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Original Article

Relationship of serum netrin-1 levels with breast masses

Serum netrin-1 seviyesinin meme kitleleriyle ilişkisi

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ABSTRACT

Introduction: Netrin-1 has been shown to induce angiogenesis and is considered to function as a proto-oncogene. We aimed to evaluate the relationship of serum netrin-1 level with the presence of breast mass and the nature of mass.
Methods: A total of 84 patients, including 27 patients with benign mass, 31 patients with malignant mass and 26 healthy controls, were enrolled in the study.
Results: In our study, the mean serum netrin-1 level was 479±325 pg/ml in the patients with malignant mass, 336.9±178.2 pg/ml in the patients with benign mass and 264.7±112.4 pg/ml in the healthy controls, respectively. There was a statistically significant difference in mean serum netrin-1 level between these three groups (p=0.007). When the patients participating in the study were divided in to two groups as those with (the benign and malign groups) and without (the control group) mass, the mean serum netrin-1 level was 264.7±112.4 pg/ml in those without mass and 412.8±274.2 pg/ml in those with mass, respectively. It was seen that the mean serum netrin-1 level of those with mass was statistically significantly higher than that of those without mass (p=0.016).
Conclusion: The mean serum netrin-1 level was found to be significantly higher in the patients with breast mass, especially those with malignant mass.
Keywords: Netrin-1, breast masses, breast cancer

ÖZ

Giriş: Netrin-1'in anjiyogenezi indüklediği ve proto-onkogen olarak işlev gördüğü gösterilmiştir. Serum netrin-1 düzeyinin meme kitlesi varlığı ve kitlenin yapısı ile ilişkisini değerlendirmeyi amaçladık.
Yöntem: Çalışmaya 27 benign kitlesi olan, 31 hasta malign kitlesi olan ve 26 sağlıklı kontrol olmak üzere toplam 84 hasta alındı.
Bulgular: Çalışmamızda malign kitlesi olan hastalarda ortalama netrin-1 düzeyi 479 ± 325 pg / ml, benign kitlesi olanlarda 336.9 ± 178.2 pg / ml ve sağlıklı kontrollerde 264.7 ± 112.4 pg / ml bulundu. Bu üç grup arasında ortalama serum netrin-1 seviyesinde istatistiksel olarak anlamlı bir fark vardı (p = 0.007). Çalışmaya katılan hastalar (benign ve malign gruplar) ve kontrol grubu olmak üzere iki gruba ayrıldığında, ortalama netrin-1 düzeyi kitlesi olmayanlarda ortalama 264.7 ± 112.4 pg / ml ve kitlesi olanlarda 412.8 ± 274.2 pg / ml. Kitle saptananların ortalama netrin-1 düzeyi kitle olmayanlara göre istatistiksel olarak anlamlı yüksek olduğu görüldü (p = 0.016).
Sonuç: Ortalama serum netrin-1 düzeyi, meme kitlesi olan, özellikle de malign kitle olan hastalarda anlamlı olarak daha yüksek bulundu.
Anahtar kelimeler: Netrin-1, meme kitleleri, meme kanseri

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Introduction

Breast cancer is the most common cancer among women in developed and developing countries in the world [1]. The number of women who are diagnosed with breast cancer is increasing day by day in the world. It has been reported that breast cancer affected more than 360,000 women and caused more than 90,000 deaths in the EU in 2012 [2]. Therefore, methods which will be developed for the early diagnosis of breast cancer are very important for women's health.

Netrins are a family of extracellular proteins that regulate cell and axon migration during embryogenesis. Three secreted netrins (netrins 1, 3 and 4) and two glycosylphosphatidylinositol (GPI)-anchored membrane proteins (netrins G1 and G2) have been described until now [3]. Netrin-1 is a laminin-related protein consisting of 640 amino acids. The main function of netrin-1 is to control neuronal navigation during development of the nervous system. Moreover, netrin-1 is not only present in the central nervous system and neuroepithelial cells, but also in other non-neuronal tissues [4-6]. Netrin-1 and its receptors DCC (deleted in colorectal cancer) and the UNC5 orthologues (human UNC5A-D and rodent UNC5H1-4) are identified as a new mechanism in the regulation of apoptosis [7]. The interaction of netrin-1 with these receptors regulates tumorigenesis by regulating inflammation, angiogenesis, and apoptosis [8,9]. Angiogenesis is a rate-limiting step for tumor growth [10]. Netrin-1 has been shown to induce angiogenesis. Netrin-1 can be considered to function as a proto-oncogene [11]. In many studies, the expression of netrin-1has been found to be elevated in breast cancer, lung cancer, colorectal cancer, pancreatic cancer, and glioblastoma [12-16]. Although it is not specific for a cancer type, it has been shown that netrin-1 may be a biomarker for the early detection of cancer [9].

The literature review we conducted shows that there are a limited number of studies on the relationship between serum netrin-1 levels and breast cancers. We aimed to evaluate the relationship of serum netrin-1 level with the presence of breast mass and the nature of mass. Thus, we want to find the relationship between serum netrin-1levels and breast tumors.

Methods

This study was carried out between October 1, 2016 and March 1, 2017 at the Breast and Family Medicine Outpatient Clinics, Konya Training and Research Hospital, University of Health Sciences. We evaluated 31 patients with breast cancer and 27 patients with benign mass who were admitted to the Breast Outpatient Clinic and 26healthy controls without any breast complaint and mass who were admitted to the Family Medicine Outpatient Clinic. A total of 84 patients satisfying the inclusion and exclusion criteria were enrolled in this study. The patients were informed about our study and then signed the informed consent form. The patients were asked to fill out the questionnaire form we prepared. Informed consent forms compatiple with the Helsinki Declaration of World Medical Association were recevied from each participant before the study. Permission was obtained from the Ethics Committee of Selcuk University for this study (2016/233).

Those who were not between the age group of 20-85 years, who were male, who underwent radiotherapy, chemotherapy or surgery for breast mass, who had benign or malignant masses in other body regions except for breast, who had cerebrovascular and neurological diseases, who had infectious diseases, and who received anticonvulsants were excluded from the study.

Laboratory Analysis

Blood Collection and Netrin-1 Analysis

Peripheral venous blood samples (5 cc) were collected into serum separator tubes (Vacuette, Greiner Bio-One, Kremsmuenster, Austria). The tubes were centrifuged at 1500×g for 10 min. For Netrin-1 analyses, 1,5 mL of serum were transferred to eppendorf tubes and stored at -80°C until analysis. Netrin-1 level was analyzed with a commercially available human enzyme-linked immunesorbent assay (ELISA) kit (catalogue no. SEB827Hu; USCN Life Science, Wuhan, China) by using Bio-Tek ELx800 Universal Microplate Reader and Bio-Tek ELx50 Microplate Strip Washer. The minimal limit of detection is 31.2 pg/mL.

Statistical Analysis

The categorical data were shown using frequency and percentage values, and the numerical data were shown using mean and standard deviation values. In analyzing the numerical data, the Mann-Whitney U test was used to compare two independent groups and the Kruskal-Wallis test (a non-parametric analogue of One-Way ANOVA) was used to compare three independent groups. The Chi-square test was used to compare the categorical data.For comparison of multiple groups, the One-Way ANOVA test was performed with the Bonferroni post hoc test.All statistical analyzes used in the study were performed bidirectionally within a 95% confidence interval andat a 5% significance level. SPSS® 21 (IBM Inc, USA) software was used for analysis of the data.

Results

The data for a total of 84 patients were evaluated in the study. The study comprised of 27 (32.1%) patients with benign mass, 31 (36.9%) patients with malignant mass and 26 (31%) healthy controls. The mean age was 41.81±2.12 years in the control group, 45.11±2.41 years in the benign group and 56.29±2.21 years in the malignant group, respectively. There was a statistically significant difference in mean age between the groups (p=0.000).The age of the malignant group was statistically significantly higher than those of the benign and control groups. BMI was 29.54±0.92kg/m² in the control group,27.93±1.11 kg/m² in the benign group and 30.35±1.01 in the malignant group, respectively. There was no statistically significant difference in BMI between the groups (p=0.238). The presence of menopause (p=0.001) and the duration of menopause (p=0.001) showed statistically significant differences between the groups; however, the number of births (p=0.259), the duration of breastfeeding (p=0.927) and the age at menarche (p=0.508) did not show statistically significant differences between the groups. 71% of the patients with

malignant mass had menopause for an average of 12.7 ± 8.6 years, while the presence of menopause was significantly lower in the other two groups. The comparison of the clinical characteristics of the study groups is presented in Table 1.

Table 1. Comparison of clinical characteristics of study groups

	Control (n=26)	Benign mass (n=27)	Malignant mass (n=31)	p
	Mean±SD	Mean±SD	Mean±SD	
Age	41.81±10.83	45.11±12.56	56.29±12.31	<0.001
BMI	29.54±4.72	27.93±5.80	30.35±5.65	0.238
Number of births	2.4±1.2	2.5±1.8	3.1±2.1	0.259
Duration of breastfeeding (month)	27.4±21.6	25.1±18.4	36.8±44.3	0.927
Age at menarche (year)	13±1	13±1.2	13.3±1.2	0.508
Presence of menopause				<0.001
No	19 (73.1)	18 (66.7)	9 (29)	
Yes	7 (26.9)	9 (33.3)	22 (71)	
Duration of menopause (year)	3±3.2	5±7.2	12.7±8.6	<0.001

When the netrin levels of the study groups were examined, the mean serum netrin-1 level was 479±325 pg/ml in the patients with malignant mass, 336.9±178.2 pg/ml in the patients with benign mass, and 264.7±112.4 pg/ml in the healthy controls, respectively. There was a statistically significant difference in mean serum netrin-1 level between these three groups (p=0.007) (Table 2). The mean serum netrin-1 level of the malignant group was statistically significantly higher than that of the control group (p=0.020). There was no statistically significant difference in mean serum netrin-1 level between the benign and malignant groups (p=0.065). There was no statistically significant difference in mean serum netrin-1 level between the benign and control groups (p=0.074).

Table 2. Netrin values of study groups

	Control (n=26)	Benign mass (n=27)	Malignant mass (n=31)	p
	Mean±SD	Mean±SD	Mean±SD	
Netrin	264.7±112.4	336.9±178.2	479±325	0.007

SD;Standard deviation

When the patients participating in the study were divided into two groups as those with (the benign and malign groups) and without (the control group) mass, the mean serum netrin-1 level was 264.7±112.4 pg/ml in those without mass and 412.8±274.2 pg/ml in those with mass, respectively. It was seen that the mean serum netrin-1 level of those with mass was statistically significantly higher than that of those without mass (p=0.016) (Table 3).

Table 3. Netrin values of patients with and without mass

	Mass (-) (n=26)	Mass (+) (n=58)	p
	Mean±SD	Mean±SD	
Netrin	264.7±112.4	412.8±274.2	0.016

SD;Standard deviation

Discussion

Different biomarkers have been evaluated to diagnose breast cancer. Despite advances in Medicine in recent years, there are still significant difficulties in the diagnosis of breast cancer. For this reason, this study aimed to realize possible new targets to diagnose breast cancer.

The role of netrin-1 and its receptors on cancer development and survival is well known. Increased expression of netrin-1 or loss of netrin-1 receptor has been shown to play an important role in cancer development and progression [9]. The apoptosis-mediated regulatory function of netrin-1 receptor in adults is related to the initiation and progression of breast and bowel tumors. Ramesh et al. [9] reported that serum netrin-1 levels significantly increased in breast, renal, prostate, liver, meningioma, pituitary adenoma and glioblastoma cancers compared with control group. In our study, the mean serum netrin-1 level was statistically higher in those with breast mass than in those without breast mass.

In the study where Fitamant et al. [13] examined human and mouse breast cancer tissues, they reported that netrin-1 can be considered as a marker of the breast cancer's ability to spread around the body and that the expression level of netrin-1 in the majority of primary breast tumors capable

of metastatic spread was elevated. In our study, the mean serum netrin-1 level of the malignant group was statistically significantly higher than that of the control group. The mean serum netrin-1 level of the malignant group was statistically significantly higher than that of the benign group, but the difference between them was not statistically significant.

Yıldırım et al. [17] found that serum netrin-1 level of patients with non-small-cell lung cancer was statistically significantly higher than that of control group. The results of our study show that there is a strong association between the serum netrin-1 levels and the presence or absence of tumors. For this reason, netrin-1 can be used as a biomarker in breast tumors.

Limitations of the Study

Our study has some limitations. Firstly, there were a small number of patients in the study(n=84). Secondly, we do not know if it is associated with other serum biomarkers. To answer all these questions, there is need for more comprehensive studies including large number of patients.

Conclusion

The mean serum netrin-1 level was found to be significantly higher in the patients with breast mass, especially those with malignant mass. Increased serum netrin-1 levels suggest that it may be a new universal biomarker for human breast cancer, although it is not specific for a cancer type.

Conflict of interest: None.

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