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RESEARCH ARTICLE

EVALUATION OF NAPSIN A EXPRESSION IN LUNG CANCERS AND ITS COMPARISON WITH THYROID TRANSCRIPTION FACTOR 1

Dr. R. Manibarathi[1], Dr. U. Suganya[2] and Dr. C. Suganthi[3]

1. Assistant professor of Pathology, Govt. Karur Medical College, Karur.
2. Assistant professor of Pathology, Govt. Mohan Kumaramangalam Medical College, Salem.
3. Assistant Professor of Pathology, Govt. Dharmapuri Medical College, Dharmapuri.

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Abstract

Introduction: Lung cancer is the leading cause of cancer mortality in the world. With the development of new, successful treatments for adenocarcinoma, it is essential to subtype Non- Small Cell Lung Carcinoma whenever possible. Thyroid Transcription Factor-1 is a promising marker for lung adenocarcinoma but its sensitivity and specificity is limited. Napsin A is a new marker that may be useful as a part of immunohistochemistry panel to classify poorly differentiated lung carcinoma on small biopsies.

Aim: To study the expression of Napsin A in lung cancer and to compare it with TTF-1 that has already recognized as a useful marker for lung adenocarcinoma.

Methodology: This is a retrospective study. By analyzing the histopathology records during the period of 2015 - 2017, histopathological slides of biopsy proven 50 malignant lung cases were collected. Napsin A expression was tested and compared with TTF1.

Results: Adenocarcinoma is the commonest type. Most common age group is 51-60 years with male female ratio is 2.7:1. Smokers have increased risk for malignancy. Napsin A and TTF1 showed similar sensitivity and the specificity of Napsin A was higher than TTF1 to lung adenocarcinoma. Napsin A showed higher sensitivity than TTF1 to exclude lung small cell carcinoma.

Conclusion: Napsin A is almost limited to adenocarcinoma, whereas TTF-1 is not so specific. Although TTF-1 is expressed in majority of adenocarcinomas, its expression is also noted in most of the small-cell lung carcinomas. Hence Napsin A can be used as an exclusionary marker for small cell carcinoma. Key words: Lung cancer, Napsin A, Small biopsy, Thyroid transcription factor 1.

Introduction:-

Lung cancer is the most frequently diagnosed major cancer and leading cause of cancer mortality in the world [(1)]. Previously, it was sufficient to diagnose primary lung carcinoma as either NSCLC or SCC for treatment purpose. With the development of new, successful treatments for adenocarcinoma, it is essential to diagnose the type of NSCLC whenever possible [(1)]. Routine sections stained with Hematoxylin and Eosin remain the most common

Corresponding Author:- Dr. R. Manibarathi
Address:- Assistant Professor of Pathology, Govt. Karur Medical College, Karur, Tamilnadu, India.

method by which lung cancers are classified; however typing of NSCLC and the more poorly differentiated tumors is often hard to achieve by H&E alone. Immunohistochemistry is an adjunctive tool for the subtyping and differential diagnosis of lung carcinomas [1]. TTF-1 is a mostly preferred marker for lung adenocarcinoma in most of the laboratories but the sensitivity and specificity of TTF1 is limitted. Napsin A is a newer marker that may be a useful marker for primary lung adenocarcinoma as well as in the subtyping of poorly differentiated lung carcinoma [1, 2].

Aims And Objectives:-

To study the expression of Napsin A in lung cancer, compared with another marker, thyroid transcription factor-1 (TTF-1), which has already recognized as a useful marker for lung adenocarcinoma

Materials and methods:-

The present study was a two years retrospective study conducted in department of pathology from September 2015 to July 2017. The total number of lung small biopsy specimens received was 309. Only histopathologically diagnosed lung malignancies were included in this study. Cases with non-neoplastic lung lesions and cases with inadequate material were excluded from the study.

All relevant clinical data were obtained from the histopathology records. Formalin fixed Paraffin embedded Hematoxylin and Eosin stained sections along with immunohistochemistry slides done for subtyping were examined in light microscopy. 50 cases were selected randomly from the total cases and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry with a marker Napsin A.

Histopathological diagnosis was done according to the recent WHO terminology for lung carcinomas in small biopsy specimens.

Immunohistochemical analysis of marker for Napsin a was done in paraffin tissue blocks using super sensitive HRP polymer system based on non bioton polymeric technology. Sections with a thickness of 4 microns were cut from the paraffin tissue blocks. They were transferred to gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with rabbit monoclonal EP 205 antibody (PATHNSITU) against Napsin and then the addition of secondary antibody conjugated with horse raddish peroxidase – polymer and diaminobenzidine substrate.

The immunohistochemically stained slides were analyzed and was read semi quantitatively on a scale from negative to 3+. A granular cytoplasmic staining was accepted as positive for Napsin A. If staining was absent or <10% tumours were considered as negative. 10%-25%, 26%-50% and 51%-100% immunoreactivity were considered as 1+, 2+ and 3+ respectively.

The statistical analysis is performed using IBM statistical package for social science software (SPSS) version 20. The comparison of Napsin A expression with TTF1 expression was calculated by Pearson Chi Square test and P value less than 0.05 are considered statistically significant.

Observation and results:-

Of total 309 lung specimens, there were 100 non neoplastic cases and 209 malignant cases. Out of 209 malignant cases, 57 cases were diagnosed and subtyped based on morphology alone. For rest of the 151 cases, because of the lack of typical specific features of subtypes, we proceeded with IHC. The markers used for subtyping were TTF1 for adenocarcinoma, P63 or CK5/6 for squamous cell carcinoma and any of the neuroendocrine markers (Synaptophysin, Chromogranin or, NSE) for small cell carcinoma were used. That slides were reviewed. From those cases 50 cases were randomly chosen and subjected to immunohistochemistry with Napsin A marker. Those 50 cases constitutes 10 cases of adenocarcinoma, 6 cases of Squamous cell carcinoma, 3 cases of small cell carcinoma, 16 cases of Non-Small Cell Carcinoma Lung – NOS and 15 cases of positive for malignancy in those cases definitive diagnosis was made out with the aid of IHC (Table 2). Immunostaining expression of Napsin A was compared with TTF1 in these cases.The results were summarized in Table 3

Table 1:- Frequency of lung carcinoma categories (n= 209).

Diagnosis (N=209)	On histopathology	After IHC

	No of cases	Percentage	No of cases	Percentage
Adenocarcinoma	40	19	90	43
Squamous cell carcinoma	17	8	61	29
Small cell carcinoma	19	9.1	25	12
Adenosquamous carcinoma	2	1	2	1
Nonsmall cell carcinoma - NOS	90	43	7	3.3
Poorly differentiated carcinoma	30	14.4	8	6.7
Carcinoid	2	1	2	1
Plasmacytoma	1	0.5	1	0.5
Synovial sarcoma	1	0.5	1	0.5
Mesenchymal malignancy (Fibrohistiocytic origin)	1	0.5	1	0.5
Metastatic carcinoma	6	2.9	10	4.8

Table 2:- Correlation of histopathological diagnosis of selected cases with IHC proven final diagnosis: (n=50). Pearson Chi-Square=34.938** p<0.001

Histopathological diagnosis	Diagnosis AFTER IHC			TOTAL
Diagnosis	ADCC	SCC	SQCC	
Adenocarcinoma	9	0	1	10
Non-Small cell Lung Carcinoma	11	0	5	16
Positive for malignancy	3	5	7	15
Small cell carcinoma	0	2	1	3
Squamous Cell carcinoma	0	0	6	6
TOTAL	23	7	20	50

Among the 10 cases diagnosed as adenocarcinoma based on morphology, 9 cases were proven by IHC as adenocarcinoma and one case was turned to be squamous cell carcinoma. Among the 3 cases diagnosed as small cell carcinoma based on morphology 2 were confirmed with IHC as small cell carcinoma and one turned to be squamous cell carcinoma. Among 6 cases diagnosed as squamous cell carcinoma, all cases were confirmed by IHC as squamous cell carcinoma. Among the 31 cases of Non-small cell carcinoma – NOS and Poorly differentiated malignancy, 14 were found to be positive for adenocarcinoma markers, 5 were found to be positive for small cell carcinoma markers and 12 were diagnosed as squamous cell carcinoma. Hence the study group of 50 cases comprised of 23 cases of adenocarcinoma, 20 cases of Squamous cell carcinoma and 7 cases of small cell carcinoma.

Table 3:- Comparison of Napsin A and TTF1 immunostaining in lung cancer subtypes.

	IHC (positive cases/ total cases)	
Diagnosis	Napsin A	TTF1
ADCC	23/23(100%)	23/23(100%)
SQCC	0/20(0)	0/20(0)
SCC	0/7 (0)	3/7 (43%)
	Pearson Chi-Square=50.000** p<0.001	Pearson Chi-Square=43.132** p<0.001

TTF1 was positive in all adenocarcinoma (23/23) and 43% of small cell carcinoma (3/7). But it was negative in all squamous cell carcinoma. Napsin A was positive in all adenocarcinoma (23/23) and negative in almost all cases of small cell carcinoma and squamous cell carcinoma (Fig 1, Fig 2 and Fig 3). The expression of Napsin A in lung cancer is statistically significant. (p<0.001).The sensitivity and specificity of Napsin A and TTF1 in each subtype were summarized in table 4

Table 4:- Comparison of sensitivity and specificity of Napsin A and TTF in lung cancer subtypes.

	Adenocarcinoma	Squamous cell carcinoma	Small cell carcinoma

Statistical data	Napsin A	TTF1	Napsin A	TTF1	Napsin A	TTF1
Sensitivity	100%	100%	100%	100%	100%	57%
Specificity	100 %	89 %	77 %	86.6 %	53.5 %	53.5 %
Positive Predictive Value	100%	88.5%	74%	83%	26%	16.6%
Negative Predictive Value	100 %	100 %	100 %	100%	100 %	88.5 %

The sensitivity of Napsin A was similar to TTF1 in lung adenocarcinoma subtying and to exclude Squamous cell carcinoma and was higher than TTF1 to exclude small cell carcinoma. The specificity of Napsin A was higher than TTF1 in the lung adenocarcinoma subtyping and it was comparable to TTF1 to exclude squamous cell carcinoma and small cell carcinoma.

Discussion:-

70% of lung cancers patients present in advanced stages and are inoperable; the diagnosis is based primarily on small biopsy and cytology specimens[3]. Therefore, a new classification for lung cancer in small diagnostic samples was introduced in the 2011 IASLC/ATS/ERS Lung Adenocarcinoma Classification [3] and adopted in the 2015 WHO Classification.Without the use of immunohistochemistry markers, it is difficult to subtype a lung cancer on small biopsies that may not show differentiation because of poor sampling, small amount of tumor tissue, crush artifact [4, 5]. In that situation we can use panel of IHC markers to subtype. The basic panel should include at least one marker specific for adenocarcinoma and one specific marker for squamous cell carcinoma. The commonly used basic panel of markers for subtyping includes TTF 1, P63 and CK 5/6. In most of the cases these basic panel of markers are enough for sub categorization.

With the use Thyroid Transcription Factor-1, lung primary can be separated from a metastasis. Another newly discovered lung specific marker is Napsin A that complements TTF-1 to identify primary lung carcinoma, to subtype NSCLC, and helps to distinguish NSCLC, particularly poorly differentiated adenocarcinoma from small cell carcinoma(SCC)[1,6].

In the present study, lung carcinoma was most common in the age group of 51-60 years (37.5%) and more common among males (76.5%) with male to female ratio was 2.7:1. In this study adenocarcinoma had a maximum incidence of 43% followed by squamous cell carcinoma (29%) and Small cell carcinoma (12%) (Table 1). Our results were in concordance with Malik PS. et al (2013)[7]Sundaram V et al(2014)[8], Mahendrakumar. et al(2016)[9]

In our study, Lung adenocarcinoma showed the IHC profile of NapsinA + ve/ TTF1 + ve ; lung squamous cell carcinoma showed Napsin A –ve / TTF1 –ve ; lung small cell carcinoma showed Napsin A – ve / TTF1 +ve IHC profile.We found that TTF1 was positive in adenocarcinomas and in half of the small cell carcinomas (43%). But it was invariably negative in squamous cell carcinomas. So with TTF1 we cannot distinguish adenocarcinoma from small cell carcinomas. Napsin A was positive in all adenocarcinomas and invariably negative in almost all cases of small cell carcinomas and squamous cell carcinomas. So napsin A can be used as a specific marker for distinguishing adenocarcinoma from small cell carcinomas. With the Napsin A positivity we can exclude small cell carcinoma. So Napsin A can be used as exclusion marker for small cell carcinoma.

In the 50 samples, Napsin A and TTF1 had similar sensitivity and Napsin A had higher specificity to lung adenocarcinoma than TTF1. Napsin A is more sensitive than TTF1 and as specific as TTF1 in excluding small cell carcinoma. Napsin A had similar sensitivity and lesser specificity in excluding squamous cell carcinoma than TTF1. The results were in concordance with Bradley M. Turner et al. that study reported that Napsin A was more sensitive than TTF1 for primary lung adenocarcinoma (87% versus 64%: p value<0.001) [1]. Napsin A was more specific than TTF1 for primary lung adenocarcinoma versus all metastatic tumours. (p value <0.001). The results were also in concordance with T Ueno et al (2003). That study reported that napsin A is as sensitive as TTF1 and more specific than TTF1 [10].

Conclusion:-

A tremendous advance in the lung cancer treatment necessitates the accurate subtyping of lung cancer. This study finds that Napsin A showed the same sensitivity as TTF1and higher specificity than TTF1 for lung adenocarcinoma.

To distinguish pulmonary small cell carcinoma from other poorly differentiated lung carcinoma with similar morphology especially those with concomitant TTF 1, Napsin A can be used as an exclusionary marker. So the addition of Napsin A in the basic IHC panel will improve the lung cancer subtyping.

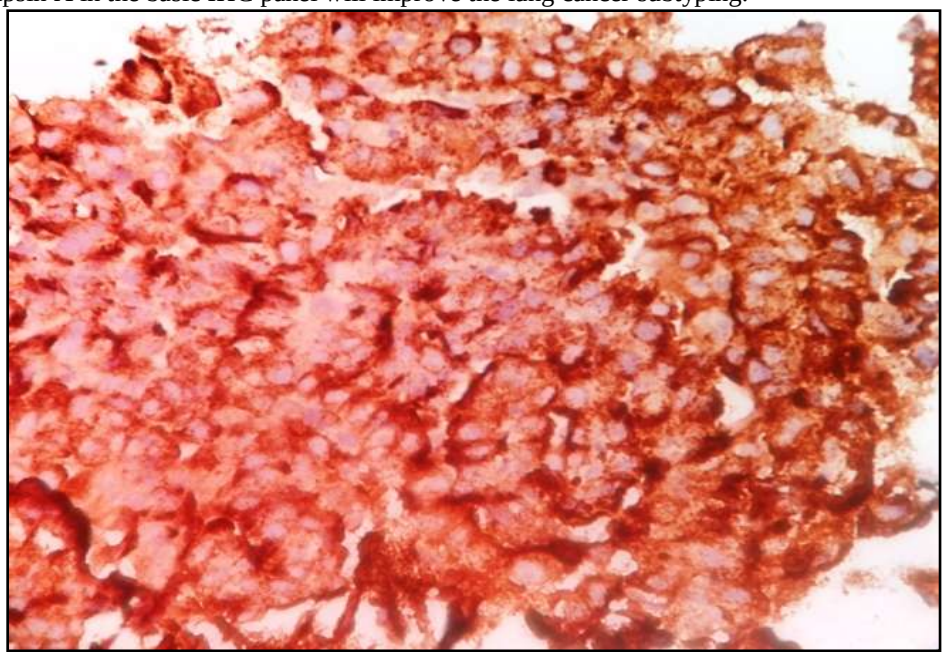

Fig 1:- Napsin A positive in tumor cells of Adenocarcinoma.

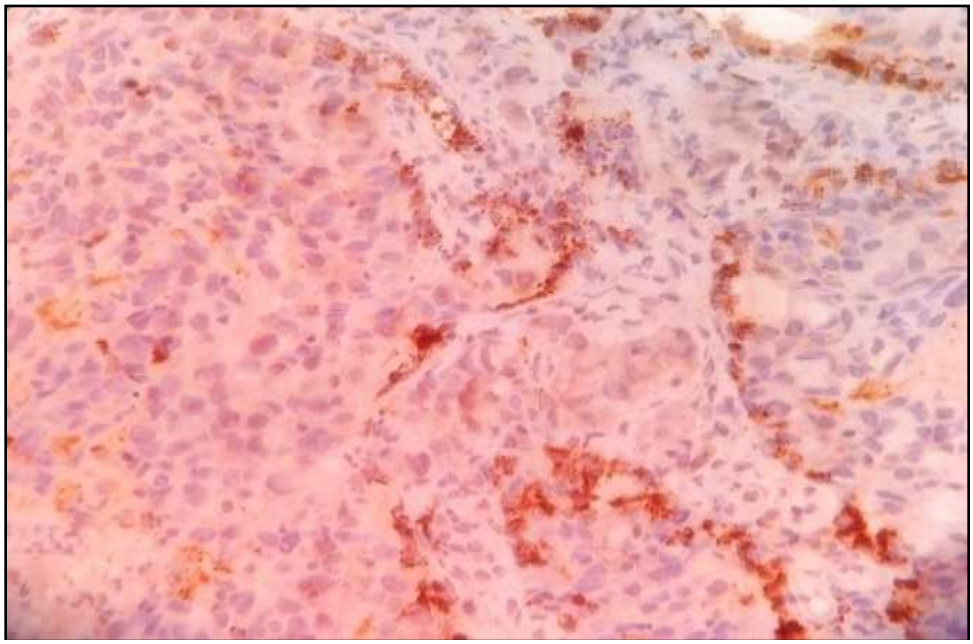

Fig 2:- Napsin A negative in tumour cells of squamous cell carcinoma.

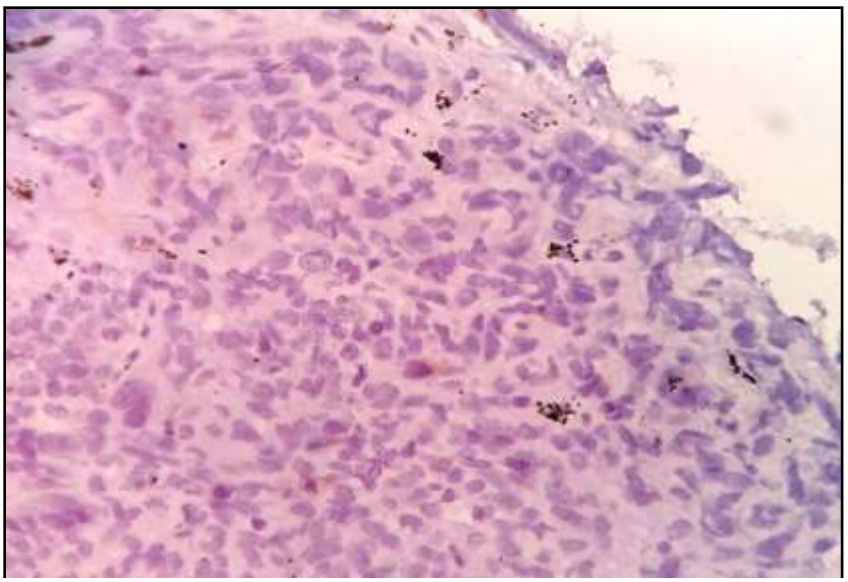

Fig 3:-Napsin A negative in tumour cells of small cell carcinoma.

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