Assesment of ovarian stromal artery Doppler characteristics and serum hormone levels in patients with Behçet's Disease

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ORIGINAL ARTICLE

Assessment of ovarian stromal artery Doppler characteristics and serum hormone levels in patients with Behçet disease

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PURPOSE
The aim of the study was to examine serum hormone levels, ovarian volume, stromal artery Doppler parameters of patients with Behçet disease (BD) to assess whether there are vascular changes in the gonads of these patients.

MATERIALS AND METHODS
Twenty patients with BD and 31 healthy controls aged between 18–45 years were examined in the early follicular phase of the menstrual cycle (day 2–3) with transvaginal ultrasound to evaluate ovarian volume and ovarian stromal artery Doppler parameters. On the same day, blood was drawn for determining serum hormone levels.

RESULTS
Patients with BD and the controls were comparable with regard to age and body mass index at study inclusion. Although comparison of the ovarian stromal artery Doppler velocimetric parameters did not show significant differences, resistivity, pulsatility indexes and systolic/diastolic ratio were higher, while peak systolic and end diastolic velocities were lower, in BD patients compared to controls. The mean ovarian volume of patients with BD was smaller than the controls but this difference did not reach statistical significance. There were no statistically significant differences between serum hormone levels of either group. We did not find any correlations between hormone levels and mean ovarian stromal artery Doppler parameters of patients with BD.

CONCLUSION
Ovarian stromal artery Doppler parameters of patients with BD did not show any significant differences compared to healthy controls. Therefore, we conclude that ovarian stromal artery is not involved in patients with BD as assessed by transvaginal Doppler ultrasound and serum hormone levels do not differ from the levels of healthy controls.

Key words: • *Behcet disease* • *ovary* • *Doppler ultrasound* • *hormones*

Behçet disease (BD) is a multisystem condition characterized by mainly mucocutaneous findings including recurrent oral and genital ulcers, ocular, articular, neurologic, urogenital, vascular, intestinal, and pulmonary manifestations (1). Classified as a systemic vasculitis, it can involve both the arteries and veins of any size in almost any organ. Epidemiological findings suggest that both genetic, immunologic and environmental factors influence the pathogenesis of the disease, but its accurate etiology and pathogenesis are still unknown (2). Interactions between the neuroendocrine and immune system may also play an important role in maintaining and restoring homeostasis in BD. Experimental evidence suggests that sex-linked hormonal factors influence the immune response and modify the expression of autoimmunity in animals and humans. However, the mechanism of sex hormone action is not precisely understood (3, 4).

There are little data on serum hormone concentrations of female patients with BD and most of these studies are about possible relationship of prolactin on BD pathogenesis or disease activity (5–9). Although some of these studies suggest a possible role for this immunoregulatory hormone in the disease expression and pathogenesis (5, 6), no such correlation has been found in several others (7–9).

Folliculogenesis in the human ovary is a complex process regulated by a variety of endocrine and paracrine signals (10). The availability of an adequate vascular supply to provide endocrine and paracrine signals may play a key role in the regulation of follicle growth (11). It is postulated that increased ovarian stromal vascularity may lead to a greater delivery of gonadotrophins to the granulosa cells of the developing follicles. Therefore, it would be valuable to study ovarian stromal vascularity, endocrine hormones, and their relationship in BD.

Ovarian stromal vascularity can be assessed by color or power Doppler ultrasound. Power Doppler is better suited to the study of the ovarian stromal vascularity, as it is more sensitive to lower velocities and essentially angle-independent (12).

In this study, we aimed to examine ovarian volume and stromal vascularity of female patients with BD to assess whether there are vascular changes in the gonads of these patients and if there is any relation to endocrine hormones. Therefore, we compared ovarian stromal artery Doppler characteristics and endocrine hormone levels of female Behçet patients with healthy controls.

Materials and methods

Patient recruitment and evaluation

Female patients with BD and healthy controls, aged between 18–45 years who were informed and willing to participate in the study, were included in this prospectively designed controlled study. All patients

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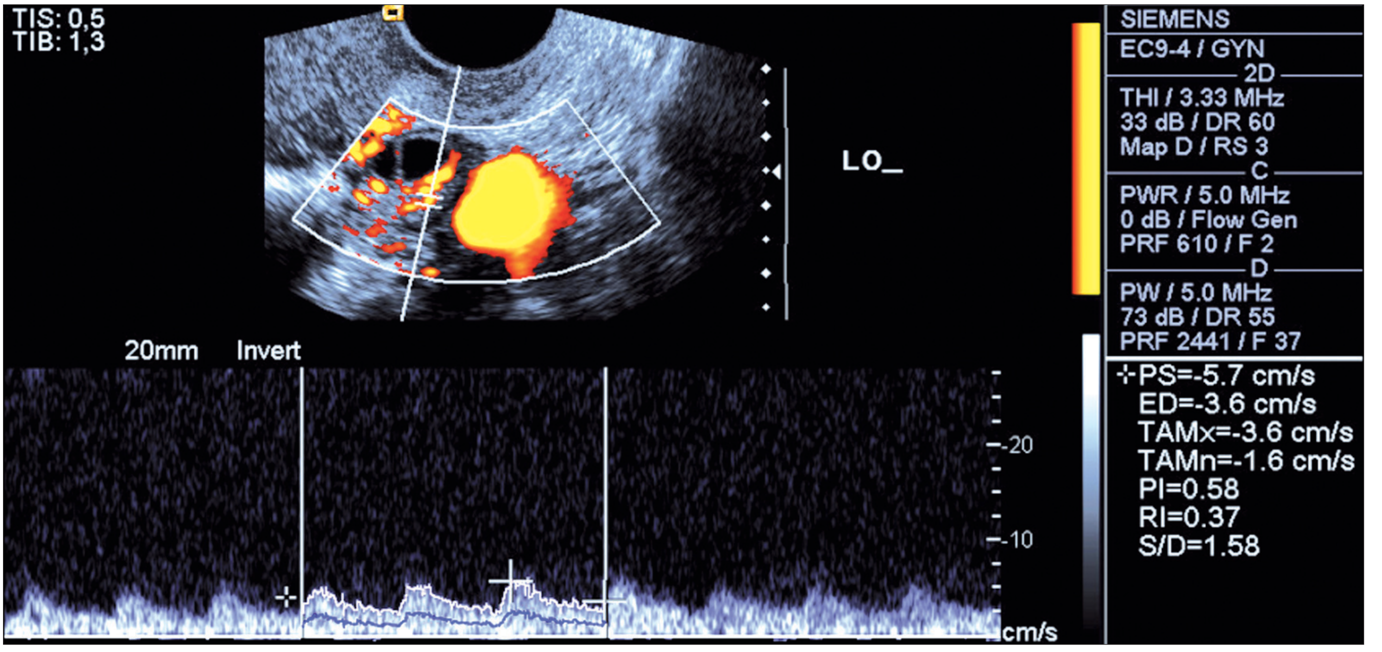

Figure. Power Doppler analysis of the ovarian stromal artery of a patient with BD in the early follicular phase of the menstrual cycle.

gave informed consent prior to study inclusion. The study was approved by the ethical committee of our university 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).

Patients with BD were referred from our dermatology clinic to the gynecology clinic. All patients fulfilled the diagnostic criteria of the 1990 International Study Group for BD (13). All patients were inactive who had responded to the systemic or local therapy (corticosteroid or immune-suppressive) and had achieved a period of remission during the last 1 month and had been taking colchicine (0.5–1 mg daily) at the time of study inclusion. Control group was recruited from healthy women who presented to the gynecology clinic for routine gynecologic follow-up.

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 onset of symptoms, presence of any systemic disease including diabetes mellitus, hypercholesterolemia, atherosclerosis, hypertension, and polycthemia, history of ovarian surgery and bilateral tubal ligation, use of oral contraceptive pills or hormonal therapy for the last 6 months, smoking and alcohol abuse. A detailed physical examination as well as an initial laboratory examination, including complete blood count and lipid profiles, was performed. Pregnant and menopausal patients, patients with a history or diagnosis of any of the aforementioned diseases or habits, were not included in the study.

After the initial work-up, 20 female patients with BD and 31 healthy controls were accepted eligible for the study group. Additional information of age at diagnosis, treatment for BD, duration of therapy, were recorded for the included patients. Patients were examined in the early follicular phase of the menstrual cycle (day 2–3) with transvaginal ultrasound to evaluate ovarian volume and ovarian stromal artery Doppler velocimetric parameters. On the same day, blood was drawn for determining serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), thyroid stimulating hormone (TSH), total testosterone (TT) and dehydroepiandrosterone sulphate (DHEAS).

Hormonal evaluation

All hormone levels were measured using electrochemiluminescence immune assay with a Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostic, Mannheim, Germany), using the Roche kit.

Ultrasound evaluation

All ultrasound examinations were performed on day 2–3 of the menstrual cycle at 9–10 a.m. with a Siemens Acuson Antares (Mountain View, California, 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 a 90° rotation of the transducer. Ovarian volumes were calculated as $d1d2d3\pi/6$, where d1, d2 and d3 are the three maximal longitudinal, anteroposterior, and transverse diameters. Both ovaries were then scanned with the power Doppler mode (Figure). Power Doppler gain settings were set to achieve maximum sensitivity to detect low velocity flow without noise. Other settings were: frequency, 5MHz; 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 (PS) and end-diastolic velocity (ED) were automatically calculated from three consecutive flow velocity waveforms. Both ovaries were identified in all participants except for one patient in whom the left ovary was not identified. The same investigator (A.P.C.) performed and videotaped all examinations.

Statistical analysis

Shapiro-Wilk test was used to assess normal distribution of continuous data. Except for age and total testosterone levels, continues variables were not normally distributed. Normally distributed variables were compared using Student's t-test, all other continuous data were compared with nonparametric Mann–Whitney U test.

Wilcoxon signed ranks test was used to assess differences between the right and left ovarian Doppler velocimetric parameters. There were no statistically significant differences between the right and left ovaries; therefore, mean values of Doppler velocimetric parameters were used to compare groups. Spearman's rank method was used to assess correlations between hormonal values and all quantitative ultrasound Doppler data.

Data were processed using SPSS 15.0 software (SPSS Inc., Chicago, USA). A P value of <0.05 was considered as statistically significant.

Results

Patients with BD (n=20) and the controls (n=31) were comparable with regard to age and BMI at study inclusion (Table 1). All of the patients with BD had been taking colchicine (0.5–1 mg daily) and had inactive disease at the time they were included in the study. Mean duration of disease was 78.10 ± 70.07 months (range, 4–240), and mean length of drug usage was 65.72 ± 59.34 months (range, 4–240).

We were able to identify both ovaries and examine Doppler parameters in all patients except for one. This 35-year-old patient had an abdominal aortic aneurysm due to vascular involvement of BD, and an endovascular aneurysm repair with an aortic stent-graft placed to the infrarenal segment of the aorta was performed 4 months previously. We could not identify the left ovary of this patient and there were no power Doppler signals on the right ovary, which was smaller (5.4 mL) than the mean average volume of patients with BD.

PRL, TSH, LH, E2 levels of patients with BD were higher and FSH, TT and DHEAS levels were lower, respectively, despite being statistically insignificant (Table 1). The right and left ovarian stromal artery Doppler velocimetric parameters did not show any significant differences between BD and the controls (Mann Whitney-U test, $P > 0.05$). There were no statistically significant differences between ovarian volume and stromal artery Doppler velocimetric parameters of the right and left ovaries of the subjects (Wilcoxon signed ranks test, $P > 0.05$). Therefore, we were able to use mean values of the two ovaries for comparison of these parameters.

Comparison of mean ovarian volume and ovarian stromal artery Doppler velocimetric parameters of BD and the controls is given in Table 2. Although comparison of ovarian stromal artery

Table 1. Comparison of demographic and hormonal parameters of patients with Behçet disease and controls

	Behçet disease	Controls	*P* value
Age (y)[a]	34.60 ± 7.40 34.5 (18–45)	32.39 ± 5.64 32 (19–42)	0.233
BMI (kg/m^2)	27.00 ± 4.78 26.17 (17.90–34.15)	23.92 ± 4.28 23.43 (19.00–34.53)	0.147
PRL (ng/mL)	11.35 ± 8.96 8.6 (4.30–34.88)	14.08 ± 10.24 10.06 (3.80–43.06)	0.267
TSH (μIU/mL)	1.82 ± 0.96 1.58 (0.61–4.26)	2.17 ± 1.24 2.02 (0.45–6.07)	0.391
FSH (mIU/mL)	7.07 ± 1.75 6.91 (3.58–11.30)	7.63 ± 2.66 6.91 (4.23–14.37)	0.741
LH (mIU/mL)	6.16 ± 2.78 5.35 (1.34–11.75)	5.47 ± 1.99 5.03 (2.37–12.69)	0.509
E2 (pg/mL)	74.34 ± 120.02 41.45 (16.83–539)	38.21 ± 15.71 33.74 (17.61–75.00)	0.358
TT (ng/mL)[a]	0.26 ± 0.15 0.24 (0.05–0.58)	0.35 ± 0.16 0.35 (0.04–0.79)	0.099
DHEA-S (ng/mL)	171.9 ± 77.15 138.2 (113–336.7)	231.71 ± 123.78 219.30 (31.80–559)	0.140

[a] Conducted by Student t-test, all other comparisons were done by Mann Whithney-U test.
Data are mean ± SD; median (range).
BMI, body mass index; PRL, prolactin; TSH, thyroid stimulating hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol; TT, total testosterone; DHEAS, dehydroepiandrosterone sulphate.

Table 2. Comparison of mean ovarian stromal artery Doppler velocimetric parameters of patients with Behçet disease and controls

	Behçet disease	Control	*P* value
RI	0.48 ± 0.09 0.45 (0.36–0.62)	0.46 ± 0.07 0.45 (0.34–0.61)	0.526
PI	0.85 ± 0.29 0.73 (0.58–1.50)	0.77 ± 0.18 0.74 (0.56–1.17)	0.496
S/D	2.08 ± 0.49 1.83 (1.56–3.22)	1.92 ± 0.31 1.86 (1.51–2.68)	0.467
PSV (cm/s)	8.06 ± 4.06 6.75 (4.70–18.10)	10.16 ± 4.87 7.23 (5.05–20.20)	0.117
EDV (cm/s)	4.61 ± 2.85 3.35 (2.00–11.25)	5.69 ± 3.26 3.98 (2.25–13.40)	0.159
Ovarian volume (mL)	7.48 ± 2.41 7.42 (4.37–14.04)	9.00 ± 3.10 7.97 (4.31–15.79)	0.095

Data are mean ± SD; median (range).
Statistics were conducted by Mann Whithney-U test.
RI, resistance index; PI, pulsatility index; S/D, systolic/diastolic ratio; PSV, peak systolic velocity; EDV, end diastolic velocity.

Doppler velocimetric parameters did not reveal any significant differences, RI, PI and S/D ratio were higher, while PS and ED velocities were lower, in BD patients compared to the controls. The mean ovarian volume of patients with BD was smaller than that of the controls but this difference did not reach statistical significance.

We did not find any correlations between hormone levels and mean ovarian stromal artery Doppler velocimetric parameters or ovarian volume of patients with BD.

Discussion

To our knowledge, this is the first report of a study in which ovarian sonographic and stromal artery Doppler characteristics are assessed in patients with BD. Although BD can involve both the arteries and veins of almost any organ, ovarian vascular involvement and its relation to endocrine hormones has never been studied to date. BD usually affects young adults between 20 and 40 years of age (14), mainly women in their reproductive years. Therefore, the information concerning ovarian vascular involvement and endocrine hormone levels in female patients with BD may be helpful in better understanding the BD pathogenesis and in consulting BD patients dealing with infertility.

Ovarian stromal blood flow in the early follicular phase of spontaneous cycles has been studied and was found to be related to ovarian follicular response (15). Kim et al. reported a higher PI of the ovarian stromal artery with a lower pregnancy rate of the corresponding in vitro fertilization (IVF) cycle (16). These results were attributed to the availability of good quality oocytes in case lower PI values revealing increased blood flow were detected in the ovarian stromal artery which implies an improved supply of oxygen, nutrients, hormones and growth factors in turn (16). Likewise, Tinkanen et al. reported that infertility patients had higher PIs in the ovarian arteries compared to the control group (17).

In this study, we could not find any significant differences in terms of ovarian volume, ovarian stromal artery Doppler velocimetric parameters or endocrine hormones between patients with BD and healthy controls. However, ovarian volume, PS, and ED velocities were lower, whereas RI and PI measurements were higher, in patients with BD compared to age-matched healthy controls, indicating a higher resistance and lower blood flow in the ovaries of patients with BD.

In the pathogenesis of BD, genetic factors, environmental agents and autoimmune basis together play a role in a susceptible host (18). In histopathologic examination of organs affected by BD, the presence of occlusive vasculitis is frequently seen and the cause of tissue damage was proposed to be the accumulation of immune complex in related tissues (19). According to the results of our study, ovarian stromal vascular changes are not present in patients with BD as assessed with transvaginal power Doppler ultrasound. In our study population of BD, we were not able to identify the left ovary of one patient and nor were able to find any power Doppler signals on the right ovary. She was diagnosed with BD four months earlier, after she had aortic stent placement in infrarenal part of the abdominal aorta for the treatment of an aortic aneurysm, and this stent-graft probably blocked the blood flow to the ovarian arteries, which originated from the abdominal aorta. Her FSH and E2 levels were within normal limits as of this writing, but follow-up of this patient will show whether there will be any changes in ovarian hormones after chronic exposure to diminished ovarian blood flow.

Sex hormones were proposed to have effects on the immune system both in normal and autoimmune situations (4). Although the effect of sex hormones on the immune system has long been recognized, several questions regarding the precise mechanism of their action have remained unanswered. Target organs of sex hormone action are lymphatic organs, T cells, B cells, natural killer cells, thymic epithelium, macrophage monocyte system, central nervous system, endocrine organs, and complement producing cells (4). Since impairment in some of these systems play a role in BD, hormonal differences might be expected between patients with BD and healthy controls; however, we did not find any significant differences. However, PRL, TSH, LH, and E2 levels in patients with BD were higher, and FSH, TT and DHEAS levels were lower, than in the controls, but not in significant levels, and we were not able to find any significant correlation between hormone levels and Doppler parameters.

PRL has been implicated as an important in vivo modulator of cellular and humoral immunity and may play a role in the pathogenesis and persistence of several autoimmune diseases (20–22). The relationship between BD and PRL had also been studied (5–9) and were found to be related to disease activity in some of the reports (5, 6), but the results are conflicting. None of these studies categorized the patients considering their sex, making it impossible to compare the difference of PRL levels between female and male patients. Therefore, based on those results, it is questionable to relate the attributed features of PRL to BD pathogenesis or activity. It is also not accurate to compare our results with those studies, because our patient population consists of females whereas others have included both sexes. Our results on PRL levels are in accordance with a recently published report in a large group of patients with BD (23).

It is reported that in most cases physiologic endogenous sex hormones, rather than sex chromosomes, contribute to the susceptibility or resistance to autoimmune diseases. There is female predominance in the occurrence of many autoimmune diseases, and this finding is related to the hypothesis that male sex hormones consistently protect against several types of autoimmune diseases which presumably differ in their induction and pathogenic pathways. On the other hand, the effects of estrogen appear to vary among the models, although, in general, estrogen promoted many autoimmune diseases (5). In our study, although not in statistically significant levels, E2 levels were higher and TT levels were lower in patients with BD, supporting this hypothesis.

Our findings are nevertheless preliminary. The most important limitation of our study is that the study population is small, and although we found some differences in ovarian volume, stromal artery Doppler velocimetric parameters and endocrine hormones of female patients with BD compared to healthy controls, these differences did not reach any statistical significance.

In conclusion, our data, as assessed by transvaginal Doppler ultrasound, did not show any significant differences in the ovarian stromal artery Doppler parameters to suggest any ovarian vascular involvement of BD. However, insignificantly higher resistance and lower blood flow in the ovaries of patients with BD need further investigation including ovarian reserve assessment with a large group of patients. It must be kept in mind that stent grafting of abdominal aortic aneurysms may result in the loss of ovarian vascular supply from the ovarian arteries. Therefore, patients with BD having aortic stent placement should be informed about the consequences they can face such as infertility or early menopause related to a diminished ovarian blood flow.

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