ORIGINAL REPORT

Menstrual Irregularities and Related Plasma Hormone Levels in Multiple Sclerosis Patients Treated with Beta Interferone

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Abstract- Multiple sclerosis is a chronic inflammatory disease of central nervous system.Women are more susceptible to this disease. One of the obvious clinical complaints in women with multiple sclerosis specially treated with Beta Interferones is menstrual cycle irregularity. The aim of this study was to determine the prevalence of menstrual irregularities and probable changes in blood levels of related hormones (FSH, LH, PRL, TSH, T_4, T_3) in 58 females with definite MS treated with beta interferones versus 58 healthy women. In comparison to the control group, the patients had higher prevalence of irregular menstruation (P=0.001), oligomenorrhea (p=0.03), abnormal amount of menstrual blood flow (P=0.001), abnormal duration of menstrual flow (P=0.01) and missed period (P=0.04). Mean LH level in patients group was higher than control group (P=0.04).Hyperprolactinemia (>25.5ng/ml) was more prevalent in patients group .There were not a significant difference in plasma levels of FSH and thyroid hormones between two groups. There were some relations between the type of Beta interferones and the subtype of menstrual irregularities in the patients. In conclusion, the results of this study emphasized the high rate of menstrual problem and changes of related plasma hormone levels in MS patients.

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Key words: Multiple sclerosis; menstruation disturbances; progesterone; hormones

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of central nervous system (CNS) that usually occurs in young adults, leads to different levels of disability and is characterized by multiple areas of white matter inflammation, demyelination, glial sclerosis, axonal degeneration and finally atrophy (1, 2). The cause of this disease is unknown, although autoimmune mechanisms, genetical and environmental factors are considered to be involved in pathogenesis (1). Women are more susceptible to the diseases probably because of carrying more susceptibility genes, more immunologic Th1 cell mediated immunity disorders or different hormonal properties (particularly sex ones). One of the obvious clinical complaints in women with multiple sclerosis is menstrual cycle problems that is a matter of concern in fertility or sexual activity of the patients. Epidemiological data have suggested that spontaneous fecundity might be reduced; several endocrine and sexual disturbances potentially interfering with reproduction have been evidenced in MS patients of both sexes.

The exact causes of these irregularities may be complex and could be due to the disturbances in hypothalamic-pituitary axis as a result of demyelinating lesions or axonal loss and even atrophy in these regions, effects of different therapeutic agents specially beta interferones or disorders of target organs (3-5).

The following mechanisms have been suggested as probable causes of menstrual irregularities in MS:

-Disorders of hypothalamic-pituitary axis due to destructive and demyelinating lesions (5-9)

-Effects of immune system on hypothalamic-pituitary-gonad axis (10-12).

-Hormonal resistance (4,5,22).

-Effects of specific or symptomatic medications of MS on hypothalamic-pituitary-gonad axis (13,14).

-Effect of stress on menstruation and hypothalamic-pituitary axis (15,18,23).

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The results of previous studies were not organized and could not clarify the prevalence rate and the cause(s) of menstrual irregularities in MS .There are only some case reports and some case series study in literatures up to the present time.

The aim of this survey was determination of relative prevalence of different kinds of menstrual irregularities and blood levels of related hormones (FSH, LH, PRL, T_3, T_4) in 58 female MS patients treated with different beta interferone products as a sample of Iranian MS patients.

Patients and Methods

Subjects

Fifty-eight females suffer from MS treated with beta interferons according to the revised criteria of McDonald *et al.* (2005) versus 58 healthy women were enrolled in this study (16). The clinical characteristics of subjects are summarized in table 1. Both groups were matched in age, BMI, parity, delivery statuses, history of abortion and contraception methods which they used. Control and patients groups were not taking steroid hormones during the period of study. Both groups were matched in the days of their menstrual cycles. Some information that were collected by filling in the questionnaire were about the first sign and duration of disease, history of taking related medications and information about menstrual cycles. If there was any evidence of menstrual irregularities, pelvic examination, *pap smear-test* and pelvic ultrasonographic assessment were done. All patients were evaluated and examined for determination of clinical signs of MS and expanded disability status scale number (EDSS). All women gave informed consent and the study was approved by the local ethical committee.

Determination of hormones plasma levels

Blood samples were obtained from days 1 to 6 of the menstrual cycle in the morning (8am-10am) after overnight fasting from controls and patients by venipunctures. After clotting, serums were prepared by centrifugation at 3000 rpm for 20 minutes. Serums were stored at-20^{o}c until hormonal analysis was conducted. Selected hormones including FSH, LH, TSH, T_4, T_3 and prolactin were measured by Elisa method. All hormone analysis kits were provided by Monobind, Inc (USA).

Statistical analysis

The results were analyzed by SPSS (version 13) statistical software and different statistical tests such as *t*-test (independent, paired *t*-test), *chi*-square and one-way ANOVA were used for analysis of information. Differences were considered statistically significant at $p<0.05$.

Table 1. Clinical characteristics in healthy subjects and patients affected with MS. the characters are similar to each other and the disease duration is 5.5years

	Healthy Subjects (n=58)	MS. Patient (n=58)
Age	32.3±7.2	31.5 ±7.7
Weight	62±9.8	62±10.7
BMI	23.2±3.2	24.1±4.4
Disease duration (years)	-----	5.5±4.5

Mean ± SEM are shown.

Results

Mean age of the patients and control groups were 32.3 and 31.5 years respectively. The most common form of MS in our patients was relapsing-remitting form (R-R) (75.9%) followed by secondary progressive in 20.7% and primary progressive in 3.4%. The most common symptom at onset of disease was sensorimotor (56%) followed by visual in 30%.

Most of the patients had EDSS score between 0 to 2 (65.5%) and only 10.3% had EDSS between 4-6. Mean duration of the disease was 5.5 years.

The most common medications that were prescribed for treatment of the patients were beta interferon and among them Avonex® was the most common (72.4%), followed by Rebif® (15.5%) and Betaferon® (12.1%) respectively. 53.4% of the patients had history of taking corticosteroid during recent 6 months and not to take it in 1 month before study as therapy for attack of disease.

In patients group 81% of cases have regular menstrual cycle before onset of clinical manifestations of the disease, in compare with 84.5% in controls, though after diagnosis of MS only 55.2% of the patients reported to have a regular menstrual cycles. Duration of menstrual cycle in the patients was completely different in time before and after onset of MS, cycle more than 35 days has been reported in 24.7% after and only 5.25% before the onset of the disease.Cycle less than 21 days has been claimed in 5.2% after onset of MS. Missing periods was detected in 31% of MS patients and 10% of controls (Table 2).

The rate of inter menstrual bleeding were similar in two groups. Dysmenorrhea was reported in 32.8% of MS patients and 46% of controls (29.3% in the patients before the onset of MS). Both groups had significant differences in regularity of cycles (P=0.001), duration of cycles (P=0.03), amount

Table 2. Comparison of menstrual irregularities in MS patients before and after the onset of MS. Only 44.8% reported regular pattern .The most common abnormality is oligomenorrhea(Cycle more than 35 days)

	Before the onset (n=58)	After the onset (n=58)
Regular menstrual pattern	81%	44.8%
Irregular menstrual pattern	19%	55.2%
Polymenorrhea (<21 days)	0	5.2%
Oligomenorrhea (>35 days)	5.2%	27.6%
Hypomenorrhea	8.6%	22.4%
Hypermenorrhea	13.8%	22.4%
Decrease in duration of menstrual blood flow (< 3 days)	1.7%	10.3%
Increase in duration of menstrual blood flow (> 7 days)	17.2%	25.9%

of menstrual blood flow (*P*=0.001), duration of menstrual blood flow (*p*=0.01) and missed period (*P*=0.04).

In comparison to the control group the patients had higher prevalence of oligomenorrhea (*P*=0.03), abnormal amount of menstrual blood flow (*P*=0.001), abnormal duration of menstrual flow (less than 3 or more than 7 days) (*P*=0.01) and missed period (*P*=0.04).

Hormonal analysis revealed that mean plasma level of LH in patient group was higher than control group and it was statistically significant (*P*=0.04). Although there was not a significant difference between mean level of prolactin in the patients and control group (*P*=0.09) though hyperprolactinemia was more prevalent in the patients than control group (*P*=0.04). There were not significant differences between plasma levels of thyroid hormones in the patients and control group (Table 3).

Also there were relationship in the different subtype of menstrual irregularities and the type of interferences which were used by the patients .In the patients who were useing Avonex® the amount of blood flow was higher (*P*=0.03). Taking Rebif® was related to irregular cycles (*P*=0.03), abnormal duration of cycles (less than 21 or more than 35) (*P*=0.01) and missed period (*P*=0.02).Taking Betaferon® was related to irregular cycle (*P*=0.05) and excessive amount of blood flow (*P*=0.005). There weren't any correlation between irregular menses and EDSS score, duration of the disease, type of MS and the existence of lesions in hypothalamus or around the third ventricle that only were found in 5 MS patients.

Discussion

The present study reveals that before the onset of MS there is no significant difference between the patients and healthy subjects in reported menstrual disturbances but after the beginning of the disease, patients reported more menstrual irregularities such as oligomenorrhea, amenorrhea and hypo and hyper menorrhea. Though, because of the probability of recall bias in the patients (mean of disease duration of 5.5 years) the claim must be interpret with caution but as the interview and statistics showed there were a uniform concept among the patients that MS leaded to irregular menstruation.

Table 3. Comparison of hormonal parameters in healthy subjects and patients with multiple sclerosis (MS.) LH is increased (*P*=0.04)

	Healthy Subjects	MS. Patients	P value
Number of subjects	N=58	N=58	
FSH (mIu/mL)	6.7±8.7	8.7±12.5	0.41
LH (mIu/mL)	6.9±5.6	11.8±14.2	0.04
TSH (µIu/mL)	1.2±1.7	0.6±0.8	0.09
T4 (µg/dL)	7.8±2.3	8.5±2.4	0.22
T3 (ng/mL)	0.5±0.3	0.5±0.4	0.11
PRL (ng/mL)	21.2±10.7	26.3±17.2	0.09

Mean ± SEM are shown

Its worth full to mention that we studied the treated MS patients in a cross sectional cohort groups so we can only promptly tell that MS patients treated with disease modifying therapies (DMDs) have more menstrual irregularities because we couldn't clarify the exact time of onset of menstrual irregularities after the onset of the disease and also the exact time of onset of irregularities after starting treatment with DMDs. Any way, significant differences were detected in our patients groups, than in the controls. Although these differences in menstrual characteristics and level of related hormones in plasma may be a reflection of DMDs but could be at least partly related to MS. To distinguish these two possibilities from each other we need a prospective follow up cohort in two different MS groups: treated and untreated ones.

In regard to different type of menstrual disturbances In our study likewise the results of some other studies oligomenorrhea (patients versus control group: 27.6% and 6.9% respectively) and amenorrhea (patients versus control group: 31% and 10.3%respectively) were remarkably more common in patients group.

Prevalence of oligomenorrhea and amnorrhea in Falaschi *et al.* study on 76 women with MS and 50 healthy women have been reported 20% and 17% respectively (17). Interestingly the patients in this study were not taken any DMDs and the statistics are very close to our reported rate in spite of treatment with DMDs in our patients.

Golovkin *et al.* studied 36 women with MS and reported that 16 patients had amnorrhea (18). Also Miayamoto, Tanaka, Davies, Linssen and Klapps in separated studies have reported premenopausal female patients suffering from multiple sclerosis with secondary or primary amnorrhea (6-8, 13, 19).

In the present study, elevated levels of FSH and LH were associated with amenorrhea, hypomenorrhea and oligomenorrhea in the patient group.

We observed 5 patients with amnorrhea that according to hormonal analysis 3 patients had become menopause and because of being under the age of 40, two of them must be considered as premature ovarian failure (POF).

Tonacchera *et al.* also has observed the relationship between premature ovarian failure and elevated levels of FSH and LH in MS patients and has suggested that blocking antibodies against gonadotropin receptors are responsible for this event (10). Also Hansen and Moller et al have reported autoantibodies against pituitary peptides in sera from patients with multiple sclerosis (11, 12). In two another case report studies, Miayamoto and Davies reported two cases of multiple sclerosis with amnorrhea that were associated with reduction of FSH and LH levels because of hypothalamic damage (7, 8).

In the present study, among cases that complained about oligomenorrhea, there were 6 cases in patient group and 3 cases in control group that were suspected to have poly-cystic ovary (CPCO). Further evaluations revealed that only one case in patient group had not PCO, but because of insufficient number of cases, this finding could not be interpreted. There is no similar report about prevalence of PCO in multiple sclerosis.

Among the patients who complained about amnorrhea 1 case had hyper prolactinemia, otherwise, among the controls, there was not any case of hyperprolactinemia.

In the other studies, there are only some reports of relationship between amenorrhea and hyperprolactinemia in patients with MS, such as Tanaka *et al.*, that n this study they refer to a woman with MS who manifested with galactorrhea-amnorrhea syndrome and hyperprolactinemia. Subthalamic lesion has been considered to be the cause of this problem (6).

Golovkin *et al.* has also reported cases of amnorrhea associated with hyperprolactinemia in patients with MS (21).

In our study, a significant relationship between specific medications of MS and menstrual irregularities was observed and there are some reports of such problem in literatures for example:

Pakulski and DiMarco have observed a relationship between taking Betaferon and hypermenorrhea (14). The causes and probable mechanisms of relationship between Beta interferons and menstrual disturbances in MS are unknown and must be investigated.

According to Our study there was higher average levels of LH and prolactin in the patients.

Likewise, Grinsted had reported elevated levels of prolactin, LH and FSH hormones in MS patients in comparison to healthy subjects. He has suggested that peripheral resistance to gonadotropins and abnormal central regulation as probable reasons but the conclusive reason is still unknown (5).

Linssen *et al.* explained increased levels of FSH and LH hormones in MS patients who were taking immunosuppressive therapy (cyclophosphamide) and suggested that it might be a complication of this medication (13).

Other studies have revealed an increase in serum prolactin level in MS patients (3, 4, 6, 18). In a case report, a MS patient has been reported with low serum LH, FSH and normal serum prolactin level with third ventricle dilatation (7).

Also Davies *et al.* has reported low serum levels of FSH and LH in a MS patient with hypothalamic plaque

(8). In our study, we didn't observed any relationship between the hormone levels and menstrual disturbances with the existence of the lesions in hypothalamic area though, the number of these lesions in our patients was very low. Likewise study of Wei and Lightman study (20), we didn't observed any significant differences between thyroid hormones levels (TSH, T_3, T_4) in patients and control group. Though, Zych and Wajgt have observed normal serum levels of T_4, TSH and low serum T_3 level in MS patients and suggested that the most likely reason is impaired peripheral T_4 conversion to T_3 (4). In one study hypothyroidism with unknown origin has been reported (19).

These multiple conflicting data in different studies and case reports could be important and emphasize to the probability of different causes and mechanisms for these abnormalities in each MS patient. These facts must be considered in MS that many etiological factors must be involved even in individual patient. In our point of view MS itself or specific medications used in MS could have different effects in menstrual regulations, It means that MS itself may also have direct hormonal and gonadal effects as a part of widespread autoimmune disturbances or metabolic and biochemical changes. The most possible and simplest reason or a main cause of menstrual irregularities or even changes in hormones in our patients could be medications, but as we earlier mentioned the onset of menstrual irregularities begun after the onset of disease but probably before starting of any treatment for MS. Unfortunately we could not find any organized published retrospective cross sectional study in MS similar to our study for better comparison of the data.

Why do MS patients complain about menstrual irregularities? Is it related to MS or the medications such as DMDs? If we accept that the main cause is DMDs, by which mechanism the drugs can affect hormones and menstrual cycles? Are there any similar results in interferone users in other diseases such as hepatitis B? Is it important to correct the irregularities of menses in MS patients? Is it possible to select or to produce the drugs with lesser menstural and hormonal side effects? Finally if accepting the menstrual disturbances and changes of the hormones as distinct symptom of MS, can we change and balance these problems and even MS course with hormonal manipulation?

These all are the facts and the questions that should be answered. In conclusion, according to our data, menstrual irregularities are more common in MS patients. Though we can not properly interpret the findings.. we thought several mechanisms should be involved, it might be due to the effects of the disease on hypothalamic-pituitary axis followed by secondary changes in gonadotrophin levels or effects of specific medications like beta interferons on these structures. The results of another studies and case reports and suggestive mechanisms are also different and sometimes conflicting. For more accurate estimation of the prevalence of these abnormalities and also clarification of the cause(es) and the mechanisms Another comparison studies with the higher sample size, on different groups such as a cohort of new diagnosed MS patients without taking medications and of chronic patients who are taking medications are needed.

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