EPSTEIN-BARR VIRUS SEROLOGICAL MARKERS FOR NASOPHARYNGEAL CARCINOMA IN THAILAND

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Abstract. The present study reports on the prevalence of specific IgA and IgG antibodies to EBV viral capsid antigen in nasopharyngeal carcinoma (NPC) patients with different histological types of carcinoma and their age-matched controls by the indirect immunofluorescence test, using the B-95-8 lymphoblastoid cell line as source of viral capsid antigen.

EBV specific IgG was found in almost all the study cases, and antibody titers were significantly higher in the NPC patients than in non-cancer controls. GMT of anti-EBV IgG in NPC patients, patients with other malignant diseases, and those with non-malignant diseases were 371.5, 97.7 and 35.5, respectively. Anti-EBV specific IgA was more specific to NPC than was IgG, and was present in 86.5% (83 of 96) cases of NPC patients, 6.6% (2 of 30) of patients with other cancers, and 3.1% (3 of 97) cases of non-malignant diseases. A weak correlation between level of anti-EBV IgA in NPC patients was observed (r = 0.3). EBV IgA was found in all histological types of NPC, ie, WHO types 1, 2 and 3, but WHO type 1 was rare among NPC patients in Thailand. Use of anti-EBV IgA for monitoring cancer therapy is to be further investigated.

INTRODUCTION

Epstein-Barr virus (EBV) is associated with various clinical manifestations of non-malignant and malignant origin eg, infections mononucleosis (IM), hematologic diseases, acute neurologic disorders, Burkitt's lymphoma (BL), and nasopharyngeal carcinoma (NPC) (Jones, 1989; Meeting report, 1985). EBV infection is common in Thailand. Our previous report showed that approximately 90% of children aged 5-15 years had EBV IgG antibody as screened by indirect immunofluorescence at dilution 1:10 (Puthavathana *et al,* 1980). IM is uncommon and BL is a rare disease in this country. In contrast, NPC has occupied the second to the sixth rank among the ten leading sites of adult cancer in recent years (Department of Statistics, Ministry of Public Health, Thailand, personal communication).

An association between EBV and NPC has been established by indirect evidence; including the demonstration of EBV DNA in nasopharyngeal biopsies from NPC patients (Raab-Traub *et al,* 1987; Zhang *et al,* 1989), and the presence of high EBV antibody titers of various specificities eg, antibody to virus specific DNase, DNA polymerase, early antigen (EA), and viral capsid antigen (VCA) in the patients'sera (Chen *et al,* 1989; Liu *et al,* 1989; Lynn *et al,* 1985; Neel, 1984). Among these serological markers, EBV-specific IgA to VCA and EA has been widely used as a sensitive diagnostic aid for NPC (especially in cases with occult primary tumor), and also as a tool to monitor clinical management of the patients. EBV IgA antibody to VCA is more specific to NPC than is EBV IgG. (Neel, 1986; Gurtsevitch *et al,* 1986; Lynn *et al,* 1985). However, most reports have shown that the diagnostic and prognostic value of serological markers is applicable only to nonkeratinizing carcinoma and undifferentiated carcinoma (WHO types 2 and 3, respectively), but not to the well or moderately differentiated squamous cell carcinoma type (WHO type 1) of NPC (Neel, 1986; Gurtsevitch *et al,* 1986; Lynn *et al,* 1985).

The present study therefore aimed to investigate

the prevalence and levels of EBV IgG and IgA to VCA among NPC patients of different histological types in Thailand by indirect immunofluorescence (IFAT). Positive findings would provide additional diagnostic and prognostic tools for Thai patients.

MATERIALS AND METHODS

Subjects

Subjects in this study comprised NPC patients, a group of age-matched controls with non-malignant diseases, and patients with malignant diseases other than NPC. Demographic details of each group were as follows:

1. NPC patients included 96 cases, 75 males and 21 females, aged between 16 and 80 years with a mean of 46.5 years. Based on WHO criteria, these patients were classified into 3 different histological types : WHO type 1 (well or moderately differentiated squamous cell carcinoma, or keratinizing carcinoma), WHO type 2 (nonkeratinizing or poorly differentiated squamous cell carcinoma), and WHO type 3 (undifferentiated carcinoma) (Shanmugaratnam and Sobin, 1978).

2. Age-matched controls were 97 cases of 60 males and 37 females aged 15-80 years (mean 45.8) with non-malignant diseases including pneumonia, rubella, meningitis, encephalitis.

3. A total of 30 cases including 23 males and 7 females aged 12-70 years (mean 47.7) with malignant diseases other than NPC: 5 cases of lymphoma, 6 cases of acute lymphocytic leukemia, 3 cases of Hodgkin's lymphoma, 3 cases of cancer of larynx, 2 cases of cancer of nasal cavity.

Specimens

Five ml clotted blood was collected from each study case prior to any clinical management. Sera were kept frozen at -20°C until they were tested.

Cell culture

The EBV lymphoblastoid cell line designated B-95-8, obtained from Dr Petcharin Srivatanakul, National Cancer Research Institute of Thailand was used as source of the viral capsid antigen. The cells were cultured in RPMI 1640 (Grand Island Biological Company, GIBCO, NY, USA) supplemented with 8% fetal calf serum (GIBCO), 10 μg/ml HEPES, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1 μg/ml fungizone.

Immunofluorescence test

The floating B-95-8 cells in culture media were centrifuged, pelleted, smeared on glass slides, then air-dried and fixed in precooled acetone at -20°C for 10 minutes. The fixed slides were kept at -70°C until used. Each lot of fixed slides was standardized with our EBV reference sera to ensure that at least 5-10% of the cell population expressed the viral antigen.

The test sera were serially diluted 2 fold in PBS by starting from dilution 1:10. The fixed slides were then covered with various dilutions of the test sera and incubated in a moist chamber at 37°C for 30 minutes for EBV IgG detection, and 60 minutes for EBV IgA. After washing in PBS and rinsing in distilled water, the slides were reincubated with either FITC-conjugated goat anti-human IgG or IgA heavy chain specific antibodies (Hyland Diagnostics, IL, USA) for 30 minutes. Washing was repeated, then the stained slides were read under a fluorescence microscope (Optiphot, Nikhon, Japan). The antibody titer was read when the cell deposit showed 1-2 + fluorescence intensity after reacting with the highest dilution of the test serum (Gardner and McQuillin, 1980).

RESULTS

NPC was most prevalent in old persons. Sixty-six of 96 cases (68.8%) were older than 40 years. We did not find any case younger than 16 years of age.

The data in Table 1 show that most of the study patients in all 3 groups had anti-EBV IgG antibody, while anti EBV IgA was more specific to NPC patients. In addition, no statistical difference in level of anti-EBV IgG antibody titer was found between NPC patients and patients with other malignancies (unpaired *t*-test, $p > 0.5$). However, a statistical difference in level of anti-EBV IgG was found between NPC patients and those with non-malignant diseases (unpaired *t*-test, $p < 0.001$). Anti-EBV IgG geometric mean titers in NPC patients, patients with other malignant diseases and those with non-malignant diseases were 371.5, 97.7, and 35.5, respectively.

Table 1

EBV specific IgG and IgA in NPC and their controls

Ig class	Group	No. tested	No. +ve (%)	No. cases at reciprocal titer of											GM titer
				<10	10	20	40	80	160	320	640	1280	2560	5120	
IgG															
	NPC	96	96 (100)	0	2	1	2	5	17	26	25	12	5	1	371.5[a, b]
	Non-cancer	97	95 (97.4)	2	14	25	27	17	9	3	0	0	0	0	35.5
	Other cancers	30	30 (100)	0	0	4	7	5	7	5	1	1	0	0	97.7
IgA															
	NPC	96	83 (86.5)	13	11	19	16	11	15	9	2	0	0	0	38.7[c, d]
	Non-cancer	97	3 (3.1)	94	3	0	0	0	0	0	0	0	0	0	5.1
	Other cancers	30	2 (6.7)	28	2	0	0	0	0	0	0	0	0	0	5.2

a = Significant difference in EBV IgG antibody titer between NPC and non-cancers (unpaired *t*-test; $p < 0.001$).
b = No significant difference in EBV IgG antibody titer between NPC and other cancers (unpaired *t*-test; $p > 0.5$).
c = Significant difference in proportion of EBV IgA positive cases between NPC and non-cancer cases (X^2-test with Yate's correction, $p < 0.005$).
d = Significant difference in number of EBV IgA positive cases between NPC and other cancer cases (X^2-test with Yate's correction, $p < 0.005$).

Table 2

EBV specific IgA and IgG in NPC of different histological types.

Ig class	Histological type	No. tested	No. +ve (%)	No. cases at reciprocal titer of											GM titer
				<10	10	20	40	80	160	320	640	1280	2560	5120	
IgA															
	Squamous cell (WHO type 1)	1	1 (100)	0	1	0	0	0	0	0	0	0	0	0	10
	Nonkeratinizing (WHO type 2)	49	45 (91.8)	4	4	13	7	6	11	4	0	0	0	0	44.2
	Undifferentiated (WHO type 3)	46	37 (80.4)	9	6	6	9	5	4	5	2	0	0	0	34.9
	Total	96	83 (86.5)	13	11	19	16	11	15	9	2	0	0	0	38.7
IgG															
	Squamous cell (WHO type 1)	1	1 (100)	0	0	0	0	1	0	0	0	0	0	0	80
	Nonkeratinizing (WHO type 2)	49	49 (100)	0	1	1	0	3	8	14	16	4	2	0	353.3
	Undifferentiated (WHO type 3)	46	46 (100)	0	1	0	2	1	9	12	9	8	3	1	407.4
	Total	96	96 (100)	0	2	1	2	5	17	26	25	12	5	1	371.5

Regarding detection of EBV IgA, this antibody was present in 83 of 96 NPC cases (86.5%), but in only 2 of 30 cases (6.6%) with other cancers and 3 of 97 cases (3.1%) of non-malignant diseases. The proportion of anti-EBV IgA positive cases was statistically higher in the NPC group than in the group with other malignancies (x^2-test with Yate's correction, $p < 0.005$), (Table 1).

WHO type 2 and WHO type 3 were the most commonly found histological types, while WHO type 1 was seen only in 1 of 96 NPC cases. The percentages of cases with WHO types 1, 2 and 3 were 1.0, 51.0, and 47.9%, respectively (Fig 1).

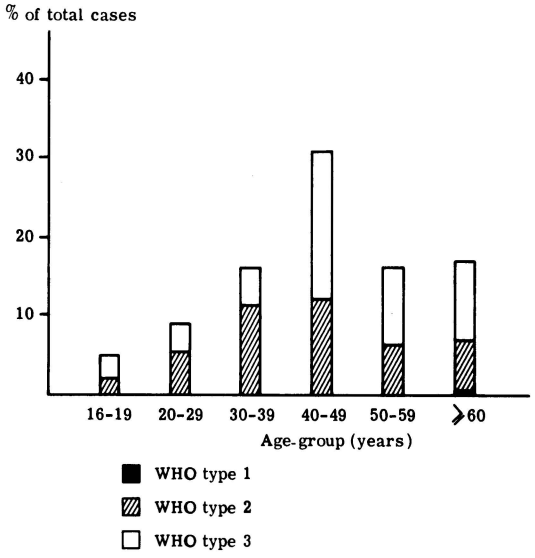

Fig 1—Age and histological types of NPC patients.

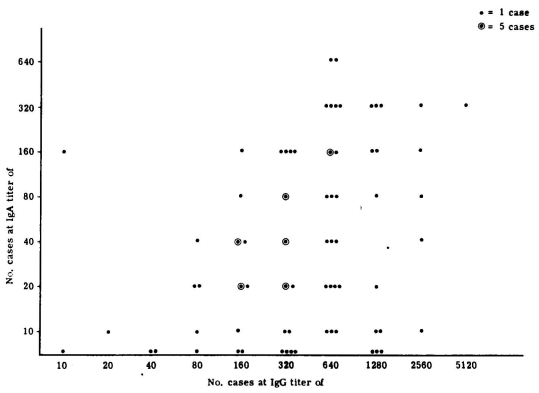

Fig 2—Correlation between serum EBV IgA and IgG titers in NPC patients.

However, EBV IgA was seen in all histological types, and high levels of EBV IgG was seen in both WHO type 2 and 3 (Table 2).

The present study also examined for the correlation between level of EBV IgA and IgG in individuals with NPC. However, only poor correlation was observed as shown in Fig 2 (linear regression : correlation coefficient r = 0.3).

DISCUSSION

An association between EBV and NPC was first proposed by Old *et al* in 1966, and supported by subsequent findings of many investigators (Hader *et al,* 1986; Wara *et al,* 1975; Zeng, 1985). The present study has confirmed our previous finding that EBV infection is common in our population as it has shown that nearly all the study cases had EBV IgG antibody. However, NPC patients had significantly higher antibody titers (Table 1).

The anti-EBV IgG geometric mean titer was also high in patients with other types of cancer, which may be due to reactivation of the latent EBV infection in the cancer patients. This study also supports the finding of many other investigators that anti-EBV IgA is more specific than IgG, since it was particularly common in NPC patients but rarely found in other study groups (Table 1). Anti-EBV IgA was found in one case of Hodgkin's lymphoma, one case of cancer of nasal cavity, one case with sudden hearing loss and two pneumonic patients. Histories concerning risk factors for NPC development in these patients were not obtained. Anti-EBV IgA was found in 86.5% of our NPC patients, a figure slightly higher than those of 78-79% reported by Srivatanakul *et al* (1981, 1988) in Thai NPC patients.

As the present study found only a poor correlation between the level of anti-EBV IgA and IgG titers in NPC patients, it is not possible to predict that NPC patients with high anti-EBV IgG antibody titer will possess high EBV IgA antibody levels.

NPC is most commonly found among the age-group of 40-50 years, and is more prevalent in males than females. Data from the Division of Epidemiology, Ministry of Public Health of Thailand, show a prevalence of NPC in Thai males of 0.72 per 100,000 or approximately 4% of all types of the ten leading sites of cancer. The male : female sex ratio of NPC usually ranges about 3:1 ; in our NPC study population the male:female ratio was 3.4 : 1. However, we have found no statistical differences in frequency and level of specific antibody titer by sex. This was demonstrated statistically when male NPC were analysed against female NPC; and also when males with non-malignant diseases were compared to females in the same study group (unpublished observations).

Regarding histopathological types of NPC, EBV IgA was observed in all of them, ie WHO

type 1, 2 and 3. However, it was surprising that NPC of WHO type 1 was rare in Thailand as was earlier shown by Lunchanavanich *et al* (1988). Our study showed a 1% prevalence of WHO type 1, while studies from North America have shown a prevalence of 24.5% (Neel, 1986).

NPC development is partly based on genetic predisposition. The disease is prevalent in southern China and Southeast Asia (Zeng 1985). In 1965, it was found that the risk ratio of developing NPC in Chinese : mixed Thai-Chinese : Thai was 3.4 : 2.2 : 1.0 (Garnjana-Goonchorn and Chantarakul, 1965). For decades, intermarriage between people of Thai and Chinese descent has been common in urban areas of Thailand. The NPC patients in our study comprised Chinese, Thais and many mixed blood patients of unknown genetic background. Thus, it is difficult to say that ethnic Thai persons are also more prone to disease development. However, it has been reported that NPC is prevalent in native Thai farmers in the northeast part of the country (Lunchanavanich *et al,* 1988).

Our study concludes that NPC of any histological type in Thailand is associated with EBV infection. Usage of anti-EBV IgA as a diagnostic aid for NPC seems promising, but its efficacy as a prognostic tool requires a long term follow-up study. Its application to various histological type of NPC is to be further investigated.

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