The Role of IFN gamma and IL-10 in Breast Cancer

LaleMaulin Prihatina[1,2], Willy Sandhika[3,4], Grace Ariani[3,4]

[1]Resident, Departement of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [2]Resident, Dr.Soetomo General Academic Hospital, Surabaya, Indonesia, [3]Lecturer, Departement of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [4]Lecturer, Dr.Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Inflammation in the tumor microenvironmentisanessentialaspectof tumor biologicalactivity. Interferon γ and IL-10 are pro-inflammatoryand anti-inflammatorycytokinesthatplay a crucialrole in regulatingthehost'simmuneresponseto cancer cells. IFN-γ and IL-10 expression are associatedwithpoor prognosis andlowsurvivalrate in breastcarcinomapatients.This cross-sectional study was performed on the 60 paraffin-embedded samples of radical mastectomy during January 2016-December 2019 at Anatomical Pathology Laboratory of Dr.Soetomo General Academic Hospital Surabaya. The samples were divided based on tumor size into four groups (T1, T2, T3, T4). TheanalyzedwasusingKruskal Wallistest. Immunohistochemical staining was performed to detect the expression of IFN-γ and IL-10. There was a significant difference in IFN-γ expression in the four groups (p=0.005) and no significant difference in IL-10 expression in the four groups (p=0,191). Interferon gamma and IL-10 were important in determining prognosis and targeted therapy in breast cancer patients. These results may contribute to the development of breast cancer research.

Keywords: *Breast cancer, IFN-γ, IL-10, T stage.*

Introduction

Inflammation in the tumor microenvironment is an essential aspect of tumor biological activity because it is associated with tumor initiation, tumor development, response to therapy, and prognosis. Most cancers arise associated with inflammation that occurs continuously.[1,2]

The incidence of breast cancer increases with age; 80% of breast cancers appear in women over 50 years. Breast cancer is rare in young women, but it becomes more aggressive, with 5-years survival rates reaching 81% at less than 45 years. Early detection with immunoregulatory cytokines including interferon-alpha, beta, gamma, interleukins 2, 6, and 10, and alpha tumor necrosis factor (TNF), which is often associated with breast cancer and can help determine the patient's prognosis.[3]

Interferon-gamma and interleukin 10 are pro-inflammatory and anti-inflammatory cytokines that regulate immune responses and inhibit pro-inflammatory function to antigen-presenting cells (APCs) through the expression of antagonist molecules. Interferon-gamma and interleukin expression are associated with poor prognosis and low survival rates[4]. IFN-γ has an antitumor function, and a reduced amount of IFN-gamma in the tumor environment is associated with a worse prognosis in breast cancer patients.[3]

IL-10 overexpression triggers a pro-inflammatory effect by increasing IFN-γ, IL-10, and other IFN-γ-induced releases of monokines. Therefore, giving IL-10 antagonists is expected to be a more effective targeted therapy.[4]

Corresponding author:
Willy Sandhika
Email: willysand@fk.unair.ac.id

This research aimed to analyze the expression of IFN-γ and IL-10 and investigate differences in expression at various stages of breast cancer

Materials and Methods

This study was analytic observational research with a cross-sectional approach performed on the 60 paraffin-embedded samples of radical mastectomy during January 2016-December 2019 at Anatomical Pathology Laboratory of Dr.Soetomo General Hospital Surabaya. The samples were grouped based on tumor size into four groups (T1, T2, T3, and T4); each group was 15 samples.

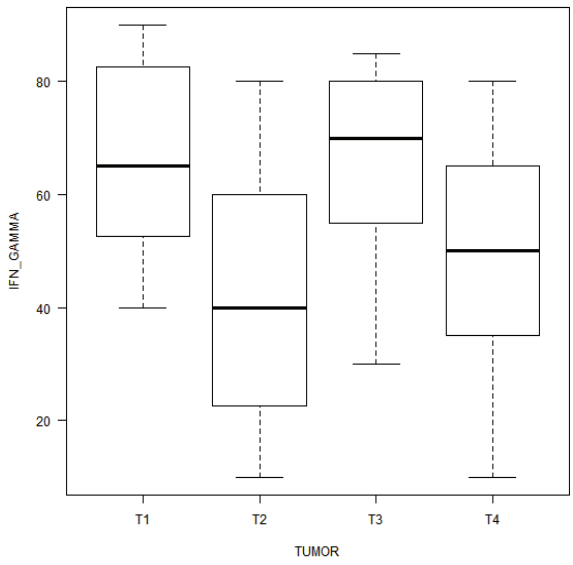
Immunohistochemistry staining was performed to detect the expression of IFN-γ and IL-10. The tissues were cut into four mm sections, deparaffinized three times with xylol for five minutes each, and rehydrated through graded alcohol. Antigen retrieval was achieved by microwave treatment in sodium citrate buffer (pH 6.0) for ten minutes. The tissue sections were then incubated with monoclonal antibodies for IFN-γ (LLO6Z: sc-74108; dilution 1:200; Santa Cruz Biotechnology) and IL-10 (GT5111; dilution 1:100; GeneTex) overnight, followed by the secondary antibody for 10 minutes at room temperature. Sections were then counterstained with hematoxylin and dehydrated with alcohol.

Cytoplasmic staining for IFN-γ and IL-10 were evaluated on tumor cells.IFN-γ and Il-10 are considered positive if expressed in tumor cells' cytoplasm.[5]

Two pathologists evaluated all samples in a blinded fashion. Any discordant was solved by interobserver agreement. The expression and comparison of IFN-γ and Il-10 expression in any T stages of breast carcinoma was tested using Kruskal- Wallis and Mann-Whitney U test.

Results and Discussion

Kruskal-Wallis test showed difference IFN-γ expression in any T stage breast carcinoma (p=0.005) (Figure 1), and the Mann-Whitney U testfound a significant difference in IFN-γ expression between T1 - T2 and T2 - T3 (p=0.03; p=0.04) meanwhile there is no considerable difference IFN-γ expression in T3 - T4 (p=0.2) or other T stages. Interferon-gamma and IL-10 expressed at cytoplasm (Figure 2 and 3). Kruskal-Wallis test showed no difference in IL-10 expression in any T stages breast carcinoma (p=0.191) (Figure 3). Interleukin-10 is expressed in thecytoplasm (Figure 1).



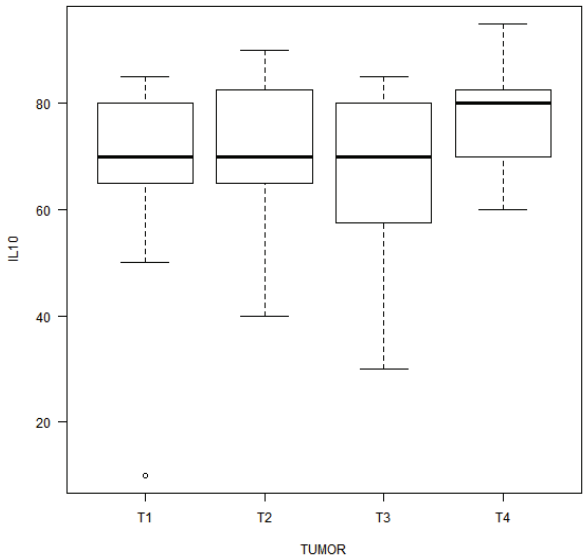


Figure 1. A Differences of IFN-γ expression in any T stages of breast carcinoma. B No differences of IL-10 expression in any T stages of breast carcinoma.

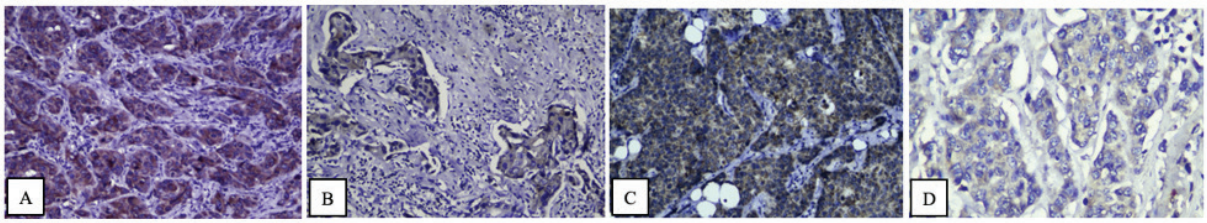


Figure 2. Immunohistochemical expression ofIFN-γ in any T stage breast carcinoma, 200× magnification. A: Expressed in 93% tumor cells; B: Expressed in 50% tumor cells; C: Expressed in 80% tumor cells; and D: Expressed in 70% tumor cells

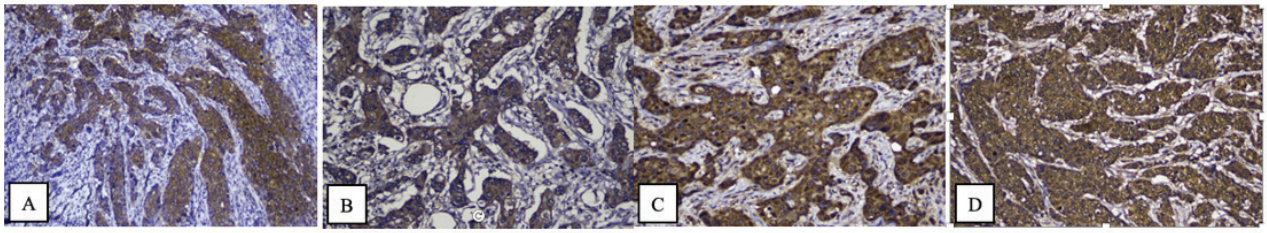


Figure 3. Immunohistochemical expression ofIl-10 in any T stage breast carcinoma, 200× magnification. A: Expressed in 80% tumor cells; B: Expressed in 80% tumor cells; C: Expressed in 85% tumor cells; and D: Expressed in 90% tumor cells

Interferon-γ (IFNγ) has a vital role in activating cellular immunity and stimulation of the antitumor immune response. Based on its cytostatic, proapoptotic, and antiproliferative functions, IFNγ is considered potential adjuvant immunotherapy for various cancer types. IFNγ may inhibit angiogenesis in tumor tissue, induce regulatory T-cell apoptosis, and stimulate pro-inflammatory M1 macrophage activity to support tumor cells proliferation.[6]

The statistical analysis results showed a significant difference between IFNγ expression in any T stages breast carcinoma (p = 0.005). The most significant

difference between each group on the IFNγ expression was performed using the Mann-Whitney U statistical test. This test shows that the T1 and T2 groups are the groups that have the most significant difference (p = 0.03), and the T2 and T3 groups (p = 0.04) while the T3 and T4 groups did not have a substantial difference with p = 0.203 as well as other groups.

High IFNγ expression was found at stage T1, then decreased at stage T2 and increased again at T3 and T4. In T1 tumors, IFNγ will be secreted in large quantities by pro-inflammatory cytokines to support tumor cell proliferation; at this early stage, the body is still able to adapt to inhibit tumor cell proliferation so that IFNγ levels can be suppressed in T2 tumors, hance inflammatory agents are not eliminated (tumor). The inflammatory process continues, causing an increase in pro-inflammatory cytokines and an accelerated proliferation of tumor cells; as a result, the body cannot keep up with the speed of tumor cell proliferation and loses its homeostasis ability, the tumor size increases, and IFNγ levels increase again at stages T3 and T4.[2,7,8]

These results were consistent with the study by Tuñón et al., who found differences in IFNγ expression at various T stages of breast carcinoma, and the highest expression was found at stages T1 and T2.[9]He et al. stated that continuous exposure to IFN-γ had been shown to increase the growth of hepatoma tumors, breast tumors, adenocarcinoma, and melanoma.[10] These results indicate that the higher levels of IFN-γ in the tumor microenvironment can increase tumor cell growth and proliferation.

Interleukin-10 is known to have an inhibitory effect on T-cell function and proliferation. Interleukin-10 has also been shown to inhibit antigen presentation by macrophages and Langerhan cells and manifest tumor-related antigens by tumor cells. IL-10 production is associated with the induction of anergy in T lymphocytes so that the tumor utilizes IL-10 production in the tumor microenvironment to escape from the immune system.[11]

The statistical analysis results showed no difference in IL-10 expression at various stages of T breast carcinoma with a p-value = 0.191. Expression of IL-10 tends to be high in all T stages, and the highest is found in staging T4 of breast carcinoma. This can occur because tumor cells naturally produce large amounts of IL-10 themselves. Many human cancer cell lines have been shown to secrete IL-10 into their supernatants. Breast tumor cells have been shown to express high levels of IL-10 mRNA. This elevated expression causes high levels of IL-10 to be expressed in the tumor microenvironment and breast tumor cells.[12]These results are in line with Bhattacharjee et al., which stated that there was no correlation between IL-10 expression and patient age, tumor size (T), and ER status, PR.[5]

Gonzalez-Garza et al. Examined samples from breast cancer patients and normal peritumoral breast tissue. Found a correlation between IL-10 expression and a worse prognosis in breast carcinoma patients, and no IL-10 was expressed in normal breast tissue samples. The determination of IL-10 expression in breast cancer patients suggests that IL-10 can be used as a biological marker to differentiate between advanced cancer.[13]

Lianes-Fernandez et al. obtained strong IL-10 expression in 23 of 27 breast cancer patients based on immunohistochemical examination. In another study of 105 samples of breast cancer patients and 13 samples of healthy breast tissue, IL10 expression was only seen in breast cancer tissue and no normal healthy tissue.[11,14]

The high concentration of IL-10 in the serum of cancer patients does not appear to be related only to the expression of this protein by the immune system. Evidence suggests that cancer cells can synthesize themselves, thereby causing an imbalance in the homeostasis of the immune system and causing tumors to escape.[13]

Conclusion

There were differences of IFNγ expression in any T stages of breast carcinoma, and there was no difference of IL-10 expression in any T stages of breast carcinoma.

Conflict of Interest : The authors declare that they have no conflict of interest.

Source of Funding : This study is supported by the Ministry of Education and Culture of the Republic of Indonesia.

Acknowledgments : We thank Dr. Budi Utomo, Department of Public Health and Preventive Medicine, Universitas Airlangga, for statistical analysis.

Ethical Approval : This study had been approved by the Health Research Ethics Committee of Dr.Soetomo General Academic Hospital, Surabaya, Indonesia 0337/ LOE/301.4.2/II/2021.

References

1. DeNardo DGand Coussens LM. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Research 2007, 9:212 doi:10.1186/ bcr1746
2. Goldberg JE,and Schwertfeger KL. Pro-inflammatory cytokines in breast Cancer: Mechanisms of action and potential targets for therapeutics. Bentham Science Publishers. Current Drug Targets. 2010, 11(9):1133-46,doi: 10.2174/138945010792006799.
3. Borj, M.R., Andalib, A.R., Mohammadi, A., Hoseiniharouni, S.M., Pourghadamyari, H., Golmohammadi, T., Ghahfarokhy, K.M., 2017. 'Evaluation IL-4, IL-17, and Interferon γ levels in patients with breast cancer'. Int J Basic Sci Med. 2017;2(1):20-24**,** doi: 10.15171/ijbms.2017.05.
4. Sheikhpour E, Noorbakhsh P, Foroughi E, Farahnak S, Nasiri R, Neamatzadeh N. A Survey on the role of interleukin-10 in breast cancer: a narrative. Reports of Biochemistry & Molecular Biology. 2018*,* 7(1) 30-37, http://rbmb.net/article-1-157-en. html
5. Bhattacherjee HK, Bansal VK, Nepal B, Srivastava S, Dinda AK, Mistra MC. Is interleukin 10 (IL10) expression in breast cancer a marker of poor prognosis?'. Indian Association of Surgical Oncology. 2016; 15(June), doi: 10.1007/s13193-016-0512-6
6. Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN-γ in tumor progression and regression: a review. Biomarker Research. 2020; Vol 8:49, doi : 10.1186/s40364-020-00228-x.
7. Castro F, Cardoso AP, Gonçalves RM, Serre K, dan Oliveira MJ. Interferon-gamma at the crossroads of tumor immune surveillance or evasion. Frontiers in Immunology. 2018; 9(May), pp. 1–19, doi: 10.3389/fimmu.2018.00847.
8. Zaidi MR. The Interferon-gamma paradox in cancer. Journal of Interferon and Cytokine Research. 2019;Vol 39, No 1, doi: 10.1089/jir.2018.0087
9. García-Tuñón I, Ricote M, Ruiz A, Fraile B, Paniagua R, Royuela, M. Influence of IFN-gamma and its receptors in human breast cancer. BMC Cancer.2007; **7**:158 doi:10.1186/1471-2407-7-158
10. He YF, Wang XH, Zhang GM, Chen, HT, Zhang H, Feng ZH. Sustained low-level expression of interferon-gammapromotes tumor development: potential insights in tumor prevention and tumor immunotherapy. Cancer Immunol Immunother. 2005; Vol 54(9):891–897, doi: 10.1007/s00262-004-0654-1
11. Llanes-Fernandez L, Goyanes RIA, Prado MCA, Gonzalez JMA, Mojarrieta JC, Perez XE, Lopez, MO, Odio SF, Rodrıguez RC, Guerra-Yi ME, Marina VM, Guerra RT, Padilla CR. Relationship between IL-10 and tumor markers in breast cancer patients. Original Article The Breast BElsevier Ltd. All rights reserved. 2006; Vol 15, pp: 482–489, doi:10.1016/j.breast.2005.09.012.
12. Hamidullah, Changkija B, Konwar R. Role of Interleukin-10 in Breast Cancer. Breast Cancer Res Treat. 2012; 133(1), 11-21, doi: 10.1007/s10549-011-1855-x
13. Gonzalez-Garza MT, Cruz-Vega DE, Maldonado-Bernal C. IL10 as Cancer Biomarker. IntechOpen. 2020, doi: 10.5772/intechopen.90806.
14. Chavey C, Bibeau F, Gourgou-Bourgade S, Burlinchon S, Boissière F, Laune D, Roques S, Lazennec G. Estrogen receptor negative breast cancers exhibit high cytokine content. Breast Cancer Research BioMed Central. 2007; Vol 9 (1), pp.R15, doi:10.1186/bcr1648 .inserm-00143810