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Examining the Effect of and Relationship Between Grade and Stage of Tumor with Neutrophil to Lymphocyte Ratio in Women with Breast Cancer

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Abstract

Introduction and purpose: Cancer is the third leading cause of mortality after coronary disease and incidents. Breast cancer is a malignant proliferation of epithelial cells of the breast ducts or lobules. Breast cancer is one of the main problems of the health system for women in all countries, such as Iran. Annually, more than 1.3 million people are diagnosed with breast cancer in the world. The present study examined the effect of and the relationship between degree and stage of the tumor with neutrophil to lymphocyte ratio in patients for sooner diagnosis. **Materials and methods:** the sample was all female patients, 178 patients, with breast cancer aged 18-70 admitted to Hematologist Clinic from 2010 to 2015 for diagnosis and treatment. The initial CBC and the results of hormonal and respiratory analyses as well as the pathologic results of these patients were examined and analyzed and after studying the patients' records, their information entered to Spss18.0 software and analyzed. **Results:** The results indicated a relationship between neutrophil to lymphocyte ratio with sickness stage. **Discussion and conclusion:** the overall results showed that with increase in the rate of tumor progression and its metastasis, NLR increases, but the degree of tumor or grade has no relationship with NLR.

Keywords: neutrophil to lymphocyte ratio, prognostic, tumor grade, tumor stage, breast cancer

Introduction

Malignancies are a group of diseases where the cells grow uncontrollably (Enayatrad and et al, 2015). Breast cancer is a malignant proliferation of the epithelial ducts or breast lobes (Kasper and et al, 2015). Breast cancer is one of the main problems of the health system for women in all countries, including Iran (Hajizadeh and et al, 2015; Sharifian and et al, 2015). Over 1.3 million people are diagnosed with breast cancer in the world every year (Ghoncheh and et al, 2015), of which 502,000 people die due to the disease (Hajizadeh and et al, 2015; Sharifian and et al, 2015).

The incidence of breast cancer in women is about one third of all their cancers, which causes the disease to be the second most common malignancy in women after lung cancer and the first malignancy in terms of mortality among women. Moreover, the prevalence of this disease among Iranian women is increasing (Hajizadeh and et al, 2015). According to a report by World Health Organization (WHO), the incidence of breast cancer increases by 2% annually (Otaghvar and et al, 2015). In Iran, breast cancer is the most common cancer among women's malignancies, accounting for 24.4% of malignancies (Sharifian and et al, 2015; Taheri and et al, 2012; Radmard, 2010) . Breast cancer affects Iranian women at least 10 years earlier than women in developed countries (Otaghvar and et al, 2015; Radmard, 2010) and most of the patients in Iran are between the ages 40-49 (Otaghvar and et al, 2015)

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The patient or the doctor usually diagnoses breast cancer by mammography or after touching a lump in the chest (Benjamin and et al, 2015). Tumor findings like the size, grade of the tumor and the amount of lymph nodes involved are as the most significant prognostic factors in breast cancer. The factors related to the disease like obesity, smoking, alcohol and aging can affect the outcome of the disease as well (Vostakolaei and et al, 2012).

Neutrophil to lymphocyte ratio (NLR), obtained by counting the exact number of neutrophils to the exact number of lymphocytes in the blood, can be connected both with prognosis and chemical susceptibility (Asano and et al, 2016). The combined ratio of these two, indicating neutrophil count to lymphocyte as NLR, is a simple and cost-effective yardstick for the evaluation of systemic inflammation and stress (Noh and et al, 2013; Kumar and et al, 2015). Stage tells the physician how big the tumor is and how much it has released in the body. In which stage the tumor is most likely to be determined by imaging studies. There are several systems for staging bone cancer. One of the most widely used ones is Ennaking System. Patient staging information (tumor size, involved lymph nodes and the presence of metastases in them) with examination and ultrasonography, mammography, chest CT scan, abdominal CT scan, Brain CT-scan and biopsy at the beginning of the stage-based diagnosis are approved by the American Oncology Hematology Association. Grade of a cancerous tumor is determined by its microscopic examination. A grade one tumor tells the physician how it behaves. Low-grade tumors are the ones that are somewhat abnormal in the microscopic study of tumor cells. These tumors are less active and the probability of their release is low. In contrast, high-grade cancers are the ones whose cells are so abnormal in microscopic study. These tumors grow fast and spread fast in the surrounding and distant tissues.
The rate of tumor progression and its prognosis are largely affected by the inflammatory response of the host cells in the areas around the tumor (Koh and et al, 2015). Cancer-related inflammatory responses contribute to proliferation, the survival of malignant cells, angiogenesis and metastasis of cancer cells and affect the disease response to chemotherapy drugs. Severe inflammatory responses are due to a weaker immune system created due to an imbalance between the inflammatory response and the malignant cells, which in overall increases the survival of the malignant cells and reduces the overall survival of the patient (Chen and et al, 2015).

In a study conducted in Turkey in 2017 on 255 patients with breast cancer admitted from 2010 to 2014, the results were as follows: there is no relationship between neutrophil to lymphocyte ratio and grade of the disease as well as lymph node and metastasis and size. According to the results, there was no relationship between the value of NLR in the treatment and prediction of the status of patients with breast cancer (Yersal and et al, 2017). In a retrospective cohort study in 2015, 1570 breast cancer patients at Stage I-III admitted to San Yat-sen Memorial Hospital in China from 2000 to 2010 were studied. The results of the study and follow-up of the patients during these years showed that NLR is an important inflammatory factor in predicting OS and DFS in patients with breast cancer, especially in TNBC (Jia and et al, 2015). In another retrospective study by Pistelli et al. in 2015, 90 patients with stage I-III breast cancer undergoing treatment in clinics from 2006 to 2012 were examined. There was no significant correlation between NLR and pathologic factors in the study. TNBC patients with high NLR had significantly lower DFS and OS compared to the patients with NLR (Pistelli and et al, 2015).

The mentioned points and the studies conducted due to the increasing prevalence of breast malignancies in Iran, the resulting mortality, and its therapeutic burden on the health system were the cases prompting the study. Moreover, considering the effect of inflammatory factors like neutrophil to lymphocyte ratio in the prognosis of many malignancies, as well as the importance of grade and stage of disease in prognosis and selective breast cancer treatment, this research studied the difference in inflammatory factor (NLR) in breast cancer patients in Semnan.

Methodology

Type of study:
The study was retrospective.
Population and the sample: The population was female patients with breast cancer. The sample was all female patients with breast cancer aged 18-70 who referred to a hematologist clinic in Semnan from 2010 to 2015 for diagnosis and treatment that entered the study after considering the inclusion and exclusion criteria. The number of these patients was estimated 178.
Sampling method: The sampling method was convenience sampling.
Inclusion criteria: All female patients were aged 18-70, who had cancer according to the biopsy of breast masses in the past five years.
Exclusion criteria:
1) The patients with metastases at the time of diagnosis
2) The patients with active simultaneous infection
3) The patients with inflammatory cancer
4) The patients treated with steroids
5) Patients with autoimmune or hematic diseases
6) The patients who were pregnant at the diagnosis time
7) The patients with CHF
8) The patients with CAD (IHD)

9) Smokers
10) The patients treated with lithium

Research procedure:

This study began after obtaining permission from the Ethics Committee and the University's Research Deputy. The study examined the records of all women with breast cancer between aged 18-70 in Semnan who had been admitted to hematologist clinic for diagnosis and treatment and lacked the exclusion criteria. In each patient, NLR was calculated according to the CBC test performed at the beginning of the diagnosis. Then the patients were divided according to grading and TNM and the NLR was calculated and compared in each group. Patients' staging and metastasis were determined by ultrasound, mammography, chest CT scans, abdominal CT scan, Brain CT-scan, and biopsy at the beginning of the diagnosis, and written down in each patient's record. Furthermore, according to the existing papers and other studies, NLR was considered with cutoff=2.5. The patients with the probability of an inflammatory disease and an increase in NLR or the patients using drugs such as corticosteroids that had an effect on NLR were excluded from the study. Finally, all the information was included in the data collection forms already prepared by the researcher for this purpose. This was carried on until the specified sample size was completed.

Data collection tool: Data collection was done using direct observation records of patients located in the office's digital archives, recorded in the data collection form previously prepared for this purpose.

Data analysis method: Kolmogorov-Smirnov, Mann-Whitney, Kruskal-Wallis tests and Spearman correlation coefficient were used for data analysis. The significance level was 5% and the software used was SPSS 18.0.

Ethical considerations:
1) The information about each patient was completely confidential.
2) Excessive costs were not imposed on the patient for examinations.

Results

The degree of tumor differentiation was excellent in 6.7% (12 subjects) of the patients, 66.9% (119 subjects) moderate, and 26.4% (47 subjects) with very little differentiation. There was no significant correlation between NLR level and tumor grade (p =0.287) (r = 0.08) (Table 1).

Table 1- Mean, standard deviation, median, and Interquartile range of NLR in women with breast cancer with varying degrees of tumor

Tumor degree	Sample	Mean	SD	Median	Interquartile range
Excellent differentiation	12	1.43	0.61	1.34	0.49
Moderate differentiation	119	1.83	0.87	1.55	0.90
Little differentiation	47	1.90	1.02	1.56	0.96

Out of 177 patients whose tumor stage was mentioned, the most prevalent stage was n2 (27%). There was a significant correlation between NLR and tumor stage (r = 0.206, p = 0.006), meaning that with increasing tumor stage, NLR increased (Table 2).

Table 2- Mean, standard deviation, median, and Interquartile range of NLR in women with breast cancer at different stages of tumor

Tumor stage	Sample	Mean	SD	Median	Interquartile range
0	1	1.55	-	-	-
IA	26	1.50	0.65	1.35	0.61
IIA	48	1.67	0.85	1.42	0.57
IIB	36	2.04	1.00	1.83	1.15
IIIA	30	1.89	0.89	1.52	1.44
IIIB	2	0.98	0.40	0.98	-
IIIC	8	2.14	0.90	2.01	1.45
IV	26	2.04	1.01	1.98	0.91

Discussion and Conclusion

Malignancies are a group of diseases where the cells grow uncontrollably. Breast cancer is a malignant proliferation of the epithelial ducts or breast lobes. Breast cancer is one of the main problems of the health system for women in all countries, including Iran. Inflammation and cancer are closely related to one another, so that the inflammation causes the cancer and contraction of the inflammation. Neutrophil to lymphocyte ratio can be used as a parameter reflecting the immune response against inflammation. In various studies, the association of this ratio with various cancers has been proven, but its relationship with breast cancer is still under discussion (Liu and eta l, 2017)

Neutrophils are known as the most abundant leukocytes in the blood and as the first line of defense in infections and inflammation. Moreover, neutrophils are considered as an increasing marker in neutrophil-based tumors, which can increase the possibility of tumor metastasis and bad prognosis and can be useful to the patient through anti-tumor activity to some extent. How neutrophils increase in tumors is not completely clear, but probably due to Granulocyte Macrophage Colony-Stimulating Factor (GMCSF) and other inflammatory cytokines such as interleukin one and 6 released by tumor cells. The neutrophil to lymphocyte ratio increases in tumors (Enayatrad and et al, 2015).

Tumor results such as the size, grade of the tumor, receptor status, and the rate of lymph nodes involved are considered as the most important prognostic factors in breast cancer. Patient-related factors such as obesity, smoking, alcohol consumption and age can also affect the outcome of the disease (Vostakolaei and et al, 2012). There were no significant relationships between tumor grade and neutrophil to lymphocyte ratio ($p=0.287$) in the present study that was in line with a study conducted in Turkey in 2017 on 255 patients with breast cancer admitted from 2010 to 2014. The results were as follows: There was no relationship between neutrophil to lymphocyte ratio and the grade of disease. According to the results of this study, NLR value have no value in the treatment and prediction of the condition of patients with breast cancer, inconsistent with the current study. This may be because the study they performed had different results with other studies and did not even consider NLR as a predictor for breast cancer (Yersal and et al, 2017).

In the present study, tumor stage had a direct and significant relationship with neutrophil to lymphocyte ratio ($r = 0.206$ $p= 0.006$), showing that neutrophil to lymphocyte ratio increases with increase in tumor stage. In a study by Yersal et al. (2017), there were no relationships between neutrophil to lymphocyte ratio and lymph node and metastasis, and tumor size, which was unable to predict the status of patients with breast cancer (Yersal and et al, 2017). In addition, in a study by Eliasinia et al. (2017) on 195 patients under the supervision of the Tehran University of Medical Sciences, there was a significant relationship between NLR and vascular invasion, which is inconsistent with the present study maybe because they had just studied invasive vessels and not examined all stages of the tumor (Elyasinia and et al, 2017).

Thus, in the present study, there were no significant correlations between the receptors examined in breast cancer with neutrophil to lymphocyte ratio. There was no significant relationship between neutrophil to lymphocyte ratio and tumor grade, but the relationship between NLR and disease state, showing localized and metastatic progression of the disease, was significant. Accordingly, using NLR in early tests and follow-up of the patients with breast cancer can be a contributing factor in determining the degree of progression of the disease. Specifically, in the periodic follow-up of treated patients, using NLR and considering its changes is a good guide to the possibility of relapse or metastasis, or local tumor progression. However, considering the sample size used in this study, similar studies with more samples in this regard are needed.

Among the limitations of the study, one can state that the number of patients examined was less than expected. Furthermore, the patients in Semnan are limited and known and other intervening factors should be taken into account and analyzed separately.

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