The Contribution of Paraoxanase Enzyme Activity Measurement in Serum and Bronchoalveolar Lavage Liq to Distinguish Benign and Malignant Pulmonary Diseases

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Abstract

Lipid per oxidation final products and free oxygen radicals are thought as initiator role for developing of cancer in the body. Level of paraoxonase in the patients with cancer varies. In this research, we investigated the contribution of level of serum and bronchoalveolar lavage paraoxonase in the differentiation of benign and malignant lung disease patients. This research includes the patients that are diagnosis of lung cancer (research group) and benign pulmonary disease (control group) participated by accepted fiberoptic bronchoscopy (FOB). Twenty five patient with lung cancer and 19 patients with benign lung disease participated in the research. Level of serum paraoxonase in patients with lung cancer was significantly lower than the patients with benign lung disease (p = 0.005). This low level was found not to be related with smoking, age and gender parameters. Paraoxanase enzyme levels in bronchoalveolar lavage liq in both of two groups were not significantly different (p = 0.924). Serum paraoxanase enzyme level in the patients with lung cancer is significantly distinguishing lower than the patients with benign pulmonary disease, however measurement of paraoxanase level in bronchoalveolar lavage liq can’t help the differentiation.

Keywords: Lung Cancer; Paraoxanase; Bronchoalveolar Lavage; Benign Pulmonary Diseases

Abbreviations

PON: Paraoxanase; HDL: High Density Lipoprotein; FOB: Fiberoptic Bronchoscopy; BAL: Bronchoalveolar Lavage; (SO2): Oxygen Saturation

Introduction

Definition and Structure of the Enzyme

Paraoxanase (PON) which is calcium-dependent and structure of glycoprotein is an ester hydrolase with paraoxonase activity. Enzyme which has been first discovered by Abraham Mazur in 1946 has been defined as human serum PON 1. Paraoxanase gene family consists of three members in the form of PON1, PON2 and PON3 [1,2].

PON1 is localized on high density lipoprotein (HDL) in the plasma and released into the bloodstream by synthesizing in the liver. It’s an agent that protects the nervous system from the neurotoxic effects of organophosphates which is included in circulatory system and
its activity does not change depending on the age and sex. However, diet, smoking, acute phase proteins and pregnancy effects the serum PON1 level and its activity [3-6].

Paraoxonase and Cancer Relations

Oxidative stress is one of the important etiologic factor in carcinogenesis. Serum PON1 activity was found to be significantly lower in patients with lung cancer than healthy people by Elkiran., et al [7]. Also, gastroesophageal cancer, meningioma, high-grade glioma and ovarian epithelial was found to be significantly lower in tumour patients according to control groups in plasma [8]. The relationship between serum PON1 level and the occurrence of cancer is still unknown [9]. PON1 polymorphism has been reported to be related with an increased risk of occurrence of cancer in relation to environmental chemicals [10].

Reactive oxygen radicals can usually lead to tissue damage by attacking all cell components. Increased free oxygen radicals and oxidative stress which is formed in the body are one of the important risk factor in increasing various types of cancer. Lipid peroxidation’s final products and scavenger system components are thought to have a starter role in oncogenesis [11,12]. Carcinogenic radicals that are liposoluble are formed as a result of lipid peroxidation and PON1 binds with radicals that are liposoluble. PON1 are thought to be capable to metabolize these radicals that are liposoluble. PON1 activity and oxidative stress has been suggested to be inversely related in serum and macrophages. Loss of paraoxonase protective effect may play a role in increasing the sensitivity to genomic damage which inflammatory oxidants and diets carcinogens caused [10].

Purpose of this research is to compare serum and bronchoalveolar lavage liqs PON contrasts between patients with lung cancer and the control group and to determine if the enzyme is lower in plasma and bronchoalveolar lavage liqs in case of malignant.

Materials and Methods

Patient Selection

All consecutive patients with lung cancer (research group) and respiratory diseases without lung cancer (control group) who approved fiberoptic bronchoscopy (FOB) by accepting to participate in research and are monitored by diagnosed between May 2012 -December 2013 participated in research. Inclusion and exclusion criteria of the research are as follows:

**Inclusion criteria for the research group:**
1. There are not any medical contraindications for FOB and bronchoalveolar lavage (BAL).
2. Bronchoscopic symptom does not constitute an impediment status to performing.
3. The following method has been obtained in accordance with FOB and BAL material.

**Exclusion criteria for the research group:**
1. The presence of a medical contraindication to FOB and BAL
2. During FOB not detecting or not technically making the suitable segment bronchus for BAL
3. Not to be duly provided BAL materials.

**Inclusion criteria for the control group:**
1. Have lung disease without malignancy
2. The absence of addition disease
3. The absence of medical contraindications for FOB and BAL
4. The following method has been obtained in accordance with FOB and BAL material.

Exclusion criteria for the control group:

1. Detection of a new addition disease or the presence of the addition disease
2. To have a medical contraindication to FOB or BAL
3. Not to be duly provided BAL materials.

The research has been approved by Local Ethics Committee of our Hospital (approval date: 17\textsuperscript{th} May 2012 approval code: 5572 serial: 304). A written informed consent form was provided by each patient.

**Fiberoptic Bronchoscopy**

Bronchoscopy was performed under local anaesthesia and sedation by mouth as hungry by performed and informed and got the consent of patients. Process was performed with PENTAX EB1970K fiberoptic bronchoscopy. Lidocaine HCL (2 - 4\%) was applied to oral cavity and pharynx in the form of third puff for upper respiratory local anaesthesia. 0.07 mg/kg dose of midazolam was applied for sedation. Before FAB, vital symptom which are oxygen saturation (SO\textsubscript{2}), heart rate and arterial blood pressure were recorded and during the process monitorized with Philips Intelli VUE MP 20. Topical anaesthesia was administered to cases with stable vital symptom. Bronchoscopy was applied by the oral route to the patients who are participated in the research in supine position.

**Bronchoalveolar Lavage (BAL)**

BAL was applied to where the lesion was detected by radiologically, usually the right middle lobe and in the left upper lob lingula. After making wedge to segment or subsegment where is planned to perform by using tip of the bronchoscope with 50 cc syringe and closing aspirator duct, the process was performed in giving the serum physiologic liqs which is previously prepared in body temperature from duct of the bronchoscope to the segment or subsegment slowly and then immediately aspirating 120 cc BAL liq with gentle suction and syringe.

Before FOB operation, the lung cancer was diagnosed by transthoracic fine needle aspiration biopsy, BAL was performed by preferring the right middle lobe of lung / left lingula where occurrence of malignancy is. BAL was performed to middle lobe/lingula in patients with pre-diagnosed as lung cancer or undiagnosed yet. BAL was performed to right middle lobe/lingula segment by selecting in the patients with control group.

After the operation, if vital symptoms of the patients are stable, after being rested for 30 minutes the patient was discharged.

**Paraoxonase Measurement**

Before bronchoscopy, 10 cc of blood were drawn from all patients via the cubital vein in sterile conditions. 16 x 100 mm Ayset tube was placed in two biochemistry tube. BAL material and a tube of blood sample were sent to The Microbiology Laboratory after the operation. The other one was sent to The Biochemistry Laboratory.

Whole blood and BAL samples were centrifuged at 3500 rpm for 5 minutes in the laboratory and stocked at -200\degree C until the test run. Serum total cholesterol, HDL, LDL levels were researched at whole blood samples which are placed in another tube in biochemistry laboratory by using OLYMPUS AU 2700 autoanalyzer. For the measurement of PON levels in serum and BAL, Human Paraoxonase ELISA kit (Nova Tein Inc. Biosience. USA) was used.

ELISA kit stored at +40\degree C, whole patient serum and BAL samples stored at -200\degree C was introduced to room temperature (18 - 25\degree C) in the research. The samples that are completely dissolved were allowed to mix by vortexing for one minute in vortex mixer. 10 \mu L lysis buffer was allowed to stand at room temperature for one hour by adding on 100 \mu L BAL sample. This operation was not applied for the patient serum just because of required for tissue samples, body fluids and cell culture samples etc. ELISA's work was performed with the automated ALISEI (Radim-Italy).

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The following test steps: pipetted the 100 μL patient samples and standards without dilution into the MICROELISA plaque wells; after adding 50 μL conjugate, incubation for 60 minutes; washing the wells (350 μL/to be well); adding 50 μL chromogenic substrate A and 50 μL chromogenic substrate B; at the end of 10 minutes waiting time, stopping the reaction by adding 50 μL stop solution and reading by 450 nm optical reader. Test results of the patients were determined by plotting a curve according to reading the Standard of the test. Standard I is 0, Standard II is 10, Standard III is 25, Standard IV is 50, Standard V is 100 and Standard VI is 250 ng/ml concentration.

Statistical Analysis

SPSS 16 software was used for data analysis. Continuous variables are presented as median (interquartile ratio), categorical variables (%) was defined as number. Group comparisons was made by using Mann Withney U test for continuous variables and using Fischer's exact test for categorical variables. Logistic regression method was used for multivariate analysis. p < 0.05 was considered significant.

Results and Discussion

Results

The research includes the patients with lung cancer and 19 patients that diagnosed with benign lung disease as control group (Table 1).

<table>
<thead>
<tr>
<th>Lung cancer group</th>
<th>Count (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Median (min-max) 64 (61 - 71)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 20  Female 5</td>
</tr>
<tr>
<td>Type of tumor cells</td>
<td>NSCLC 21  SCLC 4</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes 24  No 1</td>
</tr>
</tbody>
</table>

Control Group (n = 19)

| Age               | Median (min-max) 49 (34 - 67) |
| Sex               | Male 10  Female 9 |
| Smoking           | Yes 10  No 9 |

Benign lung disease

| Pneumonitis 6  COPD 6  Sarcoidosis 3  Tuberculosis 1  Pneumoconiosis 1  Bronchiectasis 1  Langerhans cell histiocytosis 1 |

Table 1: Characteristics of the patients.

SCLC: small cell lung cancer
NSCLC: non-small cell lung cancer
COPD: chronic obstructive pulmonary disease

Serum PON1 level was found to be significantly lower in the lung cancer group than the control group (Table 2). BAL PON1 level was found to be significantly lower in patients with lung cancer than the control group, but no statistically significant differences. The result of Serum PON1 / BAL PON1 was found to be significantly lower in the cancer group than the control group. Also, serum PON1 level were compared by standardized with HDL, levels in the patients with lung cancer was found to be statistically significantly lower than the control group. After determination of low level of PON1 enzyme levels according to the patients with lung cancer than the control group in univariate analysis, to check the low levels if it’s affected by other variables or not, multivariate analysis (linear regression analysis) were evaluated. Age, gender, and smoking were included in the model. The only independent factor that affect the rate of BAL PON1/Serum PON1 was lung cancer (p: 0.019) OR: -0.2 (-0.36, -0.034). Age, gender, and smoking in PON1 levels were determined to be ineffective.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lung cancer group (n = 25) (median, min-max)</th>
<th>Control group (n = 19) (median, min-max)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL PON1 (ng/ml)</td>
<td>154 (141 - 175)</td>
<td>158 (118 - 178)</td>
<td>0.924</td>
</tr>
<tr>
<td>Serum PON1 (ng/ml)</td>
<td>53 (42 - 70)</td>
<td>89 (55 - 130)</td>
<td>0.005</td>
</tr>
<tr>
<td>Serum HDL (mg/dl)</td>
<td>35 (29 - 43)</td>
<td>39 (34 - 45)</td>
<td>0.379</td>
</tr>
<tr>
<td>Serum / BAL PON1</td>
<td>0.37 (0.27 - 0.53)</td>
<td>0.61 (0.37 - 0.81)</td>
<td>0.004</td>
</tr>
<tr>
<td>Serum PON1/HDL</td>
<td>1.46 (1.14 - 2.02)</td>
<td>2.46 (1.67 - 3.01)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Table 2: Paraoxonase levels of serum and bronchoalveolar lavage of patients.

BAL: bronchoalveolar lavage
PON: paraoxonase
HDL: high density lipoprotein

*P values were calculated using the Mann-whitney U tests

Discussion

Serum PON1 levels of the patients with lung cancer were found not to be related to smoking, age and sex parameters and was found to be significantly lower than benign lung disease. Benign and malignant lung disease can not to be distinguished with PON1 levels measurements in bronchoalveolar lavage. Increase in incidence of various types of cancer are related with each other by increasing free oxygen radicals and charge of oxidative stress. Final products of lipid peroxidation is thought to be initiator role during development of occurrence of cancer. Fat-soluble radicals which are carcinogenic are formed as a result of lipid peroxidation and at this point radicals that are fat-soluble of PON1 enzyme were thought to inhibit during the starting point of oncogenesis for metabolizing. Lipid peroxidation which is formed due to free oxygen radicals that released as a result of the oxidative stress is closely related to the destruction of the cell membrane [11,12]. Increased lipid peroxidation in serum and erythrocytes of patients with cancer was observed [13-15].

Antioxidant enzyme activities and erythrocyte malondialdehyde, nitric oxide, total glutathione levels, erythrocyte superoxide dismutase, catalase, xanthine oxidase activities were found to be significantly higher in the patients with lung cancer than the control group. The effect of oxygen radicals and lipid peroxidation is increasing while reducing the effect of antioxidant in the patients with lung cancer [16]. Malondialdehyde levels in final products of lipid peroxidation is higher in patients with lung cancer [13].

PON1 activity is decreased in gastro-esophageal and cancer-related anemia and lymph node metastases were associated with a decrease in serum PON1 [17]. Brain and epithelial ovarian tumors is lower than the serum PON1 activities in tumor patients according to control group.
Q/Q genotype of PON1 gene was higher in patients with lung cancer [18]. Serum PON1 arylesterase levels is lower in patients with cancer than healthy people. Lower enzyme levels are related to HDL levels or not were researched and a positive correlation were determined between serum PON1 activities and HDL concentration. Changes of PON1 activity was determined not to be affected by the serum levels of HDL. In the same research PON1 enzyme activity was determined to be lower in patients with lung cancer who are smoking than control group who are smoking and those results show that smoking is not related to low of PON1 enzyme in patients with lung cancer [7]. The low of PON1 enzyme activity which is detected in patients with lung cancer has been suggested that it is related to increase in reactive oxygen radicals [19].

Serum PON1 and arylesterase level have been identified as low in patients with cancer than the healthy people in Elkiran’s and her friends’ research. The low of PON1 activities of patients with lung cancer was examined to be related to the low of HDL. For this purpose, PON1/HDL which is stardandized for HDL concentration was found to be significantly lower in patients with lung cancer than the control group. The positive correlation was determined between serum PON1 activities and HDL concentration. The change of PON1 activities is not related to be caused by HDL level [7]. In the research, serum P01 levels in control group which are diagnosed as lung cancer and benign lung cancer were compared and serum PON1 level was found to be statistically significantly lower in lung cancer group. This results in diagnosis of benign and malignant disease is very promising for separation. In addition, our study group of patients with lung cancer and benign lung disease do not differ in terms of levels of HDL and PON1/HDL ratio has found to be significantly lower in patients with lung cancer.

Low of Serum PON1 level is determined in smokers [20]. PON1 activity was found not to vary depending on sex and age [6]. When we examined the diminution of serum PON1 which is detected in patient with lung cancer, the decrease was found not to be related to smoke, age and gender parameters.

A limitation of our research is not reviewed of the whole members of paraoxonase gene family. Antioxidant enzymes which is started the process of cancer development is one of the great importance factor. Serum PON1 activity is very complex and variable. PON1 activity and the decrease in lung cancer patients is still not fully elucidated. This reduction may be related to increase in serum of reactive oxygen species. Measurement of PON1 level in BAL fluid was considered one of the examination as explaining the local mechanism. BAL PON1 level which is researched was not to be observed in patients with lung cancer in the literature. PON2 level in BAL fluid in children with cystic fibrosis has been researched and PON2 level in children with infected with pseudomonas aeruginosa was found to be lower [21]. We have tried to make the distinction of benign lung disease with BAL PON1 level, but we found similar levels in the two groups.

Conclusion

Serum PON1 levels in patients with lung cancer were found to be significantly lower than the patients with benign lung disease. These differences are not related to smoking, age and sex parameters and in the differentiation of benign and malignant lung disease shows promise. PON1 levels in bronchoalveolar lavage fluid are not helpful in the differentiation of benign and malignant disease.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript and own full responsibility in case any conflict arises after publication of the same.

Disclosure of Funding

As this study does not involve receiving of any kind of funding from anybody for its conduction so no disclosure of any kind of funding is required for the same.

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