Nasopharyngeal carcinoma in Brunei Darussalam: low incidence among the Chinese and an evaluation of antibodies to Epstein-Barr virus antigens as biomarkers

Hsien Y C, Abdullah M S, Telesinghe P U, Ramasamy R

ABSTRACT

Introduction: Little or no information is available on the prevalence of nasopharyngeal carcinoma (NPC) among different ethnic groups in Brunei, or how useful plasma IgA antibodies are against viral capsid antigen (VCA) and early antigen (EA) in the diagnosis of NPC, even though they are routinely measured in patients suspected to have NPC.

Methods: The National Cancer Registry at Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital was used to identify NPC patients diagnosed between 2000 and 2006. Clinical data and antibody test results for 38 NPC patients and for nine patients suspected of NPC but later diagnosed as negative for NPC by biopsy (control group) were obtained from the Oncology and Histopathology Departments at RIPAS Hospital.

Results: The annual incidence rates for NPC among the major ethnic groups in Brunei were determined and compared to data from Singapore and Peninsular Malaysia. The most significant finding was that the average annual incidence of NPC among Bruneian Chinese males (4.1 per 100,000 persons) was significantly lower than that for Chinese males from Singapore (15.9) and Peninsular Malaysia (19.6). IgA anti-VCA and IgA anti-EA were sensitive and specific to NPC in Brunei in accordance with studies elsewhere. The measurement of IgA antibodies against VCA by ELISA was the better serological test for NPC. However, many stage IV NPC cases did not possess IgA anti-VCA and IgA anti-EA.

Conclusion: Determining the factors that are responsible for a lower incidence of NPC among Chinese males in Brunei Darussalam may be useful for formulating measures to reduce NPC incidence elsewhere. The possible tendency for the loss of IgA antibodies against VCA and EA in advanced stages of NPC needs to be established with a larger number of patients, and the causes elucidated, in order to better understand the disease process in NPC.

Keywords: cancer biomarkers, cancer epidemiology, Epstein-Barr virus antigens, IgA antibodies, nasopharyngeal carcinoma

Singapore Med J 2009;50(4):371-377

Institute of Medicine, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong BE1410, Brunei Darussalam

Hsien YC, BHSc
Medical Student

Ramasamy R, MA, PhD
Professor of Immunology

Department of Oncology, Raja Isteri Pengiran Anak Saleha Hospital, Bandar Seri Begawan BA1710, Brunei Darussalam

Abdullah MS, MBChB, MSc, MRCP
Specialist Oncologist

Department of Pathology

Telesinghe PU, MBBS, FRCPath
Specialist Pathologist

Correspondence to:
Prof Ranjan Ramasamy
Tel: (673) 246 3001 ext 1965
Fax: (673) 246 081
Email: ramasamy@im.ubd.edu.bn

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is common among populations in southern China, Southeast Asia, the Arctic, the Middle East and parts of Africa, but is rarely reported in Western countries and Japan.[1] NPC is an epithelial neoplasm arising from the fossa of Rosenmüller that has been classified into three histopathological subsets, viz. keratinising squamous cell carcinoma, non-keratinising carcinoma and undifferentiated carcinoma.[2] There is no published information on the incidence of NPC among the different ethnic groups in Brunei. Current data suggest three main aetiological factors for NPC, viz. the environment, heredity and infection with Epstein-Barr virus (EBV). Chinese descendents who migrated to Western countries have lower incidence rates than do the Chinese in Asia. For example, the annual incidence among people of Chinese descent in Los Angeles is reportedly 6.5 cases per 100,000 men and 3.7 cases per 100,000 women,[3] compared to a reported annual incidence among the Chinese in Singapore of 18.1 cases per 100,000 men and 7.4 cases per 100,000 women in one study.[4] Hence, the environment must contribute in some way to the development of NPC. Several factors including cigarette smoking, occupational exposure

to wood and formaldehyde, and salted fish consumption have been implicated.[5]

Incidence rates among the Chinese in Western countries, however, remain higher than those of the Caucasian populations in Western countries, indicating that an inherited predisposition for NPC may also exist.[3] In Hawaii, for instance, the annual incidence among Caucasians is 0.7 per 100,000 men and 0.9 per 100,000 women. On the other hand, the incidence among the Chinese in Hawaii is 8.9 cases per 100,000 men and 3.7 cases per 100,000 women.[4] Indeed, familial clustering of NPC has been documented in Chinese populations and even in low-risk populations. Recent studies have linked genetic polymorphisms of some metabolic enzymes (CYP2E1 and GSTM1), some DNA repair enzymes (XRCC1 and hOGG1), and certain human leucocyte antigen groups to susceptibility to NPC.[5] Major susceptibility loci have also been identified on chromosomes 4, 3 and 14 in at-high-risk families from southeast China, southern China and Taiwan, respectively.[5]

EBV, a member of the herpes virus family, is a ubiquitous infectious agent that infects more than 90% of the world's population. It has been established that EBV is closely associated with the development of NPC.[6] EBV DNA has been detected in virtually all NPC samples of the non-keratinising and undifferentiated carcinoma subtypes obtained worldwide. EBV is also linked to the development of several other malignancies besides NPC, including African Burkitt's lymphoma, AIDS-associated B-cell lymphoma and some cases of Hodgkin's lymphoma.[2] Patients who present with relevant nasal or ear symptoms have a biopsy done to obtain a definitive diagnosis of NPC. A biopsy is an invasive procedure, however, and can cause complications to patients, and therefore other biomarkers of NPC have been sought to screen high-risk patients in high-incidence areas. EBV-specific antibodies are one such biomarker set.

Patients with NPC have elevated plasma IgG and IgA antibody titres to various EBV antigens, including the early antigen (EA) and viral capsid antigen (VCA). Some early studies suggested that these titres correlated with tumour burden, remission and recurrence, and may therefore be of diagnostic and prognostic value.[6] EAs are produced during initial stages of viral replication before viral DNA synthesis. In contrast, VCAs are late antigens produced after viral DNA synthesis. IgG antibodies have been shown not to be specific to NPC and were found in almost all study cases, including in healthy blood donors and patients with other cancers.[7-9] This reflects the fact that more than 90% of individuals are infected with EBV. Infection with EBV usually persists throughout a lifetime and IgG antibodies to EBV antigens are likely to be generated in almost every infected individual. On the other hand, these studies showed that IgA antibodies are not only as sensitive as IgG antibodies, but that they also show greater specificity to NPC.[7-9] IgA anti-VCA and IgA anti-EA are conventionally used in the diagnosis of NPC patients worldwide. A serum profile of elevated anti-VCA and anti-EA IgA titres usually presents a significant positive diagnosis for the presence of malignancy.[10] No previous study in Brunei has looked critically into determining how useful the measurement of plasma IgA antibodies is in the diagnosis of NPC in Bruneian patients. In this study, the sensitivity and specificity of anti-VCA and anti-EA antibodies, as well as their relationship with the NPC tumour stage, were examined retrospectively in Bruneian patients.

METHODS

A retrospective study of the 127 patients diagnosed with NPC between 2000 and 2006 was performed with details obtained from the National Cancer Registry. The age at detection of NPC in patients ranged from 12 to 83 years, with a majority in the age range of 40–60 years. Only two cases were squamous cell carcinomas, while the rest were undifferentiated carcinomas. Complete medical records for 87 patients were available from the Oncology Department at Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital. Results of IgA anti-VCA and IgA anti-EA tests were retrieved from laboratory reports stored in the hospital's computerised information system. The results of these tests, within a month of diagnosis, were traceable for only 38 out of the 87 NPC patients whose relevant clinical details were also available.

Tests for IgA anti-VCA and IgA anti-EA were not performed in Brunei Darussalam. Instead, the sera collected from Bruneian patients were tested for IgA anti-VCA and IgA anti-EA by overseas commercial laboratories under contract with the Ministry of Health, Brunei. Samples collected between 2000 and 2005 were sent to two separate laboratories in Singapore while samples collected in 2006 were sent to a Malaysian laboratory. The Singaporean laboratories used the immunofluorescence assay (IFA) technique. However, one laboratory measured titres with an upper limit of 100 whereas the other had an upper limit titre of 640. Results beyond these upper limits are thus reported as titres of >100 and > 640 by the respective laboratories. Samples with titres < 10 are considered negative for IgA antibodies. On the other hand, the laboratory in Malaysia utilised enzyme-linked immunosorbent assay (ELISA). Calibrated spectrophotometry was then used to report

Table I. Average annual incidence of nasopharyngeal carcinoma in Brunei Darussalam, Singapore and Peninsular Malaysia by ethnic group and gender.

Ethnic group/gender	Incidence per 100,000 (no. of cases)	95% confidence interval
Brunei*		
Malay		
Male	7.2 (61)	5.5–9.3
Female	4.6 (37)	3.3–6.4
Chinese		
Male	4.1 (6)	1.5–8.9
Female	5.5 (7)	2.2–11.2
Others		
Male	4.1 (12)	2.1–7.1
Female	1.6 (4)	0.4–4.0
Overall		
Male	6.2 (79)	4.9–7.7
Female	4.1 (48)	3.0–5.4
Singapore†		
Malay		
Male	5.7 (65)	4.4–7.2
Female	2.1 (24)	1.4–3.2
Chinese		
Male	15.9 (992)	14.9–16.9
Female	5.7 (359)	5.1–6.3
Indian		
Male	1.8 (12)	nd
Female	0.2 (1)	nd
Overall§		
Male	13.2 (1,079)	nd
Female	4.7 (386)	nd
Malaysia‡		
Malay		
Male	3.4 (203)	3.0–3.9
Female	1.2 (71)	1.0–1.5
Chinese		
Male	19.6 (516)	18.0–21.4
Female	8 (201)	6.9–9.2
Indian		
Male	1.8 (16)	nd
Female	0.6 (5)	nd
Overall§		
Male	8.5 (825)	nd
Female	3.2 (300)	nd

The respective studies were conducted in *2000–2006, †1998–2002, and ‡2003. Data for Singapore was adapted from the Singapore Cancer Registry Report No. 6.[14] Data for Peninsular Malaysia was adapted from the Second Report of the National Cancer Registry Cancer Incidence in Malaysia.[12] §The overall numbers of cases include ethnic groups other than Chinese, Indians and Malays.
nd: not done

the IgA levels in international units per millilitre (IU/ml). The laboratory considered levels of < 8 IU/ml as negative, levels between 8 and 12 IU/ml as borderline and levels > 12 IU/ml as positive. For the purpose of this study, borderline results are taken as being negative. Results from all three laboratories were occasionally only reported in terms of positivity, without actual titres or levels provided.

Population statistics were obtained from the following sources: Department of Economic Planning and Development, Prime Minister's Office, Brunei; Singapore Resident Population Report 1990–2006;[11] and the Second Report of the National Cancer Registry Cancer Incidence in Malaysia.[12] The terms, sensitivity and specificity of the screening tests, were used according to standard definitions.[13] The sensitivity of IFA IgA anti-VCA and IFA IgA anti-EA in detecting NPC was calculated for 31 NPC cases (diagnosed between 2000 and 2005), where results within a month of diagnosis were traceable.

A total of 84 nasopharyngeal biopsies were done for patients suspected of having NPC in 2006. Data on these patients was obtained from the histopathology department. 16 patients were diagnosed as positive for NPC. The rest were found to have other non-NPC-related conditions such as inflammation and lymphoid hyperplasia. IgA anti-VCA and IgA anti-VCA results, within a month of the biopsy being obtained, were traceable only for ten NPC patients and nine non-NPC patients, who made up the control group. The sensitivity and specificity of ELISA IgA anti-VCA and ELISA IgA anti-EA were calculated.

IFA IgA anti-VCA and IFA IgA anti-EA results for

Table II. Frequencies of positive and negative results for IgA anti-VCA and anti-EA by immunofluorescence assay in the nasopharyngeal carcinoma and control groups.

	Biopsy positive	Biopsy negative
IgA anti-VCA +ve	25	nd
IgA anti-VCA −ve	6	nd
IgA anti-EA +ve	20	nd
IgA anti-EA −ve	11	nd

nd: not done

Table III. Frequencies of positive and negative results for IgA anti-VCA and anti-EA by ELISA in the nasopharyngeal carcinoma and control groups.

	Biopsy positive	Biopsy negative
IgA anti-VCA +ve	9	1
IgA anti-VCA −ve	1	8
IgA anti-EA +ve	8	1
IgA anti-EA −ve	2	8

31 patients (diagnosed between 2000 and 2005) were examined for correlation. Reported results without actual values were classified as follows: negative was given a titre of 0; positive was given a titre of 10; > 100 was given a titre of 100; and > 640 was given a titre of 640.

A regression analysis was performed using the Microsoft Excel statistical package. Frequencies of positive and negative results of IgA anti-VCA and IgA anti-EA for each NPC stage were examined in 38 patients (diagnosed between 2000 and 2006) whose relevant clinical details, as well as IgA anti-VCA and IgA anti-EA results within a month of diagnosis were available. Exact 95% confidence intervals of incidence rates, based on a Poisson distribution, were computed using the statistical software, STATA version 9.2 (Stata Corp, College Station, TX, USA). The significance of the differences in incidence rates for the two populations was determined as the mid-probability value for a two-tailed, exact binomial test using STATA. The chi-square test was used to test the statistical significance of differences in proportions in the serological tests for EBV antigens. Cells that included values of 0 or when the calculated expected value was < 5 were tested by Fisher's exact test.

RESULTS

Table I shows the average annual incidence rates of NPC among males and females of the different ethnic groups in Brunei Darussalam, Singapore and Peninsular Malaysia. In general, the incidence for females was lower than that for males except for the Chinese in Brunei, where the gender difference was not significant. In Brunei Darussalam, there are a number of indigenous minorities, e.g. Dusuns and Ibans, and resident foreigners, who are classified under the category of others in Table I. There was no significant difference in the NPC incidence between Chinese males and Malay or other males in Brunei Darussalam. No significant difference was observed between the NPC incidence among Malay men in Brunei Darussalam and Singapore, while the incidence in Malay men in Peninsular Malaysia was significantly lower than in Brunei Darussalam (relative risk 0.5, $p < 0.0001$) and Singapore (relative risk 0.6, $p = 0.0007$). The incidence among Chinese men in Brunei, however, was markedly lower than the incidence among the Chinese in both Singapore (relative risk 0.26, $p < 0.0001$) and Peninsular Malaysia (relative risk 0.21, $p < 0.0001$). Chinese females in Singapore had a significantly lower incidence of NPC than their counterparts in Peninsular Malaysia (relative risk 0.7, $p = 0.0001$), but not in Brunei. The incidence of NPC in Peninsular Malaysians and Singaporeans among Indians was lower than that for the Chinese or Malays in both countries, probably reflecting the Caucasoid nature of the Indian populations.

The sensitivity of IgA anti-VCA by IFA was 81% and that of IgA anti-EA by IFA was 65% (Table II). The specificity of IgA anti-VCA and of IgA anti-EA by IFA could not be calculated because the data on patients, in whom histological diagnosis was negative, was unavailable. ELISA IgA anti-VCA has a sensitivity of 90% and ELISA IgA anti-EA has a sensitivity of 80%. The specificity of IgA anti-VCA by ELISA is 89% and that of IgA anti-EA by ELISA is also 89% (Table III). The detection rates of IgA anti-VCA and IgA anti-EA in the control group were significantly lower ($p < 0.001$ and $p < 0.005$, respectively) than those in the NPC group. The correlation coefficient by regression analysis of IgA anti-VCA and IgA anti-EA titres was 0.66. The significance of the correlation calculated by analysis of variance is $p = 4.6 \times 10^{-05}$.

We also examined the relationship between the detection of EBV-specific IgA antibodies to EA and VCA, and the stage of the tumour. Patients were grouped according to stages using the American Joint Committee on Cancer-Union Internationale Contre le Cancer (2002) classification system. The distribution of the NPC patients included in this analysis was as follows: Stage I (1), Stage IIA (0), Stage IIB (4), Stage III (11), Stage IVA (8), Stage IVB (13) and Stage IVC (1). Due to a significant disparity in the number of patients in the various subgroups, patients belonging to subgroups of a particular stage were combined to form only four major groups consisting of Stages I, II, III and IV. The results showed that a number of Stage IV cases

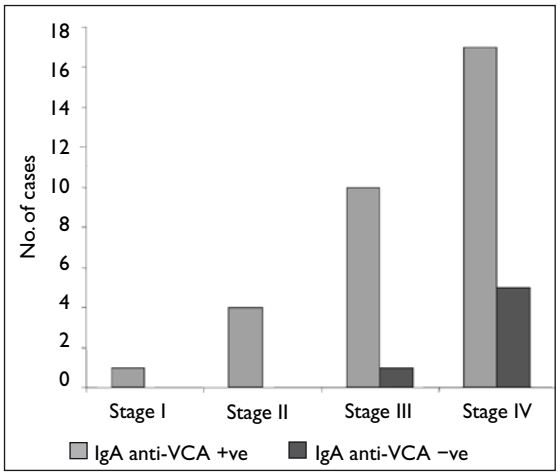

Fig. 1 Bar chart shows the frequencies of positive and negative results for IgA anti-VCA in patients with different tumour stages.

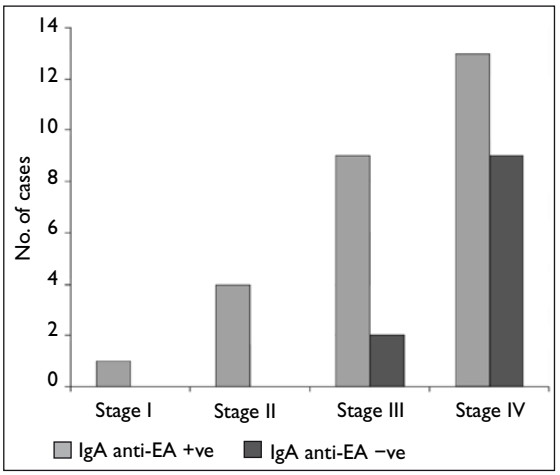

Fig. 2 Bar chart shows the frequencies of positive and negative results for IgA anti-EA in patients with different tumour stages.

were negative for IgA anti-VCA antibodies, and a higher number of cases were negative for IgA anti-EA antibodies (Figs. 1 and 2). The proportion of IgA-anti-VCA-negative cases in Stage IV is not significantly different ($p = 0.370$) from the combined proportion of IgA-anti-VCA-negative cases in Stages I–III. However, the higher proportion of IgA-anti-EA-negative Stage IV cases compared to the combined proportion in the other stages showed a trend towards statistical significance ($p = 0.078$).

DISCUSSION

In Brunei Darussalam, the average NPC incidence (between 2000 and 2006) in males is about 1.5 times that in females. This is consistent with findings in other countries. Smoking has been found to be a moderate risk factor for NPC,[15] and it has been suggested that the higher incidence in males is partly accounted for by an increased incidence of smoking.[16] Our data for the incidence of NPC in Southeast Asians of Chinese, Indian and Malay origin is consistent with the influence of genetic factors in the development of NPC. The most notable finding was that the incidence rate in Bruneian Chinese males is significantly lower than the incidence rates among Chinese males in Singapore, determined here and by others,[4] as well as in Peninsular Malaysia. This is an unusual finding and genetic factors are unlikely to play a role in causing this difference, as most Chinese in Southeast Asia would have descended from populations originating in southern China. It is unlikely also to be due to Bruneian Chinese seeking medical treatment overseas, as the first point of detection and investigation is the RIPAS Hospital in Brunei Darussalam, although some patients may opt for subsequent treatment abroad. Variations in social and environmental factors seem more likely to be the cause of the difference. At present, only speculations are possible.

The lifestyle, healthcare, eating habits, incidence of smoking, exposure to air pollutants, and characteristics of exposure to EBV among the Chinese population in Brunei may differ from their counterparts in Singapore and Peninsular Malaysia. These factors may in fact be more similar to those that Chinese populations in America are exposed to, thereby producing a comparable lowering of the incidence of NPC. Further investigations on larger sample populations of the relevant socioeconomic and environmental factors that have led to the reduction of NPC in Brunei Darussalam may provide information that may be useful in reducing the incidence of NPC in Asian countries. Our data also suggests that Malay men in Peninsular Malaysia are less prone to developing NPC than Malay men in the other two countries, and that Chinese women in Malaysia may be more susceptible to NPC than Chinese women in Singapore. These findings have to be confirmed with larger samples over a longer time period, but it is possible that behavioural and environmental factors also contribute to these differences.

IFA has been conventionally used to test for IgA anti-VCA and IgA anti-EA antibodies. The sensitivity of IgA anti-VCA by IFA averages 85% based on a number of studies.[7-9,17,18] The sensitivity of IFA IgA anti-VCA (81%) in detecting NPC in Brunei patients is in accordance with values reported by these studies. Evidence on the sensitivity of IgA anti-EA by IFA is less clear, however, with one study reporting a sensitivity of 73%[18] and another reporting 48%.[9] The sensitivity of IFA IgA anti-EA (65%) in detecting NPC in Brunei seems to corroborate more with the former study although it is lower. This may possibly be

related to the high number of cases of Stage IV NPC that were not detected by IFA IgA anti-EA. ELISA has recently become a more popular method for testing for anti-EBV antibodies. The sensitivity of ELISA IgA anti-VCA (90%) in this study is slightly higher than that (83.5%) reported by others.[8] ELISA anti-EA detected 80% of NPC patients in Brunei and this supports the findings in the above study,[8] where the sensitivity of ELISA anti-EA was 79.1%. The sensitivity of ELISA IgA anti-VCA (90%) appears to be higher than that of IFA IgA anti-VCA (81%). ELISA IgA anti-EA (sensitivity 80%) also appears to be more sensitive than IFA IgA anti-EA (sensitivity 65%). The low detection rates of ELISA IgA anti-VCA (11%) and ELISA IgA anti-EA (11%) in the control group, compared to the corresponding IgG antibodies,[7-9] are consistent with other reports.[8,10]

Regardless of whether IFA or ELISA is used for testing, it has generally been established that IgA anti-VCA and IgA anti-EA antibodies are useful biomarkers for the diagnosis of NPC. NPC is an epithelial neoplasm that is very closely associated with EBV. IgA antibodies are predominantly produced in the mucosa, including the epithelial linings of the respiratory, digestive and genitourinary tracts. Individuals who are infected with EBV but do not have malignancy are unlikely to generate IgA antibodies against EBV antigens, probably explaining the specificity to NPC. There is a significant correlation between IgA anti-VCA and IgA anti-EA, suggesting that it may be necessary to perform only one of the two tests, thereby economising on resources. IgA anti-VCA is apparently better than IgA anti-EA because it has a higher sensitivity in detecting NPC and similar specificity in our studies. However, others have reported that IgA anti-EA has a higher specificity for NPC.[10] Some laboratories only test for IgA anti-EA when an IgA anti-VCA test is positive to further validate the chances of the individual being screened having NPC.[10]

Our findings are notable in that a relatively large number of Stage IV cases tested negative for IgA anti-VCA and IgA-anti-EA antibodies, although these findings were not statistically significant at the $p = 0.05$ level. However, the higher frequency of IgA-anti-EA-negative Stage IV cases tended to approach significance ($p = 0.078$). If the proportion of cases negative for EBV-specific antibodies is indeed higher in advanced stages of the tumour, it indicates that the association with EBV replication decreases as the tumour becomes more advanced. We suggest that it is possible that advanced tumours have undergone many sequential genetic alterations, so that EBV replication is no longer needed for tumour growth. Mutations in multiple growth regulatory genes are indeed characteristic of many types of advanced cancers.[19] We also propose that increased regulatory T-cell activity and/or immunosuppression in patients with advanced disease may be another possible explanation for the decreased production of antibodies to EBV antigens in patients with advanced tumours. Inhibition of tumour-specific immunity through multiple immunosuppressive mechanisms is characteristic of many types of advanced tumours,[20] but such mechanisms have not been well studied in NPC. These possibilities need to be investigated in subsequent immunological and molecular studies using larger numbers of Stage IV cases.

An alternative marker to plasma EBV-specific IgA antibodies, for the diagnosis of NPC patients, is plasma EBV DNA load. One study demonstrated that cell-free EBV DNA was detectable in 96% of NPC patients and only 7% of controls, suggesting that cell-free EBV DNA is highly sensitive and specific to NPC.[21] Undetectable plasma EBV DNA load after completion of radiotherapy was associated with tumour regression, whereas consistently high EBV DNA load correlated with disease persistence and progression. Other recent studies have supported the earlier findings.[22,23] EBV DNA is therefore a promising marker for the presence of NPC and for predicting treatment outcome.

ACKNOWLEDGEMENTS

We are grateful to Prof MM Hossain and Dr L Naing of Universiti Brunei Darussalam for performing some of the statistical calculations.

REFERENCES

1. Chang ET, Adami HO. The enigmatic epidemiology of NPC. Cancer Epidemiol Biomarkers Prev 2006; 15:1765-77.
2. Kumar V, Abbas AK, Fausto N. Robbins and Cotran Pathologic Basis of Disease. 7th ed. Philadelphia: Saunders, 2004.
3. Sun LM, Epplein M, Li CI, Vaughan TL, Weiss NS. Trends in the incidence rates of nasopharyngeal carcinoma among Chinese Americans living in Los Angeles County and the San Francisco metropolitan area, 1992–2002. Am J Epidemiol 2005; 162:1174-8.
4. Lo S, Lee N, Karimi S, et al. Nasopharynx, squamous cell carcinoma. In: eMedicine from WebMD [online]. Available at: www.emedicine.com/radio/topic551.htm. Accessed March 14, 2007.
5. Yang XR, Diehl S, Pfeiffer R, et al. Evaluation of risk factors for nasopharyngeal carcinoma in high-risk nasopharyngeal carcinoma families in Taiwan. Cancer Epidemiol Biomarkers Prev 2005; 14:900-5.
6. Raab-Traub N. Epstein-Barr virus in the pathogenesis of NPC. Semin Cancer Biol 2002; 12:431-41.
7. Hadar T, Rahima M, Kahan E, et al. Significance of specific Epstein-Barr Virus IgA and elevated IgG antibodies to viral capsid antigens in nasopharyngeal carcinoma patients. J Med Virol 1986; 20:329-39.
8. Puthavathana P, Kositanont U, Chongkolwatana C, et al. Prevalence of IgA specific antibodies to Epstein-Barr virus capsid and early antigens in nasopharyngeal carcinoma. Asian Pac J

Allergy Immunol 1993; 11:39-43.
9. Cai WM, Li YW, Wu B, et al. Serologic diagnosis of nasopharyngeal carcinoma. A double-blind study of four EB virus antibodies with evaluation by sequential discrimination. Int J Radiat Oncol Biol Phys 1983; 9:1763-8.
10. Gan YY, Fones-Tan A, Chan SH, Gan LH. Epstein-Barr viral antigens used in the diagnosis of nasopharyngeal carcinoma. J Biomed Sci 1996; 3:159-69.
11. Kim WW. Singapore Resident Population Report 1990-2006. In: Department of Statistics, Ministry of Trade & Industry, Singapore [online] 2006. Available at: www.singstat.gov.sg/pdtsvc/pubn/demo.html#pop. Accessed April 18, 2007.
12. Lim CC, Yahaya H. Second Report of the National Cancer Registry Cancer Incidence in Malaysia [online] 2004. In: National Cancer Registry, Ministry of Health, Malaysia. Available at: www.acrm.org.my/ncr/. Accessed March 20, 2007.
13. Petrie A, Sabin C. Medical Statistics at a Glance. Oxford: Blackwell, 2000.
14. Seow A, Koh WP, Chia KS, et al. Singapore Cancer Registry Report No. 6 [online] 2004. In: Singapore Cancer Registry, Ministry of Health Singapore. Available at: www.hpb.gov.sg/data/hpb.home/files/edu/Appendix_C&D.pdf. Accessed March 20, 2007.
15. Yuan JM, Wang XL, Xiang YB, et al. Non-dietary risk factors for nasopharyngeal carcinoma in Shanghai, China. Int J Cancer 2000; 85:364-9.
16. Hirayama T. Descriptive and analytical epidemiology of nasopharyngeal cancer. IARC Sci PubL 1978; 20:167-89.
17. Puthavathana P, Sutthent R, Vitavasiri A, et al. Epstein-Barr virus serological markers for nasopharyngeal carcinoma in Thailand. Southeast Asian J Trop Med Public Health 1991; 22:326-31.
18. 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.
19. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70.
20. Melief CJ. Cancer immunotherapy by dendritic cells. Immunity 2008; 29:372-83.
21. Lo YM, Chan YS, Lo KW, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer Res 1999; 59:1188-91.
22. Lin JC, Wang WY, Chen KY, et al. Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. N Eng J Med 2004; 350:2461-70.
23. Tan EL, Selvaratnam G, Kananathan R, Sam CK. Quantification of Epstein-Barr virus DNA load, interleukin-6, interleukin-10, transforming growth factor-β1 and stem cell factor in plasma of patients with nasopharyngeal carcinoma. BMC Cancer 2006; 6:227.