RESEARCH COMMUNICATION

Significance of Plasma IgA and IgG Antibodies to Epstein-Barr Virus Early and Viral Capsid Antigens in Thai Nasopharyngeal Carcinoma

Danai Tiwawech[1, 3], Petcharin Srivatanakul[1], Anant Karaluk[2], Takafumi Ishida[3]

Abstract

Epstein-Barr virus (EBV) is an important causal factor of human nasopharyngeal carcinoma (NPC). High levels of serum IgA and IgG antibodies to EBV early and viral capsid antigens (IgA/EA, IgA/VCA, IgG/EA and IgG/VCA) have been reported in NPC patients. Since specific serum IgA/EA, IgA/VCA and IgG/EA are claimed to be useful serological markers for NPC. In order to evaluate whether plasma IgA/EA, IgA/VCA, IgG/EA and IgG/VCA antibody levels are useful markers for diagnosis and prognosis of Thai NPC, we examined the prevalence of these antibodies in 79 NPC patients, and 127 age-matched controls (47 healthy subjects (HS), 32 cases of other disease (OD) and 48 cases of other cancer (OC)) by using an indirect immunofluorescence assay. The prevalence of plasma IgA/EA, IgA/VCA, and IgG/EA in NPC patients (55.7, 68.4 and 68.4%) was significantly higher than in the HS (0.0, 0.0 and 20.5%,), OD (0.0, 0.0 and 3.1%) and OC (0.0, 0.0 and 20.8%) groups ($p<0.05$). The prevalence of plasma IgG/VCA in NPC patients (93.7%) was significantly different from those for the OD and OC groups (71.9 and 43.8%) but not for the HS group (89.4%). In NPC patients, the geometric mean titers (GMT) of plasma IgA/EA, IgA/VCA and IgG/EA were increased with an advanced clinical stage of disease but not IgG/VCA. In contrast, GMT of IgG/VCA was increased with aggressive type of disease (histological type) but not IgA/EA, IgA/VCA, and IgG/VCA. The results of our study suggest that plasma IgA/EA, IgA/VCA and IgG/EA antibodies may be useful markers for diagnosis and assessing prognosis of Thai NPC.

Key Words: nasopharyngeal carcinoma - Epstein-Barr virus - plasma - antibody - Thailand

Asian Pacific J Cancer Prev, **4**, 113-118

Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy that is specifically endemic in Southern China (age-standardized incidence rate: ASR = 30-50/10^5 per year) and Southeast Asia (ASR = 9-12/10^5 per year) but it is uncommon in the rest of the world (McDermott et al., 2001). NPC is one of the top ten cancers in Thailand (ASR = 3-10 /10^5 per year) and causes dramatical loss of human resources and economic status in Thailand annually (Parkin et al., 1997; Cancer Statistics, 1999). However, NPC patients respond well to radiotherapy with overall 5 years survival rates of 70-80% for stage I disease and 20-30% for stage IV disease, respectively (Lee et al., 1992; Sham et al., 1990). NPC is thus a curable disease and a mass screening of a high-risk population for identifying the early stage NPC patients followed by effective therapy is an important step to fight against this serious cancer.

Several epidemiological studies have demonstrated that NPC is a multi-factorial disease caused by individual or in combination of the Epstein-Barr virus (EBV) infection (Young et al., 1988; Raab-Traub et al., 1983; Pearson et al., 1993; Kottaridis et al., 1996 and Niemhom et al., 2000), environmental carcinogens (Zheng et al., 1994) and genetic susceptibility (Lu et al., 1990; Golovleva et al., 1997). However, the mechanism of NPC development induced by

[1]Research and [2]Pathology Division, National Cancer Institute, Bangkok 10400, Thailand. [3]Unit of Human Biology and Genetics, Department of Biological Sciences, School of Science, University of Tokyo, Tokyo, Japan.
Correspondence to Danai Tiwawech, Research Division, National Cancer Institute, Bangkok 10400, Thailand. Tel:[06] 2 2460061 ext. 1417, 1418 Fax:[06] 2 2465145 E-mail: tdanai@hotmail.com

these factors is still unclear. Regarding to EBV, a well accepted causative agent of NPC, it has been shown that EBV infection is accompanied by the synthesis of typical antibodies to EBV including anti-EBV-associated nuclear antigen (EBNA), anti-EBV early antigen (EA), and anti-EBV viral capsid antigen (VCA). IgA antibody to EBV-EA (IgA/EA) and EBV- VCA (IgA/VCA) for the undifferentiated carcinoma of epithelial tumors arising in the nasopharynx has been well documented (Kottaridis et al., 1977; Henle et al., 1976; Zong et al., 1992). IgA/EA and IgA/VCA are thus considered as useful markers for early detection of NPC.

A number of successful mass screening programs for early detection of NPC in China have been reported (Desgranges et al., 1978; Zeng et al., 1982, Zeng et al., 1983a; Pi et al., 1985). The results of those studies revealed that IgA/EA was a better screening tool than IgA/VCA for the diagnosis of NPC. Furthermore, high levels of IgG/EA were also detected in sera of NPC patients suggesting that IgG/EA may be used as a serological marker for NPC (Desgranges et al., 1979; De Schryver et al., 1972).

Several case-control studies have demonstrated that the levels of serum IgA and IgG antibodies to EBV-EA and EBV-VCA in NPC patients were higher than in healthy controls and other cancers (Henle et al., 1976; Desgranges et al., Ho et al., 1976). The levels of these antibodies in NPC patients increase with advancing stages of the disease and tend to decrease to normal values when successful responses to treatments are achieved. In addition, it was found that elevated IgA/VCA antibody levels could be observed for several months prior to the onset of NPC and in patients with primary or recurrent cancer (Ho et al., 1978; Zeng, et al., 1985; Rowe et al., 1987; Ho et al., 1978; Coates et al., 1978). Moreover, it was claimed that not only serum but also saliva IgA/EA and IgA/VCA could have clinical values for diagnosis and prognosis of NPC patients (de-Vathaire et al., 1988).

In Thailand, serum IgA/VCA levels were recognized as a useful serological marker for early detection and diagnosis of NPC (Srivatanakul et al., 1988; Srivatanakul et al., 1985; Puthavathana et al., 1991). Recently, we have preliminary demonstrated that serum IgA/EA and IgG/EA levels are also more useful markers for the diagnosis and prognosis of Thai NPC (Tiwawech et al., 1996). In this study, we aimed to establish plasma IgA/EA, IgA/VCA, IgG/ EA and IgG/VCA levels to be serological markers for diagnosis of NPC and to determine their relationships with clinical status of NPC in Thais.

Materials and Methods

Plasma

Plasma samples from a total of 79 untreated NPC patients (49 males and 30 females, ranging from 24-79 years with a mean age of 50 years) who were admitted at the National Cancer Institute of Thailand (NCIT) were examined. All patients were pathologically proven to have NPC based on the criteria of the World Health Organization (WHO). There were 12 cases of WHO type I (squamous cell carcinoma), 35 cases of WHO type II (non-keratinising carcinoma) and 32 cases of WHO type III (undifferentiated carcinoma). NPC staging was based on the TNM tumor classification by the 1997 AJCC system (Fleming et al., 1997). There were 2 cases of stage I, 9 cases of stage II, 19 cases of stage III and 49 cases of stage IV.

At the same time, plasmas from 127 age-matched ($\pm$ 5 years) controls which consisted of 47 healthy subjects (24 males and 23 females, ranging 31-69 years with a mean age of 48 years), 32 cases of other disease (14 males and 18 females, ranging 26-72 years with a mean age of 51 years) and 48 cases of other cancer (23 males and 25 females, ranging 33-63 years with a mean age of 53 years) who attended the NCIT for general check-ups and cancer investigations were used as controls. All samples were kept at -80^{o}C prior to assayed.

Preparation of EA and VCA-positive cells

Raji and P3HR-1 cells treated with 4 mM n-butyric acid (Sigma, St. Louis, Mo., USA) and 20 ng/ml 12-tetradecanoylphorbol-13-acetate (Sigma, St. Louis, Mo., USA) for 48 hours were prepared for EBV-EA and EBV-VCA antigen positive cells, respectively (Ishida et al., 1993). Both of treated cells were washed twice with phosphate-buffered saline, smeared on glass slides, fixed with acetone for 3 minutes at room temperature, air dried, and stored at -30 ^{0}C until use.

Titration of anti-EA and anti-VCA

The detailed procedures for the indirect immunofluorescence assay to detect IgA/EA, IgA/VCA, IgG/EA and IgG/VCA have been described elsewhere (Ishida et al., 1993). Briefly, for titration of EA and VCA specific IgA and IgG antibodies, acetone-fixed smears of treated Raji and P3HR-1 cells were used. Ten µl of serially diluted plasma were added on treated Raji and P3HR-1 cell-smears, then incubated at room temperature for 30 minutes. After incubation, slides were washed twice with PBS (soaked 5 minutes each time) and air dry. Then, 10 µl of fluorescein isothiocyanate conjugates (goat antibodies to either human IgA (alpha chain-specific) or human IgG (gamma chain-specific) purchased from Medical & Biological Laboratories Co., Ltd., Nagoya, JAPAN) was dropped on the cell-smears and they were incubated at room temperature for 30 minutes. The slides were then washed twice with PBS (soaked 5 minutes each time), air dried, and mounted in buffered glycerol. IgA/EA, IgA/VCA, IgG/EA and IgG/VCA-positive Raji and P3HR-1 cells were measured under a fluorescence microscope.

Statistical Analyses

The difference in the prevalence of anti-EBV antibodies between NPC patients and other groups was calculated by Chi-Square test or Fisher's exact test. The difference in the distribution of titers of anti-IgG/EA and -IgA/VCA between

NPC patients and other groups was tested by Mann-Whitney U test. P value less than 0.05 was considered as significantly different. Geometric mean titers were calculated to compare NPC status between the clinical stage and histological type.

Results

The results of the indirect immunofluorescence assay of plasma IgA/EA, IgA/VCA, IgG/EA and IgG/VCA of cases and controls are shown in Table 1. None of the 47 healthy subjects (HS), 32 other disease (OD) cases and 48 other cancer (OC) cases tested was IgA/EA positive (titer ≥1:10), whereas IgA/EA was positive in 44 (55.7 %) out of the 79 NPC cases. The geometric mean titers (GMT) of IgA/EA of HS, OD, OC and NPC groups were 1:5.0, 1:5.0, 1:5.0 and 1:20.0, respectively.

IgA/VCA was detected in 12.5 and 68.4% of OD and NPC groups (GMT=1:5.7 and 1:42.2) but not in HS and OC groups (GMT=1:5.0 and 1:5.0). Meanwhile, IgG/EA was detected in 17.0, 3.1, 20.8 and 68.4% of HS, OD, OC and NPC groups with GMT = 1:6.4, 1:5.1, 1:6.4 and 1:39.0, respectively. The prevalence of plasma IgA/VCA and IgG/EA in NPC group was distinctive from and significantly higher than those of other groups ($p<0.05$).

IgG/VCA was detected in 89.4, 71.9, 43.8 and 93.7% of HS, OD, OC and NPC groups with GMT=1:46.4, 1:23.3, 1:13.0 and 1:257.0, respectively. However, the prevalence of plasma IgG/VCA in NPC patients was significantly higher than those of OD and OC groups ($p<0.05$) but not HS group.

The relationship between the clinical stage and histological type of NPC and GMT of IgA/EA, IgA/VCA, IgG/EA and IgG/VCA is shown in Figure 1 and Figure 2. The GMT of IgA/EA, IgA/VCA and IgG/EA of NPC patients increased with the stage of the disease (GMT of IgA/EA=1:5.0 for stage I, 1:18.5 for stage II, 1:20.7 for stage III and 1:21.1 for stage IV; GMT of IgA/VCA=1:5.0 for stage I, 1:40.0 for stage II, 1:44.6 for stage III and 1:45.4 for stage IV and GMT of IgG/EA=1:14.1 for stage I, 1:37.0 for stage II, 1:38.6 for stage III and 1:41.2 for stage IV), whereas the GMT of IgG/VCA did not (GMT of IgG/VCA=1:160.0 for stage I, 1:296.3 for stage II, 1:192.0 for stage III and 1:285.7 for stage IV) (Figure 1). On the other hand, the GMT of IgG/VCA showed a tendency to increase with an aggressiveness of the disease or histological type (GMT of IgG/VCA=1:169.5 for WHO type I, 1:273.1 for WHO type II and 1:281.0 for WHO type III) but not IgA/EA, IgA/VCA, and IgG/EA (GMT of IgA/EA=1:20.0 for WHO type I, 1:16.7 for WHO type II and 1:24.3 for WHO type III; GMT of IgA/VCA=1:50.4 for WHO type I, 1:29.7 for WHO type II and 1:57.8 for WHO type III and GMT of IgG/EA=1:67.3 for WHO type I, 1:26.9 for WHO type II and 1:47.6 for WHO type III) (Figure 2).

Discussion

The results of our study clearly showed that high levels of IgA/EA, IgA/VCA, IgG/EA and IgG/VCA could be detected in the plasma of Thai NPC patients. The prevalence

Table 1. Prevalence and Geometric Mean Titers of Plasma Anti-EBV Antibodies in NPC Patients and Controls

Antibody/	Number with anti-EBV titers of											% [a]	Total	GMT [b]
Group	< 1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	(≥1:10)		
IgA/EA														
HS	47	0	0	0	0	0	0	0	0	0	0	0	47	1:5.0
OD	32	0	0	0	0	0	0	0	0	0	0	0	32	1:5.0
OC	48	0	0	0	0	0	0	0	0	0	0	0	48	1:5.0
NPC	35	2	8	13	11	4	5	1	0	0	0	55.7	79	1:20.0
IgA/VCA														
HS	47	0	0	0	0	0	0	0	0	0	0	0	47	1:5.0
OD	28	2	2	0	0	0	0	0	0	0	0	12.5	32	1:5.7
OC	48	0	0	0	0	0	0	0	0	0	0	0	48	1:5.0
NPC	25	5	4	7	11	9	10	5	2	1	0	68.4*	79	1:42.2
IgG/EA														
HS	39	2	3	3	0	0	0	0	0	0	0	17.0	47	1:6.4
OD	31	1	0	0	0	0	0	0	0	0	0	3.1	32	1:5.1
OC	38	5	3	2	0	0	0	0	0	0	0	20.8	48	1:6.4
NPC	25	2	6	12	10	12	5	4	1	2	0	68.4*	79	1:39.0
IgG/VCA														
HS	5	2	3	16	11	9	1	0	0	0	0	89.4	47	1:46.4
OD	9	9	3	2	2	4	0	2	1	0	0	71.9	32	1:23.3
OC	27	6	0	5	5	5	0	0	0	0	0	43.8	48	1:13.0
NPC	5	2	2	4	9	6	17	17	12	2	3	93.7@	79	1:257.0

[a]Percentage of anti-EBV antibody titers was compared between NPC group and other groups.
[b]All the cases less than 1:10 were taken as 1:5.
*Significant difference between NPC group and other groups ($P<0.05$).
@Significant difference between NPC group and OD and OC groups ($P<0.05$).

of plasma IgA/EA, IgA/VCA and IgG/EA in NPC patients were significantly higher than those of HS, OD and OC groups, whereas IgG/VCA was significantly higher than only those of OD and OC groups (Table 1). The high prevalence of plasma IgA/EA, IgA/VCA and IgG/EA (55.7, 68.4 and 68.4 %) from NPC patients in this study is comparable to that of 56.5, 83.4 and 89.5% reported by Desgranges and de The' (Desgranges et al., 1979). However, elevated IgA and IgG antibodies to EBV-EA and EBV-VCA in our study are slightly lower than those in the previous studies using sera. In addition, the titers of plasma IgA/EA, IgA/VCA and IgG/EA in NPC group are significantly higher than those of other groups and such titers were increased with advancing stage of the disease (Table 1 and Figure 1). These results reveal that plasma IgA/EA, IgA/VCA and IgG/EA may be a useful tool for diagnosis and prognosis in Thai NPC patients.

Regarding the clinical usefulness of the studied markers, plasma IgA/EA seems the best marker for diagnosis of patients with NPC because it shows the highest specificity when compared with other markers (cut off titer at ≥1:10). However, plasma IgA/VCA and IgG/EA may also be good markers for diagnosis of NPC since the specificity of these markers will increase into 100 % with cut off titers at ≥1:40 and ≥1:80 respectively. We suggest that the cut off titer at ≥1:40 for IgA/VCA and ≥1:80 for IgG/EA may be useful indicator for detection of NPC in suspected people.

The presence of IgG/EA in healthy individual is observed in the present study. The prevalence of IgG/EA in healthy individuals varies by their living conditions and ethnic origins (unpublished data); for example, pregnant or breast-feeding women sometimes show IgG/EA in their sera. Also, it is readily conceivable that reactivation of latently infected EBV in healthy individuals gives rise to an elevation in EA-antibody levels. The use of IgA/EA and IgA/VCA as sero-diagnostic measures is more appropriate than IgG/EA at this moment as the rare presence of IgG/EA gives false positive NPC cases on the diagnosis. Since the number of healthy controls is limited in this study, a large screening test should be carried out to obtain basic data such as cutting off values, on the Thai population.

It is notable that 35 (44.3%), 25 (31.6%), 25(31.6%) and 5 (6.3%) out of 79 NPC patients had no plasma IgA/EA, IgA/VCA, IgG/EA and IgG/VCA, even their tumors were clearly evident. It is important to remind that the use of single sero-diagnostic marker misses this group of NPC patients and detection and monitoring of treatment of NPC ends in vain. Therefore, investigations of other specific and sensitive techniques for identifying and monitoring of anti-EA/VCA sero-negative NPC patients are awaited. Since it was found that anti-ZEBRA (Matthew et al., 1994; Cheng et al., 2002), neutralizing antibodies against EBV DNase (Chen et al., 1987; Chen et al., 1989; Chien et al., 2001), and anti- EBV BamHI A rightward open-reading frame-1 (BARF1) protein (Tanner et al., 1997) were highly specific for NPC. Thus, we propose that these markers may be useful for this group of NPC. Interestingly, we observed that the combined use of plasma IgA/EA, IgA/VCA and IgG/EA markers for NPC diagnosis gave more accuracy than using each of markers alone (data not show). With this evidence, we recommend the use of a combination of these 3 markers for improving the out come of NPC examination. Diagnostic techniques other than serological means are molecular diagnostic measures such as cell free EBV-DNA in the patients' plasma or serum. Although the high sensitivity of molecular methods is proved by the PCR techniques, the low specificity derived from the high sensitivity subsides its practice (Kantakamalakul et al., 2000).

With respect to the relationship between clinical stage of NPC and plasma IgA/EA, IgA/VCA and IgG/EA, it has been reported that the GMT of these antibodies in patient was increased with the stages of the disease (Ida et al., 1973; Henle et al., 1977). The results in this study are in agreement with the former studies. In addition, we found no relationship

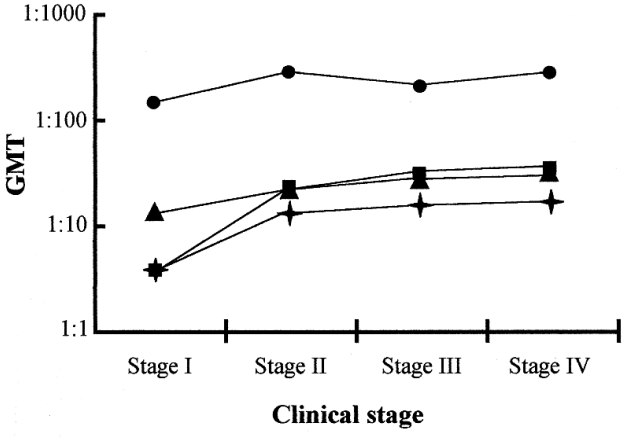

Figure 1. Geometric Mean Titer of Anti-EBV Antibodies in NPC Patients by Clinical Stage. IgA/EA (✦), IgA/VCA (■), IgG/EA (▲) and IgG/VCA (●).

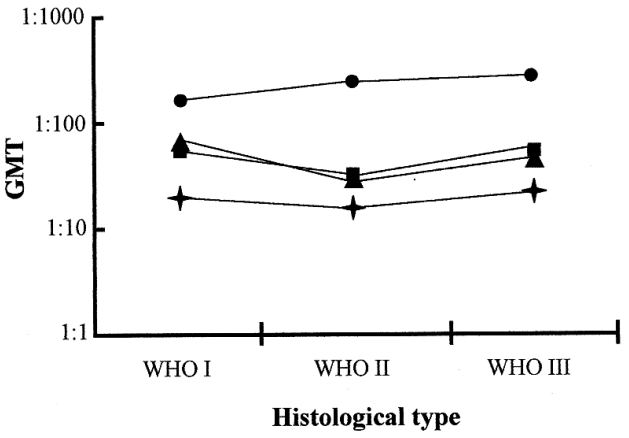

Figure 2. Geometric Mean Titer of Anti-EBV Antibodies in NPC Patients by Histological Type. IgA/EA (✦), IgA/VCA (■), IgG/EA (▲) and IgG/VCA (●).

between clinical stage of NPC and GMT of IgG/VCA that similar to the previous study (Ida et al., 1973). Concerning the relationship between histological type of NPC and plasma IgA/EA, IgA/VCA, IgG/EA and IgG/VCA, it has not been reported on this issue. We here noticed that only GMT of IgG/VCA increased with the aggressiveness of the disease. Thus the monitoring of these titers has substantial meaning in following up the EBV sero-positive NPC patients.

Early detection of NPC and its treatment with radiotherapy are the major components of a strategy, however, most NPC patients are diagnosed after metastases or at late stages of disease. Any method able to detect tumors at an early stage of development with higher sensitivity should be suitable for such a large scale screening. It should also be simple and inexpensive. Our present method fulfills these criteria and it would therefore be valuable for Thai public health screening program in the near future.

In conclusion, we found that an association between the levels of plasma IgA/EA, IgA/VCA and IgG/EA and NPC development existed. In addition, the levels of these anti-EBV antibodies were tending to increase with advancing stages of the disease. Therefore, we conclude that plasma IgA/EA, IgA/VCA and IgG/EA may be useful serological markers for early detection, diagnosis and prognosis of NPC in the Thai population.

Acknowledgments

We gratefully acknowledge MEXT, Japan and the National Research Council of Thailand for grant support. This study was partly supported by Core University System Exchange Programme under Japan Society for the Promotion of Science, coordinated by the University of Tokyo and Mahidol University.

References

Cancer Incidence in Five Continents. Vol. VI, edited by Parkin DM, Whelan SL, Ferlay J Raymond and Young J. IARC Scientific Publications No. 143: Lyon, 814-815, 1997.

Cancer Statistics, National Cancer Institute, Department of Medical Services, Ministry of Public Health, 1999.

Chen JY, Chen CJ, Liu MY, et al (1987). Antibodies to Epstein-Barr virus specific DNase in patients with nasopharyngeal carcinoma and control group. *J Med Virol*, **23**, 11-21.

Chen JY, Chen CJ, Liu MY, et al (1989). Antibody to Epstein-Barr virus specific DNase as a marker for field survey of nasopharyngeal carcinoma in Taiwan. *J Med Virol*, **27**, 269-73.

Cheng WM, Chan KH, Chen HL, et al (2002). Assessing the risk of nasopharyngeal carcinoma on the basis of EBV antibody spectrum. *Int J Cancer*, **97**, 489-92.

Chien YC, Chen JY, Liu MY, et al (2001). Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. *N Eng J Med*, **345**, 1877-82.

Coates HL, Pearson GR. Neel III HB, et al (1978). An immunologic basic for detection of occult primary malignancies of the head and neck. *Cancer*, **41**, 912-8.

de-Vathaire F, Sancho-Garnier H, de-The H, et al (1988). Prognostic value of EBV markers in the clinical management of nasopharyngeal carcinoma (NPC): a multicenter follow-up study. *Int J Cancer*, **42**, 176-81.

De Schryver A, Klein G, Henle G, et al (1972). EB-virus associated serology in malignant disease: antibody levels to viral capsid antigens (VCA), membrane antigens (MA), and early antigens (EA) in patients with various neoplastic conditions. *Int J Cancer*, **9**, 353-64.

Desgranges C, de The'G (1978). IgA and nasopharyngeal carcinoma. In: Oncogenesis and Herpesviruses III (de The'G, Ito Y, Rapp F, eds.), 883-891. Lyon: IARC.

Desgranges C, de The' G (1979). Epstein-Barr virus specific IgA serum antibodies in nasopharyngeal and other respiratory carcinomas. *Int J Cancer*, **24**, 555-9.

Fleming ID, Cooper JS, Henson DE, et al (1997), eds. AJCC Cancer Staging Manual. 5th edition. Philadelphia: Lippincott-Raven.

Golovleva I, Birgander R, Sjalander A, Lundgren E, Beckman L (1997). Interferon-alpha and p53 alleles involved in nasopharyngeal carcinoma. *Carcinogenesis*, **18**, 645-7.

Henle G and Henle W (1976). Epstein-Barr virus-specific IgA serum antibodies as an outstanding feature of nasopharyngeal carcinoma. *Int J Cancer*, **17**, 1-7.

Henle W, Ho JHC, Henle G, et al (1977). Nasopharyngeal carcinoma: Significance of changes in Epstein-Barr virus-related antibody patterns following therapy. *Int J Cancer*, **20**, 663-72.

Ho HC, Ng MH, Kwan HC, Chau JCW (1976). Epstein-Barr virus specific IgA and IgG serum antibodies in nasopharyngeal carcinoma. *Br J Cancer*, **34**, 655-60.

Ho HC, Kwan HC, Ng MH, de TG (1978). Serum IgA antibodies to Epstein-Barr virus capsid antigen preceding symptoms of nasopharyngeal carcinoma. *Lancet*, **1**, 436.

Ho HC, Kwan HC, Wu P, et al (1978). Epstein-Barr virus antibodies in suspected nasopharyngeal carcinoma. *Lancet*, **2**, 1094-5.

Ida S, Hinuma Y, Chu CT, et al (1973). Further studies on antibodies to early antigens induced by Epstein-Barr virus in nasopharyngeal carcinoma patients. *Gann*, **64**, 545-53.

Ishida T, Suzuki J, Yamamoto K (1993). Serological features of infection with an Epstein-Barr-Virus-Like antigen in Japanese Macaques (Macaca fuscata). *Folia Primatol*, **61**, 228-33.

Kantakamalakul W, Chongkolwatana C, Naksawat P, et al (2000). Specific IgA antibody to Epstein-Barr viral capsid antigen: a better marker for screening nasopharyngeal carcinoma than EBV-DNA detection by polymerase chain reaction. *Asian Pac J Allergy Immunol*, **18**, 221-6.

Kottaridis SD, Dafnou M, Besbeas S, et al (1977). Antibodies to Epstein-Barr virus in nasopharyngeal carcinoma and other neoplastic conditions. *J Natl Cancer Inst*, **59**, 89-91.

Kottaridis SD, Panotopoulou E, Diamantis I, et al (1996). Nasopharyngeal carcinoma: Epstein-Barr virus significance. *Anticancer Res*, **16**, 785-9.

Lee AWM, Poon YF, Poo W, et al (1992). Retrospective analysis of 5037 patients with nasopharyngeal carcinoma-treated during 1975-1985, overall survival and patterns of failure. *Int J Radit Oncol Biol Phys*, **23**, 261-70.

Lu SJ, Day NE, Degos L, et al (1990). Linkage of a nasopharyngeal carcinoma susceptibility locus to the HLA region. *Nature*, **346**, 470-1.

Matthew A, Cheng HM, Sam CK, et al (1994). A high incidence of serum IgG antibodies to the Epstein-Barr-virus replication

activator protein in nasopharyngeal carcinoma. *Cancer Immunol Immunother*, **38**, 68-70.
McDermott AL, Dutt SN, Watkinson JC (2001). The aetiology of nasopharyngeal carcinoma. *Clin Otolaryngol*, **26**, 82-90.
Niemhom S, Kitazawa S, Murao S, Kunachak S, Maeda S (2000). Co-expression of p53 and bcl-2 may correlate to the presence of Epstein-Barr virus genome and the expression of proliferating cell nuclear antigen in nasopharyngeal carcinoma. *Cancer Lett*, **160**, 199-208.
Pearson GR (1993). Epstein-Barr virus and nasopharyngeal carcinoma. *J Cell Biochem Suppl*, **17F**, 150-4.
Pi GH, Zeng Y, de The' G (1985). Enzyme-linked immunosorbent assay (ELISA) for the detection of nasopharyngeal carcinoma by Epstein-Barr virus IgA/EA antibodies. *Ann Virol Inst Pasteur*, **136E**, 131-40.
Puthavathana P, Sutthent R, Vitavasiri A, et al (1991). Epstein-Barr virus serological markers for nasopharyngeal carcinoma in Thailand. *Southeast Asian J Trop Med Public Health*, **22**, 326-31.
Raab-Traub N, Hood R, Yang, CS, Henry B 2nd, Pagano JS (1983). Epstein-Barr virus transcription in nasopharyngeal carcinoma. *J Virol*, **48**, 580-90.
Rowe M, Evans HS, Young LS, et al (1987). Monoclonal antibodies to the latent membrane protein of Epstein-Barr virus reveal heterogeneity of the protein and inducible expression in virus-transformed cells. *J Gen Virol*, **68**, 1575–86.
Sham JS and Choy D (1990). Prognostic factors of nasopharyngeal carcinoma: a review of 759 patients. *Br J Radiol*, **63**, 51-8.
Srivatanakul P, Tiwawech D, Boonyaratavej C, et al (1988). Early detection of nasopharyngeal carcinoma by using serum IgA antibody toEpstein-Barr virus as a screening test. *Thai Cancer J*, **14**, 18-24.
Srivatanakul P, Boonyaratavej C, Tiwawech D, et al (1985). Serum IgA antibodies to Epstein-Barr virus determination as a diagnostic test for nasopharyngeal carcinoma. *Thai Cancer J*, **11**, 115-8.
Tanner JE, Wei MX, Alfieri C, et al (1997). Antibody and antibody-dependent cellular cytotoxicity responses against the BamHI A rightward open-reading frame-1 protein of Epstein-Barr virus (EBV) in EBV-associated disorders. *J Infect Dis Jan*, **175**, 38-46.
Tiwawech D and Ishida T (1996). Presence of IgA and IgG antibodies against Epstein-Barr virus early antigen in Thai nasopharyngeal carcinoma. *Thai Cancer J*, **22**, 68-75.
Young LS, Dawson CW, Clark D, et al (1988). Epstein-Barr virus gene expression in nasopharyngeal carcinoma. *J Gen Virol*, **69**, 1051-65.
Zeng Y, Hang LG, Li HY, et al (1982). Serological mass survey for early detection of nasopharyngeal carcinoma in Wu-Zhou City, China. *Int J Cancer*, **29**, 139-41.
Zeng Y, Gong CH, Jan MG, et al (1983a). Detection of Epstein-Barr virus IgA/EA antibody for diagnosis of nasopharyngeal carcinoma by immunoautoradiography. *Int J Cancer*, **31**, 599-601.
Zeng Y, Zhang LG, Wu YC (1985). Prospective studies on nasopharyngeal carcinoma in Epstein-Barr virus IgA/VCA antibody-positive persons in Wuzhou City, China. *Int J Cancer*, **36**, 545–7.
Zheng YM, Tuppin P, Hubert A, et al (1994). Environmental and dietary risk factors for nasopharyngeal carcinoma: a case-control study in Zangwu County, Guangxi, China. *Br J Cancer*, **69**, 508-14.
Zong YS, Sham JS, Ng MH (1992). Immunoglobulin A against viral capsid antigen of Epstein-Barr virus and indirect mirror examination of the nasopharynx in the detection of asymptomatic nasopharyngeal carcinoma. *Cancer*, **69**, 3-7.