8-Isoprostane in Exhaled Breath Condensate of Patients with Non-small Cell Lung Cancer: The Effect of Chemotherapy

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Abstract. Aim: The aim of the study was to evaluate the exhaled breath condensate (EBC) levels of a valid oxidative stress marker, 8-isoprostane, before and after chemotherapy, in patients with non small cell lung cancer (NSCLC) in correlation with the extent of the disease and response to treatment. Patients and Methods: Forty-five patients with inoperable NSCLC were initially enrolled in the study. Twenty-nine of them were finally evaluated in regards to 8-isoprostane levels in EBC before and after chemotherapy. Results: 8-Isoprostane levels were significantly lower after chemotherapy (p=0.014). Further analysis showed that the differences were mainly attributed: a) to the extent of the disease, with patients diagnosed with up to locally advanced disease (stages IB–IIIB) having significantly lower EBC 8-isoprostane levels post-chemotherapy (p=0.031); and b) to the response to treatment, with patients evaluated with partial response to treatment having significantly lower EBC 8-isoprostane levels post-chemotherapy (p=0.02). Conclusion: In this prospective study, we showed that 8-isoprostane might represent a biomarker in NSCLC, reflecting both response to chemotherapy, as well as the extent of the disease.

Lung cancer is a leading cause of global cancer death with oxidative stress playing an important role in its pathogenesis (1). The use of biomarkers to detect for the disease, as well as to identify both the at-risk population and the extent of disease, has been the goal of many investigations but none have so far managed to reach the clinical setting (2). Exhaled breath condensate (EBC) is a simple, safe and non-invasive procedure sampling the lower respiratory tract which has gained much interest in recent years (3). Studies on EBC of patients with lung cancer have shown increased levels of inflammatory and oxidative stress biomarkers, as well as DNA alterations and mutations (4).

8-Isoprostane belongs to the broader family of isoprostanates, characterized as the most specific biomarkers of lipid peroxidation, and represents a valid marker of oxidative stress in lung diseases, including lung cancer.

None of the previous studies have addressed the use of EBC as a screening tool for biomarkers to evaluate the response-to-treatment strategies. In order to investigate this issue in inoperable patients with non-small cell lung cancer (NSCLC), we measured the levels of 8-isoprostane in EBC before and after chemotherapy (5, 6). Furthermore, we evaluated whether baseline and post-chemotherapy levels of 8-isoprostane could predict for survival outcomes.

Patients and Methods

The study was approved by the Ethics Committee of Sotiria General Hospital, Athens, Greece (approval protocol number: 16382) and written informed consent was obtained from all patients. Forty-five patients (men: 38, women: 7; mean±SD age: 65±9 years; mean±SD Body Mass Index (BMI): 26±4 kg/m²; all current or ex-smokers, mean±SD pack years: 62±36) were initially recruited, all with histologically or cytologically documented NSCLC. The tumor histological types were: adenocarcinoma in 14 patients (31.1%), squamous cell carcinoma in 15 (33.3%) and nonspecified NSCLC in 16 patients (35.6%). All patients underwent staging procedure according to the staging system of American Joint Committee on cancer TNM classification and all were characterized as inoperable (7). According to the TNM staging, there were two patients with stage IB disease, five with stage IIA, 11 with stage IIB, and the majority: 27 patients with stage IV. In total, there were 18 patients (40%) with limited/locally advanced disease (stages IB-IIB) and 27 patients (60%) with extensive/metastatic disease (stage IV). Patients with current or recent infections/sepsis were not recruited to prevent any confounding effect of inflammation, nor were patients with comorbidities such as diabetes mellitus, immune-related diseases, liver disorders or other malignancies.

EBC was collected according to standard procedures (EcoScreen; Viasys, Hoechberg, Germany). 8-Isoprostane concentration in EBC was determined by a specific enzyme immunoassay kit (8-Isoprostane 5143 5144 5145 5146 5147 5148 5149).
Results

8-Isoprostane levels in EBC of patients with NSCLC decreased significantly after chemotherapy (Figure 1). This significant difference in 8-isoprostane levels seemed to be influenced by two independent factors: the response to chemotherapy and the extent of the disease (Figure 1).

Specifically, the baseline EBC 8-isoprostane levels of the 45 patients were statistically significantly ($p=0.014$) higher when compared to those of the 29 patients of the follow-up ($13.5±6.2$ pg/ml vs. $10.4±5.0$ pg/ml).

Regarding the correlations with response to chemotherapy, only patients with partial response (PR) to treatment presented a statistically significant decrease ($p=0.002$) in 8-isoprostane after chemotherapy (falling from $17.2±5.0$ pg/ml to $10.9±5.1$ pg/ml) and there were no statistically significant differences in patients with stable disease (SD) or disease progression (PD).

Interestingly, in patients who had tumor stages IB-IIIB, there was a statistically significant decrease ($p=0.031$) of 8-isoprostane after chemotherapy ($14.2±7.0$ pg/ml vs. $11.2±4.2$ pg/ml), in contrast to patients with advanced/metastatic disease (stage IV) who presented no statistically significant differences in 8-isoprostane levels after chemotherapy ($p=0.2$).

Baseline 8-isoprostane levels $>18$ pg/ml presented a sensitivity of 0.63 and a specificity of 0.89 for the prediction of one-year survival, with an AUC of 0.759 (95% confidence interval=0.56-0.9, $p=0.008$). No significant predictive value was found for 8-isoprostane levels after chemotherapy.

Discussion

Oxidative stress has been widely explored recent years, as it has been implicated in cancer pathogenesis including that of the lung. Direct exposure of lungs to both external (cigarette smoke, environmental pollutants or carcinogens) and internal (free radicals generated from metabolic processes) oxidants results in disequilibrium between oxidants and anti-oxidants (such as glutathione, ascorbic acid, and β-carotene), leading to increased oxidative stress (9). Among the different markers that have been investigated, isoprostanes seem to be ideal for exploring oxidative stress, being specific products of free radical-catalyzed lipid peroxidation of arachidonic acid, independently of cyclooxygenase – as their levels are modulated by anti-oxidant status (10). From the broad family of isoprostanes, 8-isoprostane is a reliable marker and is considered a gold standard for lipid peroxidation (10).

Over the past decade, breath tests, and in particular ECB, have been widely used for screening potential lung cancer biomarkers due to specific advantages: being safe, convenient, repeatable and non-invasive (3, 4). There are few ECB studies that have evaluated the levels of 8-isoprostane in patients with lung cancer. Chan et al. confirmed higher levels of 8-isoprostane in patients with lung cancer compared to healthy smokers, ex-smokers and non-smokers, but the differences were not statistically significant (9). In a similar study by Dalaveris et al., the conclusion was that there was no significant difference regarding the level of 8-isoprostane in EBC between patients with lung cancer and controls, and any differences in the levels of 8-isoprostane were not significantly related to clinicopathological parameters (12).
On the very interesting topic of how EBC oxidative stress markers correlate with treatment, such as chemotherapy, there is no scientific evidence. Current knowledge acquired through blood testing, confirms that use of antineoplastic drugs (alkylating agents, platinum etc.) may increase oxidative stress in patients with lung cancer by producing reactive oxygen and nitrogen species. In addition many anticancer drugs cause peroxidative damage through generation of free radicals (11). The human anti-oxidant system (superoxide dismutase, catalase, glutathione peroxidase, etc.) interacts with the increased level of oxidative stress generating during chemotherapy in a critical way, thus the antioxidant status of patients seems to play an important role in response to treatment (11).

It is well-known that in patients with lung cancer, levels of some oxidative stress markers increase (such as malondialdehyde and H2O2) and levels of some antioxidant markers (including glutathione, β-carotene, vitamins A and C) decrease as the disease progresses (13, 14). Based on this hypothesis, it was scientifically interesting to evaluate the levels of a valid oxidative stress marker such as 8-isoprostane in EBC before and after chemotherapy, in correlation with the burden of disease and with respect to response to treatment, which was the aim of our study (with the limitation of a relatively small number of patients).

In the current study, we show that 8-isoprostane levels in EBC are significantly influenced by chemotherapy in patients with NSCLC. This influence was mainly attributed to the underlying extent of the disease, as well as to the response to chemotherapy. Furthermore, baseline values are predictors of one-year survival. Compared to existing studies, our study raises a possible role for 8-isoprostane in EBC as a biomarker of NSCLC that is responsive to therapy and may represent a predictor of one-year survival. This latter finding, however, needs to be prospectively evaluated in long-term survival studies.

Conflicts of Interest

Nothing to declare.

References


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