

SHORT COMMUNICATION

Epstein-Barr Virus Serology in the Diagnosis of Nasopharyngeal Carcinoma

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SUMMARY The antibody levels to viral capsid antigen (VCA) and early antigen (EA) of Epstein-Barr virus (EBV) in 164 nasopharyngeal carcinoma (NPC) patients from Sarawak, East Malaysia were significantly higher than those in 147 sex, age and ethnically matched healthy controls. As diagnostic markers of NPC, IgG/VCA at reciprocal titers ≥ 160 was the most sensitive (89%, with 98% specificity), while IgA/EA at ≥ 5 was the most specific (100%) but the least sensitive (75%). The sensitivity and specificity of IgA/VCA at reciprocal titers ≥ 10 were 84% and 97%. IgA/VCA has an advantage over IgG/VCA despite the slightly lower sensitivity due to its consistently more distinct fluorescence reaction. The sensitivity and specificity can be marginally improved by a combination of two tests.

The Epstein-Barr virus (EBV) is closely associated with nasopharyngeal carcinoma (NPC). The role of EBV in NPC remains unclear but antibodies to EBV proteins especially the viral capsid antigen (VCA) and early antigens (EA), are useful diagnostic markers of NPC.^{1,2} IgA/VCA is the most sensitive marker while IgA/EA is more specific but is only detected in individuals positive for IgA/VCA.^{3,4}

The profiles of EBV antibodies in NPC patients from East Malaysia are not well documented. East Malaysia consists of Sabah and Sarawak, each with a different ethnic distribution that differs from that in West Malaysia. Since NPC is known to be associated with specific ethnic groups, we investigated the EBV antibody profiles in NPC patients from Sarawak.

MATERIALS AND METHODS

Serum samples were obtained from 164 histologically confirmed NPC patients prior to radio-

therapy in the Radiotherapy Unit (RTU) of Kuching General Hospital, Sarawak. Control sera (147) from sex, age and ethnically matched individuals were collected from non-NPC patients of the Kuching General Hospital. Informed consent was obtained from all the individuals from whom blood was collected. Sera were inactivated at 56°C for 30 minutes and stored at -20°C until tested.

IgA/VCA, IgG/VCA, IgA/EA and IgG/EA were tested by using the indirect immunofluorescence assay (IFA) modified after Sam *et al.*² The P3HR-1 and Raji cells were induced with 20 ng/ml of 12-*o*-tetradecanoyl phorbol 13-acetate (TPA, Sigma) and 3 mM sodium butyrate (NBA, Merck) for the expression of VCA and EA respectively. The

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Table 1 Sensitivity and specificity of EBV antibodies

Assay	Reciprocal titer	Sensitivity ^a (%)	Specificity ^b (%)
IgA/VCA	≥ 5	92.7	85.1
	≥ 10	83.6	97.3
	≥ 40	60.4	100.0
IgG/VCA	≥ 5	100.0	0.0
	≥ 10	100.0	31.3
	≥ 40	96.9	86.4
	≥ 160	89.0	98.0
IgA/EA	≥ 5	75.0	100.0
	≥ 10	64.0	100.0
	≥ 40	40.2	100.0
IgG/EA	≥ 5	94.5	67.3
	≥ 10	89.0	82.3
	≥ 40	76.8	99.3
	≥ 160	51.8	100.0

^aSensitivity is the proportion of NPC positive to a marker

^bSpecificity is the proportion of non-NPC controls negative to a marker

secondary antibody used was goat-antihuman antibody from Kirkegaard and Perry Laboratory, USA.

RESULTS

Table 1 shows the sensitivity and specificity of IgA/VCA, IgG/VCA, IgA/EA and IgG/EA. Sensitivity is the proportion of NPC patients positive to a marker and specificity is the proportion of non-NPC controls negative to a marker. The results showed that IgA/VCA ≥ 10, IgG/VCA ≥ 160, IgA/EA ≥ 5 and IgG/EA ≥ 40 are elevated titers that are useful cut-off titers for the sensitive and specific diagnosis of NPC. Of the 164 NPC patients, 150 (91%) had titers higher than the cutoff titers in one or more of the markers.

The sensitivity and specificity of IgA/VCA ≥ 10, IgG/VCA ≥ 160, IgA/EA ≥ 5 and IgG/EA ≥ 40 are marginally improved when two markers are combined (Table 2).

DISCUSSION

The 164 NPC patients studied belonged largely to 4 major ethnic groups in Sarawak of East Malaysia: Bidayuh, Chinese, Iban and Malay. The treatment center from which the blood samples were

Table 2 Sensitivity and specificity of combination of two anti-EBV antibodies (N = 164)

Combination of markers	Sensitivity (%)	Specificity (%)
IgA/VCA with IgA/EA	83.6	97.3
IgA/VCA with IgG/EA	87.2	96.6
IgA/VCA with IgG/VCA	90.9	95.2
IgG/VCA with IgA/EA	89.6	98.0
IgG/VCA with IgG/EA	90.2	96.3
IgA/EA with IgG/EA	82.9	99.3

randomly collected caters to all ethnic groups, but 42% of the NPC patients in this study were Ibans, although Ibans make up less than 30% of the population in Sarawak.⁵ On the other hand, only 23% of the patients in this study were Chinese, although Chinese make up 27% of the population in Sarawak. The prevalence of NPC is well known to be highest in Southern Chinese, including the Chinese in Malaysia.⁶ Within the limitations of the small sample size, the Ibans in Sarawak have a higher incidence of NPC even compared to the Chinese. Genetic predisposition and cultural factors both contribute to the development of a cancer and the apparent high incidence of NPC among the Ibans requires further documentation and studies.

Our results showed that EBV serological immunofluorescence tests using IgA/VCA ≥ 10 , IgG/VCA ≥ 160 , IgA/EA ≥ 5 or IgG/EA ≥ 40 are sensitive and specific for the diagnosis of NPC. In practice, IgA/VCA ≥ 10 is often preferred despite being slightly less sensitive than IgG/VCA ≥ 160 , because of its consistently more distinct fluorescence reaction in the IFA.

The discrimination power of the serological markers is slightly improved through the combination of two markers but the marginal improvement in sensitivity or specificity has to be balanced by the higher cost. In particular, IgA/EA does not increase the sensitivity of IgA/VCA since the patients positive for IgA/EA were also the same patients positive for IgA/VCA.

In conclusion, the ethnic distribution of the 164 unselected NPC patients showed that the Ibans have the highest incidence of NPC in Sarawak. Anti-EBV antibodies can be used as diagnostic markers of NPC in Sarawak. The sensitivity and specificity being 84% and 97% for IgA/VCA ≥ 10 ; 89% and 98% for IgG/VCA ≥ 160 ; 75% and 100% for IgA/EA ≥ 5 ; 77% and 99% for IgG/EA ≥ 40 . The combined use of two markers marginally improved the discriminating power but that has to take into consideration the higher costs.

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