Assessment of ovarian reserve and Doppler characteristics in patients with multiple sclerosis using immunomodulating drugs

İmmünmodülatör ilaç kullanan multiple sklerozlu hastalarda over rezervinin ve Doppler karakteristiklerinin değerlendirilmesi

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Abstract

Objective: There is limited data about fertility in multiple sclerosis (MS) patients using immunomodulating drugs and no data exists regarding the ovarian reserve of these patients. Therefore, we aimed to evaluate,the ovarian reserve and doppler characteristics of MS patients using immunomodulating drugs.

Material and Methods: MS patients using immunomodulating drugs (interferon (IFN) β and glatiramer acetate) and age-matched healthy controls were included in the study. Subjects were examined in the early follicular phase of the menstrual cycle with transvaginal ultrasound to evaluate ovarian volume, antral follicle count (AFC) and ovarian stromal artery Doppler. On the same day, blood was taken for determining serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) levels. A subgroup analysis was also carried out between MS patients using only IFN β and controls to compare the same parameters.

Results: Mean ovarian volume and total AFC were lower in MS patients using immunomodulating drugs than in the controls. FSH and E2 levels did not show any differences between the groups, but LH levels were significantly higher in MS patients. All the Doppler parameters of the ovarian stromal artery were higher in MS patients but not significantly. In the subgroup analysis, the same significant differences were found for ovarian volume, AFC and LH levels. In addition, MS patients showed significantly higher mean pulsatility index measurement than the controls.

Conclusion: The findings of this study demonstrated diminished ovarian volume and follicular reserve in MS patients using immunomodulating drugs compared to age matched healthy controls. However, further studies are required to elucidate whether compromised ovarian reserve in MS patients is due to drugs or the disease itself.

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Key words: Multiple Sclerosis, immunomodulating therapy, interferon-beta, ovarian reserve, Doppler

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Özet

Amaç: İmmünmodülatör tedavi kullanan Multipl Skleroz (MS) hastalarının fertiliteleriyle ilgili sınırlı, over rezervleriyle ilgili ise hiç data mevcut değildir. Bu çalışmadaki amacımız, immünmodülatör tedavi kullanan MS hastalarının over rezervleri ve stromal arter doppler karakteristiklerini incelemektir.

Gereç ve Yöntemler: Çalışmaya immünmodülatör tedavi (interferon-beta ve glatiramer asetat) kullanan MS hastaları ve yaş uyumlu sağlıklı kontrol grubu alındı. Hastalar erken foliküler dönemde transvajinal ultrasonografi ile incelenerek over hacmi, antral folikül sayısı (AFS) ve overyan stromal arter Doppler ölçümleri açısından değerlendirildi. Aynı gün serum folikül stimüle edici hormon (FSH), luteinize edici hormone (LH) ve estradiol (E2) ölçümleri için kan alındı. Sadece interferon tedavisi alan hastaları kontrol grbuyla karşılaştırmak için subgrup analizi de yapıldı.

Bulgular: İmmünmodülatör tedavi kullanan MS hastalarının ortalama over hacmi ve total AFS ölçümleri kontrol grubuna göre düşük bulundu. Gruplar arası FSH ve E2 seviyeleri bir fark göstermemekle birlikte LH seviyesi MS hastalarında anlamlı olarak yüksekti. Overyan stromal arter Doppler parametrelerinin tümü MS hastalarında yüksekti ancak bu yükseklik anlamlı değildi. Sırf interferon kullanan hastaların dahil edildiği subgrup analizinde, over hacmi, AFS ve LH seviyeleri arasında yine anlamlı fark bulundu. Buna ek olarak interferon kullanan MS hastalarının PI ölçümleri kontrol grubuna göre anlamlı olarak yüksekti.

Sonuç: Bu çalışmada immünmodülatör tedavi kullanan MS hastalarının over hacmi ve folikül rezervi aynı yaştaki sağlıklı kontrol grubuna göre daha düşük bulundu. Ancak over rezervindeki bu düşüklüğün MS hastalığına mı, yoksa kullanılan immunmodülatuar tedaviye mi bağlı olduğunun, daha geniş hasta serileriyle araştırılması gerekmektedir.

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Anahtar kelimeler: Multipl Skleroz, immunomodulatuar tedavi, interferon-beta, over rezervi, Doppler

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Introduction

Multiple sclerosis (MS) is the most common disabling central nervous system disease of young adults, and is estimated to affect 2.5 million people worldwide, with a prevalence of ~1 in 1000. Among adults with MS, there is a clear gender bias towards females, with a female-to-male ratio of 3-2:1 with the majority (80%) being diagnosed between the ages of 20 and 45 years (1). Since women of reproductive age are commonly affected, future reproductive performance is likely to be of great importance to these women.

There are limited data available concerning fertility in MS, but spontaneous female fecundity appears to be decreased (2). A recently published review suggested reduced spontaneous fecundity due to endocrine, autoimmune and sexual dysfunction, as well as gonadotoxic therapies in MS patients (3). Alterations in tubal function due to neuronal dysfunction may also affect fertility in patients with MS. In the studies where pregnancy outcome of MS patients was evaluated, a higher frequency of low birth weight infants was observed compared to healthy women. This is suggested to result from alterations in the uterine function due to neuronal dysfunction in pelvic organs, which may produce suboptimal intrauterine conditions influencing fetal growth, or due to neuronal-mediated dysfunction of blood circulation in pelvic organs (4, 5). However, there has never been any implication that the reduced fertility in MS patients could be due to diminished ovarian reserve.

Although prospective studies evaluating fertility and ovarian follicular reserve in these patients are lacking, some of the immunosuppressive drugs such as mitoxantrone and cyclophosphamide used in MS are cited as highly gonadotoxic based on animal and human studies (6-8). However, those studies evaluated the incidence of amenorrhea, instead of evaluating ovarian reserve markers such as follicle stimulating hormone (FSH), estradiol (E2), inhibin B, anti-müllerian hormone (AMH) or antral follicle count (AFC) to determine diminished ovarian function.

On the other hand, to date, such an effect has not been attributed to the immunomodulating drugs [IFN beta (β) (IFN-β1a (Avonex®, Rebif®), IFN-β1b (Betaseron® /Betaferon®)), and glatiramer acetate (GA) (Copaxone®)]., An International consensus statement on the use of disease-modifying agents in MS published in 2002 advised physicians that they should warn patients of the effect of disease-modifying agents on fertility, and their safety in pregnancy or breastfeeding has not yet been established (9).

There is limited data about fertility in MS patients using immunomodulating drugs and no data exists regarding ovarian reserve of these patients. Therefore, we aimed to evaluate ovarian reserve and Doppler characteristics of MS patients using immunomodulating drugs and compared antral follicle count, ovarian volume, ovarian stromal artery Doppler indices and basal hormonal levels of MS patients using immunomodulating drugs with age-matched healthy controls.

Material and Methods

Patient Recruitment and Evaluation

In this prospectively designed controlled study MS patients, who were informed of the study and wished to participate, were referred from the neurology clinics of Kirikkale and Ankara Universities and consent was obtained from each patient. The study was approved by the Ethical Committees of Kirikkale and Ankara University School of Medicine and conducted according to the guidelines for clinical studies described in the Declaration of Helsinki (as revised by the World Medical Association, http://www.wma.net).

The control group was recruited from healthy women who presented at the gynecology clinic of Kirikkale University School of Medicine for routine follow-up and who wished to participate in the study.

The demographic features, including age and body mass index (BMI), were recorded for all subjects. A detailed medical history was elicited from all patients, including the presence of any systemic disease including diabetes mellitus, hypercholesterolemia, atherosclerosis, hypertension, and polycythemia, history of ovarian surgery and bilateral tubal ligation, use of oral contraceptive pills or hormonal therapy in the six months prior to the study inclusion, and smoking and alcohol abuse. A detailed physical examination was performed and an initial laboratory examination, including complete blood count and lipid profiles were performed. Patients with a history or diagnosis of any of the above mentioned diseases or habits were excluded from the study for both MS and the control groups. In addition, infertility and having polycystic ovary syndrome according to Rotterdam criteria (10) were accepted as exclusion criteria for the control group.

Patients eligible for the study were examined in the early follicular phase of the menstrual cycle (day 2-3) with transvaginal ultrasound to evaluate ovarian volume, AFC, and ovarian stromal artery Doppler indices. Serum samples were obtained simultaneously to determine FSH, luteinizing hormone (LH) and E2 levels.

At this visit, the age at the first MS attack, menstrual cycle pattern, treatment for MS, treatment period, previous pregnancies and pregnancies while on therapy, and type of contraception were also recorded.

Ultrasound Evaluation

All Ultrasound examinations were performed on day 2-3 of the menstrual cycle at 9-10 am with the Siemens Acuson Antares (Mountain View, CA, USA) ultrasound machine equipped with a 4-9 MHz transvaginal probe after the bladder had been emptied. B-mode transvaginal sonography was applied first to localize the ovaries and determine any existing ovarian pathology. The length and height of the ovaries were measured in the sagittal section and width in the transverse section after 90° rotation of the transducer. Ovarian volumes were calculated as: d1xd2xd3x $\pi/6$, where d1, d2 and d3 are the three maximal

longitudinal, anteroposterior, and transverse diameters. The numbers of small antral follicles (2-5 mm) and of larger antral follicles (6-10 mm) were counted as the transducer was moved from the outer to the inner margin of the ovary. The follicle diameter was calculated as the mean of two perpendicular measurements.

Doppler Sonography

Both ovaries were then scanned with the power Doppler mode. Power Doppler gain settings were set to achieve maximum sensitivity to detect low velocity flow without noise. Other settings were as follows: frequency: 5 MHz and Filter: 2.

Ovarian stromal artery blood flow was then evaluated in pulsed Doppler mode to obtain flow velocity waveforms by examining vessels in the ovarian stroma (searching for any small artery in the ovarian stroma not close to the surface of the ovary or located near the wall of a follicle). For each examination, the mean value of three consecutive waveforms was obtained. Resistance index (RI), pulsatility index (PI), systolic/diastolic (S/D) ratio and peak systolic velocity (PSV) were automatically calculated from three consecutive flow velocity waveforms. Both ovaries were identified in all participants. The same investigator (A.P.C.) performed and videotaped all examinations.

Hormonal Assays

Hormone levels were measured using electro chemiluminescence immunoassay with MODULAR ANALYTICS E170 (Elecsys module) immunoassay analyzer (Roche Diagnostic, Mannheim, Germany), using the Roche kits. The intra- and interassay coefficients of variation (CV) for FSH ranged between 0.6%-2.4% and 2.5%-3.9%, respectively. The intra-assay CV range for LH and Estradiol were 0.8%-1.8%, and 2.7%-6.5%, respectively. The minimum detectable limits for FSH and LH were <0.10mIU/ml and for Estradiol this was 10 pg/ml.

Statistical Analysis

Shapiro-Wilks test was used to assess normal distribution of continuous data. Comparison of the continuous data between the MS and control groups was estimated using Student's t-test and non-parametric Mann Whitney-U test according to the distribution. Categorical data were compared using the chi-square test.

Mean ovarian volume, RI, PI, S/D, PSV and AFC measurements of right and left ovaries were compared using Paired samples t-test or Wilcoxon signed ranks test. Hence, there were no statistically significant differences between right and left ovaries, mean values of these parameters were used in comparing the MS and control groups.

A P-value ≤0.05 was considered as statistically significant for all tests used. Statistical analysis was performed using the SPSS 15.0 statistical package (SPSS Inc, Chicago, IL, USA).

Results

Patient Characteristics

Twenty-two patients with MS were referred to the gynecology clinic. All of the patients had a normal regular menstrual cycle pattern. Of the 22 patients, 20 presented with relapsing-remitting, 1 with primary progressive, and 1 with secondary progressive multiple sclerosis. Of the 22 patients, 21 patients used immunomodulating drugs (17 patients IFN-β, 2 patients GA, and 2 patients IFN-β1a and GA) and 1 patient used the immunosuppressive drug azathioprine.

Three patients were excluded from the study because one was using Azathioprine, one was 46 years old, and the other due to non-compliance. Thus, the study group for comparison purposes included 19 patients with a mean age of 33.21±5.72 (range 23-41). Twenty-five healthy women with a mean (±SD) age of 33.04±5.07 (range 21-42) were evaluated as controls.

Mean duration of disease was 88.2 ± 57.3 months (range 8-192) and mean length of drug usage was 38.7±32.3 months (range 5-96) in 19 MS patients using immunomodulating drugs.

Fertility Before and After MS Diagnosis

Of the 22 patients, 4 patients had no desire to become pregnant. Apart from these 4 patients, 11 MS patients had been using an intrauterine device, condom or coitus interruptus for contraception at the time of study inclusion. None had had pelvic surgery other than cesarean section (4 patients). One patient experienced infertility prior to the diagnosis of MS and conceived after ovulation induction with clomiphene citrate. After the diagnosis of MS, the patient remained infertile and could not conceive for 5 years despite having unprotected intercourse. Eighteen patients (after excluding patients with no desire to become pregnant) had 57 pregnancies in total; 5 resulted in spontaneous abortion and 15 ended in termination of pregnancy. Eleven of these pregnancies were terminated because of a recent MS diagnosis or being on MS treatment. The total number of pregnancies after the diagnosis of MS was 17, and 7 of these pregnancies occurred while the patients were on treatment, all of which were terminated. In the control group, 5 patients had no desire to become pregnant. The total number of pregnancies was 53, eleven of which resulted in spontaneous abortion and 7 ended in termination of pregnancy. There were no statistically significant differences between miscarriages and induced abortions between the groups (Table 1).

Table 1. Fertility outcomes of MS patients and the control group

	MS patients (n=22)	Control (n=25)	P value*
Never had a desire to get pregnant	4	5	0.623
Total pregnancies	57	52	
Pregnancies before MS diagnosis	37	-	
Pregnancies after MS diagnosis	17	-	
Pregnancies while on treatment	7	-	
First trimester miscarriages	5 (8.8%)	11 (21.2%)	0.068
Induced abortions	15 (26.3%)	7 (13.2%)	0.086
*Conducted by Chi-Square test			

Ovarian Reserve Assessment

MS patients (n=19) and the controls (n=25) were comparable with regard to BMI and age at study inclusion (Table 2). Ovarian sonography revealed no pelvic pathology in any of the participants performed on cycle day 2-3. FSH and E2 levels did not show any significant differences between the groups, but mean LH level was significantly higher in MS patients than in controls. No significant differences were found in ovarian volume, AFCs and Doppler indices of the left and right ovaries between the MS and control groups (p>0.05). Therefore, mean values of right and left ovarian volume, AFCs and Doppler indices were used for comparison. Although the mean number of small antral follicles (2-5 mm) were lower in MS patients, this difference was not statistically significant. Nevertheless, mean number of larger antral follicles (6-10 mm), mean number of total antral follicles (2-10 mm) and mean ovarian volume were significantly lower in MS patients than in controls. The results of hormonal and sonographic markers of ovarian reserve are given in Table 2.

The mean ovarian stromal artery RI, PI, S/D and PSV values of MS patients were higher, but this was not statistically significant (Table 3).

Analysis of patients using only IFN- β

Of the 19 MS patients, 15 used IFN-β, 2 patients GA, and 2 patients both IFN-β and GA. In order to evaluate a more homogeneous group, a subgroup analysis was conducted excluding the 4 patients who had been using GA, and comparing MS patients using only IFN-β (n=15) with controls (n=25). MS and control groups were comparable regarding age and BMI. There were no significant differences between the groups with respect to FSH and E2 levels, but mean LH levels were significantly higher in MS patients. MS patients had a significantly lower mean ovarian volume, mean number of larger antral follicles (6-10 mm) and of total antral follicles (2-10 mm). Although not statistically significant, mean number of small antral follicles (2-5 mm) was also lower in MS patients (Table 4).

The PI of MS patients were significantly higher than the controls. Other Doppler parameters were also found to be higher; however they did not reach statistical significance (Table 3).

Discussion

To our knowledge, this is the first study in which ovarian reserve and ovarian stromal artery Doppler indices are assessed

Table 3. Comparison of mean Doppler indices between MS and control patients

Patients using IFN-ß ±GA	MS (n=19)	Control (n=25)	P value
RI	0.49 (0.39-0.80)	0.46 (0.34-0.60)	0.195
PI	0.79 (0.65-1.28)	0.74 (0.56-1.14)	0.098
S/D	1.94 (1.68-3.20)	1.89 (1.51-2.68)	0.222
PSV (cm/s)	10.88 (4.20-16.40)	7.78 (5.05-20.20)	0.601
Patients using only IFN-ß	**MS (n=15)**	**Control (n=25)**	**P value**
RI	0.51 (0.41-0.80)	0.46 (0.34-0.60)	0.070
PI	0.85 (0.69-1.28)	0.74 (0.56-1.14)	0.038
S/D	1.94 (1.68-3.20)	1.89 (1.51-2.68)	0.111
PSV (cm/s)	11.23 (4.20-16.40)	7.78 (5.05-20.20)	0.515

Note: Data are median (range)

RI: Resistance index. PI: Pulsatility index. S/D: Systolic/diastolic ratio. PSV: Peak systolic velocity

Statistics were conducted by Mann Whitney-U test

Table 2. Comparison of hormonal and sonographic markers of ovarian reserve in MS patients using immunomodulating therapies (IFN-ß + GA) and healthy controls

	MS patients (n=19)	Control (n=25)	P value
Age (y) *	33.21±5.72	33.04±5.07	0.917
BMI (kg/m^2)	24.38 (19.1-33.1)	23.83 (19.0-34.5)	0.318
FSH (mIU/ml)	7.16 (4.87-17.92)	6.96 (4.21-14.37)	0.935
LH (mIU/ml)	6.26 (3.49-11.9)	4.96 (3.75-8.29)	0.008
E2 (pg/ml)	47.99 (15.11-148.3)	36.54 (17.6-192.8)	0.521
AFC (2-5 mm)	5 (0-13)	6 (2-16.5)	0.144
AFC (6-10 mm)	2 (0-10)	4 (2-9.5)	0.004
Total AFC (2-10 mm)	7.5 (1.5-20)	11 (6-23)	0.012
Ovarian volume (ml)	6.13 (3.41-12.24)	7.61 (3.61-15.79)	0.040

Note: Data are mean ±SD; median (range)

BMI: Body mass index. FSH: Follicle stimulating hormone. LH: Luteinizing hormone E2: Estradiol. AFC: Antral follicle count

*Conducted by Student t-test, all other comparisons were performed by Mann Whithney-U test

Table 4. Comparison of hormonal and sonographic markers of ovarian reserve in MS patients using only IFN-ß with healthy controls

	MS patients (n=15)	Control (n=25)	P value
Age (y)	36 (23-39)	32 (21-42)	0.720
BMI (kg/m^2)	24.39 (19.05-33.10)	23.83 (19.0-34.5)	0.408
FSH (mIU/ml)	6.56 (4.87-10.96)	6.96 (4.21-14.37)	0.820
LH (mIU/ml)	6.26 (3.49-11.90)	4.96 (3.75-8.29)	0.031
E2 (pg/ml)	48.04 (15.11-148.3)	36.54 (17.6-192.8)	0.604
AFC (2-5 mm)	5 (0-10)	6 (2-16.5)	0.074
AFC (6-10 mm)	2 (0-10)	4 (2-9.5)	0.001
Total AFC (2-10 mm)	7 (1.5-20)	11 (6-23)	0.002
Ovarian volume (ml)	5.34 (3.41-12.24)	7.61 (3.61-15.79)	0.009

Note: Data are median (range)
BMI: Body mass index. FSH: Follicle stimulating hormone. LH: Luteinizing hormone E2: Estradiol. AFC: Antral follicle count
Statistics were conducted by Mann Whitney-U test

in MS patients using immunomodulating drugs. In this study, we found that ovarian volume, larger AFC and total AFC of MS patients using immunomodulating drugs (IFN-β, GA) were significantly lower compared to age-matched healthy controls. However, no significant differences were found between the groups regarding serum FSH and E2. In order to homogenize the MS group; we excluded patients who had been using GA and compared ovarian reserve markers of MS patients using only IFN-β with controls, and found the same significant differences in the mean number of total AFC and in larger AFC (6-10 mm) between the groups. Subgroup analysis of the MS patients using GA was not possible because of the small patient number (n=4), 2 of whom had also used IFN-β. In ovarian stromal artery Doppler analysis, we found no significant differences between the groups.

In the current study we also evaluated the menstrual pattern and pregnancy outcomes of MS patients using immunomodulating drugs, and observed that none of the MS patients had menstrual irregularities or amenorrhea. There are a few studies that have evaluated the menstrual pattern and ovarian endocrine function in MS women of reproductive age (11-13). In an earlier study, 14 MS patients were compared with 14 regularly menstruating controls. Consistent with our results, all of the patients in that study had normal menstrual cycles, having no fertility problems. In contrast to that study, one of the 22 MS patients in our study had infertility and conceived after induction with clomiphene citrate. Those investigators found that basal FSH, LH and prolactin levels were significantly higher in patients with MS than in controls and suggested the possibility of lowered dopaminergic tone, since dopamine is a central inhibitor of prolactin and gonadotropin secretion. They concluded that the increase in FSH levels demonstrated reproductive aging. In this early study, which was undertaken in 1989, the authors did not emphasize the gonadotoxic effects of drugs used by MS patients (11). Furthermore, the significant increase in FSH levels could also have been due to the 3.5-year difference in the ages of MS and control patients (36.4 vs. 32.9), In contrast to their study, there was no significant differences in FSH and E2 levels between the groups in our study.

Another study evaluating menstrual irregularities in 57 MS patients found that after IFN-β use, 23.5% of the patients had menstrual irregularities, most commonly in the form of hypermenorrhea (33.3%) and oligomenorrhea (33.3%) (12). In another study comparing menstruel pattern and hormone levels of 58 MS patients with 58 matched healty female, they found that 55% of MS patients compared to 20% of normal women had menstruel disturbances but the type of drugs used was not defined. The LH levels in this study were significantly higher in MS patients than in controls (13).

The latter two studies were abstract presentations and thus the details of these studies were not available to make a full comparison with our results. In those studies, the authors did not mention whether the subjects were using oral contraceptives or had any gynecologic abnormalities that could have a potential effect on the menstrual pattern of these patients. However, none of the MS patients using immunomodulating drugs reported menstrual irregularities related to drug usage in our study, although one of our MS patients had an intramural myoma measuring 4 cm.

There are several studies evaluating pregnancy outcomes of MS patients using IFN-β (14-22) but it is difficult to give a pregnancy rate based on these studies because none of them evaluated the intent to conceive while on treatment or the time interval to pregnancy. We did not compare the pregnancy rates of MS patients under IFN-β treatment with controls, assuming it would be inaccurate, since most of the pregnancies in our study were not planned. In order for our comparison to reflect accurate results, both groups should have included patients who did not use any contraceptive methods and who wished to become pregnant. The number of induced abortions was higher in MS patients due to the disease and MS treatment, only four of these patients had termination of their pregnancies unrelated to MS diagnosis.

In the ovary, primordial follicles make up 95% of the ovarian reserve (23). With increasing age there is a decline in a woman's reproductive function, which is assumed to be determined by the decline of the ovarian follicle pool and the quality of the oocytes within (ovarian reserve) (24). Therefore, various endocrinological and sonographic markers have been investigated and proposed as accurate predictive markers of the primordial follicle pool. These include FSH, E2, Inhibin B, AFC and anti-Müllerian hormone (AMH) (25). A serum FSH >12IU/L, or E2 >75 pg/ml measured on the 2nd or 3rd day of the menstrual cycle reflects diminished ovarian reserve. The size of the antral follicle cohort can be directly assessed by ultrasound (26), and the observed pattern of its decline appears to correspond with that of the primordial follicle pool (27). AMH is strongly correlated to AFC, both predictors have a linear relationship with age and these markers are currently believed to be the best predictors of ovarian reserve (28-31). When assessing ovarian reserve, it should be underlined that amenorrhea is the last event of reproductive aging, and that a normal regular menstrual pattern may still persist despite extremely diminished ovarian reserve (23).

Doppler assessment of ovarian stromal blood flow in the early follicular phase of spontaneous cycles has also been studied and related to ovarian follicular response (32). Kim et al. reported a higher PI of ovarian stromal artery with a lower pregnancy rate of the corresponding IVF cycle. They related these results to the availability of good quality oocytes by better circulation when the PI of the ovarian stromal artery is lower, which results in improved supply of oxygen, nutrients, hormones and growth factors (33). Tinkanen et al. reported that infertility patients, excluding those with male factor infertility, had higher PIs in the ovarian arteries compared to the control group (34). We also found higher PIs in the ovarian stromal arteries of patients with MS in accordance with these results. The finding of significantly higher Doppler indices might have importance in MS patients apart from diminished AFC.

The presence of normal FSH and E2 levels with diminished AFC may appear to be paradoxial, but there are other studies supporting our findings (35-38). Recently, Nardo et al. (35) published their study aiming to find the best marker for ovarian response to controlled ovarian stimulation. They found that for patients with poor response, AMH was significantly a better predictor than FSH but not AFC. Studies on female cancer survivors with regular menses and normal serum FSH levels showed that they had significantly smaller ovaries along with a lower AFC as compared to age-matched controls (36, 37). A recent study on gonadal function in childhood cancer survivors showed that regular menstrual cycles and normal early follicular phase FSH did not confirm the absence of damage to the ovary (38). These observations indicate that parameters such as cycle length and FSH serum levels are not accurate markers of ovarian function. Serum AMH levels, AFC and ovarian volume appear to be more reliable predictors (31, 36, 38).

Our results show that currently the most reliable sonographic marker of ovarian reserve, AFC, is significantly decreased in MS patients using immunomodulating drugs compared to age matched regularly menstruating controls. In addition, mean ovarian volume of MS patients is significantly lower compared to controls. Therefore, these patients are at increased risk of diminished ovarian reserve. The ovarian stromal artery Doppler findings showing high resistance also suggest decreased ovarian function in MS patients using immunomodulating drugs. We have determined that MS patients under treatment with immunomodulating drugs with normal menstrual cycles have sonographic changes suggesting impairment of ovarian potential. These results are important because most MS patients are of childbearing age and might desire to become pregnant.

Our findings are nevertheless preliminary. One of the weak points of our study is that the study population is small and the other is that we had no information regarding the ovarian and endocrine functions of MS patients who had never been treated. As a result we cannot determine whether the observed alteration in ovarian reserve and function is due to the treatment or the disease itself. We are currently conducting another prospective study on newly diagnosed MS patients, to compare baseline ovarian reserve markers including AMH and Doppler assessment of the ovaries with age-matched regularly menstruating controls and MS patients using immunomodulating drugs. This study may determine whether this difference in ovarian reserve and function is derived from the drugs or the disease itself. However, collaborating studies take a long time to complete and we wish to publish our preliminary results assuming that this study would be a guide to physicians for future studies and a beginning of investigation of the ovarian reserve in MS patients using different types of therapies.

In conclusion, AFC, the most reliable sonographic marker of ovarian reserve, is diminished in MS patients undergoing treatment with IFN-β compared to age matched regularly menstruating controls. The significant increase in resistivity marker PI supports the potential decrease in ovarian function. Future studies with larger groups of MS patients and more sensitive ovarian reserve markers are needed to enhance our understanding of the impact of immunomodulating drugs on ovarian function. Accordingly, MS patients should be counselled about their reproductive potential.

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