ORIGINAL AERTICLE

Bali Medical Journal (*Bali MedJ)* 2022, Volume 11, Number 3: 1548-1552
P-ISSN.2089-1180, E-ISSN: 2302-2914

Association between serum TNF-α level with the incidence of metastases in women with breast cancer in Dr. Soetomo General Hospital, Indonesia

Rachman Efendi[1], Husnul Ghaib[2], Iskandar Ali[2*]

[1]Department of Surgery, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;
[2]Oncology Surgery Division, Department of Surgery, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;

*Corresponding author:
Iskandar Ali;
Oncology Surgery Division, Department of Surgery, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;
iskandarali1983@yahoo.com

Received: 2022-08-02
Accepted: 2022-10-06
Published: 2022-11-13

ABSTRACT

Background: Breast cancer is the most common cancer in women. The occurrence of metastasis in breast cancer should be detected early. Tumor necrosis factor-α (TNF-α) is involved in every step of the carcinogenesis process. A rise in TNF-α level is linked to the advancement of breast cancer. We aimed to determine the association between TNF-α levels and breast cancer metastases.

Methods: An analytic cross-sectional study was conducted on women with breast cancer treated at the Oncology Surgery Center between November 2021 and April 2022. The patient's data were collected from the medical record, including age, menopausal status, tumor size and lymph node involvement, and tumor subtype. The serum TNF-α level was measured using ELISA. The optimal cut-off value was determined using the receiver operating characteristics analysis. The association between serum TNF-α and the incidence of breast cancer metastases was analyzed using SPSS version 26 for Windows.

Results: We analyzed a total of 50 patients. The mean age was 48.8 ± 8.3 years. The serum TNF-α level was divided into high-TNF-α and low-TNF-α using a cut-off value of 22.755 pg/mL. There were 27 patients (27/50; 54.0%) with high serum TNF-α levels and 23 patients (23/50; 46.0%) with low TNF-α levels. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 73.9%, 63.0%, 63,0%, 73,9%, and 68%, respectively. There was a statistically significant association between serum TNF-α levels and metastatic breast cancer (p=0.009).

Conclusion: There was a significant association between TNF-α levels and metastatic breast cancer. Serum TNF-α may be a potential biomarker to detect metastasis in breast cancer patients.

Keywords: advanced-stage cancer, breast cancer, metastases, TNF-α.
Cite This Article: Efendi, R., Ghaib, H., Ali, I. 2022. Association between serum TNF-α level with the incidence of metastases in women with breast cancer in Dr. Soetomo General Hospital, Indonesia. *Bali Medical Journal* 11(3): 1548-1552. DOI: 10.15562/bmj.v11i3.3611

INTRODUCTION

The most prevalent disease in women, breast cancer, is a prominent cause of death in both developed and developing nations. According to the International Agency for Research on Cancer (IARC), the number of new cases of breast cancer diagnosed worldwide in 2018 was roughly 2.1 million (11.6%).[1,2] Breast cancer affects 0.5 out of every 1000 women in Indonesia.[3] Approximately 70% of breast cancer patients at Dr. Soetomo General Hospital were in an advanced stage.[4,5] In 2002-2006, Cancer Research UK found that patients who present at an early stage have a 90% chance of survival, whereas those who present at an advanced stage have just a 15% survival rate.[6] Metastatic breast cancer is a primary cause of increased mortality and morbidity. The most common metastases in breast cancer patients at Dr. Soetomo General Hospital were bone, lung, and liver.

More than 30% of breast cancer patients die from metastatic breast cancer. Understanding the mechanism of tumor cell invasion and metastasis is one of the most challenging tasks in cancer research.[7] The occurrence of metastases in breast cancer must be detected early for the patient to receive the best treatment to increase the survival rate and the quality of life. Chest X-rays, bone scans, ultrasonography (USG), Computed Tomography scans (CT scans), and magnetic resonance imaging (MRI) can all be used to detect metastases. However, most of these examination techniques are expensive, require special skills, and are only available at a limited number of healthcare facilities.[8]

One of the cytokines released by active macrophages is Tumor Necrosis Factor-Alpha (TNF-α). TNF-α may promote proliferation, migration, and metastasis in cancer cells.[9] TNF-α is involved in various steps of the carcinogenesis process. TNF-α causes the initiation and promotion of tumors by activating NF-kB, PKCa and AP-1-dependent pathways. TNF-α also activates NF-kB and increases tumor cell proliferation to increase tumor cell survival. In addition, TNF-α promotes tumor angiogenesis by interacting with IL-8 and VEGF. Through NF-kB, chemokine receptor (CXCR4), monocyte chemoattractant protein-1 (MCP-1), IL-8, and intercellular adhesion molecule-1,

TNF promotes tumor cell motility and metastasis.[10]

Currently, there is a limited number of TNF-α studies in metastatic breast cancer in Indonesia. Therefore, in this study, we aimed to examine the association between metastatic breast cancer and the level of TNF-α expression.

MATERIALS AND METHODS

Study design and participants

Our study design was an analytic cross-sectional design study. The inclusion criteria were women with breast cancer treated at the Oncology Surgery Center, Dr. Soetomo General Hospital, Surabaya, Indonesia, between November 2021 and April 2022 and willing to participate in this study. The exclusion criteria were breast cancer patients with autoimmune disease and patients with no complete medical record data. Sampling was carried out by consecutive sampling between November 2021 and April 2022. We collected patients' data, including age, menopausal status, tumor size and lymph node involvement, and tumor subtype from the medical record.

Blood samples were taken during the patient's visit to the Oncology Surgery Center. The blood samples were immediately taken to the Clinical Pathology Department, Dr. Soetomo General Hospital (Surabaya, Indonesia). The blood samples were processed to obtain the blood serum. The serum samples were aliquoted and immediately stored in the –20^{0}C refrigerator until used.

Breast cancer and metastasis diagnostic

The breast cancer diagnosis was confirmed histologically by fine needle aspiration biopsy, core needle biopsy, or incisional biopsy. In addition, we also classified the breast cancer subtypes using immunohistochemistry by examining the expression of estrogen receptor (ER), progesterone receptor (PR), HER2/neu, and Ki67. Tumor staging was determined based on the American Joint Committee on Cancer (AJCC) 2018. The presence or absence of metastases is assessed through in-depth anamnesis, clinical symptoms, physical examination, and radiologically by chest X-rays, abdominal ultrasound, and CT scan.

TNF-α measurement

The measurement of TNF-α was performed using a commercially available enzyme-linked immunoassay (ELISA) kit following the manufacturer's instruction. The optical density of the ELISA reaction was measured using ADVIA Centaur CP immunoanalyzer (Siemens), calibrated every 28 days. The serum sample was thawed and used for TNF-α measurement. The TNF-α levels were measured in pg/mL units. The result was recorded for further analysis.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software package version 26.0 (IBM Corp., Armonk, NY, USA) for Windows. The variables were analyzed and presented as frequency distribution and cross-tabulation. The associations between TNF-α level and incidence of metastasis were analyzed using Chi-squared (χ^2) test. Receiver operating characteristic (ROC) curve analysis was performed to calculate the optimal cut-off value for TNF-α to predict the occurrence of metastases in breast cancer patients. The optimum cut-off used in this study was the cut-off that produced the highest accuracy value. The optimum cut-off value was determined using the point closest to the (0,1) corner in the left upper quadrant of the ROC plane analysis. This cut-off was then used to convert the ratio scale of TNF-α into a nominal scale of high TNF-α and low TNF-α levels. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Patient characteristics

We included 50 patients with breast cancer who met the inclusion and exclusion criteria between November 2021 – April 2022. The mean age was 48.8 ± 8.3 years, with the youngest patient being 29 years old and the oldest 65 years old. There were 29 subjects (58.0%) aged less than 50 years old and 21 subjects aged more than equal to 50 years old (42%). Twenty-four subjects had menopause (48.0%). Most of the patients came with tumor sizes of more than 5 cm (74.0%). Most tumors (74.0%) were luminal subtypes, with luminal B HER 2-negative being the predominant luminal type (30.0%). There were 33 patients with lymph node involvement (66.0%). The characteristics of breast cancer patients in this study are shown in Table 1.

The association between patients' characteristics and metastatic breast cancer

We analyzed the association between various patients' characteristics and the occurrence of *metastasis*, as shown in Table 2. We found that there were 12 patients under the age of 50 years with metastasis (41.4%), and there were 11 patients aged ≥50 years with metastasis (52.3%). There was no significant association between age and metastasis status (p=0.441). We found that there were 13 menopausal patients with metastasis (54.2%), and there were 10 patients who had no menopause with metastasis (38.5%). There was no significant association between menopause status and metastasis (p=0.266). Based on the tumor size, we also found no significant association between the tumor size and metastatic status (p=0.509). We also analyzed the association between lymph node spreading and metastatic occurrence. We revealed 17 lymph node-spreading patients with metastasis (51.5%). Although not significant (p=0.276), this percentage was higher than that of patients with no lymph node spreading that had metastasis (35.3%). In addition, we also found no significant association between breast cancer subtype and metastasis (p=0.526).

Serum TNF-α level and the optimal cut-off value for metastasis

We analyzed the profiles of the serum TNF-α value in all subjects and serum TNF-α in both metastasis and non-metastasis. The mean value of serum TNF-α in this research is 164.3 ± 361.2 pg/mL. The lowest value is 5.74 pg/mL, and the highest is 1733.0 pg/mL. The median value was 26.9 pg/mL.

A *receiver operating characteristic* (ROC) analysis was used to calculate the optimal cut-off value of TNF-α to predict the metastases incidence in breast cancer. The ROC curve is shown in Figure 1. We revealed that the area

Table 1. The characteristics of the study sample.

Characteristics	Total (n)	Percentage
Age		
<50	29	58.0%
≥50	21	42.0%
Menopause		
Menopause	24	48.0%
Pre-menopause	26	52.0%
Tumor size		
<5 cm	13	26.0%
≥5 cm	37	74.0%
Lymph node (N)		
KGB negative	17	34.0%
KGB positive	33	66.0%
Subtype		
Luminal	37	74.0%
Non-luminal	13	26.0%

Table 2. The association between various patients' characteristics and the occurrence of metastasis.

Characteristics	Total (n)	Metastasis		P value
		Yes	No	
Age				
<50	29	12	17	0.441
≥50	21	11	10	
Menopause status				
Menopause	24	13	11	0.266
Pre-menopause	26	10	16	
Tumor size				
<5 cm	13	7	6	0.509
≥5 cm	37	16	21	
Lymph node (N)				
KGB negative	17	6	11	0.276
KGB positive	33	17	16	
Subtype				
Luminal	37	18	19	0.526
Non-luminal	13	5	8	

under the curve (AUC) was 0.654 (95% CI: 0.500 – 0.807; p=0.063). We found that the optimal cut-off value to determine the metastasis status was 22.755 pg/mL. Then, we classified the serum TNF-α value into high-TNF-α (≥ 22.755 pg/mL) and low-TNF-α (< 22.755 pg/mL) based on the cut-off value determined by ROC curve analysis. We found that 27 patients (54.0%) had high serum TNF-α levels and 23 patients (46.0%) had low TNF-α levels. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 73.9%, 63.0%, 63,0%, 73,9%, and 68%, respectively.

The association between serum TNF-α and metastasis

The association between TNF-α and metastases in breast cancer was analyzed and presented in Table 3. In this analysis, we divided the patients into 2 groups, high TNF-α level and low TNF-α level, using the optimum cut-off from the ROC analysis. We revealed that in the low TNF-α group, there were 17 patients with no metastasis (73.9%) and 6 with metastasis (26.1%). In the high TNF-α group, there were 10 patients with no metastasis (37.0%) and 17 with metastasis (63.0%). We found a statistically significant association between serum TNF-α levels and the incidence of breast cancer metastases (p=0.009), as shown in Table 3.

DISCUSSION

Breast cancer is the most common cancer in women and the primary cause of cancer death in women between the ages of 40 and 55. One in every eight women will be diagnosed with breast cancer, with a 3.4% chance of dying. The incidence of breast cancer fell dramatically in 2002-2003, owing to the publishing of hormone therapy research on breast cancer, and the incidence of breast cancer has remained relatively stable since early 2000.[11] Despite the high incidence of breast cancer, only 5-10% of breast cancers are thought to be caused by genetic factors, while 90-95% of breast cancers are thought to be caused by preventable environmental and lifestyle factors. Ionizing radiation, hormone therapy, late pregnancy, alcohol, a high-fat and high-sugar diet, obesity, and a lack of physical activity are all environmental and lifestyle factors that contribute to breast cancer. Primary prevention is essential in avoiding breast cancer and lowering breast cancer mortality and morbidity.[12]

The average age of the samples in this study was 48.8 years. These findings support the findings of Winters S et al., who found that the incidence of breast cancer began to rise at 40 and peaked at 60. Furthermore, it is believed that the highest incidence of breast cancer in Asian and African populations is found around the age of 40-50 years, whereas the peak incidence of breast cancer in Western populations is discovered at 60-70 years.[6] In addition, the youngest age obtained in this study was 29 years. Young breast cancer patients (< 40 years) have a worse prognosis when compared to patients > 40 years old at the time the cancer is diagnosed.[13] Previous studies reported that there were 92 cases of early breast cancer occurred in women <40 years between 2012 – 2013, in which 41.3% of the patients came in an advanced stage, 36.9% in locally advanced breast cancer

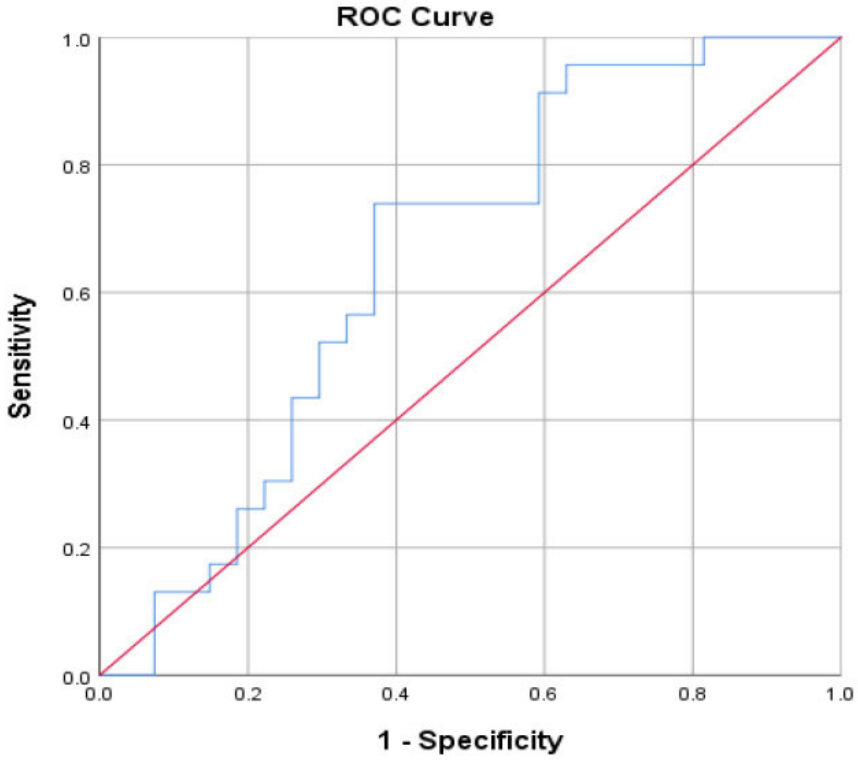

Figure 1. ROC analysis of TNF-α value.

Table 3. The association between serum TNF-α and breast cancer metastasis.

TNF-α level*	Total n	Status Metastasis		P value
		M0	M1	
High, n (%)	27 (54.0)	10 (37.0)	17 (63.0)	0.009*
Low, n (%)	23 (46.0)	17 (73.9)	6 (26.1)	

M0: No metastasis; M1: Metastasis; *Chi-Square: statistically significant if p-value less than 0.05; Cut-off value = 22.755 pg/mL

and only 21.8% came in early breast cancer stage.[14]

Breast cancer risk is influenced by menopausal status. Patients who have gone through menopause will have a lower chance of acquiring breast cancer. This is caused by the decrease in estrogen in patients who have experienced menopause. Furthermore, women who are late to reach menopause (more than 55 years) have an increased risk of breast cancer due to their lifetime exposure to estrogen.[15] Menopause usually occurs when a woman reaches the age of 45. Breast cancer risk increases by 3% each year that menopause is postponed so that by age 55, the risk of breast cancer has increased by up to 30%.[16] In this study, the number of samples who had experienced menopause and those who had not experienced menopause was the same. Because no data on the age at which the sample began to experience menopause was available in this study, the results cannot be used to determine whether menopause raises or decreases the risk of breast cancer.

The size of a tumor in breast cancer has an impact on the patient's long-term prognosis. When breast cancer is discovered while it is still ≤2 cm in diameter and there is no lymph node involvement, the best outcome can be achieved. When it is ≤2 cm in size, patients diagnosed with breast cancer have a 50% lower chance of death than those diagnosed once it is 2-5 cm in size.[17] The majority of the samples in this study had a ≥ 5 cm breast carcinoma. This indicates that most of the samples are at an advanced stage with large tumor sizes. On the other hand, samples with metastases had about the same number as those without.

Luminal A, luminal B, Erb-B2 overexpression, and Basal Like/Triple Negative are the widely known subtypes of breast cancer. Breast cancer with a high number of hormone receptors but minimal proliferation is known as Luminal A. Luminal B, in contrast to Luminal A, has a lower number of hormone receptors and a higher rate of proliferation, resulting in a worse prognosis. The presence of positive HER2 receptors characterizes the Erb-B2 overexpression subtype, which can be treated with targeted therapy such as Trastuzumab. The triple-negative breast cancer subtype has a poor prognosis due to negative results of hormone receptors (estrogen and progesterone) and HER2 receptors.[18] In this study, luminal subtypes had more participants than non-luminal subtypes. These findings support recent research that found that most breast tumors (75%) exhibited positive ER and PR or Luminal group subtypes. However, further research revealed that luminal B HER2 (-) was the most prevalent breast cancer subtype, followed by luminal B-HER2 (+), Luminal A, Erb-B2 Overexpression, and Basal-like/Triple Negative. This contradicts prior research, which claimed that the most common subtype detected was Luminal A. This result can be caused by a lack of sufficient sample size, making it unable to define breast cancer subtypes' prevalence in this population fully. The breast cancer subtype is an important factor for chemotherapy response that clinicians should consider. A previous study in Surabaya also reported that the most common breast cancer subtype was the luminal B HER2-negative.[19]

There are various biomarkers that can be used for detection purposes and to act as a prognostic factors. Tumor cells can generate TNF-α, infiltrating immune cells and stroma in the tumor microenvironment. TNF-α levels are raised in serum and tumor biopsy samples from patients with advanced cancer. TNF-α has been linked to cancer, proliferation, angiogenesis, metastasis, and a weakened immune response. TNF-α may also cause tumors to become resistant to chemotherapy.[20] Another factor that has been reported to affect the chemotherapy response is the expression of Ki67. The decrease of Ki67 expression

was associated with a positive response towards neoadjuvant chemotherapy.[21]

This study also investigated the association between TNF-α levels and the occurrence of metastases in breast cancer patients. The findings of this study revealed a statistically significant association between elevated TNF-α levels and the development of breast cancer metastases. Our findings are consistent with previous studies. In recent research, it was revealed that TNF-α has a pro-metastatic role and influences the epithelial-to-mesenchymal (EMT) process, which will promote tumor cell migration and the occurrence of metastases.[10] In addition, TNF-α was reported to increase along with the increasing cancer stage. The expression of high TNF-α was independently associated with a higher risk of death in breast cancer patients.[22,23] These results showed that the measurement of TNF-α might be a potential marker to predict metastasis and clinical outcome in breast cancer patients.

The limitation of this study is not multicentered; this study was done only in Dr. Soetomo General Hospital Surabaya. Therefore, a further study conducted in several locations in Indonesia might be important to represent the result in the Indonesian population as a whole.

CONCLUSION

There was a significant relationship between elevated TNF-α levels and the incidence of metastases in breast cancer. Serum TNF-α may be a potential biomarker to detect metastasis in breast cancer patients.

CONFLICTS OF INTEREST

No competing interests were declared.

ETHICAL CLEARANCE

This study was reviewed and approved by the Medical Ethical Committee of Dr. Soetomo General Hospital (Ref. No. 0692/LOE/301.4.2/XI/2021), following the guidelines of the Declaration of Helsinki.

AUTHOR CONTRIBUTION

Conceived the study: RE. Designed the study: RE, HG, and IA. Analyzed the data: RE, HG, and IA. Wrote the manuscript: RE and HG. Review the manuscript: HG and IA.

FUNDING

The author(s) received no financial support for this article's research, authorship, and publication.

REFERENCES

1. Agustina J, Sinulingga D, Suzanna E, Andinata B, Ramadhan R, Kadir A. Epidemiology of Female Breast Cancer in West Jakarta, Indonesia. J Glob Oncol. 2018;4(Suppl 2):65s-65s.
2. Gondhowiardjo S, Christina N, Ganapati NPD, Hawariy S, Radityamurti F, Jayalie VF, et al. Five-Year Cancer Epidemiology at the National Referral Hospital: Hospital-Based Cancer Registry Data in Indonesia. JCO Glob Oncol. 2021;7:190–203.
3. Solikhah S, Lianawati L, Matahari R, Rejeki DSS. Determinants of Breast Cancer Screening Practice among Women in Indonesia: A Nationwide Study. Asian Pac J Cancer Prev. 2021;22(5):1435–1441.
4. Hidayat YH, Ishardyanto H, Anniwati L. High Sensitive Troponin I and Extended Range C-Reactive Protein as Markers to Predict Cardiotoxicity in Locally Advanced Breast Cancer with Neoadjuvant CAF (Cyclophoshpamide, Adriamycin/Doxorubicin, 5Fluorouracil) Therapy. Folia Medica Indones. 2020;6:56(2):91–98.
5. Vidiyanti C, Fauziah D, Suprabawati DGA. ER, PR, and HER-2/NEU Profile on Young Breast Cancer Carcinoma Patients. Folia Medica Indones. 2014;50(1):1-10.
6. Winters S, Martin C, Murphy D, Shokar NK. Breast Cancer Epidemiology, Prevention, and Screening. Prog Mol Biol Transl Sci. 2017;151:1-32.
7. Dillekås H, Rogers MS, Straume O. Are 90% of deaths from cancer caused by metastases? Cancer Med. 2019;8(12):5574–5576.
8. Nakahara H, Namba K, Wakamatsu H, Watanabe R, Furusawa H, Shirouzu M, et al. Extension of breast cancer: comparison of CT and MRI. Radiat Med. 2002;20(1):17–23.
9. Wei B, Wang J, Bourne P, Yang Q, Hicks D, Bu H, et al. Bone metastasis is strongly associated with estrogen receptor-positive/progesterone receptor-negative breast carcinomas. Hum Pathol. 2008;39(12):1809–1815.
10. Mercogliano MF, Bruni S, Elizalde P V., Schillaci R. Tumor Necrosis Factor α Blockade: An Opportunity to Tackle Breast Cancer. Front Oncol. 2020;10:584.
11. Libson S, Lippman M. A review of clinical aspects of breast cancer. Int Rev Psychiatry. 2014;26(1):4–15.
12. Kolak A, Kamińska M, Sygit K, Budny A, Surdyka D, Kukiełka-Budny B, et al. Primary and secondary prevention of breast cancer. Ann Agric Environ Med. 2017;24(4):549–553.
13. Fu J, Wu L, Xu T, Li D, Ying M, Jiang M, et al. Young-onset breast cancer: A poor prognosis only exists in low-risk patients. J Cancer. 2019;10(14):3124–3132.
14. Devi DG, Ishardyanto H. Young Age Breast Cancer: Profiles and Overall Survival in Dr. Soetomo Oncology Center, Surabaya, Indonesia. EJSO. 2020;46(2):63–64.
15. Hamajima N, Hirose K, Tajima K, Rohan T, Friedenreich CM, Calle EE, et al. Menarche, menopause, and breast cancer risk: Individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012;13(11):1141–1151.
16. Surakasula A, Nagarjunapu G, Raghavaiah K. A comparative study of pre- and post-menopausal breast cancer: Risk factors, presentation, characteristics and management. J Res Pharm Pract. 2014;3(1):12-18.
17. Narod SA. Tumour size predicts long-term survival among women with lymph node-positive breast cancer. Curr Oncol. 2012;19(5):249–253.
18. Teichgraeber DC, Guirguis MS, Whitman GJ. Breast cancer staging: Updates in the AJCC cancer staging manual, 8th edition, and current challenges for radiologists, from the AJR special series on cancer staging. Am J Roentgenol. 2021;217(2):278–290.
19. Winantyo VI, Tanggo VVCM, Ali I. Association of neutrophil-lymphocyte ratio (NLR) with the anthracycline-based neoadjuvant chemotherapy (NAC) clinical response in locally advanced breast cancer (LABC) in young women. Bali Medical Journal. 2022;11(2):602-608.
20. Zhang Z, Lin G, Yan Y, Li X, Hu Y, Wang J, et al. Transmembrane TNF-alpha promotes chemoresistance in breast cancer cells. Oncogene. 2018;37(25):3456–3470.
21. Devi DGAP, Purwanto H, Sandhika W. Changes in Ki67 expression and clinical response to neoadjuvant chemotherapy in locally advanced breast cancer (LABC). Bali Medical Journal. 2021;10(3):925–931.
22. Sheen-Chen S-M, Chen W-J, Eng H-L, Chou F-F. Serum concentration of tumor necrosis factor in patients with breast cancer. Breast Cancer Res Treat. 1997;43(3):211–215.
23. Tripsianis G, Papadopoulou E, Anagnostopoulos K, Botaitis S, Katotomichelakis M, Romanidis K, et al. Coexpression of IL-6 and TNF-α: prognostic significance on breast cancer outcome. Neoplasma. 2014;61(2):205–212.