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Meme Kanseri Olan Hastalarda Serum Homoarjinin ve İskemi Modifiye Albumin Düzeyleri [Serum Homoarginine and Ischemia Modified Albumin Levels in Patients with Breast Cancer]

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Öz

Amaç: Homoarjinin ve iskemi modifiye albumin üzerinde çalışmaların yoğun olarak devam ettiği moleküllerdendir. Meme kanseri hastalarında homoarjinin, ve iskemi modifiye albumin seviyeleri hastalıkların patogenezinin anlaşılması, bu molekülleri tedavi ile ilişkilendirmek, yeni tedavi protokollerinin geliştirilmesi ve hatta hastalıklar oluşmadan sağlıklı kişilerin risk faktörlerinin elimine edilmesi açısından son derece önemlidir.
Bu çalışmada amacımız, meme kanserli hastaların serumlarında homoarjinin ve iskemi modifiye albumin düzeyleri ve hastalıkla ilişkisinin saptanmasıdır.
Gereç ve Yöntemler: Çalışmaya 27 kontrol, 27 meme kanseri olan birey dahil edilmiştir. Serum metilarjinin ve homoarjinin düzeyleri kütle spektrometrik, iskemi modifiye albümin düzeyleri kolorimetrik yöntemle Selçuk Üniversitesi Tıp Fakültesi Biyokimya Laboratuvarında çalışılmıştır.
Bulgular: Kontrol grubu meme kanseri grubu ile karşılaştırıldığında L-monometilarjinin, Total Metil Arjinin, Homoarjinin seviyeleri istatistiksel olarak düşerken, asimetrik dimetilarjinin ve iskemi modifiye albümin seviyeleri istatistiksel olarak artış göstermiştir ($p<0.05$).
Sonuç: Artan iskemi modifiye albümin seviyeleri meme kanserinde ortaya çıkan oksidatif stresi gösteriyor olabilir. Asimetrik dimetilarjinin düzeylerindeki artış azalmış nitrik oksit sentezi ve vasküler yapının bozulmasına yol açabilir.
Anahtar Kelimeler: Meme kanseri; Homoarjinin; İskemi modifiye albümin; Kütle spektrometre

Abstract

Objectives: Homoarginine and ischemia modified albumin are among the molecules that are intensely studied on. Homarginine and ischemia-modified albumin levels in breast cancer patients are extremely important in understanding the pathogenesis of diseases, associating these molecules with treatment, developing new treatment protocols, and even eliminating risk factors in healthy people before diseases occur.
Our aim in this study is to determine homoarginin and ischemia-modified albumin levels in the serum of breast cancer patients and its relation with the disease.
Material and Methods: 27 controls, 27 breast cancer were included in the study. Serum methylarginine and homoarginine levels were studied by mass spectrometric method, and ischemia modified albumin levels by colorimetric method in Selcuk University Faculty of Medicine Biochemistry Laboratory.
Results: While the levels of L-monomethylarginine, Total Methyl Arginine, Homoarginine statistically decreased, asymmetric dimethylarginine and ischemia modified albumin levels significantly increased in patients compared to controls ($p <0.05$).
Conclusions: Increased ischemia modified albumin levels may indicate the oxidative stress that occurs in breast cancer. Increase in asymmetric dimethylarginine levels can lead to decreased nitric oxide synthesis and deterioration of the vascular structure.
Keywords: Breast cancer; Homoarginine; Ischemia modified albumin; Mass spectrometry

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INTRODUCTION

Ischemia Modified Albumin (IMA)is frequently used among cardiac markers and free radicals formed during ischemia cause chemical changes in albumin. This new albumin molecule is also called ischemia-modified albumin and is measured spectrophotometrically by the binding capacity of albumin to cobalt. Increased levels have been found in cardiovascular diseases. IMA serum was first used by Bar-Or et al. Although shown by Sinha et al. It was found to be increased in chest pain and high sensitivity [1].

Tissue damage, ischemia-reperfusion injury, infection, and reactive oxygen derivatives released from the tissue increase the formation of IMA by causing a difference in the albumin structure. Under normal circumstances, IMA is about 1-2% of albumin. It has been observed that serum IMA levels increase with different clinical pictures associated with oxidative stress such as cerebrovascular ischemia, acute mesenteric ischemia, asphyxia, hypoxia, and diabetes[2]. Due to its relatively easy working principle and low cost, the relationship between IMA and many acute and chronic diseases has been frequently questioned in many studies. Ischemia Modified Albumin was found to be significantly higher in patients with severe preeclampsia, with a sensitivity of 80% showing a specificity of 77%[3].

L-Homoarginine ($C_7H_{16}N_4O_2$) is a basic, impure cationic amino acid containing an excess of CH_2 group, which is a longer carbon chain than L-arginine, homolog to L-arginine. L-arginine from L-lysine is assumed to be formed by transamination by the enzyme glycine-amidinotransferase (AGAT, EC 2.1.4.1)[4]. Given its structural similarity to L-arginine, it can interact with the metabolism of L-homoarginine and arginine and interrupt signaling [5]. L-Homoarginine is a substrate for nitric oxide synthase (NOS) that produces nitric oxide (NO) from L-arginine and acts as a competitive inhibitor of NOS. Therefore, competing of L-homoarginine with L-arginine for binding to the active site of the NOS can affect endothelial function. However, compared to arginine, the K_m value of homoarginine is higher as it reflects a lower catalytic efficiency of NOS using homoarginine as substrate. Therefore, NO production can be reduced at a high homarginine / arginine ratio. It remains unclear whether high or low levels of L-homoarginin are beneficial in relation to cardiovascular disease. Recently, low levels of L-homoarginin in plasma have been associated with cardiovascular mortality and stroke in a large cohort study. In contrast, many studies have reported that high rather than low levels of homarginine are associated with ADMA plasma concentrations and cardiovascular disease[6]. L-homoarginine is a non-proteinogenic amino acid, structurally related to L-arginine, with the potential to interfere with L-arginine / NO metabolism in vivo. Its molecular weight is 188.228 g / mol. L-homoarginin has been detected in small amounts in all body fluids and organs examined (eg serum, urine, cerebrospinal fluid, liver, kidney, and brain), but its function has not yet been clearly elucidated [7].

In this study, we aim to determine whether there is any change in homocysteine and ischemia-modified albumin levels in breast cancer patients and explain the relationship of these proteins with cancer, since we think that this information may be determinant in cancer patients.

MATERIALS AND METHODS

Twenty seven controls, 27 breast cancer patients were included in the study. IMA levels are taken into a 200 µl sample tube and 200 µl into a blank tube. Put 50 µl of 1g / L cobalt-2-chloride in both the sample tube and the blank tube. The prepared mixture was mixed with vortex and left to incubate for 10 minutes at room temperature. After 10 minutes, just add 50 µl of 1.5 g / L dithiothretiol to the sample tube. To observe the color change, it is left at room temperature for 2 minutes. After 2 minutes, put 1 mL of Serum Physiological (0.9% NaCl) into both sample and blank tubes. Absorbances are measured at 470 nm in the spectrophotometer. Sample and blank tube results are recorded separately. Results are recorded as Absorbance Unit (ABSU)[1].

Levels of homoarginin were analyzed in Shimadzu LC-20AD system MDS SCIEX (USA) API 3200 mass spectrometer device. Serums stocked in eppendorf tubes at -80 ° C were kept at room temperature to dissolve. Dissolved serums were vortexed for 3-5 seconds to ensure homogeneity. After the internal standard (d7-ADMA) dissolved in 100 µL methanol was added to the 200 µL serum sample, the precipitated proteins were removed by centrifugation at 13000 rpm for 10 minutes. The supernatant was taken into a clean tube and evaporated under nitrogen gas at 60 0C. The derivatization process. Freshly prepared 200 µL of 5% (v v − 1) butanol / acetyl chloride solution was added and kept at 60 ° C for 20 minutes. The

solvent was evaporated again at 60 ° C under nitrogen gas. Thawing process 100 µL water-methanol (90:10, v v − 1) containing 0.1% (v v − 1) formic acid. was carried out with. 40 µL is injected into the analytical column[6]. Statistical analyzes were performed using Microsoft Excel and SPSS 16 computer programs. The distribution of the data was examined with the Kolmogrov Smirnov test, and comparisons were made using the Mann Whitney U test for non-parametric distribution in both groups, and Student t test if it was parametric.

RESULTS

L-NMMA, Total Methyl Arginines, HomoArgine levels decreased statistically, while IMA levels increased statistically when compared to the control group breast cancer group (Table 1).

Table 1. Levels of serum methylated arginine and IMA levels. ABSU: Absorbance unit. Results were expressed as mean±SD

	Controls (n=27)	Breast Cancer (n=27)
ADMA (µmol/L)	0.69±0.20	0.60±0.18
SDMA (µmol/L)	0.75±0.18	0.74±0.24
L-NMMA (µmol/L)	0.08±0.02	0.07±0.02
Homoarginine (µmol/L)	3.62±1.07	3.19±0.77
Total methylated arginine (µmol/L)	1.52±0.30	1.41±0.34
IMA (ABSU)	0.44±0.13	0.57±0.15

DISCUSSION

Today, breast cancer is the most common cancer among women and the second in female cancer deaths. Breast cancer is more common in women who have a family history of breast cancer, advanced age, had a prolonged reproductive stage, and women who have not had a child. Similarly, having the first child at an advanced age, obesity, use of exogenous estrogen and oral contraceptives, and presence of cancer in the other breast increase the possibility of breast cancer. There are mutations in the suppressors BRCA1 and BRCA2 genes responsible for transcriptional regulation in familial cancers [8]. In addition, endogenous estrogen excess is thought to play a significant role [9]. In particular, obesity is a risk factor for breast cancer in postmenopausal women [10,11]. Obesity-related hormonal changes are thought to be a side effect, particularly due to an increase in estrogen production [12]. High endogenous estrogen levels have been shown to increase the progression of postmenopausal carcinoma in the chest6. Production and estrogen of androstenodione increase in obese women, and postmenaposal plasma estrogen concentration increases. The obesity-related increase in circulating estrogens may be associated with an increased risk of breast cancer and increases the progression of estrogen receptor positive breast cancers [13].

Methylarginines are formed by the methylation of arginine residues found in proteins. These proteins are commonly found in the nucleus. Protein-arginine methylation is a post-translational modification that transfers 1 or 2 methyl groups to the guanido nitrogen of arginine in proteins. In humans, it is performed by PRMTs (Protein Arginine Methyl Transferase). The product formed as a result of Type 1 PRMT activity is Asymmetric Dimethylarginine and N-Monomethyl L-Arginine. These molecules have the ability to inhibit Nitric oxide synthase (NOS). In the synthesis step, S-adenosyl methionine (SAM) is used as the methyl group donor. Type 2 PRMT plays a role in the formation of SDMA (symmetrical dimethylarginine). SDMA does not inhibit NOS. If SDMA is injected intravenously, it will urinate at 60%, but the rate of ADMA to urinate in this way is 5%. Therefore, in renal failure, SDMA is found at much higher levels in the circulation than ADMA. Methyl arginines are metabolized by the methyl transferase enzyme of dimethylarginine in the kidney and acetylation in the liver [14].

A defect in any of the steps in ADMA metabolism affects the serum level. The main causes are increased methylation of proteins with increased PRMT enzyme activity, increased degradation of methylated proteins, decreased excretion as a result of renal failure, and decreased DDAH enzyme activity [15,16].

NO secreted by the endothelium has protective effects on vascular structure and functions. Among these effects, it prevents smooth muscle proliferation, leukocyte adhesion and platelet aggregation. ADMA selectively inhibits the synthesis of endogenous anti-atherogenic molecule (NO) with these important functions, and shows its pathophysiological effects by preventing the vascular system from benefiting from the protective effects of NO [17].

L-NMMA along with ADMA is an endogenous inhibitor of NOS. Vascular mediators released from the endothelium play an important role in maintaining vascular tone and structure, and one of

the most important mediators is Nitric Oxide. Nitric oxide is synthesized by isoforms of the NOS enzyme found in endothelial, neuronal and macrophages. Arginine and ADMA play important roles in the control of nitric oxide synthesis. Nitric oxide plays a role in platelet adhesion and aggregation in the regulation of vascular tone. SDMA does not have an inactivating effect on the NOS enzyme, but has an indirect effect on the rate of NO production by affecting the cell entry pathway with arginine and ADMA. ADMA, SDMA and L-NMMA enter endothelial cells through cationic amino acid transporters called the Y carrier protein. Methylarginines compete with each other and with the amino acid arginine for entry into the cell. High concentrations of ADMA reduce NO synthesis by inhibiting L-Arginine transport into the cell as well as NOS inhibition [18].
ADMA has been one of the molecules that have been frequently researched recently because it inhibits a molecule such as NO, which has many regulatory effects on the cardiovascular system. When the literature is reviewed, it is seen that the activities of enzymes that play a role in ADMA synthesis and degradation change in various diseases. Some diseases increase the ADMA levels by increasing PRMT1 activity and increasing its synthesis; some of them appear to be caused by reducing the destruction of ADMA. The number of studies showing the close relationship between oxidant / antioxidant system and ADMA is increasing. There is evidence that oxidative stress acts by altering enzyme activities in both the production and degradation of ADMA. Answers are sought for the question of "can it be a marker" in cancer diseases, especially cardiovascular diseases. However, the fact that it causes endothelial dysfunction and that endothelial dysfunction is important in the pathogenesis of many diseases indicate that research on ADMA will continue in the near future [17].
It is known that the arginine / ADMA ratio reflects the NO bioavailability [19]. It is generally mentioned that three conditions are important in increasing plasma ADMA levels. 1) Increased ADMA synesthesia 2) Decrease in kidney excretion 3) Decreased enzymatic hydrolysis of ADMA. It has been shown that PRMT 1 and DDAH enzymes that hydrolyze ADMA are regulated in a sensitive manner to redox balance [19].
Until now, SDMA was thought to have a minor role in NO physiology and was mostly thought to be an indicator of kidney function, but recent studies show that SDMA inhibits NO synthesis and increases the production of reactive oxygen species in endothelial cells in a dose-dependent manner. Some studies have reported that the Arginine / methylarginine ratio is associated with survival and improves with survival [20].
In a previous study, it was reported that ADMA levels may increase with increased protein catabolism in hematological malignancies [21].
In our study, the increase in ADMA levels with treatment in the cancer group is striking. In addition, it has been reported that ADMA levels may affect vascular endothelial growth factor levels as well as regulating nitric oxide concentrations. In addition, ADMA has an important role in breast cancer growth and angiogenesis. Again, Akyol et al. In his study, an increase in ADMA levels was reported after cancer chemotherapy intake, that is, after treatment. They attributed this to the ability of chemotherapeutics to increase oxidative stress, apoptosis, and endothelial dysfunction [22]. In addition, the formation of both NO and arginine analogs by protein methylation and proteolysis are intracellular processes. Circulating ADMA and SDMA originate from the cells destruction cycle [23].
In conclusion, we think that these biomarkers may be useful in monitoring diagnosis and treatment for breast cancer patients. We aim to repeat this study with more patient groups.

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Ethical approval: The local Institutional Review Board deemed the study exempt from review.

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