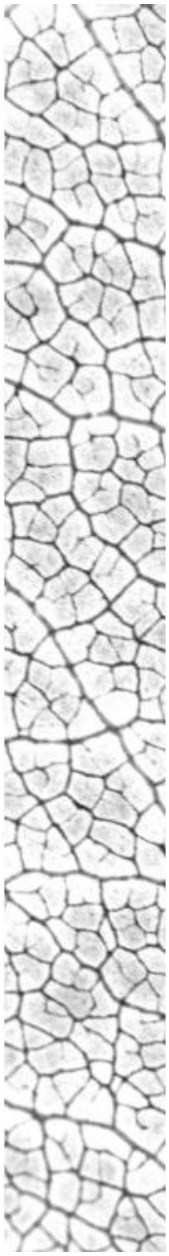

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The immunity analysis on osteosarcoma as the basic development of therapy follow-up

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Abstract

Background: The immune system conditions of patients with osteosarcoma correlate with the neoadjuvant therapy administration. NK cell (natural killer cell) of macrophages are known as proinflammatory macrophages (macrophage-1/M-1). If IFN-γ decreases, NK cells will be inactivated so that they cannot destroy cancer cells. This condition occurs due to the activities of anti-inflammatory macrophages, known as macrophage-2 (M-2). **Purpose:** This study aims to determine the correlation between M-1 and M-2 in patients with osteosarcoma, ranging from non-metastatic to metastatic osteosarcoma. **Method:** There were 26 patients with osteosarcoma, consisting of 13 samples with stage IIB osteosarcoma and 13 samples with stage III osteosarcoma. TNF-α and IL-10, which became intermediate variables for the M1/M2 ratio, were immunohistochemically stained and counted in 10 visual fields (visual field: 15625μ^2) with 400 times magnification. The data analysis was performed using a statistical test, i.e., difference test using the Mann-Whitney U test between groups of patients with stage IIB and stage III osteosarcoma on IL-10 and TNF-α. **Results:** M1/M2 ratio on osteosarcoma, in terms of anti-inflammatory aspect, i.e., based on the number of macrophages that express IL-10 between patients with stage IIB and stage III osteosarcoma, reached 1:6.4, rounded to 1:6. Meanwhile, the M1/M2 ratio on osteosarcoma in terms of proinflammatory aspect amounted to 5:1. **Conclusion:** The result of the M1/M2 ratio on stage IIB osteosarcoma tissue is five times greater on M-1 macrophages, while the M1/M2 ratio on stage III osteosarcoma is six times greater on M-2 macrophages.

Keywords: osteosarcoma, immunity, macrophages, natural killer cells

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INTRODUCTION

One of the efforts to treat osteosarcoma is by administering a combination of neoadjuvant chemotherapy, a combination of cisplatin, ifosfamide, and doxorubicin. In administering the chemotherapy, out of 36 patients evaluated, there were 20 patients (55.6%) who provided good responses, while the rest did not respond. This situation might be due to the correlation between the immune system condition of patients with osteosarcoma and neoadjuvant therapy administration. Patients' conditions, especially related to a psychological condition, will affect the immune system condition.

NK cells (natural killer cells) cells hold a crucial role in destroying cancer cells by utilizing interferon-gamma stimulation (IFN-γ). In addition to macrophages, one of the cells producing IFN-γ is Th-1 osteosarcoma. However, these cells will produce IFN-γ when there is an interleukin-1 (IL-1) stimulation secreted by macrophages. These macrophages are known as proinflammatory macrophages (macrophages-1/M-1). If IFN-γ decreases, NK cells will be inactivated so that they cannot destroy cancer cells. This condition occurs due to the activity of anti-inflammatory macrophages, known as macrophages-2 (M-2). At present, the ratio difference between M-1 and M-2 at the osteosarcoma stages is unidentified yet. (Sorbi, & Farrokhnia, 2018).

The bond between CD95 (APO-1/Fas) and its ligand, CD95R, on the cancer cell surface, will cause protein activity in cancer cells, known as Fas-associated Protein with Death Domain (FADD). Then, the FADD activates the caspases, and subsequently, the cancer cells will die (apoptosteosarcoma). Macrophage cells that secrete proinflammatory cytokines are known as macrophage-1 (M-1). TNF-α and IL-6 cytokines are produced by M-1.

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Table 1. The data distribution of the mean value and standard deviation of variables: IL-10 and TNF-α

No.	Number of Samples	Stage	Variable	Average	Standard Deviation
1	13	IIB	IL-10	0.5538	0.24703
2	13	IIB	TNF- α	4.5308	1.08042
3	13	III	IL-10	4.2615	1.23054
4	13	III	TNF- α	0.9154	0.22674

On invasive cancers, macrophages polarize into tumor-associated macrophages (TAMs), which then secrete interleukin-10 (IL-10). Macrophage cells that secrete anti-inflammatory cytokines (IL-10) are known as M-2. TAMs will induce tumor cell proliferation, invasion, and metastasis, as well as stimulate tumor angiogenesis and inhibit the immune response to the tumors.

This study aims to determine the correlation between M-1 and M-2 in patients with osteosarcoma, ranging from non-metastatic to metastatic osteosarcoma. This study involved samples consisting of patients with stage IIB (non-metastatic) and stage III (metastatic) osteosarcoma.

MATERIAL AND METHOD

This study was an observational study with a crosteosarcomas sectional analytic study. There were 26 patients with osteosarcoma, consisting of 13 samples with stage IIB osteosarcoma and 13 samples with stage III osteosarcoma. The sample criteria were patients with 10-45 years old (stable immune system), male, and stage IIB and III osteosarcoma. The immunohistochemical staining method was used in 10 visual fields (visual field: 15625μ2), with 400 times magnification. The tissue, sliced with a microtome and then placed on the object glass, was then deparaffinized, i.e., pulling out/removing the paraffin contained in the tissue.

TNF-α is a cytokine expressed by macrophages in osteosarcoma tissue incision, which provides a posteosarcomative reaction to anti-TNF-α. The tissue, slashed with a microtome and placed on the object glass, was then deparaffinized, which is to pull/remove paraffin contained in the tissue. The immunohistochemical staining method was used and counted in 10 visual fields (visual field: 15625μ^2) with 400 times magnification. The data analysis was performed using a statistical test, i.e., difference test using the Mann-Whitney U test between groups of patients with stage IIB and stage III osteosarcoma on IL-10 and TNF-α.

RESULTS

After evaluating each sample, both in the stage IIB and stage III osteosarcoma groups, an observation was conducted using immunohistochemical methods in 10 visual fields (visual field: 15625μ^2). Then, the data obtained were tested statistically using the Mann-Whitney U test, which was previously tested by the Kolmogorov-Smirnov normality test. Based on IL-10 statistical analysis in osteosarcoma tissue using the normality test (Kolmogorov-Sminornov), it was found that the data to analyze had an abnormal distribution due to the p-value = 0.001, therefore the Mann-Whitney U test was performed. Based on the Mann-Whitney U test, IL-10 between stage IIB and stage III osteosarcoma groups obtained significant results, i.e., p = 0.000 (X-IIB = 0.5538; SD = 0.24703) and (X-III = 4.2615; SD = 1.23054). These results indicated that the number of macrophages expressing IL-10 in stage III osteosarcoma tissue was greater than that in stage IIB osteosarcoma tissue.

Based on the TNF-α statistical analysis in osteosarcoma tissue using the normality test (Kolmogorov-Sminornov), the data to analyze was found to have an abnormal data distribution with a p-value of 0,000. Based on this result, the difference test was carried out using the Mann-Whitney U test. The analysis results showed that the macrophages expressing TNF-α in stage IIB and stage III osteosarcoma groups indicated significant results with a p-value of 0.000 (X-IIB = 4.5308; SD = 1.08042) and (X -III = 0.9154; SD = 0.22674). These results indicated that the number of macrophages expressing TNF-α in stage III osteosarcoma tissue was smaller than stage IIB osteosarcoma tissue.

DISCUSSION

Based on the results in **Table 1**, the number of macrophages expressing IL-10 in stage III osteosarcoma tissue was greater than that in stage IIB osteosarcoma tissue. In stage III, it was caused by the existence of cancer cell activity to induce macrophages into TAMs, so the number of anti-inflammatory macrophages producing IL-10) increased. The increased number of anti-inflammatory macrophages (M-2) in stage III osteosarcoma tissue indicated that, in this condition, cancer cell proliferation, angiogenesis, and cancer cell metastasis increased.

Malignant cells could engage peripheral monosteosarcoma. In an effort to foster its development, these monosteosarcomas were known as tumor-associated macrophages or TAMs (10). These TAMs play crucial roles in the developmental prosteosarcoma and metastases of cancer cells because they can release several mediators, including growth factors, proteolytic enzymes, and Monocyte Chemoattractant Protein-1 or MCP-1, which have a role in angiogenesis

prosteosarcoma and migration/invasion factors of cancer cells.

The number of macrophages that expressed IL-10 and TNF-α in stage III osteosarcoma tissue was smaller than that of macrophages that expressed TNF-α in stage IIB osteosarcoma tissue. This condition was cancer cell activity did not occur to induce macrophages into TAMs in stage III. Therefore, the number of anti-inflammatory macrophages (producing IL-10) did not increase, and the proinflammatory macrophages (M-1) did not experience inhibition. This condition was marked by an increase in the number of macrophage cells expressing TNF-α, thus, under these conditions, IFN-IF would also increase.

If abnormal cells experienced uncontrolled division, macrophages would release an interferon-gamma to activate NK cells, in which the active NK cells could kill the abnormal cells. If there was a bond between the FasL of NK or CTL cells and fas of target cells, then in the target cells' bodies, protein activity would occur as apoptosteosarcoma signal or FADD. Therefore, the target cells would die. An increase in the number of macrophage cells expressing IFN-γ would occur and trigger an increase in NK cell activities. The increase of NK cells would prevent cancer cells' development or damage. Therefore, in stage IIB osteosarcoma, there was no cancer cells' progression or metastasis.

Based on the analysis results, the M1/M-2 ratio in terms of IL-10 expression aspect suggested that M-1 in patients with stage IIB osteosarcoma was smaller than M-2 in patients with stage III osteosarcoma. The related studies found the increase of IL-10 indicated that the suppression of the proinflammatory immune system occurred, thus IL-6, TNF-α, IL-1, and IFN-γ decreased. Cytokine decrease caused a decrease in the number of NK cells and/or NK cell functions. As a result, the obstacles in cancer cells' development and metastasis could not be overcome. Therefore, this condition showed that cancer cells' progression increased in patients with stage III osteosarcoma.

In terms of the proinflammatory aspect, the results of the M1/M2 ratio in osteosarcoma reached 5:1, which was based on the number of macrophages expressing TNF-α between patients with stage IIB and stage III osteosarcoma calculated based on (+2 SD). The results of M1/M2 ratio analysis based on TNF-α expression indicated that the M-1 in patients with stage IIB osteosarcoma was greater than M-2 in patients with stage III osteosarcoma. Previous research stated that the increase of TNF-α indicated that the activities of proinflammatory immune systems increased. Therefore, IL-6, TNF-α, IL-1, and IFN-γ would increase as well. The increase in cytokines caused the number of NK cells and NK cell functions to increase. Therefore, patients with stage IIB osteosarcoma would face obstacles in the development and metastasis of cancer cells.

CONCLUSION

The result of the M1/M2 ratio on stage IIB osteosarcoma tissue is five times greater on M-1 macrophages, while the M1/M2 ratio on stage III osteosarcoma tissue is six times greater on M-2 macrophages. The number of macrophages expressing TNF-α in stage IIB osteosarcoma tissue is greater than patients with stage III osteosarcoma. Meanwhile, the number of macrophages expressing IL-10 in stage IIB osteosarcoma tissue is smaller than in stage III osteosarcoma.

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