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CD4-T LYMPHOCYTES IN CERVICAL CANCER PATIENTS ON PRE- AND POST-CHEMOTHERAPY

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ABSTRACT

Cervical cancer is a gynecologic cancer with the highest incidence in the Dr.Soetomo Hospital, Surabaya. Neoadjuvant chemotherapy with cisplatin has been used to increase the radiosensitivity of cancer cells before radiotherapy in advanced cervical cancer patients. The $CD4^+$ T lymphocytes act as the main regulator in antitumor immunity system. This research aimed to determine the differences of CD4-T lymphocyte profile in stage IIIB patients before and after chemotherapy administration. This research was performed in February-September 2018. 17 subjects of 31 patients with stages IIIB cervical cancer planned to receive neoadjuvant chemotherapy with cisplatin every 3 weeks for 3 series and $CD4^+$ T lymphocyte count and percentage were measured f. The examinations were done before the first and after the third chemotherapy administration. Mean±SD of the CD4-T lymphocyte count and that of CD4-T lymphocyte percentage before chemotherapy were 817±314 cells/μL and 38.96±8.47%, respectively. Furthermore, mean±SD of the CD4-T lymphocyte count and that of CD4-T lymphocyte percentage after chemotherapy were 881±335 cells/μL and 39.01±8.50%, respectively. There was no significant difference of CD4-T lymphocyte count between before and after chemotherapy (p=0.471). There was no significant difference of CD4-T lymphocyte percentage between before and after chemotherapy (p=0.866). Both the CD4-T lymphocyte count and percentage tended to increase in post-chemotherapy condition. There was no significant difference of CD4-T lymphocyte count and percentage between before and after chemotherapy administration in stage IIIB cervical cancer patients. Both the CD4-T lymphocyte count and percentage tended to increase in post-chemotherapy condition.

Key words: CD4, neoadjuvant, advanced stage, chemotherapy, cervical cancer

INTRODUCTION

Cervical cancer is the second most common cancer in woman and the first leading cause of death among all gynecological cancers in the world.[1] Cervical cancer is also the most common cancer in Indonesia which makes approximately 36% of all cancer cases, based on cancer data in 13 pathology laboratory centers. About 40,000 new cervical cancer cases are found every year in Indonesia.[2]

Limited access to health facilities and the unimproved cervical cancer screening program in a developing country make cervical cancer the most frequent cause of death.[3] A study conducted in developing cancer showed that 80% of new cervical cancer cases were found in advanced stage.[4] Thirty-seven percent of advanced cervical cancer consisted of stage IIIB cervical cancer cases which have spread into the pelvic wall.[2]

Cervical cancer is the most commonly found gynecologic cancer with the highest incidence in Dr. Soetomo General Hospital, Surabaya. Its incidence in 2015-2016 was 69.6% of all gynecological cancer. More than 4000 new cases were found between 2011-2016. Most cases (approximately 43%) were first diagnosed as stage IIIB cervical cancer.

Cervical cancer stage is mainly determined based on the International Federation of Gynecology and Obstetrics (FIGO) staging classification. The stage of this disease is one of the considerations in choosing therapy in addition to age and other medical conditions.[5] The main cervical cancer therapy in early-stage (stage I-IIa) is surgical therapy, while in advanced stage (IIb-IVb) is chemotherapy and radiotherapy that can be given as a single or combination therapy. Those therapy modalities are given with its side effects.[6,7]

Cancer patients including cervical cancer patients are prone to immune system disorder. Invasive cervical cancer patients have a lower $CD4^+$ T-lymphocyte count compared to Cervical Intraepithelial Neoplasia (CIN) patients. Invasive cervical cancer patients with metastatic conditions have a significant decrease of $CD4^+$ T lymphocyte count compared to stage I-III cervical cancer patients. Cancer itself can cause a condition called T cell

exhaustion that can cause a further decrease of both T lymphocyte count and function progressively.[8] A study conducted in types of cancers found a significant decrease of lymphocyte count in post-chemotherapy condition compared to pre-chemotherapy condition.[9]

The $CD4^+$ T lymphocyte roles in antitumor immunity have been shown in many previous studies. These cells play a crucial role in antitumor immunity as the main regulator of the immune system against the tumor cells. These cells produce various protein mediators, the lymphokines, that will interact with many cells in the immune system and bone marrow.[10] Other immune cells in antitumor immunity system cannot play their role properly without the lymphokines secreted by $CD4^+$ T lymphocytes.[11,12]

The therapy modalities may have cytotoxic and nonspecific effects on the patient's healthy cells that may worsen the immune system disorder occurred in cancer patients. Furthermore, this condition can also worsen the cancer patient's quality of life. Chemotherapy is one of nonspecific therapy modalities. Chemotherapy agents generally kill cells that rapidly divide such as the blood cells.[11,13]

The cisplatin chemotherapy in stage IIIB cervical cancer is given to the patients as neoadjuvant chemotherapy. Neoadjuvant means the chemotherapy is given before radiation as the main therapy. The purpose of administrating neoadjuvant therapy administration is to reduce the size and number of cancer cells to enable complete eradication by the main therapy. The National Cancer Institute recommends cisplatin-based chemo-radiotherapy instead of a radiotherapy in invasive cervical cancer patients.[14] Cisplatin-based chemo-radiotherapy is also recommended by ESMO for therapy of advanced-stage cervical cancer.[15]

Cisplatin is a commonly used regiment for neoadjuvant chemotherapy in advanced stage cervical cancer.[14,15] This regiment has myelosuppressive effects that are presumed to influence immune system mediated by lymphocytes.[9] However, a subclinical study conducted by de Biasi *et al* found that this agent also showed an antitumor immunomodulation effect.[16]

A recent study is a preliminary study aimed to determine the $CD4^+$ T lymphocyte profile that comprises of $CD4^+$ T lymphocyte count and percentage in stage IIIB cervical cancer patients in both pre and post-chemotherapy conditions. The differences of the $CD4^+$ T lymphocyte profile between the two conditions will also be analyzed. The information about those conditions has not been studied yet in previous research.

METHODS

This research was an observational analytic study. Study subjects were patients diagnosed with stage IIIB cervical cancer and planned to receive neoadjuvant chemotherapy (Cisplatin 50mg/m^2 every 3 weeks). The diagnosis and therapy were determined independently by clinicians in the Obstetrics and Gynecology Department of Dr. Soetomo Hospital. Subjects were informed about the study objective and procedure before asked for informed consent. All research subjects had signed informed consent. This study had been approved by the ethical committee in Dr. Soetomo Hospital with ethical clearance number of 0059/KEPK/II/2018.

This study was conducted from February until August 2018. The subjects were recruited consecutively and followed for 3 series of chemotherapy. Each chemotherapy series was 3 weeks apart. The first CD4- T lymphocyte count and percentage were measured before the first cisplatin chemotherapy administration. The second CD4- T lymphocyte-profile measurement was performed 3 weeks after the third cisplatin administration. The study flowchart is presented in Figure 1.

Inclusion criteria were stage IIIB cervical cancer patients based on FIGO classification that planned to receive chemotherapy, patients who had not received any chemotherapy before, agreed to join the study, and accessible to get a vein blood sample. The patient's conditions should be stable based on physical and laboratory examination including hemoglobin, albumin, BUN, serum creatinine, and glucose levels before stated feasible to receive chemotherapy. The feasible conditions were determined independently by clinicians. Patients with associated chronic diseases, HIV infection history, and received less than 3 series of chemotherapy were excluded.

The $CD4^+$ T lymphocyte profile was measured from venous blood collected in EDTA anticoagulant tube. The measurement was performed by skilled laboratory technologists using FACS Calibur flow cytometry and BD TritestTM CD3/CD4/CD45 reagent. All samples were measured within 24 hours of blood collection time. All measurement was performed in the Clinical Pathology Laboratory of Dr. Soetomo Hospital.

The study result was statistically analyzed using SPSS 23.0. Data normality was tested using the Kolmogorov-Smirnov test. The differences of $CD4^+$ T lymphocyte count and a percentage between pre-and post-chemotherapy administration were analyzed using paired t-test and significance level of 0.05 was used.

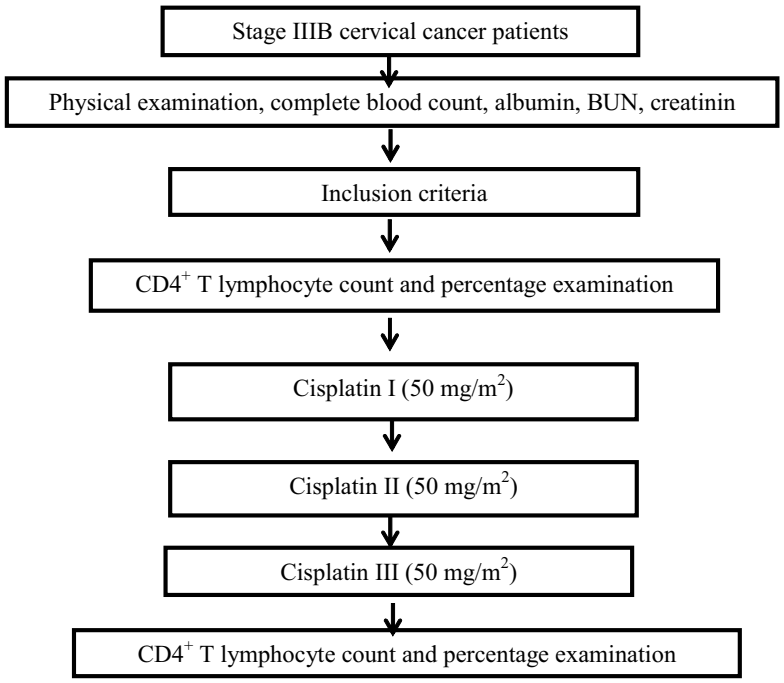

Figure 1. Study design and flow chart

RESULT AND DISCUSSION

Thirty-one patients diagnosed with stage IIIB cervical cancer and planned to receive chemotherapy were recruited in this study. Fourteen patients were excluded because of receiving chemotherapy less than 3 series. Seventeen out of 31 patients who completed 3 series of chemotherapy were analyzed for the differences of $CD4^+$ T lymphocyte count and a percentage between pre- and post-chemotherapy administration. The patient's ages ranged from 44 to 67 years old with the mean age of 54 years old. The characteristics of subjects including age, $CD4^+$ T lymphocyte count and percentage before and after chemotherapy administration were presented in Table 1. Statistical analysis showed normal data distribution that enabled the analysis with the parametric test.

Statistical analysis showed no statistically significant difference of age, $CD4^+$ T lymphocyte count and percentage in pre-chemotherapy condition, with p-values of 0.944 and 0.966, respectively.

In pre-chemotherapy condition, the mean (SD) of $CD4^+$ T lymphocyte count was 817 (314) cell/µL while that of CD4+ T lymphocyte percentage was 38.96 (8.47)%. Meanwhile, the mean (SD) of $CD4^+$ T lymphocyte count and percentage in post-chemotherapy condition was 881 (335) cell/µL and 39.01 (8.50)%, respectively (Table 1).

Statistical analysis showed no significant difference of $CD4^+$ T lymphocyte count between pre- and post-chemotherapy (p=0.471). In addition, there was no significant difference of $CD4^+$ T lymphocyte percentage between pre- and post-chemotherapy (p=0.866) (Table 2).

This study showed no significant correlation between age and $CD4^+$ T lymphocyte count in cervical cancer patients before chemotherapy administration. Aging has been known to have an effect on a decrease of immune system function. The decreased immune cell production in the thymus

Table 1. Subject characteristics

Characteristics (n=17)	Mean (SD)	p-value
Age (years old)	54 (7)	0.200
$CD4^+$ T lymphocyte count pre-chemotherapy (cell/µL)	817 (314)	0.200
$CD4^+$ T lymphocyte percentage pre-chemotherapy (%)	38,96(8,47)	0.200
$CD4^+$ T lymphocyte count post-chemotherapy (cell/µL)	881 (335)	0.200
$CD4^+$ T lymphocyte percentage post-chemotherapy (%)	39,01(8,50)	0.200

Table 2. The differences of $CD4^+$ T lymphocyte profile between pre- and post-chemotherapy administration

Variable (n=17)	Pre-Chemotherapy mean(SD)	Post-Chemotherapy mean(SD)	p-value
$CD4^+$count (cell/µL)	817 (314)	881 (335)	0.471
$CD4^+$percentage (%)	38.96(8.47)	39.01(8.50)	0.866

among the elderly can cause a compensation effect that will increase the naïve $CD4^+$ T lymphocyte life span in the peripheral circulation.[17] This study also showed no correlation between age and $CD4^+$ T lymphocyte percentage in stage IIIB cervical cancer patients before chemotherapy administration.

Normal range for $CD4^+$ T lymphocyte is 410-1590 cell/µL. This study found normal $CD4^+$ T lymphocyte count in stage IIIB cervical cancer patients before chemotherapy administration with mean (SD) of 817 (314) cell/µL. This result was consistent with the study conducted by Gemignani *et al.* that found normal $CD4^+$ T lymphocyte count in stage I-III cervical cancer patients. A significant $CD4^+$ T lymphocyte count decrease will occur in stage IV cervical cancer.[12]

$CD4^+$ T lymphocyte count after chemotherapy administration was 881 (335) cell/µL. Based on statistical analysis, there were no significant differences of $CD4^+$ T lymphocyte count between pre- and post-cisplatin chemotherapy administration. However, the $CD4^+$ T lymphocyte count tended to increase in post-chemotherapy condition. This result was similarly consistent with the study conducted by Agustiansyah *et al.* that found an increased $CD4^+$ T lymphocyte count in invasive cervical cancer patients after receiving neoadjuvant chemotherapy.[17] The $CD4^+$ T lymphocyte count was not different between pre and post-chemotherapy conditions because all the patients recruited in this study were completely in a good condition based on physical and laboratory examination as one of the chemotherapy conditions required by patients to receive chemotherapy.

$CD4^+$ T lymphocyte percentage is the percentage of $CD4^+$ T lymphocyte count from total lymphocyte count. Normal $CD4^+$ T lymphocyte percentage range is 31-60%. $CD4^+$ T lymphocyte percentage is a more stable measurement compared to $CD4^+$ T lymphocyte count to evaluate the immune system due to its less variability.[18] This study found normal CD4+ T lymphocyte percentage both in pre and post-chemotherapy condition. Statistical analysis also showed no significant difference of $CD4^+$ T lymphocyte percentage in both pre and post-chemotherapy conditions (p-value = 0.866).

Adaptive immune response to tumor cells is mainly regulated by a cytotoxic T cell ($CD8^+$ T lymphocyte). These cells will destroy tumor cells through their binding to tumor cell antigen presented by MHC class I. $CD4^+$ T lymphocyte is the main regulator that secretes cytokines required in antitumor immunity. Those cells will release cytokines for naïve $CD8^+$ T lymphocyte differentiation into the effector $CD8^+$ T lymphocyte.[19]

Cisplatin works by forming cross-links between two guanines from the same DNA of tumor cells. This binding will disrupt the cell replication process that results in cell death and their inability to grow. Actively dividing cells are more sensitive to cisplatin. A preclinical study conducted by de Biasi *et al.* showed cisplatin ability to suppress immunosuppressive milieu formed by cancer cells and increase the involvement and proliferation of effector cells.[16] A tendency of $CD4^+$ T lymphocyte increase in post-chemotherapy condition may be related to cisplatin ability to induce antitumor immunomodulation process.

Other lymphocyte subpopulations that play roles in antitumor immune system such as NK cell and $CD8^+$ T lymphocyte were not measured in this study. The limitation of this was no evaluation of the overall immune system status of the patients. However, this preliminary study was able to present data about the immune condition of stage IIIB cervical cancer patients in pre and post-chemotherapy conditions.

CONCLUSION AND DISCUSSION

There was no significant difference of $CD4^+$ T lymphocyte count and percentage in pre and post-chemotherapy conditions. However, there was a trend of increase in $CD4^+$ T lymphocyte count and percentage in post-chemotherapy condition.

Further research measuring other immune parameters e.g. NK cell count and function parameters were considered necessary. $CD4^+$ T lymphocyte profile can be considered as a parameter to evaluate immune status in cervical cancer patients due to the wide availability of its measurement.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, *et al*. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN

2012. Int.J. Cancer. 2014; 136: E359-E386.
2. Rasjidi I. Epidemiologi kanker serviks. Indonesian Journal of Cancer, 2009; 3: 103-108.
3. Mathers C, Boerma T, Fat DM. The global burden of disease: Update 2004. Geneva, WHO Press, 2004; 2-3: 12-37.
4. Khuhaprema T, Srivatanakul P, Sriplung H, Wiangnon S. Cancer in Thailand volume V. Bangkok, National Cancer Institute, 2010; 2: 52-55.
5. Belhadj H, Berek J, Bermudez A, Bhatla N, Cain J, *et al*. FIGO staging for carcinoma of the vulva, cervix, and corpus uteri. International Journal of Gynecology and Obstetrics, 2014; 125: 97-98.
6. Kokka F, Bryant A, Brockbank E, Powell M, Oram D. Hysterectomy with radiotherapy or chemotherapy or both for. Cochrane Database of Systematic Reviews, 2015; 4: 1-24.
7. Kesic V. Management of cervical cancer. European Journal of Surgical Oncology, 2006; 32: 832-837.
8. Gerloni M, Zanett M. CD4 T cells in tumor immunity. Springer Semin Immun. 2005; 27: 37–48.
9. Anazoeze M, Sunday O, Obike I, Awelw C, Kenechi M. Comparison of absolute neutrophil to CD4 lymphocyte values as a marker of immunosuppression in cancer patients on cytotoxic chemotherapy. African Health Science, 2015; 15(2): 581-589.
10. Abbas A, Lichtman A, Pillai S. Cellular and molecular immunology. 8th Ed., Philadelphia, Elsevier Saunder, 2015; 18: 383-396.
11. Adam JK, Odhav B, Bhoola KD. Immune respond in cancer. Pharmacology & Therapeutics, 2003; 99: 113-132.
12. Gemignani M, Maiman M, Fruchter RG, Arrastia CD, Gibbon D, Ellison T. CD4 lymphocytes in women with invasive and preinvasive cervical neoplasia. Gynecologic Oncology, 1995; 59: 364-369.
13. Madu AJ, Ocheni S, Ibegbulam OG, Aguwa EN, Madu KA. Pattern of CD4 T-lymphocyte values in cancer patients on cytotoxic therapy. Annals of Medical and Health Sciences Research, 2013; 3(4): 408-503.
14. Lapresa M, Parma G, Portuesi R, Colombo N. Neoadjuvant chemotherapy in cervical cancer: An update. Expert Rev. Anticancer Ther, 2015; 15(10): 1171-1181.
15. Marth C, Landoni F, Mahner S, McCormack M, A. Gonzalez-Martin, Colombo N. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment, and follow-up. Annals of Oncology, 2017; 28(4): iv72-iv83.
16. De Biasi A, Villena-Vargas J, Adusumilli PS. Cisplatin-induced antitumor immunomodulation: A review of preclinical and clinical evidence. Clin Cancer Res, 2014; 20(21): 5384-5391.
17. Agustiansyah P, Sanif R, Thodorus, Nurmila N. Cluster of differentiation as predictor of therapy response to neoadjuvant chemotherapy in advanced-stage cervical cancer. J Res Med Dent Sci. 2018; 6 (1): 209-212.
18. Taylor JM, Fahey JL, Detels R, Giorgi JV. CD4 percentage, CD4 number, and CD4: CD8 ratio in HIV infection: Which to choose and how to use. J Acquir Immune Defic Syndr, 1989; 2(2): 114-24.
19. Ostroumov D, Fekete-Drismusz N, Sabarowski M, Kuhnel F, Woller N. CD4 and CD8 T lymphocyte interplay in controlling tumor growth. Cell Mol Life Sci, 2018; 75(4): 689-713.