

Changes in plasma müllerian-inhibiting substance and brain-derived neurotrophic factor after chemotherapy in premenopausal women

Eight premenopausal women with cancer had blood drawn for analysis of brain-derived neurotrophic factor (BDNF) and müllerian-inhibiting substance (MIS) before and 3 months after receiving chemotherapy. Unlike MIS, BDNF levels were not reduced after chemotherapy. (Fertil Steril® 2010; ■: ■–■. ©2010 by American Society for Reproductive Medicine.)

Key Words: Anti-müllerian hormone, brain-derived neurotrophic factor, cancer, chemotherapy, müllerian-inhibiting substance, ovarian reserve

Accelerated ovarian aging (1, 2) and ovarian failure are common consequences of the treatment of malignant disease in women (3–5). At present, fertility prediction in women is imprecise, but müllerian-inhibiting substance (MIS) shows particular promise in this regard (6, 7). Müllerian-inhibiting substance, expressed in the granulosa cells of follicles from initiation of growth until the early antral stages (8, 9), more closely reflects ovarian reserve than do other hormones (10) and has been shown to be useful for assessing the toxicity of chemotherapeutic agents (11–13). Decanter et al. (14) showed that MIS concentrations fell dramatically just after the start of chemotherapy and were near their detec-

tion limit at the end of the treatment. It has also been shown that among childhood cancer survivors MIS can be used to identify patients who are at risk for decreased fertility (15).

Other markers that have been linked to ovarian function, but not ovarian reserve, are neurotrophins. Brain-derived neurotrophic factor (BDNF), one of the neurotrophin family, has been shown to be expressed in human ovaries and in human plasma (16–19). Additionally, BDNF has been shown to be present in the follicular fluid of women with normal cycles and in the preovulatory follicles of women undergoing in vitro fertilization (17, 18, 20, 21). It is interesting that plasma BDNF levels decrease steadily after menopause (22), and the levels tend to be lower in women with diminished ovarian reserve (23). The relationship of BDNF to chemotherapy has not been previously reported. Because BDNF decreases with age in women, we wanted to determine whether circulating BDNF is affected by chemotherapy in premenopausal women. Therefore, we measured MIS and BDNF levels in women of reproductive age, diagnosed with cancers, before and after they underwent chemotherapy.

Premenopausal women who attended the Cancer Center from January 2008 till January 2009 were screened for eligibility. Women who agreed to participate were enrolled in a prospective study evaluating pre- and postchemotherapy MIS and BDNF plasma levels. Approval for the study was obtained from the local institutional review boards at Maimonides Medical Center and the Women and Infants Hospital of Rhode Island (site of the laboratory). Written informed consent was obtained from each participant.

Women were included if they were premenopausal and receiving chemotherapy for any malignancy. Menopause was defined as the absence of periods for 1 year in a woman with a uterus and ovaries. Patients receiving radiotherapy were excluded. To assess the changes in hormone levels attributable to the passage of time, two women who were not undergoing any treatments and were not diagnosed with cancer were enlisted as controls for each case. The controls had no fertility problems and were matched for age, ethnicity, body mass index (BMI), and smoking history. The samples from the controls were obtained at 6-month intervals (although the cases had samples attained at 3 months);

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TABLE 1

Demographics and clinical profiles of a cohort of premenopausal women undergoing chemotherapy treatment and controls.

Participants	Age (y)	Race	BMI (kg/m ²)	Menstrual cycle	Type of malignancy	Stage of cancer	Chemotherapy	Surgery	Serum BDNF levels (ng/mL)			Serum MIS levels (ng/mL)						
									Cases		Controls		Cases		Controls			
									Baseline	3 mo	Baseline	3 mo	6 mo	Baseline	3 mo	Baseline	3 mo	6 mo
Case 1	43	Black	32	Irregular	Breast	3A	Doxorubicin Paclitaxel Cyclophosphamide	Modified radical mastectomy	57	1,681				<0.10	<0.10			
Control 1A	43		32								1,505	2,475	2,717			<0.10	<0.10	<0.10
Control 1B	43		33								3,368	3,306	3,379			0.33	0.38	<0.10
Case 2	44	Caucasian	33	Irregular	Gastric	3	Cisplatin 5FU Epirubicin	None	2,568	2,077						<0.10	<0.10	
Control 2A	45		33								2,531	3,095	3,034			0.41	0.27	0.62
Control 2B	44		32								3,251	2,795	2,494			<0.10	<0.10	<0.10
Case 3	42	Caucasian	27	Irregular	Breast	2A	Docetaxel Carboplatin Trastuzumab	Radical mastectomy	5,178	5,453						<0.10	<0.10	
Control 3A	43		24								1,840	2,171	1,565			1.93	1.59	1.35
Control 3B	43		23								2,041	1,593	2,211			0.25	<0.10	0.13
Case 4	47	Caucasian	27	Regular	Breast	2A	Carboplatin Docetaxil Trastuzumab	Lumpectomy	836	860						<0.10	<0.10	
Control 4A	46		27								1,674	1,049	2,055			<0.10	<0.10	0.14
Control 4B	47		26								3,236	2,106	2,587			<0.10	<0.10	<0.10
Case 5	43	Black	33	Regular	Breast	2A	Docetaxel Carboplatin Trastuzumab	Mastectomy	1,392	218						0.37	<0.10	
Control 5A	43		35								2,106	1,514	1,520			0.22	0.2	0.2
Control 5B	43		33								2,723	1,714	1,608			0.17	<0.10	<0.10
Case 6	30	Black	28	Regular	Breast	3	Doxorubicin Cyclophosphamide Trastuzumab	Modified radical mastectomy	1,069	2,479						10.2	<0.10	
Control 6A	30		30								1,769	2,326	1,288			2.03	2.85	2.11
Control 6B	29		28								3,319	2,980	2,787			2.83	3.20	3.03
Case 7	29	Asian	21	Regular	Lymphoma	3A	Cyclophosphamide Adriamycin Vincristine Prednisolone Rituximab	None	877	540						1.34	<0.10	
Control 7A	29		24								989	907	1,256			1.61	2.49	0.84
Control 7B	29		25								1,402	940	713			4.69	5.32	5.29
Case 8	22	Caucasian	28	Regular	Breast	3A	Adriamycin Cyclophosphamide Taxol	Radical mastectomy	2,098	1,142						5.15	<0.10	
Control 8A	23		30								3,718	4,035	2,252			3.83	2.84	2.64
Control 8B	23		29								1,465	1,568	1,023			1.44	0.96	0.74

Note: BDNF = brain-derived neurotrophic factor; BMI = body mass index; MIS = müllerian-inhibiting substance.

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we assumed that in only 3 months there would not be any change in marker levels among women without any extrinsic toxic treatment like chemotherapy.

Before chemotherapy and surgical therapy (if required because of disease stage), the participants gave a blood sample and completed a questionnaire that included information regarding demographics, medical and social history and obstetric and gynecologic history. Blood samples were collected before starting chemotherapy and 3 months after finishing chemotherapy.

Blood was drawn into 7-mL Vacutainer tubes containing 0.5 mL of 3.8% sodium citrate. The blood samples were processed by centrifuge after collection, and then all samples were frozen at -80°C until they were analyzed for plasma MIS and BDNF levels. There were no differences in the pre- and postchemotherapy sample collection processes.

The MIS values were measured using an enzyme-linked immunosorbent assay (ELISA) kit (DSL-10-14400; Diagnostic Systems Laboratories, Webster, TX) according to the manufacturer's recommendations. The ELISA kit is specific for MIS and does not recognize luteinizing hormone (LH), follicle-stimulating hormone (FSH), activin, inhibin, or transforming growth factor β (TGF- β). The lower limit of sensitivity was 0.10 ng/mL, and the interassay and intra-assay coefficients of variation were $<15\%$. The samples were run in duplicate without operator knowledge of group status. Concentrations were extrapolated from the standard curve using ELISA-AID (Robert Maciel Associates, Concord, MA) software.

For BDNF, we preferred to analyze plasma rather than serum BDNF to avoid possible variations due to physiologic or pathologic alterations in platelet count, as platelets are known to be one of the major storage sites for BDNF (24). Brain-derived neurotrophic factor levels were determined using the commercially available BDNF Emax immunoassay system (Promega Corp., Madison, WI). We performed the ELISA according to the manufacturer's protocol. The BDNF assay's lower limit of sensitivity was 16 pg/mL, and the assay coefficients of variation were $<15\%$.

For BDNF, exact Mann-Whitney tests were used to analyze differences between cases and controls with regard to prechemotherapy, postchemotherapy, and change scores. Wilcoxon's signed rank test was used to assess the change over time among cases. Since MIS had such a large proportion of subthreshold results, it was decided to dichotomize these as >0.10 versus ≤ 0.10 ng/mL. Fisher's exact test was used to assess the differences between cases and controls on pre- and postchemotherapy levels, and on change over time from >0.10 to ≤ 0.10 ng/mL.

The study included eight patients with cancer and 16 control subjects. One woman had diabetes and was using diabetic medications, but no other participants had comorbidities, and the only other participant using medications was taking oral contraceptives. The age range of participants was 22 to 47 years. Among the cases, six patients had breast cancer, one had lymphoma, and one had gastric cancer. There were no other differences between the cases and controls in any of the assessed demographic factors.

Among the cases, patients 1, 2, 3, and 4 had undetectable MIS levels before chemotherapy. Baseline MIS levels in the remaining women with cancer ranged from 0.37 to 10.2 ng/mL. After chemotherapy,

the MIS levels in these patients became undetectable (<0.1 ng/mL). At baseline, the proportion of cases (50%) with detectable MIS (four out of eight participants; 75%) ($P=.363$). At follow-up evaluation, there was a significantly smaller proportion of cases (none out of eight) than controls (10 out of 16 participants; 63%) with detectable MIS levels ($P=.006$). In addition, the MIS levels changed from detectable to undetectable at follow-up evaluation in more women with cancer treatment (four out of four participants; 100%) than in controls (2 out of 12 participants; 17%) ($P=.008$).

Women with cancer and matched controls showed no statistically significant difference in BDNF levels either before therapy ($P=.093$) or at the follow-up evaluation ($P=.214$). Cases and controls also showed no statistically significant difference in terms of change score ($P=.881$), and no statistically significant change over time among cases ($P=1.000$) (Table 1).

Our study, the first to our knowledge to assess changes in plasma BDNF in a cohort of premenopausal women after chemotherapy treatment, suggests that there is no change in plasma BDNF after chemotherapy.

A negative correlation between serum BDNF and age has been shown in several studies (22, 25–28). We have recently found that the levels of BDNF in the follicular fluid of patients with decreased ovarian reserve tend to be lower (though not statistically significant) than among control women with normal ovarian reserve (23). Therefore, it was tempting to hypothesize that chemotherapy, which has been shown to affect ovarian reserve, would also adversely affect plasma BDNF levels. However, chemotherapy did not seem to affect these levels in our study.

In breast cancer patients, MIS has been shown to decline after chemotherapy (12, 13). In all our cancer patients who had detectable baseline MIS levels, the MIS levels became undetectable after chemotherapy. This finding is consistent with other studies that have reported that MIS levels are close to the detection limit after completion of chemotherapy (14). The drop to undetectable levels cannot be simply attributed to the passage of time, because the controls had no change in their levels after 6 months of follow up. Su et al. (29) showed cancer patients had significantly lower MIS levels than age-matched controls, which was consistent with the dramatic drop in MIS levels seen in our patients.

Most studies have demonstrated a decline in BDNF with advancing chronologic age in women (22, 23, 26, 27), but we did not find an effect of chemotherapy on those levels. Among women with cancer in our study, the MIS levels declined after chemotherapy, but the plasma BDNF levels were not altered. Our study was limited by a small sample size and relatively short follow-up period. It is possible that BDNF levels may become undetectable in women receiving chemotherapy in an earlier timeframe than in controls if evaluated over several years. Alternatively, serum BDNF and MIS levels likely reflect different cellular sources and/or follicle groups. Although BDNF and MIS levels do decline with reproductive aging in women, BDNF (unlike MIS) does not seem to reflect ovarian function in cancer patients receiving chemotherapy.

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