THE ROLE OF IMMUNOHISTOCHEMISTRY IN DIAGNOSIS OF LUNG CANCER WITH CORRELATION OF SERUM TUMOUR MARKER CARCINOEMBRYONIC ANTIGEN

Neha Pandya¹, Vijay Popat²

¹3rd Year Resident, Department of Pathology, Shri M. P. Shah Government Medical College, Jamnagar, Gujarat, India.

²Professor and HOD, Department of Pathology, Shri M. P. Shah Government Medical College, Jamnagar, Gujarat, India.

ABSTRACT

BACKGROUND

Immunohistochemistry is a less challenging, widely available technique which provides clinically meaningful results quickly and allows for cellular localization of proteins in the context of tumour structure. Lung cancer is the leading cause of cancer-related deaths worldwide, regardless of gender. In an era of precision medicine, pathologists are required to classify lung cancer into specific subtypes and assess biomarkers relevant to molecular targeted therapies but overlapping of microscopic features of different types of tumours sometimes makes diagnosis difficult for pathologists. The objectives of this study were- 1. to accurately diagnose small and non-small cell lung carcinoma for targeted therapy. 2. to differentiate between adenocarcinoma lung and malignant mesothelioma. 3. to determine primary site of origin in patients who present with metastatic disease. 4. to correlate serum CEA level and lung carcinoma.

MATERIALS AND METHODS

A descriptive study of 60 cases which have been diagnosed histopathologically and cases which were difficult to diagnose due to inadequate material, was carried out for six months duration from July 2018 to December 2018 in the Department of Pathology, Shri M. P. Shah Government Medical College, Jamnagar. We have studied histopathology slides with different IHC panel markers like TTF-1, Napsin A, CK 7, p63 and EGFR. Results were correlated with serum tumour marker Carcinoembryonic Antigen (CEA) with the help of Enzyme Linked Immunosorbent Assay (ELISA) technique.

RESULTS

In our study of 60 cases, 24 (40%) cases of squamous cell carcinoma, 20 (33%) cases of adenocarcinoma and 15 (25%) cases of small cell lung carcinoma and 1 (1.66%) case of metastasis from colon adenocarcinoma were confirmed. Most of the patients belonged to older age group and out of 60 cases, 39 (65%) were males, and 21 (35%) were females. Most common age group was 40-60 years.

CONCLUSION

Squamous cell carcinoma is more common than other subtypes of lung cancer with male predominance and was associated with smoking. Serum CEA level is raised (>7 ng/ml) in all the cases of lung carcinoma. Immunohistochemical techniques along with serum tumour marker play critical roles as diagnostic and screening tools for lung cancer.

KEY WORDS

Immunohistochemistry, Serum Tumour Marker CEA, Small Cell Lung Carcinoma, Non-Small Cell Lung Carcinoma

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BACKGROUND

Immunohistochemistry is a widely available technique that is technically less challenging and can provide clinically meaningful results quickly and allows for cellular localization of proteins in the context of tumour structure with rapid turnaround time. Lung cancer being a leading cause of cancer related deaths worldwide, regardless of gender, in the era of precision medicine, pathologists are required to classify lung cancer into specific subtypes and assess biomarkers relevant to molecular targeted therapies.¹

But overlapping of microscopic features of different types of tumours sometimes makes diagnosis difficult for pathologists. Squamous, adenocarcinoma and small cell carcinoma of lung are common malignancies affecting lung and for that basic IHC panel markers like P63, TTF-1, Napsin A, CK 7, CK 20 and EGFR should be performed. Serum tumour marker carcinoembryonic antigen (CEA) also plays an important role in diagnosis and prognosis of lung cancer. Normal value for serum CEA in smokers is <7ng/ml and in non-smoker <5 ng/ml.

Objectives

1. To accurately diagnose small and non-small cell lung carcinoma for targeted therapy.
2. To differentiate between adenocarcinoma lung and malignant mesothelioma.
3. To determine primary site of origin in patients who present with metastatic disease.
4. To correlate serum CEA level and lung carcinoma.

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Corresponding Author:

Dr. Neha Pandya,

301, Dwarkesh Apartment,

Silver Park, Noble City,

Zanzarad Road, Jamnagar-362001,

Gujarat, India.

E-mail: drchapandya47@gmail.com

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MATERIALS AND METHODS

Type of Study

Descriptive study.

Study Period

Six months (from July 2018 to December 2018).

Study Setting

Department of Pathology, Shri M. P. Shah Government Medical College, Jamnagar.

Sample Size

60 cases.

Sample Size

Sample size was taken conveniently.

Study Procedure

Histopathology slides have been studied with different IHC panel markers like TTF-1, Napsin A, CK 7, P63 and EGFR. A general IHC protocol consists of four main steps: (1) fixation – to keep everything in its place, (2) antigen retrieval – to increase availability of proteins for detection, (3) blocking and (4) antibody labelling and visualization. Cases have been correlated with serum tumour marker CEA (Carcinoembryonic antigen) also. All of IHC stains were performed at our clinical immunohistochemistry laboratory as previously described. Briefly, the specimen were sectioned at 5 micron size, deparaffinised and incubated with primary and secondary antibodies. Staining characteristics were reviewed and considered along with intensity and distribution of staining patterns. A case was considered to be positive if greater than 5% of tumour cells with an appropriate staining patterns were identified; otherwise case was considered to be negative. In terms of staining pattern, coarse granular cytoplasmic staining was considered positive for Napsin A, nuclear staining for TTF-1, cytoplasmic staining for CK 7, diffuse nuclear staining for P63 and membranous staining for EGFR. Care was taken not to interpret entrapped normal bronchial epithelium or pulmonary macrophages as a positive staining. For serum CEA, sandwich ELISA technique was used with help of Calbiotech company kit insert having standard range from 5-250ng/ml.

Inclusion Criteria

Patients who attended TB- Chest Department, Guru Gobind Singh Government General Hospital, and Jamnagar with radiologically diagnosed lung mass. Also, cytopathologically and histopathologically diagnosed or suspicious of malignancy of lung carcinoma or any metastatic carcinoma and with raised value of serum CEA.

Exclusion Criteria

Benign, inflammatory, infective aetiology of lung pathology by radiology, cytopathology or histopathology and with inadequate material for immunohistochemistry and inadequate data.

Data Management and Analysis

The data was entered in Microsoft Excel and further statistical analysis was done using SPSS software (Version

17). The statistical methods used were mean, frequency, proportion and non-parametric test (chi-square test). The level of significance was indicated as 'p' value < 0.0001.

RESULTS

Most of the patients belonged to older age group and most common age group was 40 to 60 years. In our study of 60 cases, 24 (40%) cases of squamous cell carcinoma being the most common with P63 positivity, 20 (33%) cases of adenocarcinoma with TTF-1, CK 7, EGFR and Napsin A positivity with negative P63 and 15 (25%) cases of small cell lung carcinoma with TTF-1 positivity and Napsin A negativity and 1 (1.66%) case of metastasis from colon adenocarcinoma with CK7 negativity and CK20 positivity were confirmed and out of 60 cases, 39 (65%) were males and 21 (35%) were females. So, male to female ratio was 1.8:1. Serum tumour marker CEA level was raised (>7 ng/ml) in all the cases. The smallest tumour diameter was 1 cm and the largest 10 cm, with a mean diameter of 4.01 ± 2.09 cm. We found a statistically significant difference in favour of adenocarcinoma versus SqCC ($P < 0.0001$) in the analysis of Napsin-A staining and in favour of SqCC versus adenocarcinoma ($P < 0.0001$) in the analysis of P63 staining tumour type. There was no statistically significant correlation between the distribution of cases by stages or tumour types ($P > 0.05$).

Histology	Positive Markers
Squamous Cell Carcinoma	CK5/6, P63, Vimentin, CEA, S-100, EMA
Adenocarcinoma	TTF-1, Napsin A, CK7, Keratin 7, MUC Family
Small Cell Carcinoma	TTF-1, CD56, Synaptophysin, Chromogranin
Large Cell Carcinoma	Cytokeratin, TTF-1, CD56, NSE (Rare)
Neuroendocrine Tumours	TTF-1, CD56, Synaptophysin, Chromogranin
Adenosquamous Carcinoma	Both Markers of Adeno and Squamous
Sarcomatoid Carcinoma & Carcinosarcoma	Cytokeratin, Vimentin, CEA, EMA
Pulmonary blastoma	CD117, Surfactant Protein B, Vimentin, Desmin
Metastatic Malignancy from Colon And Other GIT	CDX2, CK7-/CK20+
Metastatic Malignancy from Prostate	PSA
Metastatic Malignancy from Breast	ER, PR, Her2/Neu
Metastatic Malignancy from RCC, Mullerian, Thyroid, Thymus	PAX8
Metastatic Malignancy from Germ Cell	SALLA4
Metastatic Malignancy from Urothelial, Squamous, Thymus	P63
Metastatic Malignancy from Mesothelioma, Mullerian	WT1
Metastatic Malignancy from Hepatocellular Carcinoma	HepPar1 Ab

Table I. Common IHC Markers Used in Diagnosis of Lung Cancer

We performed following basic IHC panel to differentiate between adenocarcinoma and squamous cell carcinoma of lung.

Positive IHC Marker	Peh Sun Loo et al ²	Argon A et al ³	Present Study
p63 (SqCC)	25	72	24
Napsin A, TTF-1, EGFR, (Adeno)	16	19	20
TTF-1, Synaptophysin, Chromogranin (Small cell)	00	00	15 (TTF 1+ve)
CK7-ve/CK20+ve (Colon Mets)	00	00	01

Table II. Comparison of IHC Markers with Other Studies

The results of this study were consistent with studies done by Peh Sun Loo et al² and Argon A et al.³

IHC Marker	SqCC	Adeno-Carcinoma	Small cell Carcinoma	Metastatic Carcinoma
p63	24	00	00	00
Napsin A	00	20	00	00
TTF-1	00	20	15	00
CK7-ve/ CK20+ve	00	00	00	01

Table III. Correlation of Types of Carcinoma with IHC Marker

Above table gives Chi square value 177 with DF of 9 and p value <0.0001.

Study	H & E Diagnosis	Total No. of Cases	IHC Diagnosis			
			SqCC	Adeno Ca	Small Cell Ca	Mets
Peh Sun Loo et al ²	SqCC	25	25	-	-	-
	Adeno Ca	16	-	16	-	-
Argon A et al ³	SqCC	72	72	-	-	-
	Adeno Ca	19	-	15	-	-
Present Study	SqCC	24	24	-	-	-
	Adeno Ca	20	-	20	-	-
	Small Cell Ca	15	-	-	15	-
	Mets from Colon	01	-	-	-	1

Table IV. Correlation of Histopathological Diagnosis and IHC Diagnosis

Above table gives Chi square value of 177.143 with DF of 9 and P value <0.0001 and contingency coefficient of 0.830.

This tumour was also negative for EGFR.

DISCUSSION

Primary lung carcinoma is the most common carcinoma in the world today. Immunohistochemistry gives confirmatory diagnosis for various subtypes of lung carcinomas which is necessary for targeted therapies. The present study was conducted on 60 cases of radiologically diagnosed lung cancer at Department of Pathology, Shri M. P. Shah Government Medical College, Jamnagar from July 2018 to December 2018.

64% of patients presented with cough, 62% with dyspnoea, 36% with haemoptysis, 26% with pleuritic chest pain and 1% case had features of metastasis as initial

presentation. Mean age group in present study was 40 to 60 years.

TTF-1 (Thyroid transcription factor-1) is a nuclear transcription factor necessary for development of thyroid and pulmonary tissue. This DNA binding protein was first identified in thymocytes (Hence its name) and later in pneumocytes.⁴ It is expressed in most cases of lung carcinoma (Especially adenocarcinoma) including small cell neuroendocrine type and has become one of the most useful markers in differential diagnosis between lung carcinoma and carcinomas of other sites in one side and mesotheliomas on the other, both in histologic sections and cytologic preparations.⁵ As usual, there is a catch: high grade neuroendocrine carcinomas of nonpulmonary origin (but not Merkel cell carcinomas), nephroblastoma, ovarian carcinomas and endometrial cancers can also stain in occasional cases.^{6,7,8}

P63 (A member of the P53 family involved in the development of epithelial tissues) is expressed in nearly all pulmonary squamous cell carcinomas but also in a significant subset of the other types, including the majority of small cell carcinomas.⁹

Napsin A (TA02) is a marker resulting in granular cytoplasmic staining. It is an aspartic proteinase (Homologous with polypeptide TA02 that was detected by gel electrophoresis of lung adenocarcinomas) involved in the maturation of surfactant protein B and expressed in type II pneumocytes, in alveolar macrophages and in epithelium of proximal and convoluted tubules of kidney. Napsin A has slightly higher sensitivity for pulmonary adenocarcinomas when compared with TTF-1. Like for the latter, Napsin A positivity decreases with increasing tumour grade.¹⁰

Although both CK7 and CK20 are intermediate filaments, they are located in different epithelial cells. CK7, a 54-kDa basic protein, is typically located in epithelia from lung and breast; CK20, a 46-kDa acidic cytokeratin protein, is common in epithelia from the intestinal tract.¹¹ CK7 is known to label several types of normal and neoplastic glandular epithelia; carcinomas from the GI tract and prostate generally are negative, whereas breast, lung, and subtypes of adenocarcinomas of the ovary generally are positive.¹² Since the early 1990s, the clinical utility of monoclonal antibodies directed against CK20 paired with anti-CK7 antibodies in the differential diagnosis of primary lung cancers and secondary lung cancers has been reported.¹³ Adenocarcinoma from the colon is usually CK20+ and CK7-, whereas adenocarcinoma from the lung is usually CK20- and CK7+.¹⁴ In current study we found a case of adenocarcinoma metastasis from colon to lung with CK7- and CK20+.

EGFR (Epidermal growth factor receptor) belongs to the erbB family of closely related receptor tyrosine kinases, which include erbB1 (Also known as EGFR), erbB2 (HER2), erbB3, and erbB4. Although their basic structures are similar, each one has distinct properties, including variation in tyrosine kinase activity. It has an extracellular ligand binding domain, a transmembrane portion, and intracellular tyrosine kinase and regulatory domains. Upon binding of a specific ligand (e.g. epidermal growth factor), the normally functioning EGFR undergoes conformational change and phosphorylation of the intracellular domain occurs, leading to downstream signal transduction by various pathways. These include the Raf1-extracellular signal-regulated kinase,

PI3K/Akt, and signal transducer and activator of transcription (STAT) factors. Depending on the pathway, the end result is cell proliferation or cell maintenance by inhibition of apoptosis.¹⁵ It is having a prognostic role in targeted therapies mainly for adenocarcinoma of lung. Though utility of EGFR overexpression detection by IHC is controversial in various studies, it is helpful in prediction of response to tyrosine kinase inhibitor therapies.

Serum tumour marker CEA (Carcinoembryonic antigen) is a glycoprotein of heterogeneous composition (MW 200000) normally detected in glycocalyx of fetal epithelial cells, mainly those of mucin secreting glandular nature.¹⁶ It is detectable only in small amounts in normal adult cells and benign tumours but is present in large quantities in carcinomas, particularly adenocarcinomas of lung and gastrointestinal tract along with medullary thyroid carcinoma. Because of the fact that it is primarily expressed by fetal tissues and malignant tumours, it is referred to as an oncofetal antigen. Monoclonal antibodies offer a greater degree of tumour specificity of tumour than conventional antisera.¹⁷

CONCLUSION

Squamous cell carcinoma is more common than other subtypes of lung cancer with male predominance and positive p63 and is strongly associated with smoking. Due to paucity of material in lung biopsy, immunohistochemical techniques play critical role as diagnostic and screening tool for lung cancer in current era of medicine along with CEA serum tumour marker. Histopathology, serum tumour marker and immunohistochemistry correlation will lead to perfect diagnosis for any lung cancer, serving as diagnostic and prognostic tools for better outcome of patients. In current WHO classification, immunohistochemical analysis is indispensable to the determination of lung cancer subtypes.

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