ORIGINAL ARTICLE

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Predictive Value of Serum Calprotectin Level in Response to Treatment, a New Inflammatory Marker in Patients with Breast Cancer Requesting Neoadjuvant Treatment

Neoadjuvan Tedavi Alan Meme Kanserli Hastalarda Yeni Bir Enflamatuvar Belirteç Olan Serum Kalprotektin Düzeyinin Tedaviye Yanıtta Prediktif Değeri

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ABSTRACT

Aim: There is a close relationship between inflammation and cancer. Calprotectin is a protein released during inflammation. The aim of this study is to investigate the relationship between breast cancer and calprotectin levels in breast cancer patients receiving neoadjuvant therapy the predictive role of calprotectin in response to treatment.

Materials and Methods: In our prospective study, a patient group with 69 breast cancer patients and a control group with 20 patients were formed. Calprotectin was studied from the blood tests taken from the whole sample. Patient data were obtained from the electronic record system. In our study, statistical evaluations were made using a package program called IBM Statistical Package for the Social Sciences Statistics 24.

Results: Eighty-nine patients (69 cancer, 20 controls) were included in the study. The median age of breast cancer patients was 48 [minimum (min): 24-maximum (max): 73], the control group was 44.5 (min: 19-max: 68) and the ages of the two groups were similar (p=0.599). Mean calprotectin levels in breast cancer patients were 28.63±30.5, median 16.5 (min: 6.7-max: 160.7). The mean in the control group was 16.09±6.1 (min: 8.7-max: 27.4) and there was no statistical difference between the 2 groups (p=0.072). A statistically significant difference was found in terms of calprotectin values according to Ki67 classes (Z=-20.043; p=0.041). Calprotectin values of those with Ki67 class >20 were statistically significantly higher than those with ≤20. Parameters that could predict complete chemotherapy response were evaluated with logistic regression analysis. There was no correlation between calprotectin level and complete response. There was a positive correlation between age increase and complete response.

Conclusion: There was no significant difference between serum calprotectin levels of the patient and control groups, but calprotectin level was found to be associated with Ki67 level. There was no relationship between calprotectin and chemotherapy response. Studies with larger sample numbers may make a significant difference.

Keywords: Breast cancer, serum calprotectin, inflammation, complete response

ÖZ

Amaç: Enflamasyon ile kanser arasındaki yakın ilişki vardır. Kalprotektin enflamasyon sırasında salınan bir proteindir. Bu çalışma ile neoadjuvan tedavi alan meme kanserli hastalarda kalprotektin seviyesi ile meme kanseri ilişkisi ve tedavi yanıtı için kalprotektinin prediktif rolünün araştırılması amaçlanmıştır.

Gereç ve Yöntem: Prospektif bir araştırma olarak dizayn edilen çalışmamızda 69 meme kanseri tanılı hasta ile hasta grubu ve 20 hasta ile kontrol grubu oluşturuldu. Örneklemin tamamından alınan kan tetkiklerinden kalprotektin çalışıldı. Hasta verileri elektronik kayıt sisteminden elde edildi. Çalışmamızda istatistiksel değerlendirmeler IBM Statistical Package for the Social Sciences Statistics 24 adlı paket program kullanılarak yapıldı.

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Bulgular: Çalışmaya 89 hasta (69 kanser, 20 kontrol) dahil edildi. Meme kanseri hastalarının median yaşı 48 [minimum (min): 24-maksimum (maks): 73], kontrol grubunda 44,5 (min: 19-maks: 68) olarak saptandı ve iki grubun yaşları benzerdi (p=0,599). Meme kanseri hastalarında kalprotektin düzeylerinin ortalaması 28,63±30,5, median 16,5 (min: 6,7-maks: 160,7) saptandı. Kontrol grubunda ortalama 16,09±6,1 (min: 8,7-maks: 27,4) olarak görüldü ve 2 grup arasında istatistiksel fark yoktu (p=0,072). Ki67 sınıflarına göre kalprotektin değerleri açısından istatistiksel olarak anlamlı farklılık tespit edildi (Z=-20,043; p=0,041). Ki67 sınıfı >20 olanların kalprotektin değerleri, ≤20 olanlara göre istatistiksel olarak anlamlı düzeyde daha yüksekti. Kemoterapi tam yanıtını ön görebilecek parametreler lojistik regresyon analizi ile değerlendirildi. Kalprotektin düzeyi ile tam yanıt arasında ilişki yoktu. Yaş artışı ile tam yanıt arasında pozitif bir ilişki vardı.

Sonuç: Hasta ve kontrol grubunun serum kalprotektin düzeyleri arasında anlamlı fark saptanmadı fakat kalprotektin seviyesi Ki67 düzeyi ile ilişkili bulundu. Bu önemli ilişkisine rağmen kalprotektin ile kemoterapi yanıtı arasında ilişki yoktu. Daha büyük örneklem sayıları ile yapılacak çalışmalar anlamlı bir fark oluşturabilir.

Anahtar Kelimeler: Meme kanseri, serum kalprotektin, enflamasyon, tam yanıt

INTRODUCTION

Breast cancer was reported to be the second most frequently diagnosed cancer in 2018, and it is the most common cause of cancer-related death in women[1]. Neoadjuvant therapy, which is an important modality in the treatment of breast cancer, is defined as all systemic treatments applied to the breast tumor before surgical operations[2]. Although discussions continue, it has been found that patients with a complete response to neoadjuvant chemotherapy, especially in HER2-positive and triple-negative breast cancers, experience better results, have a longer disease-free survival, and have a higher overall survival compared to those without any response[3,4].

Calprotectin is a heterodimeric calcium-binding protein consisting of S100A8 and S100A9 subunits from the family of S100 proteins[5]. Calprotectins are expressed in a wide variety of cell types, but are particularly abundant in myeloid cells such as macrophages in the early differentiation stage, neutrophils, monocytes, and keratinocytes. After being released from activated granulocytes, S100A8/S100A9 binds to cell surface receptors that trigger signaling pathways associated with inflammatory processes, with a cytokine-like behavior pattern, and plays a critical role in numerous cellular processes such as cell cycle progression, cell survival, proliferation, differentiation and cell migration[6,7].

In this study, it was aimed to compare the serum calprotectin levels of patients with breast cancer planned to receive various neoadjuvant treatments and the serum calprotectin levels of patients without breast cancer, to try to determine the cut-off value for calprotectin if found to be associated with the diagnosis, to investigate the relationship between the calprotectin level and the response to the treatment in the breast cancer group, and to evaluate the relationship between calprotectin and other blood parameters, already known to be related to the inflammatory processes.

MATERIALS AND METHODS

In this study, which is planned to be a specialty thesis in medicine, the screening model, one of the quantitative research models, and the relational screening model, one of the sub-types of survey models, were used.

Selection and Definition of Cases

Our study included 89 patients who were admitted to Tekirdağ Namık Kemal University Hospital, Department of Internal Medicine Diseases, Unit of Medical Oncology and Department of General Surgery, Breast Outpatient Clinic between 15.03.2019 and 19.10.2020. While the "patient arm" of our study was formed with 69 patients diagnosed with breast cancer as a result of their application to the relevant polyclinic and planned to receive neoadjuvant treatment in the multidisciplinary council of our hospital, the "control group" of our study was also formed with 20 patients who applied to the same polyclinics with the suspicion of breast cancer but were not found to have malignant pathology. Patients over the age of 18 years, who gave the consent form prepared for participation in the study, were included in the study. Those who were pregnant, who did not sign the voluntary consent form, who had additional malignancies, who had a diagnosis or sign of infection during sampling and evaluation, and who had hematological or rheumatological disorders were excluded from the study. Patient data were obtained from the hospital archive and the hospital electronic recording system.

Calprotectin Measurement

After the decision was made to include the patients in the study and their written and verbal consents were obtained, 5 mL of venous blood sample was taken to study the calprotectin levels of the patient in addition to the routine blood tests. These tubes were centrifuged at 2000 G for 20 minutes and at room temperature. After centrifugation, the supernatant was aliquoted into two microtubes as it helped to partition the separator gel in the tube. These serum samples, which were taken into microtubes, were labeled and stored at -20 °C to be stored until the time of batch analysis. Analysis of serum calprotectin levels from samples was performed with a commercially produced kit based on the enzyme-linked immunosorbent analysis method (Bioassay Technology

Laboratory, Cat. No: E4010Hu). It was ensured that the person performing the experiments was blinded in terms of the knowledge of the groups in which the studied blood samples were included. After all samples were collected, they were analyzed with the same kit. Evaluation was made by following all the instructions and experimental processes specified in the commercial kit.

Statistical Analysis

In our study, statistical evaluations were made using a package program called Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics 24). Categorical measurements were summarized as numbers and percentages, and continuous measurements as median and standard deviation. Comparison between two qualitative clinical variables was analyzed with the "Mann-Whitney U test", "independent sample-t test" and "Kruskal-Wallis H test". The "Fisher's exact" and "Pearson-χ^2" crosstabs were used according to expected value levels in the examination of the relationships between two qualitative variables. The relationship of calprotectin level with treatment response was evaluated with ROC curve and ROC-AUC analysis. Logistic regression model was used in predictive factor analysis for chemotherapy response.

RESULTS

Eighty-nine patients (69 cancer, 20 control) were included in the study. The median age was 48 [minimum (min): 24-maximum (max): 73] years in patients with breast cancer and 44.5 (min: 19-max: 68) years in the control group, and the ages of the two groups were similar (p=0.599). Of the cancer patients, 33% were ER negative, 46% were PR negative, and 54% were HER2 negative (Table 1).

In breast cancer patients, the mean calprotectin level was found to be 28.63±30.5, the median level was 16.5 (min: 6.7-max: 160.7). In the control group, the mean calprotectin level was detected to be 16.09±6.1 (min: 8.7-max: 27.4) and there was no statistical difference between the two groups (p=0.072). A statistically significant difference was found in terms of calprotectin values according to Ki67 categories (Z=-20.043; p=0.041). Calprotectin values of those with Ki67 level >20 were statistically significantly higher than those with ≤20.

Calprotectin level, which predicted complete response, was not diagnostically predictive of complete response when analyzed with ROC-curve (p=0.587, AUC: 0.453) (Figure 1).

Parameters that could predict complete chemotherapy response were evaluated with logistic regression analysis. There was no correlation between calprotectin level and complete response [Odds ratio (OR): 1.049 95% confidence interval (CI): 0.982-1.120, p=0.153]. There was a positive correlation between increasing age and complete response (OR: 1.092,

Table 1. Clinical and pathological characteristics of the patients included in the study

	Cancer (n=69)	Control (n=20)
Age		
<45	30 (43%)	12 (60)
≥45	39 (57%)	8 (40%)
Estrogen		
Negative	23 (33%)	
Positive	46 (67%)	
Progesterone		
Negative	32 (46%)	
Positive	37 (54%)	
Ki67		
<20	26 (38%)	
≥20	43 (62%)	
HER2		
Negative	37 (54%)	
Positive	32 (46%)	
Grade		
1	12 (17%)	
2	30 (43%)	
3	27 (39%)	
Tumor type		
Invasive ductal	59 (85.5%)	
Other	10 (14.5%)	

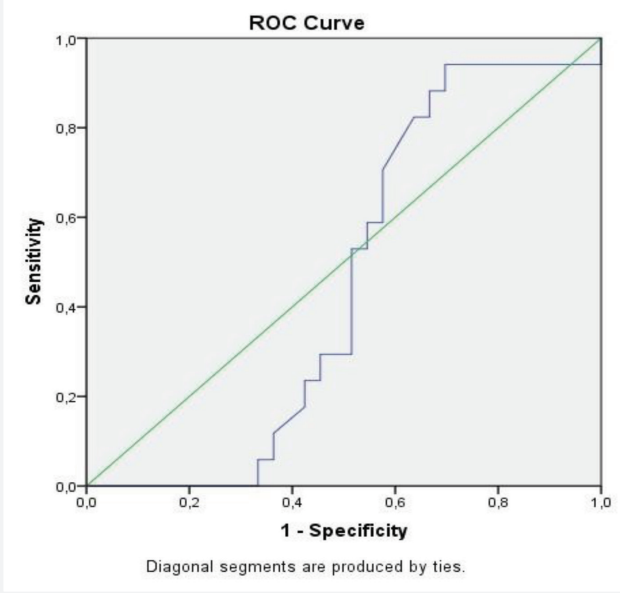

Figure 1. Cut-off determination of calprotectin level, which can predict complete response, with ROC-curve

95% CI: 1.022-1.168, p=0.010). Estrogen negative (OR: 0.284 95% CI: 0.082-0.984, p=0.047) and progesterone negative (OR: 0.238 95% CI: 0.067-0.841, p=0.026) were more likely to have complete responses (Table 2).

DISCUSSION

In our study, the value of serum calprotectin level in the diagnosis of breast cancer and response to treatment was investigated. In our analyses, we found that the calprotectin level was higher in those with high Ki67. Although there was a numerical difference in calprotectin levels between the included patient and control groups, no statistically significant difference was found, whereas pre-treatment calprotectin level could not predict treatment response. Age, estrogen receptor and progesterone receptor were predictive for complete response to neoadjuvant chemotherapy.

In the study of Khorrami et al.[8] investigating the value of calprotectin for the diagnosis of breast cancer, it was reported that serum calprotectin level could be a valuable marker in the diagnosis of cancer patients. According to the study of Gunaldi et al.[9], which was published in 2015 and examined the diagnostic importance of S100A9 and S100A12 proteins for breast cancer, the S100A9 protein, one of the 2 sub-units of the calprotectin protein, could not distinguish breast cancer from the control group. In our study, calprotectin levels were similar in cancer and control groups, and there was no statistical difference between the two groups. These differences between the studies may be related to the histological types of the patients included. While Gunaldi et al.'s[9] study included different histological types as in our study, Khorrami et al.'s[8] study consisted of only invasive ductal types. Future studies involving homogeneous patients will clarify this situation.

There was no significant difference between the serum calprotectin levels of the patient and control groups. However, while the mean serum calprotectin value was 28.63±30.46 in the patient group, it was found as 16.09±6.12 in the control group. The mean serum calprotectin value was found to be 40.03±1.54 for the control group consisting of 30 healthy volunteers in the study of Zaki et al.[10], who used exactly the same serum calprotectin kit we used in our study with all procedures. Although statistical comparison analysis was not performed for the control group in our study, we think that it was relatively high. However, unlike our study, the rate of male volunteers was 66.7% and the mean age was 32.30±11.43 years in the control group of this study, which was quite different in design from our study in terms of sample distribution[10]. In the current publication of Shaik et al.[11] in 2021, it was reported that even though benign breast disease was diagnosed, there was a significant increase in inflammatory markers in the benign diagnosis periods of patients with a high risk of breast cancer afterwards. It also comes to mind that this may be the reason why the inflammatory markers, including the calprotectin value, of the patients in the control group of our study were similar to the patient group because in the design of our study, clinical follow-up of the patients diagnosed as benign was not carried out to determine whether these pathologies converted to malignancy within a specified period.

Table 2. Logistic regression analysis of variables to predict complete response after neoadjuvant chemotherapy

		Univariate analysis	
Variable	**Category**	**OR (95% CI)**	**p**
Calprotectin	Continuous	1.049 (0.982-1.120)	0.153
Age	Continuous	1.092 (1.022-1.168)	**0.010**
Ki67	≤20/>20	0.392 (0.113-1.364)	0.141
NLR	Continuous	0.635 (0.336-1.200)	0.162
Estrogen	-/+	0.284 (0.082-0.984)	**0.047**
Progesterone	-/+	0.238 (0.067-0.841)	**0.026**
HER2	-/+	1.714 (0.525-5.603)	0.372

Statistically significant p values were marked in bold.
NLR: Neutrophil/lymphocyte ratio, CI: Confidence interval, OR: Odds ratio

Analyses were made in order to evaluate the patient and control groups in our study in terms of our main variable, and in these analyses, a difference was determined in terms of age. The age of our patient group was higher than that of the control group. Due to this difference, when the change in serum calprotectin value with age variable is examined from the literature, it is seen that many studies indicate that age is not an effective parameter. In the study of Oosterwijk et al.[12] in 2020, in which they examined variables that might be determinants of serum calprotectin levels in patients with diabetes mellitus, the age variable was determined as an insignificant parameter in this respect (r=-0.035 for age, p=0.051). In addition, in a study by Zaki et al.[10] in 2019, evaluating the relationship between clinical severity and serum calprotectin levels in psoriasis patients, the age variable was not found to be associated with serum calprotectin levels (r=0.214; p=0.136).

In our study, when two groups were formed according to the 20 cut-off value of Ki67 level, serum calprotectin value was found to be significantly higher in those with Ki67 above 20. Ki67 is one of the most well-known predictors of chemotherapy response[13,14]. Ki67 is a parameter that is generally thought to be expressed less than 3% in healthy breasts and has been used in routine pathology reports in the last 10 years[15]. At the same time, the number of studies reporting that Ki67 is prognostic has been increasing recently[16,17]. However, in our treatment response analysis in this study, Ki67 did not show predictive properties for treatment response. Nevertheless, the results of our study are promising for serum calprotectin values in response to treatments or in follow-up, since it is known that there is a significant correlation between the increased percent

expression of Ki67 value and the clinical aggressiveness of the tumor.

Current oncology guidelines report that luminal breast cancer subtypes have a worse response to chemotherapy[18]. Chou et al.'s[19] study in 2019 and Verdial et al.'s[20] study in 2022 evaluated the predictors of response to neoadjuvant chemotherapy in locally advanced breast cancers, and young age was determined to predict complete response. In our study, age and hormone receptors were found to be predictive for treatment response. Hormone receptor positive patients and older patients gave worse response to the treatment.

Study Limitations

There were some limitations regarding our study. The most important limitation was the small number of patients included in the study and the single center design. In addition, the heterogeneity of the included breast cancer patient groups was our other limitation. However, it is important that this study is the first to our knowledge to evaluate calprotectin for breast cancer treatment response.

CONCLUSION

In conclusion, the level of calprotectin did not differ significantly between the normal population and breast cancer patients, and there was no relationship between calprotectin and chemotherapy response. Further studies should investigate the relationship between calprotectin and breast cancer treatment response, with a larger number of patients and a more homogeneous patient population.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University of Local Ethics Committee (protocol no: 2019.206.11.03, date: 26.11.2019).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.B., S.Ö.G., Concept: E.B., S.Ş., Design: E.B., Data Collection or Processing: E.B., A.Ç., S.Ö.G., Analysis or Interpretation: E.B., A.Ç., Literature Search: E.B., S.Ş., Writing: E.B.

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