Ischemia Modified Albumin Levels in Invasive Ductal Carcinoma and Fibroadenoma Patients

İnvaziv Duktal Karsinom ve Fibroadenom Hastalarında İskemi Modifiye Albümin Düzeyleri

Sibel Bilgili* **Özlem Uğurlu*** **Giray Bozkaya***
Nuriye Uzuncan* **Baha Zengel****

* SBÜ İzmir Bozyaka Eğitim ve Araştırma Hastanesi, Tıbbi Biyokimya, Izmir, Türkiye
** SBÜ İzmir Bozyaka Eğitim ve Araştırma Hastanesi, Genel Cerrahi Kliniği, Izmir, Türkiye

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ABSTRACT

Purpose: Impaired oxidative-antioxidative balance plays an important role in the pathogenesis of many diseases like cancer. Ischemia modified albumin (IMA) is altered albumin by free radicals generated from ischemic tissues. The aim of this study was to evaluate the levels of the IMA in newly diagnosed patients with invasive ductal carcinoma and fibroadenoma before the surgery or medical treatment.

Methods: IMA levels were measured in 30 patients which were diagnosed as "invasive ductal carcinoma"(Group 1), 30 patients as "fibroadenoma"(Group 2) and in 28 "healthy controls"(Group 3). An albumin-cobalt binding test was used to define serum IMA in absorbance units (ABSU).

Results: There were statistically significant differences between three groups. IMA levels were significantly higher in the invasive ductal carcinoma group (0.434±0.056 ABSU) compared to fibroadenoma group (0.344±0.135 ABSU) and control group (0.196±0.050 ABSU) ($p<0.05$), also fibroadenoma group values were significantly higher than the control group ($p<0.05$).

Conclusion: These findings indicate that serum IMA measurements can be demonstrative of the severity of oxidative stress among patients with breast pathologies. Highest IMA levels in invasive ductal carcinoma group than fibroadenoma and control groups may reflect disease severity in newly diagnosed patients and in the future it may be a new marker in breast cancer.

Key Words: breast cancer; fibroadenoma; invasive ductal carcinoma; ischemia modified albümin; oxidative stress

Sibel Bilgili : https://orcid.org/0000-

Yazışma adresi: Sibel Bilgili
Sağlık Bilimleri Üniversitesi İzmir Bozyaka Eğitim ve Araştırma Hastanesi, Tıbbi Biyokimya, izmir, Türkiye
e-mail: sibel.bilgili@yahoo.com.tr

ÖZET

Amaç: Bozulmuş oksidatif-antioksidan denge, kanser gibi birçok hastalığın patogenezinde önemli bir rol oynar. İskemi modifiye albümin (IMA), iskemik dokulardan üretilen serbest radikallerle değişime uğramış albümindir. Bu çalışmanın amacı, invaziv duktal karsinomlu ve fibroadenomlu yeni tanı alan hastalarda cerrahi veya medikal tedavi öncesi IMA düzeylerini değerlendirmektir.

Gereç ve Yöntem: IMA düzeyleri invaziv duktal karsinom tanısı alan 30 hastada (Grup 1), fibroadenom tanısı alan 30 hastada (Grup 2) ve 28 sağlıklı kontrol grubunda (Grup 3) ölçüldü. Serum IMAdüzeyleri albümin-kobalt bağlanma testi ile absorbans ünitesi (ABSU) olarak ölçüldü.

Bulgular: Üç grup arasında istatistiksel olarak anlamlı fark vardı. IMA düzeyleri invaziv duktal karsinom grubunda (0.434 ± 0.056 ABSU) fibroadenoma grubuna (0.344 ± 0.135 ABSU) ve kontrol grubuna (0.196 ± 0.050 ABSU) göre anlamlı derecede yüksekti ($p<0.05$). Aynı zamanda fibroadenoma grubu değerleri de kontrol grubundan anlamlı olarak yüksekti ($p<0.05$).

Sonuç: Bu bulgular serum IMA ölçümlerinin meme patolojileri olan hastalarda oksidatif stresin ciddiyetinin göstergesi olabileceğini göstermektedir. İnvaziv duktal karsinom grubunda fibroadenoma ve kontrol gruplarına göre tespit edilen en yüksek IMA seviyeleri, yeni tanı alan hastalarda hastalık şiddetini yansıtabilir ve gelecekte meme kanserinde yeni bir belirteç olabilir.

Anahtar Sözcükler: meme kanseri; fibroadenom; invaziv duktal karsinom; iskemi modifiye albümin; oksidatif stres.

INTRODUCTION

Free radicals are known to be involved in carcinogenesis (1,2). There are potentially hundreds of different types of chemical changes in DNA resulting from reactive oxygen species (ROS) that could be mutagenic and involved in the etiology of cancer (3). A large body of evidence suggests important roles for ROS in the expansion of tumor clones and acquisition of malignant properties (4,5). Damage to the breast epithelium by chemical carcinogens such as ROS can lead to fibroblast proliferation, hyperplasia of epithelium, cellular atypia and breast cancer (6,7).

Albumin has a binding site of transitional metal ions, including cobalt and copper, on its N-terminal region (8). Ischemic and oxidative stress (free radicals and ROS) modifies this terminal peptide irreversibly to a dysfunctional form, known as ischemia-modified albumin (IMA) (9,10).

IMA levels are higher in many inflammatory and oxidative stress-associated diseases. In recent years, different studies have described the role of IMA as a new marker for diseases related to inflammation. The generation of reactive oxygen species (ROS) and free radicals can transiently modify the N-terminal region of albumin and produce an increase in the concentration of IMA. IMA is indicated a marker of ischemia and oxidative stress originating as a consequence of tissue hypoxia. There have also been studies describing the relationship between cancer and inflammation. This relationship may include both an extrinsic and an intrinsic pathway. The extrinsic pathway is related to inflammatory circumstances, which increase cancer risk, whereas the intrinsic pathway is maintained by genetic changes that cause inflammation, which may also induce oncogenesis (11).

In recent years, studies have shown that IMA is a marker of myocardial ischemia and a marker related to critical pathologies, including cancers as well (12-17). The impaired oxidative/antioxidative balance was the absolute impact in the pathogenesis of many, perhaps every human disease, including the cancer (11). Also, serum IMA levels increased in patients with gastric, prostate, soft tissue cancer and

neuroblastoma in some studies. But there is no published report about serum concentrations of IMA in patients with breast cancer. Breast cancer is common in modern societies, and the management of breast cancer is crucial. Breast carcinoma is related to the increase of lipid peroxidation in plasma with concomitant decrease of antioxidant defense capacity in blood cells, which becomes more pronounced during aging of the patients. Chemotherapy and radiotherapy promote further oxidative shift, which potentiate already existing chronic oxidative stress linked to breast cancer. In these effects, impaired capacity for H(2)O(2) detoxification (CAT, GPX and GSH) seems to have major contribution (18).

The relationship between the IMA levels and breast pathologies, also the degree of severity in patients with breast cancers and IMA correlations are unknown. So, the aim of this study was to evaluate the levels of IMA in patients with newly diagnosed as invasive ductal carcinoma (IDC) and fibroadenoma (FA) before the surgery or other treatments.

MATERIALS AND METHODS

There were three groups in the study; Group 1 was "invasive ductal carcinoma group" including 30 women, Group 2; "fibroadenoma group" with 30 women and Group 3; "healthy control group"with 28 women.

All subjects had full physical examination and were asked to complete a questionnaire and gave informed consent before the onset of study. The study was approved by the local ethics committee.

Patients with alcoholism, smoking, diabetes, viral hepatitis, cardiac disease, renal, hepatic or endocrine disease, HIV were excluded. None of the participants in the present study were using drug medications including vitamins or antioxidant drugs. Patients were diagnosed by surgery and pathology clinics using histopathologic evidence and also healthy control group was choosen after mammographic and ultrasonographic examinations.

Blood samples were collected by venous puncture into vacutainer tubes with gel. Specimens were routinely centrifuged within 1 hr of collection for 15 min at 1000 g, and aliquots of serum samples were stored at -80 ^{0}C for a maximum of 4 weeks before IMA measurement.

An albumin-cobalt binding test was used to define serum IMA. The decreased binding capacity of cobalt to albumin was assessed using the rapid colorimetric detection method developed by Bar-Or et al. (19). Briefly, 200 μL of patient serum was transferred into glass tubes and 50 μL of 0.1% $CoCl_2 \times 6H_2O$ (Sigma-Aldrich, Missouri, USA) was added. After gentle shaking, the mixture was incubated for 10 minutes to ensure sufficient cobalt to bind to albumin. Then, 50 μL of 1.5 mg/mL dithiothreitol (DTT) (Sigma-Aldrich, Missouri, USA) was added as a colouring agent. After 2 minutes, 1 mL of 0.9% NaCl was added to halt the binding between the cobalt and albumin. A blank was prepared for every specimen. At the DTT addition step, 50 μL of distilled water was used instead of 50 μL of 1.5 mg/mL DTT to obtain a blank without DTT. The absorbances were recorded at 470 nm. Colour formation in specimens with DTT was compared with colour formation in the blank tubes, and the results were expressed as absorbance units (ABSU). Serum albumin levels were measured with Olympus AU 2700 autoanalyser (Beckman Coulter, Tokyo, Japan).

All data were analysed using the statistical software package SPSS Statistics version 21. Kolmogorov Smirnov test was used to determine the normality of the distributions. Data were expressed as mean±standard deviation. The Student t test was used to evaluate the difference between groups. For comparison of IMA and characteristics of patients Man Whitney U test was used. $p<0.05$ was considered as statistically significant.

RESULTS

There were significant differences between three groups. IMA levels were significantly higher in the invasive ductal carcinoma group (0.434±0.056 ABSU) compared to fibroadenoma group (0.344±0.135 ABSU) and control group (0.196±0.050 ABSU) ($p<0.05$), also fibroadenoma group values were significantly higher than the control group ($p<0.05$). Serum albumin levels were normal in all groups. Demographic properties of groups were shown in Table 1.

Also, histopathologic findings of IDC patients were evaluated (Table 2). There were no significant correlation between these characteristics and IMA values ($p>0.05$). According to AJCC (American Joint Committee on Cancer) TNM Classifications of patients were as follows; Stage IA: 9 (%30), Stage IB: 4 (%13.33), Stage IIA: 6 (%20), Stage IIB: 9 (%30), Stage IIIA: 1 (%3.33), Stage IIIC: 1 (%3.33). Our patients tumor sizes were between 0.8 and 4.5 cm in diameter (mean:2.25± 0.96 cm).

DISCUSSION

The presence of high amounts of reactive metabolites in the cancer tissue may have a significant role in both carcinogenesis and spread of cancerous tissue (6). Cancer development is a multi-stage process and ROS-mediated DNA damage plays an important role in these stages, especially in initiation and promotion processes (20).

Table 1. Demographic characteristics of groups

Characteristics	IDC (n=30)	FA (n=30)	Control (n=28)
Age	44.6±13.1	41.8±7.6	40.2±9.6
Family history (+/-)	10/20	11/19	0/28
Birth (+/-)	26/4	25/5	22/6
Breast feeding (+/-)	26/4	25/5	22/6

(IDC: invasive ductal carcinoma, FA: fibroadenoma)

Table 2. Histopathologic findings of IDC patients

	Characteristics	**n**	**%**
Tumor size	<2cm	11	36.66
	≥2cm	19	63.33
Lymph node	Negative	20	66.66
	Positive	10	33.33
Stage	1	13	43.33
	2	15	50
	3	2	6.66
HG	1-2	25	83.33
	3	5	16.66
ER	Positive	25	83.33
	Negative	5	16.66
PR	Positive	21	70
	Negative	9	30
c-erbB-2	Positive	11	36.66
	Negative	19	63.33

(HG: Histologic grade, ER: Estrogen receptor, PR: progesterone receptor)

IMA levels are higher in many inflammatory and oxidative stress-associated diseases (11). IMA is elevated in most patients with liver cirrhosis, acute infections and advanced cancers; all these conditions are potent producers of free radicals (21,22).

It is well established that oxidative stress is linked to cancer formation and patients with several types of cancers can have high IMA levels (23-25). Kasapovic et al. (18) performed a study in patients with breast cancer and examined antioxidant status and lipid peroxidation. Fidan et al. (24) showed that serum IMA levels were increased in patients with gastric cancer. Mastella et al. (25) found increased serum IMA levels in patients with prostate cancer, but this increase was not statistically significant. Stachowicz-Stencel et al. (11) found that serum IMA levels were statistically higher in pediatric patients with neuroblastoma and soft tissue sarcomas. In the other study, the oxidative/antioxidant status was impaired in favor of oxidative stress in colorectal carcinoma patients but this observation was not confirmed by IMA measurement (23). Abusoğlu et al. reported that serum IMA concentrations statistically higher in breast cancer patients than in the control group (26).

The results of the present study indicate that IMA levels in invasive ductal carcinoma patients were higher than fibroadenoma patients and healthy subjects. Also fibroadenoma patients IMA levels were higher than healthy control group values. So we thought IMA levels may reflect the severity of the breast pathologies and this condition may be associated with the impairment of oxidant antioxidant balance. There were no correlation of histopathologic findings with IMA levels in terms of tumor size, grade, lymph node involvements, histologic grade, c-erbB-2, estrogen and progesteron receptor positivity. We think the reason of this may be the small number of our patients.

To the best of our knowledge this was the first study evaluating serum IMA measurements in fibroadenoma and also breast cancer patients. Also the relationship between the IMA and the degree of severity in patients with breast cancers is unknown. The findings of this study indicate that serum IMA measurements can be demonstrative of the oxidative stress among patients with breast pathologies. The preoperative IMA levels may help clinicians in assessment of these patients. Highest IMA levels in invasive ductal carcinoma group may reflect disease severity in preoperative patients and it may be a new biomarker in the future. The small number of patients may be the limitation of this study and therefore further studies are necessary to confirm these results.

Conflict of Interest Statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/ or affiliations relevant to the subject matter or materials included.

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