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Original Article

Associations of serum CD62P and IL-6 levels with nasopharyngeal carcinoma staging and prognosis

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Abstract: Background: Serum CD62P and IL-6 levels in nasopharyngeal carcinoma (NPC) patients were measured and correlated with NPC staging and prognosis. Patients and methods: Sixty-eight NPC patients (study group) and 40 healthy subjects (control) were enrolled, and serum CD62P and IL-6 levels were measured. Statistical significance was assessed by one-way ANOVA. Correlation analysis was carried out by Spearman correlation test; $P<0.05$ was considered statistically significant. Results: A positive correlation was observed between CD62P and IL-6 expression levels ($P<0.05$). CD62P and IL-6 amounts were significantly higher in the study group ($P<0.01$) and positively correlated with the clinical stage of NPC ($P<0.05$). Patients showing improvement after radiotherapy had significantly lower CD62P and IL-6 levels compared with those without improvement ($P<0.01$). Conclusion: NPC patients had significantly higher serum CD62P and IL-6 levels, which were increased with NPC progression and closely associated with the prognosis of NPC patients.

Keywords: Interleukin-6, CD62P, nasopharyngeal carcinoma, cancer staging, prognosis

Introduction

Nasopharyngeal carcinoma (NPC) is a rare malignant tumor, with a higher incidence in China compared with that observed worldwide [1-3]. With NPC symptoms being obscure, e.g. nasal, aural, and neurologic symptoms, timely diagnosis is a challenge faced by clinicians [4]. The etiology of NPC is influenced by multiple factors, including genetic, environmental, and dietary factors [5, 6]. A major contributor to NPC pathogenesis is Epstein-Barr virus (EBV) infection. *In vivo* studies have established that EBV is a necessary factor for malignant growth; it facilitates NPC development by preventing apoptosis, and affects host immunity by modulating cellular signaling pathways [7, 8].

Several studies employed a proteomics approach to identify potential biomarkers for NPC [9, 10], with recent findings identifying prognostic markers that could lead to timely diagnosis and treatment of NPC [11-15]. However, disease prognosis remains poor in patients with same TNM stage, with treatment regimen often exhibiting variable clinical outcomes. Further, complications like metastasis and recurrence despite treatment result in suboptimal treatment efficacies [16, 17]. Therefore, early diagnosis and prognosis of nasopharyngeal carcinoma is of great importance.

CD62P is an adhesion factor mainly synthesized in platelets and blood vessels. The levels of CD62P in tissues are generally very low; however, after stimulation by thrombin or in tumor cells, abnormal expression of CD62P is observed [18]. A previous study showed that surface expression of CD62P and CD44v6 in peripheral blood and NPC tissues is higher in later stages of NPC, and survival of patients with lower expression of both molecules is higher [19]. IL-6 is produced by monocytes, endothelial cells, or glial cells at low levels in healthy individuals. However, the expression of IL-6, which participates in tissue cell differentiation and functioning, is increased abnormally in patients with inflammation or tumor [20].

In the present study, we measured CD62P and IL-6 serum levels in NPC patients at different clinical stages, as well as before and after

Table 1. Baseline characteristics of study participants

	Study group	Control group	*P*-value
Total/male	68/45	40/25	>0.05
Age	58±4	56±3	>0.05
BMI (kg/m^2)	22.17±2.18	22.39±2.19	>0.05
CD62P	41.5±23.53	2.95±0.17	<0.001
IL-6	56.7±33.86	4.3±0.21	<0.001
Pathology type			
Low-differentiated squamous cell carcinoma	60		
Adenosquamous carcinoma	2		
Undifferentiated carcinoma	6		
Staging			
I	20		
II	26		
III	15		
IV	7		

Note: Serum concentrations in ng/mL and depicted as $\bar{x}$±SD. *P<0.05, compared to the control group.

radiotherapy. The study objective was to assess the associations of serum CD62P and IL-6 levels with NPC staging and/or prognosis.

Materials and methods

Patients

Sixty-eight patients diagnosed with nasopharyngeal carcinoma by biopsy between January 2011 and June 2014 were enrolled in this study. Inclusion criteria were: age range, 41-72; first time diagnosis; no previous surgical treatment; no previous radiotherapy.

Exclusion criteria were: poor compliance; other nasal and pharyngeal diseases.

Age, sex, body mass index, and staging and pathological type of nasopharyngeal carcinoma were recorded for each patient.

The 68 patients included 45 males and 23 females, with age of 58±4 years (ranging from 41 to 72 years). Forty healthy subjects (25 males and 15 females; mean age of 56±3 years; age range of 45 to 71 years) referred to our hospital for physical examination in the same time period were included as the control group. No significant difference in age and sex between the two groups was found (P>0.05). The present study was approved by the Ethics Committee of our hospital, and all subjects provided signed informed consent.

Sample collection and measurement

CD62P and IL-6 levels were measured in control subjects and nasopharyngeal carcinoma patients, one week before radiotherapy and 2-week after 3 cycles of radiotherapy. Fasting venous blood (5 mL) was obtained from all subjects in the morning for serum preparation. CD62P and IL-6 levels were measured by enzyme linked immunosorbent assay (ELISA) using specific kits from Nanjing Zhongjian Company, according to the manufacturer's instructions. CD62P and IL-6 levels in serum samples were obtained after generation of standard curves.

CD62P and IL-6 levels were compared between the study and control groups, among nasopharyngeal carcinoma patients at different clinical stages, as well as before and after radiotherapy.

Treatment efficacies were evaluated 2 weeks after 3 cycles of radiotherapy. Improvement was defined as neither tumor nor metastatic tumor; no improvement was reflected by tumor and/or metastatic tumor identification [4, 5].

Statistical analysis

Quantitative data are mean ± standard deviation ($\bar{x}$±SD). The SPSS 18.0 software (SPSS, Chicago) was used for statistical analysis. One-way ANOVA was used to compare groups. Spearman correlation test was used for correlation analyses. P<0.05 was considered statistically significant.

Results

General and clinical characteristics of study participants

Between January 2011 and June 2014, 68 carcinoma and 40 healthy controls were enrolled

Table 2. CD62P and IL-6 levels in patients with different stages of nasopharyngeal carcinoma

Group	Stage I	Stage II	Stage III	Stage IV	*P*-value
N	20	26	15	7	-
CD62P	19.5±5.27	31.6±12.63	43.8±24.62	63.7±31.5	0.001
IL-6	21.6±13.72	43.8±26.23	59.3±31.21	76.9±37.57	<0.001

Note: Serum concentrations in ng/mL and depicted as $\bar{x}$±SD.

Table 3. CD62P and IL-6 levels in nasopharyngeal carcinoma patients with/without improvement after radiotherapy (ng/mL, $\bar{x}$ ±SD)

Group	Improvement	No improvement	*P*-value
N	28	40	-
CD62P	23.5±12.41*	58.9±27.95	<0.001
IL-6	31.6±23.22*	65.8±32.76	<0.001

Note: Serum concentrations in ng/mL and depicted as $\bar{x}$ ±SD. *P<0.05, compared to the no improvement group.

Table 4. Correlation of CD62P and IL-6 levels with patient age, clinical stage, and prognosis

Parameters	CD62P		IL-6	
	R	*P*	r	*P*
Age	0.019	0.897	0.082	0.751
Clinical stage	0.418	0.011	0.416	0.014
Prognosis	-0.397	0.021	-0.412	0.018
CD62P	-	-	0.401	0.016
IL-6	0.401	0.016	-	-

in this study. Sex, age and body mass index were recorded, and no significant difference was found between the two groups (P>0.05) (**Table 1**). Serum levels of CD62P and IL-6 in the study group were significantly higher than control group values (both P<0.05). Staging and pathology types of the study group are also presented in **Table 1**.

Serum CD62P and IL-6 levels in patients at different nasopharyngeal carcinoma stages

Serum levels of CD62P and IL-6 were measured in the study group, and compared among subjects at different clinical nasopharyngeal carcinoma stages. A significant elevation in both CD62P and IL-6 levels was observed with increasing disease stage (**Table 2**). Stage I subjects had the lowest levels, and stage IV nasopharyngeal carcinoma patients the highest CD62P and IL-6 serum levels (P<0.05).

Comparison of CD62P and IL-6 levels with treatment efficacy post-radiation therapy

Serum levels of CD62P and IL-6 were measured before radiotherapy and 2 weeks after the study group underwent 3 cycles of radiotherapy. No significant changes in CD62P and IL-6 amounts were observed post-radiotherapy compared to pre-radiotherapy levels. Improvement (absence of tumor and metastatic tumor) was observed in 28 subjects in the study group; however, 40 subjects showed no improvement (presence of tumor and/or metastatic tumor). The serum levels of CD62P and IL-6 after radiotherapy were compared between the two patient groups ("improvement" and "no improvement" post-radiotherapy). Interestingly, levels of both CD62P and IL-6 were significantly lower in patients showing improvement than in those without improvement (P<0.05) (**Table 3**).

Correlation of CD62P and IL-6 levels with patient age, clinical stage, and prognosis

A correlation analysis of CD62P and IL-6 levels with patient age, clinical stage, and prognosis revealed that CD62P expression was positively correlated with the IL-6 level (P<0.05). Further, CD62P and IL-6 levels were positively correlated with the clinical stage of nasopharyngeal carcinoma (P<0.05) and negatively associated with nasopharyngeal carcinoma prognosis (P<0.05) (**Table 4**).

Discussion

In this study we investigated whether serum levels of CD62P and IL-6 are associated with nasopharyngeal carcinoma staging and prognosis. Our findings revealed higher levels of both these markers in the study group (nasopharyngeal carcinoma patients) compared to the control group (healthy subjects). Correlation analysis showed that CD62P and IL-6 serum levels are positively correlated with clinical stage but negatively associated with disease prognosis, thus establishing their associations with nasopharyngeal carcinoma staging and prognosis.

Nasopharyngeal carcinoma is one of the most common malignancies in China with a high

morbidity. The major presentations of nasopharyngeal carcinoma include nasal bleeding, bloody nasal discharge, nasal obstruction, and even headache and hearing loss. The lesions of nasopharyngeal carcinoma being hard to detect, with early stage of nasopharyngeal carcinoma remaining asymptomatic, early diagnosis rate of nasopharyngeal carcinoma is low [4, 5]. Moreover, primary lesions in advanced nasopharyngeal carcinoma are adjacent to many important blood vessels and nerves; in addition, the metastasis rate is very high, prohibiting resection surgery. These are limitations to the timely treatment of nasopharyngeal carcinoma; thus, radiotherapy is the only available treatment method [21, 22]. As a lowly-differentiated cancer, nasopharyngeal carcinoma is relatively sensitive to radiation and both the regions of primary and metastatic nasopharyngeal carcinomas could be targeted by radiotherapy. Although advanced nasopharyngeal carcinoma could be treated, therapeutic effectiveness is poor. Therefore, tumor markers have been introduced into clinical practice to ensure treatment effects for nasopharyngeal carcinoma [9-15]. In the present study, serum levels of both CD62P and IL-6 were measured in patients with nasopharyngeal carcinoma, and compared with those of control subjects. As shown above, the study group levels were significantly higher than those of the control group, corroborating a report by Jie*et al.* [9]. These findings suggest that nasopharyngeal carcinoma patients express abnormal levels of CD62P and IL-6, indicating their potential use as biomarkers for nasopharyngeal carcinoma.

Further, we measured and compared the levels of CD62P and IL-6 in patients at different clinical stages of nasopharyngeal carcinoma. We found that CD62P and IL-6 levels increased with cancer progression, in agreement with previous studies [9]. These findings suggest that CD62P and IL-6 expression levels in the body are positively correlated with nasopharyngeal carcinoma stage. In addition, the present study showed that CD62P and IL-6 levels were significantly lower in patients with improvement after radiotherapy compared with the non-improvement group, indicating that CD62P and IL-6 levels could be used to predict patient prognosis. Previous studies have shown that CD62 could promote binding of platelets and tumor cells to form micro cancerous thrombi [19], which could result in adhesion to vascular endothelial cells and penetration through blood vessel walls, forming metastatic lesions. In addition, advanced tumor patients actively express CD62P, which promotes tumor cell metastasis. IL-6 could induce cell migration and promote tumor cell proliferation, enhancing the expression of adhesive factors, and resulting in tumor cell diffusion and metastasis [20]. In conclusion, high levels of CD62P and IL-6 reflect poor prognosis and vice versa. Therefore, CD62P and IL-6 levels are negatively correlated with nasopharyngeal carcinoma prognosis.

The present study has a few limitations. First, the data presented were from NPC patients diagnosed through biopsy at a single hospital; for this reason, sample size was limited. Second, a single follow-up was carried out 2 weeks after 3 cycles of radiotherapy to assess improvement in patients, and no long-term follow-up was performed. Larger sample size studies with patients from multiple hospitals in areas of high incidence in China as well as longer follow-up would are warranted to confirm these findings. Further, our data showed a correlation between serum levels of CD62P and IL-6 in patients with NPC; however, we did not investigate the possible reasons for this correlation. It is worthwhile exploring the possible underlying mechanism for such correlation in future studies.

In summary, serum levels of CD62P and IL-6 in nasopharyngeal carcinoma patients are significantly higher than those of healthy subjects. Further, CD62P and IL-6 levels are positively correlated with clinical stage of nasopharyngeal carcinoma, and negatively correlated with disease prognosis. Further studies with larger sample sizes will help identify whether CD62P and IL-6 can be used for clinical staging of nasopharyngeal carcinoma and predicting disease prognosis.

Disclosure of conflict of interest

None.

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