Published in final edited form as:
Fertil Steril. 2011 April ; 95(5): 1790–1793. doi:10.1016/j.fertnstert.2010.10.033.

CHANGES IN PLASMA MULLERIAN INHIBITING SUBSTANCE AND BRAIN-DERIVED NEUROTROPHIC FACTOR AFTER CHEMOTHERAPY IN PREMENOPAUSAL WOMEN

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Abstract

Eight premenopausal women with cancer had blood drawn for brain-derived neurotrophic factor (BDNF) and Mullerian Inhibiting Substance (MIS) before and three months after receiving chemotherapy. Unlike MIS, BDNF levels were not reduced following chemotherapy.

Keywords

Ovarian reserve; chemotherapy; cancer; Mullerian inhibiting substance; Anti mullerian hormone; brain-derived neurotrophic factor

Accelerated ovarian aging (1,2) and ovarian failure are common consequences of the treatment of malignant disease in women (3,4,5). At present, fertility prediction in women is imprecise. Mullerian inhibiting substance (MIS) shows particular promise in that regard (6,7). MIS is expressed in 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 (11,12,13). Decanter et al (14) showed that MIS concentrations fell dramatically just after the start of the chemotherapy and were near their detection 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 (16,17,18,19).

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CONFLICTS OF INTEREST: UMDNJ/MGH receives royalties from Beckman Coulter for licensing their MIS/AMH patent of which part is received by D. Seifer as a coinventor. UMDNJ owns a patent on BDNF and D. Seifer is the coinventor. There are no royalties for BDNF. No other authors have any conflict of interest.

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). Interestingly, 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. Since 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 undergoing 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 post- chemotherapy 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 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 one year in a woman with a uterus and ovaries. Patients receiving radiotherapy were excluded. In order to assess the changes in hormone levels attributable to the passage of time per se, two women who were not undergoing any treatments and were not diagnosed with cancer were enlisted as controls for each case. Controls had no fertility problems and were matched for age, ethnicity, body mass index (BMI) and smoking history. The samples from controls were obtained at six month intervals (although cases had samples attained at three months), since we assumed that in only three months there would not be any change in marker levels among women without any extrinsic toxic treatment like chemotherapy.

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

Blood was drawn into 7ml 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 post-chemotherapy sample collection processes.

MIS values were measured using an enzyme-linked immunosorbent assay (ELISA) kit (DSL-10-14400, Diagnostic Systems Laboratories, Webster, TX) according to manufacturer recommendations. The ELISA is specific for MIS and does not recognize LH, FSH, activin, inhibin, or TGF-β. The lower limit of sensitivity was 0.10 ng/mL and inter- and intra-assay coefficients of variation were < 15%. 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). The ELISAs were performed according to the manufacturer's protocol. The BDNF assay lower limit of sensitivity was 16 pg/mL and assay coefficients of variation were <15%.

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

The study included eight subjects with cancer and sixteen control subjects. One woman had diabetes and was using diabetic medications but no other participants had co-morbidities, and the only other participant using medications was taking oral contraceptives (OCP). The age range of participants was 22–47. 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 prior to chemotherapy. Baseline MIS levels in the remaining women with cancer ranged from 0.37 to 10.2 ng/ml. Following chemotherapy, MIS levels in these patients became undetectable (< 0.1 ng/ml). At baseline, the proportion of cases (50%) with detectable MIS (4/8 participants) did not differ from that of controls (12/16 participants; 75%) (p=0.363). At follow-up, there was a significantly smaller proportion of cases (0/8) than controls (10/16 participants; 63%) with detectable MIS (p=0.006). In addition, MIS levels changed from detectable to undetectable at follow-up in more women with cancer treatment (4/4 participants; 100%) than in controls (2/12 participants; 17%, p=0.008).

Women with cancer and matched controls did not differ significantly in BDNF levels either pre-therapy (p=0.093) or at follow-up (p=0.214). Cases and controls also did not differ significantly in terms of change score (p=0.881) and there was no significant change over time among cases (p=1.000) (table).

This 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 (22,25,26,27,28). We have recently found that levels of BDNF in 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, in our study chemotherapy did not seem to affect these levels.

MIS has been shown to decline after chemotherapy in breast cancer patients (12,13). In all our case subjects who had detectable baseline MIS levels, 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 can not be simply attributed to the passage of time, since the controls had no change in their levels after six months of follow up. Su et al (29) showed cancer patients had significantly lower MIS levels than age- matched controls consistent with the dramatic drop in MIS seen in our patients.

In conclusion, while most studies demonstrate a decline in BDNF with advancing chronological age in women (22,23,26,27), we did not find an effect of chemotherapy on those levels. Among women with cancer, while MIS levels declined after chemotherapy in

our study, plasma BDNF levels were not altered. This study was limited by a small sample size and relatively short duration of follow-up. It is possible that BDNF levels may become undetectable in women receiving chemotherapy in a earlier time frame than in controls if followed for several years. Alternatively, serum BDNF and MIS 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.

Acknowledgments

FUNDING: This project was funded by the NIH (U01-AI-31834) and the Maimonides Research Foundation.

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Table

Demographics and clinical profile of participants

Participants	Age (years)	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 m	Baseline	3m	6m	Baseline	3m	Baseline	3m	6m
Case#1	43	Black	32	Irregular	Breast	3A	Doxorubicin	Modified	57	1681				<0.10	<0.10			
Control#1A	43		32				Paclitaxel	Radical			1505	2475	2717			<0.10	<0.10	<0.10
Control#1B	43		33				Cyclophosphamide	Mastectomy			3368	3306	3379			0.33	0.38	<0.10
Case# 2	44	Caucasian	33	Irregular	Gastric	3	Cisplatinum	None	2568	2077				<0.10	<0.10			
Control#2A	45		33				5FU				2531	3095	3034			0.41	0.27	0.62
Control#2B	44		32				Epirubicin				3251	2795	2494			<0.10	<0.10	<0.10
Case# 3	42	Caucasian	27	Irregular	Breast	2A	Docetaxel	Radical	5178	5453				<0.10	<0.10			
Control#3A	43		24				Carboplatin	Mastectomy			1840	2171	1565			1.93	1.59	1.35
Control#3B	43		23				Trastuzumab				2041	1593	2211			0.25	<0.10	0.13
Case# 4	47	Caucasian	27	Regular	Breast	2A	Carboplatin	Lumpectomy	836	860				<0.10	<0.10			
Control#4A	46		27				Docetaxil				1674	1049	2055			<0.10	<0.10	0.14
Control#4B	47		26				Trastuzumab				3236	2106	2587			<0.10	<0.10	<0.10
Case# 5	43	Black	33	Regular	Breast	2A	Docetaxel	Mastectomy	1392	218				0.37	<0.10			
Control#5A	43		35				Carboplatin				2106	1514	1520			0.22	0.2	0.2
Control#5B	43		33				Trastuzumab				2723	1714	1608			0.17	<0.10	<0.10
Case# 6	30	Black	28	Regular	Breast	3	Doxorubicin	Modified	1069	2479				10.2	<0.10			
Control#6A	30		30				Cyclophosphamide	Radical			1769	2326	1288			2.03	2.85	2.11
Control#6B	29		28				Trastuzumab	Mastectomy			3319	2980	2787			2.83	3.20	3.03
Case# 7	29	Asian	21	Regular	Lymphoma	3A	Cyclophosphamide	None	877	540				1.34	<0.10			
Control#7A	29		24								989	907	1256			1.61	2.49	0.84
Control#7B	29		25				Adriamycin Vincristin Prednisolone Rituximab				1402	940	713			4.69	5.32	5.29

Participants	Age (years)	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 m	Baseline	3m	6m	Baseline	3m	Baseline	3m	6m
Case# 8	22	Caucasian	28	Regular	Breast	3A	Adriamycin	Radical	2098	1142				5.15	<0.10			
Control#8A	23		30				Cyclophosphamide	Mastectomy			3718	4035	2252			3.83	2.84	2.64
Control#8B	23		29				Taxol				1465	1568	1023			1.44	0.96	0.74