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CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF PLEURAL NEOPLASMS

Respiratory Medicine

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ABSTRACT

Background: Broadly, pleural neoplasms can be classified into primary and metastatic. Biopsy followed by immunohistochemical analysis of pleural tissue plays a pivotal role in identification and classification of carcinomas of unknown primary siteWe enrolled 40 study subjects admitted at IRD, SMS Medical College, Jaipur who are suitable candidates of pleural biopsy. These patients underwent image guided closed needle pleural biopsy followed by histopathology and immunohistochemistry. Patients who could not be diagnosed via closed needle pleural biopsy underwent either medical thoracoscopy or CT guided biopsy. The Clinicopathological, histomorphological findings and immunohistochemical profile of the study subjects was determined. **Results:** Mean age of the patients in the present study was 58.1+-12.4 years(range 25-76) with male predominance of 62.5%(n=25).Shortness of breath and chest pain were found to be the most common symptom. Pleural fluid cytology was positive in 52.50% (n=21). Image guided closed pleural biopsy using Abrams needle was conclusive in 80% (n=32). In 35% of patients, histopathological examination was metastatic adeno carcinoma or malignant mesothelioma followed by metastatic carcinoma (32.5%).In our study 87.5% (n=35) of the pleural neoplasms were metastatic in origin amongst which metastatic adenocarcinoma (lung origin) was found to be the most common (77.14%). Primary pleural neoplasms were found in 12.5% of cases (n=5), amongst which diffuse pleural mesothelioma was seen in 3 cases, one case of synovial sarcoma and Ewing's sarcoma were also diagnosed. **Conclusion:** Image-guided closed pleural biopsy is a readily accessible, minimally invasive, rapid, and low-cost method for patients with pleural neoplasms. This can reduce the need for VATS and pleuroscopy in resource-limited settings. Among various armamentarium collection of tools, immunohistochemical marker analysis not only helps in establishing a confirmed diagnosis but also helps in determining the origin of the tumor, its prognosis and guide the clinician to manage accordingly.

KEYWORDS

pleural neoplasms, image guided closed needle pleural biopsy, histopathology and immunohistochemistry, Abrams needle

INTRODUCTION:

Pleural neoplasms are found to be one of the significant causes of morbidity and mortality in cancer patients**.** Pleural neoplasms can be classified into primary and metastatic, the incidence of which is 42.9% and 57.1% respectively. Synovial sarcoma (SS), diffuse pleural mesothelioma (DPM), Ewing's sarcoma (EW), neuroendocrine carcinoma, solitary fibrous tumor (SFT) and inflammatory myofibroblastic tumor are few of the primary pleural neoplasms, out of which DPM is the most common type.[1] The Global Cancer Observatory (Globocan) estimated 30, 870 new cases of DPM and 26,278 deaths in 2020 worldwide, for both sexes and all ages.[2] Various works of literature show that metastatic pleural neoplasm greatly outnumbers primary neoplasm of the pleura. The origin of the metastatic pleural neoplasm is lung cancer, breast cancer, lymphoma, cancer of the female genital tract, and gastrointestinal tract in the descending order of frequency.[1]

Owing to the wide range of clinical and radiological manifestations, pleural neoplasm remains a diagnostic challenge to treating physicians even after extensive workup. Thoracoscopy is usually considered the investigation of choice in pleural effusions where a diagnostic pleural aspiration is inconclusive. The diagnostic yield of closed pleural biopsy via USG and CT guided is estimated to be 80% and 88% respectively particularly if pleural nodularity or thickening is present. The diagnostic yield of thoracoscopy, either medical (pleuroscopy) or surgical (video-assisted Thoracoscopic surgery, VATS), is shown to be 91-95% for pleural malignancy and up to 100% for pleural tuberculosis (TB) considering to be gold standard in this setting.[3,4,5]

Pleural biopsy followed by histopathological and immunohistochemical analysis of the tissue can clinch to the final diagnosis. Hence this study aimed to determine the clinicopathological and immunohistochemical profile in patients of pleural neoplasms undergoing pleural biopsy (either image guided or via thoracoscopy).

MATERIAL & METHODS:

This was a hospital based observational study carried out at Department of Respiratory Medicine, Institute of Respiratory Diseases, SMS Medical College, Jaipur, over a period of one year (2020-2021).This study was initiated after seeking permission from Research Review Board and enrolled 40 patients who had exudative pleural effusion other than those with granulomatous inflammation, possible tuberculosis, and nonspecific inflammation on histomorphological examination. In addition patients with pleural-based mass lesion on radiology and who had given written informed consent were also included in this study.

All selected patients were subjected to clinical history, physical examination and routine investigations. Radiological investigations were performed. Malignant cell cytology was done for all pleural effusions. The patients with pleural effusion first underwent image guided closed pleural biopsy via Abrams needle and the samples were sent for histopathology. Then FFPE (formalin fixed paraffin embedded) sections was stained by manual Immunohistochemical procedure using pressure cooker and HRP polymerase system and Immunohistochemical analysis was performed and thereby differentiating primary and secondary pleural neoplasm. The panel of markers used in the current study included thyroid transcription factor 1(TTF-1), P-40, wilms tumor protein 1 (WT-1), calretinin, cytokeratin 7 (CK-7, K7), p-63, napsin A, synaptophysin, vimentin, pancytokeratin, transducin like enhancer of split 1(TLE-1), hector battifora mesothelial 1 (HBME), GATA-3, cluster of differentiation (CD-99), NKX2.2 ,SMA/STAT6/CD-34.

Suspected malignant cases with negative closed pleural biopsy underwent thoracoscopic biopsy. Collected data was entered into Microsoft excel data sheet which was tabulated for data interpretation. This was then subjected to appropriate statistical tests. Results were obtained to determine the histopathological and immunohistochemical profile in differentiating primary and secondary pleural neoplasms.

RESULTS:

The mean age of patients was 58.1 ± 12.4 years and amongst 40 study subjects, 25 were males and 15 were females. Amongst 40 study subjects 25 were found to be smokers and 15 were non-smokers. Thirty-five (87.50%) patients presented with shortness of breath and chest pain as major complaints. Other presenting complaints of the study subjects were cough (82.50%), loss of appetite (80.00%), loss of weight (75.00%), fever (27.50%), blood in sputum (22.50%), and hoarseness of voice.(Table no. 1)

Image-guided closed pleural biopsy using Abrams needle was conclusive in 32 patients accounting for 80% yield in diagnosis. Out of 8 patients with inconclusive findings, thoracoscopic pleural biopsy was performed and was conclusive in 7 patients. One case was diagnosed through computed tomography (CT) guided biopsy. Hence distribution of final diagnosis of study subjects through pleural biopsy was primary neoplasm in 5(12.5%) patients and metastatic in rest 35(87.5%). (Table No. 2)

In 35% of patients, histopathological examination was metastatic adeno carcinoma or malignant mesothelioma followed by metastatic carcinoma (32.5%), metastatic adeno carcinoma (17.50%), poorly differentiated Malignant Neoplasm (7.50%). Histopathological examination was low grade spindle cell neoplasm, malignant round cell tumor and solitary fibrous tumor in only 1 out of 40 patients (2.50%) each. (Table No. 3)

Primary pleural neoplasms were diagnosed in 5 study subjects (12.5%) of the total malignant pleural tumors. Diffuse pleural mesothelioma was diagnosed in 3 cases with the aid of IHC markers like CK 7, HBME, calretinin, Vimentin and WT 1 (1 case through closed needle 1 via thoracoscopy and 1 through CT guided biopsy). One case of suspected low-grade spindle cell neoplasm on histopathology following thoracoscopic pleural biopsy was confirmed to be diagnosed as synovial sarcoma with the application of TLE 1, vimentin. In our study, one case of primary Ewing's sarcoma of pleura could be diagnosed with the aid of IHC analysis (vimentin, CD 99, and NKX 2.2) through thoracoscopic pleural biopsy. (Table no. 4)

Amongst metastatic pleural tumors, adenocarcinoma of lung origin was established as the final diagnosis in 77.14 %(n=27) of study subjects with the help of IHC markers TTF 1, CK 7, Napsin A (25 cases through closed needle and 2 via thoracoscopy). Among 3 cases of metastatic malignant pleural tumors, GATA 3 helped to identify the breast as the site of origin (1 case through closed needle and 2 via thoracoscopy). The diagnosis of squamous cell and small cell carcinoma of lung origin could be established in 2 patients each with help of p40, p63, and synaptophysin, TTF 1 respectively. One case of sarcomatoid carcinoma (carcinosarcoma) of lung origin could be identified solely with the help of vimentin, pancytokeratin, and p 63 marker study. (Table no. 5)

DISCUSSION:

The mean age (years) of the male and female patients in the present study was 58.1 ± 12.4 years (range 25-76 yrs). **Guinee DG et al.**[6] reported synovial sarcoma as a rare aggressive sarcoma occurring commonly in the age group between ages 20 and 40 years and a similar age group was found in one diagnosed case of synovial sarcoma in our study.

In this study 65% of study subjects were male and 35% were females. A similar male predominance of 76.7 % was reported by **Farag et al.**[7] in their study. This may be due to the predominance of smoking habits, occupational exposure in males and tendency of males to seek medical attention more frequently than females in the Indian scenario.

The majority of study subjects in this study were found to be smokers. Smoking leads to chronic inflammation which in turn triggers the process of oncogenesis. Majority of the study subjects [35(87.50%)] presented with shortness of breath and chest pain as major concern. Dyspnoea is usually found to be the most common symptom of malignant pleural effusion, due to the reduced compliance of the chest wall and diaphragm leading to reduced lung volume. As patients with malignant pleural effusion have advanced-stage disease, it is not uncommon to have patients present with other systemic symptoms like fatigue, anorexia, and weight loss.[8]

In our study subjects with effusion, malignant cell cytology was positive in 52.50%. Overall sensitivity of cytological analysis varies from 16 to 60%. Amongst various malignancies, cytology has the highest diagnostic yield for adenocarcinoma in comparison to mesothelioma where the sensitivity can be as low as 16 %.[9,10] Tumor type, tumor load, sample quality, the expertise of the cytologist, and availability of specific ancillary tests are few of the factors determining the diagnostic yield of pleural fluid cytology.

In 80.00% of study subjects, image guided closed pleural biopsy using Abrams needle was conclusive, similar to the observation made by **Chandrakantham UM et al.**[11] Current data suggests that CT assisted and ultrasound assisted closed pleural biopsy when combined with image assisted thoracocentesis can have high diagnostic yields ranging from 85 to 87% for all diagnosis.[12] Thus, in developing countries like India, image guided closed pleural biopsy not only gives definitive diagnosis but is considered to be economical, quick and a technique to be imparted by younger physicians with bare minimal learning curve. **Venkatachala et al.**[1] observed metastatic pleural neoplasms (57.1%) to be more common as compared to primary pleural neoplasms (42.9%). In our study, out of 40 study subjects, 35(87.5%) were metastatic pleural neoplasms and only 5(12.5%) were identified as primary pleural neoplasms. The histopathological features suggested the possibility of various neoplasms and could not ascertain the final diagnosis.

In 35% of patients, histopathological examination was suggestive of metastatic adeno carcinoma or malignant mesothelioma followed by metastatic carcinoma (32.5%). Similarly in a study by **Farag et al.**[7] amongst 30 cases, histopathological examinations for malignant pleural tumors (MPT) revealed that metastatic adenocarcinoma was suspected in 50% of cases followed by malignant undifferentiated carcinoma in 33.3% and mesothelioma in 16.7%. Hence the application of a more confirmatory test like immunohistochemical analysis becomes mandatory for the final diagnosis and further management of the patients.

In an era of precision and evidence based medicine, immunohistochemistry plays a critical role in the classification of tumors into subtypes and for assessing biomarkers for timely and accurate therapeutic decision making. In our study amongst metastatic pleural tumors, adenocarcinoma of lung origin was diagnosed in majority of study subjects with the help of IHC markers {TTF 1, CK 7, napsin A} similar to the study done by **Venkatachala et al.**[1]

In three cases of metastatic malignant pleural tumors, GATA 3 helped identify the breast as the site of origin. For the detection of metastatic breast carcinomas in effusion specimens GATA3 is considered to be highly sensitive marker, though this marker is not 100% specific for malignancies of breast origin. Hence GATA3 should be not be used solely but used with additional immunohistochemical markers and with clinical correlation during the cytologic evaluation of malignant effusions.[13] The diagnosis of squamous cell and small cell carcinoma of lung origin could be established in two cases each with help of p40, p63, and synaptophysin, TTF 1 respectively.

One rare case of sarcomatoid carcinoma (carcinosarcoma) of lung origin could be identified solely with the help of marker study. The initial histopathology could only establish diagnosis of poorly differentiated malignant neoplasm. **Terra S.B. et al.**[14] reported that distinguishing pulmonary sarcomatoid carcinoma from pleural sarcomatoid mesothelioma is challenging due to overlapping histology, clinical features and immunophenotyping. IHC markers mainly vimentin pancytokeratin and p 63 was helpful in confirming the diagnosis as observed by **Terada T.et al.**[15]

Primary pleural neoplasms considered to be a rare entity were diagnosed in 5 cases. Amongst these, diffuse pleural mesothelioma was diagnosed in 3 cases which is usually a difficult challenge faced by the pathologist to establish a diagnosis only by HPE. The aid of IHC markers like CK 7, HBME, calretinin, Vimentin and WT-1 played a crucial role in differentiating DPM from adenocarcinoma.

Though immunohistochemical marker is crucial in establishing the primary, no IHC marker is completely specific for each type of tumor. Hence the International Mesothelioma Interest Group recommends at least two mesothelial and two carcinoma markers to be analyzed in addition to cytokeratin. Mesothelial markers include calretinin (nuclear and cytoplasmic staining), podoplanin (clone D2-40; membranous staining), CK5 or CK5/6 (cytoplasmic staining), and WT1 (nuclear staining).[16] For differential diagnosis between MM and lung adenocarcinoma, TTF-1, Napsin A, CEA, claudin 4 (CLDN4), Ber-EP4, and MOC31 are useful markers suggesting lung adenocarcinoma.[17]

One case of synovial sarcoma was established as diagnosis with application of TLE 1, vimentin. Sarcomatoid MM, SFT with its malignant variant, malignant peripheral nerve sheath tumor (MPNST), and metastatic spindle cell melanoma are a few of the diagnostic mimics of

Synovial sarcoma. Immunoreactivity to CD 34 clinches the diagnosis of SFT. In our case SMA, STAT 6, and CD 34 were non-reactive, ruling out the possibility of malignant SFT. Transducer-like enhancer of split (TLE1) helps distinguish SS from its histologic mimics only when strong nuclear staining is observed. This was reported by **Farag et al.**[7] and a similar observation was found in our study.

Ewing's sarcoma is a rare neoplasm overall, with less than 1% occurrence amongst soft tissue sarcoma.[18] In our study, one case of primary Ewing's sarcoma of pleura could be diagnosed with the aid of IHC analysis (vimentin, CD 99, NKX 2.2). The initial histopathological examination revealed diffuse infiltration of small round cells with a high nuclear-cytoplasmic ratio on light microscopy favoring to be malignant round cell tumor similar to study conducted by **Venkatachala et al.**[1]

Patients with undiagnosed exudative pleural effusion following thoracocentesis may be stratified based on initial imaging using ultrasound and computed tomography as having diffuse pleural thickening (>10 mm) and nodularity, insignificant pleural thickening or radiologically normal pleura or an associated mass lesion.[19] Closed pleural biopsy using Abrams needle could be utilized in patients with malignant pleural effusion having pleural nodularity or thickening under imaging guidance to improve the yield and safety.

The histopathological examination can provide valuable diagnostic information in many cases with MPT but in challenging cases pathologic differentiation and types could not be established due to various overlapping histopathological features, demanding the utilization of IHC. Thus correlation of histological patterns along with a panel of suitable IHC markers with the background of clinical and radiological presentation helps in clinching the final diagnosis in patients with pleural neoplasm.

CONCLUSION:

Image-guided closed pleural biopsy is a readily accessible, minimally invasive, rapid, and low-cost method for patients with pleural neoplasms. This can reduce the need for VATS and pleuroscopy in resource-limited settings. Among various armamentarium collection of tools, immuno histochemical marker analysis not only helps in establishing a confirmed diagnosis but also helps in determining the origin of the tumor, its prognosis and guide the clinician to manage accordingly.

Table 1:- Demographic And Clinical Characteristics Of Study Subjects.

Age(years)	Frequency(n=40)	Percentage
Mean ± SD	58.1 ± 12.4	
Gender		
Female	15	37.50%
Male	25	62.50%
Smoking History		
Non Smoker	15	37.50%
Smoker	25	62.50%
Presenting complaints		
Shortness of breath	35	87.50%
Chest pain	35	87.50%
Cough	33	82.50%
Blood in sputum	9	22.50%
Wheeze	6	15.00%
Fever	11	27.50%
Hoarsness of voice	9	22.50%
Loss of appetite	32	80.00%
Loss of weight	30	75.00%

Table 2:- Distribution Of Final Diagnosis And Yield Of Closed Pleural Biopsy

Image guided Abrams Closed pleural biopsy	Frequency(n=40)	Percentage (%)
Inconclusive	8	20.00%
Conclusive	32	80.00%
Final Diagnosis		
Primary Pleural neoplasms	5	12.5%
Metastatic Pleural neoplasms	35	87.5%

Table 3:- Distribution Of Histopathological Examination Of Study Subjects.

Histopathological examination	Frequency (n=40)	Percentage (%)
Low grade Spindle Cell Neoplasm	1	2.5%
Malignant round cell tumor	1	2.5%
Solitary Fibrous Tumor	1	2.5%
Metastatic Adeno Carcinoma	7	17.5%
Metastatic Adeno Carcinoma/ Malignant Mesothelioma	14	35%
Metastatic Carcinoma	13	32.5%
Poorly differentiated Malignant Neoplasm	3	7.5%

Table 4:- Distribution Of Primary Pleural Neoplasms Among Study Subjects

Primary pleural neoplasms	Frequency	Percentage
Malignant mesothelioma{CK7, HBME/Calretinin}	3	60%
Synovial sarcoma{TLE 1, Vimentin}	1	20%
Ewing's sarcoma{Vimentin, CD 99, NKX 22}	1	20%
Total	5	100%

Table 5:- Distribution Of Metastatic Pleural Neoplasms Among Study Subjects

Metastatic pleural neoplasms	Frequency	Percentage
Metastatic Adeno carcinoma, Lung origin {TTF 1, CK 7, Napsin A }	27	77.14%
Metastatic carcinoma, Breast origin {GATA 3}	3	8.57%
Metastatic Squamous cell carcinoma, Lung origin{p40, p63}	2	5.72%
Sarcomatoidcarcinoma (Carcinosarcoma),Lung origin {Vimentin , Pancytokeratin, p 63 }	1	2.85%
Metastatic Small Cell carcinoma, Lung origin {Synaptophysin, TTF 1}	2	5.72%
Total	35	100%

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