Original Article

Investigating neutrophil to lymphocyte ratio associated with the prognostic factors in women with breast cancer

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ABSTRACT

Cancer is the third cause of death after coronary diseases and accidents. 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, including our country. Annually more than 1.3 million people with breast cancer are recognized worldwide, with hormones playing an important role in the development of breast cancer. Investigating the factors and tumor markers are highly effective in diagnosis. Therefore, in this study, the effect and relationship between tumor markers and the neutrophil to lymphocyte ratio in patients were investigated for earlier diagnosis. The statistical sample consisted of all female patients with breast cancer aged 18-70 years who referred to Semnan hematologic clinic during 2009-2015 for the diagnosis and treatment. The number of these patients was 178. The initial CBC of these patients and the results of the hormonal and receptor analysis as well as the pathologic outcomes of the patients were investigated and analyzed by the Spss18.0 software after examining the patient records. The results showed that there was no significant relationship between the ratio of neutrophil to lymphocyte and ER receptor ($p = 0.871$), PR ($p = 0.893$), HER2 ($p = 0.259$). The overall results showed that with increasing the tumor progression and metastasis, the NLR increases. There is also no relationship between the ER / PR hormone receptors and the HER2/neu with the NLR.

Keywords: neutrophil to lymphocyte ratio, prognostics, receptor, breast cancer

Introduction

According to the statistics of Ministry of Health and Medical Education (MOHME), the cancer is the third most common cause of death after coronary diseases and accidents [1]. Breast cancer is a malignant proliferation of epithelial cells in the breast ducts or lobules [2]. More than 1.3 million people worldwide are diagnosed with breast cancer in the world [3], of which 502,000 die from this disease [4,5].

Although the breast malignancies are less common in men, over 2000 men in the United States are diagnosed with the breast cancer [2,6]. The prevalence ratio of the breast cancer malignancies in women to men is 150: 1. In Iran, the breast cancer is the most common cancer among the malignancies in women, about 24.4% of malignancies [1.5, 7]. Breast cancer affects Iranian women at least 10 years earlier than women in developed countries [7,8], and most patients in Iran are at the age range of 40-49 years [8].

Tumor findings such as size, grade of tumor, receptor status, and the rate of lymph nodes involved are considered as the most important prognostic factors in the breast cancer. The patient-dependent factors, such as obesity, smoking, alcohol and age, can also affect the outcome of the disease [9]. Breast cancer is generally diagnosed by the mammography or after touching a lump in the chest by the patient or her doctor [6].

HER2 / neu is one of the important prognostic and predictors of the breast cancer, which alone regulates the other family members of the Eidermal Growth Factor Receptor (EGFr), whose increasing expression increases the status of the negative estrogen receptor [10]. The neutrophil to lymphocyte ratio or NLR, which is obtained by counting the exact number of

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neutrophils to the exact number of lymphocytes in the peripheral blood, can be associated with both prognosis and chemical sensitivity [11]. The two composite ratio, which shows the number of neutrophil to lymphocyte as NLR, is a simple and cost-effective criterion for the evaluation of systemic inflammation and stress [12,13].

Excessive expression of the human Epidermal Growth Factor Receptor 2 (HER2 / neu), or the excessive strengthening of its genes, plays an important role in the choice of treatment and prognosis of the disease. So the amount of these factors must be checked and determined [6]. Hormones play an important role in the development and progression of breast cancer. Steroid hormones such as estrogen and progesterone are the hormones that affect breast cancer. The patients with breast cancer with a negative receptor status for these two hormones (ER - estrogen receptor / PR - progesterone receptor) are not candidates for hormonal treatment. Response to the hormonal treatment in patients who are positive for both receptors is >50%. To determine the status of ER / PR, there is a need for a new biochemical evaluation of the tumor tissue; of course, we can now use the samples of FNA (Fine Needle Aspiration) or Core-needle biopsy; determining these receptors plays an important role in choosing the method of treating these patients [10].

The rate of progression of the tumor and its prognosis are largely influenced by the inflammatory response of the host cells in the areas around the tumor [14]. Cancer-dependent inflammatory responses help the proliferation, the survival of malignant cells, angiogenesis and the metastasis of cancer cells, and also affect the response of the disease to chemotherapy drugs. Severe inflammatory responses result from a weaker immune system, created by an imbalance between the inflammatory response and the malignant cells, which overall increase the survival of the malignant cells and reduce the overall survival of the patient [15].

187 patients with HER2 / neu positive breast cancer, referred to the relevant center in 2009-2014, were studied in a retrospective study conducted by Ulas et al. in Turkey in 2015. The results indicated that the patients with positive HER2 / neu with higher NLR had lower DFS than those with lower NLR [16]. In a retrospective study in China in 2014, Yao et al. examined 608 patients with primary breast cancer during 2009-2011. This study showed that NLR is a prognostic and inexpensive factor for predicting prognosis of breast cancer patients, especially in the TNBC group, in whom the high rate of this ratio is reversely associated with the prognosis [17].

Considering the mentioned cases and the investigations due to the increasing prevalence of breast malignancies in our country and the resulting mortality and also its therapeutic burden on the health system and considering the effect of inflammatory factors such as neutrophil to lymphocyte ratio in the prognosis of many malignancies, as well as the importance of the factors of ER / PR and HER2 / neu in the prognosis and the selected breast cancer treatment, we dealt with determining the difference of the rate of inflammatory factor (NLR) in the patients with positive and negative HER2 / neu in Semnan.

Research Method

This study was retrospective and the statistical population of the patients was women with breast cancer. The statistical sample was all female patients with breast condoms aged 18-70 years who referred to the hematologist clinic in Semnan during 2009-2915 for diagnosing and treating; after considering the inclusion criteria, all female patients who were aged between 18-70 years during the last 5 years and had the breast cancer based on the biopsy done, were participated in the study. The number of these patients was estimated to be 178. Convenience sampling method was used in the study.

Exclusion criteria included: 1- The patients who have metastasized at the time of diagnosis. 2. The patients who have had active infection at the same time. 3- The patients with inflammatory cancer. 4- The patients treated with steroids. 5. The patients who have been diagnosed with the blood or autoimmune disease. 6. The patients diagnosed during pregnancy. 7- The patients with CHF. 8. The patients with CAD (IHD). 9 – The smokers and 10. The patients treated with lithium.

This research began after obtaining permission from the Ethical Committee and the University's Research Vice-Chancellor. For each patient, according to the CBC test, the NLR was calculated at the beginning of the cancer diagnosis. The patients were divided into positive and negative HER2 and positive and negative ER / PR, and the NLR was calculated and compared in each group. The patient information and the metastasis were identified by sonography, mammography, CT scan of rib cage, abdominal CT scan, brain CT-scan and biopsy at the beginning of the diagnosis and were recorded in each patient's file. Also according to the available articles and other studies, NLR was considered to be of the cutoff =2.5. The patients who had an inflammatory disease and an increase in NLR, or the patients who used drugs such as corticosteroids that had an effect on NLR, were excluded. Finally, all information was included in the data collection forms, which was previously prepared by the researcher for this purpose. This work continued until the specified sample size was completed. The data collection was done using the direct observation of patients records located in the digital library of the office. The information was recorded in the data collection form previously prepared for this purpose. Also, the information about each patient was kept completely confidential and no additional costs were incurred for conducting tests and examinations. To analyze the data, Kolmogorov-Smirnov, Mann-Whitney, Kruskal-Wallis and Spearman correlation coefficient were used. The level of significance was 5% and the software used was SPSS 18.0.

Research Findings

In this study, 178 women with breast cancer were studied. The mean ± standard deviation of neutrophil to lymphocyte ratio in women with breast cancer was 1.82 ± 0.9. The lowest value was 0.48 and the highest was 5.71. The median and inter-

quartile domain of neutrophil to lymphocyte ratio were 1. 52 and 0.95, respectively.

In terms of the ER receptor, 74.7% (133 people) were positive and the rest were negative. The mean, standard deviation, the inter-quartile domain of neutrophil to lymphocyte ratio have been given in Table 1. The level of NLR in positive and negative patients was not significantly different in terms of the ER receptor (p=0.871).

Table 1. Mean, standard deviation, median, NLR inter-quartile domain in women with breast cancer with positive and negative ER receptor

Status of ER receptor	Sample value	Mean	standard deviation	median	inter-quartile domain	p-value
Positive	133	1/82	0/88	1/55	0/92	0/871
Negative	45	1/84	0/97	1/47	0/98	

In terms of the PR receptor, 68.5% (122 people) were positive and the rest were negative. The level of NLR in patients with positive and negative PR receptors was not significantly different (p = 0.893) (Table 2).

Table 2. Mean, standard deviation, median, NLR inter-quartile domain in women with breast cancer with positive and negative PR receptor

Status of PR receptor	Sample value	Mean	standard deviation	median	inter-quartile domain	p-value
Positive	122	1/80	0/84	1/55	0/91	0/893
Negative	56	1/87	1/03	1/47	1/00	

21.9% (39 patients) had HER2 positive and the rest were negative. NLR level in patients with HER2 positive and negative were not significantly different (p = 0.259) (Table 3).

Table 3: Mean, standard deviation, median, NLR inter-quartile domain in women with breast cancer with positive and negative HER2 receptor

Status of HER2 receptor	Sample value	Mean	standard deviation	median	inter-quartile domain	p-value
positive	39	2.63	0.67	1.43	0.87	0.259

Final Discussion and Conclusion

Malignancies are a group of diseases in which the cells grow uncontrollably. Breast cancer is a malignant proliferation of the epithelial cells of the ducts or lobules of the breast. Breast cancer is one of the main problems of the health system for women in all countries, including our country. Inflammation and cancer are closely related to one another, so that the inflammation causes the cancer and the cancer causes the inflammation. As a parameter reflecting the immune response against inflammation, the neutrophilic to lymphocyte ratio can be used. In various research, the association of this ratio with various cancers has been proven, but its relation with breast cancer is still discussed [18].

Neutrophils are known as the most abundant leukocytes in the blood and as the first line of defense in infections and inflammations. Additionally, the neutrophils are considered as an increasing marker in neutrophilic tumors, which in two ways can increase the possibility of tumor metastasis and bad prognosis, and may be useful to the patient through anti-tumor activity. How the number of neutrophils increases in tumors is not completely clear, but probably due to the GMCSF (Granulocyte Macrophage Colony-Stimulating Factor) and other inflammatory cytokines such as IL-1 and 6 that are released by tumor cells, the number of neutrophils increases that as a result the neutrophil to lymphocyte ratio increases in tumors [19].

Hormones play an important role in the development of the breast cancer. Steroid hormones such as estrogen and progesterone are those that affect the breast cancer. The patients with the breast cancer and with a negative receptor status are not candidate for these two hormones (ER/PR - Estrogen Receptor/ Progesterone Receptor) for the hormone therapy. Response to the hormonal treatment in patients who are positive for both receptors is >50%. In this study conducted on the PR receptor surface and its relationship with the neutrophil to lymphocyte ratio, there was not found any significant relationship (p=0.893) [20]. Pistelli et al. who had studied ER / PR and its relationship with the NLR, did not find any correlation between them [21]. Yao et al. examined the ER / PR and its relationship with the NLR and the evaluation of the prognostic factor. The results indicated that NLR was a prognostic factor, but there was not found to be related to ER / PR [17]. Chihan et al. investigated the relation between ER/PR and NLR and evaluation of prognostic factor, which was performed by Kalmogrov-Smimov test; the results showed that between them was not any relationship [22]. Noh et al., who also investigated ER/PR and its relationship with the NLR, and its prognosticity for the breast cancers, did not find any relationship between them. In a study done by Yercal et al. in Turkey on 255 patients with the breast cancer, there was no significant relationship between NLR and positive ER and did not report a factor effective in cancer prediction [23].

Breast cancer is divided into various phenotypes based on the analysis of the linear arrangement of cDNA, in which a kind of estrogen receptor called ER becomes positive [11]. In the present study, there is not any significant relationship between the patients with ER receptor and the neutrophil to lymphocyte ratio (P = 0.871); it was consistent with the JiaW's study conducted in 2015 on 1570 patients with the breast cancer in stage3 from 2000 to 2010 in China; for the neutrophil to lymphocyte ratio the cut-off-2 has been considered. They also did not find a significant relationship with the patient's prediction and follow up in the positive ER / PR [20]. Pistelli et al., in a retrospective study on 90 patients examined the age, involvement of lymph of nodes, tumor size, histology, and ER/PR status; they were divided into two groups with cut off 3. The results showed that there was no significant relationship between the survival and disease-free period for them and ER/PR ratio and NLR status; they did not find the ER/PR relation to be effective [21]. Yao et al. also studied 608 patients with the breast cancer in 2014; the patients evaluated in the

group of ER/PR positive were evaluated 3-6 months with the cut-off 2.57 after diagnosis of the disease. Compared to different groups, there was not found any relationship between this relation with NLR and the prognosis of cancer [17]. In a study conducted in 2014 on 350 patients undergoing mastectomy and chemotherapy, Chihan evaluated the age, ER/PR status with cut-off 3, and its relation with NLR and prognosis of the patients; and he did not find any relationship between them. Even he did not know the NLR as a prognostic factor for the breast cancer [22]. In 2013, Noh et al. who studied 442 patients with the breast cancer, and in whom examined the age and status of tumor size and ER/PR status, did not find any significant relationship between this ratio and the NLR [12]. Ethier et al., who investigated the data from 15 different studies, reported a significant relationship between negative ER and its predictive role in the breast cancer; it may be due to the fact that the number of the samples of this review were very high [24]. Elyasinia et al., who had studied 195 patients in Imam Hospital, had not found any meaningful relationship between NLR and estrogen receptor, and did not find it as the effective factor in predicting cancer [25].

HER2 / neu is one of the important prognostic factors for the breast cancer, and its expression increases the status of negative estrogen receptor. Clinical studies have shown that the breast cancers are poorly differentiated with the increase in the HER2 / neu expression, and have high proliferation potential; they are of positive lymph nodes, and have lower expression of receptor hormone, and higher risk of recurrence and mortality. Therefore, in recent years, the HER2 / neu status is determined routinely in patients by the immunohistochemistry and the patients with positive HER2 / neu are candidates for anti HER2 / neu treatment [10]. In this study, the HER2 receptor surface did not have a significant relationship with the neutrophil to lymphocyte ratio (p=0.259). With the study conducted by JiaW et al. who examined the relationship between HER2 / neu and NLR and its prognosticity, it became clear that NLR was a prognostic factor in the prediction of OS and DFS, but it did not have a significant relationship with HER2 [20]. In a study on the status of HER2 / neu and its relationship with NLR and its prognosticity in the patients, Pistelli et al. found that NLR was a prognostic factor in these patients, but had no relation to HER2 / neu [21]. In a study in which the HER2 / neu has been determined, Yao et al. found that NLR was a prognostic factor in the evaluation of the breast cancer but did not have a signifocant relationship with HER2 / neu to determine the prognosis of disease [17]. Chihan et al., who performed a study on patients with the breast cancer in Canada, and determined the inflammatory factors and their relationship with disease prognosis, found that NLR had significant relationship with disease prognosis but was not associated with HER2 / neu in the prognosis [22]. Noh et al., who investigated the HER2 / neu and its relationship with NLR and their prognosticity, found that NLR was a prognostic factor for the breast cancer, but had no relation to the evaluation of the cancer with HER2 / neu [12]. In a retrospective study conducted by Elyasinia and Keramati in 2016 at Tehran University, 195 women with the breast cancer were examined. There was no significant relationship between the neutrophil to lymphocyte ratio and HER2 / neu [25]. In a study conducted in Turkey between 2010 and 2014, there was not found any significant relationship between NLR and HER2. They also did not know HER2 as a predictive factor for the breast cancer [23]. In a study that reviewed 8563 cases, a significant relationship was found between NLR and HER2; the negative HER2 was evaluated as a prognostic factor for the breast cancer, which appears to be due to the high number of cases under study [24].

Therefore, in the present study, no significant correlation was found between the receptors in breast cancer with the neutrophil to lymphocyte ratio. Accordingly, the use of NLR in early tests and follow up of patients with breast cancer seems to be a contributing factor in determining the degree of progression of the disease. Particularly in the periodic follow-up of treated patients, the use of the NLR and considering changes to it is a good guide to the probability of relapse or metastasis, or local tumor progression. However, according to the sample size used in this study, the similar studies are needed with more samples in this regard.

One of the limitations of this study is that the number of patients under examination was less than expected. The patients in Semnan are also well known and limited; so other intervening factors should be taken into account and analyzed separately.

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