Chemotherapy: Open Access
ISSN: 2167-7700

Ullah, et al., Chemo Open Access 2016, 5:2
DOI: 10.4172/2167-7700.1000194

Research Article Open Access

Prognostic Role of Microvascular Density and Mucin Production in 56 Cases of Adenocarcinoma of Lung- An Experience from a Center in Lahore, Pakistan

Ehsan Ullah[1*], Abdul Hannan Nagi[1], Muhammad Ashraf[1], Ahmed Khurshid Pasha[2], Keri Renee Maher[3] and Faiz Anwer[3]
[1]Department of Morbid Anatomy and Histopathology, University of Health Sciences Lahore, Pakistan

[2]*Department of Internal Medicine, University of Arizona, Tucson, Arizona, USA*

[3]*Department of Medicine, Division of Hematology/Oncology, University of Arizona, Tucson, Arizona, USA*

[*]**Corresponding author:** Ehsan Ullah, Auckland District Health Board Auckland, New Zealand, Tel: +64220141390; E-mail: e_ullah@yahoo.com

Received date: January 21, 2016; **Accepted date:** February 16, 2016; **Published date:** February 20, 2016

Abstract

Introduction: The relative incidence of adenocarcinoma of the lung is increasing, and it is now the most common primary lung malignancy. This study examines certain clinical and pathological factors which may affect patient survival in lung adenocarcinoma in the developing country of Pakistan.

Methods: This is a descriptive study involving 56 patients with biopsy-proven primary adenocarcinoma of lung. Biopsy tissues were reviewed along with medical record case histories. Formalin-fixed, paraffin-embedded tissue specimens were used for mucin histochemistry with mucicarmine, periodic acid Schiff (PAS) and alcian blue stains. Immunohistochemistry (IHC) with monoclonal antibodies against CD34 was used for assessment of microvascular density. Mast cell density was measured on Toluidine blue stained sections.

Results: Mean age of patients was 55.96 ± 1.67 years. Male to female ratio was 3:2. 51.8% of patients were smokers, and weight-loss was seen in 46.4% of the patients. A majority (66.1%) had stage III/IV tumors with positive lymph nodes, and 26.8% tumors were mucinous. High mast cell density was found in 32.1% and high microvessel density in 44.6% tumor specimens. Weight-loss, stage, nodal status, mucin production and microvessel density showed significant deleterious effect on patient survival.

Conclusions: The current study illustrates that for this population, weight loss, advanced stage of tumor, nodal status, mucin production by the tumor, and increased angiogenesis are predictors of poor survival in patients with primary pulmonary adenocarcinoma. Further prospective work will need to be performed to determine if these factors can assist in prognostication or formulation of treatment plan in newly diagnosed individuals in the general population.

Keywords: Adenocarcinoma; Angiogenesis; Lung cancer; Mast cell density; Microvascular density; Mucin; Survival

Introduction

The relative frequency of adenocarcinoma as compared to squamous cell carcinoma of lung has increased 8-fold in North America since 1970, and is now the most common histological type of lung cancer [1]. Therefore, it is imperative to determine clinical and pathological factors related to prognosis. Published data on the subject in South East Asian population is limited, however, as the area's population is rapidly expanding, understanding unique patient and disease characteristics of this population is important. This study examines several possible prognostic factors from a small medical center in Pakistan, including: certain patient demographics, tumor stage, mucin production, measures of angiogenesis, and mast cell density.

Mucins are high molecular weight glycoproteins synthesized, stored and secreted by the epithelial mucosal cells of various tumors, including adenocarcinoma of lung. While previously studied by immunohistohemisty, effective histochemical stains have recently gained favor as an easy, cost-effective alternative [2]. It is widely accepted that mucinous adenocarcinomas of lung, colon, ovary and other body organs have worse prognosis as compared to their non-mucinous site-specific counterparts [3,4].

Tumor angiogenesis is a multifaceted phenomenon regulated by the delicate balance between a number of pro- and anti-angiogenic molecules released by tumor cells and host cells, including endothelial cells, macrophages, mast cells and stromal components reviewed by a number of studies [5-8]. Measured in terms of microvascular density, angiogenesis is indispensable in order to supply adequate oxygenation and nutrition to tissues, and, it is widely accepted that increased angiogenesis is necessary for tumor growth and metastasis. Angiogenesis is believed to depend on an 'angiogenic switch', a cascade of events starting with the discharge of proangiogenic factors including fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), ultimately leading to the activation of endothelial cells, proteolytic enzymes such as matrix metalloproteinases (MMPs), digestion of the basement membrane, followed by endothelial cell migration, proliferation, and capillary tube formation [9]. The neo-capillaries formed in tumors are deficient in the similar supporting architecture as their parent vessels: they are thin walled and leaky, providing access to the circulation for tumor cells to metastasize [10]. This forms the basis for use of antiangiogenic chemotherapeutic agents for the treatment of a variety of human cancers including non-small

cell lung cancers (NSCLC) such as adenocarcinoma. Angiogenesis has been associated with poor survival in patients with pulmonary adenocarcinoma. Incorporating anti-angiogenic agents (like bevacizumab) for the treatment regimens of these patients have significantly improved patient survival [11-13].

Mast cells are widely known for their pivotal role in allergic, inflammatory and immune reactions. In addition, they are implicated in pain, tissue damage, and healing. In recent past, mast cells have been shown to have a strong link with cancer progression and mediate their effect through a torrent of cytokines, chemokines, pro-angiogenic factors and heparin release [14]. High mast cell density may be simply a consequence of tumor invasiveness, or, mast cells themselves may play a role in tumor progression [15]. Tumorigenic effects of mast cells may be mediated through modulation of immunosupression, angiogenesis, degradation of extracellular matrix and mitogenesis [16]. In last two decades, many investigators working on NSCLC have failed to show a direct relationship between mast cell density and patient survival, likely due to the extremely complicated role of mast cells in tumor biology [17].

Materials and Methods

This is an observational, descriptive study in which 56 consecutive patients presenting to Gulab Devi Chest Hospital in Lahore, Pakistan between the dates of September 2010 and May 2013 with biopsy confirmed primary adenocarcinoma of the lung were reviewed. Biopsy slides were reviewed by two independent pathologists (AHN, MA), and were diagnosed according to WHO classification 2004 for adenocarcinoma. Of note, since 2011, a new multidisciplinary classification of adenocarcinoma has been proposed by the European Respiratory Society, the American Thoracic Society and the International Association for the Study of Lung Cancer [2,18]. TTF 1 was used for confirmation of adenocarcinoma of the lung. ALK, KRAS, and EGFR mutations were not assessed in this study. No patient had received chemotherapy or radiation therapy prior to the initial diagnostic biopsy. However, patients were offered adjuvant chemotherapy, and surgical excision was offered for resectable tumors. Paraffin blocks containing sufficient formalin-fixed tumor samples were obtained from a total of 56 patients. Figure 1 depicts representative stained sections of tumor in hematoxylin and eosin, mucicarmine, PAS, and Anti-CD34 staining.

Mucin was observed in Hematoxylin and Eosin, Periodic Acid Schiff, Mucicarmine and Alcian Blue stained sections of adenocarcinoma, details on histochemical methods is given by Ali et al. [19].

Microvascular density (MVD) or angiogenesis per section was measured using immunostaining with a CD34 monoclonal antibody (Clone QBEnd10; BioSB, USA), which required phosphate buffer saline based antigen retrieval and 30 minutes incubation of the primary antibody (prediluted). Vessel count was assessed in areas of the biopsy containing the highest numbers of capillaries and small venules, based on the criteria of Weidner et al. [20]. Vessels in five high-power fields (200× magnifications) were counted by two independent experienced pathologists without knowledge of the patient outcome. An average value of the two scores was used in the current study. MVD was defined as the mean number of CD34+ vessels per section, and was then classified as either high or low compared to the mean observed in this study, which were 13 micro-vessels per high power field.

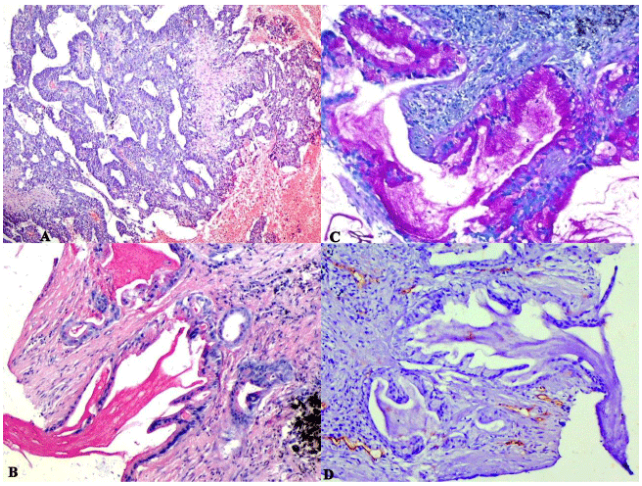

Figure 1: Selected microphotographs: A. Adenocarcinoma of lung (Haematoline and Eosin x10); B. Mucin in adenocarcinoma of lung (Mucicarmine x20); C. Mucin in adenocarcinoma of lung (PAS, x40); D. Microvessels (low microvascular density in a case of mucinous adenocarcinoma of lung (CD34, x20).

Mast cell counts per high power field (HPF) were calculated in the same manner as MVD as described above, as counted in toulodine blue stained sections. The mean mast cell density (MCD) was 3.27 mast cells per HPF.

Data was collected and stored on Microsoft excel, and subsequently analyzed using SPSS version 17 (IBM Inc. USA). Mean ± S.E. were given for quantitative variables, whereas frequencies and percentages were given for qualitative variables. Means were compared using Independent Samples t-test and ANOVA. Correlation between continuous variables was determined with Pearson's correlation. Spearman's rho was used to measure the correlation co-efficient when one of the variables was rank-scale. Kaplan-Meier survival curves were plotted and Log Rank tests were applied observe the effect of various factors i.e. age, gender, stage, nodal status, mucin production, angiogenesis and mast cell density on survival. P value <0.05 was considered to be statistically significant.

Results

Average age at time of diagnosis was 55.96 ± 1.67 years. Male (n = 34, 60.7%) to female (n = 22, 39.3%) ratio was nearly 1.5:1. About half of the patients (n = 29, 51.8%) were current smokers. A significant proportion (n = 26, 46.4%) of the patients had significant unintentional weight loss (≥10%) before diagnosis was established. Thirty-seven (66.1%) patients had locally advanced (defined as any tumor 5 cm (T3) or greater, or positive lymph nodes) or distant metastatic disease. 36 (64.3%) had biopsy-proven involvement of ipsilateral supraclavicular lymph nodes. Regarding histological grading, 15 (26.8%) were well-differentiated, 25 (44.6%) were moderately differentiated and remaining 16 (28.6%) were poorly differentiated adenocarcinomas. Out of total 56, forty-one (73.2%) tumors did not show mucinous material within the glandular structures whereas almost 1/4th (n = 15, 26.8%) tumors showed mucin by one or more histochemical methods. High mast cell density was

found in 18 (32.1%) and high microvessel density was observed in 25 (44.6%) tumor specimens.

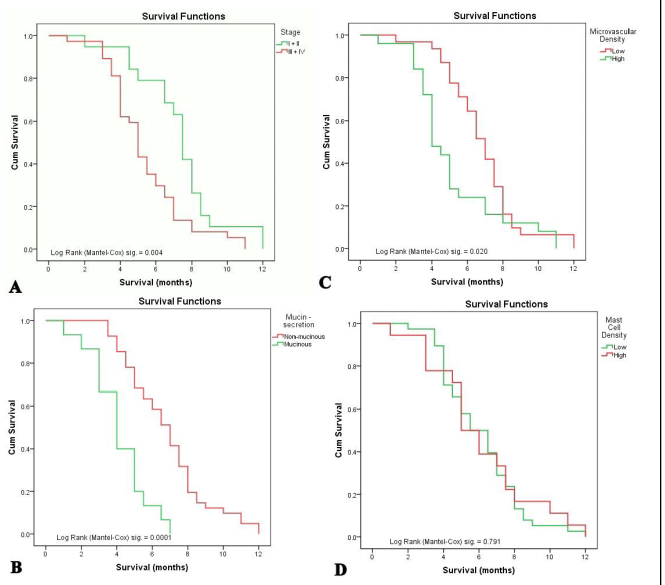

Figure 2: Selected Kaplan-Meier survival curves for: A. Stage of cancer; B. Mucin production; C. Microvascular density; D. Mast cell density.

While a third (n = 19, 33.1%) of patients presented with early stage (Stage I or II) disease, only 7 (12.5%) underwent surgical resection i.e. lobectomy (2 cases), pneumonectomy (5 cases). Other patients could not undergo resection due to various reasons (such as significant medical comorbidities) and were offered chemotherapy. The patients who underwent surgical resection were given chemo-radiotherapy with curative/therapeutic intent. The average disease progression free survival (PFS) was 6.08 ± 0.32 months (Range: 1-15 months). Table 1 shows the effect of various parameters on the patient survival in adenocarcinoma of lung. It is evident from the p values that significant weight loss, advanced stage (III or IV), positive supraclavicular lymph node(s), positive mucin production and high micro vessel density are associated with worse prognosis. However, gender, age, smoking status, mode of therapy, and mast cell density did not show statistically significant effect on patient survival in this study population. The decreased survival with relation to stage, micro vascular density, and mucin production along with the lack of difference in survival with respect to mast cell density is well visualized in the Kaplan-Meyer curves in Figure 2A-2D.

Parameters	No. of Patients	Survival in months (Mean ± S.E)	P value
Gender			
Male	34	6.35 ± 0.43	0.315
Female	22	5.66 ± 0.50	
Age			
<55 years	31	6.52 ± 0.41	0.299
55 years and above	25	5.54 ± 0.49	
Smoking			
Yes	29	5.67 ± 0.46	0.320
No	27	6.52 ± 0.46	
Weight loss			
Present	26	4.38 ± 0.45	0.007
Absent	30	6.13 ± 0.33	
Stage of Cancer			
Early (I and II)	19	7.37 ± 0.55	0.004
Late (III and IV)	37	5.42 ± 0.36	
Supraclavicular nodes			
Positive	36	4.18 ± 0.29	0.0001
Negative	20	7.38 ± 0.27	
Mode of treatment			
Surgery followed by chemotherapy	07	6.71 ± 1.26	0.277
Chemotherapy alone	49	5.99 ± 0.33	
Histological Grade			
Well differentiated	15	7.13 ± 0.57	0.154
Moderately differentiated	25	5.50 ± 0.43	
Poorly differentiated	16	6.00 ± 0.71	
Mucin Production			
Present	15	4.13 ± 0.42	0.0001
Negative	41	6.79 ± 0.36	
Mast cell density			
High	18	6.08 ± 0.35	0.791
Low	38	6.07 ± 0.69	
Microvessel density			
High	25	6.84 ± 0.37	0.020
Low	31	5.14 ± 0.51	

Table 1: Predictors of patient survival in adenocarcinoma of lung.

Discussion

Lung cancer is the second most common malignancy, and leading cause of cancer death worldwide [21]. Adenocarcinoma has now surpassed other histologic types as the most common [1]. Adenocarcinoma of lung is a disease commonly afflicting younger patients, females, and non-smokers – in drastic distinction to squamous cell carcinoma of lung which frequently occur in older, male, smokers [22-25].

Similarly to this small observational study, A recent study by Ma et al. (2010) involving 100 patients of all histological types of non-small cell lung cancer (NSCLC) including 36 patients of adenocarcinoma of

lung showed that age, gender and histological grade has insignificant effect on the patient survival [26].

The observed result in this study may be confounded due to the limited number of patients studied, as well as the low percentage of patients proceeding with curative surgical intervention despite tumor resectability at the time of diagnosis. Lack of advanced radiodiagnostic measures such as positron emission tomography (PET) scans in small centers, such as this one, may also play a role. Furthermore, there is dearth of skilled thoracic oncologic surgeons not only in the city, but, in Pakistan as a whole. While this is an important limiting factor of this study, it is an important epidemiologic reminder that a very large number of individuals world-wide with various cancers are diagnosed and managed by centers with limited resources compared to those so often taken for granted in larger institutions in developed countries.

Average age of lung cancer patients has increased in developed countries leading to changes in the management of disease [27]. Asmis et al. working with National Cancer Institute of Canada Clinical Trials Group concluded that age more than 65 years was associated with poor survival [28]. However, in the current study, the mean age of patients was only 55 years. Effect of patient age on survival was conducted by separating the patients into two groups: below 55 vs. 55 and older. None of the patients in current study were older than 57. The relative lack of variability in age likely contributed to age not having a significant effect of overall survival in our study. Moreover, previous work by Båtevik et al. demonstrated that female gender has a positive prognostic implications following surgical resection of primary NSCLC [29]. Due to very small number presenting for surgical resection in our study, this was not observed here.

As discussed previously, mucinous adenocarcinomas are generally expected to have a poorer prognosis than their non-mucinous counterparts based on previous studies. Our study lends further support to these findings, as we demonstrated decreased survival, 4.13 ± 0.42 months vs. 6.79 ± 0.36 months ($p = 0.0001$) among mucinous and non-mucinous pulmonary adenocarcinomas. Similarly, weight loss, stage of tumor and lymph node status are well-known predictors of survival in patients of adenocarcinoma of lung and the same has been demonstrated here.

Conclusions

We conclude that weight loss, tumor stage and nodal status, mucinous characteristics, and increased angiogenesis (as determined by vessel density) are predictors of poor survival in this group of patients with pulmonary adenocarcinoma. In this small cohort, age, gender, smoking status, histologic grade, and mast cell density did not reach statistical significance with respect to survival.

Acknowledgements

Authors are grateful to the laboratory staff of Department of Morbid Anatomy and Histopathology, University of Health Sciences and Gulab Devi Chest Hospital Lahore for their technical assistance. Authors also acknowledge the University of Healthy Sciences Lahore for the financial grant of this project.

Funding

University of Health Sciences, Lahore for financial grant for this project.

References

1. Vazquez M, Carter D, Brambilla E, Gazdar A, Noguchi M, et al. (2009) Solitary and multiple resected adenocarcinomas after CT screening for lung cancer: histopathologic features and their prognostic implications. Lung Cancer 64: 148-154.
2. Brambilla E (2011) 8IN How to sub-classify adenocarcinoma of the lung. Lung Cancer 71: S6.
3. Kanemitsu Y, Kato T, Hirai T, Yasui K, Morimoto T, et al. (2003) Survival after curative resection for mucinous adenocarcinoma of the colorectum. Dis Colon Rectum 46: 160-167.
4. Riquet M, Foucault C, Berna P, Assouad J, Dujon A, et al. (2006) Prognostic value of histology in resected lung cancer with emphasis on the relevance of the adenocarcinoma subtyping. Ann Thorac Surg 81: 1988-1995.
5. Sun S, Schiller JH (2007) Angiogenesis inhibitors in the treatment of lung cancer. Crit Rev Oncol Hematol 62: 93-104.
6. Zhao M, Gao FH, Wang JY, Liu F, Yuan HH, et al. (2011) JAK2/STAT3 signaling pathway activation mediates tumor angiogenesis by upregulation of VEGF and bFGF in non-small-cell lung cancer. Lung Cancer 73: 366-374.
7. Bertino EM, Otterson GA (2010) Benefits and limitations of antiangiogenic agents in patients with non-small cell lung cancer. Lung Cancer 70: 233-246.
8. Bremnes RM, Camps C, Sirera R (2006) Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood. Lung Cancer 51: 143-158.
9. Herbst RS, Onn A, Sandler A (2005) Angiogenesis and lung cancer: prognostic and therapeutic implications. J Clin Oncol 23: 3243-3256.
10. Kerbel RS (2000) Tumor angiogenesis: past, present and the near future. Carcinogenesis 21: 505-515.
11. Shijubo N, Kojima H, Nagata M, Ohchi T, Suzuki A, et al. (2003) Tumor angiogenesis of non-small cell lung cancer. Microsc Res Tech 60: 186-198.
12. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, et al. (2006) Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355: 2542-2550.
13. Ullah E, Nagi AH, Lail RA (2012) Angiogenesis and mast cell density in invasive pulmonary adenocarcinoma. J Cancer Res Ther 8: 537-541.
14. Nechushtan H (2010) The complexity of the complicity of mast cells in cancer. Int J Biochem Cell Biol 42: 551-554.
15. Galinsky DS, Nechushtan H (2008) Mast cells and cancer--no longer just basic science. Crit Rev Oncol Hematol 68: 115-130.
16. Ch'ng S, Wallis RA, Yuan L, Davis PF, Tan ST (2006) Mast cells and cutaneous malignancies. Mod Pathol 19: 149-159.
17. Carlini MJ, Dalurzo MC, Lastiri JM, Smith DE, Vasallo BC, et al. (2010) Mast cell phenotypes and microvessels in non-small cell lung cancer and its prognostic significance. Hum Pathol 41: 697-705.
18. Daniels M, Wright G, Russell P, Williams R, Conron M, (2010) Comprehensive Adenocarcinoma Sub-typing Predicts Outcome After Lung Cancer Resection. Heart, Lung and Circulation 20:269.
19. Usman Ali, Nagi AH, Nadia Naseem, Ehsan Ullah (2012) Mucin Histochemistry in Tumours of Colon, Ovaries and Lung. J Cytol Histol 3:163.
20. Weidner N, Semple JP, Welch WR, Folkman J (1991) Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. N Engl J Med 324: 1-8.
21. Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. CA Cancer J Clin 60: 277-300.
22. Gasperino J (2011) Gender is a risk factor for lung cancer. Med Hypotheses 76: 328-331.
23. Dresler CM, Fratelli C, Babb J, Everley L, Evans AA, et al. (2000) Gender differences in genetic susceptibility for lung cancer. Lung Cancer 30: 153-160.

24. Guo NL, Tosun K, Horn K (2009) Impact and interactions between smoking and traditional prognostic factors in lung cancer progression. Lung Cancer 66: 386-392.
25. Hung RJ, McKay JD, Gaborieau V, Boffetta P, Hashibe M, et al. (2008) A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 452: 633-637.
26. Ma J, Liu L, Che G, Yu N, Dai F, et al. (2010) The M1 form of tumor-associated macrophages in non-small cell lung cancer is positively associated with survival time. BMC Cancer 10: 112.
27. Higton AM, Monach J, Congleton J (2010) Investigation and management of lung cancer in older adults. Lung Cancer 69: 209-212.
28. Asmis TR, Ding K, Seymour L, Shepherd FA, Leighl NB, et al. (2008) Age and comorbidity as independent prognostic factors in the treatment of non-small-cell lung cancer: a review of National Cancer Institute of Canada Clinical Trials Group trials. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 26:54-59.
29. Båtevik R, Grong K, Segadal L, Stangeland L (2005) The female gender has a positive effect on survival independent of background life expectancy following surgical resection of primary non-small cell lung cancer: a study of absolute and relative survival over 15 years. Lung Cancer 47: 173-181.