Malondialdehyde and Nitric Oxide Levels in the Plasma of Patients with Advanced Laryngeal Cancer

SEYITHAN TAYSİ1, CELİL USLU2, FATİH AKÇAY1, and MEHMET YAVUZ SÜRBAYAZ2

1 Department of Biochemistry, School of Medicine, and 2 Department of Otorhinolaryngology–Head and Neck Surgery, Atatürk University, Medical School, 25240 Erzurum, Turkey

Abstract
Purpose. We investigated alterations in plasma nitrite, nitrate, total nitric oxide (NO•), and malondialdehyde (MDA) levels in patients with advanced stage laryngeal cancer.

Methods. We measured the levels of MDA and total NO2, as well as the NO3 marker for NO• generation, in 25 patients with advanced laryngeal cancer and 15 healthy control subjects.

Results. The levels of plasma MDA, NO•, and NO3 were significantly higher in the patients with laryngeal cancer than in the healthy controls (P < 0.001 for all). On the other hand, although plasma NO2 was increased in the patient group, the statistical difference was not significant (P > 0.05). Moreover, the MDA and NO• levels did not differ significantly between patients with stage III and stage IV laryngeal cancer.

Conclusion. These findings demonstrate that oxidative stress is increased in patients with advanced laryngeal cancer.

Key words Lipid peroxidation · Malondialdehyde · Nitric oxide · Laryngeal cancer

Introduction

Nitric oxide (NO•) is synthesized endogenously by the enzyme, nitric oxide synthase, from its precursor L-arginine. It has been reported that the NO• biosynthetic pathway is involved in physiological processes such as vasodilatation, neuroprotection, immune defense, and various endocrine and exocrine secretions in a number of different systems.1–3 It possesses either antioxidant or pro-oxidant properties, the mechanisms of which remain poorly defined. The concentrations of NO• in a nonpathological state are within a nanomolar range, whereas NO• levels under conditions of oxidant injury are within a micromolar range.4

NO• is a “double-edged sword” and has gained much attention, being found in a multitude of seemingly diverse cellular populations. Either NO• or its metabolic by-products may contribute to mutational events that lead to the development of cancer.5 On the other hand, it plays a role in many pathologic conditions in the cardiovascular, nervous, and immunologic systems. It is also well known that NOS activity increases in association with some invasive tumors.6

The process of lipid peroxidation is one of oxidative conversion of polyunsaturated fatty acids to products known as malondialdehyde (MDA) or lipid peroxides. It is the most studied, biologically relevant, free radical reaction.7 Due to its high cytotoxicity and inhibitory action on protective enzymes, it has been suggested that MDA itself acts as a tumor promoter and a cocarcinogenic agent.8 The aim of this study was to investigate alterations in plasma nitrite, nitrate, total NO•, and MDA levels in patients with advanced stage laryngeal cancer.

Patients and Methods

Patients

The patient group comprised patients with stage III–IV laryngeal cancer and the control group comprised 15 healthy subjects. The age range was 37–63 years, with a mean age of 56.2 ± 8.8 years for the laryngeal cancer group and 29–56 years, with a mean of 49.7 ± 7.4 years for the control group. The control and laryngeal cancer subjects were smokers, but they did not have a history
of alcohol abuse. All of the patients and control subjects were men. Tumor stage was determined according to the criteria of the American Joint Committee, as stage III in 12 patients and stage IV in 13 patients. Two patients had T2, 12 had T3, and 11 had T4 disease according to the classification of the AJCC (1992). There were 12 patients without lymph node metastasis, 10 with N1, 2 with N2, and 1 with N3. Distant metastasis was not found in any of our patients. None of the subjects had pneumonia, diabetes mellitus, or any other inflammatory diseases leading to increased plasma NO• products.

Blood Sampling

All subjects from both groups were recruited into the study after informed consent had been obtained. We collected 10 ml of venous blood from the patients before surgery and from all of the controls. The blood samples were centrifuged, and plasma samples were obtained and stored at −80°C until the analysis date.

Biochemical Measurements

Plasma NO• levels defined as total nitrite plus nitrate levels, measured with the Griess reagent (consisting of sulfanilamide and N-(1-naphthyl) ethylenediamine) as previously described. This method was based on a two-step process. The first step is the conversion of nitrate to nitrite using nitrate reductase, and the second step is the addition of Griess reagent, which converts nitrite into a deep purple azo compound, resulting in photometric measurement of the absorbance at 540nm. This azo chromophore accurately determines the nitrite concentration using sodium nitrate as a standard. Protein interference was eliminated by treatment of the reacted samples with zinc sulfate and centrifugation for 5 min at 10 000 × g.

The measurement of MDA by thiobarbituric acid (TBA) reactivity is the most widely used method of assessing lipid peroxidation. Plasma MDA was measured by the method of Deeper and Hadley. Briefly, 1 ml of plasma and 1 ml of a saturated solution of TBA reagent were heated at pH 1.5 for 30 min in a boiling water bath. After cooling, the absorbance of the supernatant was determined at 532nm. Biochemical measurements were carried out at room temperature using a spectrophotometer (Cecil CE 3041, UK). The Total TBA-reactive materials were expressed as MDA, using a molar extinction coefficient for MDA of 1.56 × 10^5 cm⁻¹ M⁻¹.

Statistical Analysis

Data are expressed as mean ± SD. Statistical and correlation analyses were undertaken using the Mann-Whitney U-test and Pearson’s rank correlation test, respectively. A P value of less than 0.05 was accepted as statistically significant. SPSS for windows (version 10.0) was used for statistical analyses.

Results

Our findings on assessed parameters and correlations between the parameters in patients with stage III and IV laryngeal cancer and the healthy control subjects are shown in Tables 1 and 2, respectively. Plasma MDA, NO•, and NO3 levels were found to be significantly higher in the patients with laryngeal cancer than in the healthy controls (P < 0.001). However, there was no significant difference between the patients with stage III and those with stage IV laryngeal cancer (P > 0.05). There were positive significant correlations, at different levels, between MDA and NO•, and/or NO• products in the stage III, IV, and total patient groups (Table 2).

Discussion

To our knowledge, this is the first study to examine plasma NO• and MDA levels together in patients with advanced laryngeal cancer and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>MDA (µmol/l)</th>
<th>NO2 (µmol/l)</th>
<th>NO3 (µmol/l)</th>
<th>Total NO• (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5.3 ± 0.7</td>
<td>33.5 ± 10.4</td>
<td>90.6 ± 22.5</td>
<td>124.2 ± 25.9</td>
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<tr>
<td>Stage III group</td>
<td>5.9 ± 0.4a</td>
<td>41.9 ± 18.8</td>
<td>135.3 ± 32.2b</td>
<td>177.3 ± 31.3b</td>
</tr>
<tr>
<td>Stage IV group</td>
<td>6.1 ± 0.2b</td>
<td>43.0 ± 14.0</td>
<td>145.0 ± 19.2b</td>
<td>188.1 ± 28.5b</td>
</tr>
<tr>
<td>Total patient group</td>
<td>6.0 ± 0.3b</td>
<td>42.5 ± 16.1</td>
<td>140.3 ± 26.2b</td>
<td>182.9 ± 29.8b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD
MDA, malondialdehyde; NO•, nitric oxide

aP < 0.01, bP < 0.001, compared to control group