COMPARATIVE STUDY OF PARAFFIN EMBEDDED SPUTUM AND ROUTINE SPUTUM CYTOLOGY IN THE DIAGNOSIS OF BRONCHOGENIC CARCINOMA

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ABSTRACT

BACKGROUND

Lung cancer though known for its high mortality has a good prognosis in its early stages.[1] For early detection of lung cancer sputum cytology has been used as a screening method along with radiology.[2] But its pick up rate depends on the quality of preparation and the patience of the examining person. This can be overcome by paraffin embedding the sputum sample as cell block. This makes sputum valuable in mass screening of high-risk group for the early detection of bronchial carcinoma.[3] In this context this study is done to assess the diagnostic efficacy of sputum cell block compared to ordinary sputum smear in the diagnosis of Bronchogenic carcinoma.

The aim and Objective of the study is to compare the sensitivity and specificity of sputum cell block and routine sputum smear cytology in the diagnosis of Bronchogenic Carcinoma and its efficacy in tumour typing.

MATERIALS AND METHODS

Sputum samples of patients with a clinical diagnosis of bronchogenic carcinoma were examined during a period of one year to compare both smear and cell block for their sensitivity, specificity, positive predictive value and negative predictive value in correlation with the gold standard (The diagnostic result influencing the management of the patient- which could be either cytology /biopsy of lung /lymph node).

RESULTS

Of the total number of 143cases studied definite diagnosis (Gold standard) was obtained only for 131 cases. On analysis paraffin embedded sputum cell block specimens had a higher sensitivity of 48% compared to 29% in routine sputum smears. But 2 cell block positive cases which had radiological proof were not confirmed to be cancer by other tests. As these cases had radiological proof further follow up may be needed before considering cellblock to have low specificity.

CONCLUSION

In a country with economical constraints this simple technique may help to avoid costly investigative measures for early detection of a tumour with high mortality

KEYWORDS

Sputum smear, Paraffin embedding, Bronchogenic carcinoma.

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BACKGROUND

Lung cancer is one of the most commonly diagnosed cancer worldwide.[4] With 1.61 million new cases of lung cancer per year, and 1.38 million deaths, it becomes the leading cause of cancer related mortality. In India, approximately 63,000 new lung cancer cases are reported each year.[5]

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Sputum cytology is a simple, cheap and noninvasive test for the diagnosis of bronchogenic carcinoma especially in elderly patients where bronchoscopy is contraindicated or where this facility is not readily available.

Cellblocks can be a useful substitute to smears for establishing a more definitive cytopathologic diagnosis. These paraffin embedded cellblocks have an advantage over smears since they can be reported like biopsy specimens and further studies like immunohistochemistry (IHC) and molecular tests may be done with it if needed.[6]

Aims and Objectives

1) To compare the efficacy of paraffin embedded sputum and sputum smear cytology in the diagnosis of Bronchogenic Carcinoma.

2) To compare the efficacy of paraffin embedded sputum and sputum smear cytology in tumour typing of Bronchogenic Carcinoma.

Setting and Design

This was a prospective study which included all patients who had a clinical diagnosis of bronchogenic carcinoma and underwent sputum smear examination during a period of 1 year in Government Medical College hospital, Kozhikode.

Inclusion Criteria

Patients who had a clinical diagnosis of bronchogenic carcinoma based on their symptoms, signs and radiological features.

Exclusion Criteria

1. Cases in which a definitive diagnosis was not obtained even after a gold standard investigation such as biopsy.
2. Patients with clinical diagnosis other than lung cancer.

MATERIALS AND METHODS

Early morning sputum samples of eligible subjects on three consecutive days were collected in a wide mouthed container after deep inhalation or preferably after steam inhalation. (Samples were taken after rinsing the mouth, before intake of food). A portion of these samples were initially examined for their quality, colour and consistency (mucoid, hemorrhagic or purulent). Smear preparation was done by the Pick and smear method and then stained by Papanicoloau method.

Second portion of collected sample was fixed in Bouin's fluid (Saturated picric acid + glacial acetic acid + formalin). Next day the whole sample was filtered and processed .After that it was paraffin embedded and multiple sections were taken and stained with Hematoxylin and Eosin stain.

Sputum Examination

Both sputum cytology smear and paraffin embedded histopathology sections were examined by two observers (principal investigators) blind to the clinical status of the patient and blind to the result of each other.

The gold standard for diagnostic confirmation was either cytology, biopsy of lung or lymph node (Lung FNAC, Lymph node FNAC/Biopsy, Bronchial brushings, Bronchioloalveolar lavage or Pleural fluid cytology.)

Final Evaluation

The reports of sputum smear and paraffin embedded sections were tabulated and results were compared with that of the gold standard investigation result.

Data Analysis

For the analysis, patients were divided into two groups:

Group A: 'Cancer' group: Patients confirmed to have bronchogenic carcinoma on follow up.

Group B: 'No cancer' group: All other patients found not to have cancer despite repeated investigations. The groups A and B were compared for both the sputum smear and paraffin embedded cell block results to find-

1) Sensitivity and specificity of the tests and
2) Whether the lung cancer cell types could be identified.

Statistical Analysis

The results of both smear and cell block were analyzed to calculate their sensitivity, specificity, positive predictive value and negative predictive value in correlation with the gold standard (Diagnostic result influencing the management of the patient).

Ethical Clearance

This study has been approved by Institutional Ethics Committee of Government Medical College, Kozhikode.

RESULTS

Patient Data

The distribution of the total number of cases and the number of samples examined per case are given in the tables 1 and 2. A total of 281 samples were studied in the 131 cases.

Total No. of Cases Studied	143
Total No. with Gold Standard Diagnosis	131

Table 1. Distribution of Cases included in Study

No. of Samples	No. of Cases	Percentage
1	38	29%
2	39	30%
3	51	39%
4	3	2%
Total	131	100%

Table 2. Number of Samples Obtained Per Patient in Study

The studied patients varied in age from 40 yrs. to 90 yrs. The Fig. 1 gives the age distribution within the study. Major group belonged to the age group 60- 69yrs.

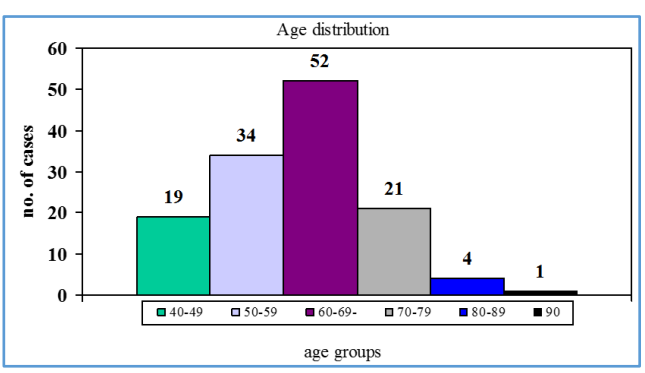

Figure 1. Age Groups with Number of Cases

Most of the patients admitted with features of lung cancer were males (Table-3).

Sex	Frequency	Percentage
Male	128	97.7%
Female	3	2.3%
Total	**131**	**100%**

Table 3. Sex Distribution of the Cases Included

Final Diagnosis	Frequency	Percentage
Cancer	100	76.3%
No Cancer	31	23.7%
Total	**131**	**100%**

Table 4. Number of Cases in the Final Diagnostic Groups

All the cases included were divided into two groups on follow up A) 'Cancer' group: Patients treated as bronchogenic carcinoma B) 'No cancer' group: Patients found not to have cancer despite repeated investigations. Table 4 reveals that 23.7% cases belonged to non cancer group in this study. This included those finally diagnosed as tuberculosis, lung abscess, interstitial lung disease or those who could not be diagnosed positively as lung cancer. One of the cases was diagnosed as mediastinal lymphoma.

The results obtained with sputum smear and cell block were broadly grouped into 4 categories either inadequate, negative, positive or suspicious for malignant cells.[3] Figure 2 and 3 presents the distribution of sputum smear and cell block results obtained in the 131 patients.

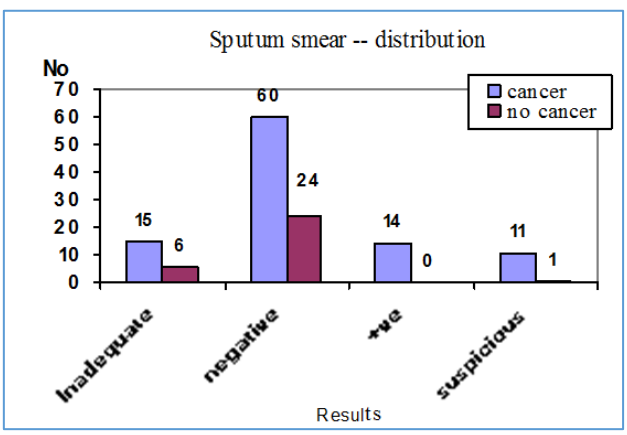

Figure 2. Sputum Smears with Number of Cases in each Diagnostic Group

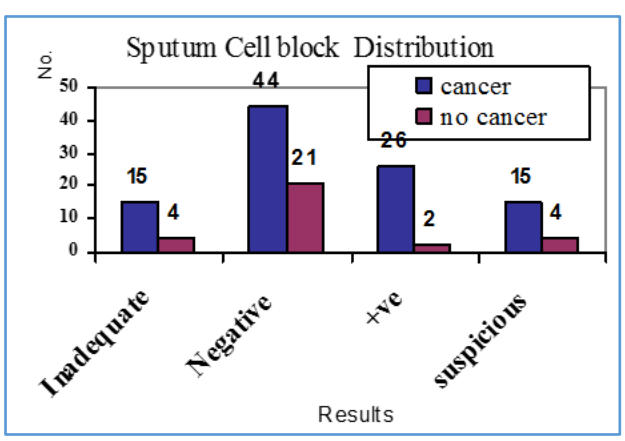

Figure 3. Sputum Cell Blocks with Number of Cases in Each Diagnostic Group

Correlating the results of smear and cell block with the gold standard investigation result obtained on follow up the sensitivity specificity, positive predictive value and negative predictive value was calculated To calculate the sensitivity and specificity the 4 groups (inadequate, suspicious, positive and negative) were regrouped into 2 groups (positive and other than positive) In other than positive were the inadequate, suspicious and the negative. In previous studies the sensitivity was calculated by omitting the inadequate samples.[3] Also as the suspicious results were significant when the radiology was diagnostic they were included in the positive. Thus their sensitivity was calculated by eliminating the inadequate samples and including the suspicious within the positive. Comparative results for sputum smear and cell block are given in table 5.

	With Inadequate Samples Included in the Analysis		Excluding Inadequate Samples &. with Suspicious Samples included in Positive	
	Smear	**Cellblock**	**Smear**	**Cell Block**
Sensitivity	14%	26%	29%	48%
Specificity	100%	93.5%	96%	78%
Positive Predictive Value	100%	92.8%	96%	87%
Negative Predictive Value	26.5%	28%	28.6%	32%

Table 5. Statistical Analysis of Sputum Smear and Cell Block Results

On analysis paraffin embedded sputum cell block specimens had a higher sensitivity of 48% compared to 29% in routine sputum smears. (Table 5). But 2 cell block positive cases which had radiological proof were not confirmed to be cancer by other tests. As these cases had radiological proof further follow up may be needed before considering cellblock to have low specificity.

Since type of lung cancer has to be identified for definitive treatment an analysis of the types of lung cancer picked up in smear and cell block was compared. From figure 4 we can make out that squamous cell carcinoma is the most commonly diagnosed entity followed by adenocarcinoma. Small cell carcinoma was picked up only in cell block. (Figure 4) This is very important as the mode of treatment is different for small cell carcinoma.

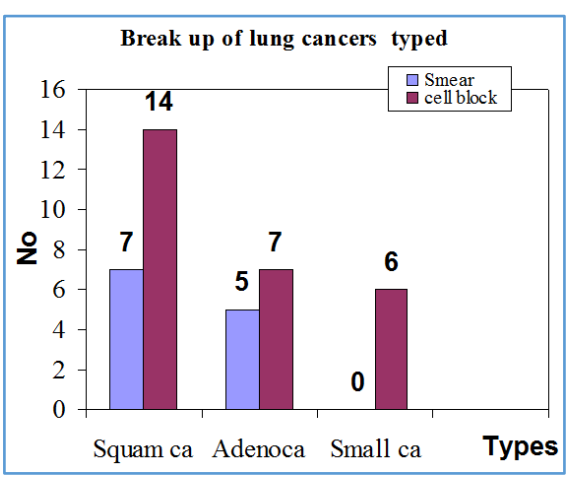

Figure 4. Number of Cases of Lung Cancer Typed in Smear and Cell Block

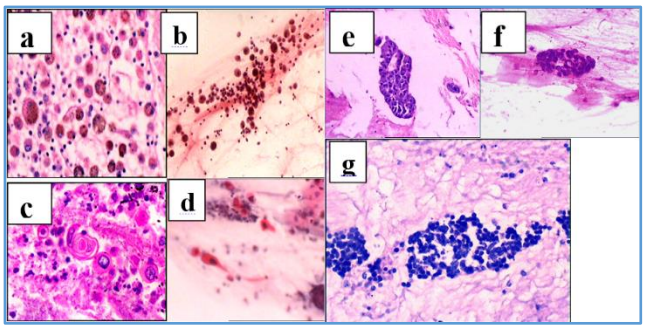

Figure 5. a) Adequate Sputum Sample- Carbon Laden Macrophages in - Cell Block. b) and in Smear. c) Squamous Cell Carcinoma – (Malignant Pearl)- Cell Block. d) Tadpole Cells - Sputum Smear e) Adenocarcinoma Cell Cluster -Cell Block f) Adenocarcinoma in Smear g) Small Cell Carcinoma Cells in Cell Block

The figure 5 bring out the normal and abnormal patterns which can be discerned in cell block and in smear preparation An important finding was that tumor type small cell carcinoma was diagnosed positively only in cell block and not in smear. (Figure 5g)

DISCUSSION

Lung cancer being a disease with high mortality its early detection is indeed important.[7] The screening method with the least discomfort to identify lung cancer would be a sputum examination.[8] Even the small amount of sample received from each patient is not completely examined in the pick and smear method. So the chances of missing a diagnostic cell are high. This is overcome by sputum cell block. The filtration method which we advocated for preparing paraffin embedded sputum cell block is a simple, quick procedure retaining the morphological patterns. The centrifugation methods previously used for preparing sputum cellblock were cumbersome procedures dispersing the cell clusters. When one does only the Pick and smear method the rest of the sample is lost where as in paraffin embedded method it can be stocked for years to do special stain/ immunohistochemistry later if necessary.

Another advantage of the embedded sample compared to that of smear is the ease with which paraffin embedded section can be screened for malignant cells. Thus the chances of not identifying a malignant cell if present are markedly reduced. This is due to the small area that need to be screened in paraffin embedded section and the fact that Hematoxylin eosin under stains the mucus providing a contrast to the malignant cells.[3]

On analyses (table 5) the paraffin embedded sputum had a higher sensitivity (48%) compared to the smear (29%). Specificity of cell block was lower than that of smear. This could be accounted. The 2 cases (Figure 3) diagnosed by paraffin embedding had all radiological features of lung cancer. One of the cases later proved to be lung cancer. So as the radiological features may be taken as gold standard for diagnosis the specificity could be considered 100% in cell block too.

Bocking[3] did a similar study on the diagnostic accuracy of sputum cytology for bronchogenic carcinoma using paraffin-embedded, serially sectioned and hematoxylin and eosin-stained specimens (tested in 4,297 sputum samples from 1,889 patients, 219 of whom had bronchial carcinoma) The diagnostic sensitivity depended mainly on the number of investigated samples and was 85.4% with three sufficient sputa. The sensitivity was not influenced by the histological types, location or TNM stage of the tumor. The specificity of the method was 99.5%.[3] This he had added is an improvement on the previously done studies on sputum by others. He had reasoned out the cause for the low sensitivity values in previous studies as being due to inclusion of inadequate samples in the calculation.

In another study by Erkilic[9] a higher sensitivity and specificity was achieved for a similar paraffin embedded cell block technique. They got a sensitivity of 69.4% and specificity of 99.5% for the smear method and a sensitivity 84.4% with specificity of 100% for the cell block method. The sensitivity of the smear and cell block together was 87.6% and specificity was 99.5%.[9]

	Present Study	**Bocking[3]**	**Erkilic[9]**
Sensitivity Smear	29		69.4
Sensitivity Cell Block	48	85.4	84.4
Specificity Smear	96		99.5
Specificity Cell Block	78	99, 5	100
Total Samples	281	4297	2524

Table 6. Comparison of this Study Results with Similar Studies

The diagnostic sensitivity of sputum cytology depends on several factors, including time, method of collection, number of samples submitted, tumor cell type, as well as size and location of the lesion. So the false-negative results in most cases can be attributed to improper specimen collection, sampling error, peripheral location, small tumor size, misinterpretation, too few specimens, tumor obstruction of bronchus or tumor type (small cell ca., metastatic carcinomas, benign tumors)[3]

The comparatively low result (table 6) in our study could be attributed to many factors-

- Fault in collection of sputum sample by patient
- Acceptance of the mere presence of carbon laden macrophages for sputum adequacy To repeat the words of Risse, "the mere presence of alveolar macrophages may not be sufficient evidence for a high-quality specimen since these cells, in lower numbers, are consistently found in sputum specimens with a false-negative cytological diagnosis."[10]
- Low number of samples- In our study we received 4 samples in 3 cases (2%) 3 samples in 51 cases (39%) 2 samples in 39 cases (30%) and 1 sample in 38 cases (29%). According to Grunze a single specimen is unreliable and Bocking says that three satisfactory

sputum specimens are necessary to detect 60% of cases of lung cancer.[11] Thus it could be a reason for false negative results.

- Inability to include post bronchoscopy sputum samples in the study as the patients underwent bronchoscopy as an out –patient procedure. .This was a disadvantage as studies have shown that post Bronchoscopy samples have the highest pickup rate and moreover it may be the only positive specimen when all other samples are negative.[12]

A smear examination of sputum has its own advantages. It is a quick procedure which does not require the extra time needed for processing and cutting as in paraffin embedding method .All that it requires is the expertise in identifying the essential area while preparing and reporting the smear. Also it has an additional advantage in that it brings out the cell morphology better as it uses the Papanicolau stain unless it is obscured by the mucus which it stains with the same intensity.

Using strict diagnostic criteria, false- positive cytological diagnoses are rare and patients may be treated on the basis of the cytological diagnosis, without tissue confirmation, in the appropriate clinical setting.[13]

The use of sputum cytology in tumor typing was examined by a multiparametric study, which included diameter, cavitation, site and histological type of the pulmonary tumors. They reported that sputum was diagnostic for neoplasms of the left upper lobe and that the sensitivity related to the histological type was not independent of the site, diameter or cavitation. In that study the overall cytological typing accuracy was 77%.[14] In this study typing accuracy of paraffin embedded method was 25% and smear method was 12%. The various types of lung cancer diagnosed in smear and cell block are seen in Figure 4.

According to previous studies[15] the accuracy in typing of lung cancer is better in squamous cell carcinoma and small cell carcinoma than in adenocarcinoma. Though squamous cell carcinoma was the one most often accurately diagnosed in our study the next in diagnostic typing correlation was adenocarcinoma rather than small cell carcinoma. The paraffin embedded sample picked up small cell carcinoma better than smear (Figure 4 & 5g). A total of 25 cases were definitely typed in paraffin embedded samples while in smears only 12 were definitely typed (Figure 4).

Histological patterns of the tumour are retained in paraffin embedded samples, which help to delineate them into particular types. This is well illustrated in the figures given with the results especially the ones which show the malignant pearl and the one which illustrates the glandular pattern (Figure 5).

Though this paraffin embedded sputum is a time consuming procedure when the samples are adequate it has a high value as a screening tool because of the ease with which even an inexperienced person can pickup diagnostic malignant cells in it.

CONCLUSION

- The results obtained with paraffin embedded sputum mainly depended on the quality of the sample It does not require additional expertise. (Sputum smear sensitivity relies highly on the ability to pick up the right area in the sample and on the slide). Though paraffin embedding consumes time in preparation of slide the actual screening procedure can be quickly done.

Paraffin embedded cellblock method positively detected small cell carcinoma (which was not diagnosed in smear) Cell blocks can be stored and if necessary may be used for special studies at a later stage.

The lower sensitivity obtained in this study (compared to literature) may be improved by requesting adequate fresh samples to replace grossly inadequate samples and by utilizing post bronchoscopy samples.

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