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Relationships of total leukocyte, neutrophil and lymphocyte levels with the menstrual cycle in patients receiving fingolimod treatment

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Abstract. – OBJECTIVE: Multiple sclerosis (MS) is a demyelinating, chronic, and progressive autoimmune disease of the central nervous system that causes the loss of axons and grey matter, and has a high prevalence in young female patients. Fingolimod is an oral treatment agent that acts by blocking the passage of the T lymphocytes responsible for the pathogenesis of MS from lymphoid tissue into the peripheral blood. We aimed to research the effects of menstrual cycles on leukocytes and lymphocyte levels in RRMS (relapsing-remitting MS) patients who received fingolimod treatment.

PATIENTS AND METHODS: This study was performed to determine the most suitable phase of the menstrual cycle in patients with RRMS for follow-up assessment of lymphopaenia levels after fingolimod treatment. The study population consisted of 41 RRMS patients receiving fingolimod therapy and 33 healthy women of reproductive age. Complete blood counts were performed in three different phases of the menstrual cycle, and the two groups were compared. Variability in the total leukocyte, lymphocyte, and neutrophil immune cell numbers between cycles was examined.

RESULTS: The results indicated that total leukocyte, neutrophil, and lymphocyte levels were decreased in RRMS patients receiving fingolimod treatment, but these changes were not related to the phase of the menstrual cycle. In our study, leukocyte levels in healthy individuals were significantly lower in the proliferative phase than in other phases.

CONCLUSIONS: The results indicated that lymphocyte monitoring in RRMS patients receiving fingolimod treatment can be performed at any stage of the menstrual cycle.

Key Words:
Multiple sclerosis, Menstrual cycle, Fingolimod.

Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease that progresses with inflammation, demyelination, and axonal degeneration of the central nervous system (CNS)[1]. This disease, which has an increased incidence between the ages of 20 and 40 years, is three times more common in women, especially those of reproductive age[2]. There are multiple subtypes of MS, and relapsing-remitting MS (RRMS) is the most common form (seen in 85% of patients). RRMS develops into secondary progressive MS (SPMS) within 10-15 years in 50% of cases[3].

Treatment includes use of multiple agents, including corticosteroids, immunosuppressants, interferons, and monoclonal antibodies. Fingolimod is of particular importance, as it was an oral agent used in the treatment of MS[4]. Pain and other injection complications are expected to decrease in patients receiving subcutaneous or intramuscular treatment with the use of fingolimod. Fingolimod (FTY720) is a sphingosine-1-phosphate (S1P) receptor agonist, and a natural bioactive sphingolipid that plays an important role in inflammation and repair of the cell membrane structure[5-7]. The active metabolite, fingolimod-phosphate, binds to, and reduces the number of, S1P1 receptors on T lymphocytes, thus preventing the passage of T lymphocytes from the lymphoid tissue into the peripheral blood[5-7]. In addition to this decrease in acquired immune cells, the numbers of neutrophils, monocytes, and macrophages, which are natural immune cells, have been shown to decrease with fingolimod treatment[8].

MS is known to show distinct courses during the menstrual cycle and pregnancy in women of

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reproductive age[9]. This is thought to be due to the interaction between immune mechanisms and sex steroid hormones in the various phases of the menstrual cycle, which is in turn likely mediated by the distribution of immune cells[10].

In this study, the relationship between drug-induced changes in the lymphopaenia level and the menstrual cycle were investigated in RRMS patients receiving fingolimod therapy, to determine the most appropriate follow-up time.

Patients and Methods

Patient Selection

Female patients of reproductive age (18-45 years) diagnosed with RRMS according to the 2010 McDonald Criteria, and who were undergoing treatment with fingolimod in the MS Outpatient Clinic of the Neurology Department of Inonu University Faculty of Medicine, were included in this study. Age-matched healthy women, without any neurological diseases and with regular menstrual cycles, were included as a control group.

Blood Samples

The mean menstrual cycles of individuals in both the case and control groups were recorded at the beginning of the study. Individuals with irregular menstrual cycles were excluded. Peripheral venous blood samples were collected into hemogram tubes with EDTA between 08:00 and 10:00 on day 2 or 3, which is accepted as the menstrual phase of the menstrual cycle, and analysed using the XN-1000 instrument (Sysmex, Istanbul, Turkey). The proliferative and secretory phases of individuals whose first samples were obtained during the menstrual phase were recorded in accordance with menstrual cycle charts. Peripheral venous blood samples were collected twice more, on days 8 and 10, during the proliferative phase, and on days 20 and 21 of the secretory phase. Complete blood counts were performed on these samples.

Statistical Analysis

Statistical analysis was performed using SPSS v.17 software (SPSS Inc., Chicago, IL, USA). Total leukocyte, neutrophil, and lymphocyte levels, and neutrophil and lymphocyte percentages, were compared between the MS and control groups using the Mann-Whitney U test. ANOVA was used to examine the differences in total leukocyte, neutrophil, and lymphocyte levels, and neutrophil and lymphocyte percentages, among the three different phases of the menstrual cycle in the MS and control groups. Moreover, group differences in the menstruation-proliferative (M&P), menstruation-secretory (M&S), and proliferative-secretory (P&S) phases of the menstrual cycle were examined using Tukey's post hoc test. Results are expressed as mean ± standard error, and p-values < 0.05 were considered statistically significant.

Results

Demographic and Clinical Data

Forty-one female patients of reproductive age with regular menstrual cycles, diagnosed with RRMS and receiving fingolimod therapy, were included in the study as the case group. In the patient group, the mean age was 33 ± 7.29 years (range: 21-45 years), the duration of disease was 104.63 ± 59.20 months (range: 12-276 months), the Expanded Disability Status Scale (EDSS) score was 3 ± 1.34 (range: 1-5), and the duration of fingolimod treatment was 18.51 ± 12.55 months (range: 1-42 months) (Table I). Symptomatic bradycardia was not observed in any of the MS patients after the first dose of fingolimod in the study. During the treatment of fingolimod, no side effects such as macular edema, encephalopathy or varicella zoster infection were observed.

The control group consisted of 33 healthy women of similar age, with regular menstrual cycles and without any chronic neurological disease or active infection. The mean age of the control group was 30.67 ± 5.33 years (range: 20-41 years), which was not significantly different from that of the case group ($p = 0.160$) (Table I).

Haematological Data of the Patients

The total leukocyte counts of the MS patients in the menstruation, proliferative, and secretory phases (4.77 ± 1.74, 4.40 ± 2.13, and 5.06 ± 1.40[10^3/μL], respectively) were significantly lower than those of the control group (6.90 ± 2.97, 6.08 ± 3.20, and 7.95 ± 1.66 [10^3/μL], respectively) ($p<0.001$) (Table II).

The neutrophil counts of the patients in the MS group were lower in the menstruation, proliferative, and secretory phases (3.59 ± 1.28, 3.86 ± 1.56, and 3.73 ± 1.18 [10^3/μL], respectively) than those of the control group (4.14 ± 1.97, 4.16 ± 1.05, and 4.65 ± 1.15 [10^3/μL], respectively). These differences were statistically significant in the menstruation and secretory phases ($p<0.05$,

Table I. Demographic and clinical data of the patients.

	MS (n = 41)	Control (n = 33)	p
Age, SD (range)	33 ± 7.29 (21-45)	30.67 ± 5.33 (20-41)	0.160
EDSS score	3 ± 1.34 (1.0-5.0)		
Disease duration, months (range)	104.63 ± 59.20 (12-276)		
Fingolimod treatment duration, months (range)	18.51 ± 12.55 (1-42)		

MS: multiple sclerosis, EDSS: Expanded Disability Status Scale.

p<0.001, respectively), but not in the proliferative phase (p=0.132) (Table II).

The percentages of neutrophils in the menstruation, proliferative and secretory phases were significantly higher in the MS group (71.63 ± 13.41%, 74.02 ± 7.56%, and 73.04 ± 6.40%, respectively) than the control group (52.27 ± 2.04%, 55.78 ± 6.13%, and 58.59 ± 6.57%, respectively) (p<0.001) (Table II).

The lymphocyte counts of the MS group were significantly lower in the menstruation, proliferative, and secretory phases (0.59 ± 0.27, 0.57 ± 0.25, and 0.62 ± 0.30 [10^3/µL], respectively) compared to the control group (2.26 ± 0.56, 2.43 ± 0.56, and 2.39 ± 0.60 [10^3/µL], respectively) (p<0.001) (Table II).

The percentages of lymphocytes of the patients in the MS group were significantly lower in the menstruation, proliferative, and secretory phases (12.26 ± 5.41%, 11.98 ± 5.32%, and 12.31 ± 4.84%, respectively) than the control group (29.54 ± 7.65%, 32.98 ± 5.88%, and 30.19 ± 5.55%, respectively) (p<0.001) (Table II).

The total leukocyte, neutrophil, and lymphocyte counts, and the percentages of neutrophils and lymphocytes, were compared among the menstruation, proliferative, and secretory phases for both the MS and control groups. There were no statistically significant differences in total leukocyte count, neutrophil count, neutrophil percentage, lymphocyte count, or lymphocyte percentage among the three phases of the menstrual cycle in the MS group (p = 0.262, p = 0.670, p = 0.553, p = 0.796, and p = 0.958, respectively) (Table III). In the control group, the leukocyte count was significantly lower during the proliferative phase than the other two phases of the menstrual cycle (p = 0.033) (Table III). There were no significant differences among phases for the other parameters (p>0.05) (Table III).

The results indicated that fingolimod significantly decreased total leukocyte, neutrophil, and

Table II. Comparison of hematological parameters according to groups in the stages of the menstrual cycle.

	MS	Control	p
Leukocyte Mens	4.77±1.74	6.90±2.97	<0.001
Leukocyte Pro	4.40±2.13	6.08±3.20	<0.001
Leukocyte Sec	5.06±1.40	7.95±1.66	<0.001
Neutrophil Mens	3.59±1.28	4.14±1.97	0.027
Neutrophil Mens %	71.63±13.41	52.27±21.04	<0.001
Neutrophil Pro	3.86±1.56	4.16±1.05	0.132
Neutrophil Pro %	74.02±7.56	55.78±6.13	<0.001
Neutrophil Sec	3.73±1.18	4.65±1.15	<0.001
Neutrophil Sec %	73.04±6.40	58.59±6.57	<0.001
Lymphocyte Mens	0.59±0.27	2.26±0.56	<0.001
Lymphocyte Mens %	12.26±5.41	29.54±7.65	<0.001
Lymphocyte Pro	0.57±0.25	2.43±0.56	<0.001
Lymphocyte Pro %	11.98±5.32	32.98±5.88	<0.001
Lymphocyte Sec	0.62±0.30	2.39±0.60	<0.001
Lymphocyte Sec %	12.31±4.84	30.19±5.55	<0.001

Mens: Menstruation, Pro: Proliferative, Sec: Secretory.

Table III. The haematological parameters of both groups according to menstrual cycle stage.

	M	P	S	F ratio	p	PostHoc Tukey Test
MS Patients						
Leukocyte	4.77±1.74	4.40±2.13	5.06±1.40	1.354	0.262	M&P: 0.623; M&S: 0.744; P&S: 0.234
Neutrophil	3.59±1.28	3.86±1.56	3.73±1.18	0.401	0.670	M&P: 0.645; M&S: 0.885; P&S: 0.912
Neutrophil %	71.63±13.41	74.02±7.56	73.04±6.40	0.596	0.553	M&P: 0.526; M&S: 0.801; P&S: 0.902
Lymphocyte	0.59±0.27	0.57±0.25	0.62±0.30	0.229	0.796	M&P: 0.956; M&S: 0.917; P&S: 0.779
Lymphocyte %	12.26±5.41	11.98±5.32	12.31±4.84	0.043	0.958	M&P: 0.971; M&S: 0.999; P&S: 0.959
Control						
Leukocyte	6.90±2.97	6.08±3.20	7.95±1.66	5.475	0.033	M&P: 0.447; M&S: 0.300; P&S: 0.025
Neutrophil	4.14±1.97	4.16±1.05	4.65±1.15	2.324	0.438	M&P: 1.000; M&S: 0.497; P&S: 0.497
Neutrophil %	52.27±21.04	55.78±6.13	58.59±6.57	3.405	0.210	M&P: 0.594; M&S: 0.185; P&S: 0.734
Lymphocyte	2.26±0.56	2.43±0.56	2.39±0.60	0.477	0.538	M&P: 0.541; M&S: 0.690; P&S: 0.968
Lymphocyte %	29.54±7.65	32.98±5.88	30.19±5.55	2.354	0.115	M&P: 0.119; M&S: 0.923; P&S: 0.249

M: Menstruation phase, P: Proliferation phase, S: Secretory phase.

lymphocyte counts, and the percentage of lymphocytes, in MS patients compared to the control group. The neutrophil percentage was increased in MS patients compared to the control group (Table II). However, these changes in haematological parameters in the MS group did not differ significantly according to the phase of the menstrual cycle. In the control group, the leukocyte count was significantly lower during the proliferative phase than the other phases, mainly driven by the difference between the proliferative and secretory phases as opposed to that between the proliferative and menstruation phases (Table III).

Discussion

MS is a neurological disease that affects the optic nerve, brain, and spinal cord, thus causing physical and mental disabilities[11]. MS occurs when the immune system attacks protective myelin sheaths that allow communication between the brain and the rest of the body[11]. Signs and symptoms of MS are very various and are dependent on the amount of nerve damage, and the nerves affected[11]. Although the pathogenesis of MS is not fully understood, environmental factors, autoimmunity, genetics, and infectious agents are thought to play major roles[12]. In particular, the prevalence of the disease is higher in women of childbearing age than in men of the same age[2]. All patients and control groups included in our study were female and the mean age was similar (20-45 years old).

Most MS patients show a relapsing-remitting disease course, and experience periods of new symptoms or relapse occurring over days or weeks, which usually improve partially or completely[13]. After this relapsing-remitting phase, most patients enter a secondary progressive phase characterized by the accumulation of irreversible neurological deficits[13]. MS is an important health problem; after trauma, it is the second most common cause of disability in young people[14,15]. Approximately 25% of patients require support to walk after having the disease for 20 years[16].

Treatment includes use of multiple agents, including corticosteroids, immunosuppressants, interferons, and monoclonal antibodies. Fingolimod is of particular importance, as it was an oral agent used in the treatment of MS[4]. Pain and other injection complications are expected to decreased in patients receiving subcutaneous or intramuscular treatment with the use of fingolimod. In our study, all of our patients are RRMS and use fingolimod therapy, and all have passed from another modifying therapy (DMTs) to fingolimod. Disease duration ranged from 12 to 276 months. When fingolimod use periods of these patients were examined, it was seen that the patient with the longest duration received this treatment for 42 months.

The pathological effects of MS, side effects of the drugs used, and physical and psychological effects of disability result in reproductive dysfunction during the chronic disease process, coinciding with the reproductive period in female MS patients[17]. Signs of sexual dysfunction, such as

decreased libido, loss of vaginal lubrication, and decreased vaginal sensation are common in these patients[17]. MS has not been reported to affect fertility in women, nor does it lead to abortion, malformation, stillbirth, or increased rates of complications in pregnancy and labour. Pregnancy has been shown to reduce the number of MS relapses, particularly in the second and third trimesters[18]. The relapse rate of MS tends to be higher during the first 3-6 months after giving birth, with an estimated risk of relapse during the postnatal period of 20-40%[18]. Pregnancy is also known to have no long-term effect on disability due to MS, but it increases the risk of attacks by threefold in the puerperal period[18].

The PRIMS study, published by Vukusic et al[19] in 2004, was the first major prospective study to investigate the effects of pregnancy and childbirth on the clinical course of MS. In that study, outcomes at the 2-year follow-up (after birth), and the probability of recurrence within 3 months after birth, were analysed. The relapse rates in each quarter until the end of the second year postpartum were compared to those in the pre-pregnancy year; the relapse rate during pregnancy showed the greatest decrease in the third trimester, and the most significant increase in the first 3 months after delivery.In the 2-year period except the second trimester and the first 3 months postpartum, the disease recurrence rate was not significantly different from that in the pre-pregnancy period[19]. Despite the increased risk of disease recurrence within the first 3 months postpartum, 72% of women did not experience relapse during that period[19]. Furthermore, the rate of disease recurrence was highest in the first 3 months postpartum in women with higher levels of disease activity, both before and during pregnancy[19]. None of our patients in our study received any disease modifying agent treatment during pregnancy.

MS is more common in premenopausal women than in other groups. Although the effects of hormonal changes on neurological symptoms in MS cannot be clearly explained, it has long been known that certain other neurological disorders, such as epilepsy and migraine, are more severe during the premenstrual and menstrual periods[20]. A study investigating correlations between neurological symptoms and the menstrual cycle in women with MS indicated an increase in MS-related complaints, especially in patients with RRMS, before or during the beginning of the menstrual cycle[21]. Fatigue, poor balance, and depression are among the most common symptoms of more severe MS[21,22]. All patients in our study have a regular menstrual cycle and there is no significant clinical worsening during the menstrual period.

New treatment options for MS are being developed. Fingolimod became the first FDA-approved oral agent for MS patients in 2010[23]. Fingolimod affects the S1P1 receptor, which is necessary for the release of lymphocytes from lymph nodes; it inhibits uptake of the receptor into the cell and reduces its synthesis, thus preventing the release of CCR7-bearing lymphocytes from the lymph nodes. Among the lymphocyte subgroups, T lymphocytes are known to be the most affected by this drug[23]. The most common side effects after the first dose of fingolimod are bradycardia and AV conduction disorders. Lymphopenia is another common side effect of fingolimod treatment. Fingolimod traps T lymphocytes in the lymph nodes, such that its use is associated with a marked decrease in T lymphocytes in the circulation[24-26]. However, fingolimod should be discontinued in cases where the lymphocyte count is below 200/ml, as the lymphocyte count below this level increases susceptibility to many opportunistic infectious agents[27]. Lymphocyte monitoring is very important, where lymphopenia resulting from use of the drug is considered a criterion for discontinuation of treatment[24-26]. There are no studies in the literature investigating the relationship between blood lymphocyte level and menstrual cycle in patients receiving fingolimod therapy.

The distribution of immune cells is known to change with changes in hormonal and immune mechanisms during the menstrual cycle[28,29]. According to the effects of hormone levels on the endometrium, the menstrual cycle may be divided into three different phases. The phase in which active menstrual bleeding occurs is called the menstruation phase; the phase in which endometrial tissue begins to proliferate after the menstruation phase is the proliferative phase; and the period of intense secretion in preparation for the next menstruation phase is the secretory phase[30,31]. In our study, we compared leukocyte, neutrophil and lymphocyte levels with each other in both MS and control groups according to the three stages of the menstrual cycle. Thus, both differences in the normal population and variability in patients using fingolimod were evaluated.

In some studies examining the distribution of immune cells, when the proliferative and secretory phases of the menstrual cycle were compared, the increase in leukocyte levels was greater dur-

ing the secretory phase[31,32]. Madhura et al[33] reported that the total leukocyte count was significantly higher during the proliferative phase compared to the menstrual and secretory phases in healthy individuals. In the present study, the leukocyte count was lowest during the proliferative phase in the control group, and highest in the secretory phase. Tikare et al[34] showed that lymphocyte levels increased in the secretory phase in comparison to the menstrual phase. Studies in healthy individuals have different results[31-34]. In our study, leukocyte levels in healthy individuals were significantly lower in the proliferative phase than other phases (p = 0.033, Table III). There was no significant difference in lymphocyte and neutrophil levels in healthy individuals. In the literature, there is no study investigating the relationship between leukocyte and lymphocyte values and menstrual cycle in MS patients receiving fingolimod therapy. In our study, there was no significant difference between leukocyte, lymphocyte and neutrophil levels in all three phases of the menstrual cycle in patients receiving fingolimod therapy. This study contributed to a limited number of studies in the literature that examined the effect of menstrual cycle on leukocyte and lymphocyte levels in healthy individuals. It also made it possible to determine the appropriate lymphocyte monitoring time in the treatment of fingolimod.

Limitations of The Study

This was a cross-sectional study. Prospective re-examination of individuals undergoing fingolimod treatment at various time points will provide a better understanding of the changes in cell numbers and functions within individuals.

Only one menstrual cycle of patients and healthy individuals was examined in this study; examination of multiple cycles will be instructive for assessing changes over time.

Studies including more subjects in both the MS and control groups are needed to validate our results.

Conclusions

There are a limited number of different results in the literature investigating the effect of the menstrual cycle on blood leukocyte and lymphocyte values. In our study, leukocyte levels in healthy individuals were significantly lower in the proliferative phase than other phases. There are no studies in the literature investigating the relationship between blood lymphocyte level and menstrual cycle in patients receiving fingolimod therapy. In our study, there was no significant difference between leukocyte, lymphocyte and neutrophil levels in all three phases of the menstrual cycle in patients receiving fingolimod therapy. In this study, the results indicated that lymphocyte monitoring in RRMS patients receiving fingolimod treatment can be performed at any stage of the menstrual cycle. This study contributed to the literature by examining the relationship between neutrophil and lymphocyte levels and menstrual cycle in healthy individuals, and provided the most appropriate lymphocyte monitoring time for RRMS patients receiving fingolimod therapy, which is not in the literature.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethical approval

This study with a protocol code of 2016/22 was approved by the Malatya Clinical Research Ethics Committee.
All procedures performed in studies involving human participants were in accordance with the Ethical Standards of the Institutional and/or National Research Committee (name of institute/committee) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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