The level of bronchoalveolar lavage fluid prostaglandine E2; is it diagnostic of bronchogenic carcinoma?

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Abstract Background: Lung cancer is the leading cause of cancer-related mortality in the United States. Cigarette smoking is the number one risk factor for lung cancer. It causes about 90% of lung cancers (14).

Objective: Evaluation of bronchoalveolar lavage level of PGE2 in patients with primary bronchogenic carcinoma.

Subjects and methods: The study was conducted on forty subjects; including twenty patients with bronchogenic carcinoma, ten patients with non-malignant lesions, and ten healthy control subjects. All subjects were submitted to fiberoptic bronchoscopy with bronchoalveolar lavage was done and examined for prostaglandin E2.

Results: The PGE2 level was significantly higher in BALF of group III (malignant group) compared to group I & II, with no significant difference between group I and group II. The cut-off value of PGE2 was 45.63 pg/ml with minimal overlap between malignant and benign lesions.

Conclusions: Bronchoalveolar lavage level of PGE2 was significantly increased in patients with bronchogenic carcinoma.

Introduction

Lung cancer is the most common cause of cancer mortality in both males and females. It constitutes 12.8% of cancer cases and causes 17.8% of cancer death worldwide. Overall, the global incidence of lung cancer is increasing at rate of 0.5% per a year [1,2].

Rising incidence of lung cancer has been shown to be closely linked to increasing cigarette smoking [3,4]. The 5-year relative survival rate from 1995 to 2001 for patients with lung cancer was 15.7%. The 5-year relative survival rate varies
markedly depending on the stage at diagnosis, from 49% to 16% to 2% for patients with local, regional, and distant stage disease, respectively [5].

A tumour marker is a substance produced by a tumour itself or produced by the host in response to a tumour, that can be used to differentiate a malignant disease from a benign one. Tumour markers can be determined easily in body fluids like blood and/or bronchoalveolar lavage (BAL) fluid, therefore many physicians consider them as useful diagnostic and prognostic tools [6].

Prostaglandins are found in most tissues and organs. They are produced by almost all nucleated cells. Prostaglandins are not endocrine hormones, but autocrine or paracrine, which are locally acting messenger molecules. They differ from hormones in that they are not produced at a discrete site but in many places throughout the human body. Also, their target cells are present in the immediate vicinity of the site of their secretion [7,8].

Prostaglandins ligate a sub-family of cell surface seven-transmembrane receptors, G-protein-coupled receptors. These receptors are termed DP1-2, EP1-4, FP, IP1-2, and TP, corresponding to the receptor that ligates the corresponding prostaglandin (e.g., DP1-2 receptors bind to PGD2) [9].

Prostaglandin E2 (PGE2) mediates several hallmark cancer: During early neoplastic transformation, PGE2 activates the epidermal growth factor receptor (EGFR) to enhance cell proliferation [13] and up-regulates surviving concentrations in tumors to decrease apoptosis [14]. At later stages of progression, PGE2 up-regulates the biosynthesis of vascular endothelial growth factor (VEGF) expression, which stimulates angiogenesis [15], increases IL-10 biosynthesis to inhibit adaptive antitumor immune responses [16] and augments protease expression, thereby promoting invasion [17].

**Objective of the study**

The aim of this work is to evaluate PGE2 level in bronchoalveolar lavage fluid to clarify its role as a diagnostic marker in patients with bronchogenic carcinoma.

**Subjects and methods**

This study was carried out on 40 subjects selected from inpatients and outpatients of Chest Department Tanta University Hospital from May 2011 to February 2012.

The subjects were classified into three groups

**Group I:** Included ten healthy non smokers volunteers (control group).

**Group II:** Included ten patients with benign pulmonary diseases; 2 patients with COPD, 2 asthmatic patients, 3 patients with pneumonia, 2 patients with lung abscess and one patient with old T.B. Their ages ranged between 45 and 58 years with a mean 51.69 ± 5.30 years.

**Group III:** Included twenty patients with bronchogenic carcinoma (confirmed by histopathological examination of BAL, brush and/or biopsy). Their ages ranged between 45 and 65 years with a mean of 54.47 ± 8.30 years.

**After an informed written consent was taken from all subjects**

Full history taking, complete clinical examination, plain chest X-ray posteroanterior and lateral view, CT Scan of the chest for some patients of benign pulmonary diseases in group II and all patients in group III were done. Bronchoalveolar lavage was done to all patients under local anaesthesia using flexible fiberoptic bronchoscopy (Olympus BF type P10) and examined for PGE2 levels by enzyme linked immunosorbent assay (ELISA).

**Principle of the immunosorbent assay (ELISA)**

This assay is based on the forward sequential competitive binding technique in which PGE2 present in a sample competes with horse radish peroxidase (HRP)-labeled PGE2 for a limited number of binding sites on a mouse monoclonal antibody. PGE2 in the sample is allowed to bind to the antibody in the first incubation. During the second incubation, HRP-labeled PGE2 binds to the remaining antibody sites and followed by a wash to remove unbound materials.

A substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of PGE2 in the sample.

**Statistical analysis**

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, unpaired student t-test, analysis of variance [ANOVA] test, by SPSS V18. The sensitivity, specificity and accuracy were calculated by using receiver operating characteristic (ROC) analysis.

**Results**

A forty subjects were included in our study, divided into three groups; Ten healthy subjects as control group (group I), ten patients with non-malignant lung diseases (group II), and twenty patients with bronchogenic carcinoma (group III). The mean values of age in group I, group II, and group III were 47.63 ± 4.63, 51.69 ± 5.30, and 54.47 ± 8.30, respectively. There was no significant difference in age in the three studied groups (P = 0.632).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean values (±SD) and Statistical comparison of BALF level of PGE2 in groups I, II and III.</th>
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<td>PGE2 level</td>
<td>ANOVA</td>
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<tr>
<td>Range</td>
<td>Mean ± SD</td>
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<td>GI</td>
<td>25–40</td>
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<tr>
<td>GII</td>
<td>40–60</td>
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<td>GIII</td>
<td>150–280</td>
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</table>
The mean values of PGE2 (pg/ml) in BALF in groups I, II and III were 32.6 ± 8.11, 47.53 ± 8.60, and 199.9 ± 22.8, respectively. PGE2 was significantly higher in group III compared to groups I & II ($F = 14.62, P = 0.001$). While there was no significant difference between group I and group II ($P = 0.01$), (Table 1).

The patients of group III were divided according to histopathological findings into three subgroups: IIIA; 8 patients diagnosed as squamous cell carcinoma (40%), with 7 males and one female, IIIB: 7 patients diagnosed as adenocarcinoma (35%), with 6 males and one female, and IIIC: 5 patients diagnosed as large cell carcinoma (25%), all of them were males.

The mean values of PGE2 (pg/ml) in BALF in groups IIIA, IIIB and IIIC were 221.60 ± 18.63, 187.22 ± 9.62 and 133.80 ± 7.10 respectively. PGE2 level was significantly higher in subgroup IIIA compared to subgroups IIIB & IIIC ($F = 6.32$, $P = 0.004$). There was no marked difference in PGE2 levels in subgroups IIIB and IIIC.

The cut-off value of bronchoalveolar lavage fluid PE2 for diagnosis of malignant lung diseases was 45.63 with sensitivity (80%), specificity (85%), +ve predictive value (88%), and –ve predictive value (16%). Analysis of the ROC curve for the PE2 in the BALF is shown in Fig. 1.

**Discussion**

In the present study, the PGE2 level in BALF was significantly higher in group III compared to group I&II, with no significant difference between group I and group II. These results are in agreement with the results obtained by Bennett et al. [18], who compared PGE2 from tumor tissues and “normal tissues” from the same lung and found that carcinomatous tissues contained a significantly larger amount of PGE2 than “normal lung tissue”. These observations suggested that tumor cells or cells associated with tumor in the tissue such as monocytes/macrophages, are the origin of the increased PGE2 observed in the tumor tissue.

Also, LeFever and Funahashi [19], who had measured the concentration of PGE2 in BALF of patients with lung carcinoma and found it to be elevated in 18 patients with primary lung cancer as compared with 6 patients with lung diseases other than lung cancer and 10 normal subjects. In the present study, PGE2 level was significantly higher in subgroup IIIA compared to subgroups IIIB & IIIC, with no marked difference in PGE2 levels in subgroups IIIB and IIIC. These results are in agreement with the results obtained by Funahashi et al. [20], who found that any malignant process, either primary lung cancer or metastatic cancer from other organs, is associated with an increased PGE2 content in BALF. Also, he found that BALF PGE2 content is significantly higher in patients with SQCA as compared with patients with ADCA or SCCA. Moreover, he found a decrease of BALF PGE2 levels observed after successful removal of cancer to the range seen in patients with pulmonary diseases other than cancer.

On the other hand, Hida et al. [21], reported that increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Also, Yoshimatsu et al. [22], who investigated the expression of PGES, a glutathione-dependent, membrane-bound enzyme that converts PGH2 into PGE2. He demonstrated that PGES levels were increased in NSCLCs relative to normal tissue and mutant ras, which is found in 30–50% of lung adenocarcinomas.

In the present study the cut-off value of PGE2 was 45.63 (pg/ml) with minimal overlap between cancer and benign lesions. Funahashi et al. [20], reported that the ability of PGE2 to differentiate malignant from benign lesion shown by the difference in the mean values of PGE2 between lung cancer and noncarcinomatous benign lesions, with a BALF PGE2 value of 51 pg/ml was used as a cut-off value between cancer and benign lesions.

**Conclusion**

The PGE2 level in BALF was significantly higher in malignant group, with the ability to differentiate malignant from benign lesions. Further studies should be carried out on a large population to study the correlation of PGE2 level with the lung cancer stage and to assess the value of this marker in monitoring the patients after therapy concerning the tumour response and its importance in early detection of tumour recurrence.
References


