eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Relationship between CD-8 Expression to Treatment Response in Nasopharyngeal Carcinoma Patient After Neoadjuvant Chemotherapy in Dr. Mohammad Hoesin Hospital Palembang

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ARTICLE INFO

Keywords:
Nasopharyngeal carcinoma
CD-8
Neoadjuvant chemotherapy
Metastasis
Carcinoma

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v6i4.455

ABSTRACT

Background. Nasopharyngeal carcinoma (NPC) is the most common malignancy in head and neck in Indonesia with 19,943 new cases in 2020 resulting 13,399 deaths. Lymphocytes are cells that play a role in the anti-cancer immune response, especially CD-8 T-cells. Neoadjuvant chemotherapy is chemotherapy given before radiotherapy that aims to kill primary tumors and micrometastasis tumors. This study aims to find out the relationship of CD-8 expression to treatment response in NPC undergoing neoadjuvant chemotherapy at Dr. Mohammad Hoesin Hospital Palembang. **Methods:** This study is an analytical observational research study on a retrospective cohort basis. Data collection from medical records using total sampling in 15 patients pilot study of NPC patients undergoing neoadjuvant chemotherapy and conducted CD-8 examination at ORLHNS polyclinic Dr. Mohammad Hoesin Hospital Palembang from December 2018 to December 2019 that met the criteria of inclusion and exclusion. **Results:** From 15 samples, the average CD-8 test result before neoadjuvant chemotherapy was 24.54 ng/µL, and after neoadjuvant chemotherapy was 193.56 ng/µL. There was a tendency to increase the average CD-8 from before to after completion of neoadjuvant chemotherapy with a statistically significant difference of p =0.001. ROC analysis found CD-8 cut-off points are 23.76 ng/µL with an area below the curve is 0.667. There were no significant relationships between CD-8 to performance status and treatment response with p values of 0.289 and 0.219, respectively. **Conclusion:** There was a significant change between CD-8 before neoadjuvant chemotherapy and after neoadjuvant chemotherapy with increased CD-8 tendencies and trends from before to after 6 series neoadjuvant chemotherapy with CD-8 cut-off points is 23.76 ng/µL. In this study, there has not been a significant relationship between CD-8 to performance status and treatment response in NPC patients undergoing neoadjuvant chemotherapy.

1. Introduction

Nasopharyngeal carcinoma (NPC) is a malignancy in the nasopharynx that can be caused by various risk factors such as genetic, environmental factors, foods containing nitrosamines or exposure to chemicals, and chronic viral infections such as Epstein Barr virus. NPC ranks fifth out of all malignancies in Indonesia and ranks first in head and neck malignancy and is more common in Asians than Caucasians where as many as 81% of cases are found in Asia. Men tend to NPC three times greater than women with the highest incidence rate at the age of 50 to 60 years.[1,2]

According to the Global Cancer Observatory

(Globocan) in 2020, there were 133,354 new cases of NPC worldwide and 31,513 in Southeast Asia. Countries with the highest NPC incidence rates include China, Indonesia, Vietnam, India, and Malaysia. NPC incidence in Indonesia amounted to 10.7/100,000 population in men and 3.0/100,000 population in women with 19,943 new cases in 2020 resulting in 13,399 deaths. In some places in Indonesia, there are varying NPC incidence rates, in the dr. Hasan Sadikin Hospital Bandung there were 2,952 NPC patients during the period 2013-2018. At the H. Adam Malik Hospital Medan in 2014-2016, there were 396 patients with NPC, while in the Dr. Mohammad Hoesin Hospital Palembang there were 284 NPC patients from the period 2013-2017.[3-7]

The immune system is the reaction of cells, tissues, and molecules that deliver and provide a coordinated immune response to pathogens, tumors, or other molecules that are considered foreign including tumor cells in NPC. There is a known immunoediting theory that encompasses the dynamic processes of immunosurveillance and tumor development that prevent malignancy by destroying tumor cells and keeping residual tumor cells dormant. This theory describes the relationship between tumor cells and the immune system through the elimination, equilibrium, and escape phases of tumor cells. The immune status of patients with malignancies is seen from oncogene activation, immune reactivity of devotees, and environmental factors.[8,9]

Lymphocytes are cells that play a role in the immune response of anti-cancer, especially T lymphocyte cells, a decrease in the number of lymphocytes in the blood will interfere with existing anti-cancer immunity and is responsible for the low number of survival observed in patients. Most evidence suggests that there is a clinical link between immune responses involving T-cells, especially cytotoxic T lymphocytes (CTL) CD-8 where CD 4 and CD-8 serve as the basis of the immune system of CTL cells. Quantitative analysis of some tumor types showed higher T-cells including CD-8, CTL, and CD 4 cells were associated with a better prognosis. A study by Onyema, et al., in 2015 in patients with chemotherapy was significantly higher in CD-8 expression and decreased after chemotherapy.[8-10]

The current principle of NPC management is radiotherapy, chemotherapy, or surgery. Radiotherapy is still a top choice in NPC treatment, but chemotherapy is also used in NPC management as neoadjuvant, concurrent, adjuvant, and palliative chemotherapy. Neoadjuvant chemotherapy is chemotherapy given before radiotherapy that aims to reduce the size of the primary tumor and kill the micrometastasis of the tumor. In the administration of neoadjuvant chemotherapy, the tumor vascular bed is still good so that the achievement of the drug to the tumor mass becomes optimal. Chang, et al., in his study using a combination of cisplatin chemotherapy and paclitaxel certain doses get therapeutic effects by minimizing immunosuppressant effects and stimulating macrophages and tumor-specific CD-8 T cell immune responses.[11,12]

Further understanding of tumor cell immunopathology in humans, particularly the mechanisms of differences in immune status in each individual as well as the identification of specific biomarkers and therapeutic targets for immunotherapy combinations need to be studied further. Based on the above it is also known that CD-8 cells are cytotoxic T-cells which are immune responses that play a role in malignancies have a clinical relationship and the response to NPC treatment. So on this consideration, this study was conducted to find out the relationship of CD-8 expression to treatment response in NPC patients undergoing neoadjuvant chemotherapy at Dr. Mohammad Hoesin Hospital Palembang.

2. Methods

This study is an analytical observational research study on a retrospective cohort basis. This study was conducted in the Dr. Mohammad Hoesin Hospital Palembang after ethical approval was signed. Data collection from medical records using total sampling in 15 patients pilot study of NPC patients undergoing neoadjuvant chemotherapy and conducted CD-8 examination with ELISA at ORLHNS polyclinic Dr. Mohammad Hoesin Hospital Palembang from

December 2018 to December 2019 that met the criteria of inclusion and exclusion. The inclusion criteria for this study is medical record data of NPC patients pilot studies that completed neoadjuvant chemotherapy as many as 6 series and CD-8 examination carried out before and after neoadjuvant chemotherapy. The exclusion criteria are patient who has undergone radiotherapy treatment, chemotherapy, or previous surgery also who have received.

Data analysis was performed to explore change in CD-8 expression before and after neoadjuvant chemotherapy and its correlation, to determine CD-8 cut-off points with the ROC curve, and to explore the relationship between CD-8 and treatment response with Chi-Square analysis. This research has an ethical exemption and was approved by the Health Research Ethics Committee of Dr.Mohammad Hoesin Palembang Hospital, No.77/kepkrsmh/2021. Ethical permits are carried out following applicable procedures and rules.

3. Results

This characteristic sample data reported in this study from 15 NPC patients that undergone neoadjuvant chemotherapy shown in table 1.

Table 1. The characteristic of nasopharyngeal patients

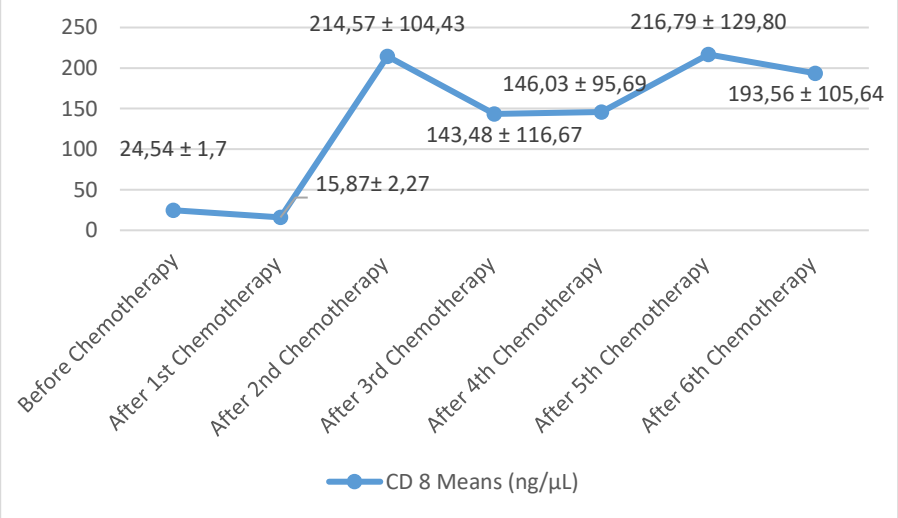
Variable	N (15)	%
Age		
≤ 50 y.o.	8	53,3 %
> 50 y.o.	7	46,7 %
Gender		
Male	12	80 %
Female	3	20 %
Body mass index		
< 18,5 / Underweight	2	13,3 %
18,5 – 24,9 / Normal weight	10	66,7 %
25 – 29,9 / Overweight	2	13,3 %
>30 / Obesity	1	6,7 %
Histophatology		
WHO Type I	0	0 %
WHO Type II	4	26,7 %
WHO Type III	11	73,7 %
TNM Classification		
T1	0	0
T2	4	26,7 %
T3	5	33,3 %
T4	6	40 %
N0	1	6,7 %
N1	5	33,3%
N2	2	13,3 %
N3	7	46,7 %
M0	15	100 %
M1	0	0
Stage		
I	0	0
II	1	6,7 %
III	2	13,3 %
IV (IV A, IV B)	12	80 %
Performance Status		
ECOG 1	12	80 %
ECOG 2	3	20 %
Smoking Habit		
Yes	9	60 %
No	6	40 %
Alcohol Consumption		
Yes	1	6,7 %
No	14	93,3 %
Salted Fish Consumption		
Yes	7	46,7 %
No	8	53,3 %
Treatment Response		
No response (Stable Disease/Progressive Disease)	3 (2/1)	20 %
Response (Complete Response/Partial Response)	12 (6/6)	80 %

From this study, patients were grouped by age where patients with < 50 years of age as many as 8 people (53.3%) and patients with > 50 years as many as 7 people (46.7%) with an average age of 49.53 years and the youngest age 32 years and the oldest age 58 years. Based on the distribution of sex obtained 12 people (80%) are male and 3 people (20%) are female. Distribution based on BMI showed the most patients in the BMI group 18.5 - 24.9 as many as 10 people (66.7%), followed by the BMI group < 18.5 and 25 - 29.9 as many as 2 people (13.3%) and the BMI group > 30 as many as 1 people (6.7%).

Based on risk factors for NPC, smoking habits were present in 9 people (60%), followed by salted fish consumption in 7 people (46.7%) and alcohol consumption in 1 person (6.7%). In the results of the most NPC biopsies with WHO histopathology type III was obtained in 11 people (73.3%) and WHO type II as many as 4 people (26.7%). From TNM classification, most patients came in T4 as many as 6 people (40%), T3 in 5 people (33.3%), and T2 in 4 people (26.7%). In this study, we did not get patients who came in T1 tumor status. In metastases to the regional neck lymph nodes (N), the most found in N3 is as many as 7 people (46.7%). It was followed by N1 as many as 5 people (33.3%), N2 as many as 2 people (13.3%), and 1 sample (6.7%) in the N0 group. As for metastases to distant organs, in this study, as many as 15 people did not get the presence of metastases to distant organs (M0).

From stage disease the most patients are in stage IV as many as 12 people (80%), followed by stage III in 2 people (13.3%), stage II in 1 person (6.7%) and we did not obtain patients in stage I. The performance status of patients assessed with ECOG, patients before chemotherapy was in ECOG 1 status in 12 people (80%) and ECOG 2 in 3 people (20%). The patient's chemotherapy response after neoadjuvant chemotherapy was obtained in the form of 3 people (20%) there was no treatment response (2 people stable disease and 1 person progressive disease) and there was a response in 12 people / 80% (partial response in 6 people and complete response in 6 people).

Table 2. CD-8 result



In the results of CD-8 examination before chemotherapy was obtained an average of 24.54 ± 1.7 ng/µL. Means CD-8 after chemotherapy 1st to 5th series which is 15.87 ng/µL, 214.57 ng/µL, 143.48 ng/µL, 146.03 ng/µL, 216.79 ng/µL, While the average CD-8 after chemotherapy completed is 193.56 ± 105.64 ng/µL where there is a trend to increase the average CD-8 from the beginning until after completion of neoadjuvant chemotherapy.

Table 3. Differences between CD-8 before and after neoadjuvant chemotherapy

Variable	Median (min-max)	p
CD-8 before chemotherapy	24,47 (21,67 – 27,02)	0.001
CD-8 after chemotherapy	183,00 (38,32 – 430,56)	

*Wilcoxon Test, there is significant differences if p < 0,05

Table 4. Correlation between CD-8 before and after neoadjuvant chemotherapy

Variable	CD-8 after chemotherapy
CD-8 before chemotherapy	r = 0,063 p = 0,823 n = 15

*Pearson Test on normal sample distribution, there is significant correlation if p < 0,05

In this study from the results of the Wilcoxon test on CD-8 examination before and after chemotherapy obtained a value of p = 0.001 so that with p < 0.05 there is a significant difference between CD-8 before and after chemotherapy. But from the results of the correlation test using the Pearson Test obtained a value of p = 0.823 which has a value of p > 0.05 so that it is concluded that there is no significant correlation between CD-8 before and after neoadjuvant chemotherapy.

Table 5. ROC curve of CD-8

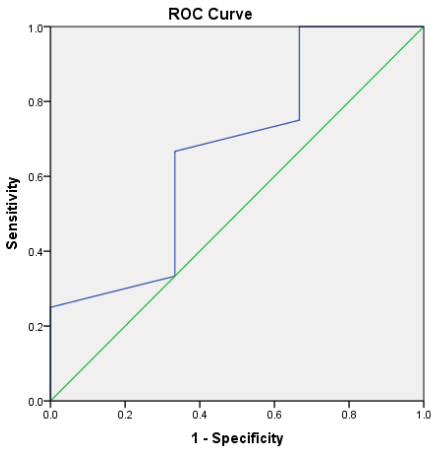


Table 6. CD-8 cut off point

CD-8 Cut-Off Point	Sensitivity	Specificity
20,67	1,000	0.000
21,90	1,000	0,333
22,60	0,917	0,333
23,18	0,833	0,333
23,41	0,750	0,333
23,76*	0,667	0,667
24,23	0,583	0,667
24,58	0,417	0,667
25,51	0,333	0,667
26,56	0,250	1.000

*Cut off point based on Youden Index

The results of ROC analysis in determining the cut point of CD-8 show the ROC curve coordinates for CD-8 based on Youden Index of 23.76 ng/µL. From the results of ROC curve analysis obtained the area under the curve of 0.667 so that it can be concluded 66.7% of the treatment response is influenced by CD-8 which is above the cut-off point value.

Table 7. Relationship between CD-8 before chemotherapy and performance status

Variable	**Performance Status**		**Total**	**p**
	ECOG 1	**ECOG 2**		
CD-8 > cut off point	6	2	8	0,268
CD-8 < cut off point	6	1	7	
Total	12	3	15	

*Chi-Square Test, significant if p < 0,05

Table 8. Accuracy CD-8 before chemotherapy to performance status

Parameter		**n**
Sensitivity	0,50	12
Specificity	0,33	3
Positive Predictive Value	0,75	8
Negative Predictive Value	0,14	7

The results of the analysis of the relationship between CD-8 and the performance status of patients using the Chi-Square test obtained a result of p = 0.268 with α = 5% and CI = 95% so that the value of p > 0.05 which gave the conclusion that there was no relationship between CD-8 and patient performance status. The analysis of the relationship between CD-8 and the performance status of patients received sensitivity results of 58% and specificity by 33%, with a positive predictive value of 75% and a negative predictive value of 14%.

Table 9. Relationship between CD-8 before chemotherapy and treatment response

Variable	**Treatment Response**		**Total**	**p**
	Response	***No Response***		
CD-8 > cut off point	7	1	8	0,603
CD-8 < cut off point	5	2	7	
Total	12	3	15	

*Chi-Square test, significant if p < 0,05

Table 10. Accuracy CD-8 before chemotherapy in predicting treatment response

Parameter		n
Sensitivity	0,58	12
Specificity	0,67	3
Positive predictive value	0,87	8
Negative predictive value	0,28	7

The results of the analysis of the relationship between CD-8 and treatment response using the Chi-Square test obtained a result of p = 0.603 with α = 5% and CI = 95% so that the value of p > 0.05 which concluded that there was no relationship between CD-8 and chemotherapy treatment response. The analysis of the relationship between CD-8 and treatment response resulted in a sensitivity of 58% and specificity of 67%, with a positive predictive value of 87% and a negative predictive value of 28%.

Table 11. Multivariate analysis factors that affect CD-8 before chemotherapy

Variable	t	p
Age	-0,244	0,815
BMI	0,942	0,383
Stage	-0,913	0,397
ECOG Smoking Habit	1,148 -0,455	0,295 0,665
Alcohol Consumption	-0,492	0,640
Salted Fish Consumption	0,002	0,998
Constanta	6,363	0,001

*Risk factors significantly affecting if p < 0,05

Table 12. Multivariate analysis factors that affecting CD-8 after chemotherapy

Variable	t	p
Age	0,095	0,933
BMI	1,161	0,365
Stage	0,892	0,466
ECOG Smoking Habit	0,324 1,027	0,777 0,412
Alcohol Consumption	-0,322	0,778
Salted Fish Consumption	-0,638	0,589
Constanta	-0,710	0,552

*Risk factors significantly affecting if p < 0,05

The results of a multivariate analysis of risk factors that affect CD-8 in this study are assessing age, BMI, stage, ECOG, smoking habits, alcohol consumption, and salted fish consumption in patients with NPC. The results of the analysis of these factors all have a p-value > 0.05 so that it does not significantly affect CD-8 before or after neoadjuvant chemotherapy in a person.

4. Discussion

This study aims to find out the cut-off point of CD-8, as well as analyze the relationship of CD-8 expression in NPC patients undergoing neoadjuvant chemotherapy to the performance status and response of neoadjuvant chemotherapy treatment at Dr. Mohammad Hoesin Palembang Hospital. From this study there are 15 samples, based on the distribution of age groups there are 8 people (53.3%) aged under 50 years and 7 people (46.7%) over 50 years with an average age of 49.53 years and the youngest age 32 years and the oldest age 58 years. Based on the distribution of sex obtained 12 people (80%) are male and 3 people (20%) are female.

According to Globocan in 2020, the incidence of NPC in Indonesia is 10.7/100,000 population in men and 3.0/100,000 population in women with 19,943 new cases resulting in 13,399 deaths. In some places in Indonesia, NPC incidence has varied numbers, at dr. Hasan Sadikin Hospital Bandung had 2,952 NPC patients during the period 2013-2018 with 57.22% in men with an average age of 47.45 years, while at Dr. Mohammad Hoesin Hospital Palembang obtained 284 NPC patients from the period 2013-2017 with 72.88% male patients and the most in the age group of 41-50 years of 33.2%.[3-7]

Salehiniya, et al., concluded in their study in 2018, men tended NPC three times greater than women with the highest incidence rate at the age of 50 to 60 years. The process of malignancy in the nasopharynx is the same as other malignancies and is widely obtained in older age because the immune system and DNA repair mechanisms in cells that have mutations have decreased. So when this DNA repair mechanism fails in carrying out its function, DNA gene mutations that have occurred will cause uncontrolled cell growth. In nasopharyngeal malignancies, there is a change in normal nasopharyngeal epithelium cells into dysplasia, hyperplasia until it develops into carcinoma cells as a result of mutations of proto-oncogene genes and tumor inhibitor genes.[1,2]

Distribution based on BMI showed the most patients in the BMI group 18.5 - 24.9 / normal weight in 10 people (66.7%), followed by the BMI group < 18.5 / underweight and 25 - 29.9 / overweight in 2 people (13.3%) and the BMI group > 30 / obesity in 1 person (6.7%). The findings in this study are following the results of a study conducted by Efranto, et al., in Malang in 2021 with the results of the study getting 17 out of 23 patients (73.91%) NPC has a normal BMI. Patients with NPC may experience changes in their nutritional status related to a variety of factors ranging from the effects of their malignancy as well as reactions from anti-malignancy therapy given. Some studies show that higher a person's BMI gives a higher life expectancy.[13,14]

Based on the risk factors for the occurrence of NPC, the risk factors examined in this study such as smoking habits were found in 9 people (60%), followed by salted fish consumption in 7 people (46.7%) and alcohol consumption in 1 person (6.7%). According to Salehiniya, et al., some of the risk factors that are strongly associated with causing NPC were EBV infection, genetics, HLA genes, and salted fish consumption. In their study also get smoking habits are related as a risk factor for the occurrence of NPC but have a strong association and are more related to the occurrence of NPC WHO type 1 where the risk of in smokers is 60% higher in nonsmokers.[2,15,16]

People who consume salted fish every day have a risk of NPC that varies 1.8 - 7.5 times greater than people who do not consume salted fish regularly. This is thought to be caused by nitrosamines in salted fish, a carcinogen that causes gene damage through the production of CYP2E1. While nicotine from cigarettes can cause DNA damage by forming carcinogenic molecules in the form of N-nitrosonornicotin and nicotine-derived nitrosamine ketone that comes into contact with the nasopharyngeal epithelium with nitrosamines in salted fish can result in mutations in the p53 gene.[2,15,16]

In this study, the results of the most NPC biopsies of WHO histopathology type 3 in 11 people (73.3%) and type 2 as many as 4 people (26.7%). In the TNM classification, the most patients in T4 were 6 people (40%), T3 in 5 people (33.3%), and T2 in 4 people (26.7%). In metastases to the regional neck lymph nodes (N) the most found in N3, in 7 people (46.7%),

followed by N1 in 5 people (33.3%) and N2 in 2 people (13.3%). Based on the examination of TNM classification above the patients determined the stage where the most patients are in stage IV as many as 12 people (80%), followed by stage III in 2 people (13.3%) and stage II in 1 person (6.7%).

The findings in these studies bear similarities to findings in NPC patients in North America where 25% have WHO type I histopathology, 12% type II and 63% type III. The population in Southern China also has the same histopathological patterns of 3%, 2%, and 95% of WHO type III. In a study conducted by Wang, et al., in 2020, 94.3% of NPC patients in the WHO classification type III. Squamous cell carcinoma with keratinization has clear growth properties on the surface of the nasopharyngeal mucosa. Cancer cells can differentiate well to moderately and produce quite a lot of keratin material, both inside the cytoplasm and outside the cell. Squamous cell carcinoma without keratinization indicates moderate differentiation and others with cells that are more toward good differentiation. Carcinoma without differentiation has a very heterogeneous pathological picture, syntitial malignanT-cells with unclear boundaries.[17-19]

The findings in this study are somewhat different from the findings in the study conducted by Wang, et al., in China in 2020 which had the classification of tumor expansion based on the most TNM, namely T3 in 46% followed by T4 in 28.6% and for N2 classification in 57.11% followed by N3 in 14.4%, and NPC patients mostly in stage III and IV in the study. While in several studies in Indonesia by Nathania, et al., conducted in Bandung and Asnir, et al., in Medan in 2020 get the characteristics of NPC patients similar to this study, the most patients in stage IV are 48.64% and 63.7%. Early-stage NPC symptoms often do not cause complaints that bother the patient to make them seek treatment that ultimately makes the diagnosis of NPC too late. Patients come seeking treatment and are diagnosed with NPC often when there has been extensive tumor expansion and metastasis to the neck lymph nodes where there have been complaints of lumps in the neck at an advanced stage that caused delays from the treatment.[5,6,19]

The performance status of patients assessed with ECOG before chemotherapy was in ECOG 1 status in 12 people (80%) and ECOG 2 in 3 people (20%). Following research conducted by Hutajulu, et al., in Yogyakarta in 2021 out of 501 NPC patients, 72.1% in the condition of ECOG performance status 0-1 and 25.2% in the condition of ECOG performance status 2. But slightly different from the results of research conducted by Xie, et al., in China in 2016 which got 47.46% in the status of ECOG performance 0-1 and 52.53% in the performance status of ECOG 2.[20,21]

In this study, the patient's chemotherapy response after neoadjuvant chemotherapy was obtained of 3 people (20%) there was no treatment response (stable disease or progressive disease) and there was the response (partial response or complete response) in 12 people (80%). This is following the results of a study conducted by Peng, et al., in 2017 where there was a treatment response to chemotherapy in NPC in 90.9% of patients (CR = 11.3, PR = 79.6%). While in the study conducted Liu, et al., who got 82.7% of his research had a complete response and 17.3% had a partial response to the administration of chemotherapy doxetaxel and cisplatin. On the examination of CD-8 before chemotherapy was obtained an average of CD-8 of 24.54 ng/µL and decreased in the examination after 1st series neoadjuvant chemotherapy which is 15.87 ng/µL, but after the 2nd series of neoadjuvant chemotherapy to completion of neoadjuvant chemotherapy CD-8 has increasing tendency where the change is statistically significant. But from this study through correlation test analysis has not been able to prove a significant relationship of this change in CD-8.[20-24]

The study also obtained a cut-off point CB 8 of 23.76 ng/µL with 66.7% of CD-8 values that were above the cut-off point affecting the outcome of neoadjuvant chemotherapy treatment response in NPC patients. However, the results of the analysis of the relationship between CD-8 and treatment response obtained a value of p = 0.603 with α = 5% and CI = 95% which concluded that there was no significant association between CD-8 and neoadjuvant chemotherapy treatment response. Where statistical analysis in predicting the results of

treatment response through CD-8 with the sensitivity of 58% and specificity by 67%, the positive predictive value of 87%, and a negative predictive value of 28%. A positive predictive value of 87% describes the accuracy of CD-8 which is above the cut-off point in predicting treatment response in neoadjuvant chemotherapy performed in NPC patients who have a high value even though statistically it has not been able to get significant results.[20,21]

Several studies on CD-8 in NPC, one of which was conducted by Santoso, et al., concluded that neoadjuvant chemotherapy caused a decrease in expression of LMP1, CD 4, and CD-8 in nasopharyngeal biopsy tissue. Chang, et al., in his research using chemotherapy with cisplatin and paclitaxel at certain doses can increase macrophage recruitment, decrease tumor microenvirontment immunosuppression and provide a specific response of CD-8 anti-tumor T-cells. In a study conducted by Chen, et al., in 2018, higher CD-8 results in NPC patients with complete and partial treatment responses. Tao, et al., got a CD-8 value before treatment in NPC patients of 19.1 ng/µL, Franke, et al. in his study got a CD value of 8 in healthy people of 3-35 ng/µL. While different CD-8 values were obtained in a study conducted by Jiang and Uppal where the average cd value of 8 in healthy people amounted to 552 cells/µL and 540 cells/µL.[12,25-29]

The decreased change from CD-8 after 1st series chemotherapy in this study may be due to the effects of neoadjuvant chemotherapy given as a result of the effects of bone marrow suppression in blood cell production, but after that, there is an increased tendency of CD-8 on examination after subsequent chemotherapy up to after completion of 6th series of neoadjuvan chemotherapy It is thought to be the body's immune response by forming CD-8 T-cells to kill tumor cells as well as the presence of long-lived CD-8 T-cells as a form of cellular immune response to malignancies thought to be affected by chemotherapy and cytotoxic reactions in tumor cells. Chemotherapy can result in immunogenic cell death by providing an immune response to malignancy and improving T cell performance. Immunogenic cell death is preceded by the stress-causing effects of the endoplasmic reticulum to be presented on the surface of the cell that signals apoptosis of the tumor cell. In this process, the inflammatory cytokine IL-1β will attract IFN-γ and CD-8 CTL to tumor cells. But in this study could not distinguish which CD-8 subsets were more dominant in the results of the examination obtained.[12,25,27,30]

Chemotherapy performed intermittently is usually less immunosuppressive than the continuous type. The function of T and B cells can return between treatment series although persistent disorders may be seen after prolonged treatment or when chemotherapy and radiation are combined. Chemotherapy drugs in low doses can inhibit suppressor cells and increase the effect of CD-8 T-cells over CD 4 T-cells. In addition, at higher doses of T-cells and CD-8 T-cells when tumor cells have stopped reproducing and lymphocytes have matured then cellular and humoral responses to cytotoxic agents can become resistant. It can also be through the mechanism of avoidance of tumor cells to the immune response. Chemotherapy also induces MHC-1 expression in tumor cells that will induce the formation of CD-8 cells and suppress some tumor microenvirontmenT-cells that can affect CD-8 production including TAMs, MDSCs and Tregs.[30-33]

A study conducted by Economopoulou, et al., stated that low CD 4 and CD-8 found in patients with neck head malignancies who are in the active phase and increased CD-8 is associated with better prognosis and survival where CD-8 T-cells can stimulate the formation of IL-2 as an activation signal and release enzyme perforin that will result in apoptosis of tumor cells. According to Oshi, et al., high CD-8 values are associated with better survival rates, this is associated with higher cytotoxic immunity activity in malignancies patients who have high CD-8. Research conducted by Krantz, et al., on urinary tract malignancies showed neoadjuvant chemotherapy improved the antitumor immune response by lowering fatigue from CD-8 in the antitumor immune response through decreased expression of PD-1 and Treg cells. According to Opzoomer, et al., CD-8 after high chemotherapy can be a predictor factor for better recurrence rates and survival rates.[34-38]

The results of the analysis of the relationship

between CD-8 and patient performance status concluded that there was no association between CD-8 and patient performance status with a sensitivity value of 58% and specificity of 33%, with a positive guess value of 75% and a negative guess value of 14%. Where a positive guess value of 75% describes CD-8 which is above the cut point in predicting better performance status of NPC patients even though it has not received a statistically significant result with a value of p = 0.268. According to Das, et al., in his study, they got CD-8 in the blood that was no different in patients with cervical malignancies based on their performance status but got CD-8 in higher tumors in patients with worse performance status.[39]

To look at the factors that influenced CD-8 values in this study, a multivariate analysis of factors such as age, sex, stage, ECOG, smoking habits, alcohol consumption, and salted fish consumption in NPC patients using linear regression. The results of the analysis of risk factors of age, stage, ECOG, smoking habits, alcohol consumption, and salted fish consumption did not have a significant and significant association as factors that affect the magnitude of CD-8 in people with NPC. Some studies link T cell counts including CD-8 to be influenced by factors such as sex, age, EBV infection, and race, as well as tumor factors i.e. NPC. Uppal, et al., concluded men had significantly higher CD-8 than women and the elderly had lower CD-8. However, there was no significant difference in CD-8 in risk factors for smoking and alcohol consumption.[29,39,40]

Limitations in this study are that the study uses secondary data taken from medical record data, so there are data limitations. Another limitation is that because it is a pilot study, the sample size of patients is small so that the study is less robust to analyze the relationships in each group. No significant association between CD-8 and factors such as age, stage, ECOG, smoking, alcohol consumption, and salted fish consumption could be due to a small sample. The study also did not have a significant association between CD-8 and performance status and neoadjuvant chemotherapy treatment response. This study is retrospective and the data is only taken from secondary medical records so it is necessary to conduct further research with prospective research methods with larger samples and other lymphocyte examination subsets such as CD 4 so that it is expected to provide a stronger relationship and get better follow-up research results.

5. Conclusion

There was a significant change between CD-8 before neoadjuvant chemotherapy and after neoadjuvant chemotherapy with an increased CD-8 tendency from before neoadjuvant chemotherapy to after neoadjuvant 6 series chemotherapy. This study also obtained CD-8 cut points before neoadjuvant chemotherapy of 23.76 ng/µL with an area under the curve of 0.667 which describes 66.7% of treatment response can be affected by CD-8. But in this study did not get a significant association between CD-8 and chemotherapy treatment response in NPC patients undergoing neoadjuvant chemotherapy and also did not get a significant association in factors of age, gender, stage, ECOG, smoking habits, drinking alcohol, and salted fish consumption against CD-8.

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