Original Article

A study of usefulness of washes and brush cytology with respect to histopathology in diagnosis of lung malignancy by using fiberoptic bronchoscopy

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ABSTRACT

Background: Examination of specimens obtained through flexible fiberoptic bronchoscope is important and often the initial diagnostic technique performed in patients with suspected malignant lung lesion. **Aims:** To evaluate the usefulness of cytological findings of bronchial washings (pre-and post-bronchoscopy) and bronchial brushing in the diagnosis of lung malignancy with histopathology of bronchial biopsy, taking the latter as the confirmatory diagnostic test. **Settings and Design:** It was a cross-sectional observational study conducted in a tertiary care center. **Subjects and Methods:** A total of fifty patients with suspected lung malignancy (clinically and radiologically) were included in this nonrandomized cross-sectional study. Bronchial brushings were obtained from all fifty cases. Prebiopsy bronchial washing (washing collected before the brushing and biopsy procedure) and postbiopsy washing (washing at the end of the procedure) were collected. **Results:** Prebiopsy (prebrushing) and postbiopsy washing showed high specificity of 92.31%, but a very low sensitivity of 32.43% and 35.14%, respectively. Sensitivity and specificity of brushing were found to be 74.36% and 81.82%, respectively. Positive predictive value of prebiopsy (prebrushing) washing, postbiopsy washing and brushing are 92.31%, 93.55%, and 92.86%, respectively. There was no significant difference in sensitivity between prebiopsy (prebrushing) and postbiopsy washing (Fisher exact probability test; $P_A = 0.99$). However, there was statistically significant difference between sensitivity of brushing with prebiopsy (prebrushing) washing (Fisher exact probability test; $P_A = 0.0012793$) and postbiopsy washing (Fisher exact probability test; $P_A = 0.00310282$). **Conclusions:** Bronchial washing cytology in combination with brush cytology aids in the early diagnosis of lung malignancy in addition to histopathology.

Key words: Bronchial washing, brushing, fiberoptic bronchoscopy, histopathology, nonsmall cell carcinoma, small cell carcinoma

INTRODUCTION

Today lung cancer is one of the most common malignancies in the world. Moreover, with an estimated increase of about 5% a year, it now represents globally the first cause of cancer-related mortality in both sexes. In India, the exact incidence of lung cancer is not known due to lack of formal epidemiological data from across the country.[1] Overall the age distribution ranges from 20 to 90 years, with a peak incidence between 50 and 70 years of age.[2] The overall therapeutic results have changed very little in the past decade in the face of an increasing incidence of this disease throughout the world. Most patients are found to have advanced disease at the time of diagnosis, and thus, treatment of this population is disappointing, very often only palliative. Several studies, however, have demonstrated that early detection, localization, and aggressive

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Access this article online

Quick Response Code:

Website: www.ccij-online.org

DOI: 10.4103/2278-0513.186107

Cite this article as: Bandyopadhyay A, Pal M, Das I, Sarkar S, Sarkar R, Taraphdar P. A study of usefulness of washes and brush cytology with respect to histopathology in diagnosis of lung malignancy by using fiberoptic bronchoscopy. Clin Cancer Investig J 2016;5:293-8.

treatment of lung cancer results in the 5-year survival rate of 70–80%.[3] Recent developments in molecular study of lung cancer along with subsequent targeted therapeutic approaches have given a new ray of hope.

Bronchoscopy is perhaps the most invaluable tool for diagnosis of lung cancer. Various diagnostic techniques have been developed using flexible fiberoptic bronchoscopy (FOB).[4] Among various bronchoscopic techniques, bronchial biopsy has the highest sensitivity and specificity for endobronchial malignant lesions. Thus, histopathological examination of bronchial biopsy specimen remains the confirmatory or the gold standard test in these situations. However, bronchial biopsies cannot be satisfactorily performed in more peripheral sites, in narrow bronchial lumen or patients at risk of hemorrhage. Hence, alternative methods for diagnosis are sometimes required. Both washing and brushing cytology are very effective in the diagnosis of lung cancers. Brushings often offer excellent specimens and accurate information about the site of the lesion.[5] Cytological assessment of specimens obtained through washing and brushing of the respiratory tract is important, and often the initial diagnostic technique carried out in a patient with suspected malignant lung lesion.[6] The utilities of cytology are extensive, and sometimes they help in planning the treatment without the requirement for an open biopsy. Imprint smears from bronchial biopsy have also been found to give a good diagnostic yield.[7]

This cross-sectional, observational study was conducted at a tertiary care center over the period of 18 months with the aim to correlate brushing and washing cytology with biopsy in the diagnosis of lung cancer.

SUBJECTS AND METHODS

It was a cross-sectional observational study conducted in the Department of Pathology in association with Department of Respiratory Medicine and Community Medicine, Nil Ratan Sircar Medical College and Hospital, Kolkata. The samples for cytological and histological examination were collected from the indoor/outdoor patients in whom clinical findings, radiological examination, and bronchoscopic examination suggested lung malignancy. Chronic cough, hemoptysis, significant weight loss, pallor, lymphadenopathy were among the most significant clinical findings that were considered. Among the radiological findings, mass with or without consolidation was the most characteristic indicator apart from pleural effusion. Among these suspicious patients, who were considered for bronchoscopy, endobronchial growth, and narrowing of bronchial lumen (due to compression from outside) were the predominant presentations. Patients with hemorrhagic diathesis, poor general condition, and sputum positive for acid fast bacilli were excluded from the study. A total of fifty cases were studied in the stipulated time frame of 18 months (January 2014–July 2015) which fulfilled our inclusion and exclusion criteria. The samples were obtained by Pentax flexible FOB done by the pulmonologists following standard protocol. Bronchial brushings were obtained by the use of a stiff-bristle disposable brush (outer diameter of brush is 2 mm, and outer diameter of sheath is 1.8 mm). Every case followed the following sequence of event: Pre-biopsy washing, brushing, biopsy, and postbiopsy washing. Brushing material smeared directly onto at least four clean glass slides. The two air-dried smears were stained with Leishman Giemsa stain and two slides are fixed with ethanol-ether mixture for Pap and hematoxylin and eosin (H and E) stain. Bronchial wash fluids taken both before brushing and after biopsy were first centrifuged (1500 rpm for 5 min) and then prepared into air dried and ethanol fixed smears (total 4 slides as before) and stained with Giemsa, H and E and Pap stain, respectively. Bronchial biopsy specimens were fixed in 10% formalin, sectioned cut at 3–4-micron thickness and stained with H and E.

RESULTS

Most patients were in their fifth and sixth decade of life with age, the range of 31–80 years. Of 50 study subjects, lung cancer was confirmed in 38 (76%) cases by histopathology of bronchial biopsy. Among patients with lung cancer, 79% were male and 21% were females [Table 1]. Squamous cell carcinoma was found to be the most common lung cancer (47.4%) [Figures 1-5], followed by adenocarcinoma (23.7%) [Figures 6-8], small cell carcinoma (15.8%) [Figures 9-10], large cell neuroendocrine (5.2%) and large cell anaplastic carcinoma [Figures 11 and 12] [Table 2]. All except two cases of bronchial biopsy could be differentiated into a specific type of nonsmall cell carcinoma (NSCC). Prebiopsy (prebrushing) and postbiopsy washing showed high specificity of 92.31%, but a very low sensitivity of 32.43% and 35.14%, respectively. Sensitivity and specificity of brushing were found to be 74.36% and 81.82%, respectively. Positive predictive value of prebiopsy (prebrushing) washing, postbiopsy washing and brushing are 92.31%, 93.55%, and 92.86%, respectively. Both sensitivity and accuracy of combined tests (postwash and brush together) increases significantly [Tables 3 and 4].

Table 1: Age-sex distribution of patients with lung malignancy

Age (years)/sex	Male (%)	Female (%)
31-40	01 (2.6)	01 (2.6)
41-50	02 (05)	02 (05)
51-60	16 (42)	02 (05)
61-70	08 (21)	01 (2.6)
71-80	03 (08)	02 (05)
Total	30 (79)	08 (21)

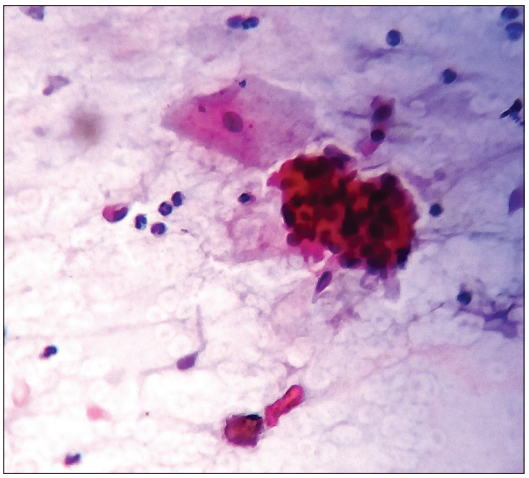

Figure 1: Photomicrograph of squamous cell carcinoma: Wash cytology smear shows clusters of polygonal cells with eosinophilic cytoplasm and hyperchromatic nuclei (Pap, ×100)

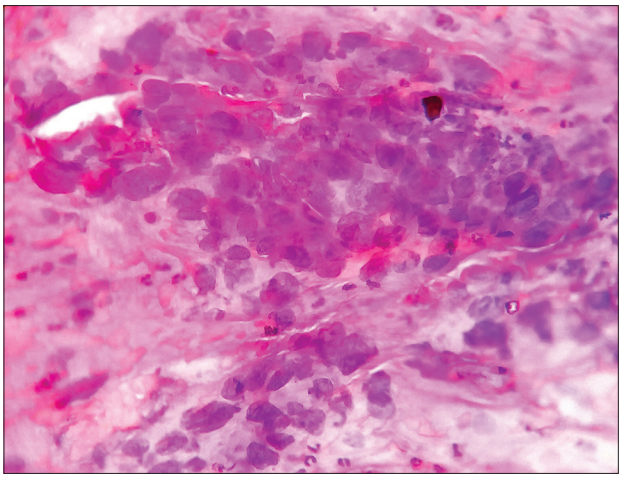

Figure 2: Brush cytology smear of squamous cell carcinoma shows similar clusters of polymorphic cells with eosinophilic cytoplasm and hyperchromatic nuclei (Pap, ×400)

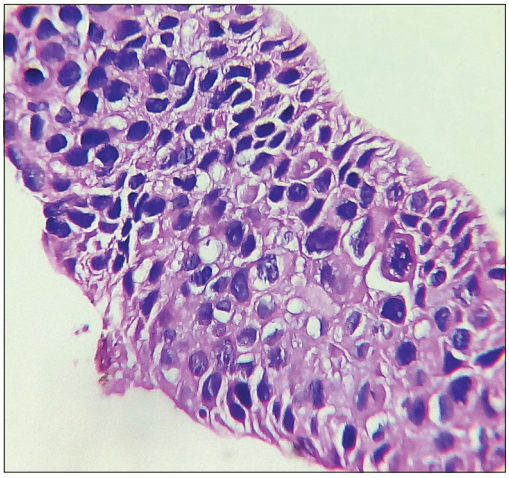

Figure 3: Histology of squamous cell carcinoma showing polygonal cells with hyperchromatic nuclei and eosinophilic cytoplasm (H and E, ×400)

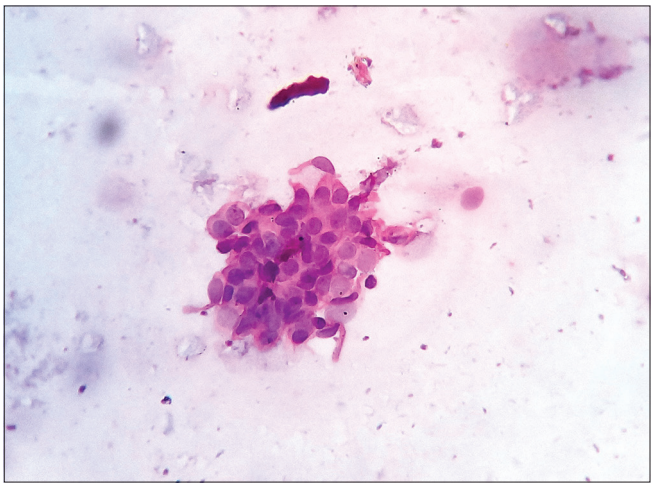

Figure 4: Photomicrograph of squamous cell carcinoma: Post wash cytology smear shows clusters of polygonal cells with eosinophilic cytoplasm and hyperchromatic nuclei (H and E, ×100)

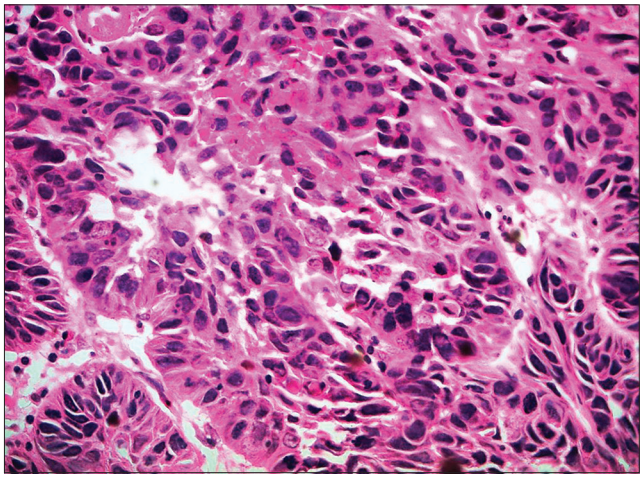

Figure 5: Corresponding histology section of squamous cell carcinoma shows tissue fragments of atypical squamous cells having polygonal shape with hyperchromatic nuclei and eosinophilic cytoplasm (H and E, ×400)

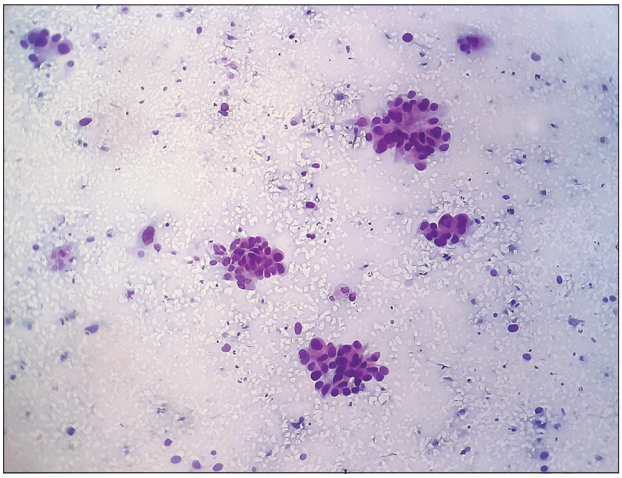

Figure 6: Photomicrograph of adenocarcinoma: Wash cytology smear showing cohesive clusters of round to oval cells with abundant vacuolated cytoplasm and hyperchromatic nuclei (Leishman and Giemsa, ×100)

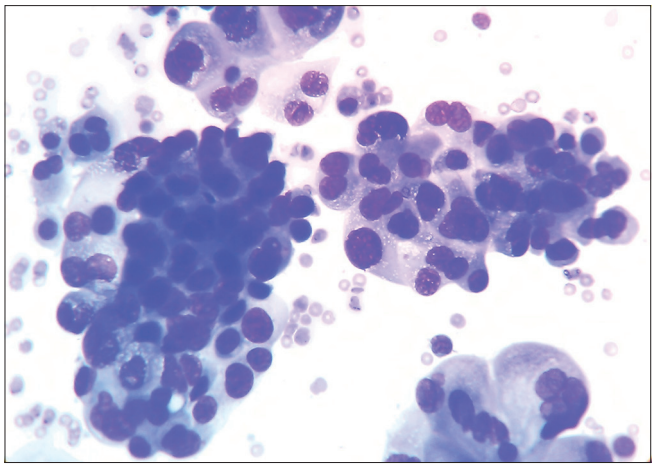

Figure 7: Brush cytology smear of adenocarcinoma showing cohesive clusters of round to oval cells with abundant vacuolated bluish cytoplasm, intracytoplasmic inclusions and hyperchromatic nuclei (Leishman and Giemsa, ×400)

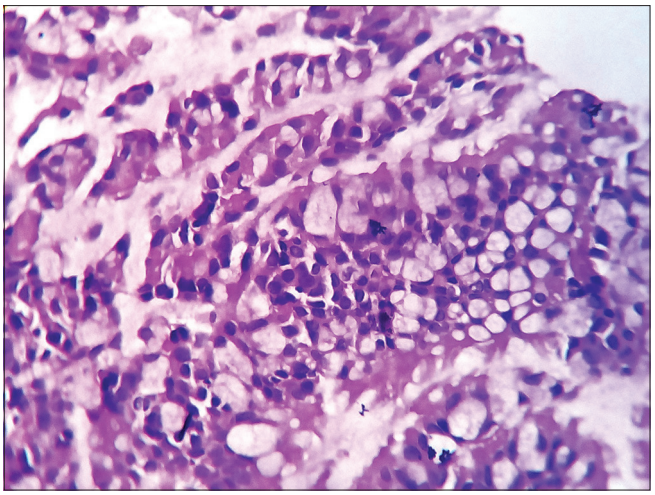

Figure 8: Corresponding histopathological section shows atypical cuboidal to low columnar cells showing an acinar architecture with pale eosinophilic intracytoplasmic mucin (H and E, ×400)

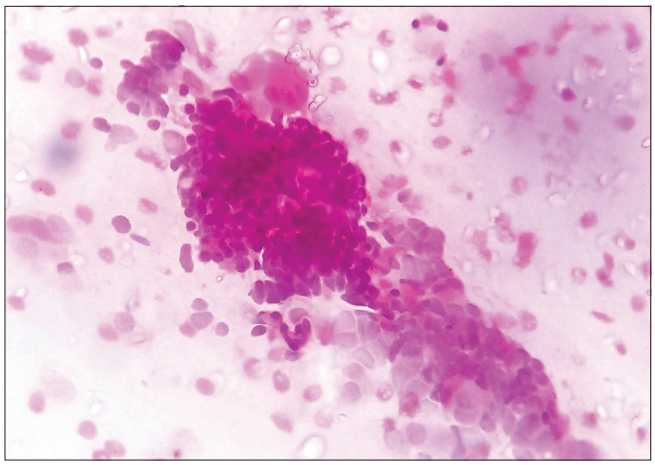

Figure 9: Photomicrograph of small cell carcinoma: Brush cytology smear showing cluster of small dark hyperchromatic cells with scanty cytoplasm (H and E, ×100)

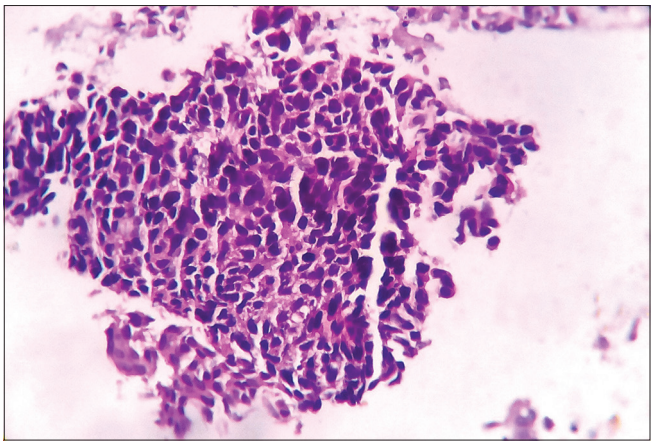

Figure 10: Corresponding histopathological section shows small dark hyperchromatic cells having very scanty cytoplasm showing necrosis and molding (H and E, ×100)

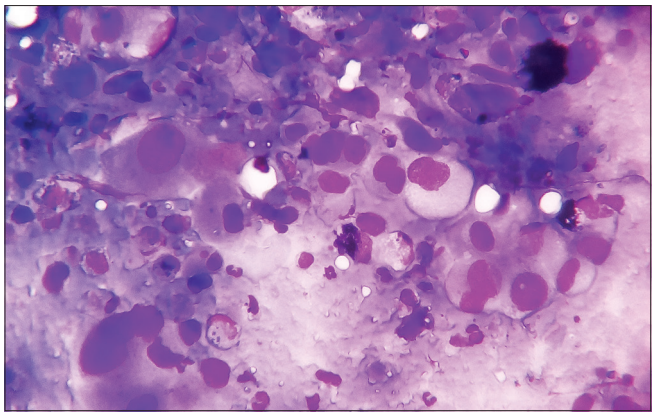

Figure 11: Brush cytology smear shows large cell anaplastic carcinoma having large bizarre looking cells. High degree of cellular atypia and pleomorphism present (MGG, ×400)

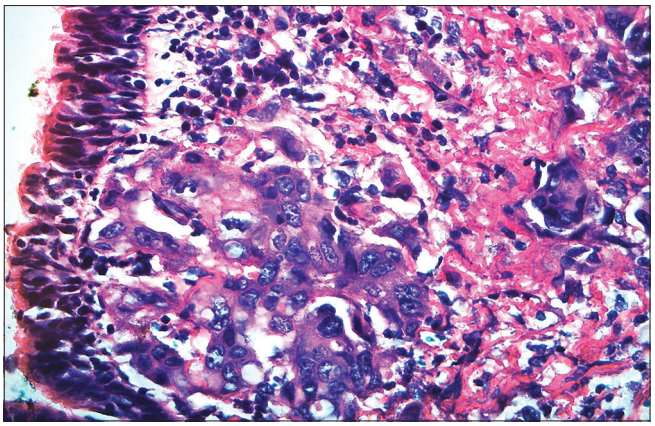

Figure 12: Corresponding histopathological section of large cell anaplastic carcinoma having large bizarre looking cells with hyperchromatic nuclei and prominent nucleoli. Few tumor giant cells are also seen (H and E, ×400)

There was no significant difference in sensitivity between prebiopsy (prebrushing) and postbiopsy washing (Fisher exact probability test; P_A = 0.99). However, there was statistically significant difference between sensitivity of brushing with prebiopsy (prebrushing) washing (Fisher exact probability test; P_A = 0.0012793) and postbiopsy washing (Fisher exact probability test; P_A = 0.00310282) [Table 5].

Table 2: Different types of lung cancers with relative distribution in percentage

Type of lung cancer	Total number	Percentage
Squamous cell carcinoma	18	47.3
Adenocarcinoma	09	23.6
Small cell carcinoma	06	15.7
Large cell anaplastic carcinoma	01	2.6
Large cell neuroendocrine carcinoma	02	5.2
NSCC NOS	02	5.2
Total	38	100

NSCC NOS: Nonsmall cell carcinoma not otherwise specified

Table 3: Results of different procedures used in the study

	Prewash	Postwash	Brush
True positive	12	13	29
True negative	12	12	09
False positive	01	01	02
False negative	25	24	10
Total	50	50	50

Table 4: Results of different cytological techniques compared to gold standard (bronchial biopsy) in percentage

Cytological technique	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
Prewash	32.43	92.31	92.31	32.43	48
Postwash	35.14	92.31	93.55	34.21	50
Brush	74.36	81.82	92.86	50	76
Combined	84.57	69	90	58.4	81

Table 5: Statistical significance among different cytological procedures

Different procedures	*P*	Significance
Prewash and postwash cytology	0.99	Not significant
Prebiopsy wash and brush cytology	0.0012793	Significant
Postbiopsy wash and brush cytology	0.00310282	Significant

Fisher exact probability test

DISCUSSION

The age distribution in our study shows a wide range, i.e., from 30 years to 80 years. Mean age of presentation in our study is 56.67 years which corroborates with other Indian and international studies.[2,8] In our study, most of the patient with lung malignancy are males (79%). We found male:female ratio in our study population 3.76:1, which is similar to sex ratios of other Indian studies.[2] Global sex ratio was found to be much lower (2.14:1) in few studies.[9] This may be due to changing smoking pattern and more accessibility and reporting of the females to healthcare facility in western countries. In our study, squamous cell carcinoma was most prevalent followed by adenocarcinoma and small cell carcinoma. This pattern is supported by other Indian studies[2] and the fact that we were dealing only with central lung malignancies. Although recently, there is an increase in adenocarcinoma cases worldwide. Bronchial washing is often used along with bronchial brushing and biopsy to diagnose lung cancer. However, the optimal timing of bronchial washing with respect to biopsy and brushing (i.e., whether before or after biopsy and brushing) has been subject to much debate. To assess the optimal sequence in which bronchial washing to be performed, the washing was obtained in two ways in this study: Prebiopsy (prebrushing) washing and postbiopsy washing.

This study was conducted with the objectives of assessing the sensitivity and specificity of bronchoscopic cytological procedures; bronchial brushing, and washing by comparing with the histopathology of bronchial biopsy obtained from lung tumors. In this study, we found that prebiopsy and postbiopsy washing showed high specificity of 92.31%, but a very low sensitivity of 32.43% and 35.14%, respectively. Sensitivity and specificity of brushing were found to be 74.36% and 81.82%, respectively. This finding of the present study is similar to the result that was observed by Mak *et al.*[10] and Chen *et al.*[11]

Previous studies by Park *et al.*[12] and Karahalli *et al.*[13] had found almost the comparable result of bronchial washing in lung cancer cases. However, in other previous studies by Solomon *et al.*[14] van der Drift *et al.*,[15] the diagnostic yield of bronchial washing was not similar with that of our study.

On comparing, no significant difference was found between sensitivity of prebiopsy washing and postbiopsy washing cytology in the current study (Fisher exact probability test; $P_A = 0.99$). A similar trend was noticed in previous studies also.[15]

False positive results were noticed in these cytological techniques used in the present study. It may be possible that some of these classified as false positives in the present study might be true positives as methods other than bronchial biopsy to confirm the diagnosis of lung cancer were not used in the present study. Majority of the previous studies[13,15] that have used other techniques such as re-bronchoscopy, surgery, transthoracic needle aspiration, CT-guided fine needle aspiration cytology, tumor markers, and autopsy, to prove the cases of lung cancer have shown that bronchial biopsy does not provide diagnostic yield in all cases of lung cancer. Chances of missing the diagnosis by bronchial biopsy are more in peripheral lung tumors. On bronchoscopic examination, the gross morphology of majority of these cases of adenocarcinoma was compression type lesion, i.e., extrinsic compression of the bronchus by the lesion[13] and thus there may be a possibility of getting less representative material by bronchial biopsy in such tumors. Furthermore, in mucinous type of adenocarcinoma, bronchial biopsy specimen may contain pools of mucin, very

few neoplastic cells with a relative lack of atypia that make the diagnosis of adenocarcinoma more difficulties observed by Butnor.[16] In our study, two cases were diagnosed as NSCC not otherwise specified as Pap stain and periodic acid schiff and diastatse (PAS-D) stains were noncontributory.

There has been a controversy as to whether bronchial washing should be routinely used or not. Many studies like Trevisani *et al*.[17] Karahalli *et al*.[13] in the past reported that the diagnostic yield did not increase significantly further by the addition of bronchial washing to bronchial biopsy and recommended that washing should not be routinely used. However, authors like Mak *et al*.,[10] Jones *et al*.[18] and Bodh *et al*.[19] have suggested that bronchial biopsy, brushing, and washing should be performed to obtain optimal diagnostic yield. Liwsrisakun *et al*.[20] have observed that the addition of bronchial washing to either biopsy or brushing is beneficial but not cost-effective. Bronchial biopsies cannot be performed in more peripheral sites or patients with luminal obstruction or at risk of hemorrhage. Hence, alternative methods for diagnosis are sometimes required.

CONCLUSION

The bronchial washing or brushing is a safer technique with much lesser risk of hemorrhage or mortality. Based on findings of the study, it may be concluded that obtaining of bronchial brushing and washing cytology specimens using bronchoscopy aids in the diagnosis of lung malignancy with a reasonable high accuracy rate and with morphological typing of neoplasms. Brush and wash cytology is particularly useful in patients with evidence of obstruction or risk of hemorrhage where bronchial biopsy is not possible. Furthermore, all these techniques may be used concurrently with bronchial biopsy to diagnose a very lethal disease like lung malignancy where early and effective diagnosis followed by appropriate treatment can reduce mortality.

Financial support and sponsorship

Department of Pathology, Department of Pulmonary Medicine and Department of Community Medicine.

Conflicts of interest

There are no conflicts of interest.

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