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STUDY OF TELOMERASE ACTIVITY IN SPUTUM AND BRONCHIAL WASHING SAMPLES OF LUNG CANCER

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ABSTRACT

Objective: Diagnosis of lung cancer with conventional methods may sometimes be difficult in therapeutictrials. Telomerase show a significant role in the maintenance of telomere length and chromosome integrity. In this study we investigate the diagnostic value of telomerase activity in sputum and bronchial washings samples of patients undergoing diagnosis of lung cancer. **Method:** Samples was collected from 58 patients whom suspected for lung cancer and had evidence of lung pulmonarydisease, the diagnosis confirmed by cytological examination. Samples were examined for telomerase activity was measured by a PCR-ELISA designed telomeric repeat amplification protocol in lung cancer patients. **Result:** Out of 58 samples 48 samples of sputum and bronchial washing from individuals of lung cancer patients and 10 from lung diseases were collected from individual. TA evaluation of sputum carried sensitivity, specificity and diagnostic accuracy of 80.9, 100%and 72.4% respectively, while that for bronchial washing was 66.6, 88.8% and 70.0%, respectively. A significant correlation ($P < 0.04$) was found between age and telomerase activity in sputum and bronchial washing samples but no positive association was perceived between gender and telomerase activity. A significant relationship found between smoking habits with telomerase activity (0.046). A positive correlation was also observed between staging and telomerase activity in sputum and bronchial washing samples ($P < 0.01$). Our findings show that telomerase activity in sputum and BW is a highly sensitive diagnostic biomarker for malignancy and it can be a complementary for cytology in the early diagnosis of lung cancer. TA test for sputum can be a noble biomarker for early and non-invasive diagnosis of lung cancer.

KEYWORDS: Telomerase activity, lung cancer, sputum, Bronchial washing.

INTRODUCTION

Lung malignancies is the most common cause of cancer mortality and death worldwide, estimated to be responsible for nearly one in five (1.8 million deaths, 19.4% of the total).[1] The restricted mortality benefit from current progress in lung cancer research highlights the need to develop early detection technique with the treatment plans; lung cancer is mostly detected at an advanced stage, resulting in a general 5-year survival of 15%.[2] Prognosis greatly improves if lung cancer is detected at an early stage.[3] In the past studies thorax x-ray screening studies have been lead for the early stage of lung cancer, sputum examination by cytological technique was part of the diagnostic procedure.[4] Sputum cytology turned out neither to be additive value in enhancing lung cancer detection nor in reducing lung cancer mortality. The average survival time increased after thorax X-ray screening because of lead time and sampling bias.[5] Earlier studies have been used morphological and functional characteristics for describing the advanced stages oflung carcinoma, but this also not help to reducing mortality of cancer. In this study we explored the investigative utility of sputum as a non-invasive biomarker and potential of defining bronchial washing telomerase activity from the same individual in high risk lung cancer patients. Telomerase is a ribonucleoprotein that synthesises telomeric sequences, telomerase provide a protective capping at the chromosomal end with the tandem repeat of TTAGGG in humans. Telomeres progressively shorten with each cell division in all somatic cells because of incomplete replication of DNA at the chromosome ends.[6,7,8] The enzyme telomerase is a ribonucleic protein with function of resynthesizing the telomeric DNA of the chromosomal ends. It maintains the telomeric length giving the cell to opportunity for uncontrolled cellular division DNA. Hence, telomere length and telomerase activity are essential for cancer initiation and the survival of tumors.[9] Using a highly sensitive polymerase chain reaction-based telomeric repeat amplification protocol assay, early studies reported finding telomerase activity in most primary lung cancer samples.[10, 11] PCR- trap assay has made it possible to detect telomerase activity with only few malignant cells in the sample.

Telomerase activity is thought to be a critical event responsible for cancer progression and immortalization.

Most human tumor express telomerase activity including lung cancer[12,13], this enzyme normally detected only in reproductive cells and cells with self-renewal capacity, but it is undetectable in normal somatic cell.[14] Most of human normal somatic cells are telomerase negative, telomere shortening progresses with each cell division. The fact directed investigators to propose that telomeres may act as a mitotic clock: once a critically short length is reached, cells encounter a proliferative barrier which may be overcome by the introduction of telomerase activity and the subsequent telomere length maintenance.[15] Modification found in the telomerase repression pathway, which cause reactivation of enzyme and maintenance of telomere length this will be essential in the immortalization process for the cancer development and survival of tumors.[16] Finally telomerase activity in and/before another mechanism that maintains telomerase is necessary for the continued proliferation of cells and is a critical, rate limiting step in cancer progression.[17] We sought to determine the role of telomerase in lung cancer diagnostics in fresh obtained lung cancer samples: sputum and bronchial washing by ELISA-PCR based trap assay.

MATERIAL AND METHOD

This study is analysis of 58 patients with cytologically/histologically confirmed lung cancer and lung diseases treated at the Cancer Hospital and Research Center Gwalior, India. The study received formal approval from the institutional ethics committee with the informed consent from all patients on proforma. There were a total of 58 samples were examined in the study with of which 48 were suspected to primary lung cancer patients. Of 58 samples 40 male, 18 female, with the mean age 55+12; 21 adenocarcinoma, 23 squamous cancers and 4 small cell lung cancer.10 cases were cytologically diagnosed negative for lung cancer as having non-malignant lung diseases and used as a control for this study of these 10 control cases, 7 male, 3 female; mean age 50 + 10 with 6 pulmonary tuberculosis, 4 Old benign Fibrosis. Sputum samples were collected before bronchoscopy in all the samples. Instruct the patient to inhale (3% hypertonic saline) deeply 2-3 times and were then asked, cough up deeply from the chest and spit in the sputum.[18] The bronchial washings samples (20 ml of 0.9% sterile saline) were aspirated via gentle suction.[19] The bronchial washings were obtained after imagining the growth. The first aspirated sample was used for the standard cytological examination and the second suction was used for telomerase assays.The sputum and bronchial washing samples were centrifuged at 2500 RPM for 5 min at 4^{o}C and washed twice with cold, sterile phosphate buffer saline (PH 7.4): during the process excess mucus was removed.[17] The supernatant was discarded and sediments were stored at -20^{o}C till further analysis. Viable count was determined. Cytological examination was also done for sputum and washing samples in blinded manner.

Telomerase Activity Assay-In our study, we have identified the telomerase activity in 48 samples by telomerase TRAP-ELISA assay and staining telomeric repeat amplification protocol.[20,21] The high sensitivity of the PCR-based TRAP assay has allowed the analysis of minimal tissue samples, such as fine-needle aspirates of the lung and liver, breast and thyroid, cervical smears, oral washings, and urine.[22-27] The sputum and bronchial washing samples pellet was washed with 1ml of PBS and the mixture was centrifuged again for 5 minutes to ensure that the cell bead complexes are all collected. And after removing the supernatant cells were re-suspended in 200µl ice cold lysis buffer and incubated for 30 minutes on ice. Cell debris was removed by centrifugation on 16000x g for 20 minutes, at 4^{o}C and then 80-90µl of supernatant was removed, taking care not to transfer any cellular debris, from the total collected extract 3 µl extract will be used for PCR reaction and remaining used for protein estimation. The protein concentration for each extract was determined by the lowery method.[28] An aliquot of extract containing 2µg of protein was used for the assay of telomerase. Two sets of aliquots of the above master mix were prepared; one was treated with heat (85^{o}C for 10 minutes) to inactivate the samples for use as a negative control, and one was the test. Then the related tubes were subjected to thermal cycling according to the protocol.[29] TRAP reaction products were analyzed on a 10 % non-denaturing DNA PAGE containing 0.5X TBE with 0.0025% methylene blue solution. The gel was run for2 hour and 15 mins at 110 volts. The gel was fixed in 10% ethanol for 10-15 min and then visualizes the 36bp DNA ladder pattern was observed on UV transilluminator.[17]

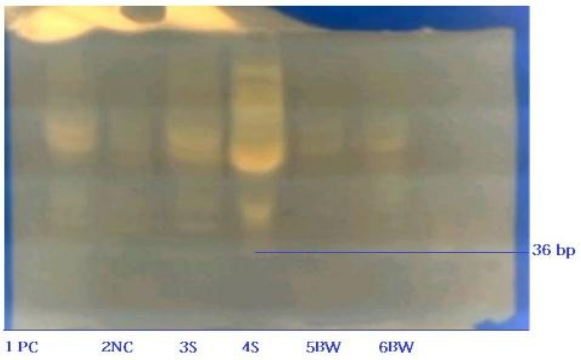

Fig1. TRAP assay. Lane I, positive control(1 RU); Lane 2, negative control (0RU)l; Lane 3, (11) sputum sample (2RU); Lane 4, (30)sputum sample (1RU); Lane 5, (6)bronchial washing sample (3RU); Lane 6,(26)bronchial washing samples (2.5).

A sample was considered as positive for telomerase activity when the 36basepairs internal control band like to that telomerase positive control lane were present in the lanes 1, 3, 4, 5 and 6 figure1.No ladders of PCR products were present considered as negative while extracts that indicates 36 base pairs internal control band lane 2. All result values were shown in RU unit (telomerase activity stated in relative unit) relative telomerase activity for samples was ordered as strong

(1), moderate (2) and weak (3). All samples were performed in duplicate.

Statistical Analysis

Results were evaluated by statistical analysis by SPSS 2.0 system, the associations between telomerase activity and the clinicopathological features were analyzed by chi square analysis. When the expected value was <5, we used Fisher's exact test. We calculated the sensitivity, specificity and diagnostic accuracy of telomerase activity in sputum and bronchial washing samples. Correlation between age and telomerase activity was done by two-sample t-test. To check relationship between telomerase activity and staging of lung cancer was analyzed by multivariate analysis (ANOVA). All P values are two-sided. Values of *P*<.05 were considered significant.

RESULT

Cytological data and telomerase activity in samples.

Cytological data.

The samples that were account for lung cancer cases subjected to cytological examination and telomerase activity but in control cases only cytological examination were performed. The data are given in Table 2. Out of 48 clinically suspected primary lung cancer patients, 42 cases finally turned out to be malignant in which 42 form NSCLC cells including, 21(50%) Squamous cell carcinomas, 17(40.5%) Adenocarcinoma and 4 (9.5%) Small cell lung cancer while 6(12.5%) were confirmed as benign by cytological examination. Most of the cases were of stages III and IV however some cases were also found of stage II. In some cases repeat cytology was done with new sputum samples in which the previous cytological examinations were non-conclusive. By the cytological examination of pre-bronchoscopic sputum diagnosed malignant cells in only 11(26.2%) in first attempt and total 15(35.7%) cases in second tries. Cytological examination of the bronchial washings could diagnose malignancy in 18 (42.8%) cases. The six cases identified negative by cytologically examination for malignancy of sputum, and bronchial washing samples respectively. Examination of the 10(100%) controls samples did not show any malignancy.

Telomerase activity

Out of 42 cytologically/histopathologically confirmed malignant cases shown in table 2, Moderate to high positive telomerase enzyme activity observed in 34 (80.95%) sputum samples and 28 (66.66%) bronchial washing samples. Telomerase activity was detected in all the 15 (35.7%) and 18 (42.8%) cytologically positive specimens. Remarkably, telomerase activity was more detected in 19 (42.2%) sputum samples and 10 (23.8%) bronchial washings, which were give negative cytology report in first attempt.10 samples which were served as control cases of patients suffering from pulmonary disease other than lung malignancy, out of the these 10 control cases low telomerase activity could be detected in 2 samples (20%), 1(50%) from sputum and 1(50%)from bronchial washing due to metastatic or inflammatory cell. But, no telomerase activity was detected in the sputum and bronchial washings samples from the six diagnostically suspected but benign cases.

The sensitivity and specificity of cytological examination of sputum were 35.7 and 62.5% while the same rate were 80.9, 100% for telomerase activity assay and cytological examination for bronchial washing carried were 42.8, 100% and telomerase activity for bronchial washing carried a sensitivity and specificity were 66.6 and 88.8% respectively. Diagnostic accuracies Cytological examination and telomerase activity were and 70.0% and 72.4%, respectively.

Statistical analysis

Using two sample t-tests, a positive significant association was seen between age and telomerase activity in sample cases (P < 0.04*). No correlation between sex and telomerase activity in sputum and bronchial washing samples was observed with the chi-square test (.851).A positive correlation was found between telomerase activity and smoking (P<.043*) with the fisher's exact test. Significantly positive relationships were established between staging and telomerase activity in sputum and bronchial washing cases (P < 0.01) using multivariate ANOVA analysis. But no significance (0.74) was found between the cell types of lung cancer and telomerase activity.

Table no. 1. Telomerase activity significance in lung cancer samples depending on various clinical and pathological factors.

Clinical and pathological Characteristics		Cytologically positive No (%)		Telomerase positive No (%)		p value
Samples		**Sputum**	**BW**	**Sputum**	**BW**	
No. of Malignant Patients	48	15	18	34	28	
Gender	Male	11	12	25	21	0.851
	Female	4	6	9	7	
Smoking status	Yes	10	12	22	19	0.043*
	No	5	6	12	9	
Cell type of lung cancer	Adenocarcinoma	4	8	13	11	0.74
	SCC	8	9	17	14	
	Small cell carcinoma	3	1	4	3	
Stage of lung cancer	II	2	3	1	4	0.01*
	III	9	10	22	18	
	IV	4	5	11	6	

#BW- Bronchial washing # SCC- Squamous cell carcinoma* p value < 0.005 (Significant value)

Table no 2: Results of cytological examination and telomerase activity with clinical features of lung cancer patient and control cases.

Sr.no	Age/sex	Smoking status	Stage	CS/TA Sputum		CS/TA BW		Final diagnosis
1	65/ M	Yes	III	+	2	-	0	Adenocarcinoma
2	42/M	Yes	IV	-	1	+	0	Adenocarcinoma
3	42/F	No	IV	+	2	+	2	Adenocarcinoma
4	37/M	No	III	-	1	-	1	SCC
5	39/M	Yes	III	-	0	-	2	Adenocarcinoma
6	42/F	No	IV	-	1	+	3	Adenocarcinoma
7	45/M	Yes	0	-	0	-	0	Negative
8	41/M	No	III	-	2	-	1	Small Cell Carcinoma
9	46/M	Yes	III	+	2	-	2	Adenocarcinoma
10	69/M	Yes	II	-	0	-	0	SCC
11	67/M	Yes	III	+	2	+	2	Adenocarcinoma
12	48/F	No	III	-	2	-	3	SCC
13	45/F	No	III	-	2	-	2	Adenocarcinoma
14	64/M	No	IV	+	0	+	1	SCC
15	38/M	Yes	III	-	2	-	2	Adenocarcinoma
16	42/F	No	III	-	0	-	0	Negative
17	50/M	Yes	II	-	2	-	0	SCC
18	66/M	Yes	IV	+	1	+	1	Small Cell Carcinoma
19	35/F	Yes	IV	+	0	+	2	Adenocarcinoma
20	47/F	No	II	-	2	-	0	Adenocarcinoma
21	59/F	No	III	-	2.5	-	0	Adenocarcinoma
22	65/F	Yes	IV	+	2	+	2	SCC
23	46/F	No	III	-	0	-	0	Negative
24	50/M	No	II	-	2	-	0	Adenocarcinoma
25	68/M	Yes	III	-	0	+	0	Adenocarcinoma
26	67/M	No	IV	+	1.5	+	2.5	Small Cell Carcinoma
27	55/M	Yes	III	-	2	-	0	Adenocarcinoma
28	65/M	Yes	III	+	2	+	2	SCC
29	52/M	No	III	-	0	-	0	SCC
30	55/M	No	IV	+	1	+	1	Adenocarcinoma
31	50/F	No	IV	-	2	-	0	SCC
32	54/M	Yes	III	-	0	+	0	SCC
33	56/F	No	IV	+	2.5	-	2.5	Adenocarcinoma
34	60/F	Yes	III	-	0	-	0	Negative
35	58/M	Yes	II	-	3	-	3	SCC
36	38/F	No	IV	+	1	+	1	Adenocarcinoma
37	54/M	Yes	II	-	2	-	2	SCC
38	42/M	Yes	III	-	2	+	2	Small Cell Carcinoma
39	67/M	No	III	-	2	-	2	SCC
40	54/M	Yes	IV	+	2	+	1	Adenocarcinoma
41	69/M	Yes	IV	+	1.5	-	2	SCC
42	46/M	No	III	-	3	-	2.5	Adenocarcinoma
43	55/F	Yes	II	-	0	-	0	Negative
44	64/M	Yes	IV	+	1	+	1.5	SCC
45	33/M	Yes	IV	+	2	+	0	SCC
46	62/M	Yes	III	-	2.5	-	3	Adenocarcinoma
47	62/M	No	IV	+	0	-	0	Negative
48	48/M	No	II	-	0	-	0	SCC
49	42/F	No	0	NA	NA	NA	NA	Tuberculosis
50	37/M	Yes	0	NA	NA	NA	NA	Tuberculosis
51	64/M	No	0	NA	NA	NA	NA	Tuberculosis
52	50/M	No	0	NA	NA	NA	NA	Benign old fibrosis
53	47/F	No	0	NA	NA	NA	NA	Tuberculosis
54	67/M	Yes	0	NA	NA	NA	NA	Benign old fibrosis

55	52/M	No	0	NA	NA	NA	NA	Tuberculosis
56	38/F	No	0	NA	NA	NA	NA	Benign old fibrosis
57	42/M	Yes	0	NA	NA	NA	NA	Tuberculosis
58	54/M	Yes	0	NA	NA	NA	NA	Benign old fibrosis

#Cytological data shown as +&-, Telomerase activity expressed in RU(1,2,3) M-Male, F-Female, SCC-Squamous cell carcinoma, CS- cytology of sputum, TS- telomerase activity of sputum, CW-Cytology of BW, TW-Telomerase activity of BW.

DISCUSSION AND CONCLUSION

The present study was undertaken to detect the telomerase activity in the sputum and bronchial washing samples of benign and lung cancer patients. The current studies which evaluated diagnostic value of telomerase activity in malignant lung cancer cases reported that the sensitivity and specificity of this tumor marker were 80.9%, 100% and 66.6%, 88.8% for sputum and bronchial washing samples respectively. Its sensitivity and specificity study was also done in order to detect the lung cancer at early stages with the help of novel biomarker telomerase.

The routine lung cancer investigation usually starts with sputum cytology, chest x-ray, CT scan, and bronchoscopy. Clinically, with the use of these methods, the early detection of lung cancer or the differential diagnosis of an abnormal shadow on X-Ray is often difficult especially when cytological examination fails to detect cancer cells. The early detection and differential diagnosis of lung cancer is rather important as some patients with early stage NSCLC can be surgically cured.[30] Malignant cells, in general, have shorter telomeres than their normal counterparts[31] as a reflection of their extended proliferation. In fact, diseases of high cellular turnover are associated with telomere shortening, telomere dysfunction and cancer predisposition.[32] Telomerase activity can be able to detected few positive malignant cells in the sample. telomerase activity has been found in approximately 85% of the most common cancers such as breast, prostate, lung, liver, pancreatic, and colon cancers[33,34], indicating that telomerase activity detection may be act as a useful marker in cancer diagnosis.

Cytology is commonly used for lung cancer screening, but a final diagnosis based on cytology alone is often difficult. It is noteworthy that, in the present study, 80% of patients with class II–IV cytology had telomerase activity detected by the extract-based TRAP assay. These observations show one of the restrictions of morphologic cytology; meanwhile cytological samples may contain only few cancerous cells and have more degenerated cells. Therefore, the combination of telomerase activity as diagnostic biomarkers with cytology may prove more reliable in the diagnosis of cancer.[19]

The present study was undertaken with the lung cancer patients. Patients were considered and attributed in two groups with two individual samples sputum and bronchial washing viz. lung cancer with 48 (82.76%) and lung diseases with 10(17.24%). Telomerase activity indicates the presence of immortal cells and can be detected even in the presence of a few positive malignant cells. In this study, we used the TRAP-ELISA assay to study and compare the telomerase activity in noninvasive pre-bronchoscopy sputum with that of bronchial washings samples which were suspected for lung cancer, to evaluate the potential of telomerase as a possible biomarker for early diagnosis of lung cancer. Telomerase activity is identified with a high frequency in lung cancer tissues.[35,36,10] Yashima et al[36] reported that 94% (32 of 34) of lung cancer samples had detectable telomerase activity however Hiyama et al[10] reported telomerase activity in 80% (109 of 136) of primary lung cancer tissues.

In this present study, the data revealed that 34 of 48(81%) sputum and 28of 48 (67%) bronchial washing samples from lung cancer patients with had telomerase activity by use of extract based TRAP-ELISA assay. So, telomerase detection in sputum and bronchial washings cells associates well with the results obtained. The present studies results approve and extend earlier reports[35,36,10] that major ratio of lung cancers has telomerase activity. Prominently, our results strongly advise that sputum samples used for the early detection of cancer cells, if they had telomerase activity, in would-be lung cancer patients. Evident telomerase activity in exfoliated cells from the urine, colon and uterus[37,38,39] of noncancerous subjects was also reported in other diseases. Moreover, Yashima et al.[36] reported that approximately 20% of normal lung tissues of current smokers hold detectable telomerase activity. Measurement of telomerase activity may be most helpful when cryptological examination fails to detect cancer cells.

Telomerase activity has been measured in a number of clinical specimen types in our study that are also relevant to early diagnosis of a range of cancers like bladder cancer, thyroid cancer, colonic cancer and cervical cancer[40,41,42,43,44,38] and in that way improving treatment outcome. In study of Yahata et al they were used extract based fluorescent TRAP assay and an in situ TRAP assay which shown telomerase activity in bronchial washing positive in 18 out of 22 patients with primary or metastatic lung cancers, compared with only one of 19 patients without cancer.[19] Xinarianos et al. found telomerase activity in bronchial lavage samples to be 70% sensitive in patients with non-small cell lung cancer.[45] There is also a report of telomerase activity being detected in aggressive immune-associated lung disease.[46]

In this study table 1 shows a significant association exists between telomerase activity and staging and age but not the sex of the patients. This approves the findings of Albanel et al.[47] and Taga et al.[48] Telomerase activity has been related to tumor staging in earlier studies of neuroblastoma, breast cancer, gastric cancer, leukemia's[49,50,51,52]as well as lung cancer tissues.[47,48,53] Table 1 data also had shown the significance between smoking status and telomerase activity(<0.043) but not shown any relevance association between cell types of lung cancer and telomerase activity in our study.

In this study we found that no cases found in stage I and stage II was only 2 cases, the major cases from stage III and IV, a statistically significant correlation was seen between staging and telomerase activity in sputum (P < 0.01) and bronchial washing (P <0.01) using multivariate ANOVA analysis. Diagnosis by assessing telomerase activity in sputum is relatively favorable in some those cases in which bronchial washing samples were not taken because of cancers are peripherally located like adenocarcinoma, and also in patients whose general condition is not good for this procedure. In our study, moderate to high telomerase activity could be noticed in sputum and bronchial washings.

Thru cytological and histopathological examination of the sputum and bronchial washings diagnose malignancy in limited cases whereas telomerase activity could be detected in a higher number of confirmed malignant cases in the first attempt. On repeating the cytology examination the samples turned out to be positive for lung cancer in these patients which were gives positive by telomerase. Table no 2 have all details of patient and studies about all clinicopathological details with telomerase activity. Sen et al[17] and Naritoku et al[54] also recognized that telomerase sensitivity is higher than the cytology in their studies. Most of our patients 35 out of 42 were prolonged smokers with smoking habits so telomerase activity was seen in nearly most the smoker patients. Using fisher's exact test significant association could be found between smoking status and telomerase activity in all samples (P = 0.043) in agreement with the study of Xinarianos et al.[46], in which a significant correlation was found between the presence of telomerase activity and current smoking status at the time of diagnosis and a trend was also found between telomerase activity and smoking exposure.

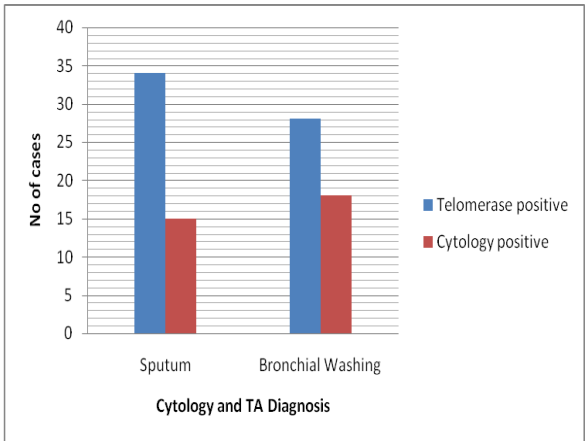

Fig 2: Comparison between cytologically and telomerase activity positive of sputum and bronchial washing samples of lung cancer.

Cytological examination was diagnostic in 15 samples of sputum and 18 samples of bronchial washing from lung cancer samples of patient, While Diagnostic yield reached up to 34 of sputum samples and 28 of bronchial washing samples of lung cancer patients when cytological examination combined with telomerase activity (Fig. 2). Telomerase activity and cytology together in lung cancer samples could detect more positive cases in the first attempt.

In our study we found 3 types of cell of lung cancer squamous cell carcinoma, adenocarcinoma and small cell lung cancer (SCLC) which were not have the significant association with the level telomerase in tumor. In 48cases, like squamous cell carcinoma was 23, adenocarcinoma was 21 and that of small cell lung cancer (SCLC) was four. During the study by cytological examination 42 cases found malignant. Cytological examination was diagnostic in 15(35.71%) of sputum samples from (8) SCC, (4) AC and (3) Small cell carcinoma. Similarly, the rate of telomerase activity in (17) SCC was greater than that of (13) AC. 34(80.9%) samples of sputum with SSC,AC and (4) Small cell carcinoma showed a positive telomerase activity, for Bronchial washing samples 28(66.6%) with (11)AC, (14)SCC and (3)small cell had a positive telomerase activity but for cytological examination only 18(42.8%) samples was shown positive for those cell types in which (8)AC, (9)SCC and (1) from small cell carcinoma. However the difference did not show statistical significance with the level of telomerase in tumor with in the group. (P=0.74) (Fig. 3).

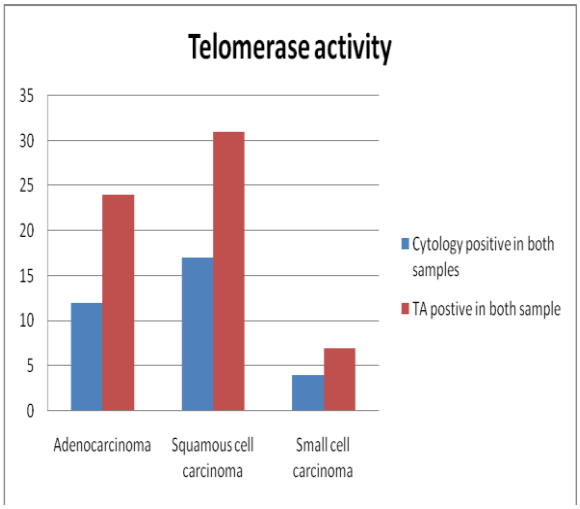

Fig 3: Comparison between Cytology and Telomerase activity regarding cell type in the lung cancer samples.

Our finding indicates in table no 1 that telomerase positivity was detected in 80.9% in lung cancer sputum and 66.6% in bronchial washing samples compared to lung disease cases (100%). Statistical analysis showed highly significant value of p<0.0001 for both samples that proved this method to be significant in identified malignant and non-malignant tissue. Previous studies reported by Several authors such as Hiyama et al[10]., Lee et al.1998[55]., Yang et al. 1998[56], Shay et al[57,58,59] and many more reported very high telomerase activity in lung cancer tissue 75-85%.[60] These results, including our study find the conclusion that telomerase activity will be used as a novel diagnostic biomarker for lung cancer. Sputum telomerase assay hold the potential to diagnose lung cancer in its early stages without any difficulty and it is also a noninvasive technique for lung cancer patients. Our finding also indicates that telomerase is a precise biomarker for lung malignancy and can complement to cytology and histopathology in the diagnosis of lung cancer.

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