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RESEARCH ARTICLE/ARAŞTIRMA YAZISI

THE RESULTS OF SCREENING WITH SELDI-TOF-MS METHOD IN BREAST CANCER DIAGNOSIS

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MEME KANSERI TANISINDA SELDI-TOF-MS YÖNTEMIYLE TARAMA SONUÇLARI

ÖZET

Amaç: Meme kanserinin tarama yöntemleriyle erken evrede saptanması hastaların sağkalımını uzatmaktadır. Tarama amacıyla en sık kullanılan yöntem mamografidir, ancak bu yöntem özellikle genç kadınlarda tarama yapılabilmesi için yeterli olmamaktadır. Bu nedenle, bu çalışmada, son yıllarda geliştirilen SELDI-TOF-MS yöntemi kullanılarak sağlıklı bireylerde meme kanseri taraması yapılması amaçlanmıştır.

Hastalar ve Yöntem: 2008 – 2009 yıllarında hastanemize başvuran sağlıklı kadınların serum örneklerinden protein profilleri SELDI-TOF-MS yöntemiyle çalışıldı. Serum örneklerinde BC1 proteininin miktarının azalması ve BC2 ile BC3 proteinlerinin miktarlarının artması meme kanseri açısından riskli olarak tanımlandı. Riskli olduğu belirlenen bireyler olası meme patolojileri yönünden radyolojik olarak tetkik edildi.

Bulgular: Çalışmaya 18 sağlıklı kadın alındı. Kadınlardan dördünde (%22.2), BC1 proteininin zirve değeri anlamlı derecede düşük ve BC2 ile BC3 proteinlerinin zirve değerleri anlamlı derecede yüksek bulundu. Riskli olduğu düşünülen kadınlardan birinde ultrasonografide kistik değişiklikler ve komplike kistler saptandı.

Sonuç: Bu çalışmada, katılan birey sayısının az olmasına rağmen, meme kanserli hastalara benzer protein profiline sahip kadınlar belirlenmiştir. Sadece bu kadınların yakın izleminin yapılması tüm kadınların izlenmesine kıyasla iş yükü ve maliyeti azaltacaktır.

Anahtar sözcükler: meme kanseri, tarama, SELDI-TOF-MS

ABSTRACT

Purpose: Diagnosis of breast cancer at an early stage with screening methods prolongs patients' survival. The most commonly used screening method is mammography, however, this method is not sufficient, especially in younger women. For this reason, in this study, it is aimed to screen healthy individuals for breast cancer with recently developed SELDI-TOF-MS method.

Patients and Methods: Protein profiles of healthy women screened between 2008 and 2009 were obtained from their serum samples using the SELDI-TOF-MS method. In serum samples, a decrease in BC1 protein level and an increase in BC2 and BC3 protein levels were identified as to carry a risk for breast cancer. Individuals carrying a risk were evaluated for possible breast pathologies.

Results: Eighteen healthy women were included in the study. In four women (22.2%), peak value of BC1 protein was found as significantly low, whereas peak values of BC2 and BC3 proteins were significantly high. Cystic changes and complicated cysts were detected in one of the patients on ultrasonography.

Conclusion: Although the number of individuals included in this study is small, women possesing a protein profile similar to breast cancer patients was detected. Following only these women who were identified will decrease labour force and expenses compared to following all women.

Key words: breast cancer, screening, SELDI-TOF-MS

Introduction

Screening of healthy individuals is important for early diagnosis in every type of cancer including breast cancer. Detection of early stage cancer in individuals during screening prolongs patients' survival. Today, the most widely used method for breast cancer screening is mammography, however, this method is not adequate for screening, especially in younger women (<40 years of age). For this reason, breast ultrasonography is used as an imaging method in younger individuals. When breast ultrasonography is used, signs which can be more effectively detected on mammography, such as microcalcifications, may be missed. Presence of certain defects of the imaging modalities used in clinical practice leads to the development of new methods in this area. Proteomics methods are utilized for this purpose. It became possible to make a risk assessment regardless of the age of the healthy individuals with the development of proteomics methods. SELDI-TOF-MS is a

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method used to detect the changes in serum protein levels of the individuals combining chromatography and mass spectrometry. In this study, it was aimed to perform breast cancer screening in healthy individuals using the SELDI-TOF-MS method.

Patients and methods

Blood samples were taken from healthy women applying to the hospital between 2008 and 2009 after obtaining consent. Serum was separated from blood samples by centrifuging and kept at -80°C until the day of study. Protein profiles of women participating in the study were analyzed from serum samples using SELDI-TOF-MS method. IMAC30 biochip (Ciphergen ProteinChip®) was used for this purpose. 20 µL of serum sample from each individual was added onto IMAC30 biochip, which was activated with 50 mmol/L $NiSO_4$ according to the manufacturer's manual, and kept at room temperature for 60 min. to enable the attachment of proteins onto the biochip surface. Afterwards, the biochip was washed with 100 µL phosphate buffer saline for 5 min. twice and with 100 µL distilled water twice removing the excess material. Analysis of the proteins attached to the biochip was performed by Protein Biosystem SELDI-TOF (Ciphergen Biosystems). Protein peaks obtained were read by ProteinChip Reader Model PBS II (Ciphergen) program and analyzed by ProteinChip Software (Ciphergen) program. Levels of BC1 (4300 Da), BC2 (8100 Da) and BC3 (8900 Da) proteins, which were reported to change in breast cancer patients, were evaluated in serum samples (5,8). Serum samples were compared to negative and positive controls. A decrease in BC1 protein level and an increase in BC2 and BC3 levels in serum samples were previously defined as risky for breast cancer. Individuals carrying a risk according to serum levels of BC1, BC2 and BC3 proteins were evaluated with bilateral mammography and breast ultrasonography for possible breast lesions. Individuals with pathologic lesions in radiologic asessments were followed.

Results

Eighteen healthy women were included in the study. Median age of the women was 33 (range, 28-38). No signs of increased breast cancer risk were detected in the past and family histories. Four women (22.2%) had significantly increased peak levels of BC2 and BC3 proteins compared to negative controls. In addition, same women showed a significant decrease in the peak level of BC1 protein. Protein profiles of the remaining 14 women were similar to negative controls.

As a result of the study, four women carrying a risk were evaluated by imaging modalities. One woman had cystic changes and complicated cysts in bilateral breast ultrasonography. Neither mastectomy nor tamoxifen prohylaxis was offered to four women carrying a risk. These women are closely followed.

Discussion

In this study, it was aimed to evaluate healthy individuals for breast cancer risk using SELDI-TOF-MS, which is one of the recently developed proteomics methods, and approximately one fourth of the studied individuals were found to carry a risk and included in follow-up program. Proteomics can be defined as the detection, identification, and quantification of all proteins present in a particular tissue, organ, and organism to provide accurate and comprehensive data about that system. Proteomics elucidates the properties of proteins, which cannot be understood by analyzing gene expressions such as post-translational modifications, compartmentalization of proteins, and formation of multi-protein complexes. In recent years, new methods have been developed in the field of proteomics. SELDI (surface-enhanced laser-desorption ionization) is one of these methods and defined for the first time by Hutchens and Yip (1). SELDI method captures proteins from various body fluids on diverse biochip surfaces using modified chromatographic techniques. Biochip surfaces can vary due to their chemical properties as hydrophobic, hydrophilic, cationic, or anionic and due to biological properties as antibody, DNA, enzyme, or receptor (2). Metal affinity biochips were used in this study. Proteins are combined with energy absorbing molecules at the surface of the biochip and pulsed with a laser into a mass spectrometer (MS). Advantages of MS technology are faster evaluation of small amount of protein samples, simple sample preparation, analysis of complex mixtures, and better data analysis.

The diagnosis of proliferative lesions such as atypical hyperplasia in the breast places the patients in a risk group, although the progression to invasive cancer is expected to occur in a small proportion of these patients. Defining the risky patients in a better way with recently developed molecular techniques will decrease the screening costs alleviating the anxiety of the individuals. SELDI-TOF MS is used to analyze proteins secreted by epithelial cells of the ductal system in the breast. Previous studies have reported different protein profiles for patients with and without breast cancer (3-5). In those studies using serum samples, diagnostic protein profiles showed sensitivities and specificities ranging between 76-93% and 90-93%, respectively (5-7). Li et al. studied 169 serum samples from patients with breast cancer (n=103), benign breast diseases (n=25), and healthy controls (n=41) and identified three protein peaks that separate breast cancer patient from non-cancer individuals with 93% sensitivity and 91% specificity (5). Proteins designated as BC1, BC2, and BC3 were used in the current study. These biomarkers were validated in a different set of serum samples from patients with breast cancer, benign breast diseases, and healthy controls (8). In this study, 61 patients with invasive breast cancer and 32 patients with in-situ breast cancer were included. Thirty-seven patients with benign breast disease and 46 healthy women formed the control group. In addition, these proteins were compared to previously identified proteins and two of these proteins were identified to be complement component $C3a_{desArg}$ and a C-terminal-truncated form of $C3a_{desArg}$ (8). Proteomic analysis of normal breast tissue and ductal carcinoma in situ revealed a difference in protein profiles between the two tissues suggesting that ductal carcinoma in situ is a preinvasive lesion (9).

In addition to the patients' serum samples, nipple aspiration fluid was used to identify breast cancer specific proteins utilizing proteomics methods. Varnum et al. identified 15 proteins that had been reported as potential biomarkers for breast cancer, but had not been previously identified in nipple aspirate fluid (10). Paweletz et al., similarly studying nipple aspiration fluid, detected two proteins unique to breast cancer and two proteins unique to normal samples (4). In addition to these studies, Sauter et al. reported on five differentially expressed proteins in nipple aspiration fluid samples and these proteins were present in 75-84% of breast cancer patients whereas this decreased to 0-9% in healthy controls (3). In contrast, in another study, nipple aspiration fluids from breast cancer patients were compared and no significant differences were identified in protein expressions between the breast with intact breast carcinoma and the contralateral noncancerous breast (11). However, nipple aspiration fluid analysis revealed several peaks that differentiate between both breasts of the cancer patients and healthy individuals. Li et al., in another study, using nipple aspiration fluid and ductal lavage fluids, identified three protein peaks, which differentiate breast cancer patients from risky women. These peaks were found to correspond to human neutrophil peptides 1 to 3 and persistent elevation of these peptides in risky women may imply early onset of breast cancer (12). Besides their functional activities in antimicrobial immunity, human neutrophil peptide expression has been shown in various tumor tissues and cell lines affecting tumor growth in a concentration dependent manner (12). Acetyl-LDL receptor is another biomarker related to early diagnosis of breast cancer. Its decreased concentration in nipple aspiration fluid compared to normal breasts indicates a strong likelihood of breast cancer or precancerous lesions. Similarly, a concentration difference of this protein between the two breasts of an individual may indicate the presence of breast cancer in the breast with lower concentration. Recently, protein profiling from serum samples was reported to differentiate highly suspicious lesions on mammography, which will result in a decrease in the number of unnecessary breast biopsies (13).

Today, despite certain restrictions, proteomics methods play an important role in the discovery of breast cancer related biomarkers and in the determination of the molecular mechanisms having a role in the conversion of proliferative or preinvasive lesions to breast cancer. In the current study, despite the small number of patients, women having a protein profile similar to breast cancer patients were determined among healthy individuals using SELDI-TOF-MS method. Following only these women closely will decrease work force and expenses compared to following all women.

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