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Interleukin-6 serum levels in metastatic breast cancer and non-metastatic breast cancer

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Abstract---Introduction: This study has investigated the correlation between IL-6 serum levels with distant metastatic breast cancer incidence. Methods: We recruited female breast cancer patients at Dr. Soetomo General Hospital from March until May 2022. The sample was divided into 2 groups that consist Metastatic Breast Cancer (MBC) and non-MBC. After the stage, the IL-6 serum levels were measured. The study design was an observational analytic study, comparative analytics by comparing IL-6 serums levels in both groups: MBC and non-MBC. Results: Sixty patients, with the Non-Specific type being the common histopathology type found in this study (85%). We found 29 patients with metastases and 31 patients without metastases. Overall analyses identify Patient with high IL-6 serum levels had a 2.54 times higher chance of having a metastasis than patients with low IL-6 serum levels (95% CI = 1.39-4.63). Conclusions: Subjects with IL-6 serum levels >15.495 pg/mL had a higher risk for incidence of metastatic. The IL-6 serum levels may be a potential biomarker to predict metastases in breast cancer patients.

Keywords---breast cancer, interleukin-6, metastases.

Introduction

Breast cancer is the most common cancer in women. It is also a leading cause of cancer-related death. Every year, breast cancer cases are increasing and about

2.2 million women in the world suffer from breast cancer. In Indonesia, a total of 17,792 new breast cancer cases were reported. (Hyuna et al, 2021; Dewi et al, 2020; Hadisuwarno et al, 2021; Dewi TK, 2018) Despite many recent advances in the detection and treatment of breast cancer, the development of metastasis remains a significant problem and the main driver of mortality. Indeed, patients with localized breast cancer have a five-year survival rate of about 98%. Meanwhile, patients with metastatic breast cancer have a five-year survival rate of only 26%. (Siegel et al, 2016)

Tumor cells depend on the tumor microenvironment to complete the process of both invasion and metastasis (Siegel et al, 2016; Gaili et al, 2019). For many years, there is increasing evidence that has shown activation of inflammation, such as Interleukin-6 (IL-6) is a crucial mechanism for both the recurrence and metastasis of breast cancer (Ji et al, 2020). Interleukin-6 (IL-6) is a pleiotropic cytokine with broad biologic activities. It provides several effects on cancer cells (Kishimoto, 2006) which play an important role in the fight against apoptosis and increase tumor aggressiveness (Ravishankaran et al, 2011).

Interleukin-6 (IL-6) that binds to its receptor activates the Janus Activated Kinase (JAK) pathway and phosphorylates Signal Transducer and Activators of Transcription (STAT). Signal Transducers and Activators of Transcription (STAT) have a role in the tumorigenesis process of breast cancer. Based on previous studies, it was found that the expression of STAT was very high in cancer cells. Either inhibiting or eliminating STAT will increase apoptosis and can reduce the angiogenesis process (Gritsko et al, 2006). Interleukin-6 induces the synthesis of mRNA from Vascular Endothelial Growth Factor (VEGF) and activates Rho protein. It plays a role in angiogenesis and cell adhesion in malignancy. Hence, it will increase the chance of tumor metastases (Pratama, 2021). Previous research by Pratama (2021), showed that IL-6 levels above 15,495 pg/ml were associated with increased severity of breast cancer, characterized by a worsening of the cancer response to chemotherapy. (Pratama, 2021) At the moment, there are no biological factors from the tumor that can be used clinically as acceptable predictive factors of distant metastases in breast cancer. Therefore, in this study we aimed to measure serum IL-6 levels which play an important role in the pathogenesis of the metastatic process of non-MBC patients compared to MBC, to assess whether serum IL-6 levels can be alternative as a marker of tumor progression.

Method

Study participant

This research is a cross-sectional comparative analytical design comparing IL-6 serum levels between MBC and Non-MBC. The subjects were breast cancer patients who were treated at Dr. Soetomo General Hospital, Surabaya, East Java, Indonesia who met the inclusion criteria. Exclusion criteria were patients with a history of acute or chronic infections and who have a history of other types of cancer.

Breast cancer diagnosis and IL-6 measurement

The diagnosis of breast cancer is based on history and clinical examination, followed by an incisional biopsy. A chest radiograph in a posteroanterior (PA) position, liver ultrasonography (USG), and bone scans were carried out to determine the stage of the tumor. Screening for brain metastases was carried out through in-depth anamnesis and clinical examinations. Venous blood samples were taken for examination of serum IL-6 levels at Clinical Pathology Laboratory, Dr. Soetomo General Hospital, (Surabaya, East Java, Indonesia). The IL-6 measurement results were recorded for further analyses. As previously described, the IL-6 serum levels were divided into two groups: High IL-6 and low IL-6, using the cut-off value of 15.495 pg/ml

Statistical analysis

The collected data is processed manually and digitally to convert data into information. Data analysis in the form of univariable analysis aims to describe the characteristics of research subjects. The statistical test used is the chi-square test

Results

We found that patients aged less than 50 years were the predominant age group in this study (55%), slightly higher than more than equal to 50 years group (45%). The average age of the study subjects was 49.3 years with a range of 28-80 years. Patient characteristics in this study included 29 patients with metastatic breast cancer and 31 patients without Metastatic breast cancer (Non-MBC) which were presented in Table 1. There were more menopause patients than non-menopause patients in this study (75% vs 25%).

Pathological results data obtained from the biopsy showed that there were 51 patients with Non-specific type breast cancer (85%) and 9 patients with specific breast cancer (15%). In this study, the most frequent breast cancer subtype was luminal (42/60; 70%) followed by non-luminal (18/60; 30%). The patient's staging was determined using the TNM system, T tumor size, N regional lymph node nodules, and M distant metastases with details as shown in Table 2. Based on the TNM table in this study sample, the highest T distribution (tumor) was in The T4b group as many as 32 patients (53.3%) followed by T4c and T3 groups. The most distribution of N (Nodules) was in the N1 group as many as 25 patients (41.7%), while the highest stage was stage IV (48.3%) (Table 2).

We found that only age had a significant relationship with breast cancer metastases incidences. The variable of menopausal status, subtypes, and regional lymph node metastases did not have a significant relationship with breast cancer metastatic incidences ($P > 0.05$). In this study, we found that 60 patients had a mean value of 20.65 ± 20.38 with a maximum level of IL-6 was 2,255 pg/mL and a minimum level of 2.85 pg/mL. The profile of IL-6 serum levels of participants in this study was shown in Table 2. This study examines the association between the subject's characteristics and the incidence of metastases. Based on tumor histopathology, 28 patients (54.9%) with Non-specific Type Breast Cancer had no distant metastases and 23 patients (45.1%) had distant metastases. In patients

with Specific types of Breast Cancer, we found 3 patients (33.3%) had no distant metastases and 6 patients (66.7%) had distant metastases. We found there is no significant relationship between histopathology and breast cancer metastases (Table 3).

Cancer subtype examination showed that in the study, 42 patients had a Luminal subtype while the remaining 18 patients had a Non-luminal subtype. In the Luminal subtype group, 19 patients (45.2%) had no distant metastases and 23 patients (54.8%) had distant metastases. In the Non-luminal group, 12 patients (66.7%) had no distant metastases and 6 patients (33.3%) had distant metastases. We found there is no significant relationship between cancer subtype and breast cancer metastases (Table 3). The cut-off value of IL-6 serum levels in this study was based on a previous study from Pratama (2021). It was obtained that a value of 15.495 pg/mL was the cut-off value for IL-6 serum levels. From the cut-off value, we divided the IL-6 serum levels into high IL-6 expression and low IL-6 expression. We found that were 28 patients (28/60; 46.7%) with high IL-6 serum levels and 32 patients (32/60; 53.3%) with low IL-6 serum levels.

The relationship between IL-6 to the incidence of MBC was examined using the chi-square test. We found that there was a statistically significant relationship between IL-6 levels to the incidence of MBC ($p=0.001$) (Table 4.). This study also calculated the correlation between the risk of elevated IL-6 serum levels with the incidence of MBC. From the analysis, we found that the group of patients with high levels of IL-6 had a 2.54 times higher risk to develop metastatic breast cancer (RR = 2.54; $p= 0.001$; 95% CI = 1.39-4.63).

Table. 1. General characteristics and demographic of study participants

Category	Non-MBC	MBC
Total	31	29
Age (years)		
< 50	21 (67.7%)	12 (41.4%)
> 50	10 (32.3%)	17 (58.6%)
Menopause status		
Non-menopause	10 (32.3%)	5 (17.2%)
Menopause	21 (67.7%)	24 (82.8%)

Table. 2. Cancer staging

No.	Category	Total (n=60)	Percentage (%)
1	TNM classification		
	T (Tumor)		
	T2	3	5
	T3	10	16.7
	T4b	32	53.3
	T4c	14	23.3
	T4d	1	1.7

	N (Nodule)		
	N0	9	15
	N1	25	41.7
	N2	12	20
	N3	14	23.3
	M(Metastasis)		
	M0	31	51.7
	M1	29	48.3
2	Stadium		
	Iia	2	3.3
	Iib	2	3.3
	IIIa	4	6.7
	IIIb	18	30
	IIIc	5	8.3
	IV	29	48.3

Table. 3. Association between pathologic examination and metastases breast cancer

No.	Characteristic	Non-MBC	MBC	P
1	Pathology			0.233
	Anatomy			
	Non-Specific type	28 (90.3%)	23 (79.3%)	
	Specific type	3 (9.7%)	6 (20.7%)	
2	Tumor Subtype			0.128
	Non-Luminal	12 (38.7%)	6 (20.7%)	
	Luminal	19 (61.3%)	23 (79.3%)	

Tabel.4 Comparison of IL-6 serum levels beetwen MBC and Non-MBC patients

Metastatic status	Interleukin-6 level		P value	RR (95% CI)
	Low (<15.49 pg/mL)	High (>15.49 pg/mL)		
MBC	9 (15%)	20 (33.3%)	0.001	2.54 (1.39-4.63)
Non-MBC	23 (38,3%)	8 (13.3%)		

Discussion

The youngest age of study participants in the MBC group was 36 years. Meanwhile, in the Non-MBC group was 28 years. The oldest age in the MBC group was 80 years and in the non-MBC group was 65 years. The mean age is 49.3 years. The result of this study found that the average age of patients with breast cancer has shifted to younger, which is under the age of 50 years. Although breast cancer can strike at any age, this age shift can be caused by several factors including lifestyle changes, such as drinking alcohol and obesity. (Breast Cancer Health Center, 2022) Several factors that put young women at high risk for breast cancer, namely a history of breast carcinoma, especially in the mother, daughter, or sister, a history of radiation therapy to the chest before 40

years old, and proven to have genetic disorders (BRCA1/BRCA2 mutations). (Breast Cancer Health Center, 2022)

We found that there is no significant relationship between histopathology and breast cancer metastases ($p=0.233$). These results showed that histopathological type was not a crucial factor affecting breast cancer metastases. Our result concordance with the research concluded by Borst, M.J. and Ingold, J.A. which concluded that rates of metastasis to the liver and central nervous system were not significantly different in invasive ductal carcinoma and invasive lobular carcinoma. (Borst et al 1993) This is consistent with the theory, which states that patients with Non-specific type and specific type breast cancer had similar overall survival. (Yang et al, 2020)

This study found there is no significant relationship between cancer subtype and breast cancer metastases. There is a very strong and significant tendency that breast cancer subtype affects metastasis. Another study conducted by Xiao et al. showed that breast cancer subtypes are associated with different metastatic patterns and confer different prognostic impacts. (Xiao et al, 2018) The contradictory result in this study can be caused by various factors including the design of this study which is not suited for examining the effects of the cancer subtype variable.

Kozłowski et al. (Kozłowski, 2003) in their study found that IL-6 serum levels in breast cancer patients were higher than in the control group with an average of 31.7 pg/mL. Ravishankaran and Karunanuthi found that IL-6 serum levels were higher in MBC (39.7 ± 9.3 pg/mL) compared to patients who had no distant metastases (17.3 ± 7.6 pg/mL) (Ravishankaran et al, 2011). The average IL-6 serum levels of the Non-MBC group were obtained from the previous study (Kozłowski, 2003). Meanwhile, compared to another study by Ravishankaran and Karunanithi (Ravishankaran et al, 2011), the results were higher than what was obtained in this study. It was >40 pg/mL. The difference in the mean IL-6 serum levels obtained by the two researchers in this study was due to differences in the inclusion and exclusion criteria applied to the study participants. The IL-6 serum levels are influenced by many factors, such as comorbidities and inflammation.

High serum IL-6 levels are associated with pathological processes that occur in the MBC group. Interleukin-6 (IL-6) secreted by breast carcinoma cells has been shown to contribute to a process called "self-seeding". Tumor cells circulate aggressively within their tumor environment (Kim et al, 2009). Strong evidence from previous studies suggests that IL-6 promotes the growth of these cells. Breast cancer cells activate STAT3 that ultimately regulates the proliferation of oncogenes, such as c-Myc and cyclin D1, as well as growth factors such as hepatocyte growth factor (HGF), VEGF, and also epidermal growth factor (EGF) (Yang et al, 2020).

In this study, a cut-off point of IL-6 serum levels in the non-MBC and MBC groups was obtained based on the previous study. Pratama et al. (Pratama et al, 2021) suggested that the limit value for IL-6 serum levels to detect the possibility of metastases was >15.495 pg/mL. From the analysis of this study, it was found that there was a statistically significant relationship between IL-6 serum levels

and the incidence of metastasis ($P = 0.001$). The interleukin-6 (IL-6) is a soluble mediator with pleiotropic effects on inflammation, immune response, and hematopoiesis. The interleukin-6 (IL-6) binds to cancer cells via the GP130 receptor. After binding to receptors on cancer cells, IL-6 can activate several cascades. The interleukin-6 (IL-6) can activate JAK and increase STAT3 then increase VEGF expression, thereby, increasing angiogenesis. (Dewi et al, 2017) The interleukin-6 (IL-6) can also activate the Erk $\frac{1}{2}$ cascade and decrease the protein E-cadherin. So, it affects the cohesion of cancer cells. The interleukin-6 (IL-6) has also been reported to bind to CSCs and activate ICAM-1 which stimulates cancer cell adhesion. These various cascades such as cohesion, adhesion, and angiogenesis will affect the increasing breast cancer metastasis. IL-6 is a promising prognostic factor in cancer patients because it is associated with cell proliferation, survival, angiogenesis, and metastasis. A high IL-6 may represent a condition to likely become distant metastases.

Conclusion

Subjects with IL-6 serum levels >15.495 pg/mL had a higher risk for incidence of metastatic. The IL-6 serum levels on MBC group are higher than on non-MBC group. The IL-6 serum levels may be a potential biomarker to predict distant metastases in breast cancer.

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