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ORIGINAL ARTICLE

Serum nitric oxide levels in patients with head and neck squamous cell carcinoma

Baş-boyun yassı epitel hücreli kanserli hastaların serumlarında nitrik oksit düzeyleri

Ahmet KIZILAY, M.D.,[1] M. Tayyar KALCIOĞLU, M.D.,[1] Fikret ÖZUĞURLU, M.D.,[3] Hüseyin ÖZYURT, M.D.,[3] İbrahim ALADAĞ, M.D.,[4] Orhan ÖZTURAN, M.D.,[1] Ömer AKYOL, M.D.[2]

Objectives: We determined serum nitric oxide (NO) levels in patients with head and neck squamous cell carcinoma (SCC) and sought correlations with TNM staging, tumor localization, and tumor grade.

Patients and Methods: Serum samples were obtained from 36 patients (mean age 63 years; range 37 to 80 years) with head and neck SCC prior to treatment and from 20 healthy individuals (mean age 56 years; range 30 to 72 years) as controls. Tumor staging was based on the criteria of the American Joint Committee of Cancer staging system in 2002. Thirteen patients had stage I-II, and 23 patients had stage III-IV tumors and all had well- or moderately-differentiated SCC (grade 1-2). Serum NO levels were analyzed by a spectrophotometric method based on the determination of total nitrite levels in serum and compared between the patient and control groups.

Results: The mean serum NO levels were 20.08±1.40 µmol/l and 13.57±0.99 µmol/l in cancer patients and controls, respectively (p=0.001). There were no correlations between NO levels and age, sex, tumor stage, localization, and histological grade.

Conclusion: These data suggest that head and neck SCC is associated with increased serum NO levels, which may play a role in tumor growth.

Key words: Carcinoma, squamous cell/metabolism; head and neck neoplasms/metabolism; nitric oxide/blood; tumor markers, biological.

Amaç: Baş-boyunda skuamöz hücreli kanser saptanan hastalarda serum nitrik oksit (NO) düzeyi belirlendi ve bunun TNM evresi, tümör yerleşimi ve derecelendirmesi ile ilişkisi araştırıldı.

Hastalar ve Yöntemler: Baş-boyunda skuamöz hücreli kanser tanısı konan 36 hastadan (ort. yaş 63; dağılım 37-80) tedavi öncesinde ve kontrol olarak 20 sağlıklı erişkinden (ort. yaş 56; dağılım 30-72) serum örnekleri alındı. Tümör evrelemesi AJCC (American Joint Committee of Cancer) 2002 ölçütlerine göre yapıldı. On üç hastada evre I-II, 23 hastada evre III-IV tümör vardı. Tüm hastalarda tümör diferensiyasyonu iyi veya orta derecedeydi (derece 1-2). Serum NO düzeylerinin analizi, serumda total nitritin saptanması esasına dayanan spektrofotometrik yöntemle yapıldı ve hasta ve kontrol gruplarının NO düzeyleri karşılaştırıldı.

Bulgular: Kanserli grupta ve kontrol grubunda ortalama serum NO düzeyi sırasıyla 20.08±1.40 µmol/l ve 13.57±0.99 µmol/l bulundu (p=0.001). Kanserli grupta NO düzeyi yaş, cinsiyet, tümör evresi, yerleşimi ve histolojik derecesi ile ilişkili bulunmadı.

Sonuç: Bu veriler baş-boyundaki skuamöz hücreli kanserlerde serum NO düzeyinin arttığını göstermektedir. Bu aktivite artışı tümör büyümesinde rol oynayabilir.

Anahtar sözcükler: Karsinom, skuamöz hücreli/metabolizma; baş-boyun neoplazileri/metabolizma; nitrik oksit/kan; tümör belirteci, biyolojik.

◆ *Departments of [1]Otolaryngology and [2]Biochemistry, Medical Faculty of İnonu University (İnönü Üniversitesi Tıp Fakültesi, [1]Kulak Burun Boğaz Hastalıkları Anabilim Dalı, [2]Biyokimya Anabilim Dalı), Malatya; Departments of [3]Biochemistry and [4]Otolaryngology, Medical Faculty of Gaziosmanpaşa University (Gaziosmanpaşa Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı, [4]Kulak Burun Boğaz Hastalıkları Anabilim Dalı), Tokat, Turkey.*

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◆ *Correspondence (İletişim adresi): Dr. Ahmet Kızılay. İnönü Üniversitesi Turgut Özal Tıp Merkezi Kulak Burun Boğaz Hastalıkları Anabilim Dalı, 44069 Malatya, Turkey. Tel: +90 422 - 341 06 60 / 4605 Fax (Faks): +90 422 - 341 07 28 e-mail (e-posta): akizilay@inonu.edu.tr*

Nitric oxide (NO) is an important biological messenger molecule involved in a wide range of biological actions, many of which are still poorly understood.[1-3] Nitric oxide is probably one of the oldest molecules thought to have developed in the primitive atmosphere of the cooling planet.[3] However, the demonstration of NO formation in the endothelial cells in 1987 opened a new area of biological research.[1] This was a crucial discovery for human biology and it soon became evident that NO was found in a variety of tissues and was involved in multiple cell functions, including immunostimulation and cytotoxicity.[1,2] It is also involved in various medical conditions, having both beneficial and detrimental roles.[3,4]

Nitric oxide is synthesized from the amino acid L-arginine by three different isoenzymes of nitric oxide synthases.[1,2] The endothelial and neuronal nitric oxide synthases (eNOS and nNOS, respectively) are calcium-dependent and calmodulin-dependent isoforms and they mediate physiological processes such as vasodilation and synaptic events. Both are constitutively expressed.[1] The third isoform, inducible nitric oxide synthase (iNOS) which is expressed during inflammatory reactions, is calcium- and calmodulin-independent. Inducible nitric oxide synthase was originally detected in activated macrophages where it produced large quantities of NO in response to endotoxin- or cytokine-mediated macrophage activation.[1,2]

In the field of tumor biology, the actions of NO are implicated in growth including mutagenicity, angiogenesis, and metastasis of solid tumors.[3] Inducible nitric oxide synthase is primarily involved in carcinogenesis. However, it also appears to have effects in cytotoxicity of macrophages against tumor cells and immunosuppression.[5] This is somewhat confounding due to both tumor-promoting and inhibiting effects of NO.[6] In this respect, in head and neck squamous cell carcinoma (SCC), the role of NO and the impact of iNOS still remain obscure .[3,6,7] Moreover, the relationship between NO levels and survival and prognosis is still controversial.[3,4,7,8]

The aim of this study was to determine serum NO levels in patients with SCC of the oral cavity, pharynx, and larynx.

PATIENTS AND METHODS

Patient population

Serum samples were obtained from 36 patients (mean age 63 years; range 37 to 80 years) with head and neck SCC prior to treatment and from 20 healthy individuals (mean age 56 years; range 30 to 72 years) comprising the control group. The clinical and radiological data of each patient were evaluated. To avoid any confounding effect on NO levels, we excluded patients who received any treatment previously for the current carcinoma, including surgery, radiotherapy, chemotherapy, or immunotherapy. All the patients had primary SCC of the larynx, oral cavity, or pharynx confirmed by histopathological examination.

Tumor staging was based on the criteria of the American Joint Committee of Cancer staging system in 2002. Thirteen patients had stage I-II, and 23 patients had stage III-IV tumors and all had well- or moderately-differentiated SCC (grade 1/2). Preoperative nutritional status and the hematological values of the patients were found within normal limits. We excluded patients with evidence for infection. Most of the patients were heavy smokers, some of them used alcohol, as well.

Ten milliliters of venous blood were taken from the patients and controls after obtaining their informed consent about the study and its aims. The samples were centrifuged and the serum stored frozen at –25 °C until analysis.

Assay of serum NO levels

Since NO is a very labile molecule, its direct measurement in biological samples is very difficult. In aqueous solutions, NO reacts with molecular oxygen and accumulates in plasma as nitrite (NO_2^-) and nitrate (NO_3^-) ions. Therefore, the stable oxidation end products of NO (namely, NO_2^- and NO_3^-) can readily be measured in biological fluids and have been widely used as *in vitro* and *in vivo* indicators of NO production.[9,10] Thus, plasma nitrite concentration was accepted as an index of NO. For total nitrite determination, deproteinized serum samples were treated with copperized cadmium granules to reduce NO_3^- to NO_2^-. Nitrite concentrations were quantified by a calorimetric assay based on the Griess reaction.[11] Briefly, a chromophore with a strong absorbance at 545 nm is formed by reaction of nitrite with a mixture of N-naphthylethylenediamine and sulphanilamide. A standard curve was established with a set of serial dilutions (10^8 to 10^3 mol/l) of sodium nitrite. The results were expressed as micromole per liter of serum (µmol/l).

Serum NO levels of the patient and control groups were compared and correlations were

TABLE I

CHARACTERISTICS OF THE PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA

Characteristics	n	%
Sex		
Male	31	86.1
Female	5	13.9
Tumor localization		
Larynx	25	69.4
Oral cavity	8	22.2
Oropharynx	1	2.8
Hypopharynx	2	5.6
Stage		
I-II	13	36.1
III-IV	23	63.9
Histologic differentiation		
Well	19	52.8
Moderate	17	47.2

sought with TNM staging, localization, and tumor grade.

Data were processed using the SPSS 10.0 for Windows computing program. The results were analyzed by independent samples t-test. Nitric oxide values were expressed as mean±standard error. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

Of 36 patients with head and neck SCC, 25 patients had larynx, eight had oral cavity, and three had pharynx cancers. The clinicopathologic characteristics of the patients are summarized in Table I. The mean serum NO levels were 20.08±1.40 µmol/l and 13.57±0.99 µmol/l in cancer patients and controls, respectively (p=0.001). There were no correlations between NO levels and age, sex, tumor stage, localization, and histological grade. No association was detected between NO levels and TNM staging.

DISCUSSION

To our knowledge, the association between serum NO levels and tumor characteristics in patients with SCC of the head and neck has not been previously assessed. The presence of iNOS activity in tumor tissues of head and neck SCC has been reported.[4,7,12] An enhanced expression of iNOS at the protein and mRNA levels and a correlation with cervical lymph node metastasis have been reported previously for human oral SCC.[13] The hypothesis that NO pathway plays a relevant role in angiogenesis and tumor progression in SCC of the head and neck were investigated and it was found that there was a significant increase in iNOS activity detected immunohistochemically during transition from hyperplastic/mild dysplastic to moderate/severe dysplastic lesions to SCC.[14] Although the local concentration of this molecule is very important, serum levels of NO may also indicate local and general progression of the disease. The results of the present study showed that serum levels of NO in patients with head and neck SCC were higher than those of healthy controls. Increased NO level in serum may result from increased production of NO in cancerous tissues by NOS activation and these changes in tumor tissue may play a role in tumor growth.

Nitric oxide is produced in malignant epithelial head and neck tumors and this molecule is synthesized locally by the epithelial cells.[7] Increased NO levels in the current study may reflect increased NO production in the squamous epithelial cells of patients with head and neck tumors. The growth of solid tumors requires a complex sequence of events including cell proliferation, formation of new blood vessels, and permeation through the basement membrane at the primary site.[3] The same processes occur again at the site of metastasis.[3] There are now at least 20 angiogenic and anti-angiogenic mediators, of which NO is a very important mediator. Nitric oxide overproduction, as a vasodilator and mediator in angiogenesis may enable tumor cells for further cell proliferation with an enhancement of vascular permeability.[12] Following the increase in NO production, hyperpermeability may occur via the interaction with vascular endothelial growth factor, which is stimulated upon NO release.[15] Based on animal experiments, this may enable tumor cells to escape into the circulation, representing the first step in the metastatic process.[3] The relationship between tumor size and iNOS expression has not been fully elucidated.[7] In our study, serum NO levels were not correlated with TNM staging.

The presence of NO and NOS has been assessed in many human malignant tumors including breast, colorectal, renal cell, pancreas, gynecologic, prostate, lung, stomach, and head and neck tumors.[3,6] As in

other studies,[3,12] we also suggest that manipulation of NOS to reduce NO production might be of therapeutic benefit in the treatment of cancer and prevention of distant metastasis. Monitoring serum NO levels may be useful in the follow-up of patients after definitive therapy. To clarify this, further studies are needed on the relationship between NO and tumor growth and metastasis.

In conclusion, the presence of a tumor in the head and neck is associated with an increase in serum NO levels. This elevation may play a role in tumor growth and metastasis. Further research on head and neck SCC may clarify the role of NO in the pathogenesis of head and neck cancers, and its probable prognostic and therapeutic role.

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