Asian Pacific Journal of Allergy and Immunology (1993) 11 : 39-43

Prevalence of IgA Specific Antibodies to Epstein-Barr Virus Capsid and Early Antigens in Nasopharyngeal Carcinoma

Pilaipan Puthavathana[1], Uraiwan Kositanont[1], Cheerasook Chongkolwatana[2], Choakchai Metheetrairut[2], Nivat Chantarakul[3], Somchit Nuntarakchaikul[1], and Chantapong Wasi[1]

SUMMARY **Ninety-one patients with nasopharyngeal carcinoma (NPC), and 164 age-matched healthy controls were tested for presence of IgA antibodies to Epstein-Barr virus capsid antigen (VCA) and early antigen (EA) in their sera by indirect ELISA using "EBViral DETECT" commercial test kit. IgA anti-VCA was found in 76 (83.5%) of NPC patients and 16 (9.8%) of the controls. Meanwhile, IgA anti-EA was found in 72 (79.1%) of NPC patients and 21 (12.8%) of the controls. In a parallel study by indirect immunofluorescence test (IIF), IgA anti-VCA was found in 77 of 91 (84.6%) NPC patients and 22 of 142 (15.5%) controls. The prevalence rates of anti-VCA as screened by ELISA and IIF were very similar suggesting that either one of the two tests can be used alternatively depending on the purpose and facilities in each individual laboratory. IgA antibodies to VCA and EA were more prevalence in NPC patients than those in the controls, the finding which again supported the association between EBV and NPC as was suggested in many other reports.**

Nasopharyngeal carcinoma is reported to be one of the ten leading sites of male cancer in Thailand.[1,2] The disease is prevalent among Chinese[3] and native Thai people in the northeast.[4] NPC was originally found to be associated with EBV infection by Old *et al,* in 1966[5] and subsequently by other investigators.[6-17] Several serological markers specific to EBV such as IgG and IgA antibodies to VCA and EA have been found preferentially in NPC patients[6-8,10-12,14-17]. Our previous data showed that approximately 86.5% of NPC patients possessed IgA antibody to VCA, while it was present in 3.1% of their age-matched controls with non-malignant diseases as tested by indirect immunofluorescence (IIF).[12] However, prevalence of EA IgA was not determined in that work because it was difficult to prepare and standardize the test antigen. The purpose of this study was to determine the prevalence of IgA antibodies to VCA and EA by ELISA; the determination of IgA anti-VCA by IIF was also performed in parallel.

MATERIALS AND METHODS

Subjects

Subjects participating in this study were 91 NPC patients whose clinical diagnosis was confirmed histologically. These patients attended the Department of Otolaryngology, Siriraj Hospital during the period between August 1989 and May 1991. Their ages ranged from 20 to 65 years. Five to 8 ml of their blood were collected prior to any clinical treatment.

The subjects also included 164 age-matched, healthy controls who visited the hospital during the same period of time for screening of antibody to human immunodeficiency virus (HIV), and all were negative for HIV antibody.

ELISA test kits

ELISA test kits designated "EBViral DETECT" for detection of IgA anti-VCA or IgA anti-EA were kindly provided by Bangkok RIA Centre Laboratory, Thailand and Singapore Biotech, Singapore. The tests are based on indirect ELISA in principle. Briefly, the test sera at dilution 1:100 were allowed to react with the antigens coating on 96 well, flat bottom microtiter plates for 1 hour at 37°C. After washing with

From the [1]Department of Microbiology, [2]Department of Otolaryngology, [3]Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Correspondence : Pilaipan Puthavathana

PBS-tween 20, the plates were then added with sheep anti-human IgA conjugated with horseradish peroxidase and incubated for 1 hour at 37° C. The test system used tetramethylbenzidine (TMB) as chromogenic substrate, and the enzymatic reaction was allowed to take place at room temperature for 30 minutes. The control set included 2 wells for blank controls, 3 wells for negative controls and 2 wells for positive controls. The test plates were read for optical density (OD) by microplates reader (Titertek Multiskan, Flow Laboratories Australasia Pty Ltd, NSW, Australia) at wave-length of 450 nm. Cut-off value according to the kit instruction was equal to the mean of OD of negative controls plus 0.1. Absorbance values within ± 5% of the cut-off values were retested. The relative cut-off values for positive IgA to EA and to VCA for all experiments were 0.311 and 0.312, respectively.

Immunofluorescence test

B 95-8, an EBV lymphoblastoid cell line was used as source of VCA. The test sera at dilution 1:10 were screened for the presence of IgA anti-VCA. FITC-conjugated goat anti-human IgA heavy chain specific (Hyland Diagnostics, IL, USA) or that derived from rabbit origin (Dakopatts AB, Alvsjo, Sweden) were used as the second antibody. Details of slide preparation and staining method were described elsewhere. [12]

RESULTS

It has been shown that 76 of 91 (83.5%) NPC patients and 16 of 164 (9.8%) age-matched healthy controls possessed IgA anti-VCA; and 72 (79.1%) of the patients and 21 (12.8%) of the controls did have IgA anti-EA as determined by ELISA (Table 1). These antibodies were more prevalent in NPC patients than the controls (X^2 with Yates correction = 134.89, $p < 0.001$ for anti-VCA; and X^2 with Yates correction = 108.24, $p < 0.001$ for anti-EA). The relative OD value for each test serum is illustrated in Fig. 1 for anti-VCA assay, and in Fig. 2 for anti-EA assay. Anti-VCA and anti-EA usually coexisted in most of the sera tested. However, there were some cases who possessed either anti-VCA or anti-EA alone (Table 2).

By immunofluorescence study, IgA anti-VCA was observed in 77 of 91 (84.6%) NPC patients and 22 of 142 (15.5%) controls (Table 3). Again, IIF showed higher prevalence of EBV IgA in the NPC patients (X^2 with Yates correction = 105.63, $p < 0.001$). There was no difference in prevalence rates of anti-VCA as determined either by ELISA or IIF, nor in any of the populations studied (X^2 with Yates correction = 0, $p = 1$ in group of NPC patients; and X^2 = 1.81, $p > 0.05$ in the control group) (Table 4).

Table 1. IgA anti-VCA and anti-EA in NPC patients and controls as determined by ELISA

Group	No. of study cases	No. of cases with IgA antibodies to VCA (%)	EA (%)
NPC patients	91	76* (83.5)	72** (79.1)
Controls	164	16 (9.8)	21 (12.8)

There are statistically significant differences between NPC patients and controls for the presence of IgA anti- VCA and IgA anti- EA

*X^2 = 134.89, $p<0.001$ **X^2 = 108.24, $p<0.001$

Table 2. Presence of IgA antibodies to VCA and to EA in NPC patients and controls

IgA anti-EBV	NPC patients (%)	Controls (%)
VCA+, EA+	70 (76.9)	11 (6.7)
VCA+, EA−	6 (6.6)	5 (3.0)
VCA−, EA+	2 (2.2)	10 (6.1)
VCA−, EA−	13 (14.3)	138 (84.1)
Total	91 (100)	164 (100)

Table 3. IgA anti- VCA in NPC patients and controls as determined by IIF

Group	No. of study cases	No. of cases with antibody (%)
NPC patients	91	77 (84.6)*
Controls	142	22 (15.5)

There are statistical significant difference between NPC patients and controls for presence of IgA to EBV-VCA,
*X^2 = 105.63, $p<0.001$

Table 4. Determination for IgA anti-VCA by ELISA and IIF

	NPC patients*		Controls**	
	ELISA	IIF	ELISA	IIF
EBV IgA positive	76	77	16	22
EBV IgA negative	15	14	148	120
Total cases	91	91	164	142

There are no statistical significant differences in numbers of cases who possess EBV IgA antibody as compared ELISA to IIF
*X^2 = 0, p = 1 **X^2 = 1.81, $p>0.05$

DISCUSSION

IgA antibodies to VCA and EA have been introduced as the screening tools for diagnosing NPC and as prognostic markers for monitoring the treatment of this cancer for more than two decades.[6] It was also demonstrated that people who had EBV IgA antibody may develop NPC within a mean time of 8-30 months.[14] On the other hand, a decrease in EBV IgA antibody titers reflected a good prognosis in long term follow-up, while an increase in these antibodies reflected a poor prognosis.[8,10,14,17]

This study demonstrated the presence of IgA anti-VCA in 83.5 and 84.6% of NPC patients as tested by ELISA and IIF, respectively (Table 1 and 3). The data indicated that IgA anti-VCA was more specific to NPC patients than the controls; these figures are similar to those of 79.2[11] and 86.5%[12] reported in Thailand previously. The determination of IgA anti-VCA either by ELISA or IIF showed no statistical difference in terms of antibody prevalence, eventhough the prevalence rate obtained by IIF in the control group was slightly higher than that screened by ELISA ie, 15.5% *vs* 9.8% (Tables-1, 3).

In parallel, IgA anti-EA has been introduced as an adjunct to IgA anti-VCA as the serological markers for NPC. Persons who possess IgA antibodies to both VCA and EA were more closely associated with NPC than those who possess IgA anti-VCA alone.[14,15] However, IgA anti-EA was detected in lower frequency.[7,14,15] No case with IgA anti-EA alone without the presence of IgA anti-VCA had been reported as determined by immunoenzyme staining.[15] In another words, IgA anti-EA is more specific, but less sensitive than IgA anti-VCA for the detection of NPC. In contrast, our study has shown the presence of IgA anti-EA antibody alone in 2.2% of NPC patients and in 6.1% of the healthy controls (Table 2). Reliability and interpretation of this laboratory result needs further investigation.

In the meantime, we can conclude that determination for IgA anti-VCA alone is adequate to confirm NPC diagnosis. Prevalence rates of IgA anti-VCA as determined by IIF and ELISA system of "EBViral DETECT" were comparable, which implied that the two tests can be used alternatively according to the convenience in each laboratory. ELISA is practical to investigate

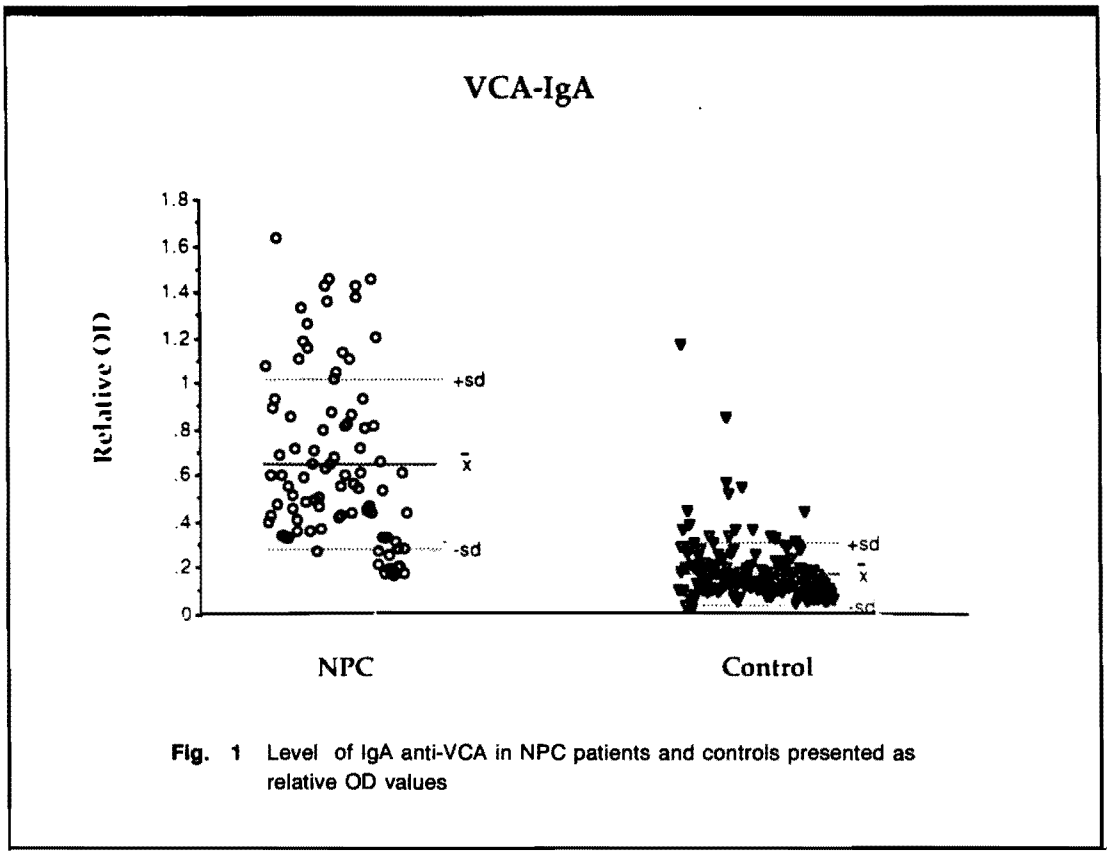

Fig. 1 Level of IgA anti-VCA in NPC patients and controls presented as relative OD values

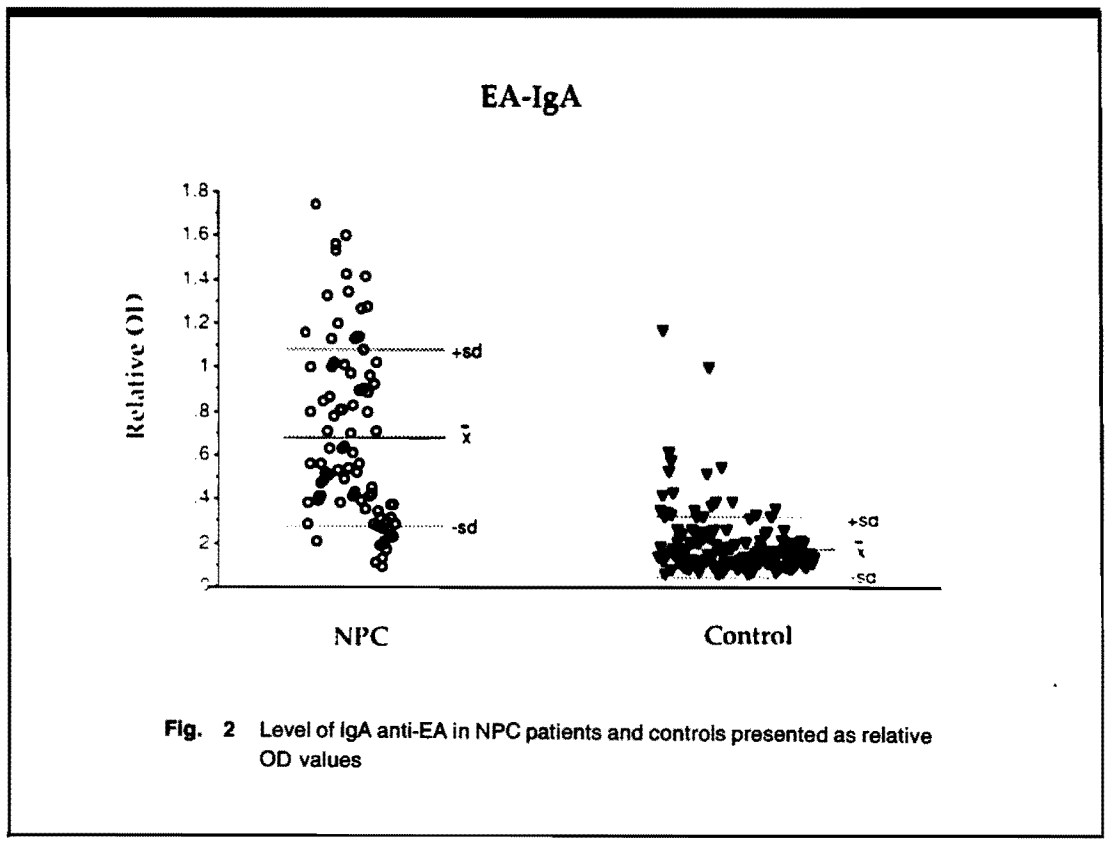

Fig. 2 Level of IgA anti-EA in NPC patients and controls presented as relative OD values

large numbers of serum specimens such as in field studies and the system is more objective than IIF. However, IIF is more economical in the laboratory where few specimens are to be investigated at a time.

ACKNOWLEDGEMENTS

The authors would like to express their since thanks to Mrs Nilawan Pitchayodhin, Bangkok RIA, Co Ltd, and Singapore Biotech for providing the ELISA *EBViral DETECT* test kits.

REFERENCES

1. National Cancer Institute, Ministry of Public Health, Thailand. Cancer Statistics 1978, 1979.
2. Titapant V, Vatanasapt V, Tangvoraponkchai V, *et al.* Cancer incidence in Khon Kaen Province 1985-1987. Thai Cancer J 1989; 15 : 55-63.
3. Garnjana-Goonchorn S, Chantarakul N. Nasopharyngeal cancer at Siriraj Hospital, Dhonburi, Thailand. UICC Monograph Series 1965; 1 : 33-7.
4. Lunchanavanich P, Sangsa-ad S, Kraitrakul S, Puapairoj A. Nasopharyngeal cancer. Otolaryngology-Head and Neck Surgery 1988; 3 : 167-76 (in Thai with English abstract).
5. Old LJ, Boyse EA, Oettgen HF, *et al.* Precipitating antibody in human serum to an antigen present in cultured Burkitt's lymphoma cells. Proc Natl Acad Sci USA 1966; 56 : 1699-704.
6. de-The G, Yi Z. Population screening for EBV markers : Toward improvement of nasopharyngeal carcinoma control. In : Epstein MA, Achong MA (eds). The Epstein-Barr virus : Recent advances. New York. John Wiley & Sons Inc. 1986; 237-49.
7. Gurtsevitch V, Ruiz R, Stepina V. Epstein-Barr viral serology in nasopharyngeal carcinoma patients in the USSR and Cuba, and its value for differential diagnosis of the disease. Int J Cancer 1986; 37 : 375-81.
8. Henle G, Henle W. Epstein-Barr virus-specific IgA serum antibodies as an outstanding feature of nasopharyngeal carcinoma. Int J Cancer 1976; 17 : 1-7.
9. Feinmesser R, Miyazaki I, Cheung R, Freeman JL, Noyek AM, Dosch HM. Diagnosis of nasopharyngeal carcinoma by DNA amplification of tissue obtained by fine-needle aspiration. N Engl J Med 1992; 326 : 17-21.
10. Lynn TC, Tu SM, Kawamura A. Long-term follow-up of IgG and IgA antibodies against viral capsid antigens of Epstein-Barr virus in nasopharyngeal carcinoma. J Laryngol Otol 1985; 99 : 567-72.
11. Srivatanakul P, Tiwawech D, Boonyaratavej C, Somboncharoen S. Early detection of nasopharyngeal carcinoma by using serum IgA antibody to Epstein-Barr virus as a screening test. Thai Cancer J 1988; 14 : 18-24.
12. Puthavathana P, Sutthent R, Vitavasiri A, Wasi C, Chantarakul N, Thongcharoen P. Epstein-Barr virus serological markers for nasopharyngeal carcinoma in Thailand. Southeast Asian J Trop Med Public Health 1991; 22 : 326-31.
13. Walter MA, Menarguez-Palenca J, Peiper SC. Epstein-Barr virus detection in neck metastasis by polymerase chain reaction. Laryngoscope 1992; 102 : 481-5.
14. Yi Z.Seroepidemiological studies on nasopharyngeal carcinoma in China. Adv Cancer Res 1985; 44 : 121-38.
15. Yi Z, Zhang LG, Li HY, *et al.* Serological mass survey for early detection of nasopharyngeal carcinoma in Wuzhou City, China. Int J Cancer 1982; 29 : 139-41.
16. Zong YS, Sham JS, Ng MH, *et al.* Immunoglobulin A against viral capsid antigen of Epstein-Barr virus and indirect mirror examination of the nasopharyngeal carcinoma. Cancer 1992; 69 : 3-7.
17. Neel HB. III. A prospective evaluation of patients with nasopharyngeal carcinoma. J Otolaryngol 1986; 15 : 137-44.