Immunohistochemical Expression of Cytokeratin-19 in Non Small Cell Lung Carcinomas - An Experience from a Tertiary Care Hospital in Lahore

Nadia Naseem, Nadeem Reyaz, A. H. Nagi, M. Ashraf and Waqas Sami

Objective:: Previous literature reveals a significant recognition of Cytokeratin (CK) 19 as a reliable tumour marker in epithelial malignancies. With the hypothesis that CK 19 is extensively released by the tumour cells in patients with non small cell lung carcinomas (NSCLCs), this study was designed to assess (1) the clinicopathological characteristics of lung cancer in this sub-region (2) the pattern of immunohistochemical expression of CK-19 in different NSCLC subtypes with respect to their histological grades.

Materials and Methods: A cross-sectional descriptive study comprising of detailed account of clinical data of 225 patients presenting with NSCLCs. After H/E staining, immunohistochemistry (IHC) was performed using prediluted ready to use mouse monoclonal antibody to CK 19.

Results: Our findings of the study revealed that the mean age of the patients was 45 years with the male to female ratio being 5:1. Most prevalent type seen was squamous cell carcinoma (SCC) and adenocarcinoma (AC) in males and females respectively. Some cases were not easily classifiable as squamous or adenocarcinoma and hence they are classified as NSCLs Unclassified. SCC was commonly associated with smoking in both genders. Immunohistochemical staining of the histological tissue sections of 93.7% NSCLCs demonstrated a varying immunopositivity while showing strong, diffuse to focal cytoplasmic staining of the tumour cells. A significant association (p=0.001) was found between the increasing grades of malignancy and the strength of CK-19 expression.

Conclusion: Supporting the previous literature, we have also come across that CK-19 is credibly expressed in patients with NSCLCs and may be applied as a reasonably reliable immunohistochemical marker. In future the patients over expressing CK-19 histologically might constitute potential candidates to be followed up through non invasive serial serum levels estimation intended for assessment of tumour burden in recurrence or advanced disease.

Key Words: NSCLC : non small cell lung carcinomas, Young Adults, Cytokeratin 19 , IHC:

Introduction

Cancer of the lung is the most common organ malignancy and is one of the most prevalent causes of cancer-related deaths worldwide with approximately 1.2 million deaths annually. According to a published report by World Health Organization (WHO) in 2005, lung cancer was the most common malignancy (age standardized incidence being 20 per 100,000) and the leading cause of cancer deaths among Pakistani men with the annual incidence and mortality rate being 42/100,000 and 19/1000 in male population which was highest among all cancers occurring in Pakistan. In the year 1999, according to the WHO classification of malignant epithelial lung carcinomas the non-small cell lung carcinomas (NSCLC) constitute 80-85% cases of all lung carcinomas. The main three histological subtypes in NSCLC are squamous cell carcinoma (SCC), adenocarcinoma (AC), and unclassifiable carcinoma (UC). NSCLC describes a histologically heterogeneous group of tumours with variable clinical behaviour. Performance status, tumour stage and histological type have important prognostic implications, but clinical outcomes in individual patients remain unpredictable. These tumours including bronchogenic carcinoma show more or less aggressive behaviour depending upon their grades and the stages but unfortunately large programs of screening in the population have failed to demonstrate...
any benefit for early detection of lung tumors and the vast majority of patients are diagnosed either by chance or through their symptoms. Currently, there are no specific tumor markers enabling detection of lung cancer at an early stage. On the other hand, there is a long list of lung specific tumour markers which are categorized into oncofetal proteins, structural proteins, enzymes, cell membrane components, secreted peptides, hormones, and other tumour-associated antigens. Cytokeratins (CK), a group comprising of at least 29 different proteins, are alpha-keratins and are water insoluble proteins of intermediate filaments of cell cytoskeleton which are further classified according to their isoelectric point into two types: acid (type I), basic (type II). Under the influence of intrinsic or extrinsic factors, each cell will express different types of cytokeratins in course of its evolution which is also affected by the growth and differentiation rate. Markers originating from cytokeratin, especially the cytokeratins, are of great practical interest to the clinicians and researchers. CK-19, a new marker of cytoskeleton, is the smallest human cytokeratin with molecular weight up to 40Kd. It is an excellent marker of epithelial differentiation regardless of its origin from endoderm, neuroectoderm or germ cell lineage. Studies have shown that it is equally potent in detecting the premalignant and malignant epithelial lesions. Also CK-19 has been shown to be a promising marker in lung carcinomas because of its correlation with tumour mass, the surveillance for post surgical relapses and patient prognosis. Keeping in view the expected importance of CK-19 as an immunohistochemical marker in lung carcinomas, this study was carried out to evaluate the clinico-pathological characteristics and immunohistochemical expression of CK-19 in various NSCLC subtypes with respect to their histological grading in our part of world.

Materials and Methods

This was a cross-sectional descriptive study comprising of two hundred and twenty five patients of NSCLCs with unilateral, operable lung cancer (mean age 45 years, age range 08-80 years) who underwent resection of primary tumour at Gulab Devi Chest Hospital Lahore from January 2004 to January 2008 were selected for this study. Cases with history of comorbidity or taking medication /therapy for their malignancy i-e follow up cases or, if any, necrotic tissue specimens were excluded. All selected patients gave written informed consent. Relevant clinical and laboratory data of these patients including age, sex, tumor location, and type of surgical procedure were recorded in separate Performa. More than 10 years history of habitual active smoking was taken as positive history and as an important correlating parameter. Lung biopsies of several types and of different sizes including trucut, core needle, wedge, pleural, endoscopic lung biopsy, excision biopsy, debulking specimen, bullectomy, segmentectomy, lobectomy, pneumonectomy, mediastinotomy, thoracotomy, CT guided biopsy etc. were included. The tissues were processed in formalin and embedded in paraffin to form tissue blocks which were collected from the hospital. Gross observations took account of size and location of tumors. Microscopic features tabulated included patterns of growth, nuclear features, mitoses, necrosis, and stromal reaction. One section from each tissue, 4-7µm for hematoxylin and eosin (H&E) staining and two sections of 3-6µm for immunohistochemistry were recut by rotary microtome and collected on poly-L-lysine-coated slides. After confirmation of the diagnosis of NSCLC, the histological grading of these tumour types was undertaken following the criterion by AJCC and WHO. After H/E staining, immunohistochemistry was performed using the standard ‘Avidin Biotin Peroxidase’ method. The primary antibody employed was prediluted ready to use IgG2a mouse monoclonal anti CK-19 antibody ([A53-B/A2] (ab7754) reacting with the rod domain of CK peptide 19 (40 kDa) in human tissue at a concentration of 1.000 mg/ml. The expression of marker was assessed as follows: 0, no staining or staining in less than 10% of the tumor cells; 1+, staining in 10% to 25% of the cells; 2+, staining in 26% to 50% of the cells; 3+, staining in 51% to 75% of the cells; 4+, staining in more than 75% of the cells. Staining of 1+ or 2+ was defined as focal staining, and staining of 3+ or more was defined as diffuse staining. For statistical analysis, cases with any degree of positive staining were grouped as positive. Paraffin sections of about 50 tissues from non neoplastic lung (10), benign (05) and malignant breast (05), oral (10) and cervical squamous cell carcinomas (10) and bladder cancers (10) were taken as positive controls whereas 50 negative controls included uterine leiomyomas (10), soft tissue lipomas, (05), gastrointestinal stromal tumours (05), neoplastic (10) and non neoplastic lymph nodes (05), sarcomatous tissues from lung, abdominal wall, various soft tissues etc. For immunohistochemical staining, 05 positive and 02 negative controls were run with each batch of 25 histological sections of NSCLCs. The entire slide was scanned for immunostaining and was scored on the basis of intensity of staining and the percentage of the cells that stained positively. Statistical Analysis: The data was entered and analyzed using SPSS 17.0 and STATA 8.2. Mean ± S.D. (standard deviation) were given for quantitative variables. Frequencies and percentages were given for qualitative variables. Pearson Chi Square and
Fisher Exact test were applied to observe associations, if any, between the qualitative variables. Diagnostic statistics (sensitivity, specificity, positive / negative predictive values and diagnostic accuracy) were applied for CK-19. A p value of < 0.05 was considered as statistically significant.

Results

Our findings of the study revealed that among n=225 cases reported at GDCH, n=185 (82%) were males and n=40 (18%) were females with an overall age range of 08-83 years (mean age: 45 years). Among 185 male patients, n=5 (2.5 %) were between the age group of 08-20 years, n=33 (18 %) in the age group 21-40 years, n=48 (26 %) in the age group 61-80 years and n=05 (2.5 %) patients were above 80 years of age. Among n=40 female patients, n=11 (27 %) were in the age group of 10-30 years, n=6 (15.3 %) in the age group 31-50 years and n=23 (58 %) patients were between the age group 51-70 years.

About n=120 (64.8%) males and n=12 (30%) female patients reported a > 10 years history of smoking. Association of lung carcinoma with positive history of smoking was observed in 79.4 % and 50% followed by 58.3 % and 41% males and females with SCC and NSCLC respectively. Maximum number presented with poorly differentiated variety (Table I).

Table I: Histological Grades of NSCLC Subtypes Included

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Tumour Grades</th>
<th>SCC n (%)</th>
<th>AC n (%)</th>
<th>UC n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WD</td>
<td>12 (7.9)</td>
<td>15 (29.4)</td>
<td>---</td>
<td>27 (12)</td>
</tr>
<tr>
<td>2</td>
<td>MD</td>
<td>37 (24.5)</td>
<td>17 (33.3)</td>
<td>---</td>
<td>54 (24)</td>
</tr>
<tr>
<td>3</td>
<td>PD</td>
<td>102 (67.5)</td>
<td>19 (37.2)</td>
<td>23 (100)</td>
<td>144 (64)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>151</td>
<td>51</td>
<td>23</td>
<td>225</td>
</tr>
</tbody>
</table>

(NSCLC: non small cell lung carcinomas, SCC: squamous cell carcinoma, AC: adenocarcinoma, UC: unclassifiable carcinomas, WD: well differentiated, MD: moderately differentiated and PD: poorly differentiated)

TABLE II: Histological Grades of NSCLC Subtypes Positive for CK-19

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Histological Grades</th>
<th>CK-19 Positive</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCC (151) n (%)</td>
<td>AC (51) n (%)</td>
<td>UC (23) n (%)</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>WD</td>
<td>8 (66.6)</td>
<td>13 (86.6)</td>
<td>---</td>
<td>0.006</td>
</tr>
<tr>
<td>2</td>
<td>MD</td>
<td>35 (94.5)</td>
<td>16 (94.1)</td>
<td>----</td>
<td>0.914</td>
</tr>
</tbody>
</table>

There was subsequent rise in percentage positivity of CK-19 with respect to the tumour grades of SCC only. However, no significant correlation was observed between the two parameters in any of the NSCLC subtypes. (Table II)

Table II: Association between Patterns of CK-19 Positivity With Respect to Histological Grades of NSCLC Subtypes

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Tumour Grades</th>
<th>SCC (143) Focal N (%)</th>
<th>SCC (143) Diffuse N (%)</th>
<th>AC (47) Focal N (%)</th>
<th>AC (47) Diffuse N (%)</th>
<th>UC (21) Focal N (%)</th>
<th>UC (21) Diffuse N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WD</td>
<td>06 (75)</td>
<td>02 (25)</td>
<td>10 (77)</td>
<td>03 (23)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>MD</td>
<td>26 (74)</td>
<td>09 (26)</td>
<td>06 (37.5)</td>
<td>10 (62.5)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>PD</td>
<td>02 (2.0)</td>
<td>98 (98)</td>
<td>03 (16.6)</td>
<td>15 (83.3)</td>
<td>03 (14.2)</td>
<td>18 (85.7)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>34 (23.7)</td>
<td>109 (76.2)</td>
<td>19 (40)</td>
<td>28 (60)</td>
<td>03 (14.2)</td>
<td>18 (85.8)</td>
</tr>
</tbody>
</table>

(NSCLC: non small cell lung carcinomas, SCC: squamous cell carcinoma, AC: adenocarcinoma, UC: unclassifiable carcinomas, WD: well differentiated, MD: moderately differentiated and PD: poorly differentiated)

As regards the clinical symptoms of the presenting patients, cough and/or expectoration was seen in 92%, chest pain in 84%, dyspnoea in 50%, hemoptysis in 41% and generalized weakness with weight loss in 67% of the patients. About n=38 (16.6%) patients, 26 males and 12 females, were on antituberculous therapy for more than two years.

We observed that left lung 59 % was the most frequent site of involvement by lung carcinoma in male population. Right lung was involved in 38 % and other locations including mediastinal, pleural or metastatic involvement by lung carcinoma was observed in 3% males. Conversely, we observed that right lung (65 %) was the most common location for lung carcinoma in female population while left lung was involved in 27%
females presenting with lung carcinoma. Other locations including mediastinal, pleural or metastatic were observed in 08% females. However, in both sexes, upper lung lobe was most frequently involved on both right and the left lung.

We have found that adenocarcinoma was the most commonly observed malignancy in female population (51.2%) while squamous cell carcinoma was the commonest malignancy in males (77.1%). 93.7% (n=211) NSCLCs with about 143 (94.7%) SCCs, 47 (92.1%) ACs and 21 (91.3%) UCs were positive for CK-19. The positive staining of all NSCLCs depicted a varying pattern, from focal (26.5%; n=56) to almost diffuse (73.5%; n=155) strong cytoplasmic staining of the tumour cells (Fig: 1, 2). We observed that of 94.7% (n=143) positive SCC, 23.7% (n=34) demonstrated focal while 76.2% (n=109) showed almost diffuse staining (p>0.01), 92.1% (n=47) and 91.3% (n=21) positive ACs and UCs respectively, 40.5% (n=19) and 30.5% (n=07) demonstrated focal positive while 59.5% (n=28) (p=0.05) and 69.5% (n=16) (p>0.01) showed almost diffuse strong positive cytoplasmic staining of tumour cells with CK-19 respectively. About 6.3% (14) NSCLC sections were CK-19 negative while showing none to only a few scattered tumour cells cytoplasm being positive. Only 2 of the mesenchymal tissues taken as negative controls were positive and that too only focally (+). Thereby the sensitivity, specificity, positive and negative predictive values and the diagnostic accuracy of CYFRA21-1 at 95% confidence intervals were 97.06%, 77.42%, 93.78%, 96% and 94.18% respectively.

As regards the tumour grades, 21 (77.7%) WD, 51 (94.4%) MD and 139 (97.2%) PD NSCLC cases were CK-19 positive. However, no significant correlation was observed between the two parameters in any of the NSCLC subtypes when immunopositivity was assessed in general (Table:2). On the other hand, when the pattern of CK-19 positivity (focal or diffuse) was assessed with respect to the histological grades, we found that all WD tumours and MD SCCs were significantly (p=0.01) focal positive as compared to the diffuse pattern seen in all PD tumours (p=0.01) (Table:3). No significant association was observed between the gender and the tumour subtypes (p=0.361), the histological grade (p=0.451) and CK-19 staining pattern (p=0.061). Similarly no significant association was observed between the age and the above mentioned variables.

Discussion

This study assessed the clinico-pathological pattern of lung carcinoma in a local hospital but also analyzed the immunohistochemical expression of an IgG2 antibody to the CK-19 in NSCLC cases especially with respect to the histological grading of these tumours. Our observations revealed that lung carcinoma is a predominating disease in males with a ratio of 5:1 with females. The most prevalent morphological subtype in males was NSCLC including SCC. 51% male patients
were between the age group of 41-60 years. In contrast, AC was observed as the most prevalent malignancy in females with 58% being in the age group 51-70 years. SCC followed by NSCLC UC was most commonly associated with smoking in both genders. Regarding the gross location of lung carcinoma, left lung was involved in males 1.5 times the right lung whereas in females right lung was involved 2.5 times the left lung. Upper lobe was most frequently involved in both genders. The major symptoms were cough and/or expectoration followed by chest pain (84%). These clinicopathological features of lung carcinoma are in accordance with reports from other studies conducted in tertiary care hospital settings. According to Chandrashekhar, bronchogenic carcinoma is the most common cancer in Nepal. In a retrospective case-series analysis of 136 cases of primary bronchogenic carcinoma treated between September 2001 and August 2005 in a tertiary care hospital of western Nepal, the median age of the male and female patients was 67 and 66 years, respectively. The maximum number of patients was in the 60–69 year age group. One-hundred and thirteen (83.1%) of these patients were smokers. Among the male patients, 83.3% (60/72) were smokers and females 82.8% (53/64) were smokers. The male to female ratio of the patients was 1:1:1. The most common histological type was SCC (51.5%), followed by small-cell carcinoma (17.6%) and AC (8.1%). Similarly according to A. K. Pathak, patterns of lung cancer in India varies from the western population. The data from lung cancer clinic of a tertiary care hospital between 1999 to 2001 revealed that for 403 cases of lung cancer, peak incidence was at 51-60 yrs (mean 56.27 yrs). Overall male to female ratio was approximately 10:1. Over all 88% were smokers of which 91% were males & 50% females. Non small cell lung cancer constituted 80%; whereas small cell lung cancer was seen in 20%. SCC was the most common (33.16%) as compared to the AC in the west. The disease tends to occur early (the peak incidence is at 51-60 years).

Nearly a half century ago, CK-19 was proposed as a possible marker for epithelial tumours by Bjorklund. Nearly a half century ago, CK-19 was proposed as a possible marker for epithelial tumours by Bjorklund. Its reproducible and highly sensitive staining characteristics make CK-19 a useful antibody to be applied as a broad epithelial marker for carcinoma detection in routinely processed paraffin sections. The highly significant sensitivity and a considerable specificity in our observations reveal that this particular CK is substantially expressed by the epithelial tumours of our population. Another important feature of this antibody as compared to other CKs is that it shows very little reactivity in mesenchymal tissues or mesenchymally derived tumours, and a few types of epithelial malignancies, known not to contain CK 19 such as cholangiocellular carcinoma. The soft tissue and the lymphoid tumours included in our study also reflected a poor to almost negative staining response by the marker.

Increased CK-19 expression in tissues of patients with NSCLC is not only due to CK release following cell lysis or necrosis but also due to degradation of CK filaments by activated proteases in the tumour cells. Several series concerning CK-19 expression in lung tumours or benign pulmonary diseases reported a high sensitivity as well as a promising prognostic significance. Koscak investigated the immunohistochemical tissue localization of CK19 in tumorous and non invaded lung parenchyma in a series of 34 patients with lung cancer. A different CK 19 expression in different histological type of cancer cells as well as in non neoplastic epithelium covering bronchial tree and alveolar surfaces was observed with the most intensive CK19 expression being expressed in SCC and AC while SCLC revealed poor staining pattern. However, in non invaded parts of the resected lungs, the strong expression of CK19 was found in the cytoplasm of regenerative type II pneumocytes occurring in large quantity in the cases of interstitial lung fibrosis concomitant with some tumors. The findings of Chyczewski and colleagues suggest that CK-19 may be a used as a marker of choice for screening and monitoring of lung cancer, particularly SCC, as its sensitivity increased significantly with the increase in clinical stage. They examined the degree of CK 19 expression in 94 patients (64 males; 30 females) with NSCLC who had undergone surgery. The mean age was 59.12 ± 8.61 years. Histological diagnosis was SCC in 59 patients, AC in 26, LCLC in 5 while NSCLC without determination of the subtype in 4 patients.

In all examined tumors they observed positive cytoplasmatic staining of CK 19 with 44% cases showing weak staining, (1+), 37% showing medium (2+) while 19% demonstrated strong (3+) cytoplasmatic staining of tumour cells. All patients were observed for 24 months among which 45 patients survived while 49 died. They demonstrated higher expression of CK 19 in patients who survived 2 years (p = 0.003) and also observed relationship between higher expression of CK 19 and survival in patients with SCC in contrast to AC. Analogous to these findings, the pattern of histological expression and sensitivity of CK-19 in our study also correlated well with the increasing tumour grades, especially in SCCs, since most of the PD NSCLCs stained diffusely and more strongly when compared to the better differentiated tumours.
Conclusion

From the above results, we have come across several findings, most important of which is that CK-19 is extensively expressed by the tumour cells in patients with NSCLC presenting in our population with a significant association with the degree of differentiation of the tumour. Therefore, it may be applied as a reasonably reliable immunohistochemical marker and for future studies in our region, patients overexpressing CK-19 might constitute potential candidates to be followed up through non invasive serial serum levels estimation intended for assessment of tumour burden in recurrence or advanced disease.

References
