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Study on the Relation Between Nasopharyngeal Carcinoma and EBV Antibody

In 1965, Old discovered high titer of EBV antibody in the serum of nasopharyngeal carcinoma patients. Since then, more EBV-related antigens such as VCA (viral capsid antigen), EA (early antigen), MA (membrance antigen), EBNA (EBV-induced nuclear antigen), and soluble antigen have been discovered[1]. Of them, EBV VCA antibody and EBV DNase antibody are related to the immunological reaction of nasopharyngeal carcinoma with high sensitivity and specificity[2]. Taiwan Area, ROC, is high in nasopharyngeal carcinoma. The relatively conservative estimate of the DOH cancer registration report in 1984 gave an annual incidence of 7.2 per 100,000 for male (the 5th cancer in male), and 3.3/100,000 for female (the 7th cancer in female). The study intends to investigate the relation between EBV and nasopharyngeal carcinoma by case-control matching method and analysis of serum antibody.

Cases are selected from the nasopharyngeal carcinoma patients of the ENT special clinic of the National Taiwan University Hospital who meet the following two criteria: (1) pathologically confirmed nasopharyngeal carcinoma patients; and (2) living in the greater Taipei Area for the convenience of home-visiting. To improve the comparison between case and control groups, the community comparison approach is used: controls should live in the same neighborhood of the matching cases, of same sex, and within five years of age difference. Suitable matching controls are identified through household registration in the same neighborhood of cases as indicated by their addresses. Six controls meeting the criteria and living closer to the case are selected as candidates for each case. In home-visiting, the candidate who is approximate in age as the case is interviewed first. If refused, other candidates will be interviewed following the order of the age differences.

Between October 1985 and May 1987, 347 pairs had been interviewed. Their age and sex distribution is shown in Table 1. The average age of the patient group is 44.92 ± 11.75 years; that of the control group is 45.12 ± 12.02 years. No statistically significant difference is found. There are 238 male and 109 female pairs.

Table 1. Age and Sex Distribution of 347 Pairs of Nasopharyngeal Carcinoma Patients and Healthy Controls

Variables	Groups	Patients		Controls	
		No.	%	No.	%
Age (years)	<30	22	6.3	22	6.3
	30 - 39	71	20.5	72	20.7
	40 - 49	96	27.7	95	27.4
	50 - 59	97	28.0	97	28.0
	60 +	61	17.6	61	17.6
Sex	male	238	68.6	238	68.6
	female	109	31.4	109	31.4

5-10 cc blood samples were taken from both cases and controls. After centrifugation, the samples were tested for antibodies by following methods[3]: (1) testing of EBV VCA-IgA antibody-using P_3HR_1 as the target cell to extract VCA antigen under low temperature. Diluted serum is added the target cell (37°C, 30 minutes), washed with buffer solution (mercapto-ethanol) for 15 minutes, dried, and acted with secondary antibody for observation under fluorescent microscope. The dilution power that is capable of staining 1% target cell is the antibody titer. (2) testing for EBV DNase antibody-the EBV DNase antigen is prepared from P_3HR_1 cells treated with IUDR. The testing of the DNase activity is done by adding ^{14}C-Ecoli as the base, harvested for one hour at 37°C, interrupt the reaction with TCA, centrifuged at 3,000 rpm for 15 minutes to test the radio-activity of the supernatant to estimate the enzyme activity. In the reaction, by adding serum, the existence and intensity of antibody can be tested. One unit of DNase activity is defined as the amount of enzyme that converts 1 μg of double-stranded DNA to acid soluble material in 10 minutes at 37°C. The level of antibody to EBV DNase activity is expressed as the units, of DNase activity neutralized by 1ml of serum.

The results of the EBV antibody of 238 male and 109 female cases are shown in Table 2.

Table 2. EBV VCA Antibody and DNase Antibody of 347 Pairs of Nasopharyngeal Carcinoma Cases and Healthy Controls

Sex	Variables	Control	Case			Ratio	95% confidence interval
Male	EBV VCA		<10	≥ 10			
	antibody(titer)	<10	86	147		1.00	
		≥10	1	4		147.00	(20.57-1050.54)
	EBV DNase		< 1	1	≥ 2		
	antibody(unit)	< 1	48	56	108	1.00	
		1	3	5	12	13.26	(5.16-34.06)
		≥ 2	2	0	4	126.58	(27.75-577.40)
Female	EBV VCA		<10	≥ 10			
	antibody(titer)	<10	46	63		1.00	
		≥10	0	0		127.00	(7.86-2052.75)
	EBV DNase		< 1	1	≥ 2		
	abtibody (unit)	< 1	28	21	48	1.00	
		1	2	4	1	15.96	(3.18-80.06)
		≥ 2	0	1	4	73.58	(9.00-601.56)

Taking EBV VCA-IgA antibody at 1:10 as the cutting point, if the antibody titer is equal to or greater than 10, the relative risk for the male group is 147 times, and for the female group, 127 times. The sensitivity of diagnosis for nasopharyngeal carcinoma is 63.45% for the male group and 57.80% for the female group; the specificity is 97.90% for the male and 100% for the female. When EBV DNase antibody is smaller than 1 unit, when the antibody titer is equal to 1 unit, the relative risk of nasopharyngeal carcinoma for male is 13.26 times, and for female, 15.96 times; if the EBV DNase antibody is equal to or greater than 2 units, the relative risk is 126.58 times for male and 73.58 times for female. When using 1 unit as the cutting point, the sensitivity is 77.70% for male and 72.50% for female; the specificity is 89.00% for male and 88.99% for female.

In the present study, either EBV VCA-IgA antibody or EBV DNase shows significant difference between the case and the control groups (McNemar's test, P<0.001); and the higher the antibody, the greater the relative risk (Mantel-Henszel linear trend, P<0.001). Though present study is not yet sufficient to suggest that EBV is the pathogen of nasolpharyngeal carcinoma, EBV antibody, for its significant relation with nasopharyngeal carcinoma, can be used for the screening of the high risk group. In particular, for the high sensitivity of EBV DNase, it can be used for primary testing, the positive ones can be further screened with EBV VCA-IgA of high sepcificity to save efforts.

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Editorial Note: Nasopharyngeal carcinoma is rarely seen in the white population. However, Chinese, either in-country or overseas, always show a high incidence. Nasopharyngeal carcinoma and hepatoma thus are called "Chinese cancers". This significant ethnical difference makes some researchers to investigate into the genetic factors with findings such as: nasopharyngeal carcinoma tends to concentrate in families; and HLA-A2, BW46, and B17 tend to appear more often, though no genes have been found yet to be related to nasopharyngeal carcinoma. Some researchers suspect that the particular Chinese ways of life, the burning of smoke-producing fuel, and the intake of some salty fishes by some Hong Kong inhabitants for instance, may have some implications. Nitrosamine, a substance considered carcinogenic, has been isolated from salty fishes. But not all salty fish eaters are prone to develop nasopharyngeal carcinoma, and there are patients among people who do not take salty fishes at all.

That EBV VCA-IgG antibody is higher in nasopharyngeal carcinoma patients than in patients of other cancers or healthy persons was discovered by Old in 1966, and subsequently by Asian, African, and European researchers as well.Lanoir and De-The proposed the relation between EBV and nasopharyngeal carcinoma as follows[4]: (1) EBV infects the epithelium cells directly to induce changes. Laboratory studies, however, show that nasopharyngeal epithelium cells cannot be induced changes through the direct infection of EBV. (2) Normal nasopharyngeal cells may not be infected by EBV, EBV, however, may infect malignant nasopharyngeal cells to accelerate the development of cancer. In other words, EBV is the promoting factor rather than the pathogenic factor of nasopharyngeal carcinoma. (3) Normal nasopharyngeal cells may not be infected by EBV, but lymphocytes carrying EBV can merge with the nasopharyngeal epithelium cells to produce hybrid cells or to transfer infection thus to induce changes on the epithelium cells. EBV is transmitted through saliva, hence, almost everyone has been infected. The age of first infection, however, differs from place to place. Uganda representing the high Burkitts Lymphoma incidence areas in Africa, seems to have an early age of EBV first infection; Singapore, representing high nasopharyngeal carcinoma incidence areas, the age for the first EBV infection stays between that of the black and the white people; and France, representing the infectious monocytosis high incidence areas, seems to be late in the first infection. These three diseases are related to EBV, though their expressions differ greatly among different ethnic groups. This may be so because different ethnic groups immunologically react differently to EBV, or there may be three different EBV sub-groups inducing different diseases. More studies are needed.

Although a definite pathogenic relation between EBV and nasopharyngeal carcinoma can not yet be established, the sepcificity and sensitivity of EBV VCA-IgA antibody and EBV DNase antibody to nasopharyngeal carcinoma can be used for early detection. Since 1981, Guangxi Province of Mainland China began to screen with EBV VCA-IgA in high risk areas each year[6]. The VCA-IgA antibody positive rate (21:10) is 0.2%, of which 16.2% are nasopharyngeal carcinoma patients. New cases have been identified after 2-3 years of follow-up, 90% of them are nasopharyngeal carcinoma of I and II phases. In Taiwan Area, between 1983 and 1986, in the nasopharyngeal carcinoma high incidence areas such as Tajen, Chechen, Kuanhsi, Yuanshan, and Hsinpu, a screen with EBV DNase of persons between 20 and 70 years of age was conducted[7]. The EBV DNase antibody positive rate (equal to or greater than 2 units) is 11.92% (1176/9869). Of the 829 antibody positive cases, 11 early nasopharyngeal carcinoma patients have been identified through ENT examinations, giving an EBV DNase antibody diagnosis rate of 1.32%. Thus, this can be used for the the early diagnosis of nasopharyngeal carcinoma.