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Correlation between sex hormones and magnetic resonance imaging lesions in multiple sclerosis

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Objective – To determine if sex hormones play a role in the pathogenesis of multiple sclerosis (MS) by correlating serum estradiol and progesterone levels with gadolinium (Gd) enhancing lesions on magnetic resonance imaging (MRI) in MS. *Methods* – Thirty patients with MS were studied with Gd enhanced brain MRI and simultaneous serum estradiol and progesterone levels either during the early follicular, late follicular or luteal phases of their menstrual cycle. Correlation between hormone levels and number of Gd enhancing lesions was determined. *Results* – Patients with high estradiol and low progesterone levels had a significantly greater number of Gd enhancing lesions than those with low levels of both these hormones. Patients with a high estrogen to progesterone ratio had a significantly greater number of active MRI lesions than those with a low ratio. *Conclusion* – Estradiol and progesterone may influence disease activity in MS. If further studies confirm these results, it may be possible to develop therapy by altering levels of these hormones.

S. Bansil[1], H. J. Lee[2], S. Jindal[3], C. R. Holtz[1], S. D. Cook[1]

Departments of [1]Neurosciences, and [2]Radiology, UMDNJ-New Jersey Medical School; [3]Center for Reproductive Medicine, Hackensack University Medical Center, and UMDNJ-New Jersey Medical School, Newark, NJ 07103

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S. D. Cook, Chairman, Department of Neurosciences, UMDNJ, 185 South Orange Avenue, MSB H-506, Newark, NJ 07103, USA

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Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system of unclear etiology. MS attacks are often unpredictable but may be affected by pregnancy with remissions occurring during pregnancy and exacerbations increasing post partum (1, 2). Likewise, there have been reports of fluctuation of MS symptoms during the normal menstrual cycle (3). This suggests that sex hormones, such as estradiol and progesterone, may play a role in influencing disease activity. Gadolinium (Gd) enhanced magnetic resonance imaging (MRI) is a sensitive method of detecting MS disease activity (4). In this study we evaluated the effects of hormonal changes during the normal menstrual cycle on Gd enhancing lesions on brain MRI in female patients with MS.

Materials and methods

Patients

Thirty pre-menopausal women with clinically or laboratory supported definite MS were entered into the study (5). Patients were eligible for the study if they met the following criteria: a) had regular menstrual cycles of 28–30 days; b) were not on oral contraceptives or any form of sex hormone replacement therapy; c) had not experienced an acute attack of MS in the previous 4 weeks; d) had not been on corticosteroids for at least 2 weeks and e) had not been on β-interferon, copolymer-1, or any other immunomodulating therapy for at least 3 months.

Consecutive patients meeting these criteria and agreeing to participate in the study were randomly assigned to receive an MRI of the brain either during days 1–3 (early follicular phase), or days 14–16 (late follicular phase), or days 21–23 (luteal phase) of their menstrual cycle. Blood was drawn for estradiol (E2) and progesterone (P4) levels on the same day that the MRI was performed. Thus, a single patient received 1 MRI scan and had 1 measurement of sex hormone level (E2 and P4) performed on the same day. In addition, all patients were examined clinically within 1 week of the time that the MRI was performed and assigned a score on the expanded disability status scale (EDSS) (6).

Magnetic resonance imaging (MRI)

All patients were examined on a General Electric 1.5 T Unit (Milwaukee). MRI of the brain was performed using the following pre-contrast

Table 1. Clinical characteristics of MS patients in groups 1, 2 and 3

	Mean age (years ± SD[1])**	Type of MS	Duration of MS (years ± SD)**	No. of attacks in the past year ± SD**	Mean EDSS ± SD**
Group 1 ($n=14$)	36.9 ± 5.4	RR[2] $n=12$ RP[3] $n=2$	7.1 ± 4.2	0.7 ± 0.8	2.2 ± 1.7
Group 2 ($n=6$)	42.5 ± 9	RR $n=2$ RP $n=4$	9.2 ± 9	0.8 ± 0.6	3.75 ± 1.8
Group 3 ($n=10$)	37.9 ± 7.4	RR $n=6$ RP $n=4$	7.9 ± 5.7	0.6 ± 0.7	2.3 ± 1.7

[1] SD: standard deviation; [2] Relapsing–remitting; [3] Relapsing–progressive.
** P: not significant between groups.

sequences: Sagittal T_1 weighted (TR/TE: 500/20) slice thickness 5 mm with 1.5 mm gap; Axial T_1 weighted (TR/TE: 500/20) and proton density and T_2 weighted (TR/TE:2000/30–80) slice thickness 5 mm with 1 mm gap. This was followed by sagittal and axial (5 mm slice thickness with 1 mm gap) and coronal (3 mm contiguous slices) T_1 weighted sequences performed after administration of double dose (0.2 mmol/kg) intravenous gadolinium (Magnevist; Schering, Germany).

All scans were performed in an identical manner on the same machine and read by a single blinded neuroradiologist who was unaware of the hormone levels and phase of the menstrual cycle of the patient.

Serum estradiol and progesterone levels

Blood samples were allowed to clot for 30 min, then centrifuged for 10 min at 500 ***g***. Serum concentrations of immunoactive E2 were assayed using a polyclonal rabbit antibody specific for E2 in a solid-phase chemiluminescent immunoassay (DPC; Diagnostic Products Corp., Los Angeles, CA, USA). The antibody is highly specific for E2 with low cross reactivity to other steroids that may be present in patient samples. The assay has a working range of 20–2000 pg/ml, and the sensitivity of the assay is 12 pg/ml. The intra- and inter-assay coefficients of variation (CV), respectively, were consistently below 10% (DPC-Cirrus Immulite E2 package insert).

Serum concentrations of immunoreactive P4 were assayed using a polyclonal rabbit antibody specific for P4 in a solid-phase, ligand-labeled competitive chemiluminescent immunoassay (DPC; Diagnostic Products Corp., Los Angeles, CA, USA). The antibody is highly specific for P4, with low cross reactivity to other steroids that may be present in patient samples. The assay has a working range of 0.2–40 ng/ml, and the sensitivity of the assay is 0.09 ng/ml. The intra- and inter-assay CV, respectively, were consistently below 10% (DPC-Cirrus Immulite P4 package insert).

Statistical analysis

Differences in hormonal levels and number of Gd enhancing lesions between groups were determined using non-parametric analysis (Wilcoxon Rank Sum test).

Results

Serum hormone levels did not, in all patients, correspond to the time of their menstrual cycle. Thus, patients were reclassified into 3 groups based on their hormone levels:

Group 1 (n=14) – Low E2 ($\leq$100 pg/ml) and low P4 ($\leq$3.5 ng/ml) levels. These levels are characteristic of the early follicular phase of the menstrual cycle.

Group 2 (n=6) – High E2 ($>$100 pg/ml) and low P4 ($\leq$3.5 ng/ml) levels. These levels are characteristic of the late follicular phase of the menstrual cycle.

Group 3 (n=10) – High P4 ($>$3.5 ng/ml) levels. E2 levels in this group were variable. These levels are characteristic of the luteal phase of the menstrual cycle.

Thus, groups 1 and 2 had low P4 levels and were distinguished from each other by low and high E2 levels, respectively. Group 3 was distinguished from Groups 1 and 2 by its high P4 level.

The clinical characteristics of each group are shown in Table 1.

Serum hormone levels. Estradiol – As expected, serum E2 levels were significantly higher ($P=0.0001$) in Group 2 patients (mean ±

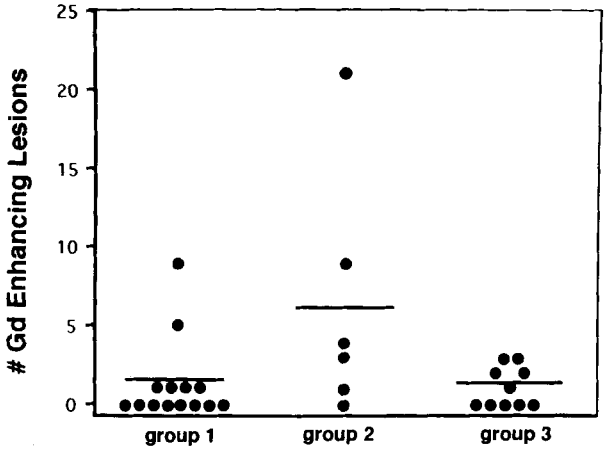

Fig. 1. Number of gadolinium (Gd) enhancing lesions in groups 1, 2 and 3. The horizontal lines represent the mean number of Gd enhancing lesions in each group.

standard deviation (SD) = 163 ± 66.4 pg/ml) as compared to Group 1 patients (mean ± SD = 61.1 ± 25.2 pg/ml). Serum E2 levels were not significantly different between Groups 2 and 3 (mean ± SD = 165 ± 158 pg/ml). E2 levels were significantly higher ($P = 0.03$) in Group 3 patients in comparison to Group 1.

Progesterone – P4 levels were significantly higher in Group 3 patients (mean ± SD = 7.0 ± 3.3 ng/ml) in comparison to Group 2 (mean ± SD = 0.8 ± 0.3 ng/ml) and Group 1 (mean ± SD = 0.8 ± 0.4 ng/ml) patients ($P = 0.001$ and $P = 0.0001$, respectively). Progesterone levels were not significantly different between Groups 1 and 2.

Thus, Group 1 patients had low estrogen and progesterone levels and a low estrogen to progesterone ratio, Group 2 had high estrogen and low progesterone levels and high estrogen to progesterone ratio, and Group 3 had high estrogen and progesterone levels and a low estrogen to progesterone ratio.

Gd enhancing lesions on MRI – The number of Gd enhancing lesions on MRI was significantly higher ($P = 0.04$) in Group 2 (mean ± SD = 6.3 ± 7.8) as compared to Group 1 (mean ± SD = 1.3 ± 2.6) (Fig. 1). There was a trend towards a greater number ($P = 0.06$) of Gd enhancing lesions in Group 2 in comparison to Group 3 (mean ± SD = 1.1 ± 1.3). There was no significant difference in the number of Gd enhancing lesions between Groups 1 and 3.

In addition, the number of Gd enhancing lesions in patients with a high estrogen to progesterone ratio (Group 2) was significantly higher ($P = 0.04$) than those with a low estrogen to progesterone ratio (Groups 1 plus 3; mean ± SD = 1.21 ± 2.11).

Discussion

The results of this study indicate that female patients with MS who have high estradiol and low progesterone levels have evidence of greater disease activity (as measured by the number of Gd enhancing lesions on brain MRI) than patients with low estradiol and progesterone levels. MRI effects of high estradiol levels may be inhibited by high progesterone levels since patients with high estradiol and high progesterone levels (Group 3), have a trend towards lower levels of disease activity than patients with high estradiol and low progesterone levels (Group 2). Further evidence for progesterone having a "protective" effect is provided by the presence of lower number of Gd enhancing lesions in patients with a low estrogen:progesterone ratio (Groups 1 plus 3) versus those with a high estrogen:progesterone ratio (Group 2). These findings, thus, suggest that disease activity in MS may be influenced by relative and absolute serum sex hormone levels.

The results of our study are consistent with another recently reported study in which the cytokine secretion profile of autoantigen specific T cells from MS patients and controls could be altered by the addition of estradiol (7). The *in vitro* effects of estradiol were evident predominantly at pregnancy associated physiologic or supra physiologic concentrations (> 5000 pg/ml). Since cytokines may be important in disease pathogenesis, this may be one of the mechanisms by which sex hormones exert an immunomodulatory effect. Unlike this study, our study examined the combined effects of physiological, non-pregnant state serum levels of estradiol and progesterone *in vivo* on MRI disease activity in MS and the results, thus, may more accurately reflect the role of these hormones in MS.

In contrast to our results, another recent preliminary study reported no correlation between the number of Gd enhancing lesions and serum hormone levels during the menstrual cycle (8). It is unclear from this study if patients were imaged in both the early and late follicular phases and if the combined effects of estradiol and progesterone were examined.

Our pilot study may be criticized for several reasons, including the small numbers of patients studied and that each patient was studied at only one time point. Clearly our conclusions need to be confirmed in a sequential study of larger numbers of patients. There was a disproportionately higher number of patients with relapsing–progressive MS in Group 2 than in the other groups, and it may be argued that this accounts for the presence of greater MRI disease activity in this group. How-

ever, in this study, the number of enhancing lesions in patients with relapsing–progressive MS (mean $\pm$ SD = 3 $\pm$ 6.1) was not significantly different from those with relapsing–remitting MS (mean $\pm$ SD = 1.9 $\pm$ 2.8). The EDSS score was higher, but not significantly different, in group 2 in comparison to the other groups. Since prognosis and MRI disease activity may be related to the EDSS this may have been a confounding factor in the study. MS lesions may show gadolinium enhancement for up to 6 weeks and the exact time that lesions developed in our study cannot be determined. Although it is possible that the current hormonal state did not lead to the observed MRI disease activity, the predominance of Gd enhancing lesions in the late follicular phase of the cycle suggests that they may be more likely to develop during this period. A sequential study performed in the early follicular, late follicular and luteal phase of the menstrual cycle would help confirm the validity of this hypothesis.

In addition, magnetization transfer imaging which may reveal changes in the white matter prior to the development of enhancing lesions may be useful in, more accurately, delineating the time of appearance of MS lesions on MRI (9).

The mechanisms by which sex hormones could exert their effects in MS are unclear. These hormones may cause changes in body temperature and worsening of MS symptoms may be associated with an increase in temperature. This is unlikely to be a factor in this study since active MRI lesions were observed predominantly during the late follicular phase and a rise in basal body temperature occurs in the luteal phase of the menstrual cycle (10). The similarity of the chemical structure of corticosteroids and sex hormones, and the well-known beneficial effects of corticosteroids in MS, suggest that sex hormones may have a similar mechanism of action as corticosteroids.

In conclusion, further studies are necessary on the influence of estrogen and progesterone on clinical and MRI disease activity in MS. If our results are confirmed, it may be possible to develop therapy, that influences sex hormone blood levels, which may be useful in some MS patients.

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