Cytological and Immunocytochemical Study of Bronchial Wash in Bronchogenic Carcinoma

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Summary:

Background: *- Carcinoma of the lung has became the most common type of cancer since 1985 & the most common cause of cancer death in both males & females.*
Aim of study: *- To assess the diagnostic accuracy of bronchial wash cytology & application of immunocytochemical methods, using two tumor markers (low molecular weight cytokeratin & epithelial membrane antigen) for more accurate & precise diagnosis of lung tumors.*
Patients, materials and methods: *- Fifty fifes suspected lung cancer cases according to their clinicoradiological examinations were included in this study.*
Bronchial wash cytology was performed for all the 55 patients. Smears were stained by conventional cytological stain inaddition to immunocytochemial staining using low molecular weight cytokeratin & epithelial membrane antigen. The final results of bronchial wash were compared to histopathological results & final clinical diagnosis as.
Results: *- Cytological smears of bronchial wash revealed the presence of malignant cells in 33 cases (60%).*
The sensitivity of bronchial wash cytology was 82.5%; the specificity was 100.0%, with overall accuracy of 87.3%.
Using cytokeratin staining, 26 cases (47%) were positive for malignant cells, & 29 were negative. The sensitivity, specificity & overall accuracy were 65%, 100%, & 74.5% respectively.
With EMA staining, 19 cases (27%) were positive for malignant cells & 36 were negative. The sensitivity, specificity & overall accuracy were 47.5%, 100%, & 61.8% respectively.
Combined use of CK & EMA raised the sensitivity to 72.5%, specificity of 100% & overall accuracy of 80%. Combined use of monoclonal antibodies & conventional cytology raised the sensitivity to 95%, specificity 100%, with overall accuracy of 96.3%.
Conclusions: *- Using more than one monoclonal antibody, or using combined conventional cytology & immunocytochemistry increase the sensitivity for detection of malignant cells in bronchial wash smears.*

Introduction: -

Bronchogenic carcinoma is the most insidious neoplasm in the whole realm of oncology, & it is usually discovered by the age of fifties ".[1] Lung cancer is the second most common type of cancer in Iraq in both males and females by the year 1998, 1999; 2000[(2)]. Bronchogenic carcinoma is presently the most common malignant disease in the industrial world an[(;)]d it become increasingly frequent during the past 50 years[(", it account for 19% of all cancer and 27% of cancer death $151.

Bronchoscope and bronchial wash were introduced by Ikeda (1964) since that; flexible fiberoptic bronchoscope has an important role in both diagnosis and staging of lung cancer. (6.7)

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Patients, Materials, and Methods: -

During the period from the first of September 2004 to the first of June 2005, fifty fife patients suspected to have lung cancer according to their clinico-radiological examinations, attending the *Cardiothoracic Department* at *AL-Kadhimya Teaching Hospital* underwent fiberoptic bronchoscopic examination, with collection of bronchial wash and brush samples from all the patients and transbronchial biopsy for 29 patients with visible bronchoscopic findings. These samples were processed in the pathology lab of college of medicine of *AL-Nahrain University.*

Bronchial wash: -

With the use of FOB, bronchial wash was performed by installation of 10 nil. of saline through the accessory port of the bronchoscope into the suspected area and reaspirated and collected into can tube, then centrifugation smearing of the sediment then immediately fixed in 95% ethanol alcohol .. at least. Eight slides were prepared, four of them in an ordinary glass slides (two for H&E stain, and two for pap stain) for

conventional cytological examination. The other four slides are positive charged slides (two for low molecular weight *CK,* and the other two for *EMA)* for immunocytochemical staining[8.9,,0,

Bronchia brush: -

Bronchial brush is done to the suspected area or to the viewed mass by *FOB* by a special brush device which is introduced through the accessory port of the FOB. Brush result in higher yield of cellular materials. Brushing specimens should be handled immediately to reduce the artifact in the specimens. After removal of the *FOB* and the brush, the brush materials are smeared directly on glass slides with circular movement about 0.5 to 1 cm. in diameter and immediately fixed in 95% alcohol for 30 min [19,11].

Statistical analysis: -

The test of significance used in this work is the "difference between two proportions"; these differences were calculated using the Chi-square analysis, the difference is regarded as significance if the P value is less than 0.05.

The suspicious cases were regarded as false negative for statistical analysis.

Results: -

Total of 55 patients presented with clinical and radiological criteria of pulmonary tumors including cough, haemoptysis, repeated chest infection and dyspnea with *CXR* and/or *CT* findings. Of *55* patients, According to the final clinical and histopathological diagnosis, 40 patients were diagnosed as having bronchogenic carcinoma. Of these 40 positive patients, there were 28 (50.9%) male patients and 12 (21.8%) female patients (table 1).

Table1 Sex distribution in correlation With fnal clinical and histopathological diagnosis

Sex	Positive cases		Negative cases		Total	
	No.	%	No.	%	No.	%
Male	28	50.9	12	21.8	40	72.7
Female	12	21.8	3	5.5	15	27.3
Total	40	72.7	15	27.3	55	100

SQCA found to be the most common type of pulmonary tumors according to the final clinical and histopathological diagnosis and representing 26/40 (65%) cases (table 2).

Table 2 *Types of lung cancer in correlation with the final clinical and histopathological diagnosis.*

Type	No.	%
SQCA	26	65
ADCA	5	12.5
LCCA	6	15
SCCA	3	7.5
Total	40	100

CytologicafResults: -

Cytological examination of bronchial wash specimens revealed malignant cells in 33/55 (60%) cases, 15 cases were negative and 7 cases were falsely negative in correlation with the final clinical and histopathological diagnosis (table 3).

Cytology result	No.	%
Positive	33	60
Negative	15	27
False negative	7	13
Total	55	100

Table 3 *Cytology staining results of the 55 bronchial wash smears*

SQCA were diagnosed cytologically in bronchial wash specimens in 2 1/33 cases (64%) of the total positive cytological cases.

The cytology result has been compared with the final clinical and histopathological diagnosis; there were 7 false negative results with no false positive (5 of them *SQCA, I* case *ADCA, I LCCA).*

The sensitivity of Bronchial wash cytology was 82.5% specificity 100%, PPV 100%, negative predicative value 68.2%, accuracy of 87.3%.

Bronchial wash smear showing SQCA stained with H&E stain. (400X)

Immunocytocizemieal results: -
All the 55 samples of Bronchial wash studied by immunocytochemical techniques using *LMW* cytokeratin *(CK)* and epithelial membrane Antigen *(EMA).*

CY, results:
Staining with CK were positive in 26/55 cases (47%) and were negative in 15 cases with 14 false negative cases which proved to be malignant by other methods of diagnosis (table 4).

Table 4 Immuocytochemichal stain (CK) of the 55 bronchial wash smears.

CK response	***No.***	***%***
Positive CK	***26***	***47***
Negative CK	***15***	***27***
False negative CK	***14***	***26***
Total	***55***	***100***

The most identifiable tumor was *SQCA* with 65.5% while small cell carcinoma shows negative staining with 0%.

The sensitivity of *CK* staining was 65.0%, specificity 100%, PPV 100%, NPV 51.7%, and over all accuracy 74.5%.

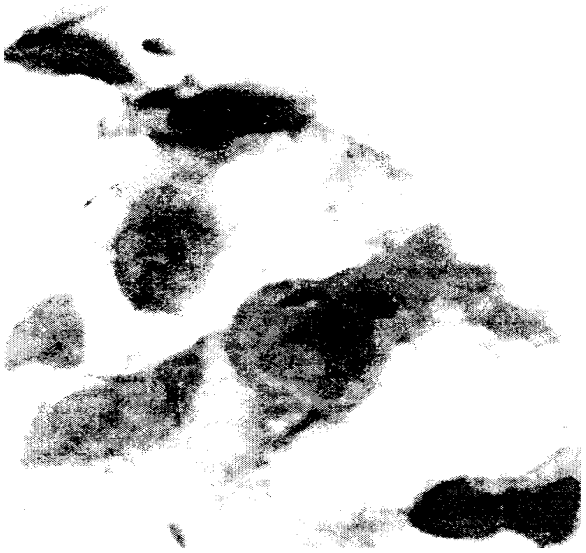


figure 2 Bronchial wash smear showing SQCA stained positively with CK stain (granular red cytoplasm). (IOOOX)

E M A Results:

Table 5 show that 19/55 cases (35%) were stained positively with EMA while 15/55 cases were negative and 21/55 cases were false negative, the results of immunocytochemical staining was compared with the final clinical and histopathological diagnosis (table 5).

Table 5 5Immunocytocheinichal stain (EMA) of the 55 bronchial wash smears.

EMA response	***No.***	***%***
Positive EMA	***19***	***35***
Negative EMA	***15***	***27***
False negative EMA	***21***	***38***
Total	***55***	***100***

The most common type of cancer encountered was SQCA with 63% while small cell carcinoma also shows negative staining. The sensitivity, specificity, PPV, NPV and accuracy were, 47.5%, 100%, 100%, 41.7%, and 61.8% respectively.

Table (6, 7) show the distribution of cases in response to positive cytology, CK and EMA staining, there is significant correlation between the immunocytochemical staining in both markers and the positive cytological results.

Table 6 Distribution of cases in response to cytology and immunocytochemical staining of cytokeratin.

Immunocytochemistry stain	***Cytology result***		***Total***
	Positive	***Negative***	
Positive CK	***22***	***4***	***26***
Negative CK	***11***	***18***	***29***
Total	***33***	***22***	***55***

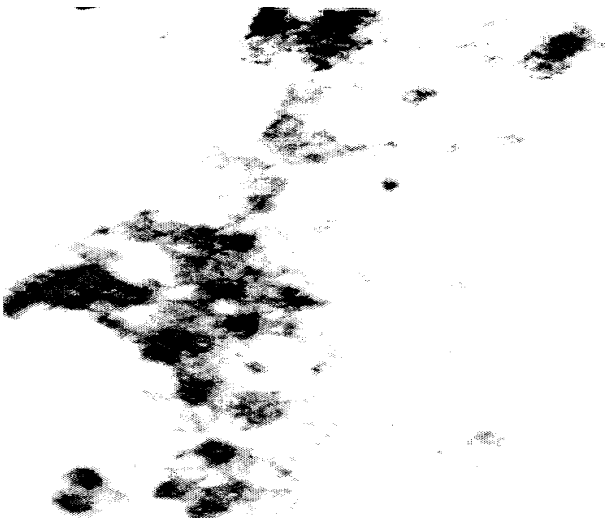
P=0.0011

Table 7 Distribution of cases in response to cytology and imnuunocytochenlical staining of EMA.

Immunocytochemistry stain	***Cytology result***		***Total***
	Positive	***Negative***	
Positive EMA	***16***	***3***	***19***
Negative EMA	***17***	***19***	***36***
Total	***33***	***22***	***55***

P=0.0176

When both cytological results and immunocytochemical staining were combined there was much more increase in the accuracy and sensitivity. The sensitivity was 95%, specificity 100%, PPV 100%, NPV 88.2%, Overall accuracy 96.3%



FIGUER 3 Bronchial wash smear showing SQCA with intense homogenous cytoplasm staining of EMA stain. (400X)

Discussion: -

Lung Tumors frequently exhibit; multiple pathogenesis of cellular differentiation, making them difficult to be diagnosed and classified by routine light microscopic examination 112), immunoeytochemical techniques have become widely used in cytopathology by using multiple tumor markers to aid in the diagnosis of different types of cancers and to increase the sensitivity of various diagnostic methods.

From total of 55 cases, most of the cases were among males 40/55 (72.7%), the rest 12/55 (27.3%) were among females. This result is similar to studies of Vincent et al "3j, and Santose et al 2005'14).

Forty cases were proved to have malignancy by final clinical and histopathological diagnosis. Most of the malignant cases were between the age of 60-69 years old with 18/40 and representing 47.3% of the total cases. This results is similar to another Iraqi studies done in Mousl and Baghdad '1'- 'G' As reported in the literature, lung cancer is relatively uncommon before the age of 50 years with rate increasing rapidly after the age of 50 years ")

. The most common type of carcinoma diagnosed in our study was squamous cell carcinoma (65%), this results is similar to finding of Santose et al '14), this may be because that most of the patients selected in this study found to have squamous cell carcinoma or because over 90% of the patient were smokers and as we know the ultimate relationship between smoking and its role in the pathogenesis of SQCA.

The sensitivity of Bronchial wash cytology was 82.5%; this finding is similar to the finding of Al-Rawi. FA., a study which gives sensitivity of 82% but differ from the results of Biob et al giving 76%, Troung et al giving 66%, Bederssian et al 76% (" Ahmed A. et al who gives sensitivity of 94.5% [8'.

The preparation of cytological specimens to be examined immunocytochemically was the same procedure of conventional cytology. Ideally it is preferable to use the cytocentrifuge (cytospin) apparatus which gives circles with concentrated cells on the slide rendering the further steps of application of immunocytochemical materials much easier and cost effective, the fixation of the smear in 95% ethanol alcohol Because the cytological smears are not routinely subjected to formalin, so one could conclude that epitope retrevial technique are unnecessary for immunocytochemical staining of these specimens. Because alcohol fixed tissue by dehydration and removed of water molecule that are non covalently bound to the side chain of amino acid, not by induction of molecular cross links in protein, which change the nature three dimensional protein configuration; there by altering the normal 3-dimensional structure of epitope, making it more difficult for the antibody to bind to its target as a formalin did (9)

By the use low molecular weight cytokeratin, 26/55 cases were positive (47%) The most common type of cancer encounter with *CK* staining was squamous cell carcinoma, because of its high percentage in the selected cases, increase expression in that type of tumor [2w] or due to the sensitivity of the markers for *SQCA* as reported by Chu PG, et al [2"] Many studies concentrate on the usefulness of antikeratin antibodies in the differentiating small cell carcinoma of the lung from other histological types of lungs cancers, [22.23,24] small cell carcinoma of the lung had no reactivity for keratin; whereas all others types of lung carcinoma did have reactivity (''). So the use of antikeratin antibody is important at least to differentiate small from non-small cell lung cancer which is an important point for treatment especially that SCCA is metatstatic at diagnosis and responsive to cytotoxic chemotherapy and radiotherapy, therefore inorder to improve the dismal prognosis of patient suffering from SCCA. It is of great important to be able to recognize accurately the SCCA in cytological preparation by rapid and reliable cyto diagnostic tests (`').

According to EMA staining, 35% of the cases were positive. The most common type present with EMA staining was squamous cell carcinoma again. The sensitivity of EMA was 47.5%; this result is close to the finding of Domagala-Kulawik et al on Bronchial wash cytology (251.

The result, show that combination of both cytology and immunocytochernical techniques will increase the sensitivity of bronchial wash cytology from 82.5% to 95%, this results similar to Weinberg et al (2004) and Rosell et al (1998) [26.27].

Combinations of both markers increase the sensitivity to 72.5%. There was highly significant correlation between cytokeratin positive staining and *EMA* positive staining .

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