·Basic Research·

Six anti-Epstein-Barr virus antibodies in healthy adults in a high risk area of nasopharyngeal carcinoma

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[**Abstract**] **Background and Objective**: Nasopharyngeal carcinoma (NPC), with a remarkable geographic distribution, is consistently associated with Epstein-Barr virus (EBV) infection, and almost all NPC patients have sustained high levels of serum antibodies against EBV. This study was to compare the levels of six anti-EBV antibodies in healthy natives of Zhongshan (a high-incidence area of NPC) with those in provisional migrants from foreign provinces (low-incidence areas of NPC), and to illustrate the relationship between EBV infection and the geographic distribution of NPC. **Methods**: The serum levels of EBNA1-IgA, EBNA1-IgG, VCA-p18-IgA, VCA-p18-IgG, Zta-IgA and Zta-IgG in 303 healthy Zhongshan natives and 92 provisional migrants were tested using ELISA, and presented by values of adjusted relative absorbance (Ar*A*). The serum levels and positive rates of the six antibodies were compared between the two groups. **Results**: The mean Ar*A* values of both Zta-IgA and VCA-p18-IgA were significantly higher in Zhongshan natives than in provisional migrants (0.84±0.03 vs. 0.42±0.04, $P<0.05$; 0.96±0.05 vs. 0.40±0.05, $P<0.05$). In addition, the positive rates of Zta-IgA and VCA-p18-IgA in subjects aged of 30–49, or of 50 and above were significantly higher in Zhongshan natives than in provisional migrants (29.27% vs. 3.03% and 48.28% vs. 6.67% for Zta-IgA, $P<0.05$; 28.46% vs. 9.09% and 43.10% vs. 13.33% for VCA-p18-igA, $P<0.05$). **Conclusion**: Zhongshan natives are likely to have an elevation of serum IgA antibodies against EBV lytic antigens (Zta and VCA-p18), which represents reactivation of EBV latency infection and implies that Zhongshan natives may have higher risk to develop NPC than provisional migrants.

Key words: Epstein-Barr virus, serum antibody, nasopharyngeal neoplasm, healthy adults

Nasopharyngeal carcinoma (NPC) is a malignancy with striking geographic distribution, and its development is associated with Epstein-Barr virus (EBV) infection. Is EBV infection associated with geographic distribution of NPC? In previous study, we had compared the antibody profile composed of EBNA1-IgA, EBNA1-IgG, VCA-p18-IgA, VCA-p18-IgG, Zta-IgA, and Zta-IgG between healthy natives of Zhongshan (a high-incidence area of NPC) with those in immigrants from foreign provinces (low-incidence areas of NPC), and found that EBV infection in Zhongshan natives is often activated, while that in immigrants is

usually in a latent way with high expression of EBNA1.[1] In this study, we compared the positive rate and content of the six antibodies between healthy Zhongshan natives and immigrants to explore the association between EBV infection and geographic distribution of NPC.

Material and Methods

Samples. A total of 395 healthy subjects aged of 20-60 years were recruited from physical examination population in Health and Fitness Center at Zhongshan City Peoples Hospital. None of them had EBV-related disease and other infectious diseases that would alter the levels of antibodies against EBV. Of the 395 subjects, 303 were Zhongshan natives, 92 were first generation immigrants from foreign provinces who live in zhongshan for no more than 20 years. In addition, 160 native Cantonese patients with pathologically confirmed NPC were enrolled. Peripheral blood samples were collected without anticoagulation from the subjects (2 mL from each subject). All serum samples were isolated and stored at -20℃ for use. The samples from NPC patients were used to determine the cutoff values of antibodies against EBV.

Antibody detection by ELSA. ELSA kit for EBNA1-IgA, EBNA1-IgG, VCA-p18-IgA, VCA-p18-IgG, Zta-IgA, and Zta-IgG detection were provided by Sinoclone Hongkong Ltd. According to the instruction, the serum samples were diluted by PBS containing 2% bovine calf serum, incubated, washed by buffer, added with peroxidase-labeled antiserum, incubated and washed again, then stained with tetramethyl benzidine (TMB) at a ratio of 1:1. The absorbance value at a wavelength of 450 nm (A_{450}) was detected.

Relative absorbance and cut-off. Serum antibody was scaled by relative absorbance (rA) defined as the A_{450} of a sample divided by the average A_{450} of two positive controls. The rA values were equally partitioned into 20 intervals. The percentages of NPC patients with rA values exceeded every interval were dotted to draw sensitivity curve; the percentages of NPC patients with rA values within or below every interval were dotted to draw specificity curve. The intersection of two curves was defined as cut-off. The samples with rA greater than or equal cut-off were defined as positive samples; those with rA less than cut-off were defined as negative samples.

Adjusted relative absorbance. To minimize the errors of rA and cut-off from intra-set variability, rA was adjusted by following formula: adjusted rA (ArA)= rA/cut-off of a given batch of samples. The samples with ArA ≥ 1 were defined as positive samples; those with ArA < 1 were defined as negative samples.

Statistical analysis. Statistical analyses were done using SPSS 13.0 software. Variance analysis and Chi-square test was performed to compare rA values and rates between two samples, respectively. A P value of < 0.05 was considered as significant.

Results

Comparison of ArA values in different age groups. The Zhangshan natives and immigrants were divided into four age groups with an interval of 10 years. No significant differences in the mean ArA values of EBNA1-IgA, EBNA1-IgG, Zta-IgG, and VCA-p18-IgG were found between Zhangshan natives and immigrants in all age groups; the mean ArA values of Zta-IgA and VCA-p18-IgA were significantly higher in zhongshan natives than in immigrants (F=53.56, P<0.05 for Zta-IgA; F=31.85, P<0.05 for VCA-p18-IgA) (Table 1).

Comparison of positive rates in different age groups. No significant differences in the positive rates of EBNA1-IgA, EBNA1-IgG, Zta-IgG, and VCA-p18-IgG were found between Zhangshan natives and immigrants. In 30-39 and ≥ 50 age groups, the positive rates of Zta-IgA and VCA-p18-IgA were significantly higher in Zhongshan natives than in immigrants (for Zta-IgA, 29.27% vs. 3.03%, 48.28% vs. 6.67%, P<0.05; for VCA-p18-IgA, 28.46% vs. 9.09%, 43.10% vs. 13.33%, P<0.05) (Table 2).

Discussion

Zhongshan city locates in west river basin. It

Table 1 Comparison of the serum levels of six anti-Epstein-Bar virus antibodies between healthy Zhongshan natives and provisional migrants from foreign provinces

Age	Resident	Cases	EBNA1-IgA	EBNA1-IgG	Zta-IgA	Zta-IgG	VCAp18-IgA	VCAp18-IgG
20–29	ZS	30	0.51±0.11	0.66±0.12	0.72±0.08	0.38±0.09	0.66±0.09	0.67±0.13
	FP	29	0.81±0.18	0.49±0.08	0.34±0.04	0.36±0.08	0.31±0.07	0.56±0.12
	F		2.09	1.43	15.87	0.38	9.6	0.38
	P		0.15	0.24	<0.01	0.54	<0.01	0.54
30–39	ZS	123	0.50±0.04	0.62±0.05	0.81±0.05	0.47±0.05	0.92±0.08	0.65±0.07
	FP	33	0.58±0.06	0.56±0.06	0.40±0.05	0.45±0.09	0.36±0.06	0.66±0.13
	F		0.83	0.32	20.01	0.06	14.19	0
	P		0.36	0.46	<0.01	0.81	0.00	0.95
40–49	ZS	92	0.62±0.05	0.59±0.06	0.8±0.05	0.53±0.06	0.90±0.09	0.71±0.09
	FP	15	0.84±0.29	0.66±0.14	0.50±0.17	0.54±0.17	0.45±0.20	0.56±0.15
	F		1.54	0.19	5.12	0.01	3.31	0.43
	P		0.48	0.66	0.02	0.94	0.07	0.51
⩾50	ZS	58	0.62±0.10	0.55±0.08	1.01±0.07	0.77±0.10	1.29±0.16	0.86±0.12
	FP	15	0.56±0.06	0.60±0.14	0.58±0.06	0.34±0.10	0.59±0.06	0.69±0.11
	F		0.10	0.07	8.29	4.71	4.97	0.49
	P		0.75	0.79	<0.01	0.03	<0.01	0.89
Total	ZS	303	0.56±0.03	0.60±0.03	0.84±0.03	0.54±0.04	0.96±0.05	0.71±0.05
	FP	92	0.69±0.08	0.56±0.05	0.42±0.04	0.45±0.05	0.40±0.05	0.62±0.07
	F		3.10	0.42	53.56	1.59	31.85	1.03
	P		0.08	0.52	<0.01	0.21	<0.01	0.31

ZS indicates Zhongshan natives while FP indicates migrants from foreign provinces. All values are presented as mean ± SD of relevant groups.

Table 2 Comparison of the positive rates of six anti-Epstein-Bar virus antibodies between healthy Zhongshan natives and provisional migrants from foreign provinces

Age	Resident	Cases	EBNA1-IgA [cases(%)]	EBNA1-IgG [cases(%)]	Zta-IgA [cases(%)]	Zta-IgG [cases(%)]	VCA-p18-IgA [cases(%)]	VCA-p18-IgG [cases(%)]
20–29	ZS	30	3(10.00)	7(23.33)	5(16.67)	2 (6.67)	6(20.00)	6(20.00)
	FP	29	5(17.24)	3(10.34)	1 (3.45)	3(10.34)	2(6.69)	4(13.79)
	P		0.42	0.18	0.09	0.61	0.14	0.52
30–39	ZS	123	11 (8.94)	25(20.32)	36(29.27)	17(13.82)	35(28.46)	24(19.51)
	FP	33	4(12.12)	5(15.15)	1 (3.03)	4(12.12)	3 (9.09)	6(18.18)
	P		0.58	0.50	<0.01	0.80	0.02	0.86
40–49	ZS	92	21(22.82)	19(20.65)	27(29.35)	17(18.48)	27(29.35)	21(22.83)
	FP	15	5(33.33)	4(26.67)	2(13.33)	2(13.33)	1 (6.67)	1 (6.67)
	P		0.38	0.60	0.19	0.63	0.06	0.15
50–59	ZS	58	5 (8.62)	9(15.52)	28(48.28)	15(25.86)	25(43.10)	19(32.76)
	FP	15	1 (6.67)	4(26.67)	1 (6.67)	2(13.33)	2(13.33)	5(33.33)
	P		0.81	0.31	<0.01	0.31	0.03	0.97
Total	ZS	303	40(13.20)	60(19.80)	96(31.68)	51(16.83)	93(30.69)	70(23.10)
	FP	92	15(16.30)	10(10.87)	5(5.43)	11(11.96)	8 (8.70)	16(17.39)
	P		0.19	0.68	<0.01	0.37	<0.01	0.13

Footnotes as in Table 1.

is one of the highest incidence region in Guangdong province as well as one of NPC investigation spots. To study the association between EBV infection and the geographic distribution of NPC, we selected Zhongshan residents as study subjects. The profiles of

antibodies against EBV are various among different subjects with different EBV infection statuses. Therefore, the profile of antibodies against EBV reflects EBV infection status. Sustainable presence of antibodies against EBV in peripheral blood indicates frequent reactivation of latent EBV, which leads to genetic variants and consequently contributes to carcinogenesis of NPC. The antibodies against EBV have been detected to predict the risk of developing NPC among EBV carriers.[2-6] Based on previous reports,[7,8] we detected six antibodies against EBV by ELISA in serum samples from Zhongshan natives and immigrants from foreign provinces, and compared the differences. The serum levels (ArA values) of Zta-IgA and VCA-p18-IgA were higher in Zhongshan natives than in immigrants except for some age groups. The positive rates of Zta-IgA and VCA-p18-IgA were all higher in Zhongshan natives than in immigrants in 30-39 and ≥ 50 age groups.

Zta protein, an immediate early transactivator, controls the switch of EBV from latent stage to replication stage and triggers the chain reactions of EBV replication.[10] The presence of Zta-IgA indicates that EBV has been activated and entered lytic infection stage. VCA is generated late in the lytic cycle of EBV.[11] VCA-p18 is a component of VCA. Both VCA-p18-IgA and VCA-IgA reflect lytic EBV infection. EBNA1-IgA is persistently expressed in latent phase of EBV infection. When infected with EBV, Zta-IgA, VCA-p18-IgA and EBNA1-IgA reflect the EBV status in body. Cheng et al.[2] have found that the serum level of Zta-IgG was higher in individuals with a family history of NPC than in healthy population, which suggested enhanced potential of EBV reactivation and high NPC risk of individuals with a family history of NPC. IgA and IgG antibodies are two classes of immunoglobulin that come from immune responses in body, and have different metabolism cycles and immune functions. Previous studies have showed that IgA antibodies were more predictive for NPC risk than IgG antibodies.[9] Our results showed that Zta-IgA and VCA-p18-IgA were higher in Zhongshan natives than in immigrants, suggested that Zhongshan natives might harbored more lytic infected EBV at reactivation status. No differences in IgG antibodies were found between the two groups, suggested the differences in detection sensitivity between IgG and IgA antibodies. Whether this is caused by various metabolism cycles or other reasons needs to be further investigated.

In 30-39 and ≥ 50 age groups, the positive rates of Zta-IgA and VCA-p18-IgA were significantly higher in Zhongshan natives than in immigrants. Our results are similar to those of previous investigation in high incidence areas of NPC.[12] In our previous study on the profile of antibodies against EBV, the positive rate of Zta-IgA alone or together with VCA-p18-IgA was higher in Zhongshan natives than in immigrants, suggested that Zhongshan natives harbored more EBV at reactivation status and have high risk of NPC,[1] which was verified by this study.

As we known, sustainable high levels of the antibodies against EBV indicate frequent reactivation of latent EBV and high risk of NPC. Only single serum detection was performed in this study, while tracking detection and dynamic analysis of serum levels of these antibodies will benefit the investigation on EBV infection statuses in these two populations.

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