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Lung tumor metabolit detection using magnetic resonance spectroscopy lung in vivo technique: Correlation of metabolite MR spectroscopy with cytology

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ABSTRACT

Background

Lung tumors are generally diagnosed by cytological examination via FNAB with guided CT Scan. Biopsy measures for cytology are still the gold standard for determining the type of lung cancer. Possible complications of FNAB CT are pneumothorax, bleeding, with the incidence of complications range between 7% and 62%. The use of MR spectroscopy allows assessing tumor function by measuring the concentration of metabolites in tumor tissue. MR spectroscopy metabolite spectrum can distinguish between benign and malignant tumors, by knowing the lung cancer tissue metabolites, the use of MR spectroscopy is expected to overcome obstacles to determine the type of lung cancer in patients when biopsy cannot be done.

Objective

To determine the correlation between MR spectroscopy metabolite values in lung tumors with lung tumor cytology.

Methods

Quasi-experimental study on MR spectroscopy of lung tumors with TE 135 ms in 19 patients with lung tumors by FNAB. Then we performed correlation and comparative analysis of the results of the metabolite values with cytology

Results

The NAA value and the NAA/Cho ratio, NAA/Cre obtained p-value < 0.001 Spearman r-value > 0.8 showed a significant correlation. Chi-Square Fisher Test with a 0.04 NAA cut-off point showed that there was no difference between metabolite values and cytology results in determining tumor types with a p-value of 0.005. NAA metabolites, NAA/Cho and NAA/Cre ratios can differentiate between benign tumors, adenocarcinoma and malignant squamous cell carcinoma in this study.

Conclusion

MR spectroscopy with TE 135 ms on NAA metabolite values, NAA/Cho ratio and NAA/Cre can be used to differentiate between benign tumors, adenocarcinoma and malignant squamous cell carcinoma in this study.

Keywords: MR Spectroscopy, TE 135 ms, Lung Tumor, Cytology

INTRODUCTION

Cancer is a major public health problem in the world and the number two cause of death in the United States. GLOBOCAN 2018 data, the International Agency for Research on Cancer (IARC) shows there are around 18.1 million new cancer cases and 9.6 million cancer deaths in the world in 2018 [1]. Lung cancer is the most diagnosed cancer with the number of new cases reaching 2.1 million cases, equivalent to almost 11.6% of new cancer cases in the world[1]. In the last century, lung carcinoma has progressed from an unusual disease to become the most common cancer in the world and the most common cause of cancer deaths [2].

The incidence of lung cancer in Indonesia is 11.6% with a total of 34,696 new cases [3]. The number of new cases of lung cancer, according to data from the Department of Pulmonology and Respiratory Medicine FKUI-Persahabatan Hospital, has increased more than 5 times in the last 10 years. Lung cancer is the most cancer in men, number 4 the most in women, and is the leading cause of cancer deaths based on the results of research from 100 hospitals in Jakarta [3]. Lung cancer is generally diagnosed by taking a tissue sample with a needle with the guiding CT scan for cytological examination. Biopsy action to take tissue samples that will be done cytology examination is still a gold standard to determine the type of lung cancer [4,10]. However, this invasive approach may not be safe in many patients with lung cancer, which often have other diseases such as empyema [11], and also the location of tumors close to the heart or aortic organ.

Possible complications of CT guided lung biopsy are pneumothorax, bleeding, rarely seeding neoplastic cells along the needle path, cardiac effusion, and lung infections (pneumonia, empyema) [11]. The incidence of complications ranges between 7% and 62%, pneumothorax is the most common complication [11,12]. With the rapid development of MRI technology, an MRI technique called MR Spectroscopy can be carried out. Magnetic resonance spectroscopy (MRS) is a new sophisticated imaging technique that can be used to reveal complementary noninvasive information about the biochemical composition of the imaged tissue [6].

MR spectrum is a plot of the intensity and frequency of chemical signals or metabolites in a voxel that is determined. In proton MR spectroscopy in vivo, the location of the water frequency is used as a reference standard to identify chemicals. The frequency or location shift of chemicals relative to water produces qualitative and quantitative information about chemicals that occur in the tissue, forming the basis of tissue characterization by MR spectroscopy [7]. To be able to display the metabolites of a network, the most important parameter in MR spectroscopy is Time Echo (TE). In MR spectroscopy the TE parameters are divided into 2 namely long TE (long TE) to display the metabolites of NAA, Choline, Creatin [8] and short TE (short TE) to display metabolites of Myoinositol, Glutamine, Lipid [8]. The advantage of the metabolites displayed on MR spectroscopy is that there are differences in the value of metabolites between malignant and benign tumors so that the results of the metabolite spectrum can distinguish between types of malignant and benign tumors [9].

As an alternative to help determine the diagnosis of lung cancer in addition to using the FNAB biopsy procedure with guiding CT scan, it may be possible to use MR Spectroscopy as a noninvasive method to assess the concentration of lung cancer tissue metabolites. Previous study dealing with in vivo proton MRS of the lung has conducted feasibility studies on the use of MR Spectroscopy in lung cancer states that MR Spectroscopy can and is feasible to be used to obtain the spectrum of metabolites in lung cancer [12]. Research on the application of MR spectroscopy in lung cancer that has been done by Yoon SH, Park CM, uses the TE parameter 30 ms. In this study the metabolites obtained in the spectrum were mostly lipids, and only a small amount of choline [13]. The research was continued by changing the TE parameters to 135 ms in order to display other metabolites such as NAA, cholin and creating so as to produce qualitative and quantitative information about chemicals that occur in cancerous tissues.

MR spectroscopy spectrum spectroscopy can distinguish between benign and malignant tumors [9], so that by knowing the metabolites of lung cancer tissue the use of MR spectroscopy is expected to overcome obstacles to determine the type of lung tumor in patients who cannot be biopsy. Based on this, the research was carried out the application of MR spectroscopy technique on lung tumors with TE (Time Echo) 135 ms to detect lung

tumor metabolites, and analyze the relationship between MR spectroscopy metabolite values with cytology results. This research was conducted using MRI Siemens Magnetom Aera 1.5 T.

MATERIALS AND METHODS

This research is a quasi-experimental study. This study has been licensed by the Health Research Ethics Committee at Doctor Soedarso Hospital Pontianak, West Borneo. All patients who agreed to take part in the study signed informed consent. Data were collected from January 2020 to March 2020 in the Radiology Installation of Doctor Soedarso Hospital Pontianak using MRI Siemens Magnetom Aera 1.5 Tesla. The sample in this study was lung tumor patients who had a CT scan of the thorax with FNAB. In total there were 19 patients (17 men and 2 women with an age range between 15-76 years) with lung tumors. This research was conducted using MRI Siemens Magnetom Aera 1.5 T with spectroscopy software at Siemens Healthcare Syngovia SyngoMR E11. The parameters in spectroscopy use multi-voxel MRS technique using point resolved spectroscopy sequence (PRESS) with TE 135 ms, TR 2100 ms, average 4, voxel of interest (VOI) 60 mm x 60 mm, voxel size 4x4x15 mm, spectral width 2500 Hz and 2048 data points. The total acquisition time is 20-25 minutes.

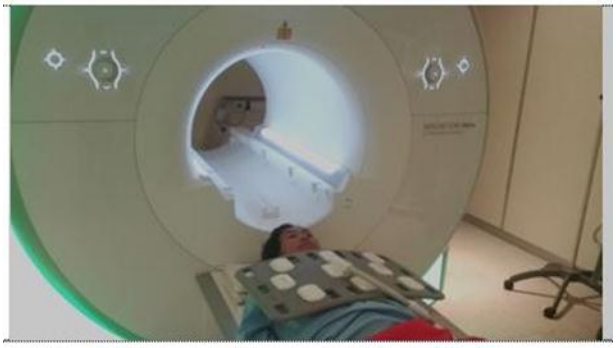

Fig 1.Patients undergoing MR Spectroscopy with MRI Siemens Magnetom Aera 1.5 T

The metabolite data obtained were then analyzed using IBM SPSS Statistics version 21.0 for Windows. Data were performed to test the correlation between the values of metabolites with the results of cytology using the Spearman test to evaluate the correlation between the values of metabolites with the results of cytology.

The next statistical analysis is to determine the cut-off point for the NAA value and then to do the Chi-Square Fisher test to evaluate if there is a difference between the MRS metabolite values and the cytological results in determining tumor malignancy. Chi-Square Fisher's test was also conducted to evaluate whether there is a difference between the value of NAA metabolites and the results of cytology in determining squamous cell carcinoma and adenocarcinoma.

RESULTS

All of 19 samples in this study, the frequency of malignant squamous cell carcinoma cases were 5 cases, benign tumor 5 cases and the highest sample frequency in this study was adenocarcinoma as many as 9 cases. Table 2 summarizes the MR Spectroscopy metabolite values and findings cytology results in the 19 patients.

The MR Spectroscopy metabolite value produced in lung cancer is quantitatively carried out to analyze the differences in the metabolite values of NAA, Cholin, Creatin in each lung tumor patient. Measurement of metabolite values in the spectrum is done by looking at the peak values of the metabolites displayed by the Siemens Syngovia E11 spectroscopy software that is available on the Siemens Magnetom Aera MRI modality. Then the NAA/Cholin, and NAA/Creatin ratios are calculated manually.

From the data that has been obtained is then carried out statistical tests. Before determining the statistical test that will be used then the data normality test is first performed to determine whether the data is normally distributed or not. Because the number of samples is less than 50, the data normality test is done using Shapiro-Wilk test.

Table 1. Shapiro Wilk data normality test results

No	Metabolit	volume	p-value	Result
1	NAA	19	< 0,001	abnormal
2	Choline	19	< 0,001	abnormal
3	Creatin	19	< 0,001	abnormal

Furthermore, to find out whether there is a relationship between MR Spectroscopy metabolite values with TE 135 ms in lung tumors with cytological results and how the relationship is necessary to do statistical tests. The test conducted in the correlative hypothesis test, because what will be tested is numerical data with ordinal then using the Spearman test.

Table2. Results of metabolite values from MR spectroscopy of pulmonary in vivo and cytological results

No	MR	Age	Sex	SH	Metabolits			NAA/Cho	NAA/Cre	Cytology
					NAA	Cho	Cre	Ratio	Ratio	
1	16334	63	♀	No	0,02	0,11	0,04	0,182	0,500	adeno ca
2	122903	50	♂	Yes	0	0,34	2,61	0,000	0,000	m. scc
3	122406	55	♂	Yes	0,08	0,95	0,45	0,084	0,178	adeno ca
4	122459	51	♂	Yes	0,03	0,08	0,19	0,375	0,158	adeno ca
5	121416	65	♂	Yes	0,54	0,3	0,19	1,800	2,842	inflammation
6	123321	60	♂	Yes	0,26	0,45	0,08	0,578	3,250	adeno ca
7	122231	61	♂	Yes	0,01	0,05	0,18	0,200	0,056	adeno ca
8	62953	76	♂	Yes	0,04	0,11	0,31	0,364	0,129	adeno ca
9	125417	60	♀	No	5,17	2,11	2,85	2,450	1,814	Colloid nodul
10	119707	69	♂	No	0,47	0,04	0,01	11,750	47,000	Hyperplasia
11	125900	48	♂	Yes	0	0,34	2,95	0,000	0,000	m.scc
12	126287	50	♂	Yes	0,05	0,54	0,06	0,093	0,833	adeno ca
13	126458	53	♂	No	0,01	0,07	0,11	0,143	0,091	adeno ca
14	126009	66	♂	Yes	0	0,06	3,65	0,000	0,000	m. scc
15	126287	58	♂	Yes	0	0,28	1,19	0,000	0,000	m. scc
16	119145	59	♂	Yes	0	0,15	0,08	0,000	0,000	m. scc
17	127588	15	♂	No	4,33	2,16	1,74	2,005	2,489	Benign
18	127196	24	♂	Yes	9,43	6,57	6,38	1,435	1,478	Benign
19	127511	36	♂	Yes	0,11	0,13	0,04	0,846	2,750	adeno ca

RM=Medical Record, SH=Smoking History, M.SCC=Malignan Squamous Cell Carcinoma

Table 3. Range of values and mean metabolite ratios

Tumor Type	Value Range			Mean Ratios	
	NAA	Choline	Creatine	NAA/Cho	NAA/Cre
Squamous Cell Ca	0	0,15-0,34	0,08-3,65	0	0,019
Adeno Ca	0,02-0,33	0,07-0,54	0,04-0,45	0,475	0,938
Benigna	0,47-9,43	0,3-6,57	0,19-6,38	1,997	7,868

To find out whether there is a relationship between MR Spectroscopy metabolite values with TE 135 ms in lung tumors with cytology results and how the relationship is necessary to do a statistical test. The test conducted in the correlative hypothesis test, because what will be tested is numerical data with ordinal then using the Spearman test.

Table4. Spearman correlation statistical test results

Metabolit	volume	p-value	r-value
NAA	19	0,000	0,936
Choline	19	0,298	0,252
Creatin	19	0,626	-0,119
Metabolit ratio	**volume**	**p-value**	**r-value**
NAA/Cho	19	0,000	0,935
NAA/Cre	19	0,000	0,842

It can be seen that the NAA value data obtained p- value of 0,000 which indicates that the correlation between NAA values with cytological results is significant. Spearman r-value correlation value of 0.936 shows the correlation with the strength of the correlation is very strong. Only the value of NAA has a significant correlation with the results of cytology while the value of choline and creatin with the results of cytology is statistically not significant. In clinical applications of MR spectroscopy, the ratio of various metabolites is used more often than the absolute metabolite concentration [18], in addition to the NAA/Choline ratio as well as the NAA/Creatin ratio. So in this study also conducted a correlation test to see the relationship between the NAA/Choline ratio and the NAA/Creatine ratio with the results of lung tumor cytology.

Table 4 we can see that the NAA/Choline and NAA/Creatin ratio values obtained p-value < 0.001 which shows that the correlation between the ratio of NAA/Choline and NAA/Creatin values with statistically significant cytology results. Spearman correlation r-value of 0.935 in NAA/Choline and 0.842 in NAA/Creatine shows the strength of the correlation is very strong.

Once it is known that there is a correlation between the values of metabolites with the results of cytology then it is necessary to do a different test to determine whether the existing metabolite values can represent the result of cytology so that the MR spectroscopy results can help determine tumor malignancy as well as cytology. Before we do the different test with Chi-Square Fisher, first we have to determine the cut-off to determine the type of tumor category according to cytology results. The cut off determination for data that is not normally distributed is done by using a median value from NAA data which has a very strong correlation with cytology results. From the statistical test, the data obtained that the cut off value for distinguishing benign tumors and lung cancer from NAA metabolites is 0,04. After the cut-off point is obtained then a Chi-Square difference test is performed for categorical data scales.

Table 5 Chi Square Fisher different test results between NAA metabolites and cytological results in distinguishing between benign tumors and lung cancer

Metabolit	Cut Off	Cytology			p- value
		Cancer	Benigna	Vol	
NAA	≤ 0,04	11 (78, 6%)	0 (0%)	11 (57, 9%)	0,005
	> 0,04	3 (21, 4%)	5 (100%)	8 (42, 1%)	
	Volume	14 (100 %)	5 (100%)	19 (100%)	

Based on the results of the NAA cross table with the results of cytology in 19 samples, it was found that the samples that had lung cancer cytology results were 14, while the benign ones were 5. Of the 14 samples, 11 samples (78.6%) had an NAA value ≤ 0.04 while 3 other samples (21.4%) had an NAA value > 0.04. Furthermore, out of 5 benign samples overall (100%) have NAA values > 0.04. The test results obtained p-value 0.005 < 0.05 so that it can be interpreted that there is no difference between the results of the NAA metabolites with the

results of cytology in determining benign tumors or lung cancer.

Chi-Square Fisher's different test was also carried out on the ratio of the value of the metabolites of NAA/Choline and NAA/Creatine to the results of cytology. The cutoff point from the NAA/Choline and NAA/Creatin ratio data is determined using the median value because the data are not normally distributed [16]. From the statistical test, it was obtained that the cut-off value for NAA/Choline ratio was 0.20 and the cut-off for NAA/Creatine was 0.17.

Table 6 Chi Square Fisher different test results between the ratio of NAA/Cho metabolites with the results of cytology in distinguishing between benign tumors and lung cancer

Metabolit	Cut Off	Cytology			p- value
		Cancer	Benign	Vol	
NAA/ Cho Ratio	≤ 0,20	12 (85, 7%)	0 (0%)	12 (63, 2%)	0,002
	> 0,20	2 (14, 3%)	5 (100%)	7 (36, 8 %)	
	Volume	14 (100%)	5 (100%)	19 (100%)	

Based on the results of the NAA/Choline cross table with the cytology results in 19 samples, it was found that the samples that had lung cancer cytology results were 14, while the benign ones were 5 people. From 14 samples, 12 samples (85.7%) had an NAA/Choline ratio value ≤ 0.20 while the other 2 samples (14.3%) had an NAA/Choline ratio value > 0.20. Furthermore, from 5 whole benign samples (100%), they have NAA/Choline value > 0.20. The test results obtained p-value 0.002 < 0.05 so that it can be interpreted that there is no difference between the results of the NAA/Choline metabolite ratio with the results of cytology.

Table 7. Chi-Square Fisher different test results between the ratio of NAA/Creatine metabolites with the results of cytology in distinguishing between benign tumors and lung cancer

Metabolit	Cut Off	Cytology			p- value
		Cancer	Benign	Vol	
NAA/ Cre Ratio	≤ 0, 17	10 (71, 4%)	0 (0%)	10 (52, 6%)	0,011
	> 0, 17	4 (28, 6%)	5 (100%)	9 (47, 4 %)	
	Vol	14 (100%)	5 (100%)	19 (100%)	

Based on the results of the NAA/Creatin cross table with the cytology results in 19 samples, it was found that the samples that had lung cancer cytology results were 14, while the benign numbered 5 people. Of the 14 samples, 10 samples (71.4%) had NAA/Creatin ratio value ≤ 0.17 while the other 4 samples (28.6%) had NAA/Creatin ratio value > 0.17. Furthermore, out of 5 benign samples overall (100%) have NAA/Creatin values > 0.17. The test results obtained p-value 0.011 < 0.05 so that it can be interpreted that there is no difference between the results of the NAA/Creatine metabolite ratio with the results of cytology in determining the type of benign tumor and lung cancer.

From the metabolite spectroscopy value of NAA, the NAA/Choline ratio and the NAA/Creatine ratio in the different test results with cytology obtained p-value < 0.05 so that it can be interpreted that there is no difference between the results of these metabolites with cytology in order to determine benign tumor or lung cancer.

In this study, two lung cancer subtypes were obtained, namely malignant squamous cell carcinoma and adenocarcinoma. For this reason, it is also necessary to test the difference between the

metabolite values in the two types of lung cancer and the results of cytology. Determination of cut-offs point on NAA metabolite values, NAA/Choline ratio and NAA/Creatine ratio using median values [16]. Statistical results show that the NAA cut off point for both types of cancer is 0.015. The cut-off point in the NAA/Choline and NAA/Creatine ratio is 0.11.

Chi-Square Fisher's different test was then performed between the values of NAA metabolites in both cancers and the results of cytology. The following are the results of Chi-Square Fisher's different tests of NAA metabolite data on squamous cell carcinoma and adenocarcinoma with lung tumor cytology results.

Table 8 Results of different Chi Square Fisher test for NAA metabolites in squamous cell carcinoma and adenocarcinoma with cytological results.

Metabolit	Cut Off	Cytology			p- value
		SCC	AC	Volume	
NAA	≤ 0, 015	5 (100%)	2 (22, 2%)	7 (50%)	0,021
	>0, 015	0 (0, 0%)	7 (77, 8%)	7 (50%)	
	Vol	5 (100%)	9 (100%)	14 (100%)	

SCC=Squamous cell carcinoma, AC=Adeno Carcinoma

Based on the results of the NAA/Creatin cross table with the cytology results in 19 samples, it was found that the samples that had lung cancer cytology results were 14, while the benign numbered 5 people. Of the 14 samples, 10 samples (71.4%) had NAA/Creatin ratio value ≤ 0.17 while the other 4 samples (28.6%) had NAA/Creatin ratio value> 0.17. Furthermore, out of 5 benign samples overall (100%) have NAA/Creatin values > 0.17. The test results obtained p-value 0.011 < 0.05 so that it can be interpreted that there is no difference between the results of the NAA/Creatine metabolite ratio with the results of cytology in determining the type of benign tumor and lung cancer.

Based on the results of the NAA cross table in squamous cell carcinoma and adenocarcinoma with cytology results in 14 samples, it was found that there were 5 samples with squamous cell carcinoma cytology results, while 9 adenocarcinomas were cytology. 5 squamous cell carcinoma samples overall (100%) had NAA values $\leq$0.015. From 9 adenocarcinoma samples, 2 samples (22.2%) had an NAA value $\leq$ 0.015 while 7 other samples (77.8%) had an NAA value> 0.015. The test results obtained p-value 0.0021 <0.05 so that it can be interpreted that there is no difference between the results of the NAA metabolites in squamous cell carcinoma and adenocarcinoma with the results of cytology in determining the type of lung cancer in this study.

DISCUSSION

In this study, the largest number of male samples was 17 people with a percentage of 89.5%. While the number of samples with female sex is only 2 people or 10.5%. Referring to the existing literature this is in accordance with the incidence of lung cancer which is the most cancer in men [4,5]. Most men become lung cancer sufferers allegedly caused by smoking. From the patient's history it was found that of the 19 samples available, 14 people had a history of smoking or 73.7% of the sample. The most common cause of lung cancer according to the literature is smoking habit [19,20]. This is consistent with the findings in this study sample that more than 70% have a history of smoking. History of smoking is closely related to the occurrence of lung cancer types of Non-Small Cell Lung Cancer, especially subtype adenocarcinoma [20, 21]. In this study of 10 samples with adenocarcinoma cytology results, 9 patients had a history of smoking. This is consistent with previous research which states that adenocarcinoma as the type of NSCLC is mostly diagnosed in patients with a history of smoking [15].

Basically, the results of the metabolite spectrum displayed from the MR spectroscopy sequence are influenced by the TE (Time Echo) parameter set on the MRI computer [14]. Previous studies conducted by Yoon SH, Park CM, et al used TE 30 ms and the spectrum of metabolites that obtained most of the

lipids and only a small amount of choline [13]. If using a short TE of 30 ms the metabolite values are shown more complex and overlapping so that the application of short TE is rarely done because the spectrum produced is complex and the results of metabolites presented are not a major component in spectroscopy evaluation [14]. By using TE 135 ms it will simplify the spectrum produced because the peak shown is a peak metabolite with a long T2 which is a major component in spectroscopy evaluation [14] and this makes it easy to identify.

In this study the TE value (Time Echo) of 135 ms was used to display the concentrations of NAA, choline and creatin metabolites in lung tumor tissue, so the metabolite results obtained in this study differed from previous studies by Yoon SH, Park CM [13]. The reason for the selection of TE 135 ms is that TE 135 ms can display the NAA, choline, and creatine metabolites which are the main components in spectroscopy evaluation [14]. The spectrum value displayed depends on the concentration of the metabolite value contained in the tissue entering the voxel spectroscopy area. Biochemical changes in tumor cell metabolism associated with malignant transformation are reflected in changes in the concentration of certain metabolites in tumor tissue. The concentration of metabolites in tumor tissue is influenced by the type of tumor.

In this research, information on the metabolic value of lung tumors was obtained from the MR Spectroscopy multi voxel technique with a TE parameter of 135 ms showing the concentrations of NAA, choline, creatine metabolites from the lung tumor tissue. The results of the metabolite values were then analyzed quantitatively by Spearman's nonparametric statistical tests to determine the extent of their relationship with the cytological results of lung tumors. The most common lung tumor cytology results in this study were malignant squamous cell carcinoma and adenocarcinoma, this is in accordance with previous studies which stated that in cytological specimens, malignant squamous cell carcinoma and adenocarcinoma are the majority of tumor types [22].

The Spearman test results showed that the correlation between NAA metabolite values and lung tumor cytology results was very strong with a Spearman correlation value of 0.936 indicating a correlation with very strong correlation strengths. However, the choline value data obtained p-value 0.298 which shows that the correlation between choline values with cytology results is statistically not significant. Likewise, the creatine value data obtained p-value 0.626 which indicates that the correlation between creatine values with cytological results is not statistically significant. The results of these statistical tests, the value of NAA metabolites has a significant relationship or correlation with the results of lung cancer cytology. The value of NAA and the presence or absence of the concentration of NAA metabolites in lung tumor tissue is closely related to the results of the cytology of the lung tumor. So by looking at the value of NAA metabolites in the MR Spectroscopy spectrum value and comparing it with the choline value can help determine whether the lung tumor is benign or malignant [9].

Metabolite ratio values can also be used to assess the level of tumor malignancy [17]; therefore a correlation test of the NAA/Choline and NAA/Creatin metabolites with cytology results is also performed. From the test results show that the level of correlation between the ratios of NAA/Choline metabolites with the results of lung tumor cytology is very strong with the Spearman correlation r-value of 0.935 indicates the strength of the correlation is very strong. In the statistical test on the ratio of NAA/Creatin metabolites also obtained results that show that the value of the metabolite ratio of NAA/Creatin has a significant relationship with p-value < 0.001.

To differentiate the value of benign tumor and lung cancer metabolites from the results of cytology, the cut-off point of metabolite values must be determined to categorize the benign tumor with lung cancer. From the statistical test the cut-off point for NAA is 0.04, if NAA $\leq$ 0.04 then it is included in lung cancer whereas if NAA > 0.04 then it is included in benign tumors. In Chi-Square Fisher's different test with a cut-off point of 0.04, a p-value of < 0.05 was obtained so that it could be interpreted that there was no significant difference between the results of NAA metabolite values and cytological results in helping to determine the type of benign tumor or lung cancer. In addition to distinguishing benign and malignant tumors, it can be seen from the ratio of NAA/Choline metabolites detected in the tumor tissue [17]. In this study after the Spearman correlation statistical test, it was found that the value of the NAA/Choline metabolite ratio had a significant relationship with p-value < 0.001 and strong correlation.

In benign lung tumors, the concentration of metabolites detected in tumor tissue shows a much higher concentration of NAA compared to choline; this is in accordance with research which states that NAA in benign tumors has higher concentrations than choline [23,24]. The peak NAA concentration in benign pulmonary tumors is 2 times higher than that of choline. The average value of NAA in the mid-tumor region was 4,867 µmol/g; this value is far compared to the average value of choline 2,785 µmol/g. Because NAA is a marker or an indication of tissue cell health, a higher concentration of NAA compared to choline indicates that cells in benign tumor tissue are still healthy tissue cells and are not destructive. The NAA/Choline ratio in tumors detected in this study can distinguish between benign tumors and lung cancer. Provided that the NAA/Choline metabolite ratio is said to be normal if > 1.6 and said to be abnormal tends to be malignant if < 1.2 [17].

In the statistical test on the ratio of NAA/Creatin metabolites also obtained results that show that the value of the metabolite ratio of NAA/Creatin has a significant relationship with p-value < 0.001 and a very strong correlation. In previous studies, it was said that the ratio of NAA/Creatin metabolites is said to be normal if > 2 and it's too abnormal tends to be malignant if < 1.6 [17]. The mean ratio of NAA/Choline metabolites in benign lung tumors is 1.997 which means that it falls into the normal category where if the concentration of the NAA metabolite value is 1.6 times higher than the choline value then the tumor tends to be benign. In tumors that tend to be malignant, the mean value of the NAA/Choline metabolite ratio is 0.475, which means < 1, 2. This is consistent with previous research which states that in malignant tumor tissue there will be an increase in the concentration of choline [23], creatin, and decrease in NAA. Decrease in NAA concentration due to tumor growth displaces or destroys healthy cells [17].

In this study, the results of pulmonary benign tumors have the characteristic that is the concentration of NAA value is 2 times higher than choline, because in benign tumors there are still many healthy cells and the process of cell metabolism is still going well. Thus, according to available data, an analysis can show that tumors containing high choline concentrations in relation to decreasing NAA concentrations are parameters that indicate indicatively the growth of malignant tumors [25] and the NAA / Choline metabolite ratio is very helpful in distinguishing between malignant and benign tumors in the lungs.

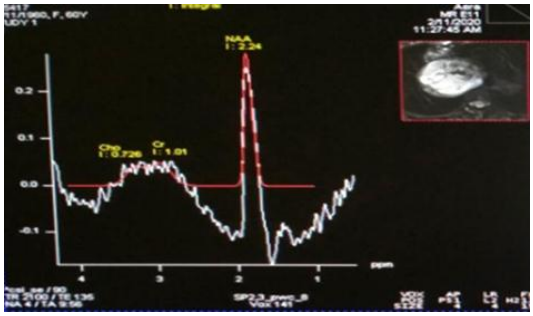

Figure 2. Spectrum spectrum of benign tumors, NAA concentration of 2.24 µmol / g is much higher than choline 0.73 µmol / g.

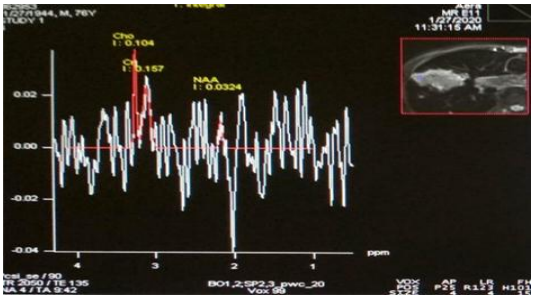

Figure 3. Spectrum spectrum of adenocarcinoma malignant tumors, NAA concentration of 0.03 µmol / g is much lower than choline 0.10 µmol / g.

The NAA/Creatine ratio can also help distinguish between malignant and benign tumors in the lungs. In this study the quantitative calculation of the NAA/Creatine ratio obtained the average value of the NAA/Creatine ratio in malignant adenocarcinoma tumors was 0.938 and in benign tumors was 7.868. Quantitatively if the calculation of the NAA/Creatin ratio > 2 then the tumor tends to be benign and if the NAA/Creatin ratio < 1.6 then the tumor tends to be malignant. This is consistent with previous research which states that in malignant lung tumors there will be a significant increase in the concentration of choline and creatin metabolites [23].

To differentiate the value of malignant squamous cell carcinoma and adenocarcinoma metabolites from the results of cytology, the cut-off point of metabolite value must be determined to categorize between malignant squamous cell carcinoma and adenocarcinoma obtained in this study. From the statistical test the cut-off point for NAA is 0.015. NAA values $\leq$ 0.015 are included in squamous cell carcinoma while NAA values > 0.015 to $\leq$ 0.04 are included in adenocarcinomas. In Chi-Square Fisher's different test with a cut-off point of 0.015, the p-value < 0.05 was obtained so that it could be interpreted that there was no significant difference between the results of the NAA metabolite values and the results of cytology in helping to determine the type of cancer malignant squamous cell carcinoma and adenocarcinoma.

The results of metabolites detected in the two types of malignant lung tumors also have differences so that the results of spectroscopy can distinguish malignant squamous cell carcinoma with adenocarcinoma. MR spectroscopy with TE parameters of 135 ms should be able to display concentrations of NAA metabolites, choline and creatin[8] in lung tumor tissue. However, in lung tumors whose cytology results are malignant squamous cell carcinoma, of the 5 samples, all spectroscopy results in the sample were not detected by any NAA metabolites in the tumor tissue. Only choline and creatine metabolites can be detected in malignant squamous cell carcinoma tumor tissue.

Squamous cell carcinoma is cytologically a polygon cell with an intercellular bridge, a crisp eosinophilia cytoplasm. Tumors also contain keratin pearls, and may have extensive necrosis[26]. This necrosis is thought to cause the absence of NAA which is an indication of tissue cell health. The absence of NAA in squamous cell carcinoma lung tumors indicates that the tumor tissue is a necrotic cell and this distinguishes malignant squamous cell carcinoma from adenocarcinoma on spectroscopy.

In adenocarcinoma lung tumor samples that are included in the type of non-small cell lung cancer. Lung adenocarcinomas are often found at the periphery of the lungs, and cytologically show larger cells with cytoplasmic lace often in glandular patterns[26]. Multi- voxel MR spectroscopy with TE 135 ms parameter can detect the presence of NAA, choline, and creatin in the tumor tissue.

The concentration of NAA in adenocarcinoma tumor tissue is in the range of 0.02 - 0.33 µmol/g with a mean of 0.108 µmol/g. Detection of NAA metabolites in non-small cell lung cancer tumors is in accordance with molecular biology research with NAT8L reagent by Lou TF, Sethuraman D, which mentions the presence of NAA concentrations in non-small cell lung cancer tumor tissue [27] and this potential can be used as biomarkers for these types of lung tumors [27]. However, in this study, NAA content was not detected in most malignant squamous cell carcinoma tumor tissue.

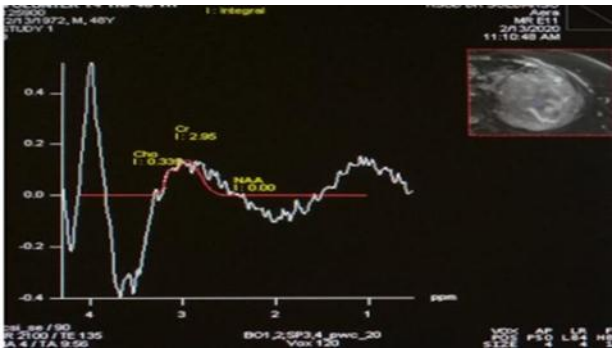

Figure 4 Spectrum spectrum of malignant squamous cell carcinoma, there is no NAA concentration detected

The difference in NAA concentration is caused by differences in the tissue that forms the tumor. Malignant squamous cell carcinoma is formed from squamous cells and has extensive necrotic tissue so that the concentration of NAA in tumor tissue is not detected. Adenocarcinoma originates from larger cells with cytoplasmic lace that are often in glandular patterns so that cells in this tumor tissue still contain small amounts of NAA metabolites. Of the three metabolite concentrations detected in the lung tumor tissue, the concentration of the NAA metabolite, the calculation of the NAA/Choline ratio and the NAA/Creatine ratio statistically have a very strong correlation and it is relatively easy to be able to distinguish between types of lung tumors, malignant squamous cell carcinoma, adenocarcinoma and tumors benign lung. This is because the concentration of NAA metabolites and the NAA/Choline ratio in the three tumor types are significantly different.

In this study, MR spectroscopy multi-voxel technique can be performed on lung tumors; using the TE 135 ms parameter can detect concentrations of NAA, choline and creatin metabolites in the lung tumor tissue. From the results of the correlation statistical test obtained a strong relationship between the values of metabolites with the results of cytology, so MR spectroscopy can have a role to help differentiate tumor types. Chi-Square Fisher's different test results showed no statistical difference between the results of metabolites with the results of cytology in helping determine tumor malignancy. Limitations in this study are the number of patients involved being relatively small samples and samples involved in data collection are patients with suspect lung cancer so that variations in cytological results found are still limited to three types of lung tumors, not all types of lung cancer can be covered in this study, there are only squamous cell carcinoma and adenocarcinoma which are epidemiologically the most cancers in the lungs and this study was only done in one modality.

CONCLUSION

MR spectroscopy with TE 135 ms on the value of NAA metabolites, the NAA/Choline ratio, and the NAA/Creatine ratio can be used to distinguish between benign tumors, adenocarcinomas and malignant squamous cell carcinomas found in this study. This research is focused to see the correlation between MR spectroscopy metabolite values in lung tumors with the results of the lung tumor cytology at TE 135 ms, for further research can be developed with various variations of TE. Further research with more samples needs to be done to be able to obtain more varied lung cytology results.

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