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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

MEME KANSERLİ HASTALARDA DİNAMİK TİYOL, DİSÜLFİT DENGESİ İLE CA-15-3 SEVİYELERİ ARASINDAKİ İLİŞKİ

THE ASSOCIATION BETWEEN THIOL-DISULPHIDE BALANCE AND CA-15-3 LEVELS IN PATIENTS WITH BREAST CANCER

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ÖZ

AMAÇ: Tiyol/disülfit dengesi birçok hastalıkta önemli bir oksidatif belirteçtir. Kanserli olgularda biyo-belirteç olması yönünde çalışmalar yapılmaktadır. Bu çalışmanın amacı meme kanserli hastalarda tiyol-disülfid homeostazisi (Dinamik tiyol [-SH], disülfit [-S-S] ve total tiyol [TT]) ile CA 15-3 (kanser antijeni 15-3) ve CEA [karsinoembriyojenik antijen], IMA[iskemik modifiye albümin], albümin arasındaki ilişkiyi araştırmak ve literatürde ilk kez yapılan sağlıklı kontrollerle karşılaştırmaktır.

GEREÇ VE YÖNTEM: Hastalar kemoterapi altında çalışmaya katıldılar. Çalışma prospektif bir çalışmadır. Çalışmaya 39 kanserli hasta ve 41 sağlıklı toplamda 80 hasta dahil edildi. Çalışmamızda, tiyol-disülfid homeostazisindeki değişiklikler ve IMA, Albumin, CA 15-3 seviyelerine bakıldı.

BULGULAR: Kontrol ile meme CA grupları arasında IMA, albumin ve CA-15-3 açısından istatiksel olarak anlamlı fark olduğu belirlendi ($p<0.05$). CA 15-3 in yüksek olduğu grupta SH/TT değerlerinde azalma, SS/TT değerlerinde artış görülmesine rağmen tiyol ve disülfid miktarlarında anlamlı bir değişiklik olmadı. Tiyol-disülfid parametreleri ile Meme Ca grubunda tümör biyo-belirteç değerleri arasında ilişki görülmedi.

SONUÇ: Tiyol-disülfid homeostazisindeki değişikliklerin CA 15-3 ile etkileşmediği söylenebilir.

ANAHTAR KELİMELER: Meme kanseri, disülfid, tiyol, tümör biyobelirteç

ABSTRACT

OBJECTIVE: Thiol/disulphide homeostasis is important in cancer. Studies are carried out to be biomarkers in cancer patients. The purpose of this analysis is to investigate the relationship between thiol-disulphide equilibrium (Native thiol[-SH], disulfide [-S-S] and total thiol [TT]) and CEA [carcinoembryonic antigen], IMA [ischemic modified albumin], albumin, CA 15-3 (cancer antigen 15-3) in patients with breast cancer and compare it with healthy controls, which is conducted for the first time in the literature.

MATERIAL AND METHODS: Patients participated in the study under chemotherapy. The study is a prospective study. A total of 80 participator including 39 patients with breast cancer and 41 wholesome individuals participated in the study. In our study, changes in thiol-disulfide homeostasis and IMA, albümin and CA-15-3 levels were examined.

RESULTS: In breast cancer group, IMA, albümin and CA-15-3 were obtained sitatiscally significant difference compared to the controle group ($p<0.05$). Although in high CA-15-3 group decreased SH/TT and increased SS/TT ratios, there wasn't a significant change in the amount of thiol and disulfide. There was not any relationship between thiol-disulphide parameters and tumor markers in the the breast cancer group.

CONCLUSIONS: It can be said that changes in the thiol-disulphide homeostasis may not be interact with CA 15-3 values.

KEYWORDS: Breast cancer, disulphide, thiol, tumor marker.

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INTRODUCTION

Cancer is one of the most common diseases in our country and in the world with its many unrevealed structural and biochemical characteristics (1). Breast cancer is among the most commonly seen cancers in women around the world and in Turkey (2). It is difficult to identify how much risk each woman carries in terms of breast cancer. In our country, the characteristics of breast cancer and a roadmap for its treatment have not yet been determined systematically in a detailed manner. Thiols, which generate the thiol/disulphide homeostasis, are organic compounds that contain a sulfhydryl (-SH) group and play a significant role in preventing the occurrence of oxidative stress in cells (1-3, 4-6).

They have significant roles in dynamic thiol/disulphide homeostasis, antioxidant defense, regulation of enzyme activities, detoxification, apoptosis and cellular signal transduction mechanisms and are known to involve in the pathogenesis of many disorders such as abnormal thiol/disulphide levels, cancer, Multiple sclerosis, Parkinson, cardiovascular diseases, rheumatoid arthritis, diabetes, chronic renal insufficiency, Alzheimer and liver diseases (5-7).

The most prominent risk factors in breast cancer cases are the female gender and the age factor - 75% of the cases are seen above the age of 50 (6-8). CA 15-3 is a product of mucin-1 (MUC-1) gene and a mucin antigen with glycoprotein structure and is responsible for metastasis. MUC-1 gene is present during lactation and in cancerous breast tissue in higher concentration concentrations. Therefore, CA 15-3 protein produced from this gene is the most frequently used serum marker in breast cancer. The serum concentration is considered high if it is >25 U/ml (9, 10). This protein is an important marker in treatment follow-up; however, it is not sufficient for diagnosis since it rises in other malign diseases, benign breast and liver diseases and is a parameter with low specificity (11).

Previous studies investigated thiol/disulphide homeostasis and oxidative damage in several diseases such as cancer (1-17). CA 15-3 and CEA are significant parameters in breast cancer follow-up and are investigated in follow-ups (18). In the present study, we designed to investigate thiol/disulphide homeostasis in breast cancer by a newly developed reliable assay as well as the association between thiol-disulphide homeostasis and breast cancer. The aim of this study was to investigate an oxidative stress marker (thiol/disulphide homeostasis) and IMA, Albumin, CEA, CA-15-3 in patients with breast cancer and compare the results with controls.

ETHICS APPROVAL

The study was conducted in accordance with the Declaration of Helsinki 2013 Brasil version and was approved by Dumlupınar University Ethics Research Committee of Kütahya in Turkey (2015-KAEK-86/08-158). Analyses of the participants were their routine parameters at the time they were included in the study and those were recorded from patient files.

MATERIAL AND METHOD

Study population

This prospective cross-sectional study was carried out from January 2017 to March 2017at the General Surgery Clinic of special hospital and at the Biochemistry Department of a University Hospital in Turkey. The study was conducted in accordance with the Declaration of Helsinki 2013 and was approved by the Ethics Research Committee of Kütahya in Turkey. Written patient consent was obtained from all participants. Current breast cancer was confirmed by the presence of the oncology department. All of the participants were over the age of 18. Participants included voluntary groups and patient groups. The control group included 41 participants with healthy volunteers, the second group included 39 patients with breast cancer. Eighty participants were included in the study. All breast cancer participants were receiving chemotherapy. Since all the stages of the patients were not recorded, the comparison of thiol-disulfide did not occur according to their stages.The control group consisted of healthy volunteers without any chronic disease and druguse (kidney failure, diabetes mellitus, liver disease, cardiovascular and cerebrovascular disease; smoking, alcohol consumption).

Blood samples and assay

Fasting blood samples were procured from the breast cancer and the controls in plain tubes. To separate serum from cells, collected blood samples were centrifuged at 1500 rpm for 10 min. Remaining serum samples were immediately stored frozen at -80 ºC until the analysis was performed for the Thiol/Disulfide homeostasis and IMA. Serum levels of native and total thiol and the ratio of disulfide to native and total thiol were measured by using a new and fully automated colorimetric method. In this method developed by Erel and Neselioglu (7), the dynamic disulfide bonds (-S - S-) were reduced to functional thiol groups (-SH) using sodium borohydride (NaBH4). This method was comprised of the 2 steps: Di-sulfide bonds were reduced to free thiol groups by NaBH4 and the residual NaBH4 materials were entirely eliminated from the environment with formaldehyde. Native thiol and total thiol were measured by using a new and fully automatic system, disulphide and disulphide/nativethiol, disulphide/total thiol and native thiol/total thiol ratios were calculated by Erel and Neselioğlu method (7).

A spectrophotometric method was used to measure the concentrations of TT, –SH, and –S–S, the ratios of SS/ (SH + SS) and SS/SH, and the ratios of SH/ (SH + SS). Half of the difference between total thiols and natural thiols gives the active disulfide level. The total thiol was measured using reagent modified Ellman (DNTB,5,5'-ditiobis). Native thiol measurements were performed at the same time. Serum IMA and albumin levels were measured by colorimetric analysis and then –S–S, the ratios of SS/ (SH + SS) and SS/SH, and the ratios of SH/ (SH + SS) were calculated. Commercially available assay kits were used for IMA and albumin analysis. Total IMA (ABSU: absorbans units) and albumin (g/dL) levels were measured in automatic Roche-Hitachi Cobas c501 analyzer with a calorimetric method with commercially available assay kits. Serum CEA and CA 15-3 were determined using an automated clinical biochemistry analyzer with original reagents (Architect CEA 7k68/ Architect CA 15-3 2K44). Analysis of the participants (breast cancer group and control group) were their routine parameters at the time they were included in the study and those were recorded from patient files.

STATISTICAL ANALYSIS

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) 17 program. Normality of distribution was evaluated using Histogram and Shapiro-Wilk test. Normally distributed numerical variables were presented as mean ± standard deviation. Differences in the continuous variables between groups were assessed by t-test for variables with normal distribution. Pearson correlation tests were used to investigate the correlations between serum Thiol/Disulfide Homeostasis Parameters and IMA and albumin levels, CEA, and serum CA-15-3 levels. A probability level of $p<0.01$ and $p<0.05$ was considered to be indicative of statistical significance.

RESULTS

Patients with 39 breast cancer (F/M:38/1) and 41 healthy controls (F/M:40/1) were included in the study. Overall, 2 (2.5%) of the cases were male and the remaining 78 (97.5%) were female. The mean age was 51.75 years(range: 37-82) in the breast cancer patient group and 50.73 years (range: 29-73) in the control group. There were no statistically significant differences regarding the ages of the patients among the control group and breast cancer group. Only one of the patients with breast cancer was bilateral lobular cancer and the others were invasive ductal carcinoma.The sex of the patient with bilateral lobular cancer was female. Two of the patients with invasive ductal carcinoma were male and the others were female. The thiol/disulfide homeostasis parameters (native thiol, disulfide, total thiol, and native thiol/disulfide) of the patients and healthy controls are summarized in **(Table 1)**.

Table 1: Comparison of thiol/disulfide homeostasis and biochemical parameters in the study and control groups.

	CONTROL	BREAST CA
SH [NATIVE THIOL] µmol/L	268.45±85.07	262.98±58.94
TT[TOTAL THIOL] µmol/L	302.06±87.61	296.01±61.06
SS[DISULPHIDE] µmol/L	16.81±7.82	16.52±7.81
SS /SH [%]	6.85±3.99	6.70±3.73
SS /TT [%]	5.83±2.86	5.72±2.83
SH /TT [%]	88.34±5.71	88.55±5.66
IMA (ABSU)	1.00±0.22	0.84±0.10*
ALBUMIN (g/dL)	3.98±0.35	4.16±0.13*
CA-15-3 (U / mL)	15.52±6.89	60.78±199.55*
CEA (ng/mL)	2.4158±1.45	2.20±2.70

*P value< 0.05 considered to be significant compared to the control.

Correlation of thiol/disulfide homeostasis and biochemical parameters in the patients with breast cancer groups are demonstrated in **(Table 2)**.

Table 2: Correlation of thiol/disulfide homeostasis and biochemical parameters in the patients with breast cancer groups.

		IMA	ALBUMİN	CA-15-3	CEA	AGE
SH[NATIVE THIOL]µmol/L	r	-,517**	,499**	,312	,164	-,069
	P	,001	,001	,064	,331	,681
TT[TOTAL THIOL] µmol/L	r	-,545**	,512**	,222	,179	-,082
	P	,000	,001	,193	,290	,626
SS[DISULPHIDE] µmol/L	r	-,181	,116	-,321	,084	-,059
	P	,278	,488	,056	,621	,727
SS /SH [%]	r	,040	-,088	-,290	-,030	-,012
	P	,810	,601	,086	,862	,945
SS /TT [%]	r	,041	-,089	-,318	-,018	-,005
	P	,807	,594	,059	,915	,976
SH /TT [%]	r	-,041	,089	,318	,018	,005
	P	,807	,594	,059	,915	,976

**. Correlation is significant at the 0.01 level, r pearson correlation P; Significant

CA-15-3, IMA, albumin, CEA, total thiol, native thiol, and disulfide as well as disulfide/native thiol and disulfide/total thiol ratios were compared between the groups. Serum disulfide levels were 16.52±7.81 µmol l−1 in the breast cancer group and 16.81±7.82 µmol l−1 in the healthy group. Native thiol levels were 262.98±58.94 µmol l−1 in the breast cancer group and 268.45±85.07 µmol l−1 in the healthy group, and total thiol levels were 296.01±61.06 µmol l−1 in the breast cancer group and 302.06±87.61 µmol l−1 in the control group. In breast cancer group, obtained levels of IMA, albumin and CA-15-3 were statistically significantly different compared to the control group ($p<0.05$).

No marked difference was detected in average native thiol and average total thiol levels between the breast cancer group and control group ($p >0.05$). Disulphide levels were similar in both groups (breast CA: 16.52±7.81; control: 16.81±7.82). A statistically significant difference was observed between control-breast CA groups in terms of IMA, albumin and CA-15-3 ($p<0.05$).

There was no observed relationship between thiol-disulphide parameters and tumor biomarker values in breast CA group.

DISCUSSION

Reactive oxygen radicals play an important role in the pathology of many diseases such as cancer(1-4-5). Thiols are organic compounds that contain a sulfhydryl (-SH) group and play a significant role in preventing the occurrence of any oxidative stress in cells. Total thiols in the body are in free form which are bound to the proteins in the body or formed as reduced glutathione. Thiol and cysteine which are found in active regions of proteins such as thioredoxin and peroredoxin activate glutathione peroxidase and glutathione S transferase enzymes and protect the cells against oxidative stress (1).

Reactive oxygen radicals causing oxidative damage and thiol groups in the medium are oxidized and converted into reversible disulphide bonds. Dynamic thiol/disulphide homeostasis plays an important role in events such as detoxification, apoptosis and regulation of enzyme activities and is impaired in many diseases, notably cancer, and, furthermore non-enzymatic antioxidants (total thiols) are important because they contribute in maintaining of normal cell structures and functions. GSH (S-glutathionylation) which has an important place in this homeostasis was studied as a potential biomarker in some diseases and was observed to increase (1, 19). Although several tumor markers are found high in cancer cases, diagnosis and treatment follow-up is difficult because changes are observed in these markers in metabolic and hormonal disorders. In this regard, Thiol-Disulphide Homeostasis has been studied for years in terms of its contribution to tumor markers and both variables can now be measured separately with the newly developed method (1-7, 20, 21). In a study regarding small cell lung cancer, it was observed that an impairment was present in thiol-disulphide homeostasis and that it can be evaluated as a biomarker in this cancer type. In a study on ischemia-induced stroke pathogenesis, on the other hand, it is stated that low native thiol can be an important marker (22-23). Breast cancer is the most commonly seen cancer type in Turkey, as in across the world, and genetic (BRCA1 and BRCA2, HER-2/neu and p53) and non-genetic factors play a role in its

formation. Several studies have been published with regard to the effect of the age of cases with breast cancer, tumor's stage, diameter, histological type, nuclear grade and the number of metastatic axillary lymph nodes on survey and tumor recurrence (6-8, 24). Thiol levels have a significant effect on the reduction of harmful effects of oxygen radicals and a decrease in these levels also leads to a decrease in antioxidant effect. In a study on patients with hyperemesis gravidarum regarding thiol-disulphide homeostasis, it was stated that thiol levels decreased and thiol-disulphide homeostasis was impaired (25). In a study conducted on patients with Alopecia Areata, no statistically significant difference was reported between the study group and the control group in terms of thiol-disulphide (26). Many albumin thiols form the plasma thiol pool. IMA is an oxidatively modified version of albumin. IMA has been studied as a sensitive biomarker in diseases such as diabetes, myocardial infarction and peripheral vascular disease (27). However, the relation between thiol-disulfide markers and IMA levels in breast cancer has not been reported yet. In breast cancer group obtained levels of IMA, albumin were statistically significantly different compared to the control group ($p<0.05$).The IMA levels were higher in the breast cancer group compared with control group.

Technical limitations occurred in our study. Serum samples were stored and thus, they were not immediately used (28). IMA spectrophotometrically is measured by albumin cobalt binding test (29). The reference range of the IMA was determined as 52.76-116.56 U/mL. IMA levels may increase after freezing in which the IMA is susceptible to being stored at low temperatures (28, 29).

In another study on thyroid cancer, results showed that thiol/disulphide homeostasis was not impaired and no statistically significant difference was present between Thyroid stimulating hormone (TSH) and thiol/disulphide (1). Reactive oxygen molecules(such as hydrogen peroxide (H2O2), hydroxyl radical (•OH) andsuperoxide anion (O2•–)) play an important role in carcinogenesis. Oxidative stress may be can be related to breast cancer (30, 31). Serum thiol / disulfide levels may be indicative of determining oxidative status in some patient populations (32).The level of disulfide under oxidative stress is anticipated to increase as the thiol level decreases (30, 31).Contrary to expectations, no relationship was observed between thiol-disulphide parameters and tumor biomarker values in breast CA group in the present study. Although disulphide /total thiol values decreased and dynamic thiol/total thiol values increased in the group with high CA 15-3, there was no statistical difference in thiol and disulphide amounts. But results of our study did not support this. There are many factors (rates of liver release of human serum albumin and glutathione (thiol-containing molecules), the rates of transport between the plasma compartment and erythrocytes and endothelial cells,) that affect the thiol-disulfide balance outside oxidative stress. These factors may have affected the results of our study. Although the results of our study dismiss the argument that there might be a biomarker in breast cancer, the presence of a few researches on the measurement of thiol(-SH), disulphide (-S-S) amounts and the determination of -SH/-S-S homeostasis makes our study special.

CONCLUSION

Non protein thiol groups contribute to protecting normal cell functions. Thiols have significant roles in dynamic thiol/disulphide homeostasis, antioxidant defense. In our work, thiol-disulfide parameters were detected with a new method in breast cancer patients. There was no relationship between thiol disulfide parameters and tumor markers. It is thought that thiol deficiency and maintenance of thiol-disulphide homeostasis is important and multi-centered studies with higher number of patients are required.

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