A Preliminary Study of Serum Apelin Levels in Patients with Head and Neck Cancer

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ABSTRACT

Objective: Treatment planning is primarily based on the tumor node metastasis (TNM) staging system for head and neck cancer (HNC). However, TNM does not give sufficient information about biological aggressiveness, treatment response, and prognosis. New molecular markers are needed for individualized cancer treatment. Apelin is a bioactive peptide and an endogenous ligand for the G protein-coupled receptor (APJ). Its expression is induced under hypoxic conditions. Apelin and its receptor APJ are important factors in physiological angiogenesis and may be novel targets for anti-angiogenic tumor therapies. This preliminary study aimed to investigate whether there was a difference in serum apelin levels between patients with HNC and control group and also to compare the serum apelin levels before and after radiotherapy.

Materials and Methods: Twenty-two patients with HNC (patient group) and 30 healthy individuals (control group) were included in the study. In the patient group, blood samples were collected before and after radiotherapy. Serum apelin-36 levels were measured by enzyme-linked immunosorbent assay.

Results: Serum apelin-36 levels were significantly higher in patients with HNC than in the control group ($p<0.001$). Coverage of the measured apelin-36 levels showed a significant decrease after radiotherapy according to the levels before radiotherapy. There was no statistically significant difference between the groups ($p>0.05$).

Conclusion: Apelin may be a potential therapeutic target and a novel biomarker. Additional studies are needed to reveal the relationships between serum apelin and radiotherapy in solid human tumors.

Keywords: Apelin, radiotherapy, head and neck cancer

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Introduction

Head and neck cancer (HNC) is a malignancy that arises from the oropharynx, oral cavity, larynx, hypopharynx, nasal cavity, nasopharynx, and paranasal sinuses [1]. For patients with early stage HNC, either surgery or radiotherapy alone is effective with a 5-year survival rate of 60%–90%. However, for advanced HNC, standard treatments are surgery, radiotherapy, chemotherapy, and their combinations. A 5-year survival rate between 10% and 30% has been observed [2].

In clinical practice, treatment planning is based on the tumor node metastasis (TNM) staging system for HNC. However, TNM classification does not provide enough information about treatment response, prognosis, or biological aggressiveness of HNC. For these reasons, new molecular markers are needed for individualized cancer treatment of HNC.

Apelin is a bioactive peptide and an endogenous ligand for the G protein-coupled receptor (APJ). The apelin gene encodes a secreted preprotein of 77 amino acids with a prodomain, a signal peptide, and a C-terminal peptide. Apelin-36 (amino acids 42-77) and apelin-13 (amino acids 65-77) are the most active and predominant isoforms. Apelin and APJ are expressed in the liver, heart, lung, bone, brain, kidney, adipose tissues, and skeletal muscle [3, 4].

Apelin and APJ are necessary to promote angiogenic blood vessel growth because they play important roles in blood vessel morphogenesis [5, 6]. Furthermore, apelin expression is induced under hypoxic conditions [7, 8]. The findings show that apelin and its receptor APJ are important factors in physiological angiogenesis and anti-angiogenic tumor therapies and may be novel targets [9].

Heo et al. [10] reported that hypoxia-induced upregulation of apelin is associated with poor prognosis in oral squamous cell carcinomas. Similarly, Berta et al. [11] showed that high levels of apelin expression stimulate the microvessel densities and tumor growth in vivo and are associated with poor prognosis in lung cancer.

This preliminary study aimed to investigate whether there was a variety in serum apelin levels between patients with HNC and control group. In addition, the present study compared the serum apelin levels before and after radiotherapy.

Materials and Methods

Subjects

A total of 22 patients with HNC as the patient group and 30 healthy individuals as the control group were included in the study. In the patient group, blood samples were collected prospectively from 22 patients with HNC before and after radiotherapy. In the control group, blood samples were selected from 30 people who attended routine hematological examinations. Table 1 shows the characteristics of patients.

Inclusion criteria in the patient group comprised [1] blood samples collected from patients with HNC before and 2 months after radiotherapy, prospectively and [2] all patients treated with radiotherapy±chemotherapy. In the control group, inclusion criteria were [1] no history of serious illness, [2] have no any chronic disease (e.g., cardiovascular or pulmonary disease or diabetes mellitus), [3] have no any cancer diagnosis, [4] >18 years old, and [5] non-smokers.

Table 1. Patient characteristics

All patients	Radiotherapy group	Control group
Gender (n)		
Male	81% (18)	50% (15)
Female	19% (4)	50% (15)
Age (years)		
Range	17-71	20-70
Median	59	56
Tumor site (n)		
Nasopharynx	36% (8)	
Larynx	41% (9)	
Oral cavity–oropharynx	23% (5)	
Concurrent chemotherapy (n)		
Yes	86% (19)	
No	14% (3)	
Radiotherapy dose (Gy)		
Range	54-72	
Median	67	

The local ethics committee approved the study. Written informed consent was obtained from all participants.

Measurements

All sampling procedures were performed in the morning after 12h of fasting. Blood samples were centrifuged at 4 °C. Serum was separated and maintained at -80 °C until analysis.

Serum apelin-36 levels were measured by enzyme-linked immunosorbent assay using Human Apelin-36 EIA Kits (EK-057-15; Phoenix Pharmaceuticals Inc., Burlingame, CA, USA). The inter- and intra-assay coefficients of variation were <12% and <10%, respectively. The minimum detection limit for apelin-36 was 0.07 ng/mL, and the analytical range for apelin-36 was 0-100 ng/mL.

Statistical Analysis

All data were entered into a database. Statistical analysis was performed using the The Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) version 18.0 software. The comparison between pre- and post-radiotherapy groups serum apelin-36 was carried out using paired sample t test and the control group by independent sample t-test. A $p<0.05$ was considered statistically significant.

Results

There were 18 (81%) male and 4 (19%) female patients. The median age of the patients was 59 (range 17-71) years in patients with HNC. There were 9 (41%) patients with larynx, 8 (36%) nasopharynx, and 5 (23%) oral cavity/oropharynx cancer. All patients were treated with radiotherapy. The median dose of radiotherapy was 67 (range 54-72) Gy. There were 19 (86%) patients who underwent concomitant cisplatin-based chemotherapy with a dose of 40 mg/m^2 every week during radiotherapy. There were 3 (14%) patients who did not tolerate and did not undergo chemotherapy.

Serum apelin-36 levels were significantly higher in patients with HNC than in the control group ($p<0.001$). Coverage of the measured apelin-36 levels showed a significant decrease after radiotherapy according to the levels before radiotherapy. However, there was no statistically significant difference between the groups ($p>0.05$) (Table 2).

Discussion

To our knowledge, we demonstrated for the first time in the present study that serum apelin levels were increased in HNC. In addition, our data showed that serum apelin levels were lower after radiotherapy than before radiotherapy.

Until now, apelin overexpression was shown in human tumor tissues [10-14].

Sorli et al. [12] reported that in a murine breast cancer model, apelin overexpressions increase tumor growth. Berta et al. [11] showed that apelin overexpression stimulates tumor growth, microvessel densities, and parameters in vivo, and in patients with lung cancer, high level of apelin expression is a poor prognostic factor and increases the aggressive behavior of human lung cancer. In a recent study, Heo et al. [10] reported that apelin expression is localized in the cytoplasm of oral squamous cell carcinoma and is upregulated under hypoxic conditions; they demonstrated that apelin expression is significantly correlated with tumor recurrence and disease-free survival.

Diakowska et al. [15] reported that serum apelin is significantly higher in patients with gastroesophageal cancer than in healthy controls and has a positive correlation between serum apelin concentrations and their levels in tumor tissue.

To our knowledge, we present the first study that serum apelin levels were higher in patients

Table 2. Serum apelin levels between the groups

Groups	n	Serum apelin (ng/mL) Mean	SD	p
Control group	30	0.53	0.19	<0.001*
Before RT	22	1.66	0.74	
After RT	22	1.51	0.68	>0.05**

RT: radiotherapy.

*Before RT group compared with the control group.

**Before RT group compared with after RT group.

with HNC than in healthy controls. We also present that serum apelin levels were decreased after radiotherapy. To the best of our knowledge, there is no study in the literature about the effect of radiotherapy on serum apelin levels in patients with cancer.

This is a preliminary study. There are some limitations in our study. First, few patients participated in the study. Second, the effect of radiotherapy on serum apelin levels showed a reduction, but it was not statistically significant. Further study with larger patient numbers can aid to determine the role of serum apelin levels in patients with cancer compared with healthy controls, and the role of the radiotherapy can be made certain.

In conclusion, serum apelin levels were significantly higher in patients with HNC, and radiotherapy may be associated with serum apelin levels. These results suggest that apelin may be a novel biomarker and potential therapeutic target. Additional studies to show the relationships between serum apelin and radiotherapy in solid human tumors are warranted.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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