*Araştırma Makalesi (Research Article)*

# Üriner Sistem Semptomları Olan Multipl Sklerozlu Hastalarda Üriner Sinir Büyüme Faktörü Düzeyleri

## *Urinary Nerve Growth Factor Levels in Multiple Sclerosis Patients with Urinary System Symptoms*

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**Özet**

**Amaç:** Multiple Skleroz (MS) hastalarında idrar sinir büyüme faktörünün (NGF) duyarlılığını ve özgüllüğünü detrüsör aşırı aktivitesinin belirteçleri olarak tahmin etmek.

**Gereç ve Yöntemler:** MS tanısı ile takip edilen üriner sistem semptomları olan 20 gönüllü hasta ve MS tanısı olan ancak üriner sistem semptomları olmayan 29 gönüllü hasta çalışmaya dahil edildi. Kişilerden kapsamlı bir anamnez ve spot idrar örnekleri alındı. Benzer yaş ve cinsiyet dağılımına sahip 27 sağlıklı gönüllü NGF düzeyleri açısından karşılaştırma yapmak üzere dahil edildi ve spot idrar örnekleri alındı.

**Bulgular:** İdrar NGF düzeyleri ve NGF/idrar Kreatinin oranları üriner semptomları bulunan MS hasta grubunda üriner semptomları bulunmayan hasta grubuna göre istatistiki olarak anlamlı olmayan şekilde daha düşüktü, üriner semptomları olmayan hasta grubunda da control grubuna göre istatistiki olarak anlamlılık içermeyen düşüklük gözlendi (p: 0,114 ve 0,833)

**Sonuç:** Üriner semptomları olan hastalarda istatistiksel olarak anlamlı olmamakla birlikte idrar NGF düzeylerinin semptomu olmayan hastalara kıyasla daha düşük olduğu tespit edilmiştir. MS hastalarında NGF ve üriner sistem şikayetleri arasındaki ilişkinin aydınlatılması için daha geniş hasta popülasyonlarında yapılacak çalışmalara ihtiyaç bulunmaktadır.

**Anahtar Kelimeler:** Multipl skleroz, Sinir büyüme faktörü, İdrar yolu disfonksiyonu

**Abstract**

**Objective:** To estimate the sensitivity and specificity of the serum and urinary nerve growth factor (NGF) in MS patients as markers of detrusor overactivity.

**Material and Methods:** 20 volunteers who were diagnosed with MS and who had urinary tract symptoms and 29 volunteers who were diagnosed with MS however who did not have urinary tract symptoms were included in the study. A comprehensive anamnesis and spot urine samples were obtained from the subjects. Twenty seven healthy volunteers with similar age and gender distribution were included for making a comparison with regard to NGF levels and spot urine samples were obtained.

**Results:** Urinary NGF levels and NGF/urinary creatinine ratios were found lower in the group composed of MS patients who had urinary symptoms compared to the group composed of MS patients who did not have urinary symptoms but the difference was not statistically significant. These parameters were found lower in patient group who did not have urinary symptoms compared to control group however the difference was not statistically significant (p: 0,114 and 0,833)

**Conclusion:** Urinary NGF levels were found to be lower in patients with urinary symptoms compared to patients without symptoms. Further studies in larger patient populations are needed to clarify the relationship between NGF and urinary tract symptoms in MS patients

**Key Words:** Multiple sclerosis, Nerve growth factor, Urinary tract dysfunction

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## INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease characterized by neuro-inflammation and neuro-degeneration in central nervous system. The disease influences the white matter, cortex, deep gray matter and characterized by inflammatory-demyelinating lesions and neuronal/axonal degeneration (1).

MS is usually seen in young adults and initial symptoms develop between ages 20-30. The disease is 1.5-3 fold more common among females. The main symptoms of the disease include hemispheric or spinal involvement-related sensory, motor, brain stem and cerebellar signs and optic neuritis (2-4).

Lower urinary system dysfunction is common in MS patients as detrussor muscle and external urethral sphincter are innervated by lateral cortico-spinal (pyramidal) and reticulo-spinal pathways and MS plaques usually influence these regions. More than 80% of MS patients exhibit lower urinary system symptoms and urologic findings are present in more than 96% of the patients who had the disease for longer than 10 years. Urgency or polyuria is seen in 31-85% of the patients, incontinence in 37-72%, obstructive symptoms presenting with urinary retention in 2-52%. Bladder symptoms become evident due to long standing disease or the other lesions like benign prostate hyperplasia, stress incontinence in patients older 50 years. The risk for upper urinary system disorder is reported to increase in patients with detrussor sphincter dys-synergia (4).

Supra-sacral, sacral or intra-cranial plaques in the white matter may lead to varying types of neurogenic bladder dysfunction; sensory, motor, sphincter functions of the bladder may be affected (5-7). However studies are also available indicating that urinary symptoms and lesion location are not inter-related in MS (8).

Kurtzke Disability Status Scale (DSS) was developed for measuring disability status of MS patients by Dr. John Kurtzke in 1950s. The aim of the scale was to establish an objective approach for measuring the magnitude of functionality that could be widely used for health service providers. The scale was changed for several times in order to correctly reflect the levels of clinically observed disability and the name of the scale was changed as Expanded Disability Status Scale (EDSS). The overall score of the scale varies between 0 and 10. The first level 1.0 and 4.5 corresponds to high ambulatory skills, and the scores between 5.0 and 9.5 corresponds to the loss of ambulation ability.

In recent years, the role of urinary neuro-trophins including nerve growth factor (NGF) and brain-derived neuro-trophic factor (BDNF) are stressed as the underlying agent and urinary bio-marker (9,10).

NGF is the first discovered member of neurotrophin (NT) family and the other NTs include BDNF, NT-3, NT-4/5 (11-14). NGF is known to be necessary for growing of various neuron types, some nerve groups, for maintenance and development of life. NF is a protein which plays a role in neuroblast proliferation, in maturation of dorsal root ganglion through affecting neuronal phenotype and as a messenger between peripheral effector tissue and the neurons innervating this tissue (11,12,15,16).

The source of NGF in peripheral tissues is estimated to be the tissues innervated by nerves. Smooth muscle cells, astrocytes, fibroblasts and the other cells synthesize NGF in culture (11,17). NGF is also known to play an important role in development, maintenance and differentiation of neural crest-originated sympathetic and sensory neurons, in obstructive, inflammatory and growing diseases (11,12,15,17).

NGF is the determinant of neuronal functions in response to physiologic or pathologic conditions, also has a modulation role defined as "neuronal plasticity". In vitro studies have revealed that NGF regulates neuro-transmitter release, triggers synaptic re-organization, increases nervous excitability through reducing activation threshold, increasing the sensitivity of nosi-septive nerves in case of hyper-sensitivity (18,19).

NGF is produced in urothelium and smooth muscle cells in urinary tract (20). Studies indicate that elevated NGF levels in bladder tissue and urine is directly correlated with lower urinary tract dysfunction like interstitial cystitis and over-active bladder (21-23).

The aim of the present study is to investigate the role of urinary NGF (uNGF) levels as an objective bio-marker in detection of urinary tract symptoms of MS patients.

## MATERIAL and METHODS

The study was planned according to the principles of the Helsinki Declaration. 20 volunteers who were diagnosed with MS and who had urinary tract symptoms and 29 volunteers who were diagnosed with MS however who did not have urinary tract symptoms were included in the study. Patients who had urinary tract infection, Diabetes Mellitus (DM), lower urinary tract operation or previous urinary tract complications were excluded from the study. A comprehensive anamnesis and spot urine samples were obtained from the subjects. Twenty seven healthy volunteers with similar age and gender distribution were included for making a comparison with regard to NGF levels and spot urine samples were obtained. The study was approved by the local Ethics Committee (2017/14) and informed consent was obtained from all patients and controls.

Urine samples were stored at -80 °C until the day of analysis and brought to room temperature at the day of analysis, centrifuged at 4000 rpm at 4 °C for 15 min. NGF levels were measured with commercial ELISA kit (CUS:10025; SunRed Biological Technology Company; (Shanghai; China), which had a minimum sensitivity of 7.336 pg/mL. The assays were performed according to the manufacturer's instructions. NGF levels (pg/mL)were normalized to the concentration of urinary creatinine (pg/mg). Urine creatinine wasmeasured using ADVİA 1800 chemistry system (Siemens Healthcare GmbH).

Statistical analyses were performed using SPSS software version 20 and the statistical significance was set as $p < 0.05$. Values were reported as mean and standard deviation. Kolmogorove Smirnov analysis was used evaluate normality. One Way ANOVA test (post hoc Tukey, Tamhane) was used for inter-group comparison of normally distributed parameters and Kruskall-Wallis test (post hoc Mann-Whitney U) was used for comparison of the parameters which did not show a normal distribution .

## RESULTS

Age distribution was similar between groups (p:0,101). Mean age was 37.7 in control group, 34.0 in MS patients who did not have urinary symptoms and 40.3 in MS patients who had urinary symptoms **(Table 1).** Control group was composed of 17 females and 10 males, MS patients without urinary symptoms group was composed of 22 females and 7 males, MS patients with urinary symptoms group was composed of 17 females and 3 males

Duration of disease and EDS scores were recorded and inter-group comparisons were made **(Table 1).**

Disease duration and EDS scores were found significantly higher in the group composed of MS patients who had urinary symptoms compared to the group composed of MS patients who did not have urinary symptoms (p: 0,001 and <0,001)**(Figure 1-2).**

Urinary NGF levels and NGF/urinary creatinine ratios were found lower in the group composed of MS patients who had urinary symptoms compared to the group composed of MS patients who did not have urinary symptoms but the difference was not statistically significant. These parameters were found lower in patient group who did not have urinary symptoms compared to control group however the difference was not statistically significant (p: 0,114 and 0,833) **(Figure 3-4).**

**Table 1. Duration of disease and EDS scores**

| Group | | Age | Disease Duratıon | EDSS |
|---|---|---|---|---|
| **Control** | **Mean** | 37,74 | | |
| | **N** | 27 | | |
| | **Std. Deviation** | 12,07 | | |
| **MS** | **Mean** | 34,03 | 2,80 | 0,52 |
| | **N** | 29 | 29 | 29 |
| | **Std. Deviation** | 10,82 | 2,10 | 0,99 |
| **MS+Incontınence** | **Mean** | 40,35 | 8,20 | 3,22 |
| | **N** | 20 | 20 | 20 |
| | **Std. Deviation** | 5,43 | 7,67 | 2,21 |

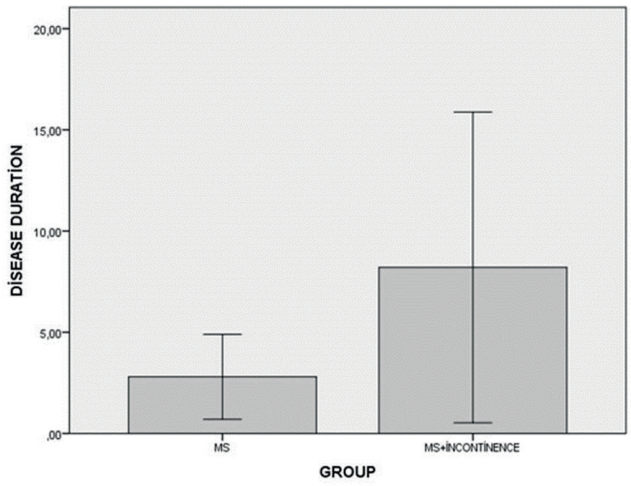


**Figure 1.** Disease Duration

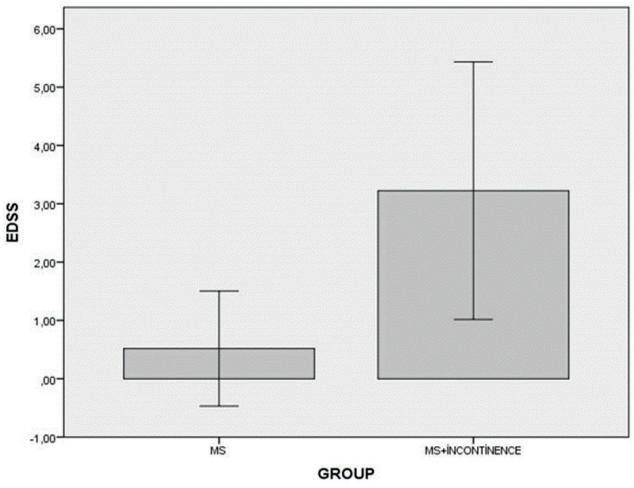


**Figure 2.** EDS Scores

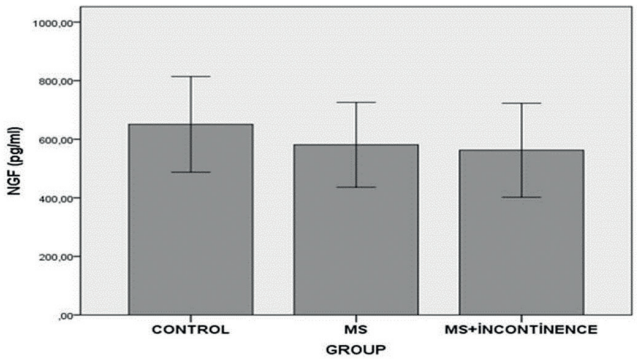


**Figure 3.** Urinary NGF levels

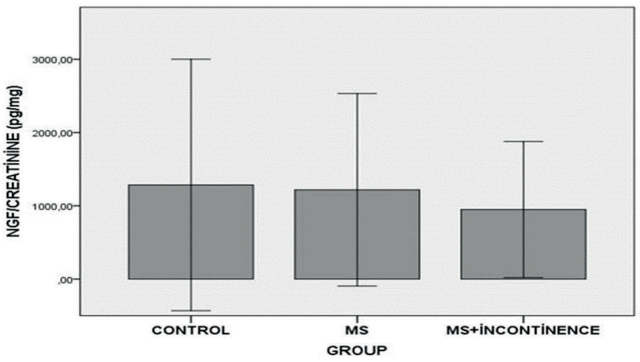


**Figure 4.** NGF/urinary creatinine ratios

## DISCUSSION

Neurotrophic polypeptides play central roles in nervous system development, from naturally occurring cell death to differentiation and neuronal outgrowth. The acknowledged prototypical example and best-characterized neurotrophin is nerve growth factor (NGF).It acts on sympathetic and neural crest-derived sensory neurons, and is also present in the central nervous system (CNS), where it serves a trophic function in the development and maintenance of basal forebrain cholinergic neurons. Serum NGF was found to play an important role in pathogenesis of auto-immune disorders and degenerative diseases. Serum NGF levels were found elevated in vernal conjunctivitis, allergic diseases and asthma (24,25). Elevated urinary NGF level was proposed as a bio-marker superior to BDNF for over-active bladder (26, 27). Elevated serum NGF levels may reduce excitatory threshold value in dorsal root ganglion of the bladder and may lead to an increase in mechanic sensitivity of bladder wall (28).

However elevated NGF levels leads to an important problem in urinary tract infections, stones and bladder tumors (29).

In our previous study, we investigated uNGF in 40 children with over-active bladder.Urine samples were examined at baseline, at 3th and 6th months after treatment and uNGF/Cr levels were measured in study group. We observed that uNGF/Cr levels were higher in over-active bladder group compared to control group and also uNGF/Cr levels decreased to control group levels at 6th month of anti-muscarinic treatment (30).

Lower urinary tract symptoms are common in MS patients. Based on the North American Research Committee on Multiple Sclerosis Registry, a large survey of more than 9700 MS patients,65% reported moderate to severe urinary complaints. Nocturia, followed by urinary urgency and frequency were the most prevalent signs. Urinary incontinence and poor bladder emptying were noted less frequently. These condition is significant morbidity and impairment of their quality of life (31). Literature data show that uNGF levels are mostly studied in lower urinary tract disorders, mainly over-active bladder. In our study, uNGF levels were found lower in MS patients who did not have urinary tract symptoms compared to control group. uNGF levels were found lower in MS patient who had incontinence compared to MS patients who did not have incontinence however the difference was not statistically significant. It is considered that reduced NGF leads to an impairment in modulation defined as neuronal plasticity and incontinence may develop.

Neuronal growth factor (NGF) was intensively studied as a neuro-protective substance in neuro-degenerative diseases and reported to take part in neuronal survival and repair processes (32). NGF was reported to protect central nervous system in experimental auto-immune encephalo-myelitis (EAE)(33). The strongest rationale of mesenchymal stem cell (MSC) transplantation which is an effective therapeutic approach in MS, Alzheimer disease (AD), Parkinson disease (PD) and Amiotrophic Lateral Sclerosis (ALS) was reported to be MSCs' ability of releasing neuro-trophic factors (34).

In vivo and in vitro studies have indicated that NGF induces axonal regeneration, survival, protection and differentiation of oligo-dendrocytes; facilitates migration and proliferation of oligo-dendrocyte precursors which play an important role in fighting with demyelinating diseases; improves clinical findings and induces the recovery of neuro-inflammatory diseases including experimental allergic neuritis and EAE (35-37).

As shown in EAE which is an animal model sharing most of the clinical and pathologic characteristics defined for human MS, neuro-trophins and receptors play a role in patho-physiology of MS (38). Various studies have indicated that administration of neuro-trophins like BDNF and NT-3 increases survival of neurons in EAE and the other neuronal injury models (39,40).

Zhao Qu et al. have found that the useful effect provided with corneal iridoid glycoside (CIG) treatment in EAE model could be achieved through prevention of the reduction in BDNF and NGF expression in medulla spinalis(41).

Similarly with the studies about the role of NGF in MS, NGF was found lower in MS groups compared to control

group, although the difference was insignificant. Finding EDS scores higher in MS patients with incontinence compared to the patients without incontinence reveals that disease is more severe in the group with incontinence. Lower NGF levels in incontinence group in which disease severity is higher reveal the presence of an inverse proportion between disease severity and NGF levels.

Urinary NGF levels were found to be lower in patients with urinary symptoms compared to patients without symptoms. Further studies in larger patient populations are needed to clarify the relationship between NGF and urinary tract symptoms in MS patients.

### Conflict of Interest and Financial Status

Our study has not been financed by an institution and institution. In this study, there is no conflict of interest among the authors on any subject.

### Research Contribution Rate Statement Summary

The authors declare that, they have contributed equally to the manuscript.

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