EXPRESSION CHANGES IN SERUM TPA, EBV AND CYFRA21-1 AND THEIR CLINICAL DIAGNOSTIC VALUE IN PATIENTS WITH NASOPHARYNGEAL CARCINOMA

Pu Wang*, Jianguo Zhang, Shengbin Zhong, Rongyue Liu, Guangli Chen
The First People's Hospital of Fuyang District, Hangzhou, Zhejiang, 311400, China

ABSTRACT

Objective: *To study expression changes in serum levels of tissue polypeptide antigen (TPA), Epstein-Barr virus (EBV), cytokeratin 19 (CYFRA21-1) and their clinical diagnostic value in patients with nasopharyngeal carcinoma (NPC).*

Methods: *158 patients with NPC admitted to our hospital between January 2016 and January 2017 were analysed retrospectively; 62 patients who were in our hospital during the same period with benign head and neck diseases were also selected. Follow-ups were conducted with NPC patients for 3 years, and patients were divided into groups according to patient prognosis—recurrence or distant metastasis (n=98) versus clinical remission (n=60)-as well as according to patients' clinical stages—stage I (n=23), stage II (n=40), stage III (n=61), stage IV (n=34). At admission, 5 ml of early morning fasting venous blood was collected from all patients; TPA and CYFRA21-1 levels were detected by enzyme-linked immunosorbent assay, whereas the quantitative real-time fluorescence method was used to detect EBV DNA. The serum levels of TPA, EBV DNA and CYFRA21-1 in each group were compared to analyse the changes in their expression in patients with NPC and thus explore their clinical diagnostic value.*

Results: *The positive rates of serum TPA, EBV and CYFRA21-1 in the NPC group were 66.46%, 51.90% and 67.90%, respectively, all of which were significantly higher (P<0.01) than those in the benign disease group. The positive rates of serum TPA, EBV and CYFRA21-1 in the recurrence or distant metastasis group were 98.98%, 79.59% and 96.94%, respectively, all of which were significantly higher (P<0.05 or <0.01) than in the clinical remission group. The positive rate of TPA, EBV and CYFRA21-1 showed an upward trend correlated with the clinical stage. In other words, the positive rate of patients in the stage IV group was significantly higher than that of stage I, II and III groups, the stage III group rate was significantly higher than that of the stage I and II groups, the stage II group rate was significantly higher than that of the stage I group (all differences significant at P<0.01). The sensitivity of TPA in diagnosing NPC patients was 95.45%, the specificity was 51.81%, the positive predictive value was 66.46% and the negative predictive value was 91.94%; the sensitivity of EBV DNA in diagnosing NPC patients was 96.47%, the specificity was 43.70%, the positive predictive value was 51.90% and the negative predictive value was 95.16%. The sensitivity of CYFRA21-1 in diagnosing NPC patients was 99.07%, the specificity was 53.98%, the positive predictive value was 67.09% and the negative predictive value was 98.39%.*

Conclusion: *Serum TPA, EBV and CYFRA21-1 levels are highly expressed in NPC patients, and these levels varied with the changes of patient's prognosis and clinical stage. Thus, they have particular value in the diagnosis of NPC and can be widely used in clinical practice.*

Keywords: *Serum, TPA, EBV, CYFRA21-1, nasopharyngeal carcinoma, expression, change, clinical, diagnosis, value.*

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Introduction

Nasopharyngeal carcinoma (NPC) refers to a malignant tumour that occurs on the top and side walls of the nasopharyngeal cavity. Such tumours can clinically manifest as nasal congestion, blood in the discharge, hearing loss, ear congestion, diplopia, headache and other symptoms[1]. NPC is one of the most common malignant tumours in China. It has the highest incidence rate among malignant tumours of the ear, nose and throat, which seriously threatens the life and health of patients[2]. NPC mostly occurs in the posterior wall of the nasopharyngeal roof and pharyngeal crypts. Its location is relatively hidden and the early symptoms are not obvious. Thus, due to clinical and technical reasons, NPC are prone to missed diagnoses, and most patients have metastases when they are finally treated. This affects follow-up treatment and endangers the life of the patient[3]. Therefore, finding a high-sensitivity method for the

detection of NPC that can facilitate early diagnosis and treatment is of great significance, as it enables clinicians to slow disease progression and thus prolong the lives of patients. The pathogenesis of NPC is not yet fully understood. It is currently believed to be closely related to genetics, Epstein-Barr virus (EBV) infection and environmental factors[4]. As early as 1999, the International Cancer Research Center listed EBV as a human carcinogen. EBV infection, combined with a variety of somatic and phenotypic genetic changes, synergistically destroys normal cell functions, resulting in the occurrence of NPC[5].

Soluble cytokeratin 19 segment (CYFRA21-1) is abnormally expressed in squamous epithelial tumours and plays an important role in the detection of epithelial tumours[6]. In recent years, studies have found that CYFRA21-1 is significantly increased in the serum of NPC patients, which may be related to the occurrence and development of NPC[7]. Tissue polypeptide antigen (TPA) is an indicator that reflects the growth of tumour cells with high sensitivity. There have been many studies on tumour diagnosis, staging and prognostic evaluation recently[8]. This study retrospectively analysed 158 NPC patients admitted to our hospital between January 2016 and January 2017 with the goal of studying the clinical diagnostic value of changes in the expression of serum EBV, CYFRA21-1 and TPA in NPC patients.

Methods

General information

We retrospectively analysed the 158 patients with nasopharyngeal carcinoma (NPC) admitted to our hospital between January 2016 and January 2017.

Inclusion criteria were as follows:

• All patients were diagnosed as NPC by pathological examination when admitted to our hospital, and no distant metastasis had occurred;

• All patients were without other malignant tumours;

• Patients and their families were informed and signed informed consent.

Exclusion criteria were as follows:

• Those with severe dysfunction in vital organs such as the heart, liver and kidney;

• Those who were pregnant or breastfeeding;

• Those with autoimmune disease;

• Those with serious infections;

• Those in whom the tumour was accompanied by nervous system and mental diseases;

• Those who refused participation in the experiment or terminated participation for other reasons. Over the same time period, 62 patients with benign head and neck diseases were selected from our hospital population.

Inclusion criteria for this group were as follows:

• The patient's diagnosis was confirmed pathologically as one of several benign head and neck diseases, including nodular thyroid disease, cervical lymphadenitis and benign tumours of the head and neck;

• The patient had no malignant tumours;

• The patient's heart, liver, kidney and other important organs had no serious dysfunction;

• The patient and their family members were informed and signed an informed consent.

Follow-ups with NPC patients were conducted for 3 years. Of the 158 patients in the NPC group, 80 were male and 78 were females, with an average age of 45.06±9.78 years and an average BMI value of 20.05±0.98 Kg/m^2. Of the 62 patients in the benign disease group, 32 were male and 30 were female, with an average age of 45.11±9.34 years and an average BMI value of 20.12±0.87 Kg/m^2. Patients in the NPC category were divided into a recurrence or distant metastasis group (n=98) and a clinical remission group (n=60) according to their prognosis. Of the 98 patients in the recurrence or distant metastasis group, 49 were male and 49 were female, with an average age of 45.01±9.98 years and an average BMI value of 20.12±0.78 Kg/m^2. Of the 60 patients in the clinical remission group, 31 were male and 29 were female, with an average age of 45.12±9.62 years and an average BMI value of 20.01±0.75) Kg/m^2.

According to clinical stage, patients were divided into stage I group (n=23), stage II group (n=40), stage III group (n=61) and stage IV group (n=34). The stage I group comprised 23 patients, 13 males and 10 females, with an average age of 45.06±9.78 years and an average BMI value of 20.05±0.98 Kg/m^2. Of the 48 patients in the control group, 28 were male and 20 were female, with an average age of 45.06±9.78 years and an average BMI value of 20.05±0.98 Kg/m^2. There were no significant differences (P>0.05) in age, sex or BMI value among the subjects in any of the groups compared in the study.

Observation indicators

Serum test

5 ml of fasting venous blood was collected in the early morning from all patients after being

admitted to the hospital; samples were kept at room temperature for 20 minutes, then centrifuged at 3000r/min for 10 minutes and the serum was separated carefully and stored in a –70°C environment to avoid repeated freezing and thawing. In all patients, enzyme-linked immunosorbent assay was used to detect TPA and CYFRA21-1 levels and real-time fluorescence quantitative method was used to detect EBV DNA levels.

Statistical methods

Data were analysed using the SPSS20.0 software package. All measurement data were compared and expressed in terms of mean and standard deviation ($\bar{x}\pm s$); count data were all represented by percentage; t tests were performed to make within-group comparisons, whereas between-group comparisons were assessed by χ^2 test. All results with P<0.05 were deemed statistically significant.

Results

Comparison of the positive rates of serum TPA, EBV and CYFRA21-1 between the NPC and benign disease groups

The positive rates of serum TPA, EBV and CYFRA21-1 in the NPC group were 66.46%, 51.90% and 67.90%, respectively, all of which were significantly (P<0.01) higher than those in the benign disease group. See Table 1.

Groups	TPA			EBV DNA			CYFRA21-1		
	+	–	Positive rates	+	–	Positive rates	+	–	Positive rates
NPC group	105	53	66.46%	82	76	51.90%	106	52	67.09%
Benign disease group	5	57	8.06%	3	59	4.84%	1	61	0.00%
χ^2	60.727			37.149			80.271		
P	<0.001			<0.001			<0.001		

Table 1: Comparison of positive rates of serum TPA, EBV and CYFRA21-1 between the NPC and benign disease groups (cases, %).

Comparison of positive rates of serum TPA, EBV and CYFRA21-1 in patients with different prognoses

The positive rates of serum TPA, EBV and CYFRA21-1 in the recurrence or distant metastasis group were 98.98%, 79.59% and 96.94%, respectively, all of which were significantly (P<0.05 or <0.01) higher than the clinical remission group. See Table 2.

Groups	TPA			EBV DNA			CYFRA21-1		
	+	–	Positive rates	+	–	Positive rates	+	–	Positive rates
Recurrence or distant metastasis	97	1	98.98%	78	20	79.59%	95	3	96.94%
Clinical remission	8	52	13.33%	4	56	6.67%	11	49	18.33%
χ^2	5.444			10.667			4.571		
P	0.020			0.001			0.033		

Table 2: Comparison of positive rates of serum TPA, EBV and CYFRA21-1 in patients with different prognoses (cases, %).

Comparison of positive rates of serum TPA, EBV and CYFRA21-1 according to patients' clinical stages

The positive rates for TPA, EBV and CYFRA21-1 demonstrated an upward trend corresponding to clinical stage. The positive rate for patients in stage IV group was significantly higher (P<0.01) than that for patients in the stage I, II and III groups, the positive rate for patients in stage III group was significantly higher (P<0.01) than that for patients in the stage I and II groups, and the positive rate for patients in the stage II group was significantly higher (P<0.01) than that for patients in the stage I group. See Table 3.

Groups	TPA			EBV DNA			CYFRA21-1		
	+	–	Positive rates	+	–	Positive rates	+	–	Positive rates
Stage I	8	15	34.78%	4	19	17.39%	10	13	43.48%
Stage II	24	16	40.00%	13	27	32.50%	21	19	52.50%
Stage III	38	23	62.30%	35	26	57.38%	49	12	80.33%
Stage IV	28	6	82.35%	30	4	88.24%	26	8	76.47%
χ^2	13.283			35.717			15.860		
P	0.004			<0.001			0.001		

Table 3: Comparison of positive rates of serum TPA, EBV and CYFRA21-1 according to patients' clinical stages (cases, %).

Value analysis of serum TPA, EBV DNA and CYFRA21-1 in the diagnosis of NPC

The sensitivity of TPA in diagnosing NPC patients was 95.45%, the specificity was 51.81%, the positive predictive value was 66.46% and the negative predictive value was 91.94%; the sensitivity of EBV DNA in diagnosing NPC patients was 96.47%, the specificity was 43.70%, the positive predictive value was 51.90% and the negative predictive value was 95.16%. The sensitivity of CYFRA21-1 in diagnosing

NPC patients was 99.07%, the specificity was 53.98%, the positive predictive value was 67.09% and the negative predictive value was 98.39%. See Table 4.

Index	Sensitivity	Specificity	Positive predictive value	Negative predictive value
TPA	95.45%	51.81%	66.46%	91.94%
EBV DNA	96.47%	43.70%	51.90%	95.16%
CYFRA21-1	99.07%	53.98%	67.09%	98.39%

Table 4: Value analysis of serum TPA, EBV DNA and CYFRA21-1 in the diagnosis of NPC.

Discussion

Because NPC patients experience no obvious discomfort in the early stages of the disease, the proportion of patients who are diagnosed and treated early is low. Most patients are in the middle and late stage when they see a doctor, treatment effects are poor and the 5-year survival rate is low, which seriously endangers the health and life of the patients[9]. Currently, radiotherapy is the main treatment method in clinical practice, and both local recurrence and distant metastasis are common indicators of treatment failure. Therefore, early diagnosis and timely treatment, as well detection of curative effect and assessment of various means of prognosis are of great significance, because these can improve prognoses and prolong the lives of patients[10]. TPA is a broad-spectrum tumour marker. Studies have shown that it can be used as a molecular indicator for detecting distant metastasis of NPC. Li Runhan et al.[11] believe that changes in TPA levels shed important light on the occurrence, development, metastasis and prognosis of NPC. EBV is a sporangial virus. Through close contact, it not only infects human B lymphocytes, but also oropharyngeal epithelial cells without causing clinical symptoms. When the body's immune function is diminished and the proportion of T cells is imbalanced, EBV spreads freely and produces EBV antibodies that induce a series of pathological changes, including tumour occurrence and development[12]. Studies have shown that EBV is one cause of malignant lymphoma and NPC, and serum levels of EBV DNA are often elevated before the clinical discovery of tumours[13].

CYFRA21-1 is a soluble fragment of cytokeratin 19, a skeletal protein derived from epithelial cells. When epithelial cells become cancerous, the activated protease degrades the tumour cells, causing a large amount of CYFRA21-1 to be released into the blood, increasing its serum level substantially[14]. Thus, CYFRA21-1 is widely used as an epithelial tumour marker in clinical practice. Because NPC is primarily derived from the covered squamous epithelium of the nasopharynx, CYFRA21-1 may be of value in the diagnosis of NPC. In this study, the positive rates of serum TPA, EBV and CYFRA21-1 in the NPC group were 66.46%, 51.90%, and 67.90%, respectively, which were significantly higher ($P<0.01$) than those in the benign disease group. The positive rates of serum TPA, EBV and CYFRA21-1 in the recurrence or distant metastasis group were 98.98%, 79.59% and 96.94%, respectively, which were significantly significant ($P<0.05$ or <0.01) higher than the clinical remission group. The positive rate of TPA, EBV and CYFRA21-1 showed an upward trend with the clinical stage. The positive rate of patients in stage IV group was significantly higher than that of stage I, II and III groups, and the positive rate of patients in stage III group was significantly higher ($P<0.01$) than that of I and II groups. The positive rate of patients in the stage II group was significantly higher ($P<0.01$) than that in the stage I group. The significant differences among groups distinguished by both prognosis and clinical stage suggests that the positive rate of serum TPA, EBV and CYFRA21-1 in may provide strong test support for the diagnosis of NPC, a finding similar to the results of Wang Kongcheng et al.[15].

In this experiment, the sensitivity of TPA in diagnosing NPC patients was 95.45%, the specificity was 51.81%, the positive predictive value was 66.46% and the negative predictive value was 91.94%; the sensitivity of EBV DNA in diagnosing NPC patients was 96.47%, the specificity was 43.70%, the positive predictive value was 51.90% and the negative predictive value was 95.16%. The sensitivity of CYFRA21-1 in diagnosing NPC patients was 99.07%, the specificity was 53.98%, the positive predictive value was 67.09% and the negative predictive value was 98.39%. The high values for sensitivity, specificity and positive and negative predictive values, for TPA, EBV and CYFRA21-1 suggest that all three have good predictive value in the diagnosis of NPC, which can help doctors diagnose NPC early and take corresponding measures, which is extremely important for effective treatment of patients.

In summary, TPA, EBV and CYFRA21-1 are highly expressed in NPC patients' serum levels, and these levels increase along with changes in the

patient's prognosis and clinical stage, which has certain value in the diagnosis of NPC and can be widely used in clinical practice.

References

1) Pisano G, Roy A, Ahmed Ansari M, Kumar B, Chikoti L, et al. Interferon-γ-inducible protein 16 (IFI16) is required for the maintenance of Epstein-Barr virus latency. Virol J. 2017 Nov 13;14(1): 221. doi: 10.1186/s12985-017-0891-5. PMID: 29132393; PMCID: PMC5683537.
2) Nawandar DM, Ohashi M, Djavadian R, Barlow E, Makielski K, et al. Differentiation-Dependent LMP1 Expression is Required for Efficient Lytic Epstein-Barr Virus Reactivation in Epithelial Cells. J Virol. 2017 Mar 29; 91(8): e02438-16. doi: 10.1128/JVI.02438-16. PMID: 28179525; PMCID: PMC5375685.
3) Faè DA, Martorelli D, Mastorci K, Muraro E, Dal Col J, et al. Broadening Specificity and Enhancing Cytotoxicity of Adoptive T Cells for Nasopharyngeal Carcinoma Immunotherapy. Cancer Immunol Res. 2016 May; 4(5): 431-40. doi: 10.1158/2326-6066.CIR-15-0108. Epub 2016 Mar 23. PMID: 27009165.
4) Fang CY, Huang SY, Wu CC, Hsu HY, Chou SP, et al. The synergistic effect of chemical carcinogens enhances Epstein-Barr virus reactivation and tumor progression of nasopharyngeal carcinoma cells. PLoS One. 2012; 7(9): e44810. doi: 10.1371/journal.pone.0044810. Epub 2012 Sep 14. PMID: 23024765; PMCID: PMC3443098.
5) Li Z, Chen X, Li L, Liu S, Yang L, et al. EBV encoded miR-BHRF1-1 potentiates viral lytic replication by downregulating host p53 in nasopharyngeal carcinoma. Int J Biochem Cell Biol. 2012 Feb; 44(2): 275-9. doi: 10.1016/j.biocel.2011.11.007. Epub 2011 Nov 17. PMID: 22108199.
6) Huang SY, Fang CY, Tsai CH, Chang Y, Takada K, et al. N-methyl-N'-nitro-N-nitrosoguanidine induces and cooperates with 12-O-tetradecanoylphorbol-1,3-acetate/sodium butyrate to enhance Epstein-Barr virus reactivation and genome instability in nasopharyngeal carcinoma cells. Chem Biol Interact. 2010 Dec 5; 188(3): 623-34. doi: 10.1016/j.cbi.2010.09.020. Epub 2010 Sep 24. PMID: 20869957.
7) Lin JC, Liao SK, Lee EH, Hung MS, Sayion Y, et al. Molecular events associated with epithelial to mesenchymal transition of nasopharyngeal carcinoma cells in the absence of Epstein-Barr virus genome. J Biomed Sci. 2009 Nov 24; 16(1): 105. doi: 10.1186/1423-0127-16-105. PMID: 19930697; PMCID: PMC2799403.
8) Fang CY, Lee CH, Wu CC, Chang YT, Yu SL, et al. Recurrent chemical reactivations of EBV promotes genome instability and enhances tumor progression of nasopharyngeal carcinoma cells. Int J Cancer. 2009 May 1; 124(9): 2016-25. doi: 10.1002/ijc.24179. PMID: 19132751.
9) Lee CH, Fang CY, Sheu JJ, Chang Y, Takada K, et al. Amplicons on chromosome 3 contain oncogenes induced by recurrent exposure to 12-O-tetradecanoylphorbol-13-acetate and sodium n-butyrate and Epstein-Barr virus reactivation in a nasopharyngeal carcinoma cell line. Cancer Genet Cytogenet. 2008 Aug; 185(1): 1-10. doi: 10.1016/j.cancergencyto.2008.03.014. PMID: 18656687.
10) Cabras G, Decaussin G, Zeng Y, Djennaoui D, Melouli H, et al. Epstein-Barr virus encoded BALF1 gene is transcribed in Burkitt's lymphoma cell lines and in nasopharyngeal carcinoma's biopsies. J Clin Virol. 2005 Sep; 34(1): 26-34. doi: 10.1016/j.jcv.2004.12.016. PMID: 16087121.
11) Sun N, Chen X, Wu F. [The transfection and morphological changes of human embryonic nasopharyngeal epithelia infected by Epstein-Barr virus cooperated with promoter in vitro and in vivo]. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 1998 Mar; 12(1): 70-3. Chinese. PMID: 12515178.
12) Kapadia GJ, Azuine MA, Takayasu J, Konoshima T, Takasaki M, et al. Inhibition of Epstein-Barr virus early antigen activation promoted by 12-O-tetradecanoylphorbol-13-acetate by the non-steroidal anti-inflammatory drugs. Cancer Lett. 2000 Dec 20; 161(2): 221-9. doi: 10.1016/s0304-3835(00)00616-9. PMID: 11090973.
13) Liu Z, Liu Y, Zeng Y. Synergistic effect of Epstein-Barr virus and tumor promoters on induction of lymphoma and carcinoma in nude mice. J Cancer Res Clin Oncol. 1998; 124(10): 541-8. doi: 10.1007/s004320050212. PMID: 9829857.
14) Chen HF, Sauter M, Haiss P, Müller-Lantzsch N. Immunological characterization of the Epstein-Barr virus phosphoprotein PP58 and deoxyribonuclease expressed in the baculovirus expression system. Int J Cancer. 1991 Jul 30; 48(6): 879-88. doi: 10.1002/ijc.2910480615. PMID: 1650330.
15) Lerman MI, Sakai A, Yao KT, Colburn NH. DNA sequences in human nasopharyngeal carcinoma cells that specify susceptibility to tumor promoter-induced neoplastic transformation. Carcinogenesis. 1987 Jan; 8(1): 121-7. doi: 10.1093/carcin/8.1.121. PMID: 3026676.

Corresponding Author:
Pu Wang
Email: vk2ayg@163.com
(China)