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

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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...
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 poly-symptomatic 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 hypothalmo-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 hyperprolactinaemia 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).

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 hyperprolactinaemia, but none of them were related to the hypothalmo-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 or sensorial (n=25), optic neuritis (n=9), myelitis (n=12), and poly-symptomatic (combination of more than one attack type) (n=20) attacks. None of the poly-symptomatic 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 diseases.
Table 2. Distribution of prolactin levels (ng/mL, mean±standard deviation) according to demographic and clinical features of MS and NMO patients

<table>
<thead>
<tr>
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<th>MS (n=255)</th>
<th>NMO (n=19)</th>
<th>p</th>
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<tbody>
<tr>
<td>Female (n=174) vs. male (n=81)</td>
<td>29.2±13.0 vs. 15.1±6.9</td>
<td>24.4±19.9 vs. 11.8±4.7</td>
<td>&lt;0.001</td>
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<td>Attack serum (n=75) vs. remission serum (n=180)</td>
<td>34.7±10.5 vs. 20.6±11.9</td>
<td>33.7±23.3 vs. 13.0±5.6</td>
<td>&lt;0.001</td>
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<tr>
<td>Myelitis attack serum (n=12) vs. non-myelitis attack serum (n=63)</td>
<td>48.3±10.1 vs. 32.2±8.4</td>
<td>27.6±14.3 vs. 35.8±26.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oligoclonal band positive (n=198) vs. negative (n=57)</td>
<td>22.2±11.7 vs. 25.5±13.7</td>
<td>16.4±12.8 vs. 23.6±20.1</td>
<td>0.103</td>
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<td>NMO (n=19) vs. female (n=15) vs. male (n=4)</td>
<td>22.4±23.5 vs. 21.0±11.9</td>
<td>24.4±19.9 vs. 11.8±4.7</td>
<td>0.438</td>
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<tr>
<td>Attack serum (n=8) vs. remission serum (n=11)</td>
<td>27.6±14.3 vs. 35.8±26.4</td>
<td>27.6±14.3 vs. 35.8±26.4</td>
<td>0.305</td>
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<td>ON attack serum (n=2) vs myelitis attack serum (n=6)</td>
<td>16.4±12.8 vs. 23.6±20.1</td>
<td>16.4±12.8 vs. 23.6±20.1</td>
<td>0.189</td>
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<tr>
<td>Oligoclonal band positive (n=5) vs. negative (n=14)</td>
<td>22.2±23.5 vs. 21.0±11.9</td>
<td>22.4±23.5 vs. 21.0±11.9</td>
<td>0.438</td>
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ON: optic neuritis; Aqp-4 Ab: aquaporin-4 antibody

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-spatial 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 hypotalamic 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-spatial 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 sucking 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 Istanbul University Istanbul 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.Girig, A.Ö.; Writing Manuscript - R.T.; Critical Review - U.A.; Other - A.Ö., U.A.

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