Ferritin and Prolactin Levels in Multiple Sclerosis

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ABSTRACT: **Background:** Multiple sclerosis (MS) is a common demyelinating disorder of the central nervous system (CNS) and although it is a well-established autoimmune disease its ethiopathogenesis has yet to be fully elucidated. The disease may present in several clinical forms that are closely associated with disease morbidity. In recent years various environmental and hormonal factors have been implicated in the pathogenesis of autoimmunity.

Objectives: To evaluate ferritin and prolactin levels in MS patients and their correlation with clinical manifestations of the disease.

Methods: Serum samples from 150 multiple sclerosis patients were evaluated for demographic characteristics, clinical parameters as well as prolactin and ferritin levels utilizing the Liaison chemiluminescent immunoassays (DiaSorin, Italy). Sera from 100 matched healthy donors were used as controls.

Results: Hyperprolactinemia was documented in 10 of 150 MS patients (6.7%) and hyperferritinemia in 12 (8%), both of which were significantly more common in this group compared with healthy controls ($P \leq 0.01$ and $P = 0.02$ respectively). Among female MS patients, elevated prolactin levels were related to the secondary-progressive type of disease ($P = 0.05$), whereas hyperferritinemia was associated with male gender ($P = 0.03$) and with the relapsing-progressive type of the disease ($P = 0.02$). An inverse association was found between hyperferritinemia and the relapsing-remitting type of MS in male patients ($P = 0.05$)

Conclusions: Our results suggest a plausible association between these biomarkers and certain clinical types and gender among MS patients. Further studies combining clinical data, CNS imaging and these markers are warranted.

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KEY WORDS: multiple sclerosis, ferritin, prolactin, iron, autoimmunity

Multiple sclerosis is a common autoimmune inflammatory demyelinating disease of the central nervous system, which affects females twice as often as males, mainly during the third and fourth decades [1]. MS is characterized clinically by the involvement of different areas of the CNS at different time points, and pathologically by multifocal areas of demyelination, loss of oligodendrocytes, astroglial scarring, and axonal injury. The latter was recently recognized as a prominent feature of this autoimmune disease [2]. Although certain clinical features are typical of MS, the disease has a highly variable pace and many atypical forms. The ethiopathogenesis of MS is unknown, but the most accepted theory is that MS is mediated by autoreactive lymphocytes [3-5] following a combination of genetic, environmental and hormonal triggers such as prolactin and ferritin [6-8].

Prolactin is a neuroendocrine peptide that belongs to the growth and lactogen hormone family and exists in several isoforms [9]. Prolactin is secreted by the anterior pituitary gland, under hypothalamus control, and is stimulated by thyrotropin-releasing hormone, stress and immune modulation, as well as by extrapituitary cells, mainly lymphocytes [10,11]. Pituitary secretion of prolactin itself was found to be not only a hormone but an immunomodulating molecule with an array of effects, including regulation of the maturation of CD4-CD8- thymocytes into CD4+ CD8+T-cells; impairment of the autoreactive B cells negative selection; anti-apoptotic effects on transitional B cells; enhancement of the proliferative response to specific antigens and mitogens; enhancement of antigen-presenting cell development expressing major histocompatibility complex class II and co-stimulatory molecules CD40, CD80, CD86; increased immunoglobulin production; upregulation of Th1 cytokines; and enhancement of interleukin-2 effects on lymphocytes [12]. Mild hyperprolactinemia has been found to be associated with autoimmune diseases in humans (e.g., systemic lupus erythematosus, rheumatoid arthritis, autoimmune thyroiditis) as well as in animal mod-

MS = multiple sclerosis
CNS = central nervous system

els [12,13]. In the experimental model of MS, experimental allergic encephalomyelitis, increased levels of prolactin were found. Furthermore, the administration of the prolactin antagonist bromocriptine led to disease suppression [10].

Ferritin is an acute-phase reactant that represents the major intracellular iron storage protein essential for various metabolic processes including myelin formation and oxidative phosphorylation [14]. Data from several biochemical and pharmacological studies indicate that free radicals participate in the pathogenesis of experimental allergic encephalomyelitis, and iron has been implicated as the catalyst leading to their formation [15]. Ferritin also has immunomodulatory effects including reduction of granulocyte phagocytosis and suppression of antibody production and of delayed-type hypersensitivity [16]. Additionally, hyperferritinemia has been found to be associated with inflammation, malignancies, infections and autoimmunity [16]. Mainly it is a characteristic of adult-onset Still´s disease but was also reported in other autoimmune diseases such as lupus, rheumatoid arthritis and dermatomyositis [16,17]. Previously we found an increased prevalence of hyperferritinemia and hyperprolactinemia in patients with MS. Thus, the aim of this study was to evaluate possible correlations with different clinical presentations of the disease.

PATIENTS AND METHODS

Serum samples were collected from 150 consecutive Hungarian patients with multiple sclerosis. We also obtained demographic and clinical data, such as gender, age, disease duration, pattern of disease (i.e., relapsing-remitting, primary-progressive, secondary-progressive and relapsing-progressive) and treatment given during the period of the study. The latter included glucocoticoids, immunomodulatory treatments (i.e., interferon-gamma), immunosuppressive drugs (azathioprine or mitoxantrone) or other treatments following immunomodulatory treatment. Sera from 100 matched healthy donors served as controls. The study received approval from the local ethics committees and adhered to the ethical guidelines of the most recent Helsinki Declaration (Edinburgh, 2000).

PROLACTIN AND FERRITIN LEVELS MEASUREMENT

We used the Liaison chemiluminescent immunoassays (DiaSorin, Italy) to measure serum concentration of prolactin and ferritin levels in the sera of patients and controls as described elsewhere [14]. Briefly, this method provides quantitative determination of these markers using a direct, competitive chemiluminescence immunoassay. Specific antibodies are used for coating magnetic particles (solid phase). After an incubation period with the subject sera, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units and is inversely proportional to the concentration hormones present in calibrators, controls, or samples.

The prolactin results are expressed in mIU/L. The normal prolactin levels in women are dependent on age. Normal levels in women of reproductive age (≤ 45 years) are 132–498 mIU/L while in postmenopausal women (> 45 years old) the normal levels are 90–392 mIU/L. In men the normal levels range between 87 and 392 mIU/L. Normal ferritin levels are also dependent on gender and age: for younger women (≤ 45 years old), levels of 4–104.2 ng/ml are considered normal, while for those > 45 years old the range is between 4.9 and 232.2 ng/ml. Normal ferritin levels in men are 18.2–341.2 ng/ml.

STATISTICAL ANALYSIS

Comparison of categorical variables between groups was performed by chi-square test and Fisher's exact test (two-tailed) as appropriate. Continuous variables are expressed as mean ± standard deviation and were compared between groups by Student's *t*-test (two-tailed). For all tests P values < 0.05 were considered statistically significant. The statistical program SPSS 13.0 (SPSS, Chicago, IL, USA) was used for all analyses.

RESULTS

We found an increased prevalence of both hyperprolactinemia (10/150 patients, 6.7%) and hyperferritinemia (12/150, 8%) among MS patients as compared with healthy controls, who exhibited no hyperprolactinemia ($P \leq 0.01$) and only 1% (1/100) had hyperferritinemia ($P = 0.02$). The mean prolactin level was 108 ± 126 mIU/L versus 83 ± 55 mIU/L in the patients and controls respectively ($P = 0.06$), while the mean ferritin level was 231 ± 187 ng/ml versus 150 ± 64 ng/ml in the patients and controls respectively ($P < 0.001$). Our MS group consisted of 96 females (64%) and 54 males (36%). The patients' mean age was 36 ± 9 years and the mean duration of disease was 6.7 ± 4 years. Age, duration of disease and its types were similar between genders except for the relapsing-progressive type of disease, which was present only in male patients [Table 1]. Most of our patients (135/150, 90%) were diagnosed with relapsing-remitting disease, and only 15 patients (10%) presented with one of the progressive types [Table 1]. At the time of the study all patients received medical treatment: 48.6% of patients were treated with glucocorticoids, 40% with immunomodulatory drugs (i.e., interferon-gamma), 6% with immunosuppressive drugs (azathioprine or mitoxantrone) and 5.3% with other medications [Table 1].

In this group of MS patients, 10/150 (6.6%) had elevated serum levels of prolactin, 8 of whom were women. No association was demonstrated between prolactin levels and age, gender, duration of disease or treatment [Table 2]. However,

Table 1. Demographics of multiple sclerosis patients

	Male	Female	P*
No. of patients	54 (36%)	96 (64%)	NS
Age (yrs, mean ± SD)	34 ± 7	37 ± 10	NS
Duration of disease (yrs, mean ± SD)	6.8 ± 4.5	6.7 ± 5	NS
Type of disease			
Relapsing-remitting	47 (87%)	88 (91%)	NS
Primary-progressive	3 (5.5%)	3 (3.1%)	NS
Secondary-progressive	1 (1.8%)	5 (5.2%)	NS
Relapsing-progressive	3 (5.5%)	0	0.04
Treatment			
Glucocorticoids	22 (40.7%)	51 (53.1%)	NS
Immunomodulatory	23 (42.5%)	37 (38.5%)	NS
Immunosuppressive	5 (9.2%)	4 (4.1%)	NS
Other	4 (7.4%)	4 (4.1%)	NS

*P was considered significant below 0.05; NS=non significant

Table 2. Characteristics of multiple sclerosis patients according to prolactin levels

	Elevated prolactin	Normal prolactin	P
No. of patients	10	140	
Age (yrs, mean ± SD)	36 ± 12	36 ± 9	NS
Duration of disease (yrs, mean ± SD)	8.3 ± 5	6.7 ± 5	NS
Gender (male)	2 (20%)	52 (37.1%)	NS
Type of disease			
Relapsing-remitting	8 (80%)	127 (91%)	NS
Primary-progressive	0	6 (4%)	NS
Secondary-progressive	2 (20%)	4 (3%)	0.05
Relapsing-progressive	0	3 (2%)	NS
Treatment			
Glucocorticoids	3 (30%)	70 (50%)	NS
Immunomodulatory	7 (70%)	53 (38%)	0.089
Immunosuppressive	0	9 (6.4%)	NS
Other	0	8 (5.7%)	NS

*P was considered significant below 0.05; NS=non significant

two patients with hyperprolactinemia (20%) had the secondary-progressive type of disease. This prevalence was higher than in patients exhibiting normal levels ($P = 0.05$). Among our eight female patients with hyperprolactinemia, 2 (25%) were diagnosed with the secondary-progressive form of disease compared with only 3/88 MS female patients (3.4%) with normal PRL levels ($P = 0.05$). Six of our female MS patients with high prolactin levels (75%) were treated with immunomodulatory treatment compared to 35% of those with normal levels of this hormone ($P = 0.05$).

Twelve MS patients (8%) were diagnosed with hyperferritinemia [Table 3]. This was associated with male gender ($P = 0.03$), and with the relapsing-progressive type of disease ($P = 0.02$) [Table 3]. Additionally, among our eight male MS patients with hyperferritinemia we documented a relatively lower prevalence with the relapsing-remitting type (5 patients, 62.5%) and a higher prevalence with the

Table 3. Characteristics of multiple sclerosis patients according to ferritin levels

	Elevated ferritin	Normal ferritin	P
No. of patients	12	138	
Age (yrs, mean ± SD)	35 ± 5	36 ±10	NS
Duration of disease (yrs, mean ± S.D)	6.4 ± 5	6.8 ± 5	NS
Gender			
Male	8 (67%)	46 (33.3%)	0.03
Female	4 (33%)	92 (66.7%)	NS
Type of disease			
Relapsing-remitting	9 (75%)	126 (91%)	NS
Primary-progressive	1 (8.3%)	5 (3.6%)	NS
Secondary-progressive	0	6 (4.3%)	NS
Relapsing-progressive	2 (16.6%)	1 (0.7%)	0.02
Treatment			
Glucocorticoids	6 (50%)	67 (48.5%)	NS
Immunomodulatory	3 (25%)	57(41.3%)	NS
Immunosuppressive	2 (16.6%)	7 (5%)	NS
Other	1 (8.3%)	7 (5%)	NS

*P was considered significant below 0.05; NS=non significant

relapsing-progressive type (2 patients, 25%), as compared to male patients with non-elevated ferritin levels, among whom 91% had the relapsing-remitting type ($P = 0.057$), and 2% the relapsing-progressive type ($P = 0.054$). Of the four female patients with elevated levels, no significant associations were found between high serum levels of ferritin and any of the clinical data evaluated.

DISCUSSION

In this study we analyzed the sera of 150 MS patients for prolactin and ferritin levels and found an increased prevalence of hyperprolactinemia among MS patients compared with healthy subjects, as well as a subtle association between hyperprolactinemia in female patients and the secondary-progressive type of disease. Previous studies that evaluated the serum levels of prolactin in MS patients yielded conflicting results. On the one hand Kira et al. [18] reported a mild to moderate increase in prolactin levels in 30% of MS patients, and Azar and Yamout [19] described high activity of the hypothalamic-pituitary axis by demonstrating a higher prolactin stimulatory capacity after thyrotropin-releasing hormone test in MS patients. In another study, Yamasaki et al. [20] found hyperprolactinemia in female patients with an opticospinal variant of MS (termed the Asian type), associated with hypothalamic lesions. In a more recent report, monthly intravenous methylprednisolone treatment was associated with a reduction of the enhancing lesions and of T2 lesion volume and a decrease in prolactin concentration [21]. On the other hand, in 1997 Wei and Lightman [22] reported normal prolactin levels and hypothalamo-pituitary-adrenal function in MS patients, and Heesen et al. [10] showed no

correlation between baseline prolactin values and MS activity or course. Thus, currently it is not clear if altered levels of prolactin documented in MS patients are a primary phenomenon in the pathogenesis of the disease, similar to the effect of prolactin on the experimental model of the disease [10], or if hyperprolactinemia occurs as a result of a specific endocrine axis involvement dependent on localization of MS plaques. Moreover, conflicting results in previous studies on prolactin levels in MS may be partially due to the different assays of detection used. Alternatively, elevated levels of prolactin may merely represent an epiphenomenon, associated with extensive and prolonged CNS involvement. In a recent study Kutzelnigg and co-researchers [23] suggested that MS begins as a focal inflammatory disease, but as the disease progresses an accumulation of diffuse brain inflammation, cortical demyelination, and slowly progressive axonal injury in the normal-appearing white matter occurs. This diffuse injury is prominent in the progressive types of disease. In this context we propose that our finding of elevated prolactin levels in females with the secondary-progressive type of MS may thus be secondary to diffuse brain involvement. Moreover, we hypothesize that high prolactin levels may serve as an earlier marker of disease progression in females.

In the present study we found significantly increased levels of ferritin among MS patients compared with healthy individuals. Furthermore, hyperferritinemia was associated with male gender and a more progressive type of MS (i.e., relapsing-progressive), whereas an inverse association was noted between the milder form of disease (relapsing-remitting) and hyperferritinemia among male MS patients. Several well-established risk factors for progression of MS were previously defined, such as male gender, younger age, and disease type at onset. Taken together, these findings suggest that hyperferritinemia may be involved in this autoimmune disease and can be regarded as a bad prognostic sign.

The synthesis of ferritin is regulated by cytokines (tumor necrosis factor-alpha and interleukin-1 alpha), while its expression is influenced by several factors such as hormones (thyroid, insulin), cytokines and growth factors (insulin growth factor-1) [14]. Ferritin and iron homeostasis have been implicated in the pathogenesis of many diseases such as primary hemochromatosis, Parkinson's disease, Alzheimer's disease [16], and some autoimmune diseases [17]. Several studies support our notion by suggesting a role for transferrin and ferritin in the pathogenesis of MS disease. Hulek et al. [24] demonstrated a disrupted distribution of ferritin binding in brain tissues from MS patients, which was absent in the MS plaque itself and in the immediate periplaque region but normalized as the distance from the lesion became greater. In contrast, in the same study transferrin binding was present in the white matter tracts, but only in the periplaque region, suggesting that the loss of ferritin binding is involved or is a result of the demyelinization associated with MS; Fagos and colleagues [25] demonstrated elevated ferritin levels in MS patients only in the chronic progressive forms of disease.

Therefore, although our study has several limitations such as the relatively small number of patients with elevated biomarkers and the lack of imaging studies, it seems that both hyperprolactinemia and hyperferritinemia may be involved in the pathogenesis of multiple sclerosis; they may coexist, or they may be a consequence of CNS demyelinization in MS.

CONCLUSIONS

Our results suggest an association between elevated titers of prolactin and ferritin biomarkers and some specific types of multiple sclerosis. Further studies with larger cohorts and combining clinical data and CNS imaging are warranted to evaluate the weight of each biomarker and the clinical features of the disease.

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References

1. Miller JR. Multiple sclerosis. In Rowland LP, ed. Merrit´s Neurology. 3rd edn. Philadelphia: Lippincott-Williams & Wilkins, 2000: 773-91.
2. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; 50 (1): 121-7.
3. Weiner HL. MS is an inflammatory T cell mediated autoimmune disease. *Arch Neurol* 2004; 61 (10): 1613-15.
4. Roach ES. Is multiple sclerosis an autoimmune disorder? *Arch Neurol* 2004; 61 (10): 1625-6.
5. Grau-López L, Raïch D, Ramo-Tello C, et al. Myelin peptides in multiple sclerosis. *Autoimmun Rev* 2009; 8 (8): 650-3
6. Shoenfeld Y, Blank M, Abu-Shakra M, et al. The mosaic of autoimmunity: prediction, autoantibodies, and therapy in autoimmune diseases – 2008. *IMAJ Isr Med Assoc J* 2008; 10: 13-19.
7. Shoenfeld Y, Zandman-Goddard G, Stojanovich L, et al. The mosaic of autoimmunity: hormonal and environmental factors involved in autoimmune diseases – 2008. *IMAJ Isr Med Assoc J* 2008; 10: 8-12.
8. Shoenfeld Y, Gilburd B, Abu-Shakra M, O, et al. The mosaic of autoimmunity: genetic factors involved in autoimmune diseases. *IMAJ Isr Med Assoc J* 2008; 10: 3-7.
9. Chikanza ZC. Prolactin and neuroimmunomodulation: in vitro and in vivo observations. *Ann NY Acad Sci* 1999; 876: 119-30.
10. 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 (1-2): 9-18.
11. Walton PE, Cronin MJ. TNF-α and INF-γ reduce prolactin release in vitro. *Am J Physiol* 1990; 259: E672-6.
12. Orbach H, Shoenfeld Y. Hyperprolactinemia and autoimmune diseases. *Autoimmun Rev* 2007; 6: 537-42.

13. Jara LJ, Benitez G, Medina G. Prolactin, dendritic cells and systemic lupus erythematosus. *Autoimmun Rev* 2008; 7 (3): 251-5.

14. Zandman-Goddard G, Shoenfeld Y. Hyperferritinemia in autoimmunity – 2008. *IMAJ Isr Med Assoc J* 2008; 10: 83-4.

15. Levine SM, Chakrabarty A. The role of iron in the pathogenesis of experimental allergic encephalomyelitis and multiple sclerosis. *Ann N Y Acad Sci* 2004; 1012: 252-66.

16. Zandman-Goddard G, Shoenfeld Y. Ferritin in autoimmune diseases. *Autoimmun Rev* 2007; 6: 457-63.

17. Orbach H, Zandman-Goddard G, Amital H, et al. Novel biomarkers in autoimmune diseases: prolactin, ferritin, vitamin D, and TPA levels in autoimmune diseases. *Ann N Y Acad Sci* 2007; 1109: 385-400.

18. Kira J, Harada M, Yamaguchi Y, Shida N, Goto I. Hyperprolactinemia in multiple sclerosis. *J Neurol Sci* 1991; 102: 61-6.

19. Azar ST, Yamout B. Prolactin secretion is increased in patients with multiple sclerosis. *Endocr Res* 1999; 25: 207-14.

20. Yamasaki K, Horiuchi I, Minohara M, et al. Hyperprolactinemia in optico-spinal multiple sclerosis. *Intern Med* 2000; 39 (4): 296-9

21. Then Bergh F, Kuemple T, Schumann E, et al. Monthly i.v. methylprednisolone in relapsing-remitting MS: reduction of enhancing lesions, T2 lesion volume and plasma prolactin concentrations. *BMC Neurol* 2006; 6: 19-28.

22. Wei T, Lightman SL. The neuroendocrine axis in patients with multiple sclerosis. *Brain* 1997; 120: 1067-76.

23. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 2005; 128: 2705-12.

24. Hulek SW, Power S, Connor Jr. Distribution of transferrin and ferritin binding in normal and multiple sclerosis human brains. *J Neurol Sci* 1999; 165 (1): 48-55.

25. Fagos C, Makis AC, Chaidos A, et al. Serum ferritin, transferrin and soluble transferrin receptor levels in multiple sclerosis patients. *Mult Scler* 2005; 11(3): 272-5.