RESEARCH ARTICLE

Utility of Peripheral Blood Parameters in Predicting Breast Cancer Risk

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Abstract

  <u>Purpose</u>: We aimed to study the inflammatory parameters of complete blood count in breast cancer cases. <u>Materials and Methods</u>: This retrospective study covered 178 breast cancer patients and 107 age and body mass index matched healthy women. Complete blood count parameters, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and MPV/platelet were analyzed. <u>Results</u>: The leukocyte, neutrophil and neutrophil/lymphocyte ratio were higher in the patient group (p values 0.001, 0.0001 and 0.0001, respectively) while haemoglobin and hematocrit were higher in the control group (p=0.0001 for both). Logistic regression analysis showed that elevated neutrophils and platelet distribution width (PDW) (OR: 0.627, 95%CI: 0.508-0.774, p=0.001 and OR: 1.191 95%CI: 1.057-1.342 p=0.003) were independent variables for predicting breast cancer. The cut-off value for the neutrophil/lymphocyte ratio was 2.56. <u>Conclusions</u>: According to our study results, neutrophil levels as part of complete blood count may be used as an independent predictor of breast cancer risk.

Keywords: Breast cancer - complete blood count - neutrophils - platelet distribution width

Introduction

The most common malignancy among women is breast cancer (Islam et al., 2014). It was estimated by The American Cancer Society (ACS) that 29% of all new malignant diseases are breast cancer and it is 16% cause of deaths (Siegel et al., 2012). Personal and family history of breast cancer, age, overweight, menstrual history, genetics, radiation to chest or face before age 30, pregnancy/breast feeding, race/ethnicity, hormone replacement therapy, alcohol consumption, dense breast, lack of exercise and smoking are the most important determinant factors of breast cancer (Majeed et al., 2014). Inflammation seems to be a shared process in the all risk factors of cancer identified till now. Activated inflammatory process is considered to be involved in invasion, tumor growth, metastasis and angiogenesis (Aggarwal and Gehlot, 2009; Pierce et al., 2009). Therefore, several inflammatory markers in the blood count such as platelets, neutrophils, lymphocytes, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), mean platelet volume (MPV) have been studied in different patients with malignant tumors (Teramukai et al., 2009; Gasparyan et al., 2011; Unal et al., 2013). As far as we know, there is only one study that compare some blood parameters (leukocyte, haemoglobin, platelet) between the breast cancer patients

and healthy controls (Ufelle et al., 2012). But it does not contain other complete blood count parameters as PLR, MPV, NLR, etc. So in this retrospective study we aimed to compare all complete blood count indices between breast cancer patients and healty controls.

Materials and Methods

This study was approved by our hospital local ethical committee. Our hospital's records between January 2011 and June 2014 were reviewed and with International Classification of Diseases (ICD) code (C50) of patients of breast cancer were determined and their post-surgery retrospective data were analyzed. In this study, a total of 320 records were scanned and 142 patients of those were excluded because of the presence at least one of the following criteria: Diagnostic mismatch between the ICD code and the histopathological results, absence of the pre-chemotherapy complet blood count results, absence of the investigations necessary for staging of the cancer such as thoracic and abdomino pelvic computed tomography (CT), magnetic resonance imaging (MRI) and positron emussion tomography (PET).

Age, weight, height, menopausal status, histopathological reports, mammography, breast MRI, thoracic and abdomino pelvic CT, PET reports, complete

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blood count parameters, NLR, PLR and MPV/platelet values results of the remained 178 patients were analyzed. Disease staging was performed according to the tumor-node-metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC) (Edge and Compton, 2010). The control group consisted of 107 age and body mass index (BMI) matched healthy women. Complete blood count was determined by a Coulter LH 750 auto analyser (Beckman Coulter, CA, USA).

Statistical analysis

Statistical analyses were performed using SPSS 22.0 statistical package for Windows. Continuous data were expressed as mean standard deviation, while categorical data were presented as number of patients. The distribution of variables was checked with the kolmogorov-smirnov test. Chi-square test was used for comparison of categorical variables, while student-t test was used to compare continuous variables. Logistic regression analysis was performed to determine the independent predictors of breast cancer. Receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off level of leukocyte, neuthrophil, NLR to predict the dipper versus non-dipper status. p values less than 0.05 were considered statistically significant.

Results

The records of 178 women with breast cancer and 107 healthy control women were analysed. There was no statistically significant difference in mean age between patient and control groups (53.8 ± 11.47 vs 53.72 ± 14.69 years, respectively, p=0.96). The staging of breast cancer patients was as follow: Stage 1 (13.4%), stage II (40.9%), stage III (36.6%) and stage IV (9.1%). Characteristics of the two groups are shown in Table 1.

The rate of NLR was significantly higher in breast cancer patients than in the control group (2.37 ± 1.4 vs 2 ± 1.26, respectively, p=0.0001). The mean haemoglobin (Hb) and haematocrit (Hct) levels in the patient group were significantly lower than the control group ($12.21\pm1,38$g/dl and $37.36\%\pm3.84$ vs 13.24 ± 0.88g/dl and $40.20\%\pm2.58$, respectively, p=0.0001 in both). There were no significant differences in PLR and MPV/platelet values between two groups (p>0.05) (Table 2).

A neutrophil count more than 2.56×10^4/mm^3 had a sensitivity of 59%, a specificity of 70.8%, a positive predictive value of 77.2%, and a negative predictive value of 50.7% in predicting breast cancer [(area under curve was 0.675 (0.611-0.740) and p=0.0001]. The cut-off values of leukocyte, neutrophil and NLR (Figure 1) levels for predicting breast cancer were determined by ROC analysis (Table 3).

The subanalysis of the patients group, platelet levels as well PLR values were higher in premenopausal sub-group than postmenopausal sub-group (298.76 ± 71.32 and 169.1 ± 87.25 vs 276.06 ± 70.94 and 141.16 ± 64.61, p was 0.037and 0.002, respectively). The difference in the leukocyte, neutrophils, lymphocytes, Hb, Hct, mean corpuscular volume (MCV), MPV, MPV/PLT ratio, platelet distribution width (PDW), NLR values
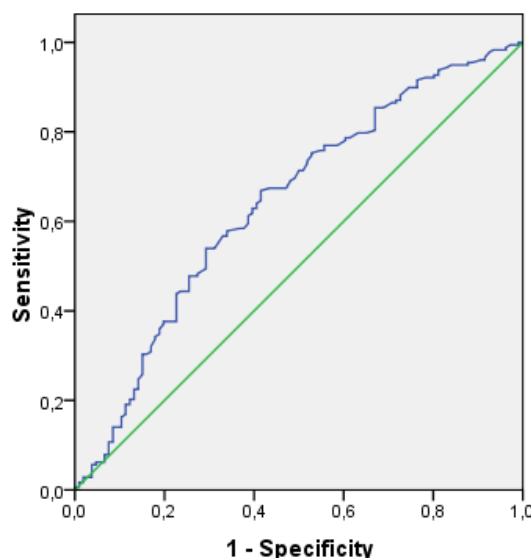
Figure 1. ROC analysis for NLR to Predict Breast Cancer (Area Under Curve 0.642)

Table 1. Characteristics of the Patient and Control Groups

Parameter	Patients n=178	Controls n=107	p value
Age (years)	53.8±11.5	53.7±14.7	0.96
BMI (kg/m^2)	28.8±5.37	29.9±4.72	0.076
Stage I	22 (13.4)		
II	67 (40.9)		
III	60 (36.6)		
IV	15 (9.1)		
Menopausal Status			
Premenopausal	78 (43.8)		
Postmenopausal	100 (56.2)		
Tumor localization			
Right	83 (49.1)		
Left	79 (46.7)		
Bilateral	7 (4.2)		
Histology			
Ductal carcinoma	143 (81.3)		
Lobular carcinoma	19 (10.8)		
Other	16 (7.9)		
Surgery			
Radical Mastectomy	101 (56.7)		
Breast-conserving	77 (43.3)		
Metastasis			
No	154 (88.5)		
Yes	20 (11.5)		

Table 2. Comparison of Inflammatory Parameters of Complete Blood Count

Parameters	Patients n=178	Controls n=107	p value
Leukocyte[1]	7.28 ± 1.99	6.58 ± 1.5	0.001
Neutrophil[1]	4.46 ± 1.51	3.68 ± 1.21	0.0001
Lymphocyte[1]	2.09 ± 0.7	2.15 ± 0.67	0.476
MPV	8.45 ± 1.09	8.68 ± 1.24	0.099
PDW	13.16 ± 1.65	16.84 ± 2.79	0.254
Platelet[1]	285.81 ± 71.79	287.26 ±75.72	0.871
NLR	2.37 ± 1.4	2.0 ± 1.26	0.0001
PLR	153.16 ± 76.21	144.95 ±57.19	0.762
MPV/Platelet	0.317± 0.1	0.325± 0.103	0.436

[1] x10^3/mm^3; MPV, mean platelet volume; NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio

Table 3. Diagnostic Values of the Study Parameters

	AUC	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Leukocyte	0.605	8.35	28.7	87.9	79.7	42.5	50.9
Neutrophil	0.675	4.09	59	70.8	77.2	50.7	63.4
NLR	0.642	2.56	30.3	84.9	77.1	42.1	50.7

*AUC: Area under curve, PPV: Positive Predictive Value, NPV: Negative Predictive Value, NLR: Neuthrophil Lymphocyte Ratio

Table 4. Univariate and Multivariate Analysis of Risk Factors for the Breast Cancer

	Univariate analysis			Multivariate analysis			
	OR	95%CI		p value	OR	95%CI		p value
Leukocyte	0.802	0,696 -	0.924	0.002				
Neutrphil	0.642	0,523 -	0.787	0.0001	0.627	0.508-	0.774	0.0001
Neutrophil/lymphocyte ratio	0.757	0,588 -	0.975	0.031				
PDW	1.158	1,032-	1.299	0.012	1.191	1.057	1.342	0.003

*Presence: Patients with breast cancer/Absence: Control group, OR: odds ratio, CI: confidence interval

of the sub-groups was not significant (p>0.05). There were no significant differences in age, BMI, leukocytes, neutrophils, lymphocytes, platelets, MPV, Hb, Hct, MCV, PDW, NLR, PLR, MPV/Platelet ratio in stage of cancer (p>0.05). There was no significant differences in leukocytes, neutrophils, lymphocytes, Hb, Hct, MCV, MPV, MPV, PDW, NLR, PLR, and MPV/Platelet between patients with metastasis (n=20) and patients without metastasis (n=158, p>0.05). The mean platelet in the group with metastatic (276.06±70.94) was lower than in non-metastatic (298.76±71.32, p=0.037).

Logistic regression analysis showed that elevated neutrophil and (OR: 0.627, 95%CI: 0.508-0.774, p=0.001) was an independent variable for predicting breast cancer (Table 4).

Discussion

As we mention above, in this pilot study we tried to evaluate and compare NLR, PLR and other inflammatory parameters of complete blood count between patients with breast cancer and healthy controls. The average values of leukocyte, neutrophil and NLR were found to be significantly higher in breast cancer patients than healthy group (Table 2). Platelet and PLR values were significantly higher in premenopausal women. There was no significant difference in age and BMI and blood parameters between stages of breast cancer. But platelet count was significantly lower in the metastatic (i.e stage IV) patients. Also there was no significant difference in the mean MPV levels between patients with breast cancer and control groups (Table 2).

It is well known that inflammation has a role in the development and progression of different cancers. Many tumors arise from sites of chronic irritation, infection, and inflammation and also inflammatory cells play important roles in the growth and progression of some tumours (Hussein and Ahmed, 2005; Aliustaoglu et al., 2010; Bae and Kim, 2014; Hoshida et al., 2014; Mitsunaga et al., 2014; Okada, 2014; Sicking et al., 2014). Infiltrates of many immune cells have been detected in malignant tissue (Yildirim et al., 2012). Presence of excess number of neutrophils in tumour stroma has been associated with poor prognosis. Neutrophils induce tumour progression

and development of metastases via secretion of cytokines and chemokines and accelerating tumor angiogenesis. It has been detected that NLR estimated from peripheral blood components predicts degree of tumor-promoting inflammation and also prognostic significance of antitumoral immune cell response has been demonstrated. Increased NLR was shown in many tumours. Inflammation is an important process in cancer development and progress (Balkwill and Mantovani, 2001). In one study, leukocyte, neutrophil and NLR were significantly higher in the patient group in accordance with the control group (Thavaramara et al., 2011). In non-malignant diseases contributed to systemic inflammation response, NRL has an independent predictive value of the outcome of such diseases. This increases reliability of NLR for the use as a potential biomarker of the systemic inflammatory response (Chua et al., 2011; Unal et al., 2013; Lee et al., 2014; Yilmaz et al., 2014). In our study patient group has higher leukosyte, neutrophil and NLR than healthy control group (Table 2). Logistic regression analysis showed that high neuthrophil levels are independent predictors of breast cancer risk (Table 3). Highness of the neutrophil level in our cancer patients also supports the previous findings of the presence of inflammatory status in malignant diseases.

The activation of coagulation and fibrinolysis is common among cancer patients. Such tumors have high risk of invasion, metastases, and negative final outcomes(Li et al., 2014b). Previous studies showed that high platelet levels associated with later stage, metastasis and higher risk of recurrence in many types of cancers (Unal et al., 2013; Li et al., 2014a) . Ovarian cancer (Lee et al., 2011), renal cell carcinoma (Cho et al., 2011), colorectal cancer and gastric cancer (Hwang et al., 2012) associated with thrombocytosis had poorer prognosis. In a study on 243.012 patients, Gnerlich J. L. et al. founded higher mortality of breast cancer in younger women than olders (Gnerlich et al., 2009). In our study platelet levels and PLR were higher in premenauposal (n=78) than postmenauposal (n=100) women which may somewhat explain this high mortality rate in younger patients (p was 0.037 and 0.002, respectively). In Ufelle et al. study, platelet levels were found to be higher in control group than pre and post-operative breast cancer patients (p<0.05 in both). In our study platelet levels were also higher in

control group but not reached statistical significance.

Our study is retrospective, cross-sectional, single-center study with a limited number of patients included. So we could not evaluate the effect of this parameters on prognosis. Another limiting point of this study is that we included only post-surgery but pre-chemotherapy data. So we could not see the effect (if any) of the presence of tumor mass on these blood parameters too. But in Ufelle and his collage's study, there was significant difference between the controls and pre and post-operative blood parameters of breast cancer but no effect of its surgery on these parameters (Ufelle et al., 2012).

In conclusion, our study results show increased inflammatory parameters of complete blood counts in breast cancer patients (in compare to healthy controls). Additionaly high neutrophil levels were an independent predictors of breast cancer risk. Whether these to be used in daily practice need to befurther studied.

Acknowledgements

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