MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2015; 21: 2105-2109
DOI: 10.12659/MSM.893415

Received: 2014.12.23
Accepted: 2015.02.23
Published: 2015.07.20

Correlation Analysis of Nasopharyngeal Carcinoma TNM Staging with Serum EA IgA and VCA IgA in EBV and VEGF-C and -D

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Source of support: Departmental sources

Background: Nasopharyngeal carcinoma often occurs in humans in the nasopharyngeal epithelium area. Ebstein-Barr (EB) virus plays a key role in the process of nasopharyngeal carcinoma lesions. Early antigen antibody (EA-IgA) and viral capsid antigen IgA (VCA-IgA) of EB virus detection in serum can effectively monitor the process of nasopharyngeal carcinoma lesions. Serum vascular endothelial growth factor (VEGF) -C and VEGF-D expression detection can reflect the distant metastases ability of human tumor cells.

Material/Methods: 153 cases of nasopharyngeal carcinoma patients in our hospital were enrolled, while 148 cases of healthy adults were selected as control. ELISA was used to detect serum EA-IgA, VCA-IgA, VEGF-C and -D expression levels. Spearman rank correlation analysis was applied to test the correlation of nasopharyngeal carcinoma TNM clinical stage and different indexes.

Results: Serum EA-IgA, VCA-IgA, VEGF-C and -D expression in nasopharyngeal carcinoma patients was 43.74±2.6 U·mL^{-1}, 62.5±2.7 U·mL^{-1}, 473.25±3.4 pg·mL^{-1}, and 498.36±2.3 pg·mL^{-1}, respectively, which was significantly higher than in the control group as 18.65±3.7 U·mL^{-1}, 23.74±1.5 U·mL^{-1}, 225.42±2.3 pg·mL^{-1}, and 257.24±3.5 pg·mL^{-1} ($P<0.05$). Nasopharyngeal carcinoma TNM clinical staging was obviously correlated with serum EA-IgA, VCA-IgA, and VEGF-C ($P<0.05$), but not VEGF-D ($P>0.05$).

Conclusions: Nasopharyngeal carcinoma patient serum EA-IgA and VCA-IgA expression levels were significantly correlated with TNM staging. The high levels of these 3 indicators suggest advanced nasopharyngeal carcinoma TNM staging and serious lesions.

MeSH Keywords: Nasopharyngeal Diseases • Neoplasm Staging • Vascular Endothelial Growth Factor C • Vascular Endothelial Growth Factor D

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/893415

1800 3 — 25

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Background

China is a high-incidence area of nasopharyngeal carcinoma, for the incidence of nasopharyngeal carcinoma in the head and neck malignant tumor accounts for 78.08% in recent years [1]. Due to its concealed disease area and early metastasis [2], it is of great significance to nasopharyngeal carcinoma prognosis by early diagnosis and treatment. Epstein-Barr (EB) virus is a carcinogenic herpes virus that is a major risk factor for nasopharyngeal carcinoma. The aggravation of nasopharyngeal carcinoma may activate EB virus and let the virus enter the replication phase. Antigens expressing the EB virus mainly include early antigen (EA) and viral capsid antigen (VCA). Previous studies have shown that EB virus could latently infect human nasopharyngeal epithelial cells, and induce lytic infection by triggering capillary expansion mutation gene activation and DNA damage response protein localization during viral replication [3]. Nasopharyngeal carcinoma cells contain EBV virus gene as well as a variety of EB virus-specific antigens and antibodies, such as EB virus early antigen EA, viral capsid antigen VCA, early antigen antibody EA-IgA, and viral capsid antigen antibody VCA-IgA, which can be used as molecular biology markers in prognosis. Thus, EA-IgA and VCA-IgA antibodies expression level in serum could reflect the EB virus physiological state in the body [4], and further indicate nasopharyngeal carcinoma severity. For recurrence of cancer, *in situ* and tumor lymphocytes metastasis are the important reasons for treatment failure. Serum vascular endothelial growth factor VEGF-C and -D expression level can effectively reflect the metastatic ability of tumor cells [5].

Clinical treatment of nasopharyngeal carcinoma mainly uses radiotherapy and chemotherapy, which have serious adverse effects and bad clinical compliance [6]. The TNM staging system is most commonly used worldwide to assess tumor invasiveness in the body and is one of the main malignant tumor prognosis evaluation indexes. Effective clinical staging and appropriate diagnosis could contribute to better clinical effect. Following its constant improvement through evidence-based medicine, the 7th edition of the International UICC/AJCC Staging and Domestic 2008 Staging are currently widely used for clinical of nasopharyngeal carcinoma clinical staging. With the deepening of nasopharyngeal carcinoma research, EB virus DNA gene antibody [7] and vascular endothelial growth factor expression levels showed great significance in assessment of nasopharyngeal carcinoma clinical stage and prognosis. The tumor has a relatively better prognosis in the early stage, and the prognosis worsens following the upgrade of clinical staging. In the present study, we tested serum EA-IgA, VCA-IgA, VEGF-C and -D expression levels in nasopharyngeal carcinoma patients and normal controls by ELISA to explore their correlation with clinical nasopharyngeal carcinoma TNM staging and to provide new insights for clinical staging standard.

Material and Methods

Clinical information

We enrolled 153 nasopharyngeal carcinoma patients (92 males and 61 females) between October 2012 and October 2013 in our hospital. We selected 148 healthy adults (80 males and 68 females) as controls. The average ages in the test and control groups were 53.6±4.8 and 49.8±3.5 years old, respectively. The mean age and sex ratio showed no significant differences between the 2 groups. Venous blood was collected and centrifuged at 3000 r/min for 10 min. Serum was isolated and stored at –20°C [8].

The study protocol was approved by the Research Ethics Committee of our hospital, and all patients gave their informed consent before study commencement.

Detection method

ELISA was used to detect serum EA-IgA, VCA-IgA, VEGF-C, and -D expression levels according to the manual. TMB stop buffer was added and OD value was read at 450-nm wavelength. A concentration-absorbance curve was drawn to calculate EA-IgA, VCA-IgA, VEGF-C, and -D levels in serum. The results are presented as mean ± standard deviation ($\bar{x}\pm s$). ELISA kits were purchased from the IBL Company (Germany) and an automatic microplate reader was purchased from Shanghai Tiancheng Technology Co., LTD (BIO-RAD model1680, Shanghai, China).

Statistical analysis

All statistical analyses were performed using SPSS17.0 software (Chicago, IL). Differences between means were analyzed using the t test, with $P<0.05$ considered to indicate a statistically significant result. T test and Spearman rank correlation test were applied to calculate the correlation coefficient R between different groups.

Results

Serum EBV EA-IgA, VCA-IgA, VEGF-C, and -D expression levels between the 2 groups

ELISA results showed that EA-IgA was expressed in 95.4% (146/153) of nasopharyngeal carcinoma patients and in 43.2% (64/148) of healthy controls; VCA-IgA was be detected in 90.8% (139/153) of nasopharyngeal carcinoma patients and in 35.8% (53/148) of healthy controls, and VEGF-C was detected in 78.4% (120/153) of nasopharyngeal carcinoma patients and 30.4% (45/148) of healthy controls. Serum VEGF-D was expressed in all of the enrolled subjects. Serum EA-IgA, VCA-IgA, VEGF-C, and -D expression in nasopharyngeal carcinoma patients was

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Table 1. Serum EBV EA-IgA, VCA-IgA, VEGF-C and -D expression levels between the two groups.

Group	n	EA-IgA/U·mL^{-1}	VCA-IgA/U·mL^{-1}	VEGF-C/pg·mL^{-1}	VEGF-D/pg·mL^{-1}
Control group	148	18.65±3.7	23.74±1.5	225.42±2.3	257.24±3.5
Observation group	153	43.74±2.6	62.5±2.7	473.25±3.4	498.36±2.3
t	–	9.54	6.36	9.76	7.32
P	–	P<0.05	P<0.05	P<0.05	P<0.05

Table 2. Serum EA-IgA and VCA-IgA expression level in nasopharyngeal carcinoma patients at different stages.

Clinical stage	EA-IgA (U·mL^{-1})	R	VCA-IgA (U·mL^{-1})	R
Stage I	22.31±4.7*	0.986	34.35±4.7*	0.949
Stage II	37.24±3.6*	0.924	46.27±3.6*	0.902
Stage III	45.17±4.3*	0.882	57.25±4.3*	0.875
Stage IV	63.37±4.2*	0.807	78.46±4.2*	0.823

* P<0.05 for correlation analysis.

Table 3. Serum VEGF-C and -D expression level in nasopharyngeal carcinoma patients at different stages.

Clinical stage	VEGF-C (pg/ml)	R	VEGF-D (pg/ml)	R
Stage I	258±5.1	0.904*	295±4.7	0.892
Stage II	317±5.4	0.897*	386±3.8	0.861
Stage III	473±7.4	0.856*	485±5.3	0.834
Stage IV	428±6.3	0.821*	432±4.3	0.805

* P<0.05 for correlation analysis.

43.74±2.6 U·mL^{-1}, 62.5±2.7 U·mL^{-1}, 473.25±3.4 pg·mL^{-1}, and 498.36±2.3 pg·mL^{-1}, respectively, which was significantly higher than in the control group as 18.65±3.7 U·mL^{-1}, 23.74±1.5 U·mL^{-1}, 225.42±2.3 pg·mL^{-1}, and 257.24±3.5 pg·mL^{-1} (P<0.05) (Table 1).

Correlation of nasopharyngeal carcinoma TNM staging with patient serum EBV EA-IgA and VCA-IgA level

Nasopharyngeal carcinoma TNM clinical staging was obviously correlated with serum EA-IgA and VCA-IgA level (P<0.05). Serum EA-IgA and VCA-IgA level increased following the upgrade of TNM stage (Table 2).

Correlation of nasopharyngeal carcinoma TNM staging with patient's serum VEGF-C and -D level

Nasopharyngeal carcinoma TNM clinical staging was significantly correlated with serum VEGF-C level (P<0.05) but not with VEGF-D (P>0.05). Serum VEGF-C and -D level was markedly elevated following the upgrade of TNM stage, and their level decreased in the patients at stage IV (Table 3).

Discussion

Nasopharyngeal carcinoma has significant genetic characteristics and can be caused by EB virus infection, heredity, and diet, but is not related to age or sex [9]. EB virus stays in a latent state in healthy people, and it can induce nasopharyngeal carcinoma lymphocyte proliferation and differentiation, mainly through expressing latent membrane protein (LMP_1) [10,11]. Jiang et al. [12] found that, in addition to virus membrane protein LMP_1, oncogene $BARF_1$ encoded by EBV can transform human epithelial cells and lymphocytes. Transformed cells present a malignant phenotype and play an important role in nasopharyngeal carcinoma. In addition, the product encoded by EB virus can interact with the related genes in the epithelial cells, resulting in a series of biological molecular events in the development of nasopharyngeal lesions. These biological macromolecules can be detected in the peripheral blood. Thus, serum EA-IgA and VCA-IgA expression could be detected clinically to evaluate nasopharyngeal carcinoma lesions level [13–15]. In this study, we tested the 2 index expression levels mentioned above in human serum to investigate its

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correlation with clinical nasopharyngeal carcinoma staging and provide basis for early clinical staging diagnosis of nasopharyngeal carcinoma in the future.

Clinically, nasopharyngeal carcinoma can be treated by radiotherapy and chemotherapy, but they have serious adverse effects and bad clinical compliance. Effective nasopharyngeal carcinoma clinical staging can help in patient classification and in choosing the appropriate diagnosis and treatment methods. Cancer recurrence *in situ* and lymphocyte metastasis were the important reasons for treatment failure [16]. Nasopharyngeal carcinoma aggravation may activate EB virus to enter the viral replication phase and express EA and VCA. Morphology changes such as nasopharyngeal epithelium squamous metaplasia, epithelial atypia, carcinoma *in situ*, and microinvasive carcinoma can be found in the process of nasopharyngeal carcinoma. In the cancerous process, the EB virus gene began to stimulate production of transcript-related EBV antigen and antibody. Therefore, clinical detection of serum EA-IgA and VCA-IgA expression levels can effectively show EB virus proliferation *in vivo*, and detect nasopharyngeal carcinoma changes earlier. The growth of blood vessels and lymphatic vessels closely participate in tumor lymph node metastasis. VEGF and its receptor (VEGFR) family factors play important roles in the formation of tumor blood vessels and lymphatic vessels. VEGF-A, -B, -C, -D, and -E have been found, of which VEGF-C and -D are directly involved in the formation of lymphatic vessels and tumor cells metastasis. Du Q et al. [17]suggested that VEGF-C and -D are the important factors for gallbladder carcinoma lymphatic vessel formation and lymph node metastasis. As a key inflammatory cytokine in gallbladder carcinoma chronic inflammation, tumor necrosis factor-α can stimulate VEGF-C and -D expression in some non-tumor cells. Also, several studies indicated that only VEGF-C and -D among the VEGF family factors can specifically bind with corresponding receptor VEGFR-3 to stimulate tumor lymphocyte proliferation, growth, and metastasis [18,19]. Thus, detecting VEGF-C and -D level in serum can effectively reflect the metastatic degree of nasopharyngeal carcinoma lymphocytes to surrounding tissues. ELISA testing of EA-IgA, VCA-IgA, VEGF-C and -D expression in patients and healthy control serum at the same time can improve both sensitivity and specificity [20–23], to provide reliable indexes for nasopharyngeal carcinoma clinical TNM staging.

According to the 2008 nasopharyngeal carcinoma TNM staging standard, nasopharyngeal carcinoma can be divided into tumor phase, lymph nodes phase, and metastatic phase. TNM staging classifies nasopharyngeal carcinomas into 4 stages [24,25]. This study selected 153 cases of nasopharyngeal carcinoma patients and 148 healthy controls. The mean age and sex ratio showed no significant differences between the 2 groups (P>0.05). Results showed that serum EA-IgA, VCA-IgA, VEGF-C, and -D expression in nasopharyngeal carcinoma patients was significantly higher than in controls (P<0.05), indicating that these 4 indexes can be used to detect nasopharyngeal carcinoma patient EB virus transfection and the tumor distant metastasis ability of tumor cells, and are therefore important markers for prognosis. In addition, VEGF-C and -D binding to corresponding receptors induced formation of nasopharyngeal carcinoma patient lymphatic vessels and blood vessels, thus promoting tumor cell proliferation, differentiation, and metastasis. They can be used as new method in treating nasopharyngeal tumor blood vessels and lymphatic generation. Nasopharyngeal carcinoma TNM clinical staging was obviously correlated with serum EA-IgA, VCA-IgA, and VEGF-C (P<0.05). Furthermore, following the progress of clinical TNM stage, the abovementioned markers increased. It indicated that the above 3 markers have important roles in monitoring disease changes and distinguishing clinical stage. Nasopharyngeal carcinoma clinical stage exhibited no significant correlation with VEGF-D (P>0.05). Thus, serum EA-IgA, VCA-IgA, and VEGF-C expression can accurately reflect EB virus transfection and tumor cell differentiation and metastasis ability in nasopharyngeal carcinoma patients.

Conclusions

Nasopharyngeal carcinoma TNM clinical staging is of great significance for disease diagnosis and treatment. Only by establishing clear clinical staging can we evaluate disease changes accurately and select appropriate means of treatment and prevention. Serum EA-IgA, VCA-IgA, and VEGF-C expression was selected after screening to provide new markers for clinical staging. To sum up, serum EA-IgA, VCA-IgA, VEGF-C, and -D expression in different TNM clinical stages can reflect EBV infection and tumor metastasis in nasopharyngeal carcinoma patients. Serum EA-IgA, VCA-IgA, and VEGF-C increased following the progress of clinical TNM stage, indicating their correlation with the later and indicating that combined detection of the abovementioned markers can assist nasopharyngeal carcinoma clinical staging. Moreover, higher expression level of EA-IgA, VCA-IgA, and VEGF-C in serum indicates later TNM staging and worse disease.

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