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Research Article

Cyto-Histological Correlation of Lung Masses with Special Reference to the Immunohistochemistry- A Hospital Based Prospective Study

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Abstract: Introduction- Primary lung epithelial malignancies are the most common neoplasms among all lung masses. Lung carcinoma is the leading cause of death for which a histological or cytological confirmation and categorization of malignancy is required before treatment. It is desirable to have both FNAC or TBNA and biopsy for exact diagnosis. Sometimes, even immunohistochemistry is required to confirm the diagnosis. **Materials and method-** Total 110 patients from the chest medicine department, having lung mass in CT scan findings, going for CT guided FNAC or trans bronchial needle aspiration and true cut biopsy or bronchial biopsy in the pathology department, are included in this prospective study. IHCs are done in some cases for exact categorization of the tumour. SPSS software is used for statistical analysis. **Result-** Out of 110 patients, 80 patients (72.73%) are male and 30 (27.27%) are female and most of them (37.27%) are in 7th decade. Among these, 42 patients (38.18%) are diagnosed to have primary lung adenocarcinoma and 38 patients (34.55%) have primary squamous cell carcinoma of lung and 13 patients (11.82%) have secondary metastatic deposit in lung from different primary malignancies. Sensitivity and specificity of cytology to diagnose primary lung mass are 76.92% and 77.78% respectively and sensitivity of cytology for secondary lung mass is 92.31%. Sensitivity of histopathology for diagnosing primary lung tumour is 92.78%. Cohen's Kappa shows Kappa is 0.741 i.e. there is a good strength of agreement between the procedures. The P value < 0.0005 indicates that the Kappa co-efficient is statistically significantly different from zero. **Conclusion-** Most of the lung masses are diagnosed properly by cytological and histopathological examination. But some cases, immunohistochemistry is required to confirm the diagnosis because diagnosis of non-small cell carcinoma or poorly differentiated carcinoma is not helpful for specific therapy.

Keywords: Lung mass, Correlation, Cytology, Biopsy, Immunohistochemistry

INTRODUCTION

Lung cancer is the leading cause of cancer-related death all over the world, with an average 5-year survival rate of 16.8% despite advancement of chemo radiation (Epidemiology and End Results(SEER). Haaga and Alfidi (Haaga, J. R., & Alfidi, R. J. 1976) reported computed tomography (CT)-guided biopsy in the 1970's, but Menetrier (Menetrier, P. 1886) probably performed the first cutting needle biopsy for histological diagnosis. The diagnostic accuracy has been reported as being >80% for benign disease and >90% for malignant disease (Tsukada, H. *et al*., 2000). Cytology is faster and more cost-effective and yields almost as good a result when compared with more invasive biopsy and immunohistochemistry. In the era of personalized medicine, small biopsies or cytological material obtained from fine-needle aspiration cytology (FNAC) may be the only available specimen for diagnosis of lung cancer, sub typing of non-small cell carcinoma (NSCC) into adenocarcinoma and squamous cell carcinoma, and for further mutation testing (Ramalingam, S., & Belani, C. 2008; Travis, W. D. *et al*., 2011) But sometimes, it is very difficult to diagnose by cytology only, then biopsy for histopathology examination is needed and a few cases in which diagnosis is not confirmed by histopathology findings, then only immunohistochemistry is required for confirmation.

Lung carcinoma is one of the most frequent cancer in human beings representing 14% and 13% with mortality rates of 28% and 26% in males and female respectively (Kumar, V. *et al*., 2014). Small biopsies and FNAC are the first step diagnostic modalities to diagnose lung cancers and cytologic smears form very important tools to diagnose lung cancers (Horn, L. *et al*., 2015).

Lung epithelial tumours are classified into squamous cell carcinoma, adenocarcinoma, small cell carcinoma and large cell carcinoma (Rossi, G., &Cavazza, A. 2015). However, it applies to resected

specimens. Previously based on FNAC, the lung carcinomas were classified into Small cell and Non small cell lung carcinomas (NSCLC) due to different management protocols (Horn, L. *et al*., 2015). No further categorization of NSCLC was needed which i.e. squamous cell carcinoma, adenocarcinoma and large cell carcinoma as all NSCLCs were treated similarly. Recently novel molecular abnormalities involving mutations in Epidermal growth factor receptor gene (EGFR) and translocations involving anaplastic lymphoma kinase (ALK) gene have been identified in adenocarcinoma and specific targeted therapy is available against them (Igbokwe, A., & Lopez-Terrada, D.H. 2011; Dacic, S. 2011). In addition, certain therapies are hazardous in patients with squamous cell carcinomas (Hasanovic, A. *et al*., 2008). Hence accurate sub typing of NSCLC into adenocarcinoma and squamous cell carcinoma is very essential so that patients can get benefit of specific targeted therapy and appropriate molecular testing may also be done. The distinction between adenocarcinoma and squamous cell carcinoma is now viewed as critical to optimal therapeutic decision making (Horn, L. *et al*., 2015).

New guidelines have been published by the International association for the study of lung cancer (IASLC) for reporting of NSCLCs in small biopsies and cytological smears (Travis, W. D. *et al*., 2011; Travis, W. D. *et al*., 2013). According to these guidelines, NSCLC should be sub typed into adenocarcinoma and squamous cell carcinoma on morphological features and limited panel of immunehistochemical and mucin stains should be used in doubtful cases. Tissue should also be preserved for molecular studies (Neeraj, D. *et al*.,).

AIMS AND OBJECTIVES

1) To evaluate the efficacy of cytology in comparison with biopsy for diagnosis of lung mass.
2) To assess the role of immunohistochemistry in diagnosis of lung mass.

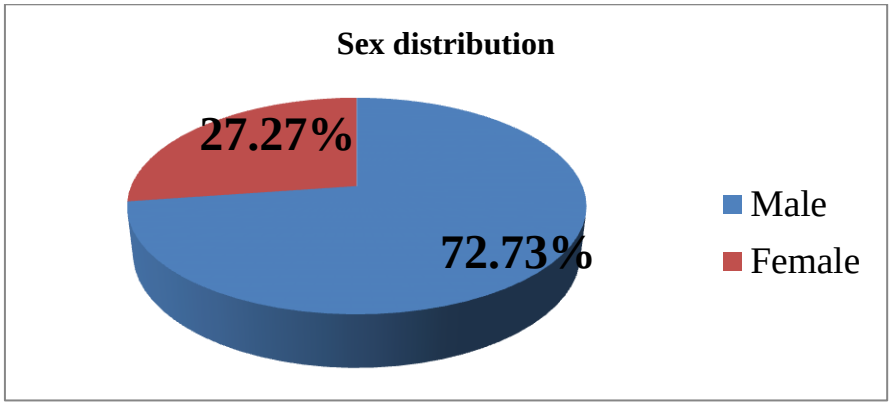
MATERIALS AND METHODS

It is a prospective study from November'18 to September'19 in the Department of Pathology, Department of Radiology, Department of Chest Medicine at Calcutta National Medical College and Hospital, Kolkata. Those patients, having lung mass, are referred to our department for CT guided fine needle aspiration cytology and true cut biopsy or have trans bronchial needle aspiration and biopsy from the department of Chest Medicine are included. IHCs are done if diagnosis is not also confirmed by biopsy examination. Cytological examination is done after staining of the slides with Leishman Giemsa stain, Pap stain and Haematoxylin and Eosin stain. Histopathological examination is done after staining of the slide with routine Haematoxylin and Eosin stain. Metastatic deposits in lung are mainly diagnosed by proper history, clinical examination and previous biopsy reports and few metastatic deposits cases are diagnosed finally by immunohistochemistry. Immunohistochemistry is also used for confirmed diagnosis of small cell carcinoma; histology diagnosed non- small cell carcinoma and poorly differentiated carcinoma. Immunohistochemistry used for epithelial tumours are CK-5/6, TTF-1, small cell carcinoma is chromogranin and malignant spindle cell tumour is S-100, CK, EMA, TLE-1, CD 34, desmin, ki-67. SPSS software is used for statistical analysis.

RESULT ANALYSIS

Table-1. Sex distribution of patients with lung mass

Sex	No. of patient	% of patient (n=110)
Male	80	**72.73**
Female	30	27.27

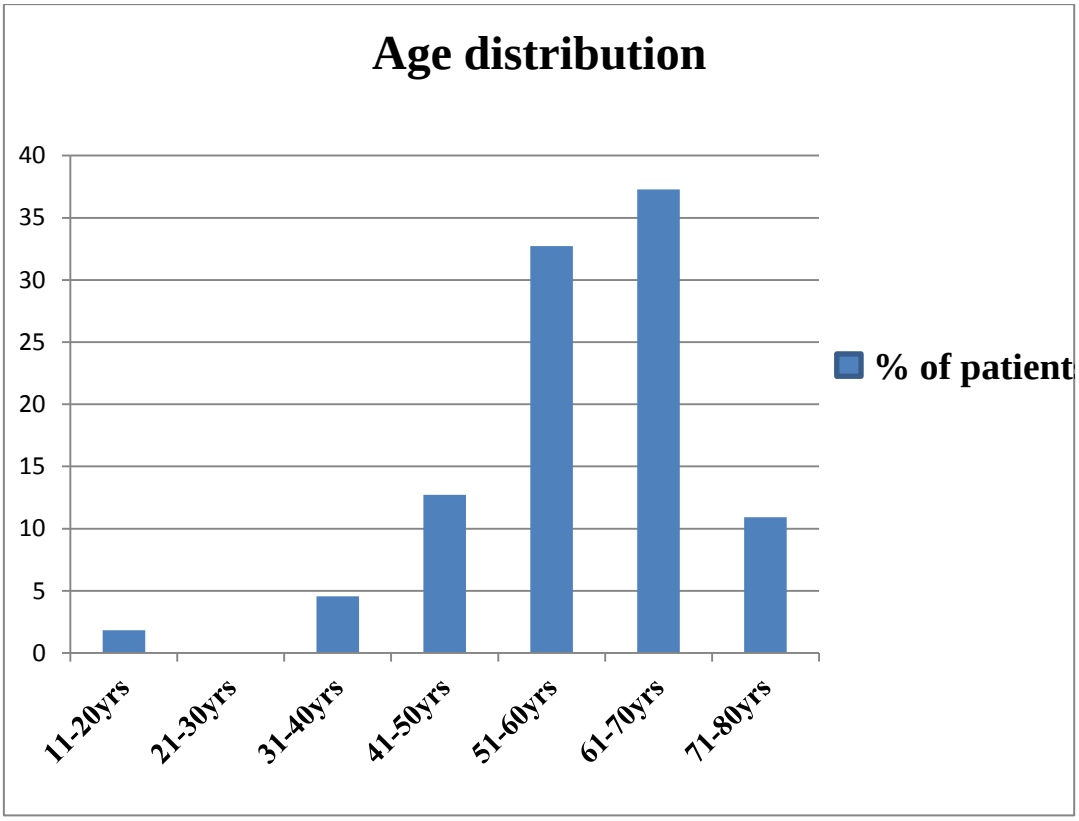


Graph-1: Sex distribution of patients with lung mass

Inference- Lung masses are more common in male patients (72.73%)

Table-2. Age distribution of patients with lung mass

Age (in years)	No. of patients	% of patients (n=110)
11-20	2	1.82
21-30	-	-
31-40	5	4.55
41-50	14	12.73
51-60	36	32.73
61-70	41	**37.27**
71-80	12	10.91

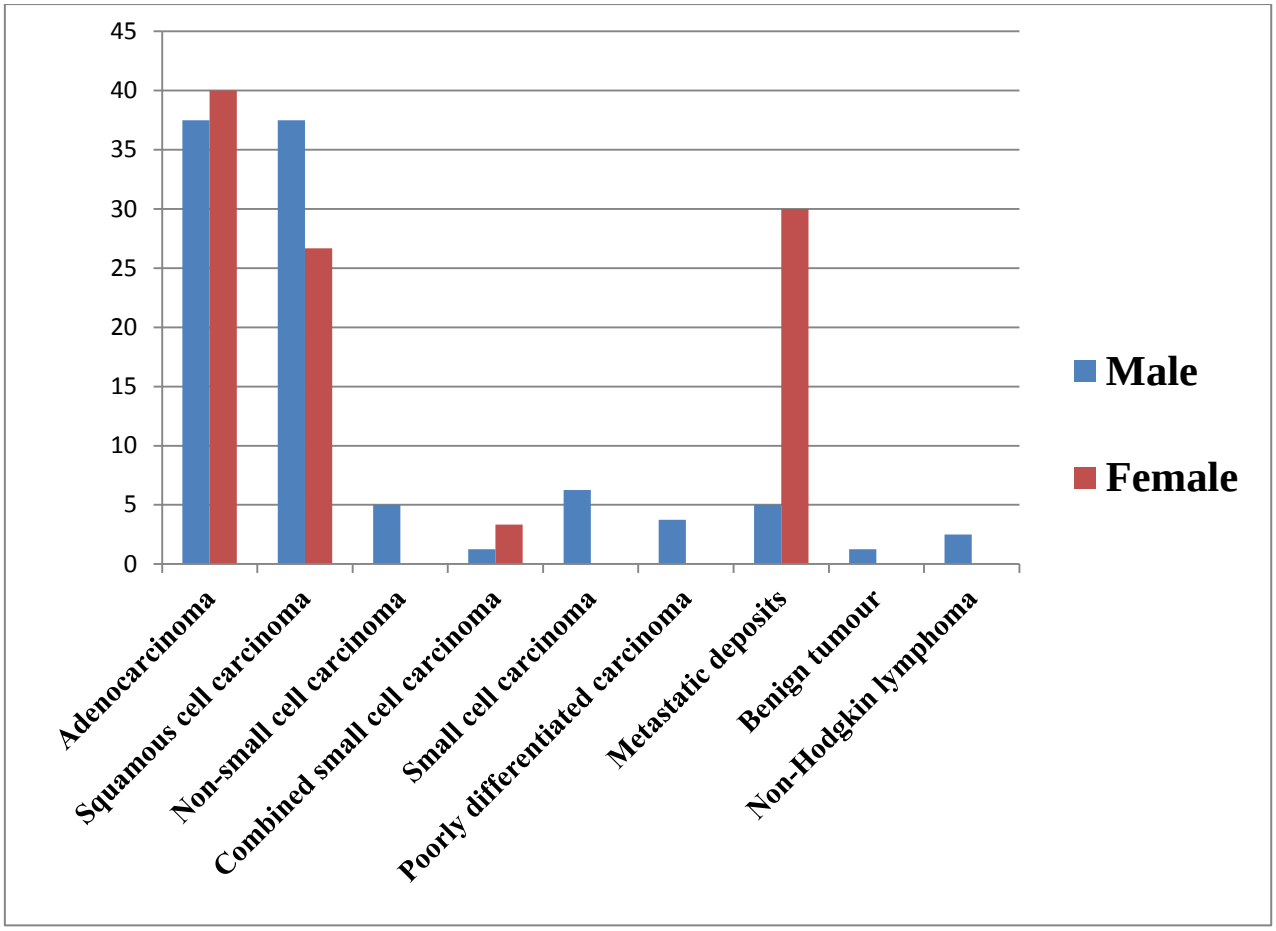


Graph-2: Age distribution of patients with lung mass

Inference- Majority of patients are in 61-70 years, followed by, 51-60 years. Lung masses are less commonly found in both extreme age groups.

Table-3. Distribution of sex in different histopathological findings of lung masses

Histopathological findings	Sex			
	Male (n=80)		Female (n=30)	
	No. of patient	% of patient	No. of patient	% of patient
Adenocarcinoma	30	**37.5**	12	**40.0**
Squamous cell carcinoma	30	**37.5**	8	26.67
Non-small cell carcinoma	4	5.0	-	-
Combined small cell carcinoma	1	1.25	1	3.33
Small cell carcinoma	5	6.25	-	-
Poorly differentiated carcinoma	3	3.75	-	-
Metastatic deposits	4	5.0	9	30.0
Benign tumour	1	1.25	-	-
Non-Hodgkin lymphoma	2	2.50	-	-

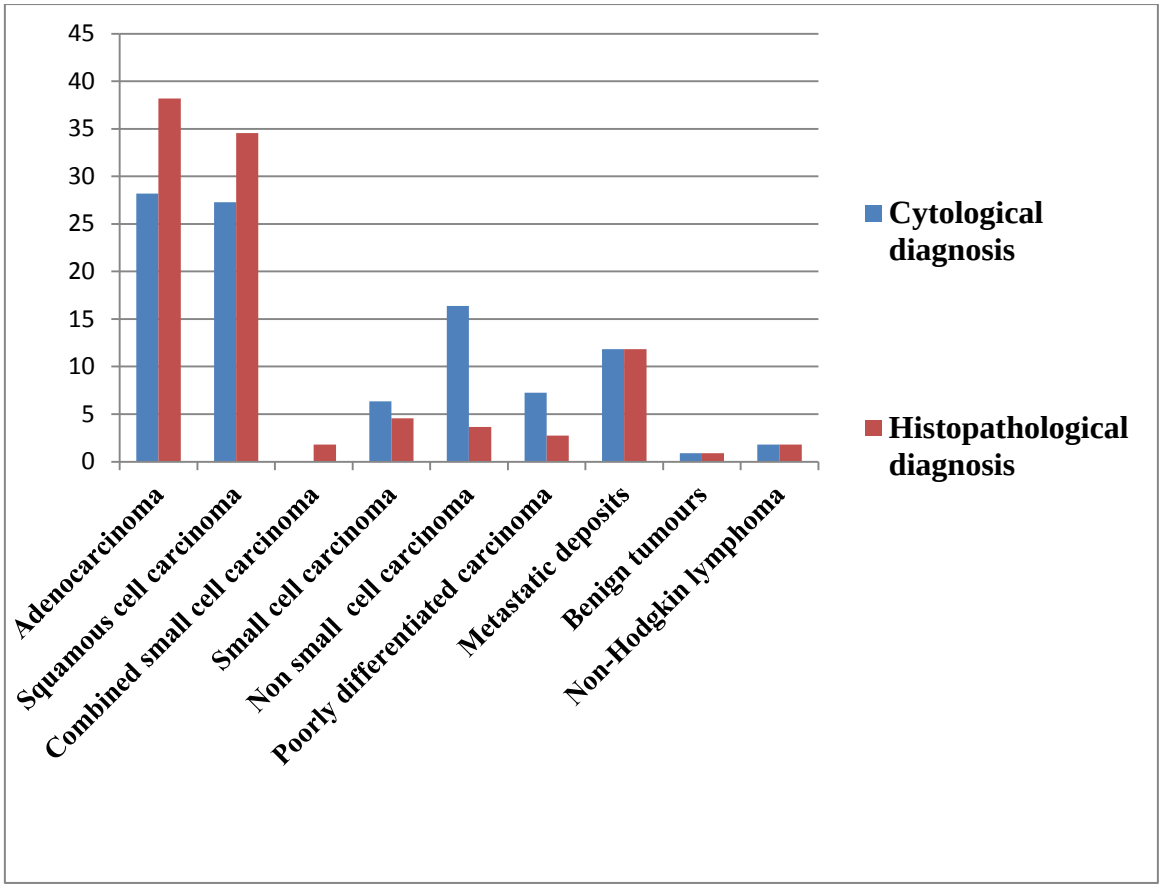


Graph-3. Distribution of sex in different histopathological findings of lung masses.

Inference- Adenocarcinoma is most common histopathological findings among the all lung masses.
40% female patients with lung mass have adenocarcinoma.
Adenocarcinoma of lung is most common in female patients.
In this study males are equally affected by squamous cell carcinoma and adenocarcinoma.
Metastatic deposits are more commonly found in female patients.

Table-4. Distribution of diagnosis of different types of lung mass in cytological and histopathological examination

Types of lung mass	**Cytological diagnosis**		**Histopathological diagnosis**	
	No. of patient	**% of patient (n=110)**	**No. of patient**	**% of patient (n=110)**
Adenocarcinoma	31	28.18	42	**38.18**
Squamous cell carcinoma	30	27.27	38	**34.55**
Combined small cell carcinoma	-	-	2	**1.82**
Small cell carcinoma	7	6.36	5	4.55
Non small cell carcinoma	18	16.36	4	3.64
Poorly differentiated carcinoma	8	7.27	3	2.73
Metastatic deposits	13	**11.82**	13	**11.82**
Benign tumours	1	0.91	1	0.91
Non-Hodgkin lymphoma	2	1.82	2	1.82



Graph-4. Distribution of diagnosis of different types of lung mass in cytological and histopathological examination

Inference- Most of the adenocarcinoma and squamous cell carcinomas are diagnosed separately in histopathological examination.
Combined small cell carcinomas may be missed in cytological examination, but can be diagnosed in histopathological examination.
Sometimes, small cell carcinomas are diagnosed falsely in cytological examination. It should be confirmed by histopathological examination.
Most of the non small cell carcinomas and poorly differentiated carcinoma, diagnosed by cytological examination, can be categorized by histopathological examination. If not possible, then immunohistochemistry is required for these cases. Metastatic deposits can be diagnosed in cytological examination, but for categorization histopathological examination and immunohistochemistry is required.

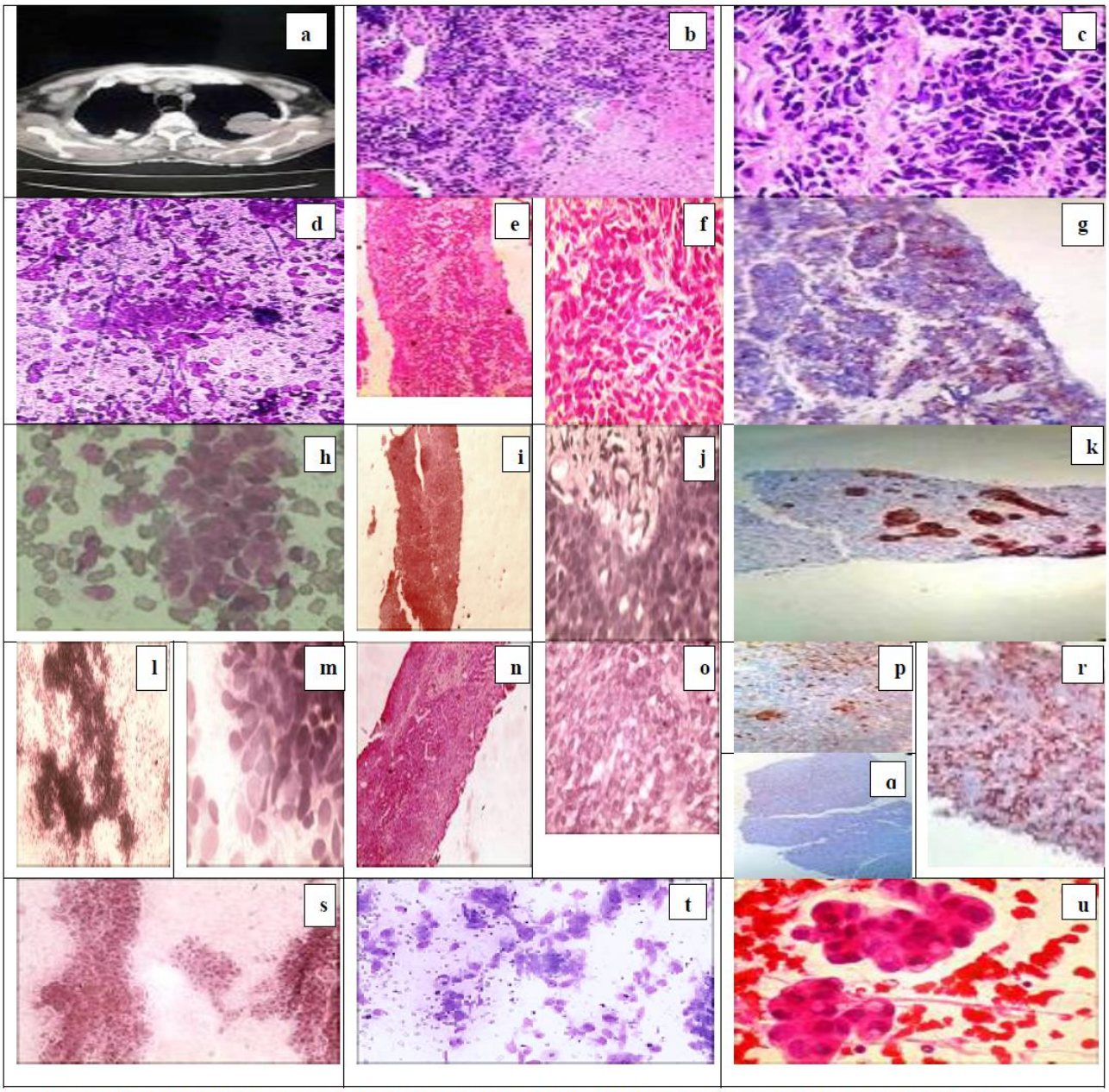
Intra class correlation coefficient

	Intra class correlation	95% confidence interval		F test with true value 0			
Average measures	0.877	**Lower bound**	**Upper bound**	**Value**	**df1**	**df2**	**Sig.**
		0.821	0.916	8.127	109	109	0.000

The inter rater reliability between the two procedures by intra class correlation coefficient is 0.8777 which is quiet good. The P value is < 0.0005 which supports the fact that there is a statistically significant inter rater reliability.

Cohen's Kappa-

Kappa- 0.741
P value < 0.0005
There is a good strength of agreement between the two procedures. The P value < 0.0005 indicates that the Kappa co-efficient is significantly different from zero.
The sensitivity and specificity of cytology to diagnose primary lung mass is 76.92% and 77.78% respectively. The sensitivity of cytology for secondary lung mass is 92.31%. The sensitivity of histopathology for diagnosing primary lung tumour is 92.78%.



Photomicrograph- (a) CT scan- true cut needle in lung mass, (b) low & (c) high power- combined small cell carcinoma, (d) low power FNAC, (e) scanner & (f) high power- biopsy of small cell carcinoma, (g)chromogranin A (+) small cell carcinoma, (h) high power- poorly differentiated carcinoma (L-G stain, FNAC), (i) scanner & (j) high power- biopsy of sarcomatoid squamous cell carcinoma, (k) CK 5/6 positive- squamous cell carcinoma, (l) scanner & (m) high power malignant spindle cell tumour (FNAC, H& E stain), (n) scanner & (o) high power- biopsy of malignant spindle cell tumour, (p) S100 (+), (q) TLE-1 (+) & (r) EMA (+) pleomorphic sarcoma, (s) low power – deposit from renal clear cell carcinoma (H&E stain, FNAC), (t) low power- deposit from malignant phylloides (L-G stain, FNAC), (u) high power- adenocarcinoma (H & E stain, FNAC)

DISCUSSION

Lung cancer is the most common cancer diagnosed worldwide (World Health Organization. 2008). Before 2004 WHO classification, there was no importance of distinguishing histological subtypes such as adenocarcinoma and squamous cell carcinoma. Therefore, tumours other than small cell carcinoma were simply called together as non-small cell carcinomas (NSCCs), without more specific histological sub typing. However, major therapeutic advances have since taken place in the lung cancer field, with profound implications for pathological diagnosis and molecular testing (Travis, W. D. *et al*.,2013; Mitsudomi, T. *et al*., 2010; Mok, T. S. et a., 2009; Rosell, R. *et al*., 2012). Patients treated with pemetrexed, with adenocarcinoma or NSCC, not otherwise specified fared better than those with squamous cell carcinoma (Scagliotti, G. V. *et al*. 2008), and patients with squamous cell carcinoma have a higher risk of life-threatening haemorrhage if treated with bevacizumab) (Johnson, D. H. *et al*., 2004).

In 2012, Noronha V *et al* have showed that out of 489 patients, there were 380 (77.7%) males and 109 (23.3%) female, i.e. a male:female ratio of approximately 3.5:1 (Noronha, V. *et al*., 2012). In this study also, out of 110 patients 80 are male (72.73%) and 30 are female (27.27%) and male:female ratio 2.7:1. In their study from January 2009 to September 2013 , Ruquiya Afrose *et al* have found 342 cases of primary lung carcinoma, including 280 (81.87%) male and 62 (18.12%) female (Afrose, R. *et al*., 2015).

In 2016, Torre, L.A., *et al*., have showed that approximately 53% of cases occur in individuals 55 to 74 years old and 37% occur over 75 years old (Torre, L. A. *et al*., 2016). Lung cancer is the leading cause of death by any means in men over 40years and in women over 59 years of age (Siegel, R.L. *et al*., 2018). In this study, out of 110 patients, 41 patients (37.27%) are in 61-70 years and 36 patients (32.73%) are in 51-60 years, 14 patients (12.73%) patients are in 41-50 years and 12 patients (10.91%) are in 71-80 years, only 7 patients are 40years or below(6.36%).

In this study by cytological and histopathological diagnosis, out of 80 male patients, 75 (93.75%) have primary lung carcinoma, 4 patients (5.0%) have metastatic deposits, and only 1 patient (1.25%) have benign fibrous tumor. From the primary carcinoma, adenocarcinoma found in 30 male patients (37.5%), squamous cell carcinoma found in 30 males (37.5%), 4 patients (5.0%) have non-small cell carcinoma and 3 patients (3.75%) have poorly differentiated carcinoma, 5 patients (6.25%) have small cell carcinoma, 2 patients (2.50%) have non-Hodgkin lymphoma and 1 patient (1.25%) has combined small cell carcinoma (small cell carcinoma + squamous cell carcinoma). Out of 30 female patients, 21 patients (70%) have primary lung carcinoma and 9 patients (30%) have metastatic deposits. Among these, 12 female patients (40%) have adenocarcinoma and 8 patients (26.67%) have squamous cell carcinoma and 1 patient (3.33%) have combined small cell carcinoma. So, in this study , it is found that adenocarcinoma is most common primary lung carcinoma, followed by squamous cell carcinoma. Most of the female patients with lung mass have adenocarcinoma. All the small cell carcinoma cases are found in male but metastatic deposits are most commonly found in female patients in this study. Although histological type varied between countries, adenocarcinoma was more common than squamous cell carcinoma, particularly among females. Generally among males, the incidence of adenocarcinoma was higher than that for squamous cell carcinoma. In some countries such as Belarus, India, the Netherlands, and the Russian Federation, however, squamous cell carcinoma had the higher incidence (Cheng, T. Y. D. *et al*., 2016). But it was also found that in men, adenocarcinoma (47.9%) was followed by small-cell carcinoma (27.7%), squamous cell carcinoma (16.8%), and large-cell carcinoma (7.6%). Likewise, adenocarcinoma was the most common histological type in women (48.2%), with slightly increased percentage of small-cell carcinoma (32.1%), followed by squamous cell (12.5%) and large-cell carcinoma (7.1%) (Milovanovic, I. S. *et al*., 2017).

The previous 2004 World Health Organization (WHO) classification of lung cancer was based on resection specimens (Travis, W. *et al*., 2004). In the recent 2015 WHO classification, recent literature-specific issues with small biopsy and cytology samples are addressed (Travis, W. D. *et al*., 2015; Roy-Chowdhuri, S. *et al*., 2016). In biopsies, the distinction between adenocarcinoma and SCC made using the routine haematoxylina and (H &E) stain is not possible in up to 30-40% of NSCLC cases, because of the small size (~1mm) and the reduced chance of sampling a differentiated part of the tumour (Thunnissen, E. *et al*., 2012). For this situation the diagnosis of NSCLC not otherwise specified (NOS) is used, which leads to the application of a panel of three IHC markers, thyroid transcription factor (TTF-1) and mucin stain for glandular lineage and p63/p40 for squamous cell lineage (Thunnissen, E. *et al*., 2012). The threshold for positivity is different for TTF-1 and p63/p40. TTF1 is considered positive, if weak but certain staining is present (Thunnissen, E. *et al*., 2012). However, in most TTF1- positive lung carcinomas the staining is +++ in the majority of the tumour cell nuclei. p63/p40 is strongly +++ positive in almost all nuclei of undifferentiated neoplastic cells in SCC, especially in the periphery of a field of tumour cells, with loss of staining in more differentiated areas.

In a study by Bevinahalli Nanjegowda Nandeesh *et al* have found that out of 39 patients 22 cases were classified as adenocarcinoma, 7 cases were squamous cell carcinoma and 9 cases were diagnosed as small cell carcinoma by cytological examination and in histopathological examination also 22 cases of adenocarcinoma, 9 cases of squamous cell carcinoma, 6 cases of small cell carcinoma and 2 poorly differentiated carcinoma were identified (Nandeesh, B. N. *et al*., 2015). In this study, by cytological examination 31 patients (28.18%) diagnosed as adenocarcinoma, 30 patients (27.27%) as squamous cell carcinoma, 7 patients (6.36%) as small cell carcinoma, 13 patients(11.82%) with metastatic deposit, 2 patients (1.82%) diagnosed as Non-Hodgkin lymphoma and 1 patient (0.91%) with benign fibrous tumour, but 18 patients (16.36%) diagnosed as non-small cell carcinoma and 8 patients (7.27%) as poorly differentiated carcinoma and by histopathological examination, 42 patients (38.18%) diagnosed as adenocarcinoma, 38 patients (34.55%) as squamous cell carcinoma, 2 cases (1.82%) as combined small cell carcinoma , 5 patients (4.55%) diagnosed as small cell carcinoma, 13 patients (11.82%) as metastatic deposits, 2 patients (1.82%) as Non-Hodgkin lymphoma, 1 patient (0.91%) as benign fibrous tumour, but 4 patients

(3.64%) diagnosed as non-small cell carcinoma and 3 patients (2.73%) diagnosed as poorly differentiated carcinoma. Combined small cell carcinoma may be difficult to diagnose by cytological examination. In this study secondary deposits in lung is found in cases primary from renal cell carcinoma, invasive ductal carcinoma of breast, malignant phylloides tumour of breast, pleomorphic sarcoma. Metastatic deposit can be diagnosed by cytological examination but for further categorization histopathological examination and immunohistochemistry is required along with proper history, clinical examination and reports of previous investigations. Most of the non-small cell carcinoma and poorly differentiated carcinoma diagnosed by cytological examination, can be categorized properly by histopathological examination, but sometimes immune histochemistry is required for final diagnosis.

The inter rater reliability between the two procedures by intra class correlation coefficient is 0.8777 which is quiet good. The P value is < 0.0005 which supports the fact that there is a statistically significant inter rater reliability.
There is a good strength of agreement between the two procedures. The P value < 0.0005 indicates that the Kappa co-efficient is significantly different from zero.

The sensitivity and specificity of cytology to diagnose primary lung mass is 76.92% and 77.78% respectively. Some studies show that diagnostic sensitivity and specificity of FNAC for lung carcinoma was 91.5% and 72.5% (Mullan, C.P. *et al*., 2004; Cox, J. E. *et al*., 1999). and accuracy of FNAC was 88.5% based on final HPE report (Modi, M. B. *et al*., 2016). In 2010, A. Fassina *et al* have showed that when correlated with histology, FNA with ROSE discriminated malignant versus non-malignant lesions (Cohen's Kappa 0.78), with three false negatives (sensitivity 96.3%, specificity 100%). Moreover, a satisfactory overall agreement of 71.4%was achieved in differentiating the cancer histological types (Fassina, A. *et al*., 2011).

The sensitivity of cytology for secondary lung mass is 92.31%. The sensitivity of histopathology for diagnosing primary lung tumour is 92.78%.FNAC is found to be highly accurate (95%) in diagnosis as compared to previous studies (Madan, M., & Bannur, H. 2010; JayaShankar, E. *et al*., 2010).

CONCLUSION

This study shows that adenocarcinoma of lung is the most common primary lung carcinoma. Though this study is too small to conclude, cytology has a good sensitivity and specificity in diagnosis of lung carcinoma, but sometimes, biopsy examination is required for categorization, specially in cases of non small cell carcinoma or poorly differentiated carcinoma, diagnosed by cytology, because exact categorization is required for the treatment purpose and only in few cases immunohistochemistry is required to confirm the diagnosis.

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