ORIGINAL ARTICLE

Role of Serum CYFRA 21-1 in the Diagnosis of Primary Lung Cancer

Haroon ur Rashid[1], Solaiman Hossain[2], Rezaul Karim Chowdhury[3], Shamimur Rahman[4]
Mohammed Momenuzzaman Khan[5]

Abstract:

Majority of the lung cancer occurs in developing countries. In case of Bangladesh it's a burden for health in both sexes. A definitive diagnosis of lung cancer can help the surgeons and physicians for making decisions about the plan of treatment. The rapidity of diagnosis also alleviates the patient's anxiety whether the lesion is nonmalignant or malignant. Several studies proposed the role of CYFRA 21-1 level in diagnosis of lung cancer and its better management. To find the sensitive, feasible and cost effective test for detection of lung cancer and to evaluate the CYFRA 21-1 with their histopathological findings. This case control study was carried out in the department of Medicine of National Institute of Diseases of the Chest and Hospital (NIDCH) Dhaka and Enam Medical College Hospital, Savar, Dhaka during the period from January 2017 to September 2017. We included a total of 80 subjects among them 40 diagnosed cases of lung cancer and 40 controls with diseases other than lung cancer. The mean normal (< 3.5) serum CYFRA 21-1 level was (Mean ±SD) 1.10 ± 1.5 with ranging from 0.87 ng /ml – 1.5 ng /ml. Normal level of CYFRA 21-1 was found in all 40 (100%) of controls. The mean of high (> 3.5) serum CYFRA 21-1 level was (Mean ±SD) 18.20 ± 13.63 with ranging from 6.9 ng /ml – 49.30 ng /ml. High level of CYFRA 21-1 level was found in maximum 35(87.5%) cases. High (> 3.5 ng/ml) serum CYFRA 21-1 level was found maximum in squamous cell carcinoma (20), next adenocarcinoma (15). Among 40 cases diagnosed as malignant by histopathology, serum CYFRA 21-1 level was compared with histopathological findings. The sensitivity of serum CYFRA 21-1 level in case of squamous cell carcinoma, adenocarcinoma and combined were 90.90%, 83.33% and 87.5% respectively. The sensitivity and accuracy of serum CYFRA 21-1 for squamous cell carcinoma was more than that of adenocarcinoma. So serum CYFRA 21-1 level was highly sensitive for squamous cell carcinoma.

Introduction:

The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviors, particularly smoking, in economically developing countries[1]. About 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008; of these, 56% of the cases and 64% of the deaths occurred in the economically developing world. Lung cancer is the leading cancer site in males, comprising 17% of the total new cancer cases and 23% of the total cancer deaths[2]. Almost 50% to 70% lung cancer is still diagnosed in advanced stages[3]. In 2008 about 1.2

1. Associate professor of Pulmonology, Enam Medical College Hospital, Savar, Dhaka.
2. Associate professor of Cardiology, Enam Medical College Hospital, Savar, Dhaka.
3. Associate professor of Heamatology, Enam Medical College Hospital, Savar, Dhaka.
4. Associate professor of Nephrology, Enam Medical College Hospital, Savar, Dhaka.
5. Associate professor of Neurology, Enam Medical College Hospital, Savar, Dhaka.

million new lung cancer cases were diagnosed (Incidence rate 34.9 per 100000 and 11.1 per 100000 male and female respectively). In the world among them 1.1 million died[4]. Lung cancer is the commonest cancer in Bangladesh (22.4%) and Pakistan (20.1%), 3rd common in India (9%) and 6th in Sri Lanka (1.9%)[5]. Lung cancer is the most common fatal malignancy in both men and women, there are 150,000 new cases diagnosed every year in United States[6].

Major risk factor of lung cancer is cigarette smoking. 90% of lung cancer is due to cigarette smoking and passive or second hand smoking is other risk factor of death from lung cancer. A variety of techniques (*eg*, sputum cytology, flexible bronchoscopy [FB], and TTNA) are available as methods of achieving a definitive diagnosis. Positron emission tomography (PET) scanning has emerged as a helpful adjunct in both the diagnosis and staging of lung cancer[7].

Histological diagnosis may be obtained with sputum cytology, thoracocentesis, accessible lymph node biopsy, bronchoscopy, transthoracic needle aspiration, video-assisted thoracoscopy, or thoracotomy, chest computed tomography, positron emission tomography, and tissue confirmation of mediastinal involvement[8].

Sometimes determination of lung cancer may be difficult because of non informative sputum cytology, absence of visible endobronchial lesion by fiber optic bronchoscope and inadequate biopsy material or inaccessible location of tumour. Bronchoscopy, thoracoscopy, or open lung biopsy is sometimes contraindicated because of poor general condition of the patient[9].

Detection of tumour marker is a new and effective way of diagnosing and assessing prognosis of bronchial carcinoma. Several tumour markers have been described for lung carcinoma including CEA, CA125, SCCAg, NSE, TPS, TPA, CA 15-3, and TAG 72.3. Among them CEA and SCCAg are widely studied tumour marker[3]. CYFRA 21-1 is currently used in USA and Eastern Europe countries for diagnosis and prognosis of lung cancer[10]. CYFRA 21-1 sensitivity in small lung cancer is 40%, non small cell lung cancer 80%. Whereas specificity was 100% and over all sensitivity was 65.7%. CYFRA 21-1 sensitivity was gradually increased with the stage of lung cancer as 75%, 78% and 100% in stage I and II, III, IV respectively[9]. CYFRA 21-1 is a cytokeratin 19 fragment with 40 kDa. Cytokeratin 19 fragment is an antigen defined by two monoclonal antibodies BM 21-1 and KS 19-1. It is detectable in blood serum, plasma, and pleural fluid. Cytokines are released into the serum owing to cell lyses and tumour necrosis[11]. Serum CYFRA 21-1 recently has been shown to be the most sensitive tumor marker in non small cell lung carcinoma, particularly the squamous cell type and carcinoma in advanced stages[12,13].

Among tumor marker tests for non-small cell lung cancer, CYFRA 21-1 has consistently been shown to have the highest diagnostic sensitivity (~70 percent). In a prospective study of 211 patients with newly diagnosed, non-small cell lung cancer (stages I-IV), the sensitivity of CYFRA 21-1 determined after diagnosis but before treatment was 76 percent Molina R et al[14]. It also reflects the extent of the disease and has an independent prognostic role along with performance status and disease stage in NSCLC[13].

The aim of the study is to determine the sensitivity and specificity of serum CYFRA 21-1 for detection of lung carcinoma in Bangladesh by comparing the level serum CYFRA 21-1 with the gold standard histopathology.

Methodology:

A case control study (40 cases and 40 controls) was conducted in National Institute of Diseases of the Chest and Hospital, Dhaka and Enam Medical

College Hospital, Savar, Dhaka during the period from January 2017 to September 2017. Patients with diagnosed bronchial carcinoma were taken as cases and patients with diagnosis other than bronchial carcinoma were taken as controls. The samples were collected by non probable and purposeful sampling. Blood collected from samples were analyzed for CYFRA 21-1 in department of microbiology and immunology, BSMMU, Dhaka. The objectives were to determine the serum CYFRA 21-1 level of both case and control, to determine the sensitivity and specificity of serum CYFRA 21-1 level, to compare serum CYFRA 21-1 level with histopathological findings of bronchial carcinoma.

Results:

Table 1 shows age distribution of the study population. Among the different age groups 51-60 age group shows the highest 19 (47.5%) cases with bronchial carcinoma. The mean age difference was statistically significant (P = 0.05) between two groups in unpaired t test

Table I: Age distribution of the study population (n=80)

Age in years	Case (n=40)		Control (n=40)		*P* value
	n	%	n	%	
<40	0	0	4	10.0	
41-50	9	22.5	13	32.6	
51-60	19	47.5	17	42.6	
61-70	8	20.0	4	10.0	
71-80	4	10.0	1	2.6	0.001
>80	0	0.0	1	2.6	
Mean± SD	58.95 ±9.381		54.4±10.66		
Range (min-Max)	41-80		40-85		

P value reached from unpaired t test

Table 2 shows the serum CYFRA 21-1 level of the study population. The range of normal (< 3.5) serum CYFRA 21-1 level was 0.87 ng /ml – 1.5 ng /ml with Mean ±SD (1.10 ± 1.5). Normal level of CYFRA 21-1 was found in all 40(100%) of controls. The range of high (> 3.5) serum CYFRA 21-1 level was 6.9 ng /ml – 49.30 ng /ml with Mean ±SD (18.20 ± 13.63). High level of CYFRA 21-1 level was found in maximum 35(87.5%) cases. The mean difference was statistically significant (P < 0.05).

Table II: Level of Serum CYFRA among case and control (n=80)

Serum CYFRA level	Case(n=40)		Control (n=40)		Range	Mean ±SD	*P* value
ng/ml	n	%	n	%			
Normal <3.5	5	12.5	40	100	0.87-1.5	1.10±0.17	
High >3.5	35	87.5	0	0	6.90-49.30	18.20±13.63	
Total	40	100	40	100			0.001

P Value is reached from unpaired t – test

The table 3 shows normal (< 3.5 ng/ml) serum CYFRA 21-1 level was found in squamous cell carcinoma (2), adenocarcinoma (3). High (> 3.5 ng/ml) serum CYFRA 21-1 level were found maximum in Squamous cell carcinoma (20), next Adenocarcinoma (15). The mean difference was statistically significant (P <0.05).

Table III : Level of serum CYFRA with Histological diagnosis

Serum CYFRA level ng/dl	**Squamous Cell Carcinoma**	**Adenocarcinoma**	***P* value**
Normal < 3.5	2	3	
High > 3.5	20	15	0.0001
Total	22	18	

P value reached from unpaired t test

For 40 controls serum CYFRA 21-1 Mean ± SD was 1.08±0.16 with Range (Min-max 0.87- 1.50). At a cutoff value of 3.5ng/ml, the specificity was 100%. 22 cases with squamous cell carcinoma had serum CYFRA was 18.31±15.53 with range 1.11 – 49.30 at cutoff value of 3.5ng/ml, the sensitivity was 90.90%. 18 cases with adenocarcinoma had serum CYFRA was 21.11±11.53 with range 2.00- 39.00 at cut off value of 3.5 ng/ml, the sensitivity was 83.33%. The sensitivity of serum CYFRA 21-1 level in cases of squamous cell carcinoma, adenocarcinoma and combined were 90.90%, 83.33% and 87.5% respectively.

Table IV: Mean CYFRA 21-1 distribution according to histological diagnosis (n =80)

Histological diagnosis	N	Mean ± SD	Range Min-max	<3.5* ng/ml n	Sp (%)	>3.5 ng/ml n	Sn (%)
Control	40	1.08±0.16	(0.87- 1.50)	40	100		
Squamous cell carcinoma	22	18.31±15.53	1.11 – 49.30			20	90.90
Adenocarcinoma	18	21.11 ±11.53	2.00- 39.00			15	83.33
Combined							87.5

* Cutoff value of CYFRA 21-1 in serum < 3.5 ng/ml

Discussion:

Lung cancer in one of the most common cancers in both men and women. An estimated 1.6 million new cancer cases were diagnosed in 2008 and ranked second overall (23% of all cancer) worldwide[1,15].

Cytokeratins, especially fragment 19, are specified epithelial tissue-proteins that show increased levels in patients with carcinomas. A good number of studies were conducted to evaluate serum CYFRA 21-1 in lung cancers. Cytokeratins are the intermediate filaments of the cytoskeleton protein located in normal epithelia, tumor, and cultured cells. Recently, a fragment of cytokeratin subunit 19, referred to as CYFRA 21-1, detected in the serum of patients with non small cell lung cancer, has been reported as a new tumor marker[12].

In our study, high (>3.5 ng/ml) serum CYFRA 21-1 level was found in 35(87.5%) among 40 cases. Esmat Ali Abd EL- Nabi et al[9] conducted a case control study to investigate the role of CYFRA 21-1 in diagnosis, differentiation and staging of lung cancer. Among seventy patients with lung cancer (56 males and 14 females), mean age was 56.17 years, were divided according to the histopathological finding into 50 patients with NSCLC and 20 patients with SCLC. Thirty apparently healthy subjects were taken as a control. Serum level of CYFRA 21-1 was estimated by ELISA. It was significantly elevated in all types of lung cancer ($p< 0.05$) with a specificity of 100 %, and sensitivity of 65.7%; significantly elevated in non-small cell lung cancer compared to small cell lung cancer with a sensitivity of 80% and 40% respectively. Therefore CYFRA 21-1 is a tumor marker, with high sensitivity to NSCLC, valuable in the diagnosis and monitoring progression of lung cancer.

The mean normal (< 3.5) serum CYFRA 21-1 level was (Mean ±SD) 1.10 ± 1.5 with ranging from 0.87 ng /ml – 1.5 ng /ml. Normal level of CYFRA 21-1 was found in all 40 (100%) of controls. The mean of high (> 3.5) serum CYFRA 21-1 level was (Mean ±SD) 18.20 ± 13.63 with ranging from 6.9 ng /ml – 49.30 ng /ml. High level of CYFRA 21-1 level was found in maximum 35 (87.5%) cases. High (> 3.5 ng/ml) serum CYFRA 21-1 level were found maximum in squamous cell carcinoma (20), next adenocarcinoma (15).

In a study of Yoshinobu Maeda[16] serum soluble cytokeratin 19 fragments (CYFRA) levels were measured in 251 patients with lung cancer and 139 patients with benign lung diseases to determine the clinical usefulness of CYFRA level

determination in the diagnosis and monitoring of lung cancer. Serum levels of CYFRA were measured with the cut-off value of 3.5 ng/ml, which was associated with a specificity of 95% for benign lung diseases, CYFRA had a high sensitivity (53%) in all patients with lung cancer. They recommended that CYFRA may be a marker of choice for screening and monitoring of lung cancer, particularly squamous cell carcinoma.

In our study among 40 cases diagnosed as malignant by histopathology, serum CYFRA21-1 level was compared with histopathological findings. The sensitivity of serum CYFRA 21-1 level in case of Squamous cell carcinoma, Adenocarcinoma and combined were 90.90%, 83.33% and 87.5% respectively.

Combined positive predictive value (PPV) of CYFRA 21-1 in this study was 100% that was consistent with the findings recorded by Kulpa et al.[3] showing positive predictive value (PPV) of CYFRA 21-1 level was 100%. The combined negative predictive value (NPV) was 88.88. According to our study, the serum CYFRA 21-1 level associated with lung carcinoma and higher positivity of CYFRA 21-1 may be considered as an important marker in diagnosis of lung cancer.. CYFRA 21-1 is better marker for squamous cell carcinoma. Because of it's high specificity, the level of it should be interpreted as evidence for the presence of lung cancer in various histopathological types.

Conclusion:

CYFRA 21-1 showed higher positivity in squamous cell carcinoma than adenocarcinoma. The sensitivity of CYFRA 21-1 was more in squamous cell carcinoma than adenocarcinoma. Preference should be given on CYFRA 21-1 as a tumor marker in diagnosis of lung cancer. It can be used in combination with other diagnostic tools like Fiber Optic Bronchoscopy (FOB), Fine Needle Aspiration Cytology (FNAC) that would be more helpful for the patients.

Limitation of the study:

This study has some limitations. The study was done in limited time and cases were selected from single centre. The sample size was small.

Referances:

1. Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E. and Forman, D. (2011), Global cancer statistics. CA: A Cancer Journal for Clinicians, 61: 69–90.
2. GLOBOCON 2008.
3. Kulpa J, Wójcik E, Reinfuss M, Kołodziejski L. Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients. Clin Chem. 2002 Nov; 48(11): 1931-7.
4. Blankenburg F, Hatz R, Nagel D, Ankerst D, Reinmiedl J, Gruber C, Seidel D, Stieber PPreoperative. CYFRA 21-1 and CEA as prognostic factors in patients with stage I non-small cell lung cancer: external validation of a prognostic score. Tumour Biol. 2008; 29(4) : 272-7.
5. Jha R, Weerakoon A. P., Karki K. B., Shrestha S.Gamage P. W. K. Lung cancer and smoking in ASIA SAARC J. TUBER. LUNG DIS. HIV/AIDS; 2008 V (1) 1-6.
6. Mika Nakayama, MD,Hiroaki Satoh, MD,Hiroichi Ishikawa, MDMasachika Fujiwara, MDHiroshi Kamma, MD Morio Ohtsuka, MD,Kiyohisa Sekizawa, MD. Cytokeratin 19 Fragment in Patients With Nonmalignant Respiratory Diseases. CHEST 2003; 123: 6: 2001–2006.
7. M. Patricia Rivera, MD, FCCP; Atul C. Mehta, MB, FCCP. Evidence-Based Clinical Practice. ACCP Guidelines Initial Diagnosis ofLungcancer.CHEST.2007; 132 (3_suppl) : 131S-148S.

8. Collins LG, Haines C, Perkel R, Enck RE, 2007. Lung cancer: diagnosis and management. American Family Physician 2007; 75 (1) : 56-63.
9. Esmat Ali Abd EL- Nabi, Nada Ezz EL-Din Gomaa, Amany, Atef EL-Karim Abo Zeid, Nagwa Abdel Aziz Kantoush, Mohammed Mahmoud Ahmed, Safwat Samir Bushra. Evaluation of Cyfra 21-1 as a Diagnostic Tool in Lung Cancer. Journal of Applied Sciences Research; 2009; 5(9) : 1195-1201.
10. Jin Hwa Lee, MD, PhDJung Hyun Chang, MD, PhD Diagnostic Utility of Serum and Pleural Fluid Carcinoembryonic Antigen, Neuron-Specific Enolase, and Cytokeratin 19 Fragments in Patients With Effusions From Primary Lung Cancer. CHEST journal 2005; (128) : 2298–2303.
11. Kim YC, Park KO, Choi IS, Kim HJ, Lim SC, Bom HS. A comparison of serum CYFRA 21-1 and SCC Ag in the diagnosis of squamous cell lung carcinoma. Korean J Intern Med 1996; 11 : 50-7.
12. Masato Muraki, M.D. YUji Tohda, M.D. Takashi Iwanaga, M.D. Hisao Uejima, M.D. Yukio Nagasaka, M.D. Shigenori Nakajima, M. Assessment of Serum CYFRA 21-1 in Lung Cancer. Cancer 1996; 77:1274-7.
13. Bram Wieskopf, Rodolphe Stenger, Pascal Gries,Harvey Kreisman and Elisabeth Quoix,Christine Demangeat, Ashok Purohit. Cyfra 21-1 as a Biologic Marker of Non-small Cell Lung Cancer: Evaluation of Sensitivity, Specificity, and Prognostic role. Chest 1995; 108 : 163-169.
14. Molina R, Filella X, Auge JM, et al. “Tumour markers (CEA, CA125, CYFRA 21-1, SCC and NSE) in patients with Non-Small Cell lung Cancer as aid in histological diagnosis and prognosis: comparison with the main clinical and pathological prognostic factors”. Tumor Biol (2003); 24: 209–218.
15. Gube M, Taeger D, Weber DG, Pesch B, Brand P, Johnen G, Müller-Lux A, Gross IM, Wiethege T, Weber A, Raithel HJ, Kraus T, Brüning T Performance of biomarkers SMRP, CA125, and CYFRA 21-1 as potential tumor markers for malignant mesothelioma and lung cancer in a cohort of workers formerly exposed to asbestos.Arch Toxicol. 2011; 85(3) : 185-92.
16. Yoshinobu Maeda, Yoshihiko Segawa, Nagio Takigawa, Ichiro Takata and Nobukazu Fujimoto Clinical Usefulness of Serum Cytokeratin 19 Fragment as a TumorMarker for Lung Cancer. Internal Medicine 1996; 35: 764-771.