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Original Research Article

Study of p40 is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma on cytological smears

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ABSTRACT

Background: Histology and genetically driven management are the current standard in carcinoma lung. Adequate tissue is a challenge in a few cases. While liquid biopsy is an, it's an always preferred to get an initial cyto/histo morphological confirmation. While the immune-histo chemistry (IHC) is a proven method to differentiate the squamous vs adeno carcinoma, the role of various "immuno-cyto-chemical" makers were not studied widely.
Methods: This is a retrospective (July 2018 to December 2018) for random sample collection and prospective (Jan 2019-August 2019) study conducted from in the Department of pathology, govt. chest hospital Osmania medical college, Hyderabad as a part of PG dissertation, 120 subjects with cytological diagnosis of non-small cell lung carcinoma were analyzed during this period and others were excluded.
Results: Out of 120 cases 80-adenocarcinoma (ADC) and 40-squamous cell carcinoma (SCC). Most of the patients are in 61-70 years, 20 are 71-80 years, 20 between 51-60 and 10 are between 40-50 years. In this study sensitivity of p63 and p40 is equal, but specificity and positive predictive value are higher for p40 for diagnosis of SCC.
Conclusions: Immuno-cyto-chemistry is still a valid option in selected cases where getting a biopsy is difficult. Our findings recommend the use of p40 immuno staining rather than p63 as a squamous cell marker.

Keywords: ADC, Immunostaining, Squamous cell marker, Carcinoma lung

INTRODUCTION

Lung cancer is the leading tumor across the globe, of which 80-85% are non-small cell lung cancer (NSCLC).[1,2] Even in India, it constitutes 6.9% of all new cases and 9.3% of all cancer related deaths.[3,4] Though SCC was the most common histology, the trend of ADC picking is observed due to lesser smoking and other poorly understood environmental factors.[5-9] While prognosis in advanced stages remained poor in the past, recent advances have made a significant improvement in disease outcomes, especially after deeper understanding of etiopathogenesis and the genetics.[10,11]

While histology and genetically driven management is coming up in big way in the management of carcinoma lung, having adequate tissue is still a challenge in a few cases. While liquid biopsy is an option in patients with clinically suspected ADC, it's always preferred to get an initial cyto/histo morphological confirmation. While IHC is proven method to differentiate squamous vs adeno carcinoma, role of various immuno-cyto-chemical makers were not studied widely. Although the commonly used squamous cell marker p63 is extremely sensitive in SCC, it is less specific due to its positivity in some cases of ADCs and other tumor types particularly in lymphomas. Therefore, we conducted this study is to evaluate if p40 is

superior to p63 for diagnosis/ differentiation of SCC on cytology smears.[12]

METHODS

The details were provided in the text. This is a retrospective (July 2018 to December 2018) for random sample collection and prospective (Jan 2019 to August 2019) study conducted in the department of pathology, Government Chest Hospital Osmania Medical College, Hyderabad as a part of PG dissertation. Ethical committee approval was taken and its as per the ICH-GCP. No active patient intervention was done and all the samples were of laboratory in nature and no subject participation happened directly or indirectly. To elaborate more the sample collection is a mix of prospective and retrospective. However, the entire analysis was done prospectively and hence subgroup analysis was not attempted a total of 120 subjects with cytological diagnosis of NSCLC or poorly differentiated carcinoma were analyzed during this period. Proven cases of neuroendocrine carcinomas, malignant carcinoids were excluded. Clinical profile of eligible cases is collected and recorded in the predefined format. Samples collected by direct needle cytology, bronchial wash and were processed to make cell blocks as per standard guidelines and stained with hematoxylin and eosin (H and E).[13] The reason for choosing p63 (a standard marker for squamous differentiation) and a detailed process of smear preparation are as per standard and described by Bishop et al schematic drawing of ΔNp63 expression in mechanisms of squamous differentiation.[28] ΔNp63 is hypothesized to sustain stem cell populations after asymmetric division of a stem cell into one daughter cell programmed for differentiation).

Statistical analysis

We have used SPSS for windows for the statistical analysis and the descriptive data is represented in Tables. We performed the sensitivity, specificity positive and negative predictive values for the various IHC markers and represented them in tabular form.

RESULTS

During the period, 120 NSCLC were diagnosed and out of them 80 cases are diagnosed as ADC and 40 cases are diagnosed as SCC. Most of the patients are in the age group of 61-70 years consists 70 cases. 20 cases in the age group of 71-80 years and 20 cases between the age group of 51-60 years and 10 cases between the age group of 40-50. When analyzed for the gender distribution, we observed that males dominated over females with a M:F ratio of 3:1.

The immuno reactivity for p63 vs p40 in SCC and ADC were described in Table 2 and the sensitivity and specificity of p63 and p40 were described in Table 3. The positive and negative predictive value were elaborated in Table 4. The sensitivity and the specificity of p63 are 100% and 80% respectively, and sensitivity and specificity of p40 are 100% and 98.7% respectively positive predictive value are higher for thep40 compared with p63. To summarize, sensitivity of p63 and p40 is equal, but the specificity and positive predictive value are higher for p40 for diagnosis of SCC. One case of ADC shows positive for p40 it may be due to adeno-squamous carcinoma misdiagnosed as ADC on cytology.

We have illustrated a few of them as to detail them, Figure 1 showing SCC cytology H and E (40x) with sheets and clusters of atypical squamous cells with moderate to abundant eosinophilic cytoplasm and pleomorphic hyperchromatic nucleus some of them having vesicular nucleus with prominent nucleoli. Similarly, Figure 2 represents the p63 stain in SCC with positive nucleus stain and Figure 3, the p40 stain. While in the ADC, Figure 4 is showing a representative smear 40x H and E-depicting pleomorphic epithelial cells arranged in sheets and clusters and acinar pattern with pleomorphic hyperchromatic nucleus and p63 and p40 stains in the same smears respectively.

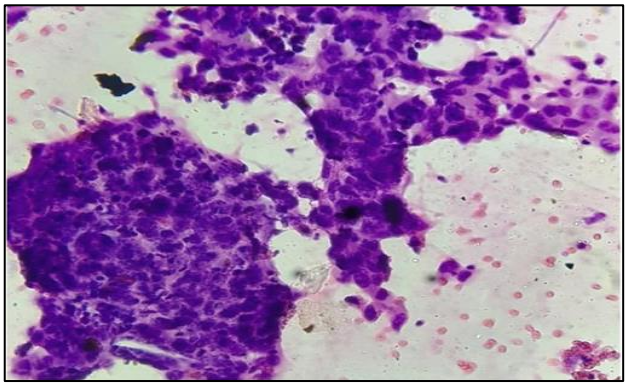

Figure 1: SCC cytology H and E (40X).

H and E smear shows sheets and clusters of atypical squamous cells with moderate to abundant eosinophilic cytoplasm and pleomorphic hyperchromatic nucleus some of them having vesicular nucleus with prominent nucleoli.

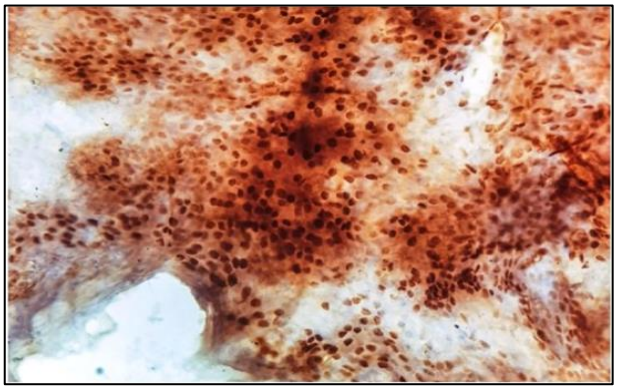

Figure 2: SCC, cytology (40X). Stain: p63, positive: nucleus.

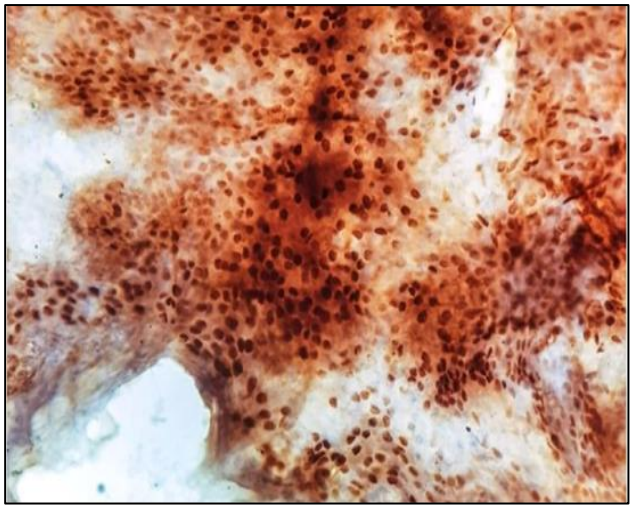

Figure 3: SCC, cytology (40X). Stain: p63, positive: nucleus.

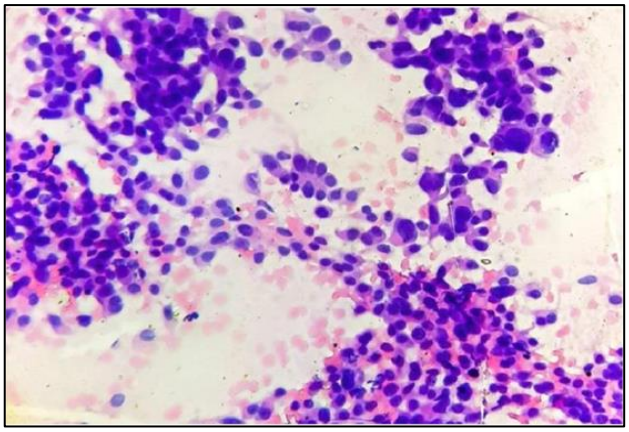

Figure 4: ADC, cytology H and E (40X).

H and E smear shows pleomorphic epithelial cells arranged in sheets and clusters and acinar pattern with pleomorphic hyperchromatic nucleus.

Table 1: Immuno-reactivity for p63 vs p40 in SCC and ADC.

Tumor type	Antibody	Number of cases	Positive	Negative
SCC	P63	40	40	0
	P40	40	40	0
ADC	P63	80	16	64
	P40	80	1	79

Table 2: Sensitivity and specificity of p63 and p40.

Marker	SCC	ADC	Sensitivity	Specificity
P63	40/40	16/80	100	80
P40	40/40	01/80	100	98.7

Table 3: Positive and negative predictive value.

Marker	SCC	ADC	Positive predictive value	Negative predictive value
P63	40/40	16/80	71.4	100
P40	40/40	01/80	97.5	100

DISCUSSION

NSCLC remains a disease with guarded prognosis and the treatment recommendations largely depend on histological type, stage and the genetic/biological behavior of cancer besides the patient fitness.[14] It's a well- known and proven fact that few drugs like gemcitabine act better in squamous cell variants compared to ADC (in which drugs like pemetrexed act better) due to variations in thymidylate synthetase expression. Even a large meta-analysis addressing "best first-line therapy for people with advanced NSCLC, performance status 2 without a targetable mutation or with an unknown mutation status" had revealed the similar results. Therefore, it is quite important to have a histological confirmation of the tumor type.[15] Emphasizing the need for tissue in NSCLC, and the challenges to get adequate tissue in lung biopsies, Zang et al observed that nearly half (46%) of patients experienced multiple biopsies prior to diagnosis, with great failure rates.[16] On occasions getting a cytology may be more practical on with better yield.[17] While the liquid biopsy remains an option in patients with clinically suspected ADC, it's always preferred to get an initial cyto/histo morphological confirmation. The use of liquid biopsy is limited due to high cost and sparse availability. Distinguishing features, such as gland formation or keratinization, are not always evident particularly when the material for review is scant (e.g., cytology).

This confusion of poorly differentiated carcinomas is often addressed with an array of IHC stains and NSCLC is no exception to this.[18] While the IHC is a proven method to differentiate the squamous vs adeno carcinoma, the role of various "immuno-cyto-chemical" makers were not studied widely. There are only a handful of studies addressing this issue. A review by Roy-Chowdhury et al found that "most predictive IHC assays

are validated on formalin-fixed paraffin-embedded (FFPE) samples and using these assays to cytologic specimens need further validation. This is due to the lack of standardized processing protocols in cytology, multiple pre-analytic variables that can impact the antigenicity of antibodies used for predictive biomarker testing.[19]

Table 4: Comparison of present study with existing literature.

Study	Number of cases	Age range (in years)	Duration	Squamous vs adeno
Present study	120	40-80	3 years	40:80
Lilo et al[25]	144	33-88	4 years	44:31
Delgado et al[26]	76	46-88	7 years	24:66
Vogt et al[27]	60	42-81	5 years	30:30
Bishop et al[28]	318	40-85	4 years	81:237

Table 5: Comparison of sensitivity and specificity of p63/ p40 in SCC.

Study	P63		P40	
	Sensitivity	Specificity	Sensitivity	Specificity
Present study	100	80	100	98.7
Vogt et al[27]	97	80	100	100
Righi et al[29]	96	80	100	96
Lilo et al[25]	77-100	32-69	64.2-92.5	83.8-100
Delgado et al[26]	82	92	100	100
Bishop et al[28]	100	60	100	98

Table 6: Comparison positive and negative predictive value of p63 in SCC.

Study	P63		P40	
	Positive predictive value	Negative predictive value	Positive predictive value	Positive predictive value
Present study	71.4	98.7	97.5	100
Vogt et al[27]	64	98	100	100
Righi et al[29]	64	98	94	100
Delgado et al[26]	82	92	100	100
Bishop et al[28]	34	100	92	100

Of the available immunohistochemical markers the notable ones are p63 and p40, while the former has emerged as the 'frontrunner' of the squamous markers.

Several studies have shown that p63 has an extremely high sensitivity (approaching 100%) for SCC.[20] A study by Pelosi et al found that p63 immuno-reactivity was seen in 109/118 SCCs, 15/95 ADCs, 2/2 adenosquamous carcinomas, 4/6 large cell carcinomas, 9/20 poorly differentiated NET, and 1/37 typical and atypical carcinoids, which indicate that its quite variable.[21] However, the main limitation of p63 is low specificity due to its unexpected reactivity in 16-65% of lung ADCs even on the cytology smears.[22]

It has been suggested primarily in laboratory studies that the predominant p63 isoforms in basal/ progenitor cells is specifically the ΔN variant, whereas the TA isoform has a wider tissue distribution. ΔNp63 is thought to function as a stem cell factor, responsible form a initialing cells in an uncommitted state with regenerative potential-a role that may be recapitulated in tumors derived from these cells.[23] In line with this functional role, it was noted that the predominant p63 transcript in SCCs of lung and other sites is ΔNp63.[27] As a corollary, these studies suggest that it is the TAp63 isoform that is responsible for the unexpected presence of p63 in certain tumors.[23]

Therefore, there was a need felt for alternative marker for the SCC lung, which was found in the form of p40. A study by Affandi et al found that p40 is an excellent marker for distinguishing lung SCC from ADC, and p40 expression is equivalent to p63 expression in lung SCC.[24]

In the present study we did a detailed evaluation of the expression of various p63 and p40 in NSCLC. The present study sample size, study duration age and the gender distribution are comparable to the published literature, which was represented in Table 4, where the age range is around 5-7th decade and the males dominated over female. Similarly, the percentage of squamous and ADC were also comparable, making our study comparable with published literature.[25-30]

When we compared the sensitivity and specificity of the present study with the published literature, we found that sensitivity of p63 and p40 is equal in predicting the SCC of the lung. However, specificity for p40 is higher for the diagnosis. The sensitivity of the parent study is compared with other similar studies.[25-30]

Similarly, we did compassion of the p63 with other literature and the sensitivity and specificity of p63 in SCC lung are similar to above studied as mentioned below in Table 5. We have a better sensitivity and specificity compared to the world literature. To conclude our results are in agreement with published literature and the sensitivity and specificity of p40 in SCC are comparable with above studies.

We also did a compassion of the p63 for the positive and negative predictive values with other literature. Comparison positive and negative predictive value of p63 in SCC was detailed in Table 6. Positive and negative predictive value of p63 in SCC is similar to above studies. Similarly, we did compassion of the P40with other literature. Comparison positive and negative predictive value of p40 in SCC was detailed in Table 6 as well. Positive and negative predictive values of p40 in SCC are comparable to above studies.[25-30]

Limitations

A double blinded review by two pathologist and the reconfirmation with biopsy as comparative gold standard could have added more strength to the present study.

CONCLUSION

The staining in cytology (immuno-cyto-chemistry) is feasible and if a standard method is followed it's as good as IHC. Although the commonly used squamous cell marker p63 is extremely sensitive, it is less specific due to its reactivity in some cases of ADC. A potentially useful new marker p40, an isoform of p63, is equally sensitive and more specific than p63 for diagnosis of SCC on cytology and is negative in ADC. The sensitivity and the specificity of p63 are 100% and 80% respectively, and the sensitivity and the specificity of p40 are 100% and 98.7% respectively positive predictive value are higher for thep40 compared with p63. Positive p63 staining may be mistakenly interpreted as squamous differentiation and result in misclassification of ADC as squamous cell lung carcinoma. That is why p40 should be used instead of p63as a squamous cell marker especially on cytology smears.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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