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Tissue oxidative stress in non small cell lung cancer

[Küçük hücreli dışı akciğer kanserinde doku oksidatif stresi]

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ABSTRACT

Aim: As the leading cause of adult mortality for many countries, lung cancer was considered to be related with free radical injury. We aimed to investigate if the oxidative stress is increased or not in malignant tissues, compared to the normal ones in non small cell lung cancer.

Methods: We compared malignant and normal lung tissues for their malondialdehyde, total thiol and vitamin E contents in 35 smoker patients with non small cell lung cancer.

Results: There was no significant difference between malignant and normal tissues for malondialdehyde and total thiol concentrations ($p>0.05$). However, vitamin E contents of the malignant tissues were significantly higher than the normal tissues ($p<0.05$).

Conclusion: Similar malondialdehyde levels in malignant and normal tissues suggest that cigarette smoke induces a diffuse increase of free radical formation in lung tissue, rather than a focal response. In malignant tissues, malondialdehyde formation might be prevented by high tissue levels of vitamin E.

Key Words: oxidative stress, non small cell lung cancer, vitamin E

ÖZET

Amaç: Birçok ülkede erişkin ölümlerinin en sık nedeni olan akciğer kanserinin serbest radikal hasarı ile ilişkili olduğu düşünülmektedir. Çalışmamızda oksidatif stresin küçük hücreli dışı akciğer kanserli dokuda normal dokuya göre artıp artmadığını araştırmayı amaçladık.

Metod: Küçük hücreli dışı akciğer kanseri olan 35 sigara içicisi hastanın malign ve normal akciğer dokuları malondialdehit, toplam tiyol ve vitamin E içerikleri açısından karşılaştırıldı.

Bulgular: Malondialdehit ve toplam tiyol konsantrasyonu açısından malign ve normal akciğer dokuları arasında anlamlı fark yoktu ($p>0.05$). Bununla birlikte vitamin E konsantrasyonları malign dokularda normal dokulara göre belirgin yüksekti ($p<0.05$).

Sonuç: Malign ve normal akciğer dokularındaki benzer malondialdehit seviyeleri, sigara içmenin sadece sınırlı alanlarda değil tüm akciğer alanlarında serbest radikal oluşumunu artırdığını düşündürmektedir. Yüksek vitamin E seviyeleri de malign dokulardaki malondialdehit oluşumunu engellemiş olabilir.

Anahtar Kelimeler: oksidatif stres, küçük hücreli dışı akciğer kanseri, vitamin E

Introduction

Living organisms are exposed to various exogenous (photochemical air pollutants, cigarette smoke, pesticides etc.) and endogenous (mitochondrial electron transport, auto oxidation of small molecules, etc.) free radical sources throughout their lifespan (1, 2). These radicals which are harmful for vital components of the cell such as DNA and lipid/ protein structures are considered to be responsible for all degenerative changes in a wide range of pathologies from aging to cancer (3).

The role of oxidant/ antioxidant systems in pathogenesis and progress of lung cancer; which is the most frequent cause of adult mortality in many countries, is one of the popular research areas (3). There are many risk factors for lung cancer. Smokers, compared with non smokers have a 10-30 times increased relative risk of developing lung cancer and smoking is considered to be the primary risk factor for this type of cancer. Exposure to asbestosis, radon, arsenic, ionizing radiation, halo ethers, polycyclic aromatic hydrocarbons and nickel are some of the other risk factors (4). Exposure to these risk factors for long durations leads to formation of free radicals in high concentrations. These radicals have the ability to cause permanent changes in DNA if defense mechanisms of the cell are insufficient. These changes increase the rate of mutations in genomes of rapidly proliferating cells, such as type II pneumocytes, bronchial epithelial and Clara cells (5). Oxidants play important roles in various stages of malignant transformation. They can make many permanent changes like point mutations, deletions, gene rearrangements and amplifications in DNA (6). Therefore we aimed to investigate if the oxidative stress is increased or not in malignant tissues, compared to the normal ones in non small cell lung cancer.

Methods

This study was performed on 35 patients (mean age 57.2±10.3 years) with non small cell lung carcinoma, who were operated in Ataturk Thoracic Diseases and Thoracic Surgery Education and Research Hospital, Ankara. Only one of them was female, the others were all males. All of them were smokers, and they smoked approximately 44.6 ± 27.3 package-year. Patients in adenocarcinoma and squamous cell carcinoma subgroups were similar for the number of cigarettes they smoke per year. Patients contributing to this study were chosen from the ones who did not recieve any chemotherapy or radiotherapy. On the basis of the histopathological tumor types, patients were grouped as follows: 9 adenocarcinoma, 23 squamous cell carcinoma, 1 large cell carcinoma, 1 pleomorphic adenoma and 1 adenosquamous carcinoma. Distribution of the cases for pathological stages was as follows: Ib (n=5), IIa (n=5), IIb (n=17), IIIa (n=2), IIIb (n=6). Lung tissue samples were taken intraoperatively and they were transferred to the pathology laboratory immediately without any fixation. Samples for malignant tissues were obtained from peripheral and non-necrotic regions of the tumor. Nonmalignant tissue samples were taken from periphery of the specimen, which is apart from the tumor borders. We could not establish an independent control group for nonmalignant tissue samples since it could not be possible to excise normal lung tissue samples in operations for causes other than malignancy (such as tuberculosis, bronchiectasia, cysts). Local ethical committee approved the study and informed consent of the patients was obtained. All tissue samples were homogenized in PBS buffer (phosphate buffered saline). Malondialdehyde (MDA), total thiol (-SH) groups and vitamin E analysis were made in tissue homogenates. MDA levels were determined with the method developed by Ohkawa et al (7) and results were given as μmol MDA/g tissue. Analysis of total -SH groups was performed with the method of Sedlak and Lindsay (8) and protein contents of tissue samples were measured with Bradford's method (9). The concentrations of total –SH groups were given as μmol –SH/g protein. Vitamin E contents were measured with HPLC method (Agilent 1100, Chromsystems). Vitamin E concentrations were also given as mg/g tissue.

Statistical evaluation of the data was performed with SPSS for windows (version 10.0). Mann Whitney U test was used for the comparison of the measurements in malignant and nonmalignant tissues. Spearman correlation test was performed for the correlation analysis. $p<0.05$ was accepted as statistically significant.

Results

In order to investigate the oxidative stress in malignant and normal lung tissues of the patients with non small cell lung cancer we have measured tissue MDA, total-SH and vitamin E contents. We observed that MDA concentrations were similar in malignant and normal tissues. There was no significant difference for total-SH levels between malignant and normal tissues, although. However vitamin E concentrations in malignant tissues were higher than the normal tissues, and this difference was statistically significant ($p<0.05$) (Table 1).

Table I. Total –SH, MDA and Vitamin E concentrations in malignant and normal lung tissues

Parameter	**Malignant tissue** Median (Interquartile range)	**Normal tissue** Median (Interquartile range)	p
Total –SH (μmol/ g protein)	652.5 ± 307.3	516.8 ± 297.8	0.145
MDA (μmol/ g tissue)	41.9 ± 28.5	51.9 ± 17.3	0.154
Vitamin E (mg/ g tissue)	6.40 ± 4.20	4.50 ± 3.62	**0.005**

We compared adenocarcinoma and squamous cellular carcinoma subgroups for the same parameters and observed that MDA and total-SH and levels in malignant tissues of the squamous cellular carcinoma cases were not different from the normal tissues of these patients (Figure 1 and 2). But vitamin E concentrations in malignant tissues of the patients with squamous cell carcinoma were higher than the normal tissues of the same cases but the difference was not statistically significant. (Figure3). The number of the cases for histopathological subtypes other than adenocarcinoma and squamous cellular carcinoma was not sufficient for statistical evaluation.

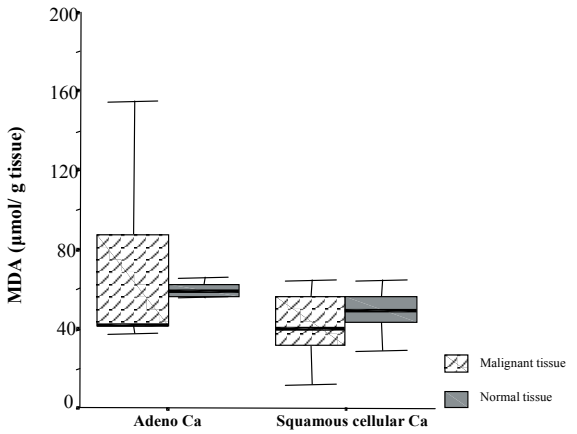

Figure 1. MDA levels of malignant and normal tissues in non small cell lung cancer and in adenocarcinoma and squamous cellular carcinoma subgroups: MDA levels were determined with the spectrophotometric method described by Ohkawa et al. The concentrations were given as µmol/g tissue. MDA levels were similar in malignant and normal tissues. (Adeno Ca: adenocarcinoma, Squamous cellular Ca: Squamous cellular carcinoma)

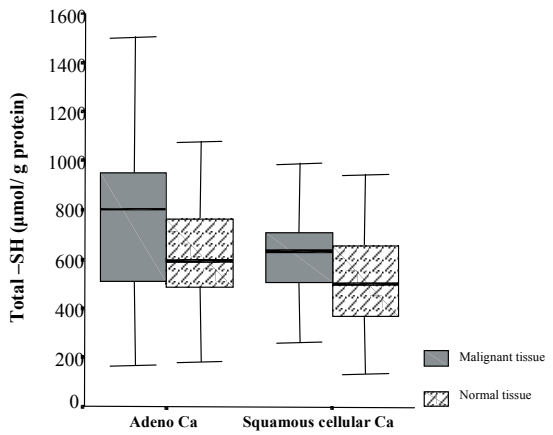

Figure 2. Total –SH (thiol) levels of malignant and normal tissues in non small cell lung cancer and in adenocarcinoma and squamous cellular carcinoma subgroups: Total –SH concentrations were measured with the method of Sedlak and Lindsay and the protein contents of tissues were determined by Bradford's method. The concentrations were given as µmol/g protein. Although tissue total thiol levels were higher in malignant tissues compared to the normal ones in either all cases or the adenocarcinoma and squamous cell carcinoma subgroups, the difference was not statistically significant. (Adeno Ca: adenocarcinoma, Squamous cellular Ca: Squamous cellular carcinoma)

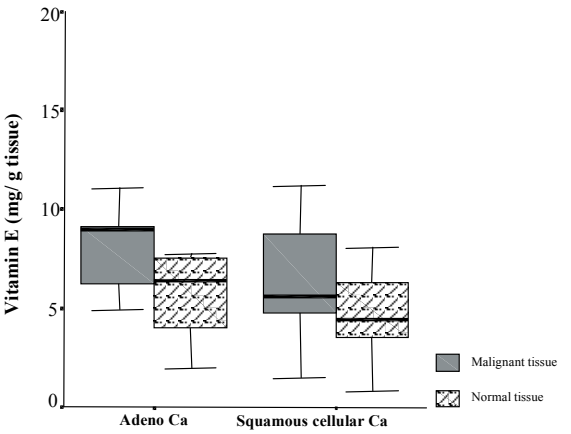

Figure 3. Vitamin E levels of malignant and normal tissues in non small cell lung cancer and in adenocarcinoma and squamous cellular carcinoma subgroups: Vitamin E analysis was performed with HPLC method (Agilent 1100, Chromsystems®). Vitamin E concentrations were given as mg/g tissue. Vitamin E levels in malignant tissues were significantly higher than the normal ones (p=0.005) and the concentrations of adenocarcinoma and squamous cellular carcinoma subgroups were similar. (Adeno Ca: adenocarcinoma, Squamous cellular Ca: Squamous cellular carcinoma)

We also found a positive correlation between MDA and vitamin E levels in normal tissues and total -SH and vitamin E levels in malignant tissues (data not shown).

Discussion

For the last two decades growing interest was focused on oxidative stress and different types of cancers. Susceptibility to lung cancer is determined by gene polymorphisms that regulate the activity of oxidant/antioxidant systems. Indeed the major burden of lung cancer in the population probably results from complex interactions between many genetic and environmental factors over time (10). But it is difficult to establish risk groups by genetic studies with large populations. The susceptibility of the body to oxidative injury depends largely on its ability to up-regulate protective free radical and reactive oxygen metabolite (ROM) scavenging systems (also known as antioxidant system) (11,12). Typical questions still exist whether free radical scavenging activities vary among different types of lung cancer and/or by other characteristics of the disease, such as stage and grade (12, 13).

Cigarette smoke, the most important risk factor for lung cancer, contains several chemicals, of which about 50 compounds are known as carcinogens. It was previously demonstrated by Garcon et al that exposure to polycyclic aromatic hydrocarbons increased MDA levels by two-fold in rat lung tissues (14).

In our study we observed that MDA concentrations were similar in malignant and normal tissues. The previous researches showed conflicting results about oxidative

stress in lung cancer. Most of the investigations referred serum levels of oxidant or antioxidant parameters. One of the previous reports about tissue levels of oxidative stress was declared by Petruzelli et al (15). They investigated MDA levels in lung tissues which seemed to be macroscopically normal in two patient groups with and without lung cancer. They did not find any significant difference between these groups (15). Our results are similar with the results of Petruzelli et al. However there are some reports claiming that oxidative stress was increased in malignant tissues. Zieba et al found that lipid peroxidation products were significantly higher in tumor tissue compared to lung parenchyma of the same patient (16). Boschetto et al demonstrated that the expression of heme oxygenase 1 decreased in tumor as compared with tumor free macrophages. This enzyme is a cytoprotective enzyme and plays a central role in the defense against oxidative stress (17). One of the previous reports about blood levels of oxidative stress in lung cancer is the investigation performed by Gencer et al (18). They determined serum ROM levels of thirty eight patients with lung cancer and twenty six healthy controls and also compared different histopathological types of the lung cancer for ROM production. ROM levels were significantly higher in patients with lung cancer than the controls. The concentrations were the highest in small cell, moderate in epidermoid and the lowest in adenocarcinoma. Esme et al recently demonstrated that blood MDA levels were increased in lung cancer and mean MDA levels were the highest in squamous cell carcinoma (19). Our results are controversial with these data. We can suggest that the normal tissues from the same patients -even they were taken from the far peripheral sides of the tumor-might have included malignant cells which can only be identified by pathological examination. This can be the reason of the similar MDA levels of the malignant and normal tissues. On the other hand, pollutants like cigarette smoke effects the whole lung tissue and induce malignant transformation. So this can be another factor contributing oxidant status in lung tissue. The previous reports declaring increased oxidative stress in lung cancer were mostly about blood levels of oxidative stress parameters and they compared patients with lung cancer and healthy controls. However, there was a positive correlation between MDA and vitamin E levels in normal tissues and total-SH and vitamin E levels in malignant tissues (data not shown).

Thiol groups are important in endogenous antioxidant defense to reactive oxygen metabolites (20). Glutathione is the major intracellular form of thiol groups (21, 22). From this point of view we analyzed the tissue total –SH levels instead of glutathione. But we did not find any significant difference between malignant and normal tissues for tissue total thiol levels. Previous reports showed conflicting results about glutathione and thiol levels in cancer cases. Petruzelli and MacKinnon did not find any significant difference between two patient groups with and without lung cancer for tissue glutathione levels (15, 23). Chung-man et al reported that there was no significant difference between malignant and normal tissues of the patients with non small cell lung cancer for tissue glutathione levels (24). On the other hand there are reports claiming an increase in glutathione levels in tumors. Blair et al and Krepela et al found significant increase in tumor glutathione levels in non small cell lung cancer patients (25, 26). Our results are similar with the data given by Petruzelli and MacKinnon but controversial with the others. The same factors we have declared for similar MDA levels would be responsible for similar total-SH levels. As an endogenous antioxidant thiol groups could be produced as a result of increased lipid peroxidation but used for cellular protection and their levels might be decreased.

It was previously shown that vitamin E decreased formation of lipid peroxidation end products (27). In rat models the preventive effect of vitamin E against lung cancer was demonstrated before (27, 28). We measured significantly higher vitamin E levels in malignant tissues than the normal ones. The studies carried on patients with lung cancer showed different results about vitamin E levels. Some studies reported that vitamin E had no effect of in lung cancer pathogenesis (29, 30). On the other hand, there are data in the literature which suggest a relationship between vitamin E and lung cancer progression (31, 32). Gromadzinska et al reported that vitamin E levels were significantly higher in the erythrocytes of the cancer patients than the healthy ones in a study carried out on patients with lung cancer (n=152) and healthy controls (n=210). They explained that this is due to increased intracellular accumulation of vitamin E for defense against oxidative stress with long duration (33). We can suggest that high tissue vitamin E levels might prevent MDA formation in malignant tissues and this can explain the similar MDA levels in malignant and normal tissues in our study. As suggested by Gromadzinska et al we can say that as a defense mechanism to oxidative stress vitamin E might have accumulated in malignant tissues.

The limited number of cases and malignant and normal tissue sampling from the same patients were the limitations of our study. We can explain the causes for these limitations: Firstly, we have included the patients with non small cell lung cancer who did not receive any radiotherapy or chemotherapy to our study, so this exclusion criterion limited the number of the cases. Secondly, excision of entirely normal lung tissue was impossible for ethical causes.

We conclude that an imbalance occurs between the levels of oxidant products and antioxidants in non small cell lung cancer. However carcinogens like cigarette smoke affect the lung tissue generally, the oxidative response against these carcinogenic factors occurs diffusely and this response is not a focal event.

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