Original Research Article

Immunohistochemical expression of Bcl 2 & Cyclin D1 in lung cancer and its correlation with histological type & clinical staging

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ABSTRACT

Introduction: Bcl 2 is one of the unique proto-oncogene, localized to mitochondria & interfering with apoptosis independent of promoting cell division. Bcl-2 is overexpressed mostly in SCLC and in a minority of the NSCLC. Cyclin D1 is an important cell cycle regulatory protein which controls the cell cycle transition from G1 to S phase.

Materials and Methods: Here, we studied immunohistochemical expression of Bcl 2 & Cyclin D1 in 40 lung cancer patients to evaluate the immunohistochemical expression of Bcl 2 & Cyclin D1 in primary lung cancer, its expression with histological subtypes of lung cancer & to correlate the expression of Bcl 2 & Cyclin D1 with the clinical stage of disease.

Results: The immunohistochemical expression of Bcl 2 & Cyclin D1 was studied in 40 cases of lung cancer and was also correlated with the histological subtypes. Out of 40 cases of lung carcinoma, 21 cases (52.5%) were positive and 16 cases (40%) were negative for Bcl 2. Among histological subtypes, Bcl 2 was significantly higher in patients with small cell carcinoma, (80%) followed by squamous cell carcinoma, (78.57%). Out of 40 cases of lung carcinoma, 16 cases (40%) were positive and 21 cases (52.5%) were negative for Cyclin D1. Among histological subtypes, expression of Cyclin D1 was significantly higher in patients with adenocarcinoma, (52.38%) as compared to Squamous cell carcinoma (35.71%). The immunohistochemical expression of Bcl 2 and Cyclin D1 was correlated with the clinical staging of lung cancer. We observed higher Bcl 2 expression in stage 3 disease (81.82%) followed by stage 4 disease (55%). Higher Cyclin D1 expression was seen in patients with stage 4 disease (80.00%). None of the stage 2 and stage 3 patients showed any expression with cyclin D1.

Conclusions: In our study, higher Bcl 2 expression was associated with small cell carcinoma of lung and among non small cell carcinoma its expression was higher in squamous cell carcinoma. Cyclin D1 expression was observed mostly in adenocarcinoma cases. On correlating the expression of these markers with clinical staging, we concluded that Bcl 2 is highly expressed in patients with stage 3 disease and Cyclin D1 expression was mostly associated with stage 4 disease.

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1. Introduction

Lung cancer is the commonest cause of cancer related deaths worldwide. It constitutes around 13% of all new cancer cases and 19% of cancer related mortality in the world. In India lung cancer accounts 6.9% of all new cancer cases and close to 9.3% of cancer related death in both

sexes.¹

Bcl 2 is one of the unique proto-oncogene, localized to mitochondria & interfering with apoptosis independent of promoting cell division.² Bcl-2 is overexpressed mostly in SCLC (small cell lung cancer) and LCNEC (large cell neuroendocrine cancer) and in a minority of the NSCLC. Bax, heterodimerises with Bcl-2 to control the degree of apoptotic susceptibility, is downregulated in the SCLC and upregulated in the NSCLC, resulting in a ratio of Bcl-2:

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Bax of 1 in 95% of the SCLC and 1/4th of the NSCLC prone to resistance to programmed cell death. This specific ratio is reversed in favour of Bcl-2 early on in tumour development, as early as minor dysplasia occurs, suggesting an early escape of the programmed cell death during the preneoplastic process.^{3,4}

Cyclin D1 is an important cell cycle regulatory protein which controls the cell cycle transition from G1 to S phase. It is also involved in the regulation of programmed cell death depending on the proliferative and differentiated state of the cell. Cyclin D1 overexpression has been reported in various types of carcinomas such as seen in the breast, esophageal and lung. When Cyclin D1 is overexpressed, it increases the risk of tumor progression and early onset of cancer. Overexpression of Cyclin D1 enhances cell proliferation and cell cycle progression.⁵ Regarding the relationship of Cyclin D1 expression in lung cancer, the results reported in the literature have been controversial.

2. Materials and Methods

This study was hospital based cross sectional study conducted in the Department of Pathology and Department of Pulmonary Critical Care and Sleep Medicine, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi. A total of 40 cases of lung carcinoma were taken. Trucut biopsy / paraffin blocks received in the department were processed as per routine protocol. Routine 4 to 5 μ m sections were cut and sections were stained with haematoxylin and eosin. Sections taken on poly-L-lysine coated slides were used to perform immunohistochemistry for BCL 2 & Cyclin D1.

2.1. Statistical analysis

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean $\pm$ SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used. Statistical tests were applied as follows-Quantitative variables were compared using ANOVA between three groups, Qualitative variables were correlated using Chi-Square test.

A p value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

3. Results

3.1. Immunohistochemical expression of Bcl 2 & Cyclin D1

The immunohistochemical expression of Bcl 2 was studied in all 40 cases of lung carcinoma. A case was considered as positive when >5% of the cells showed cytoplasmic

positivity. Out of 40 cases of lung carcinoma, 21 cases (52.50%) were positive for Bcl 2 and 16 cases (40.00%) were negative for Bcl 2. In 3 cases (7.50%) diagnosed as adenocarcinoma, the amount of tissue left in block was inadequate for performing immunohistochemistry. The immunohistochemical expression of Cyclin D1 was studied in all 40 cases of lung carcinoma. A case was considered as positive when >5% of the cells showed nuclear positivity. Out of 40 cases of lung carcinoma, 16 cases (40.00%) were positive for Cyclin D1 and 21 cases (52.50%) were negative for Cyclin D1. In 3 cases (7.50%) diagnosed as adenocarcinoma, the amount of tissue left in block was inadequate for performing immunohistochemistry.

3.2. Correlation of immunohistochemical markers with histological subtypes

The immunohistochemical expression of Bcl 2 & Cyclin D1 was correlated with histological types of lung carcinoma. We observed Bcl 2 positivity in 4/5 cases of small cell carcinoma, 11/14 cases of squamous cell carcinoma & 6/21 cases of adenocarcinoma. In 3 cases of adenocarcinoma, the amount of material left in the block was inadequate for performing immunohistochemistry. The study showed that the expression of Bcl 2 was significantly higher in patients with small cell carcinoma, showing cytoplasmic positivity (Figure 1) in 4/5 cases (80%) followed by squamous cell carcinoma, showing cytoplasmic positivity (Figure 2) in 11/14 cases (78.57%) as compared to adenocarcinoma which showed cytoplasmic positivity (Figure 3) in only 06 out of the 21 cases (28.57%) studied. This comparison was statistically significant with a p value of 0.029 (<0.05) & with Cyclin D1 we observed positivity in 11/21 cases of adenocarcinoma and in 5/14 cases of squamous cell carcinoma. All 5 cases of the small cell carcinoma were negative for Cyclin D1. In 3 cases of adenocarcinoma, the amount of material left in the block was inadequate for performing immunohistochemistry. The study showed that the expression of Cyclin D1 was significantly higher in patients with adenocarcinoma, showing nuclear positivity (Figure 4) in 11/21 cases (52.38%) as compared to Squamous cell carcinoma which showed nuclear positivity (Figure 5) in only 05 out of the 14 cases (35.71%) studied. All cases of small cell carcinoma were negative for Cyclin D1. This comparison was statistically significant with a p value of 0.048 (<0.05).

3.3. Correlation of immunohistochemical markers with clinical staging

The immunohistochemical expression of Bcl 2 & Cyclin D1 was correlated with the clinical staging. The expression of Bcl 2 was significantly higher in patients with clinical stage 3 disease in which positivity was seen in 9/11 cases (81.82%) as compared to stage 4 disease in which positivity

was seen in 11/20 cases (55%). However Bcl 2 expression was seen in only one case of stage 2 disease (11.11%). This comparison was statistically significant with a p value of 0.002 (<0.05) & the expression of cyclin D1 was significantly higher in patients with clinical stage 4 disease in which positivity was seen in 16/20 cases (80.00%). However, none of the stage 2 and stage 3 patients showed any expression with cyclin D1. This comparison was statistically significant with a p value of <0.0001 (<0.05).

Fig. 1: BCL 2 cytoplasmic positivity in small cell carcinoma of lung (400X)

Fig. 2: BCL 2 cytoplasmic positivity in squamous cell carcinoma of lung (400X)

4. Discussion

Lung cancer is one of the most common cause of cancer related deaths both in men & in women. The five year survival rate among lung cancer is poor and it varies between 6% to 14% for men and 7% to 18% for women.⁶ It is the leading cause of death worldwide with mortality rate of 19.4%. In India mortality rate is 9.3%. Thus,

Fig. 3: BCL 2 cytoplasmic positivity in adenocarcinoma of lung (400X)

Fig. 4: Cyclin D1 nuclear positivity in adenocarcinoma of lung (400X)

lung carcinoma constitutes a major burden of all the cancers ranking the fourth most common cancer in Indian population.⁷

The immunohistochemical expression of the Bcl 2 was studied in 40 cases of lung cancer and also correlated with the histological types. Out of 40 cases of lung carcinoma in our study, 21 cases (52.5%) were positive for Bcl 2 and 16 cases (40%) were negative for Bcl 2. In 3 cases immunohistochemistry could not be performed as the tissue left was inadequate. We included 35 cases of non small cell lung carcinoma (14 cases of squamous cell carcinoma & 21 cases of adenocarcinoma) and only 5 cases of small cell carcinoma lung. Out of these, we observed Bcl 2 positivity in 4/5 cases of small cell carcinoma, 11/14 cases of squamous cell carcinoma & 6/21 cases of adenocarcinoma. The study showed that the

Fig. 5: Cyclin d1 nuclear positivity in squamous cell carcinoma of lung (400X)

expression of Bcl 2 was significantly higher in patients with small cell carcinoma, showing cytoplasmic positivity in 4/5 cases (80%) followed by squamous cell carcinoma, showing cytoplasmic positivity in 11/14 cases (78.57%) as compared to adenocarcinoma which showed cytoplasmic positivity in only 6/21 cases (28.57%) studied. Among non small cell carcinoma, Bcl 2 expression was higher in SCC. This comparison was statistically significant with a p value of 0.029 (<0.05).

These observations were similar to the observation seen in studies conducted by the Jiang et al.⁸ Jiang et al observed that out of the 111 small cell lung cancers (SCLC), 104 (93.7%) showed Bcl 2 positivity, out of 64 squamous cell carcinoma, 23 (36%) showed positivity for Bcl 2 & out of 65 adenocarcinomas only 4 cases (6.2%) were positive for Bcl 2. Hence, expression of Bcl 2 was much higher in small cell carcinoma as compare to the non small cell carcinoma cases & among non small cell carcinoma, expression was higher in squamous cell carcinoma than in adenocarcinoma.

The immunohistochemical expression of the Cyclin D1 was studied in 40 cases of lung cancer and this was also correlated with the histological types. Out of 40 cases of lung carcinoma in our study, 16 cases (40%) were positive for Cyclin D1 and 21 cases (52.5%) were negative for Cyclin D1. In 3 cases immunohistochemistry could not be performed as the tissue left was inadequate. We included 35 cases of non small cell lung carcinoma (14 cases of squamous cell carcinoma & 21 cases of adenocarcinoma) and only 5 cases of small cell carcinoma of lung. Out of these, we observed Cyclin D1 positivity in 11/21 cases of adenocarcinoma and 5/14 cases of squamous cell carcinoma. None of the case of the small cell carcinoma

showed positivity for Cyclin D1. The study showed that the expression of Cyclin D1 was significantly higher in patients with adenocarcinoma, showing nuclear positivity in 11/21 cases (52.38%) as compared to Squamous cell carcinoma which showed nuclear positivity in only 05 out of the 14 cases (35.71%) studied. All 5 cases of the small cell carcinoma were negative for Cyclin D1. This comparison was statistically significant with a p value of 0.048 (<0.05). Very few studies are available in literature regarding the immunohistochemical expression of Cyclin D1 in lung cancer and its correlation with histological types. Also the results of these studies are conflicting.

The immunohistochemical expression of Bcl 2 and Cyclin D1 was correlated with the clinical staging of lung cancer. We observed Bcl 2 expression in 9/11 cases (81.82%) of stage 3 disease and 11/20 cases (55%) of stage 4 disease. Only 1 case (11.11%) of stage 2 disease showed Bcl 2 expression. This comparison was statistically significant with a p value of 0.002 (<0.05). Majority of the studies available in literature have correlated the expression of Bcl 2 with overall survival of patients. Ohmura et al⁹ observed that out of 64 cases with NSCLC, 28 (44%) showed positivity for Bcl 2. The cases with stage I & stage II disease expressed Bcl 2 immunostaining more frequently than the cases of stage III disease ($p=0.024$). However, in our study we observed Bcl 2 expression was most frequently associated with stage III disease.

The immunohistochemical expression of Cyclin D1 was correlated with the clinical staging. We observed Cyclin D1 expression in 16/20 cases (80.00%) of stage 4 disease. However, none of the stage 2 and stage 3 patients showed any expression of Cyclin D1. This comparison was statistically significant with a p value of <0.0001 (<0.05). In a study by JS Keum et al¹⁰ Cyclin D1 expression was correlated with lymph node metastasis and the stage of the tumor. 21 out of 42 NSCLCs with lymph node metastasis (50%) showed positive expression for Cyclin D1. According to the stage of the tumor, stage I tumors showed a lower Cyclin D1 positive expression than stages II and III tumors (10%, 53.8%, and 41.7% respectively). These finding were the statistically significant. ($P=0.006$ for stage I vs. II, III; $P=0.048$). In our study also we observed that Cyclin D1 expression was associated with higher clinical stage disease.

5. Conclusion

On the basis of present study, it can be concluded that there is a definite role of Bcl 2 & Cyclin D1 in Lung cancer. The expression of these markers correlated with specific histological subtypes as well as with advanced disease i.e. with higher clinical stage. In our study, higher Bcl 2 expression was associated with small cell carcinoma of lung and among non small cell carcinoma its expression was higher in squamous cell carcinoma. Cyclin

D1 expression was observed mostly in adenocarcinoma cases. On correlating the expression of these markers with clinical staging, we concluded that Bcl 2 is highly expressed in patients with stage 3 disease and Cyclin D1 expression was mostly associated with stage 4 disease. Hence, in our study these 2 markers showed higher expression with advanced stage of disease. However, Cyclin D1 was associated with more advanced stage i.e. Stage 4 diseases.

6. Source of funding

None.

7. Conflict of interest

None.

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