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Original Article

AGP1 acts as a biomarker for diagnosis of laryngeal cancer

Yi Zhang, Zhiping Wang, Xiuqing Bai, Yaosheng Xu

Department of Otorhinolaryngological, Harrison International Peace Hospital, Hengshui 053000, China

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Abstract: Background: Laryngeal cancer is a common malignancy of head and neck cancer, with increasing morbidity and mortality in our country. It is reported that *AGP1* can act as a serum marker for several diseases and cancers. In this study, we evaluated the diagnostic value of *AGP1* in laryngeal cancer. Methods: The serum *AGP1* levels of 119 laryngeal carcinoma patients and 68 healthy volunteers were detected by real-time PCR (qRT-PCR) and ELISA. Chi-square test was applied to evaluate the association between *AGP1* mRNA levels and clinical characteristics. To determine the diagnostic value of *AGP1*, ROC curve was constructed. Results: The expression level of *AGP1* was higher in laryngeal cancer patients than that in healthy group ($P<0.001$). Moreover, its mRNA level was significantly associated with tumor size ($P=0.004$), TNM stage ($P=0.006$), and distant metastasis ($P=0.018$). ROC curve demonstrated that *AGP1* could be used for laryngeal cancer diagnosis with AUC value of 0.924, combing with the sensitivity of 78.8% and specificity of 89.7%. The optimal cutoff value was 0.725. Conclusion: *AGP1* is up-regulated and correlated with tumor progression in laryngeal cancer patients. *AGP1* may be a potential diagnostic biomarker for laryngeal cancer.

Keywords: *AGP1*, laryngeal cancer, diagnosis

Introduction

Laryngeal carcinoma is one of the most common human head and neck malignant tumors, and it is also a frequently observed respiratory tract cancer [1, 2]. Given the important roles of larynx in breathing, sound production, and trachea protection, laryngeal cancer seriously affects the quality of life of the patients [3]. At the present time, the therapeutic strategies for laryngeal cancer include surgery, radiotherapy, and chemotherapy [4]. Great progress had been made in the treatments, however, the prognosis of patients diagnosed with advanced stage is still unsatisfactory [5]. Unfortunately, most of laryngeal cancer patients develop to advanced stage when diagnosed, leading to limited therapeutic effects, frequent recurrence and poor outcomes [6]. Therefore, novel biomarkers for early detection are urgently needed for laryngeal cancer.

Alpha-1-acid glycoprotein (*AGP1*), also named orosomucoid (ORM), is primarily synthesized by liver and secreted to the entire body [7]. *AGP1* is an acute phase reaction protein which can regulate the proliferation of lymphocytes, aggregation of platelets, chemotaxies, peroxidatic reaction, and so on [8]. As an immunomodifier, the abnormal expression of *AGP1* is reported to be associated with several diseases, such as cardiovascular diseases, enterovirus 71 infection in children, and systemic lupus erythematosus [9-11]. Recently, growing evidences have demonstrated that *AGP1* is involved in the tumorigenesis of human cancers. For instance, the study scheduled by Subbannayya et al. indicated that serum protein levels of *AGP1* were significantly different between gastric adenocarcinoma patients and healthy individuals, suggesting its potential as a diagnostic biomarker for the cancer [12]. Piver et al. reported that serum *AGP1* levels could show the progression of epithelial ovarian cancer, which might be a prognostic biomarker for the cancer [13]. The functional roles of *AGP1* in laryngeal cancer had also been reported in the previous studies. It was reported that serum levels of *AGP1* were obviously different between laryn-

Table 1. Association of *AGP1* expression with clinicopathological features of laryngeal carcinoma patients

Feathers	No. N=119	*AGP1* expression		*P* values
		Low (n=53)	High (n=66)	
Age (years)				
<60	50	23	27	0.785
≥60	69	30	39	
Gender				
Male	64	29	35	0.854
Female	55	24	31	
Tumor size				
<3 cm	68	38	30	0.004
≥3 cm	51	15	36	
Subsite				
Supraglottis	46	21	25	0.690
Glottis	37	18	19	
Subglottis	36	14	22	
Histologic type				
Preinvasive carcinoma	46	20	26	0.854
LSCC	73	33	40	
TNM stage				
I-II	62	35	27	0.006
III-IV	57	18	39	
Distant metastasis				
Negative	62	34	28	0.018
Positive	57	19	38	

Note: LSCC: laryngeal squamous cell carcinomas.

geal cancer patients and healthy controls, moreover, its abnormal expression was significantly correlated with tumor grade [14]. However, the diagnostic significance of *AGP1* in laryngeal cancer had been rarely reported.

In our study, we aimed to detect the serum expression level of *AGP1* in laryngeal carcinoma patients with qRT-PCR method, as well as its association with clinical characteristics. In addition, the diagnostic value of *AGP1* in laryngeal cancer was estimated in the current study.

Materials and methods

Patients and specimens collection

A total of 119 laryngeal cancer patients diagnosed by pathologists were recruited from Harrison International Peace Hospital. In addition, 68 gender- and age-matched healthy volunteers as the control group were collected in the study. In the healthy group, no one had been diagnosed with any malignancy. Our study was approved by the ethics committee of Harrison International Peace Hospital, and the written consents were obtained from all the participants. The clinicopathological features of the patients were listed in **Table 1**, including age, gender, tumor size, subsites, histological type, TNM stage, and distant metastasis.

5 mL fasting peripheral blood samples were taken from all of the participants. None of patients had received chemotherapy, radiotherapy or other treatments before blood collection. The serum was isolated from the blood specimens according to the following operations: the blood was centrifuged for 10 min at 2500 rpm and then the supernate was collected. The obtained serum specimens were stored at -20°C until RNA extraction.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from the serum specimens with Trizol reagent (Invitrogen) following the manufacturer's instruction. The first-strand cDNA was synthesized by RevertAid™ First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Then a SYBR Premix Ex Taq™ kit (Takara, Dalian, China) was applied to evaluate the relative expression of *AGP1* mRNA. *GAPDH* served as internal control. The primer sequences of *AGP1* and *GAPDH* respectively were as follows: *AGP1* forward: 5'-CAAAAACACTCCCAAACCAAA-3', reverse: 5'-CTTCAGTCGGAGAATCGG-3'; *GAPDH* forward: 5'-CATCTCTGCCCCCTCTGCTGA-3', reverse: 5'-GGATGACCTTGCCCACAGCCT-3'. The relative expression level of *AGP1* mRNA was normalized to that of *GAPDH* and calculated by the $2^{-\Delta\Delta Ct}$ method.

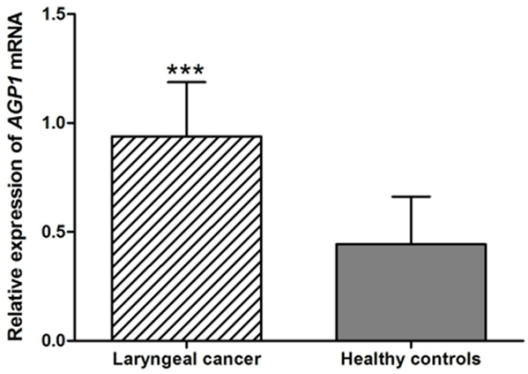

Figure 1. Serum *AGP1* mRNA levels evaluated by qRT-PCR in laryngeal carcinoma patients and healthy controls. The results suggested that *AGP1* mRNA levels were higher in patients diagnosed with laryngeal cancer than that in the healthy group. ***: indicated *P*<0.001.

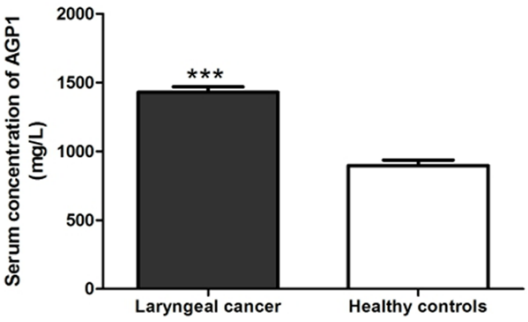

Figure 2. Different protein levels of *AGP1* between laryngeal cancer patients and healthy individuals. ELISA results demonstrated that *AGP1* protein levels were up-regulated in laryngeal cancer patients. ***: suggested *P*<0.001.

Protein levels of AGP1 in the study subjects

The protein levels of *AGP1* were evaluated by enzyme-linked immunosorbent assay (ELISA), which was performed by a commercially available ELISA kit (R&D Systems, Minneapolis, USA). The operation was according to the instructions of the manufacturer.

Statistical analysis

SPSS 19.0 and GraphPad Prism 5 were used for statistical analyses. The expression levels of *AGP1* were presented as means ± SD, and compared by student's t-tests. Chi-square tests were applied to evaluate the association between serum *AGP1* expression and clinicopathological characteristics in laryngeal cancer. In order to determine the diagnostic value of *AGP1* in laryngeal cancer, receiver operating characteristic (ROC) analysis was performed. *P* values <0.05 were considered statistically significant.

Results

Expression of AGP1 in laryngeal carcinoma

QRT-PCR was performed to examine the *AGP1* mRNA levels in 119 laryngeal carcinoma patients and 68 healthy volunteers. The results suggested that *AGP1* expression was significantly higher in laryngeal cancer tissues than that in healthy volunteers (*P*<0.001) (**Figure 1**).

The results of ELISA demonstrated that the protein levels of *AGP1* were up-regulated in the laryngeal cancer patients, which was consistent with the mRNA levels (1429.32 ± 437.11 mg/L vs 896.76 ± 324.62 mg/L, *P*<0.001) (**Figure 2**).

Association between AGP1 and clinicopathological parameters in laryngeal carcinoma patients

In order to evaluate the association between *AGP1* mRNA levels and the clinicopathological characteristics, the patients were divided into high expression group (n=66) and low expression group (n=53) according to their median mRNA levels of *AGP1*. The results demonstrated that the expression of *AGP1* was significantly associated with tumor size (*P*=0.004), TNM stage (*P*=0.006) and distant metastasis (*P*=0.018). However, the *AGP1* expression had no obvious relationship with the age, gender, subsite, or histologic type of the laryngeal carcinoma patients (all *P*>0.05) (**Table 1**).

Diagnostic value of AGP1 expression in laryngeal carcinoma patients

The ROC curve showed that the laryngeal cancer patients could be distinguished from the healthy controls according to their mRNA levels of *AGP1*, with the sensitivity of 78.8% and the specificity of 89.7%. In addition, the area under the curve (AUC) value of 0.924. The cutoff value of *AGP1* mRNA level for laryngeal cancer diagnosis was 0.725 (**Figure 3**).

Discussion

Given the functional roles of larynx, laryngeal cancer has become a great threat to human

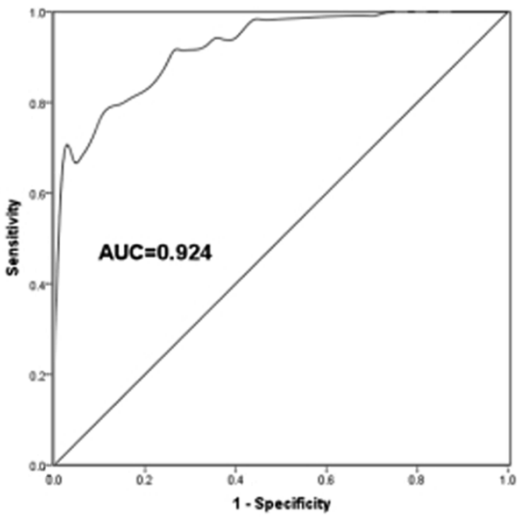

Figure 3. ROC analysis based on serum *AGP1* expression for laryngeal carcinoma patients. The curve revealed that *AGP1* mRNA levels could distinguish the laryngeal cancer patients from the healthy control with the AUC value of 0.924. The cut-off value of *AGP1* for laryngeal cancer diagnosis was 0.725, with the sensitivity of 78.8% and the specificity of 89.7%.

health. Despite of the advanced therapy strategies and interventions, the five-year survival rate and laryngectomy-free survival rate have changed slightly [15]. Lack of reliable biomarkes for early detection may be responsible for the dismal prognosis [16]. Thus in our study, we aimed to explore an efficient biomarker to early diagnose laryngeal carcinoma patients.

AGP1 is a non-specific acute phase reaction protein, and its plasma concentration will rise in the situation of acute inflammatory reaction or chronic disease [17]. *AGP1* has two mainly biological functions, one is the function of the combination of endogenous substances and drugs, and the other is the strong immune regulating function [18]. In recent years, accumulating evidences have reported that *AGP1* can be used as a tumor biomarker [19-21]. Brock et al. reported that *AGP1* could be a sensitive and non-invasive biomarker for colorectal carcinoma diagnosis [22]. Asima A et al. found that the expression of *AGP1* was higher in lung cancer tissues than in healthy controls, which might hold potential for lung cancer diagnosis [23]. All the related studies revealed that *AGP1* might be a promising biomarker for cancer diagnosis and prognosis. In the present study, we investigated the diagnostic performances of *AGP1* in laryngeal cancer.

In our study, the expression level of *AGP1* was detected by qRT-PCR and ELISA. Analysis results demonstrated that serum *AGP1* was higher in laryngeal cancer patients than that in healthy group. Moreover, the elevated expression of *AGP1* was significantly correlated with large tumor size, advanced TNM stage and positive distant metastasis. The results suggested that *AGP1* might be an oncegene in laryngeal carcinoma, and correlated with aggressive clinical characteristics of the patients. The conclusion was consistent with the previous study. Uslu et al. reported that compared with healthy individuals, the expression levels of *AGP1* were significantly up-regulated in patients diagnosed with laryngeal cancer [14]. Additionally, the study carried out by Onizuka et al. demonstrated that high serum concentration of *AGP1* after radiotherapy predicted poor survival for laryngeal cancer patients [24]. However, the mechanisms for *AGP1* regulating laryngeal cancer remained unclear. Further researches were still needed.

Until now, the traditional diagnostic methods for laryngeal cancer were based on physical examination combined with biopsy and imaging [25]. However, the methods were limited by resolution and contrast, leading to misdiagnosis [26]. Therefore, invasive and non-invasive methods were urgently needed for laryngeal cancer diagnosis. Recently, the functional roles of gene in carcinogenesis gained more and more attentions. A variety of molecular biomarkers were identified for laryngeal cancer diagnosis in the previous studies. For example, Saito et al. reported that the specific expression profile of *miR-196a* in laryngeal cancer might be a potential biomarker for early detection of the cancer [27]. Jiang et al., suggested that *BTG1* was down-regulated in laryngeal cancer and correlated with tumor progression, suggesting its function in diagnosis, treatment and prognosis of laryngeal cancer [28]. In the present study, we evaluated the diagnostic significance of *AGP1* in laryngeal cancer. ROC curve indicated that the expression of *AGP1* could serve as a diagnostic biomarker for laryngeal cancer. The molecular biomarkers provided a sensitive and non-invasive diagnostic method for laryngeal cancer patients, which might significantly improve the outcomes of the patients.

In conclusion, *AGP1* as an oncogene promotes the malignant tumor progression of laryngeal

cancer. *AGP1* may be a potential diagnostic biomarker for laryngeal cancer.

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Disclosure of conflict of interest

None.

Address correspondence to: Yaosheng Xu, Department of Otorhinolaryngological, Harrison International Peace Hospital, Hengshui 053000, China. E-mail: heiwawa7@163.com

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