MCBU SBED
MANİSA CELAL BAYAR ÜNİVERSİTESİ SAĞLIK BİLİMLERİ ENSTİTÜSÜ DERGİSİ
MANISA CELAL BAYAR UNIVERSITY JOURNAL OF INSTITUTE OF HEALTH SCIENCE
ISSN: 2147-9607

ARAŞTIRMA MAKALESİ
RESEARCH ARTICLE
CBU-SBED, 2022, 9(1): 58-62

Meme Kanseri Olan Hastalarda Serum Homosistein Düzeylerinin Araştırılması

Investigation of Serum Homocystein Levels in Patients with Breast Cancer

Funda Kosova[1*], Sedat Abuşoğlu[2], Özgü Kemal Beksaç[3], Ali Ünlü[2], Bahadır Çetin[3], Gülsüm Abuşoğlu[4]

[1*] Celal Bayar Unv Health Services Vocational School, Biochemistry, Manisa, Türkiye.
[2]Selçuk Unv. Medical faculty of departman of Biochemistry Biyokimya Abd, Konya, Türkiye.
[3]Dr. Abdurrahman Yurtaslan Oncology Hospital, General Surgery, Ankara, Türkiye.
[4]Selçuk Unv. Health Services Vocational School, Konya, Türkiye.

e-mail: fundakosova@gmail.com, sedatabusoglu@yahoo.com, kemalbeksac@yahoo.com, aliunlu@gmail.com, drbahadircetin@gmail.com, tekinglsm@gmail.com
ORCID: 0000-0001-8070-5067
ORCID: 0000-0002-2984-0527
ORCİD: 0000-0002-6339-505X
ORCİD: 0000-0002-6090-9422
ORCİD: 0000-0002-6967-4543
ORCİD: 0000-0003-1630-1257
*Sorumlu Yazar / Corresponding Author: Funda Kosova

Gönderim Tarihi / Received: 12.08.2021
Kabul Tarihi / Accepted: 06.01.2022
DOI: 10.34087/cbusbed.956450

Öz

Giriş ve Amaç: Hastalıkların tanısında kullanabilmek amacıyla birçok molekül üzerinde bilimsel çalışmalar devam etmektedir. Bu moleküllerden bir tanesinin bile tanı amacıyla kullanılabilmesi, klinisyenler açısından hastalığın tanısının konmasında oldukça önemlidir. Homosistein ve arjinin de bu doğrultuda üzerinde çalışmaların yoğun olarak devam ettiği moleküllerdendir. Bu nedenlerle biz de, bu çalışmamızda meme hastalarının kan örneklerinin serumlarında homosistein ve arjinin düzeylerinde herhangi bir değişiklik olup olmadığını belirleyebilmeyi ve bunun meme kanseri ile ilişkisini açıklayabilmeyi amaçlamaktayız.
Gereç ve Yöntemler: Selçuk Üniversitesi Tıp Fakültesi Biyokimya Metabolizma Laboratuvarında çalışmalarımız gerçekleştirilmiştir. Çalışmamıza 20 kontrol, 20 meme Ca, 20 metastaz hastası dahil edildi. Meme kanseri hastalarından alınan kan, biyokimyasal analiz yapılana kadar -80 ° C'de saklanmıştır. Alınan kan örneklerinden elde edilen serumlardan homosistein ve arginin seviyeleri ölçülmüştür Spektrofotometrede 470 nm'de protein seviyeleri ölçülmüştür. Homosistein seviyelerini ölçmek için NMRkütle spektrometresi kullanılmıştır.
Bulgular: Yapılan değerlendirmede Kontrol grubuna göre meme Ca 'li hastalarda Arginin, Arginin/ADMA, metastazlı hastalarda ise Homosistein seviyelerinde istatistiksel bir artış gözlenmiştir. Meme kanserli hata grubu, metastaz grubu ile karşılaştırıldığında ise, Homosistein seviyesi istatiksel olarak artarken, Arginin /ADMA seviyesi istatistiksel olarak azalmıştır.
Sonuç: Elde ettiğimiz sonuçlara dayanarak, meme kanseri hastalarının tanı ve tedavileri sırasında bu markerlerın kullanılabileceği düşünülmektedir.

Anahtar Kelimeler: Arginin, Homosistein, Meme kanseri, Sitrülin.

Abstract

Objective: Scientific studies are continuing on many molecules in order to be used in the diagnosis of diseases. The fact that even one of these molecules can be used for diagnosis is very important for clinicians in diagnosing the disease. For this purpose, intensive studies continue on homocysteine and arginine.

For these reasons, in this study, we aim to determine whether there is any change in homocysteine and arginine levels in the serum of blood samples of breast patients and to explain its relationship with breast cancer.
Material Materials and Methods: We carried out our work in Selcuk University Faculty of Medicine Biochemistry Metabolism Laboratory. Our study included 20 controls, 20 breast Ca, 20 metastasis patients. Blood collected from breast cancer patients stored at -80 ° C until biochemical analysis is performed. Homocysteine and arginine levels measured in the sera obtained from the blood samples taken. The levels of proteins were measured at 470 nm in the NMRspectrophotometer. To measure homocysteine levels were injected into the mass spectrometer for chromatography.
Results: In the evaluation, a statistical increase was observed in Arginine, Arginine / ADMA levels in patients with breast Ca, and Homocysteine levels in patients with metastases compared to the control group. When the breast cancer error group was compared with the metastasis group, the homocysteine level increased statistically, while the Arginine / ADMA level decreased statistically.
Conclusion: Based on the results we obtained, it is thought that these markers can be used during the diagnosis and treatment of breast cancer patients.

Keywords: Arginine, Breast cancer, Homocysteine.

1. Introduction

Asymmetric dimethylarginine (ADMA), present in tissues, urine and plasma, was first described as methylated arginines excreted in the urine in 1970. It is known as an analog of arginine. ADMA has been detected in immune system cells and meurons of animals and human endothelial cells and was first isolated in 1987 [1]. Inhibition of nitric oxide synthase (NOS) enzyme is the most important known function of ADMA [2]. Nitric oxide (NO) is known as an antiatherogenic molecule first discovered in 1995. eNOS was cloned shortly after the discovery of nitric oxide, and L-arginine was identified as the substrate of this enzyme [3,4,5,6].

ADMA is an increasingly important methylated arginine derivative, which occurs as a result of posttransional modification of methyl groups by the protein arginine methyl tranferase (PRMT) enzyme to arginine residues found in nucleoproteins and the destruction of these proteins. There are also different methylated arginine compounds in the body. These compounds are formed by the addition of 1 or 2 methyl groups to the most functional part of arginine, guanido nitrogen. ADMA and symmetric dimethyl arginine (SDMA) are methylated arginine derivatives formed by the transfer of two methyl groups and N-monomethyl-L-arginine (L-NMMA) by the addition of a single methyl group [7].

Homocysteine is an amino acid that acts like free radicals and is accepted to be involved in the oxidative system in recent years and does not enter the protein structure. Hyperhomocysteinemia causes many harmful effects in the body. Some of these include acting as free radicals and causing endothelial damage and as a result of this event, creating coagulation-enhancing effects such as platelet activation, modification of coagulation factors, thrombus formation, oxidation in biological membranes, LDL oxidation, causing atherosclerosis-enhancing effects. It has been suggested that antioxidants may be effective in preventing atherosclerosis by preventing cholesterol oxidation [8]. In this study, we aim to determine whether there are any changes in homocysteine and arginine levels in breast cancer patients and explain the relationship between these proteins and cancer, since we think that this information may be determinant in breast cancer patients.

2. Material and Methods

We carried out our work in Selcuk University Faculty of Medicine Biochemistry Metabolism Laboratory. Our study included 20 controls, 20 breast Ca, 20 metastasis patients. Blood collected from breast cancer patients stored at -80 ° C until biochemical analysis is performed. Homocysteine and arginine levels measured in the sera obtained from the blood samples taken. The levels of proteins were measured at 470 nm in the spectrophotometer. Sample and blind tube results were recorded separately. Results were recorded as Absorbance Unit (ABSU). To measure homocysteine levels, add 50 microliters of internal standard (d8 homocysteine isotope DLM-3619-1) and 50 microliters of reducing reagent (300 mmol/L 1,4 Dithiothreitol, Cat no: Merek 111474) onto 50 microliters of serum, calibrator and control samples. And kept at room temperature for 10 minutes. 50 microliters of precipitating reagent (15% trichloroacetic acid Cat no: Merck 100810) was added and vortexed for 10 seconds and centrifuged at 13000 rpm for 3 minutes. 10 microliters of supernatant were injected into the mass spectrometer for chromatography. Calibration graphs were obtained using concentrations of 100 micromol / L.

Statistical analysis carried out using Microsoft Excel and SPSS 16 computer programs. The distribution of data was compared with the Mann Whitney U test, for the non-parametric distribution in both groups.

3. Results and Discussion

In our study, we examined the relationship between homocysteine and arginine levels in breast cancer patients. There is no statistically significant difference between the ages of our errors.

Table 1. Biomarkers in breast cancer patients

Group	Arginine (umol/L)	Sitrulline (ng/mL)	Homosistein (umol/L)	Arginine /ADMA	Sitrülline /ADMA
Control	356,2±185,6	33,2±23,2	12,3±3,5	559,8±307,4	55,3±53,4
Breast ca	573,9±204,8	29,4±25,9	10,60±3,4	1018,9±371,2	50,9±46,0
Metastase	385,0±107,0	39,6±30,1	18,7±6,30	515,6±217,2	52,0±34,0

In the evaluation, a statistical increase was observed in Arginine, Arginine / ADMA in patients with breast Ca, and in Homocysteine levels in patients with metastases compared to the control group. When the breast cancer error group was compared with the metastasis group, the homocysteine level increased statistically, while the Arginine / ADMA level decreased statistically.

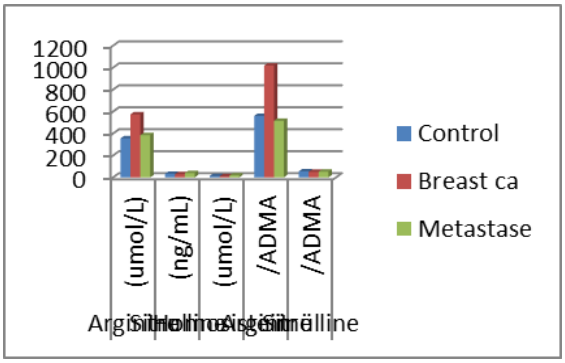

Figure 1. Biomarkers in breast cancer patients and controls.

3.1. Discussion

Arginine (2-amino-5-guanidino pentanoic acid, C6H14N4O2), R) is a positively charged (basic) (R) amino acid first discovered by Hedin in 1895. The L-arginine form is known as the form that participates in the structure of proteins. Arginine is a "semi-essential amino acid". The need for L-arginine in the body increases in some cases, these situations are trauma or infection and especially the growth period [9].

Most of the concentration of arginine in plasma comes from the protein cycle and metabolism. The major organ responsible for all arginine synthesis is the kidneys, , and they are responsible for approximately 60% of arginine synthesis [10]. Another way of arginine synthesis is citrulline, which is produced in the small intestines as a result of the metabolization of amino acids taken into the body by diet, plays the role of the primary substrate in the synthesis of arginine. After citrulline enters the systemic circulation, it is taken through the kidneys and converted into arginine in the proximal tubules [9].

The increase in IP3 levels in the cytoplasm allows the Ca2+ stored in endoplasmic reticulum to be transferred to the cytoplasm. . The NOS enzyme is activated by the Ca + 2 / calmodulin complex and thus NO and citrulline are synthesized from O2 and L-Arginine [9,10]. Tumor angiogenesis plays a very important role in tumor formation and metastasis [11]. The change in the vascular system contributes to the change in angiogenic regulatory pathways and the expression of angiogenic factors for the development of vascularization around the tumor [12,13]. Nitric oxide is one of the key enzymes in angiogenesis. It increases endothelial cell proliferation, migration and extracellular matrix destruction [14]. In addition to angiogenesis, NO plays an important role in cell cycle progression, metastasis, and life [15]. Each change in NO production plays an important role in the regulation of angiogenesis that results in tumor growth.

As a result of the studies, it is thought that the enzymatic activity of NOS is modulated by the ratio (L-Arginine / ADMA) between the concentration of ADMA (endogenous inhibitor) and the concentration of L-arginine (natural substrate) [16].

More specifically, any increase in ADMA levels in the presence of normal L-arginine levels can lead to L-arginine deficiency with respect to optimal NOS activity. Since ADMA is a competitive inhibitor of NOS, its inhibitory effect can be greatly affected by the increase in L-arginine levels [17].

Major methylarginine derivatives are N-monomethyl L-arginine (L-NMMA), Asymmetric dimethylarginine (ADMA), and symmetrical dimethylarginine (SDMA). Methyl arginines are an arginine derivate formed by methylation of arginine residues in protein with post-translational modification In order to synthesize ADMA and L-NMMA, there is a need for the protein arginine methyltransferase type I (PRMT-I) enzyme that transfers methyl groups to arginine residues. [18,19].

ADMA (C8H18N4O2) is an increasingly important derivative in clinical diagnosis, which is synthesized from the arginine residues of the arginine in

nucleoproteins by posttransional modification (post-synthesis regulation) of methyl groups via the protein arginine methyl tranferase (PRMT) enzyme and the residues of free methylarginine released as a result of catabolism of these proteins. [20].

Methylarginines are metabolized by the enzyme dimethylarginine methyl transferase / hydrolase (DDAH) in the kidney and acetylation in the liver. Since PRMT enzymes use S-Adenosyl methionine SAM as a methyl donor, the increase in this leads to an increase in PRMT activation, while the increase in SAH inhibits the PRMT enzyme. SDMA does not inhibit the NOS enzyme, ADMA, SDMA, LNMMA, all three are plasma membrane with L-arginine. They compete with arginine To enter the cell via the cationic amino acid transporter (y +), ie indirectly, SDMA also limits NO production by reducing the amount of intracellular arginine [21]. The high concentration of ADMA also inhibits the uptake of L-arginine and nitric oxide biosynthesis decreases.

It is synthesized from SAM ATP and methionine, which are used as methyl donors by PRMT enzymes. SAM loses its methyl group, converting to S-adenosylhomocysteine. The resulting homocysteine is metabolized via the trans-sulfuration pathway or remethylated to methionine. The re-methylation reaction is dependent on vitamin B and 5-methyl tetrahydrofolic acid as the methyl donor. In the ADMA synthesis reaction, two methyl groups are required and two homocysteines are formed as by-products. There are studies showing that there is a positive correlation between plasma homocysteine and ADMA levels [22].

ADMA and L-NMMA are known as endogenous inhibitors of NOS. The functions of vasoactive mediators released from the endothelial layer are important for the maintenance of vascular tone and healthy structure. Nitric oxide is one of the most important mediators. Nitric oxide is synthesized by 3 isoforms (endothelial, neuronal, and inducible forms in macrophages) of the NOS enzyme. ADMA and arginine have critical roles in the maintenance of NO synthesis. NO plays a role in platelet adhesion and aggregation. ADMA, SDMA and L-NMMA enter endothelial cells through the Y carrier protein. Methylarginines compete with each other and with arginine for entry into the cell. High concentrations of ADMA also reduce NO synthesis by preventing the transport of L-arginine into the cell [23].

In our study, a statistical increase was observed in Arginine, Arginine / ADMA levels in breast cancer patients and homocysteine levels in patients with metastases compared to the control group. When the breast cancer error group was compared with the metastasis group, we observed that the homocysteine level increased statistically, while the Arginine / ADMA level decreased statistically.

It is known that the arginine / ADMA ratio reflects the NO bioavailability Generally, it is mentioned that three conditions are important in the increase of 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 [24].

In a previous study, it was reported that ADMA levels may increase with increased protein catabolism in hematological malignancies [25].

4. Conclusion

In our study, the increase in the homocysteine group in the cancer group and the metastasis group compared to the control group suggests that there may also be an increase in the ADMA level. In addition, it has been reported that ADMA levels can 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 [26]. The methyl group is required in the ADMA synthesis reaction and two homocysteines are formed from there. There are studies showing that there is a positive correlation between plasma homocysteine and ADMA levels [22]. We plan to measure VEGF and NO in our next study. Thus, we think that these markers can be used during diagnosis and treatment in breast cancer patients.

References

1. Gough M.S, Morgan, M.A, Mack, C.M, Darling, D.C, Frasier, L.M, et al., The ratio of arginine to dimethylarginines is reduced and predicts outcomes in patients with severe sepsis, *Critical Care Medicine*, 2011, 39(6), 1351-8. doi: 10.1097/CCM.0b013e318212097c.
2. Cooke, J.P, Tsao, P.S, Arginine: a new therapy for atherosclerosis? *Circulation*, 1997, 95, 311-312.
3. Lüscher, T, Noll, G, Is it all in genes…? Nitric oxide synthase and coronary vasospasm, *Circulation*, 1999, 99, 2855-2857.
4. Furchgott, R.F, Zawadzki, J.V, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, *Nature*, 1980, 299, 373-376.
5. Palmer, R.M, Ashton, D.S, Moncada, S, Vascular endothelial cells synthesize nitric oxide from L-arginine, *Nature*, 1988, 333, 664-666.
6. Palmer, R.M, Ferrige, A.G, Moncada, S, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor, *Nature*, 1987, 327, 524-526.
7. Prefumo, F, Thilaganathan, B, Whitley, G.S, First-trimester uterine artery resistance and maternal serum concentration of asymmetric dimethylarginine, *Ultrasound Obstetric Gynecology*, 2008, 31(2) , 153-157.
8. Ganguly, P, Alam, S.F, Role of homocysteine in the development of cardiovascular disease, *Nutrition Journal*, 2015, 10,14, 6.
9. Alvarez, R, Gonzalez, P, Batalla, A, Reguero J.A, Cubero, G, Hevia, S, et al., Association between the NOS3 (2786 T/C) and the ACE (I/D) DNA Genotypes and Early Coronary Artery Disease, *Journal of Biology and Chemistry*, 2001, 5, 343-348.
10. Vidal, M.J, Romero, J.C, Vanhoutte, P.M, Endothelium-derived relaxing factor inhibits rennin release, *European Journal of Pharmacology*, 1998, 14, 401-402.

11. Veli-Pekka, V, Tomi-Pekka, T, Reijo L, DDAH gene and cardiovascular risk, Vascular Medicine, 2005, 10, S45–48.
12. Aydın, M, Fatma Erdoğmuş, F, Armutçu, F, Yiğitoğlu, M.R, Metabolism of Asymmetric Dimethylarginine (ADMA), *International Jornal of Basic Clinical Medicine*, 2013,1(1) ,61-66.
13. Bilgic¸ O, Altınyazar, H.C, Baran, H, Unlu, A, Serum homocysteine, asymmetric dimethyl arginine (ADMA) and other arginine–NO pathway metabolite levels in patients with psoriasis, *Archive of Dermatological Res*, 2015, 307, 439–444.
14. Ishıda, M, Kakuta, T, Mıyakogawa, T, Tatsumı, R, Matsumoto, C, Fukagawa, M, Association between Asymmetric Dimethylarginine and Pentosidine in Dialysis Effluent of Peritoneal Dialysis Patients, *Tokai Journal of Experimental Clinical Medicine*, 2016, Vol. 41, No. 2, pp. 97-100,
15. Reddy, K.R.K, Dasari, C, Duscharla, D, Supriya, B, Sai, N, Ram, M. et al., Dimethylarginine dimethylaminohydrolase-1 (DDAH1) is frequently upregulated in prostate cancer, and its overexpression conveys tumor growth and angiogenesis by metabolizing asymmetric dimethylarginine (ADMA), *Angiogenesis*, https://doi.org/10.1007/s10456-017-9587-0
16. Yüksel, E., , Ağır Sepsis Ve Septik Şoklu Hastalarda Asimetrik Dimetilarjinin (Adma) Ve Nitrik Oksit (No) Düzeylerinin Araştırılması, Gazi Üniversitesi Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi, 2010, Ankara, 82 s.
17. Böger, R, Asymmetric dimethylarginine (ADMA): A novel risk marker in cardiovascular medicine and beyond, *Annals of Medicine*, 2006, 38, 126-136.
18. Clarke, S, Protein methylation, *Current Opinion of Cell Biology*, 1993, 5, 977–983.
19. McBride, A.E, Silver, P.A, State of the Arg: Protein methylation at arginine comes of age, *Cell*, 2001, 106, 5–8.
20. Alaçam, H, Dikmen, Z.G, Doğan, P, Asimetrik dimetil arjinin (ADMA) metabolizması ve ADMA'n›n oksidatif hasar, endotel hasarı ve çeşitli hastalıklarla ilişkisi, *International Journal of Basic Clinic Medicine*, 2010, 41, 75-81.
21. Taner, A, 2010, Diyabetik Hastalarda Maskeli Hipertansiyonun Gelişiminde Asimetrik Dimetil Argininin Rolü, Selçuk Üniversitesi Meram Tıp Fakültesi Tıbbi Biyokimya Anabilim Dalı, Uzmanlık Tezi, Konya, 69 s.
22. Holden, D.P, Cartwright, J.E, Nussey, S.S, Whitley, G.S, Estrogen stimulates dimethylarginin dimethylaminohydrolase activity and the metabolism of asymmetric dimethylarginine *Circulation*, 2003,108,1575–1580.
23. Erdem, S, Ünlü, A, Asimetrik dimetilarjinin ve klinik önemi, *Selçuk Tıp Dergisi*, 2009, 25 (2), 107-115.
24. Arikan, E, Karadag, C.H, Guldiken, S, Asymmetric dimethylarginine levels in thyroid diseases, *Journal Endocrinology Investigation*, 2007, 30(3), 186-91.
25. Szuba, A, Chachaj, A, Wróbel, T, Dzietczenia, J, Mazur, G, Antonowicz-Juchniewicz, J, Kuliczkowski, K, Andrzejak, R, Asymmetric dimethylarginine in hematological malignancies: a preliminary study, *Leukemia Lymphoma*, 2008, 49(12), 2316-20.
26. Akyol, M, Kara, M, Alacacıoglu, A, Demır, L, Ellıdokuz, H, Kucukzeybek, Y, The Effects Of Adjuvant Chemotherapy On Serum Adma And Endothelın-1 Levels In Early Stage Breast Cancer Patıents (Izmır Oncology Group (Izog) Study, *Acta Medica Mediterranea*, 2017, 33, 557.