THE EFFECTS OF ADJUVANT CHEMOTHERAPY ON SERUM ADMA AND ENDOTHELIN-1 LEVELS IN EARLY STAGE BREAST CANCER PATIENTS (IZMIR ONCOLOGY GROUP (IZOG) STUDY

MURAT AKYOL[1], METE KARA[2], AHMET ALACACIOGLU[2], LEYLA DEMIR[3], HULYA ELLIDOKUZ[4], YUKSEL KUCUKZEYBEK[2], YASAR YILDIZ[2], VEDAT BAYOGLU[2], IBRAHIM YILDIZ[2], TARIK SALMAN[2], UMUT VAROL[2], BETUL KUCUKZEYBEK[5], UTKU OFLAZOGLU[2], HALIL TASKAYNATAN[2], ZEHRA GUMUS[2], MUSTAFA OKTAY TARHAN[4]

[11]Department of Medical Oncology, Manisa State Hospital, Manisa - [2]Department of Medical Oncology, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir-Turkey - [3]Department of Biochemistry, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey - [4]Institute of Oncology, Dokuz Eylul University, Izmir-Turkey - [5]Department of Pathology, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

ABSTRACT

Objective*: We aimed to investigate changes in body composition, serum asymmetric dimethyl arginine (ADMA) and endothelin-1(ET-1) levels before and after adjuvant chemotherapy in early breast cancer patients.*

Methods*: Seventy-two patients with early breast cancer who admitted to our outpatient clinic were included in the study. Before and after chemotherapy, the anthropometric measurements and bioelectrical impedance analysis with TANITA device, body compositions, serum ADMA and ET-1 levels were recorded.*

Results*: There was a statistically significant increase of serum ADMA levels (112.10±52.24 ng/ml vs. 206.34±128.02 ng/ml, p<0.001) after chemotherapy. On the other hand, the decrease of serum ET- 1 levels was not statistically significant (2.19±6.65 vs.1.68±3.71, p=0.57). ADMA levels was similar in both of the groups treated with anthracycline or anthracycline plus taxane regimens. There was no relationship between serum ADMA levels and disease stage/grade, hormone or nodal status. The changes in body composition and antropometric measurements were not statistically significant.*

Conclusion*: The increased serum levels of ADMA could be caused by the oxidative stress, apoptosis and endothelial dysfunction secondary to chemotherapy and the decreased estrogen levels. The decrease in serum ET-1 levels might be due to the inhibition of cell proliferation and angiogenesis due to adjuvant chemotherapy.*

Keywords*: ADMA, Endothelin-1, early stage breast cancer, adjuvant chemotherapy.*

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Introduction

Breast cancer is the most common malignant tumor seen in women worldwide and it is a major cause of mortality and morbidity[(1)]. Systemic adjuvant chemo- and hormone therapies are utilized to avoid micro-metastasis in early stage breast cancer patients[(2)]. Today, adjuvant chemotherapy protocols mostly include anthracycline and taxane[(3)].

Cardio-toxicity is the most important adverse effect of anthracyclines and it is the limiting factor for long-term or repetitive administration[(4,5)]. The organ culture and rabbit model studies showed that doxorubicin induces apoptosis of endothelial cells and causes endothelial dysfunction[(6,7)].

Docetaxel induced endothelial dysfunction through the PKCb/NADPH oxidase (protein kinase C beta/Nicotinamide adenine dinucleotide phosphate -oxidase) pathway was illustrated recently[(8)]. Another important factor for endothelial dysfunction is PKC (protein kinase C) activation during oxidative stress[(9)].

Endothelial Nitric oxide synthase (NOS) function in human endothelial cells decreases due to the up-regulation of PKC activity[10,11]. An in vivo study demonstrated that paclitaxel impairs endothelial function, but endothelial function deterioration is not related to the serum levels of inflammation markers[12].

Endothelins (ETs) are a family of genes including endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3)[13]. ET-1 is the predominant isoform, while ET-2 and ET-3 are less commonly expressed[13]. ET-1 is produced by somatic cells, including many epithelial tumors as well as healthy endothelial cells and vascular smooth muscle cells. ETs are both paracrine and autocrine proteins and bind to specific cell surface receptors[14]. The interaction of ETs and ET receptors mediates a number of functions, including vasoconstriction, pain, inflammation and most importantly, cancer[13,15].

ADMA, a methylated arginine derivative, is a comparative and specific endogenous inhibitor of nitric oxide synthase (NOSi)[16]. Cytosolic dimethyl arginine dimethyl amino hydrolase (DDAH) is responsible from the elimination of ADMA. Regulation of NO production may affect the regulation of angiogenesis as well as tumor progression. In human breast cancer, the positive correlation between NOS activity and tumor grade has been shown[17,18,19]. ADMA can regulate the concentration of nitric oxide and can affect VEGF production. Therefore ADMA plays an important role in breast cancer growth and angiogenesis[20]. Also, there are strong evidences for that ADMA is one of the most important factors for endothelial dysfunction[16].

In breast cancer prognosis, cardiovascular disease (CVD) mortality was found as common as primary cancer mortality[21]. Endothelial dysfunction, the main pathologic finding of CVD, is a result of increased oxidative stress as well as CVD risk factors[22]. The increases in cardiovascular mortality and endothelial dysfunction cannot be explained only with the most common CVD risk factors such as smoking, hyperlipidemia or hypertension. The deposition of vasoactive peptides like ET-1 and ADMA, can also cause to endothelial dysfunction[22,23].

Another CVD risk factor is obesity. In spite of improved survival with adjuvant combination chemotherapy, weight gain and unfavorable changes in body compositions are reported to be related with treatment. The previous studies suggested that 50-96% of early stage breast cancer patients gained significant weight during treatment with adjuvant chemotherapy, ranging from 2.5-6.2 kg[24,25]. Weight gain amount in response to chemotherapy is considered dependent on the chemotherapeutic agents used[26].

There are limited information and opposite results about the effects of adjuvant chemotherapy on body composition, plasma ADMA and ET-1 levels in breast cancer patients. Therefore, we aimed to investigate changes in body composition, serum ADMA and ET-1 levels before and after adjuvant chemotherapy in non-metastatic breast cancer patients.

Materials and methods

Patients Selection

After the approval of our local ethics committee, we prospectively assessed female early breast cancer participants, who admitted to our medical oncology clinic between 2012 and 2014. The patients, who were informed and gave a written consent form, were enrolled. The patients above 18-year old and with non-metastatic breast cancer were included. We excluded male patients, the patients receiving antihyperlipidemic and antihypertensive agents, and the patients with a history of diabetes mellitus, cardiovascular disease, hypo- or hyperthyroidism.

Anthropometry and Bioelectric Impedance Analysis

The body mass index (BMI) of the individuals were calculated with the formula as kg/m^2. The anthropometric measurements and the bioelectric impedance analysis with TANITA device was performed before and after chemotherapy. Body composition was determined as total body water (TBW), fat free mass (FFM), fat mass (FM) and the percentage of body fat. These variables were measured by bioelectrical impedance analysis using TANITA TBF-300A scale. The waist circumference was measured half-way between the costal arc and the iliac crest during a normal respiratory position. The hip circumference was measured over the widest part of the gluteal region, and the waist-to-hip circumference ratio (WHR) was also recorded. One nurse performed the measurements for all patients.

The serum levels of ADMA and ET-1

We took venous blood samples from the par-

ticipants between 8 and 9 a.m. after a fasting for 8-12 hours. The blood samples were stored in a tube with ethylene diamine tetraacetic acid (EDTA) and in another tube with sodium citrate. After 30 minutes, the blood samples were centrifuged for 10 minutes at 2000 g. Serum samples were put into Eppendorf tubes and were stored in -20˚C. After the blood samples were dissolved at room temperature, a vortex was used to make the sample homogenous. Serum ADMA and ET-1 levels were assessed with enzyme-linked immunosorbent assay (ELISA) kits. Serum ET-1 and ADMA levels were measured with Cusabio branded ELISA kits (Cusabio Biotech Co., Ltd. P. R. China). The ELISA kits for both of them, have coefficient of variation (CV) values less than 8% for intra-assay and less than 10% for inter-assay.

Statistical Analysis

The statistical analysis was performed with SPSS V.20. Paired- samples T test was run to detect the changes in serum levels of ADMA, ET-1, BMI, weight, FFM, FM, TBW and anthropometric measures before and after chemotherapy. A correlation analysis was performed to evaluate the correlation between serum levels of ADMA and ET-1 before and after chemotherapy, the monthly changes in BMI, weight, FFM, FM, TBW and anthropometric measures. To compare the effects of the same variables above in anthracycline and anthracycline+ taxanes chemotherapy regimens, Mann-Whitney U test was used. The relationship between disease stage, grade, serum ADMA and ET-1 was assessed with Kruskal-Wallis test. Statistical significance was set at p value <0.05 for all the statistics tests.

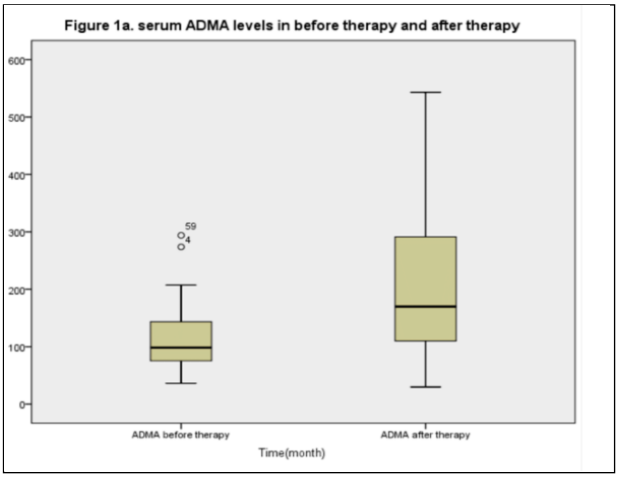
Results

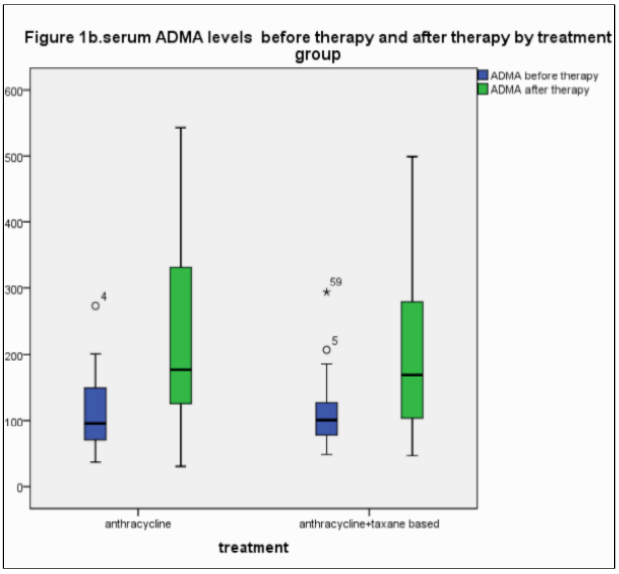
Totally, 72 patients were enrolled in the study. The demographic features of the participants were summarized in table 1. The mean age was 53.24 (±11.56). Among these patients, 17 patients (23.6%) had stage 1, 32 (44.4%) had stage 2 and 23 (31.9%) had stage 3 disease. While 29 patients (40.3%) were treated with anthracycline based chemotherapy, 43 patients (59.7%) had the anthracycline + taxanes regimen. Basal serum levels of ADMA and ET-1 were found respectively 112.10±52.24 ng/ml and 2.19±6.65 pg/ml. The mean basal values of weight, BMI and WHR were in order as follows: 74.90± 13.78 kg, 30.09± 6.19 kg/m^2 and 0.92±0.08.

Characteristics	Patient group (n=72) n (%)
Age, years (mean± SD) smoker	53.24±11.56
yes	6(8.3%)
no	60(83.3%)
Former smoker	6(8.3%)
Alcohol use	
yes	4(5.6%)
no	68(94.4%)
Surgical treatment	
Modified radical mastectomy	38(52.8%)
Simple mastectomy	5(6.9%)
Breast conserving surgery	29(40.3%)
Disease stage (TNM)	
I	17(23.6%)
II	32(44.4%)
III	23(31.9%)
Hormone receptor status	
ER/PR Positive	48(66.7%)
ER/PR negative	10(13.9%)
ER positive PR negative	11(15.3%)
ER negative PR Positive	3(4.2%)
Cerb-B2 status	
positive	17(23.6%)
negative	55(76.4%)
Chemotherapy	
Anthracycline based	29(40.3%)
Anthracycline+ taxane based	43(59.7%)
Histologic grade	
Grade I	5(40.3%)
Grade II	43(59.7%)
Grade III	24(33.3%)

Table 1: Demographic characteristics of early breast cancer patients.

We found that after adjuvant chemotherapy, there was a statistically significant increase of serum ADMA levels (112.10±52.24 ng/ml vs. 206.34±128.02 ng/ml, p<0.001) (Figure 1a). On the other hand, the decrease of serum ET- 1 levels was not statistically significant (2.19±6.65 vs.1.68±3.71, p=0.57) (table 2).





	Before therapy mean±SD	After therapy mean±SD	p value
Weight (kg)	74.9±13.78	75.17±13.06	0.57
BMI (kg/m²)	30.09±6.19	30.22±5.65	0.5
Waist-to-hip ratio	0.921±0.08	0.923±0.07	0.65
Fat mass (kg)	27.78±9.87	27.39±9.43	0.41
Percent body fat (%)	36.06±7.18	35.41±7.35	0.18
Fat free mass (kg)	47.02±5.46	47.76±5.08	0.054
Fat free mass/fat mass (kg)	1.9±0.71	1.99±0.93	0.19
Total body water(kg)	34.27±4.05	34.17±4.06	0.77
ADMA	112.1±52.24	206.34±128.02	**p<0.001**
Endothelin-1	2.19±6.65	1.68±3.71	0.57

Table 2: Serum ADMA and endothelin, body composition and anthropometric measurements at baseline and after adjuvant chemotherapy in early breast cancer patients.

BMI: Body mass index ADMA: asymmetric dimethyl arginine

ADMA levels was similar in both of the groups treated with anthracycline or anthracycline plus taxane regimens (Figure 1b). The increases of BMI, FFM, weight, WHR, waist and hip circumferences were not statistically significant. Similarly, FM and TBW decreased after chemotherapy, but it was not statistically significant (table 2). There was no relationship between serum ADMA levels and disease stage/grade, hormone or nodal status. Although baseline ADMA levels were correlated with WHR (p=0.025), this correlation was not observed after chemotherapy. No correlation was found between serum ADMA levels and BMI, FFM, FM, TBW and weight. The correlation between basal ET-1 levels and TBW was not observed after chemotherapy (table 3).

Discussion

In this study we found that after adjuvant chemotherapy, there was a statistically significant increase in serum ADMA levels. On the other hand, the decrease of serum ET- 1 levels was not statistically significant. The increase of serum ADMA levels were similar in both of the groups including anthracycline and anthracycline + taxanes.

Several studies suggested that ADMA can cause to endothelial dysfunction. The diseases characterized by endothelial dysfunction such as congestive heart failure, diabetes mellitus type 2, insulin resistance, hypertension, hyperhomocysteinemia and the end stage renal disease are also characterized by increased ADMA levels[26]. In addition, oxidative stress production increases after treatment with anti-neoplastic agents[27] and increased oxidative stress may increase ADMA level which is probably due to the decrease in DDAH enzyme activity[28]. Contrary to this situation, estrogens can negatively affect ADMA release by altering catabolism. As a result, postmenopausal endothelial dysfunction may be due to estrogen deficiency[29,30].

The study among colon cancer patients demonstrated that serum ADMA levels were higher than healthy participants[31]. ADMA is mainly generated via a metabolic process regulated by DDAH. Enhanced expression of DDAH1 has been reported to promote tumor growth in vivo, accompanied by decreased ADMA levels and increased NO synthesis[32]. However, the exact role of ADMA in tumor development is still unknown.

Variables	Basal ADMA		ADMA after chemotherapy		Basal Endothelin-1		Endothelin-1 after chemotherapy	
	R	P	R	P	R	P	R	P
Basal ADMA	1		**0.509****	**<0.001**	0.025	0.832	0.07	0.955
ADMA after chemotherapy	0.509**	**<0.001**	1		0.034	0.775	**0.315****	**0.007**
Basal Endothelin-1	0.025	0.832	0.034	0.775	1		-0.015	0.903
Endothelin-1 after chemotherapy	0.07	0.955	**0.315****	**0.007**	-0.015	0.903	1	
Weight (kg) basal	0.142	0.234	0.125	0.297	0.069	0.532	0.034	0.78
Weight (kg) after chemotherapy	0.167	0.162	0.113	0.343	0.05	0.664	0.084	0.481
BMI (kg/m^2) basal	0.091	0.447	0.015	0.901	0.084	0.483	-0.045	0.71
BMI (kg/m^2) after chemotherapy	0.114	0.341	-0.001	0.994	0.072	0.55	-0.009	0.94
Waist-to-hip ratio basal	**0.264 ***	**0.025**	0.193	0.104	0.099	0.406	0.006	0.959
Waist-to-hip ratio after chemotherapy	0.224	0.059	0.174	0.144	0.088	0.461	-0.001	0.991
Basal Fat mass (kg)	0.142	0.233	0.087	0.466	-0.091	0.466	0.014	0.907
Fat mass (kg) after chemotherapy	0.189	0.111	0.131	0.271	0.035	0.77	0.064	0.594
Basal Fat free mass (kg)	0.106	0.378	0.161	0.176	**0.341****	**0.003**	0.063	0.06
Fat free mass (kg) after chemotherapy	0.079	0.511	0.047	0.695	0.066	0.579	0.098	0.411
Basal Percent body fat (%)	0.134	0.261	0.062	0.603	-0.203	0.087	0.007	0.953
Percent body fat (%) after chemotherapy	0.203	0.087	0.166	0.163	0.028	0.813	0.048	0.692
Basal Total body water (kg)	0.095	0.426	0.16	0.179	**0.346****	**0.003**	0.039	0.744
Total body water (kg) after chemotherapy	-0.009	0.939	-0.002	0.985	0.048	0.687	0.072	0.55

Table 3: Correlations of Variables According to Pearson's Test in the Patient Group.

In human ovarian, prostate, colorectal and breast cancers, the increased secretion of ET-1 has been demonstrated. Also, in another study, compared to normal breast tissue, breast cancer patients had higher expression of ET-1[33]. Moreover, ETs may play a role in tumor recurrence and development of resistance to anti-cancer drugs[34].

There are a limited number of studies about the effects of chemotherapy on serum ADMA and ET-1 levels. A previous study demonstrated that after adjuvant treatment with taxanes, serum ADMA and ET-1 levels decreased in breast cancer patients[35]. On the other hand, another study showed that there was no change in serum ADMA levels among colon cancer patients after chemotherapy with bevacizumab[36].

In this study, after adjuvant chemotherapy, serum ADMA levels were statistically significantly increased, while there was no change in body composition. The increase in ADMA levels can be as a result of chemotherapy effects (i.e. estrogen decrease, oxidative stress and endothelial dysfunction). Although the increase of ADMA levels has been demonstrated in many studies, the effects of ADMA on prognosis and tumor progression in breast cancer are still unclear. After adjuvant chemotherapy, there was a decrease in serum ET-1 levels but it was not statistically significant. Adjuvant chemotherapy may prevent breast cancer recurrence and metastasis by decreasing ET-1 levels, which plays a role in carcinogenesis, metastasis, tumor invasion and angiogenesis.

Endothelial dysfunction in obesity-related metabolic syndrome is an evident as a failure of vasodilatation after exposure to endothelium-dependent vasodilators. However, this phenomenon may reflect the impaired NO bioavailability as well as excess vasoconstrictor tone[37]. The development of endothelial dysfunction in obesity may be due to increased concentrations of ADMA[38].

A previous study showed that WHR is probably a better estimator than BMI for endothelial dysfunction and cardiovascular risk assessment[39]. In this study, while there was a positive correlation between ADMA levels and WHR, no correlation was found with BMI. As a result, WHR may be a better predictor for endothelial dysfunction than BMI.

After breast cancer diagnosis, patients may gain weight because of decreased physical activity[40], increased food intake and modification of basal metabolic activity[41]. Food intake usually increases due to anxiety and to alleviate chemotherapy-related nausea and emesis[42,43]. Other than the reasons above, premenopausal status[41,42] and the use of corticosteroids containing chemotherapy protocols[41] are also risk factors for weight gain. Although the exact reason for gaining weight is still unclear, adjuvant chemotherapy seems to be an important factor[44]. Adjuvant chemotherapy has been found to be a strong clinical predictor of weight gain in women with early-stage breast cancer, which is independent of age, nodal status, BMI and reported calorie intake at diagnosis[45]. The significance of weight gain during chemotherapy is highlighted by its negative impact on survival: increased rates of recurrence and death have been found to be associated with weight gain[46,47]. Furthermore, weight gain in breast cancer patients tend to be accompanied by changes in body composition, with increases primarily in fat mass rather than lean body mass[40,48].

Many studies reported that the new chemotherapy regimens with shorter treatment schedules have been associated with diminished incidence of weight gain[49]. After CMF chemotherapy protocols (cyclophosphamide, methotrexate and 5-fluorouracil), significant gains in both body weight and fat mass among female patients were reported[26]. Unlikely, some studies including doxorubicin and cyclophosphamide predominantly did not find any weight gain, but demonstrated loss of lean body mass that resulted in an increased percentage of body fat[50].

In our study, despite of the increases in BMI, FFM, weight, WHR, waist and hip circumferences in breast cancer patients after adjuvant chemotherapy, we did no find any statistical significance when we compared before and after treatment. The changes of weight and body composition in our patients may be derived from diet habits, exercise, chemotherapy agents and treatment periods.

The relationship between cardiovascular mortality and, serum ADMA and ET-1 levels of our breast cancer patients which were treated with adjuvant chemotherapy could not be evaluated because of the short follow-up duration which may be an importatnt limitations of this study.

In conclusion, after adjuvant chemotherapy, a statistically significantly increase in serum ADMA levels was reported independently of body compositions. This increase might be due to endothelial dysfunction caused by chemotherapeutic agents or decreased estrogen levels and increased oxidative stress after chemotherapy. Serum ET-1 levels were decreased depending on adjuvant chemotherapy, but it was not a statistically significant. This could be as a result of inhibitor effect of chemotherapy on tumor proliferation and angiogenesis. Since there are controversial interpretations about the effects of increased ADMA levels on breast cancer progress and disease prognosis, further studies will enlighten this issue.

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Corresponding author
AHMET ALACACIOGLU, MD
Izmir Katip Celebi University
Ataturk Training and Research Hospital
Medical Oncology Clinic
35360 Izmir
(Turkey)