NÖRO PSİKİYATRİ ARŞİVİ

Brief Report

Arch Neuropsychiatry 2016; 53: 353-356 • DOI: 10.5152/npa.2016.16979

Serum Prolactin Levels in Multiple Sclerosis, Neuromyelitis Optica, and Clinically Isolated Syndrome Patients

Recai TÜRKOĞLU[1], Murat GİRİŞ[2], Mehmet GENCER[1], Uğur AKCAN[2], Arda ÖRÇEN[2]

[1]Clinic of Neurology, Haydarpaşa Training and Research Hospital, İstanbul, Turkey
[2]Department of Neurology, İstanbul University İstanbul School of Medicine, İstanbul, Turkey

ABSTRACT

Introduction: Prolactin has been discussed as a factor likely to play a mediating role in multiple sclerosis (MS). Our aim was to investigate the possible association between prolactin production and clinical features of autoimmune demyelinating central nervous system disorders.

Methods: Serum prolactin levels of 255 MS patients, 19 neuromyelitis optica (NMO) patients, 15 clinically isolated syndrome (CIS) patients, and 240 healthy controls were measured by a heterogeneous sandwich magnetic separation assay.

Results: MS and NMO cohorts had a significantly higher number of patients with hyperprolactinemia than healthy controls. Sera obtained during attacks of both MS and NMO patients displayed higher prolactin levels than those collected during remission. Prolactin level elevations were found to be more prominent in myelitis attacks in MS. No significant correlation was found between prolactin levels and age, disease duration, disability status, number of attacks, and oligoclonal band positivity. CIS patients who converted to MS had higher prolactin levels than those who did not.

Conclusion: Our findings support the possible mediating role of prolactin in the immunopathogenesis of MS, NMO, and conversion from CIS to MS.

Keywords: Prolactin, multiple sclerosis, neuromyelitis optica, clinically isolated syndrome

INTRODUCTION

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) with abnormal immune regulation and activated immune cells in the brain and peripheral blood. The etiology of MS and the factors triggering and mediating the immune response in this disease are still under examination. Previously, the dysregulation of prolactin production has been discussed as a factor likely to play a mediating role in autoimmune diseases (1). Prolactin is synthesized and secreted by the anterior pituitary gland. Peripheral prolactin, the so-called immune reactive prolactin or lymphocyte prolactin, is produced and secreted locally by lymphocytes (2). Prolactin has potent immunomodulating properties and is structurally related to members of the cytokine family. Cytokines IL-1, IL-2, and IL-6 stimulate prolactin production, while IFN-γ inhibits prolactin production**.** Prolactin plays a major role in T-cell development and influences the proliferation of pre-activated T-cells (3,4). Alterations in prolactin production have been described in systemic lupus erythematosus, Reiter's syndrome, juvenile and adult rheumatoid arthritis, autoimmune thyroiditis, and autoimmune uveitis (3,5). Increased plasma levels of prolactin have also been described in MS patients (6,7), and increased prolactin production has been associated with an optico-spinal disease variant called Asian-type MS (8), suggesting that hyperprolactinemia is involved in the pathogenesis of autoimmune demyelinating diseases of the brain.

To investigate the association of prolactin levels with clinical features of MS and neuromyelitis optica (NMO), two major autoimmune demyelinating disorders, and to delineate whether prolactin levels can be used to predict conversion from CIS to MS, we conducted prolactin-level measurements in consecutive MS, NMO, and CIS patients with or without conversion to MS.

METHODS

Subjects

A total of 255 consecutive patients with definite clinical relapse remitting MS, as per relevant diagnostic criteria (9), 19 consecutive NMO patients fulfilling the revised diagnostic criteria (10), 15 CIS patients with a minimum follow-up period of 5 years (mean±standard deviation, range; 6.9±1.6; 5-9 years), and 240 healthy control subjects were enrolled. CIS was diagnosed as indicated by relevant diagnostic criteria (9). Since it may take several years for CIS to convert to MS, a minimum follow-up rule was applied for all potential MS patients to complete their conversion from CIS to MS. We compared prolactin levels between CIS patients who converted to clinically definite MS (CIS-MS+) and

Correspondence Address: Recai Türkoğlu, Haydarpaşa Eğitim ve Araştırma Hastanesi, Nöroloji Kliniği, İstanbul, Türkiye
E-mail: recaiturkoglu@yahoo.com

Received: 29.02.2016 **Accepted:** 03.03.2016

those who did not (CIS-MS−). Six of 15 CIS patients developed clinically definite MS in a follow-up period of 2-5 years. There were no significant differences between groups by means of demographic features (Table 1).

In the MS cohort, attacks were clinically classified as brainstem, motor and/or sensorial, optic neuritis, myelitis, or polysymptomatic attacks; whereas in the NMO cohort, attacks were classified as optic neuritis or myelitis attacks. All female subjects were premenopausal, non-menstruating, non-lactating, and non-pregnant between the ages of 20 to 46. Patients with potential causes of prolactin level alteration, such as thyroid or pituitary gland disorders, other accompanying autoimmune disorders, liver or kidney disease, and major psychiatric disorders, were excluded. All subjects gave informed consent, which was approved by the local medical research ethics committee.

Disability was assessed using the Expanded Disability Status Scale (EDSS) in all patients. Magnetic resonance imaging (MRI) studies were performed in patients with attacks and hyperprolactinemia to rule out hypothalamo-pituitary axis involvement using the same 3-T scanner.

In MS and NMO cohorts, sera were obtained during either attack or remission periods, whereas all CIS sera were obtained during the attack period. All sera were immediately centrifuged and stored in aliquots at −70°C. During blood sampling, 12 patients in the NMO group and 242 patients in the MS group were under azathioprine and disease modifying drug treatment (interferon-beta or glatiramer acetate), respectively. None of the patients were receiving steroid treatments during blood sampling. In the sera of NMO patients, antibodies to the extracellular region of aquaporin-4 (Aqp-4) were measured by a cell-based assay kit utilizing human embryonal kidney cells transfected with Aqp-4 (Euroimmun, Luebeck, Germany).

Serum Prolactin Assays

Serum prolactin level was measured by a heterogeneous sandwich magnetic separation assay (Beckman Coulter, CA, USA) as per the manufacturer's recommendations. Results were calculated with a standard curve. Normal ranges were 2.6-13.1 ng/mL in males and 2.7-26.7 ng/mL in females.

Statistical Analysis

Fisher's exact test was used for comparison of incidences whereas all other parameters were compared with a non-parametric Mann-Whitney U or Kruskal-Wallis and post-hoc Dunn tests due to the abnormal distribution of data. Spearman's test was performed to seek a possible correlation between prolactin levels and clinical-demographic features of the patients. The statistical significance level was established at $p<0.05$.

RESULTS

Increased Serum Prolactin Levels in MS and NMO Patients

MS, NMO, and CIS groups had higher mean serum prolactin levels than healthy controls ($p<0.001$ by Kruskal-Wallis test). However, the post-hoc Dunn test yielded significant differences for only MS and NMO groups ($p<0.001$ and $p<0.05$, respectively). When the percentages of patients with prolactin levels higher than the normal range were compared with Fisher's exact test, MS ($p<0.001$), NMO ($p<0.001$), and CIS ($p<0.001$) groups showed significantly higher hyperprolactinemia incidences than healthy controls. There were no significant differences between MS, NMO, and CIS groups. Moreover, CIS-MS+ patients (n=6, 31.2±14.5 ng/mL) had significantly higher prolactin levels than CIS-MS− patients (n=9, 16.7±9.7 ng/mL) ($p=0.03$ by Mann-Whitney U). Five of 6 CIS-MS+ patients and 3 of 9 CIS-MS− patients had prolactin levels above the normal range ($p=0.118$ by Fisher's exact test).

Table 1. Demographic characteristics and prolactin values of the study population

	MS (n=255)	NMO (n=19)	CIS (n=15)	HC (n=240)
Age (mean±SD, range)	38.1±10.7 (20-46)	41.3±9.6 (23-42)	35.4±8.1 (21-39)	37.2±10.5 (20-44)
Sex (F/M)	174/81	15/4	11/4	172/68
Prolactin levels (ng/mL) (mean±SD, range)	24.7±13.2 (9.4-66.4)	23.5±11.4 (6.6-85.5)	20.5±13.5 (2.9-57.4)	15.2±5.7 (4.6-24.7)
Cases with increased prolactin levels (n, %)	97 (38.0%)	8 (42.1%)	8 (33.3%)	21 (8.7%)
Number of attacks (mean±SD, range)	4.8±2.1 (1-9)	5.3±5.9 (1-14)	NA	NA
Disease duration (years) (mean±SD, range)	5.4±3.6 (1-14)	7.1±5.7 (1-17)	NA	NA
EDSS (mean±SD, range)	2.3±1.4 (1.0-6.0)	3.3±1.8 (1.0-7.0)	1.4±0.5 (1.0-2.0)	NA
PI (mean±SD, range)	0.6±0.6 (0.1-2.4)	0.7±0.8 (0.2-3.0)	NA	NA

MS: multiple sclerosis; NMO: neuromyelitis optica; CIS: clinically isolated syndrome; HC: healthy controls; F: female; M: male; SD: standard deviation; EDSS: expanded disease status scale; PI: progression index (disability score divided by the duration of MS in years); NA: not applicable

Impact of Clinical Features on Prolactin Levels

Sera were obtained during attacks in 75 of 255 MS and 8 of 19 NMO patients. In both disease groups, prolactin levels in the attack sera were significantly higher than those in the remission sera (Table 2). Prolactin levels were above the normal range in 45/75 (60%) of MS attack sera and 6/9 (66.7%) of NMO attack sera. New enhancing lesions were detected in the MRI of some relapsing MS and NMO patients with hyperprolactinemia, but none of them were related to the hypothalamo-pituitary axis. There were also no chronic lesions in the diencephalon and brainstem regions of these patients. Based on predominant clinical features, MS patients' attacks were classified as brainstem (n=9), motor and/or sensorial (n=25), optic neuritis (n=9), myelitis (n=12), and polysymptomatic (combination of more than one attack type) (n=20) attacks. None of the polysymptomatic patients showed myelitis findings. While MS patients with myelitis attacks (n=12) displayed significantly higher prolactin levels than those without myelitis findings (n=63) ($p<0.001$ by Mann-Whitney U test) (Table 2), there were no significant prolactin level differences between patients with other types of attacks (not shown). Similarly, NMO patients with optic neuritis (n=2) and myelitis (n=8) attacks showed comparable prolactin levels ($p=0.305$ by Mann-Whitney U test) (Table 2).

Female NMO and MS patients had higher prolactin levels than male patients, as expected. However, no difference could be found between oligoclonal band positive and negative MS and NMO patients and Aqp-4 antibody positive and negative NMO patients (Table 2). Similarly, no significant correlation was found between prolactin levels, age, number of attacks, disease duration, EDSS, and progression index scores in MS and NMO cohorts ($R=0.041$-0.136, $p>0.05$ for all tests).

DISCUSSION

Our results have confirmed the well-known association between prolactin level changes and MS. We have also found elevated prolactin levels in NMO and CIS patients for the first time, suggesting that altered prolactin production is not exclusively seen in MS but in other autoimmune

Table 2. Distribution of prolactin levels (ng/mL, mean±standard deviation) according to demographic and clinical features of MS and NMO patients

	MS (n=255)	p
Female (n=174) vs. male (n=81)	29.2±13.0 vs. 15.1±6.9	<0.001
Attack serum (n=75) vs. remission serum (n=180)	34.7±10.5 vs. 20.6±11.9	<0.001
Myelitis attack serum (n=12) vs. non-myelitis attack serum (n=63)	48.3±10.1 vs. 32.2±8.4	<0.001
Oligoclonal band positive (n=198) vs. negative (n=57)	22.2±11.7 vs. 25.5±13.7	0.103
	NMO (n=19)	**p**
Female (n=15) vs. male (n=4)	24.4±19.9 vs. 11.8±4.7	0.027
Attack serum (n=8) vs. remission serum (n=11)	33.7±23.3 vs. 13.0±5.6	0.020
ON attack serum (n=2) vs myelitis attack serum (n=6)	27.6±14.3 vs. 35.8±26.4	0.305
Oligoclonal band positive (n=5) vs. negative (n=14)	16.4±12.8 vs. 23.6±20.1	0.189
Aqp-4 Ab positive (n=10) vs. negative (n=9)	22.4±23.5 vs. 21.0±11.9	0.438

ON: optic neuritis; Aqp-4 Ab: aquaporin-4 antibody

demyelinating disorders as well. Although prolactin is primarily known as a lactogenic hormone, it is also considered to be a potent immunomodulator (1,2,3). Prolactin abnormalities have been observed in a number of multi-organ diseases such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjögren's syndrome, and organ-specific autoimmune diseases such as uveitis (5). Our findings further confirm the notion that prolactin production is altered in autoimmune disorders presumably as a universal immunomodulating or compensating mechanism.

The findings of our study confirm previous studies that have found mild to moderate hyperprolactinemia in MS cohorts (6,7,11) and other studies that have not found an association between prolactin levels, oligoclonal band status, or disease duration (12,13,14,15). However, the association between disease activity and prolactin levels is more controversial and many previous studies conducted with relatively smaller MS cohorts have failed to show a correlation between prolactin levels and disease activity (13,14,15). In contrast to these studies, we observed higher prolactin levels in MS patients during relapse. In resemblance to our findings, prolactin levels have been elevated during MS and optico-spinal MS attacks in two separate Japanese cohorts (7,8). These conflicting results can be explained with variations in patient inclusion criteria, subject size, treatment status, and ethnogeographic features involved in the different MS studies.

Kira et al. (7) has demonstrated the presence of hypothalamic lesions in MS patients. In this study, 4 of 8 patients with hyperprolactinemia were determined to have diencephalo-hypothalamic lesions. Therefore, the authors have speculated that hyperprolactinemia in these patients can be explained by MS lesions affecting the hypothalamus that interfere with release of the prolactin inhibitory factor dopamine (7). However, we failed to find any active or old lesions in the diencephalon, hypothalamus, or brainstem regions of MS and NMO patients, indicating that hyperprolactinemia observed during attacks is not necessarily a consequence of disinhibition of prolactinergic neurons due to damaging of the dopaminergic neurons.

Notably, prolactin levels were found to be particularly elevated in the myelitis attacks of MS patients as well as in patients with NMO (our study) and optico-spinal MS (8), both of which predominantly affect spinal cord functions. Elevated prolactin levels have also been observed in other forms of spinal cord injury (16,17). The suckling stimulus from the mechanoreceptors of the nipples is delivered to the spinal cord and then projected to specific diencephalic nuclei, ultimately leading to increased prolactin secretion (18). It is thus tempting to speculate that affliction of these sensory pathways during autoimmune myelitis attacks might be dysregulating prolactin production mechanisms.

In parallel with a previous study, CIS patients did not show significantly elevated prolactin levels as opposed to MS patients in our study (14). However, prolactin production was significantly increased in CIS patients that converted to MS in due course. CIS is characterized with a single attack and objective clinical evidence for a single brain lesion. MS diagnosis is established by the demonstration of new T2 and/or contrast enhancing lesions, or a second clinical attack (9). Prediction of CIS patients that will convert to MS, or that are at high risk for conversion to MS, is critical for starting immunomodulating treatment methods as early as possible. Prolactin levels might thus serve as potential predictors of MS conversion. This assumption should be tested with further studies performed on larger CIS cohorts.

Prolactin level elevations in MS and NMO attacks, especially the more prominent elevations in myelitis attacks, support prolactin's possible mediating role in the immunopathogenesis of autoimmune demyelinating disorders. Prolactin level screening might be helpful in the differential diagnosis of pseudo-attacks and prediction of conversion from CIS to MS.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University İstanbul School of Medicine

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - R.T.; Design - M.Gencer; Supervision - U.A.; Resources - A.Ö.; Materials - U.A.; Data Collection and/or Processing - R.T.; Analysis and/or Interpretation - U.A., M.Giriş, A.Ö.; Writing Manuscript - R.T.; Critical Review - U.A.; Other - A.Ö., U.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Jara LJ, Lavalle C, Fraga A, Gómez-Sanchez C, Silveira LH, Martínez-Osuna P, Germain BF, Espinoza LR. Prolactin, immunoregulation, and autoimmune diseases. Semin Arthritis Rheum 1991; 20:273-284. [CrossRef]
2. Matera L. Action of pituitary and lymphocyte prolactin. Neuroimmunomodulation 1997; 4:171-180.
3. Chikanza IC. Prolactin and neuroimmunomodulation: in vitro and in vivo observations. Ann N Y Acad Sci 1999; 876:119-130. [CrossRef]
4. Dogusan Z, Martens N, Stinissen P, Hellings N, Demotte N, Hooghe R, Hooghe-Peters E. Effects of prolactin on cloned human T-lymphocytes. Endocrine 2003; 20:171-176. [CrossRef]

5. Orbach H, Shoenfeld Y. Hyperprolactinemia and autoimmune diseases. Autoimmun Rev 2007; 6:537-542. [CrossRef]
6. Azar ST, Yamout B. Prolactin secretion is increased in patients with multiple sclerosis. Endocr Res 1999; 25:207-214. [CrossRef]
7. Kira J, Harada M, Yamaguchi Y, Shida N, Goto I. Hyperprolactinemia in multiple sclerosis. J Neurol Sci 1991; 102:61-66. [CrossRef]
8. Yamasaki K, Horiuchi I, Minohara M, Osoegawa M, Kawano Y, Ohyagi Y, Yamada T, Kira JI. Hyperprolactinemia in optico-spinal multiple sclerosis. Intern Med 2000; 39:296-299. [CrossRef]
9. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, Lublin FD, Montalban X, O'Connor P, Sandberg-Wollheim M, Thompson AJ, Waubant E, Weinshenker B, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011; 69:292-302. [CrossRef]
10. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. Neurology 2006; 66:1485-1489. [CrossRef]
11. Moshirzadeh S, Ghareghozli K, Harandi AA, Pakdaman H. Serum prolactin level in patients with relapsing-remitting multiple sclerosis during relapse. J Clin Neurosci 2012; 19:622-623. [CrossRef]
12. Sandyk R, Awerbuch GI. Relationship of nocturnal melatonin levels to duration and course of multiple sclerosis. Int J Neurosci 1994; 75:229-237. [CrossRef]
13. Harirchian MH, Sahraian MA, Shirani A. Serum prolactin level in patients with multiple sclerosis: a case control study. Med Sci Monit 2006; 12:177-180.
14. Markianos M, Koutsis G, Evangelopoulos ME, Mandellos D, Sfagos C. Serum and cerebrospinal fluid prolactin levels in male and female patients with clinically-isolated syndrome or relapsing-remitting multiple sclerosis. J Neuroendocrinol 2010; 22:503-508. [CrossRef]
15. Heesen C, Gold SM, Bruhn M, Mönch A, Schulz KH. Prolactin stimulation in multiple sclerosis--an indicator of disease subtypes and activity? Endocr Res 2002; 28:9-18. [CrossRef]
16. Clark MJ, Schopp LH, Mazurek MO, Zaniletti I, Lammy AB, Martin TA, Thomas FP, Acuff ME. Testosterone levels among men with spinal cord injury: relationship between time since injury and laboratory values. Am J Phys Med Rehabil 2008; 87:758-767. [CrossRef]
17. Rutberg L, Fridén B, Karlsson AK. Amenorrhoea in newly spinal cord injured women: an effect of hyperprolactinaemia? Spinal Cord 2008; 46:189-191. [CrossRef]
18. Szabo FK, Snyder N, Usdin TB, Hoffman GE. A direct neuronal connection between the subparafascicular and ventrolateral arcuate nuclei in non-lactating female rats. Could this pathway play a role in the suckling-induced prolactin release? Endocrine 2010; 37:62-70. [CrossRef]