acid in relapsing-remitting multiple sclerosis: a 2-year longitudinal study. J Neurol. 2015;262(4):961-7
9. Ashtari F, Bahar M, Aghaei M, Zahed A. (2013) Serum uric acid level in patients with relapsing-remitting multiple sclerosis. J Clin Neurosci. 2013 May;20(5):676-8.
10. Zoccolella S, Tortorella C, Iaffaldano P, Direnzo V, D'Onghia M, Luciannatelli E, Paolicelli D, Livrea P, Trojano M. (2012) Low serum urate levels are associated to female gender in multiple sclerosis patients. PLoS One. 2012;7(7):e40608.
11. Liu B, Shen Y, Xiao K, Tang Y, Cen L, Wei J.(2012) Serum uric acid levels in patients with multiple sclerosis: a meta-analysis. Neurol Res. 2012 Mar;34(2):163-71.
12. Toncev G, Milicic S, Toncev S, Samardzic G. (2002) Serum uric acid levels in multiple sclerosis patients correlate with activity of disease and blood-brain barrier dysfunction. Eur J Neurol 2002; 9:221-226.
13. Drulović J, Dujmović I, Stojsavljević N, Mesaroš S, Andjelković S, Miljković Dj, et al. (2001) Uric acid levels in sera from patients with multiple sclerosis. J Neurol Feb 2001; 248(2):121-6.

14. Sotgiu S, Pugliatti M, Sanna A, Sotgiu A, Fois ML, Arru G, et al. (2002) Serum uric acid and multiple sclerosis. Nerol Sci 2002; 23:183-188.
15. Pakpoor J, Seminog OO, Ramagopalan SV, Goldacre MJ. (2015) Clinical associations between gout and multiple sclerosis, Parkinson's disease and motor neuron disease: record-linkage studies. BMC Neurol. 2015 Feb 28;15:16.
16. Hooper DC, Spitsin S, Kean RB, Champion JM, Dickson GM, Chaundrhy I, et al. (1998) Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. Proc Natl Acad Sci USA 1998; 95, pp. 675–680.
17. Dhib-Jalbut S. (1997) Mechanisms of interferon beta action in multiple sclerosis. Mult Scler 1997; 3:397-401.
18. Arnason BG, Dayal A, Qu ZX, Jensen MA, Genc K, Reder AT (1996) Mechanisms of action of interferon beta in multiple sclerosis. Springer Semin Immunopathol 1996; 18:125-148.
19. Stuve O, Dooley NP, Uhm JH Antel Jp, Francis GS, Williams G et al. (1996) Intrferon beta-1b decreases the migration of T lymphocytes in vitro: effects on matrix metalloproteinase-9. Ann Neurol 1996; 40: 853-863.
20. Rudick RA, Ransohoff RM, Peppler R, VanderBrug Medendrop S, Lehmann P, Alam J. (1996) Interferon beta induces interleukin-10 expression: relevance to multiple sclerosis. Ann Neurol 1996; 40:618-627.
21. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001; 50(1):121-7.
22. Kurtzke JF. (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983; 33:1442-52.
23. Kanabrocki EL, Third JLH, Ryan MD, Nemchausky BA, Shirazi P, Schebing LE, et al. (2000) Circadian relationship of serum uric acid and nitric oxide. J Am Med Assoc 2000; 283:2240-1.
24. Tončev G, Miletic Drakulic S, Knezevic Z.(2007) Treatment with interferon beta 1b (Betaferon) and serum uric acid levels: two year follow up. Mult Scler; 13 (Suppl 2), S7-S273, p305.
25. Massa J, O'Reilly E, Munger KL, Delorenze GN, Ascherio A. (2009) Serum uric acid and risk of multiple sclerosis. J Neurol. 2009 Oct;256(10):1643-8.
26. Koprowsky H, Spitsin S, Hooper C. (2001) Prospects for the treatment of multiple sclerosis by raising serum levels of uric acid, a scavenger of peroxinitrite. Ann Neurol 2001; 49 (1):139.
27. Spitsin S, Hooper DC, Leist T, Streletz LJ, Mikheeva T, Koprowski H. (2001) Inactivation of peroxynitrite in multiple sclerosis patients after oral administration of inosine may suggest possible approaches to therapy of the disease. Mult Scler 2001; 7(5):313-9.
28. Constatinescu CS, Freitag P, Kappos L. (1999) Increase in serum levels of uric acid, an endogenous antioxidant, in treatment with glatiramer acetate (GAA) for multiple sclerosis. Mult Scler 1999; 5(Suppl 1): S104.
29. Toncev G, Milicic S, Toncev S, Samardzic G. (2002) High-dose methylprednisolone therap in multiple sclerosis increses serum uric acid levels. Clin Chem Lam Med 2002; 5:505-508.