Journal of Medical Virology 84:768–776 (2012)

Seroreactivity Against Epstein–Barr Virus (EBV) Among First-Degree relatives of Sporadic EBV-Associated Nasopharyngeal Carcinoma in Indonesia

Susanna Hilda Hutajulu,[1] Nawi Ng,[2] Baning Rahayu Jati,[3] Jajah Fachiroh,[4] Camelia Herdini,[5] Bambang Hariwiyanto,[5] Sofia Mubarika Haryana,[4] and Jaap Michiel Middeldorp[6]*

[1]*Department of Internal Medicine, Faculty of Medicine/Dr. Sardjito Hospital, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[2]*Department of Public Health and Clinical Medicine, Division of Epidemiology and Global Health, Umeå University, Umeå, Sweden*
[3]*Field Epidemiology Training Program, Department of Public Health, Faculty of Medicine/Dr. Sardjito Hospital, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[4]*Department of Histology and Cell Biology, Faculty of Medicine/Dr. Sardjito Hospital, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[5]*Department of Ear, Nose and Throat, Faculty of Medicine/Dr. Sardjito Hospital, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[6]*Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands*

Epstein–Barr virus (EBV) infection and family history are significant risk factors associated with undifferentiated nasopharyngeal carcinoma. The presence of aberrant immunoglobulin A (IgA) antibodies against specific EBV antigens in healthy individuals can be predictive of the disease. Very limited reports explored the EBV IgA antibody presence within families of sporadic cases of nasopharyngeal carcinoma. This study aimed to determine whether EBV IgA was observed more frequently among family members of sporadic cases of nasopharyngeal carcinoma compared to community controls and evaluated the non-viral factors as determinants of antibody level. First-degree relatives of nasopharyngeal carcinoma patients (n = 520) and case-matched community controls (n = 86) were recruited. Sera from all individuals were tested in standardized peptide-based EBV IgA ELISA. Data on demographic variables and other exogenous factors were collected using a questionnaire through face-to-face interviews. A similar frequency of EBV IgA (cut-off value/CoV 0.354) was observed in the first-degree relatives of cases and in community controls (41.2% vs. 39.5%, $P = 0.770$). However, with a higher antibody level ($OD_{450} = 1.000$; about three times standard CoV), the relatives showed significantly higher frequency (36.9% vs. 14.7%, $P = 0.011$). When adjusted for all exogenous factors, the strongest factors associated with seropositivity are being a father (odds ratio/OR = 4.36; 95% confidence interval/CI = 1.56–12.21) or a sibling (OR = 1.89; 95% CI = 1.06–3.38) of a case of nasopharyngeal carcinoma. The higher level of EBV IgA seroreactivity in first-degree relatives of sporadic cases of nasopharyngeal carcinoma compared to the general population supports the use of EBV IgA ELISA for screening among family members. ***J. Med. Virol. 84:768–776, 2012.***

KEY WORDS: IgA anti-EBV ELISA; sporadic case of nasopharyngeal carcinoma; family members

Grant sponsor: Nuffic/Netherland Fellowship Program; Grant number: NFP-PhD 08–36; Grant sponsor: Dutch Cancer Society; Grant number: IN2006–21; Grant sponsor: International Agency of Research on Cancer; Grant number: IARC GEP/07/06; Grant sponsor: Universitas Gadjah Mada.

No potential conflicts of interest were disclosed.

The work was performed at the Faculty of Medicine and Dr. Sardjito Hospital, Universitas Gadjah Mada, Yogyakarta, Indonesia.

*Correspondence to: Jaap Michiel Middeldorp, VU University Medical Center and Cancer Center Amsterdam Department of Pathology, De Boelelaan 1117 1081 HV Amsterdam The Netherlands. E-mail: j.middeldorp@vumc.nl

Accepted 25 January 2012

DOI 10.1002/jmv.23263
Published online in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

Nasopharyngeal carcinoma has a unique and striking geographical distribution throughout the world [Parkin et al., 2005]. Although rare in most regions, it is very common in Southern China and South East Asia [Ho, 1972]. In Indonesia, the estimated incidence of nasopharyngeal carcinoma is 6.2 per 100,000 population [Soeripto, 1998] with vast majority of cases being undifferentiated carcinoma. Considering the large population of Indonesia, 12,000 new cases of this cancer may occur yearly, creating a significant health problem to the country. The treatment outcome of nasopharyngeal carcinoma greatly depends on the stage at diagnosis. Ninety percent 5-year overall survival can be achieved at stage I and 50–70% at stage III–IV [Lee et al., 2005]. In Indonesia, most patients come to the hospital at very late stage of disease and treatment facilities are limited. Therefore, treatment results tend to be poor. For this reason, a screening program to detect nasopharyngeal carcinoma at an early stage will be crucial for decreasing disease burden and increasing treatment efficacy. The success of screening depends on the availability of specific tests and the existence of a well-defined population at high risk for developing the disease.

Epstein–Barr virus (EBV) infection is considered to play a consistent causal role in the pathogenesis of undifferentiated nasopharyngeal carcinoma [zur Hausen et al., 1970; Raab-Traub, 1992; Chang and Adami, 2006; Secretan et al., 2009]. The close relationship between EBV and nasopharyngeal carcinoma is highlighted by the presence of viral DNA, RNA, and protein in all tumor cells, viral reactivation and the aberrant antibodies against EBV antigens in patient sera [Hildesheim and Levine, 1993; Middeldorp et al., 2003]. Aberrant antibodies against EBV antigens such as viral capsid antigen (VCA), DNase, early antigen (EA), and EBV nuclear antigen 1 (EBNA1) have benefit in clinical diagnosis [Henle and Henle, 1976; Cheng et al., 1980; Fachiroh et al., 2006; Paramita et al., 2009]. Aberrant seroreactivity in nasopharyngeal carcinoma is detectable prior to onset of clinical manifestation of the disease. Studies in China and Taiwan have shown the feasibility of using EBV serology as a predictive marker for disease development [Zeng et al., 1983; Chen et al., 1989; Chien et al., 2001; Ji et al., 2007; Ng et al., 2009; Li et al., 2010]. Although EBV is the strongest risk factor for NPC, there are additional exogenous factors, which are also closely linked with the malignancy. Food such as salted fish [Armstrong and Eng, 1983; Yu et al., 1986, 1989a; Ning et al., 1990; Zheng et al., 1994a; Armstrong et al., 1998; Jia et al., 2010], other preserved foods [Yu et al., 1989a; Armstrong et al., 1998; Yuan et al., 2000b; Gallicchio et al., 2006], and herbal medicine [Hildesheim et al., 1992; West et al., 1993; Zheng et al., 1994b] have been linked to nasopharyngeal carcinoma but not consistently. Several compounds in food have been demonstrated to induce in vitro EBV reactivation suggesting their capabilitiy of initiating enhanced in vivo virus replication [Shao et al., 1988; Poirier et al., 1989; Chen et al., 1992]. Furthermore, tobacco smoke [Nam et al., 1992; Chow et al., 1993; Cheng et al., 1999; Yuan et al., 2000a; Feng et al., 2009], passive smoke [Lee et al., 2008; Nesic et al., 2010], alcohol consumption [Nam et al., 1992; Chen et al., 2009], occupational dust [Hildesheim et al., 2001], and other inhalants [Feng et al., 2009] are among environmental factors related to nasopharyngeal carcinoma.

A family history of nasopharyngeal carcinoma has also been associated with increased risk of the disease. First-degree relatives of patients have 6- to 19-fold excess risk of developing the disease compared to those without a family linkage [Chen et al., 1990; Yu et al., 1990; Chen and Huang, 1997; Ung et al., 1999; Zou et al., 2000; Ji et al., 2011]. This effect observed among cases is the strongest compared to other cancers [Goldgar et al., 1994; Chang and Adami, 2006]. An increased risk for EBV-associated nasopharyngeal carcinoma and other infectious agent-related cancers were recorded among families with a history of nasopharyngeal carcinoma especially among the multiplex cases [Friborg et al., 2005]. This suggests an increased frequency for viral reactivation among family members of EBV-associated cases. Aberrant EBV reactivation among family members was also indicated by studies on EBV immunoglobulin A (IgA) detection in the sera among core family members of cases [Pickard et al., 2004; Cheng et al., 2009; Ng et al., 2009; Hsu et al., 2011]. These studies indicate that both genetic and environmental factors responsible for the aberrant EBV IgA are shared in the family.

Because involvement of both genetic and environmental factors in carcinogenesis has been proposed, it is reasoned that healthy individuals from families with members affected by nasopharyngeal carcinoma might have an EBV antibody profile that is distinct from that seen in healthy individuals from the community at large. A peptide-based ELISA has been developed for measuring EBV IgA in (dried) blood samples, allowing finger prick sampling on paper filters, that may serve as tool for early screening [Fachiroh et al., 2006; Fachiroh et al., 2008]. The first step to define the usefulness of this assay in a screening approach is to validate it in families with nasopharyngeal carcinoma cases versus controls without a family history. Therefore, this study aimed to evaluate whether a high level of EBV IgA antibody is more frequently observed in the direct family of patients than among community controls. This could have pivotal implications for a risk assessment. The suitability of serology for screening in multiplex high-risk families has been proven [Pickard et al., 2004; Ng et al., 2005, 2009], but very limited reports addressed the same question for relatives of sporadic carcinoma cases [Chen et al., 2009]. Therefore, the present study would help to determine the suitability of EBV IgA serological screening within families of

nasopharyngeal carcinoma patients in Yogyakarta region in Indonesia, which mostly comprises sporadic cases. Possible exogenous factors relating to aberrant EBV IgA reactivity among the first-degree relatives were also evaluated.

MATERIALS AND METHODS

Study Population

Data from two studies on nasopharyngeal carcinoma carried out in the Yogyakarta region and coordinated from the Dr. Sardjito Hospital were utilized for comparison of anti-EBV antibody titers across first-degree relatives of nasopharyngeal carcinoma cases and individuals from the local community at large. One study concerned families mostly with one affected individual per family and the other was a case–control study. The family study was conducted in collaboration with EBV group VU University medical center, Amsterdam, The Netherlands (VUmc) and the second was with VUmc, Universitas Gadjah Mada and International Agency for Research on Cancer (IARC). The human subject review committee in Yogyakarta approved both projects. Informed consent was obtained from adults or from the parents of participating children. Both studies were conducted in about the same time period and used similar data collection instruments and the same laboratory test.

In the family study, 198 cases of nasopharyngeal carcinoma diagnosed during year 2005–2009 were selected as basis for recruiting family members, most of who were of Javanese origin. Only cases that resided in and around Yogyakarta Province were recruited into the study for logistic reasons. First-degree relatives were defined as fathers, mothers, siblings, and other offspring living in the same residence or neighborhood and available for interview and blood collection. A total of 551 subjects were visited at home and recruited with consent. From the 551 subjects, 18 participants refused to give blood and subjects with lack of adequate information were deleted from analysis. Therefore, the final analysis was performed on 520 complete data sets. In the case–control study, newly diagnosed cases with histological confirmation in Dr. Sardjito Hospital during year 2007–2009 were recruited into the study. Cases who had clear addresses and who resided in Yogyakarta Province and adjacent parts of Central Java. Fifty-six cases were identified. Two healthy controls from each case's neighborhood were selected and matched to cases by gender, age, and long-term residence in same area as the corresponding case. Controls should not be related to the cases. One-hundred and one controls were recruited with consent. Only 86 out of 101 identified controls provided consent for blood sampling, and all these 86 controls with complete interview data and blood analysis were included in the final analysis.

Questionnaire

The questionnaire for the local Yogyakarta population was adapted from the original questionnaire for nasopharyngeal carcinoma patient designed by The International Agency of Research on Cancer. Interviews with each participant were administered by local trained surveyors. Information was obtained from all adult healthy controls and family members and parents of participating children in the family study. The questionnaire captured personal socio-demographic information including age, sex, and level of education, as well as exposure to behavioral risk factors such as cigarette smoking, tobacco chewing, and alcohol drinking. Information on consumption of dried salted fish a typical risk factor of nasopharyngeal carcinoma, along with other food items, which are popular in the local community, was asked during the interview. Other exposure to environmental factors was also asked, including exposure to herbal drugs, passive smoke, and household smoke. Responses to food consumption and environmental exposure questions were catagorized as never, sparingly in a year or at least once in a week.

Biological Sample and EBV Serological Method

Venous blood samples were collected from the arm of all participants. For individuals refusing to undergo venous blood collection, blood was taken using a finger prick method as described elsewhere [Fachiroh et al., 2008]. Serum obtained from blood drawn from each participant was tested for antibody reactivity against a combination of two EBV antigens associated with nasopharyngeal carcinoma. The antigens are EBNA1 as the viral latent antigen and VCA-p18 as the lytic antigen. IgA anti-EBNA1 plus VCA-p18 was analyzed in ELISA using a peptide-based antigen combination and a positive result of seroreactivity was defined as $OD_{450} > 0.354$, being the cut-off value (CoV). All assay procedures and definition of CoV were described previously [Fachiroh et al., 2006]. This test demonstrated a good sensitivity (90.1%) and specificity (85.4%) in distinguishing NPC cases from non-cancer controls. Further the assay is referred as EBV IgA ELISA.

To compensate for low-level seroreactivity, antibody levels in the seropositive subgroups were further analyzed. A cut-off point distinguishing strong from weak responders was determined accross both populations. The value was determined as OD_{450} of 1.000 (approximately three times of the CoV).

Statistical Analysis

The distribution of EBV antibody status between relatives of cases and controls and between both populations and their corresponding cases were compared using Pearson's χ^2-test. For the seropositive subpopulations, comparison was also done on the higher antibody level as defined above. To analyze demographic,

food patterns, and exogenous determinants of EBV IgA detectibility among first-degree relatives, distribution of EBV seropositivity was initially compared for each determinant using Pearson's χ^2-test. Multi-level logistic regression analysis was used to assess the strength of association of determinants of seropositivity while controlling for possible confounding factors. The variance partition coefficient (VPC) in the multi-level analysis indicated whether the residual variation in the propensity to be seropositive is attributable to unobserved family characteristics. The multi-level analysis was conducted because family members of a case with nasopharyngeal carcinoma are not independent of each other, and might share unobserved genetic or social characteristics. Maximum likelihood estimates of odds ratio (ORs) and their 95% confidence intervals (95% CIs) were obtained using STATA 11. All *P*-values presented were two-sided.

RESULTS

Seropositivity for EBV IgA above CoV was observed among 41.2% of first-degree relatives from NPC cases in the family study compared to 39.5% of community controls from the case–control study (Table I). The frequency of being EBV IgA seropositive did not differ significantly between both populations ($P = 0.770$). When compared to the corresponding cases, first-degree relatives and general healthy controls showed significantly lower mean of seroreactivity ($P < 0.001$, Fig. 1). In the family group, subjects were also analyzed according to their relationship to the corresponding carcinoma case. Fathers showed the highest frequency of positive EBV IgA seroreactivity whereas children showed the lowest, but this difference in seropositive score did not reach to the threshold of statistical significance ($P = 0.060$; Table I).

Despite the non-significant difference in overall EBV IgA seropositive score between the two populations, a difference was seen in family members when considering high-level seroreactivity. Therefore, we determined a threshold that can distinguish between strong and weak EBV IgA responders. The OD_{450} value of 1.000, almost three times the standard CoV, was set as best discriminator to yield significant difference between family and non-family controls. NPC relatives demonstrated higher proportion of elevated EBV IgA reactivity (36.9% vs. 14.7%; $P = 0.011$).

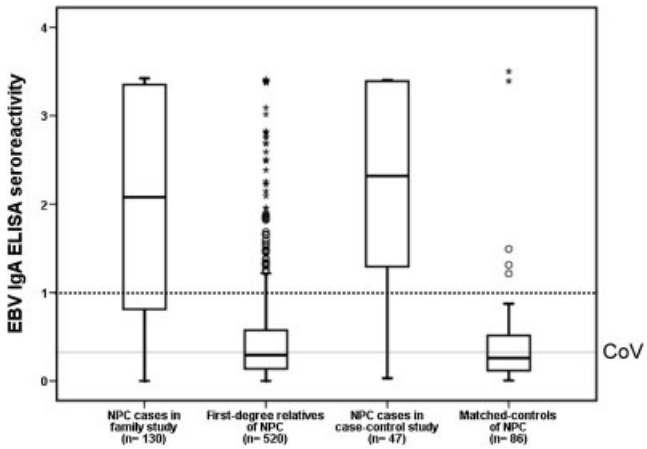

Fig. 1. Boxplot of EBV IgA ELISA seroreactivity by groups in family study and case–control study. The CoV of EBV IgA ELISA was set on 0.354. When compared to the corresponding cases, first-degree relatives of the cases and controls of the cases showed lower frequency of seroreactivity (Pearson's χ^2 test $P< 0.001$ for both corresponding groups). Relatives of nasopharyngeal carcinoma patients showed more frequent elevated EBV IgA seroreactivity than the community controls for the higher CoV (1.000) (36.9% vs. 14.7%, $P = 0.011$). Extreme and mild outliers were represented by symbols of * and °.

To evaluate further the elevated antibody levels observed between family members, we analyzed demographic factors, known risk factors associated with development of nasopharyngeal carcinoma, local food pattern, and other environmental exposures in a univariate analysis for proportional difference. For these determining factors, no significant differences in relation to having positive EBV IgA serology were observed (all $P > 0.05$; data not shown). However, family members of cases are not independent of each other and might share unobserved genetic characteristics. Hence the VPC in a multilevel multivariable logistic regression was determined. This analyisis showed that a total of 15% of the residual variation in

TABLE I. Distribution of EBV IgA Seroreactivity Among Family Members of Cases and Community Controls

Group	Number	Number (%) of positive	*P*-value	Number (%) of positive individuals with high reactivity	*P*-value
Family members of NPC cases	520	214 (41.2)	0.770	79 (36.9)	0.011
Community controls	86	34 (39.5)		5 (14.7)	
Family members of NPC cases by relationship to the corresponding case					
Fathers	41	23 (56.1)	0.060	9 (39.1)	0.557
Mothers	60	26 (43.3)		9 (34.6)	
Siblings	245	105 (42.9)		43 (41.0)	
Children	174	60 (34.5)		18 (30.0)	

First-degree relatives have higher frequency of IgA seroreactivity against EBV compared to the community controls. However the difference was not statistically different. For high level reactivity, defined as $OD_{450} \geq 1.000$, the relatives of nasopharyngeal carcinoma had a significantly higher seroreactivity against EBV than the case controls.

the propensity to be EBV IgA seropositive is attributable to unobserved family characteristics. The 85% of the remaining variance might be derived from other determining factors. In a fully adjusted logistic regression model, the data of univariate and multivariate analysis are displayed in Table II. Of note, the analysis was performed using the regular CoV (OD_{450} value >0.354) as outcome of seropositivity. The relationship among first-degree relatives associated with seropositivity is strongest for being father (OR = 4.36, 95% CI = 1.56–12.21) or sibling to a case (OR = 1.89, 95% CI = 1.06–3.38). The analysis also showed that some risk factors had a quite strong association with EBV IgA seropositivity among the family members.

TABLE II. Multilevel Logistic Regression Analysis for Risk Factors of Seroreactivity Among Family Member of Cases (Reference Categories in Bracket)

Risk factors	Univariate OR (95% CI)	Multivariate OR (95% CI)
Sex (ref: men)		
Women	1.27 (0.87–1.84)	1.54 (0.85–2.78)
Age in year (ref: ≤ 20 year)		
21–30	1.49 (0.62–3.55)	1.36 (0.53–3.47)
31–40	1.27 (0.54–3.01)	1.19 (0.46–3.09)
41–50	1.71 (0.71–4.08)	1.29 (0.45–3.64)
51–60	1.38 (0.55–3.45)	1.05 (0.35–3.19)
>60	2.03 (0.85–4.85)	1.26 (0.39–4.08)
Highest education level (ref: <6 years)		
Completed primary school	1.14 (0.52–2.48)	0.86 (0.31–2.35)
Completed secondary school	0.93 (0.44–1.94)	0.85 (0.34–2.1)
Completed high school	0.86 (0.38–1.96)	1.14 (0.44–2.92)
Completed university	0.71 (0.32–1.54)	0.85 (0.35–2.05)
Familial relationship to a NPC case (ref: child)		
Father	2.53 (1.2–5.32)	4.36 (1.56–12.21)
Mother	1.51 (0.79–2.86)	1.22 (0.48–3.15)
Sibling	1.48 (0.95–2.28)	1.89 (1.06–3.38)
Tobacco smoking (ref: never smokers)		
Ex-smokers	0.5 (0.22–1.18)	0.49 (0.18–1.36)
Current smokers—<10 cigarettes/day	1.1 (0.65–1.85)	1.31 (0.65–2.66)
Current smokers—10 or more cigarettes/day	0.78 (0.46–1.31)	0.77 (0.37–1.61)
Tobacco chewing (ref: never chewers)		
Ex-chewers	1.34 (0.16–10.98)	1.58 (0.15–17.25)
Current chewers	1.94 (0.7–5.33)	2.61 (0.79–8.6)
Alcohol drink (ref: never drinkers)		
Ex- drinkers	0.73 (0.35–1.52)	1.04 (0.42–2.54)
Current drinkers	0.51 (0.19–1.39)	0.77 (0.24–2.48)
Consumption of salted fish (ref: never consumed)		
Sparingly in a year	1.22 (0.71–2.11)	1.65 (0.87–3.13)
At least once in a week	1.46 (0.8–2.64)	1.75 (0.86–3.54)
Consumption of grilled food, ex: meat, corn (ref: never consumed)		
Sparingly in a year	0.92 (0.57–1.47)	1.21 (0.68–2.17)
At least once in a week	0.99 (0.52–1.88)	1.77 (0.79–3.94)
Consumption of other preserved food, ex: smoked fish or meat, salted meat and vegetables (ref: never consumed)		
Sparingly in a year	0.65 (0.31–1.39)	0.74 (0.33–1.67)
At least once in a week	0.56 (0.13–2.42)	0.29 ((0.05–1.51)
Consumption of meatball noodle/bakso (ref: never consumed)		
Sparingly in a year	0.6 (0.32–1.1)	0.43 (0.2–0.89)
At least once in a week	0.5 (0.26–0.96)	0.39 (0.17–0.9)
Consumption of instant noodle (ref: never consumed)		
Sparingly in a year	1.09 (0.55–2.17)	1.53 (0.68–3.45)
At least once in a week	1.05 (0.55–2.01)	1.46 (0.66–3.24)
Exposure to herbal drug from factory or self-made (ref: never exposed)		
Sparingly in a year	0.81 (0.52–1.25)	0.71 (0.43–1.16)
At least once in a week	0.95 (0.58–1.54)	0.89 (0.52–1.52)
Exposure to passive smoking during childhood (ref: never exposed)		
Sparingly in a year	1.15 (0.38–3.48)	0.94 (0.28–3.19)
At least once in a week	0.87 (0.49–1.55)	0.76 (0.39–1.48)
Exposure to passive smoking at home, at workplace, (ref: never exposed)		
Sparingly in a year	1.21 (0.36–4.01)	1.31 (0.34–5.06)
At least once in a week	1.07 (0.67–1.7)	1.12 (0.66–1.89)
Exposure to smoke in household, ex: burnt wood, mosquito coil, incent (ref: never exposed)		
Sparingly in a year	1.75 (0.78–3.94)	1.57 (0.65–3.82)
At least once in a week	0.93 (0.54–1.6)	0.75 (0.4–1.41)

In the multivariable analysis, the significant factors associated with seropositivity is being father and sibling to a case of nasopharyngeal carcinoma.

Tobacco chewing, consumption of grilled food at least once a week, and consumption of salted fish at least once in a week and sparingly in a year were associated with higher odds of seropositivity, with the ORs 2.61, 1.77, 1.75, and 1.65, respectively, although the 95% CI did not support the significance. Other factors such as gender, exposure to smoke in the household, consumption of instant noodle sparingly in a year and at least once in a week and exposure to passive smoking sparingly in a year also showed increased odds of seropositivity (ORs 1.54, 1.57, 1.53, 1.46, and 1.31), but also did not reach statistical significance.

DISCUSSION

Results of this study in the Yogyakarta region of Indonesia provides evidence for increased EBV IgA seroprevalence in families with a history of sporadic nasopharyngeal carcinoma. Although the proportion of positive seroreactivity among first-degree relatives of nasopharyngeal carcinoma cases was similar to the general community (41.2% vs. 39.5%), a significant higher proportion of family members showed EBV IgA reactivity above an elevated CoV of $OD_{450} \geq 1.000$ (36.9% vs. 14.7%, $P = 0.011$). This CoV may be suitable for starting a screening program for monitoring healthy first-degree relatives of cases, who are at known higher risk.

The EBV IgA seroprevalence of both healthy study groups observed in the present study was higher than in previous reports showing seroprevalence of 25–47.9% in first-degree relatives versus 18.4–20.5% in general controls in Taiwan and Inuits [Pickard et al., 2004; Friborg et al., 2007]. Both prior studies recruited high-risk families with multiplex cases, whereas almost 100% cases of nasopharyngeal carcinoma in Indonesia are sporadic disease cases. The differences may be partly explained by the use of different serological assays, each having a different diagnostic performance, including detection anti-VCA IgA by immunofluorecence [Pickard et al., 2004], commercial ELISA kits [Friborg et al., 2007], or DNase neutralizing activity and anti-EBNA1 IgA ELISA [Pickard et al., 2004]. The serology test used for our study is well standardized and contains a combination of two synthetic peptides representing the immunodominant epitopes of EBNA1 and VCA-p18. This test showed high sensitivity and specificity when tested in Indonesian nasopharyngeal carcinoma patients and healthy population [Fachiroh et al., 2006]. The availability of a well standardized screening test for disease risk assessment that can be cheaply produced and is suitable for finger-prick (dried) blood analysis may stimulate further field studies [Fachiroh et al., 2008].

The elevated seroreactivity observed in family members of nasopharyngeal carcinoma cases may reflect increased viral activity driven by shared genetic or environmental risk factors or both. The high seroprevalence among the general community may be due to shared environmental factors since regional cancer-controls do not share genetic predisposing factors. The high seroreactivity in both groups of Indonesian healthy persons may also reflect EBV reactivation induced by various stressors including poor general health and exhaustion as well as inflammatory cytokines known to trigger EBV reactivation [Dolken et al., 1984; Glaser et al., 2005; Coskun et al., 2010]. Observing the degree of seropositivity among both healthy populations, we found that higher level IgA reactivity ($\geq$1.000) preferentially occurs among first-degree relatives of the cases. This may reflect higher incidence of EBV reactivation in close family members of cases, in agreement with findings by others [Cheng et al., 2009; Hsu et al., 2011]. An early study on family members of nasopharyngeal carcinoma patients already reported an increased incidence of VCA IgA seropositivity, postulating that an autosomal recessive gene might be involved [Ho et al., 1978]. Importantly, increased nasopharyngeal carcinoma risk is linked to EBV IgA seroreactivity in time and level [Chen et al., 1985; Zeng et al., 1985; Chan et al., 1991; Chien et al., 2001; Ji et al., 2007; Cao et al., 2011]. Seropositive individuals, in particular the ones who showed sustained elevated seroreactivity, had a higher chance of developing the malignancy [Ji et al., 2007; Cao et al., 2011]. Therefore, we speculate that first-degree relatives showing higher titer of antibody might predispose to the disease. This underlines the importance of screening among family members of nasopharyngeal carcinoma patients. The higher EBV IgA cutoff level ($\geq$1.000) can be used for risk assessment and identifying individuals who might benefit from extensive clinical evaluation and cohort monitoring to detect the disease at early stage. Besides multiple well-established risk factors for nasopharyngeal carcinoma, family history is perhaps one of the simplest criteria for starting a case finding program. Despite the high frequency of EBV IgA seropositivity above CoV observed in the local healthy population, the level of antibody reactivity was much lower than in the corresponding nasopharyngeal carcinoma cases in this study, as demonstrated in Figure 1, confirming prior findings [Fachiroh et al., 2006].

To evaluate the reason for elevated antibody levels observed among first-degree relatives we examined socio-demographic and environmental factors as possible determinants of elevated EBV seroreactivity. There was no significant difference observed on EBV seroprevalence across the above-mentioned factors (Table II). However, the VPC in multi-level multivariate logistic regression analysis indicated that 15% of the propensity to have positive EBV IgA seroreactivity was attributable to familial characteristics. The remaining 85% relate to other influences, which signifies a role of sociodemographic and environmental factors in disease development [Hsu et al., 2011]. This indicates the potential benefit of a community education program on cancer risk factors.

Multi-variate analysis showed significant strong relationship between EBV IgA antibody detectibility and being a father or a sibling of a case, the OR being 4.36 and 1.89, respectively. The high OR of fathers can reflect the male predominance of nasopharyngeal carcinoma in combination with increased EBV reactivation with age [Zeng et al., 1982; Pickard et al., 2004]. The high OR among relatives might reflect the role of genetic factors, however, shared food and lifestyle variables among family members, in particular common exposures in early life, could not be ruled out. Siblings have been reported to have a higher risk for developing the disease compared to other relatives [Ireland et al., 1988]. A tendency was observed between female gender and elevated seropositivity rates although not being significantly different. Previous studies show anti-EBV antibody responses in females to be more robust than in males, perhaps due to hormonal influences on immunologic responses [Schuurs and Verheul, 1990; Wagner et al., 1994].

Tobacco smoking is common in Indonesia and was identified as risk factor in previous case–control studies, but the association was not consistent over multiple studies [Chang and Adami, 2006]. However, IARC monograph from 2009 pointed out tobacco smoking, as well as passive smoking, as human carcinogenic risk for some tumor sites including the nasopharynx [Secretan et al., 2009]. Other smoking and smokeless tobacco proved inconsistent risk factors for nasopharyngeal carcinoma [Chang and Adami, 2006]. The present study shows that current tobacco chewing, though not statistically significant, is related to a 2.6 times higher chance for having increased EBV IgA seropositivity compared to family member who never chew tobacco. Exposure to other smoke (OR = 1.57) and passive smoking at home and the workplace sparingly in a year also showed an association with positive seroreactivity (OR = 1.31).

Considering other exogenous factors, no significance in the multi-variable analysis was found. Consumption of certain types of food such as grilled food and instant noddles are associated with higher odds of aberrant EBV IgA antibody responses, even though the association is not statistically significant. These patterns of food consumption are very popular in the local population. However, the interpretation of the above results should be done with caution because the intervals showed considerable overlap and the number of individuals analyzed is relatively low.

Salted fish consumption is consistently observed as a moderate to strong risk predictor of nasopharyngeal carcinoma. Cantonese-style salted fish which contains high level of nitrosamines has been identified in a large number of case–control studies in Cantonese, other Southern Chinese, Northern Chinese, and Thai populations living in different regions of Asia and North America [Yu et al., 1986, 1989a; Ning et al., 1990; Sriamporn et al., 1992; Yang et al., 2005]. As a popular preserved food, more than 80% of our population of family members consumed the traditional salted fish in a frequency of sparingly in a year to at least once in a week. The frequency of antibody detectibility of these groups did not significantly differ from that of never consumers but did show an increased OR (OR = 1.75 between frequent consumers at least once a week and never consumers). Furthermore, salted fish made traditionally in Indonesia might differ to the one consumed by Cantonese population. Salted fish and other salted and preserved food contain nitrosamine and nitrosamine precursors, known as animal carcinogens [Poirier et al., 1987; Poirier et al., 1989]. Chinese salted fish can cause nasal cavity tumors in rats [Yu et al., 1989b].

This study harmonizes and utilizes data sets from two independent studies on nasopharyngeal carcinoma in the Yogyakarta province that is, the family study and the case–control study. Though these two studies did not aim to address the serological and risk hypothesis tested in this present paper, data from these two studies are comparable as both studies used similar instrument to assess behavioral and environmental risk factors for nasopharyngeal carcinoma. Only 101 healthy controls could be recruited into the current case–control study, which is much smaller than the healthy family member controls recruited in the family study. This can be viewed as a limitation of the present study and currently a larger study on this issue is planned based on the relevant lead findings presented here. In a recent independent study from China, involving a larger number of families of multiplex and sporadic nasopharyngeal carcinoma cases as well as population controls, similar findings were observed, confirming the data presented here [Qin et al., 2011]. The present study did not include follow-up examinations in the family members. Clinical investigation of individuals with elevated EBV-IgA reactivity will be done in the near future, in parallel to an ongoing study on early detection of nasopharyngeal carcinoma cases in patients with persistent head and neck complaints [Hutajulu et al., unpublished data].

In conclusion, this study confirms the presence of elevated EBV IgA seroreactivity among first-degree relatives of sporadic nasopharyngeal carcinoma cases in the Yogyakarta region, with defined high responders being considered as candidates for further NPC risk screening. A prospective cohort study is needed to evaluate whether or not these individuals with elevated levels of EBV antibodies are indeed at increased risk of carcinoma development. Additional studies including clinical follow-up and surveilance of individuals at high risk for nasopharyngeal carcinoma will provide important platform to evaluate further the utility of the peptide-based EBV IgA ELISA as population screening test.

ACKNOWLEDGMENTS

We thank Sagung Rai Indrasari for clinical support, Rosidah and Fransisca Erna Damayanti for epidemiological assistance and Sumartiningsih, Arina Prihestri

Nugraheni, Christine Fatmawati, Niken Miranti Mahaweni, Rahma Yuantari, Ferdiana Nareswari Dewi, Sri Fatmawati and Dewi Sulistyawati for technical assistance.

REFERENCES

Armstrong RW, Eng AC. 1983. Salted fish and nasopharyngeal carcinoma in Malaysia. Soc Sci Med 17:1559–1567.

Armstrong RW, Imrey PB, Lye MS, Armstrong MJ, Yu MC, Sani S. 1998. Nasopharyngeal carcinoma in Malaysian Chinese: Salted fish and other dietary exposures. Int J Cancer 77:228–235.

Cao SM, Liu Z, Jia WH, Huang QH, Liu Q, Guo X, Huang TB, Ye W, Hong MH. 2011. Fluctuations of Epstein–Barr virus serological antibodies and risk for nasopharyngeal carcinoma: A prospective screening study with a 20-year follow-up. PLoS One 6:e19100.

Chan CK, Mueller N, Evans A, Harris NL, Comstock GW, Jellum E, Magnus K, Orentreich N, Polk BF, Vogelman J. 1991. Epstein–Barr virus antibody patterns preceding the diagnosis of nasopharyngeal carcinoma. Cancer Causes Control 2:125–131.

Chang ET, Adami HO. 2006. The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 15: 1765–1777.

Chen DL, Huang TB. 1997. A case–control study of risk factors of nasopharyngeal carcinoma. Cancer Lett 117:17–22.

Chen JY, Liu MY, Chen CJ, Hsu MM, Tu SM, Lee HH, Kuo SL, Yang CS. 1985. Antibody to Epstein–Barr virus-specific DNase as a marker for the early detection of nasopharyngeal carcinoma. J Med Virol 17:47–49.

Chen JY, Chen CJ, Liu MY, Cho SM, Hsu MM, Lynn TC, Shieh T, Tu SM, Beasley RP, Hwang LY. 1989. Antibody to Epstein–Barr virus-specific DNase as a marker for field survey of patients with nasopharyngeal carcinoma in Taiwan. J Med Virol 27:269–273.

Chen CJ, Liang KY, Chang YS, Wang YF, Hsieh T, Hsu MM, Chen JY, Liu MY. 1990. Multiple risk factors of nasopharyngeal carcinoma: Epstein–Barr virus, malarial infection, cigarette smoking and familial tendency. Anticancer Res 10:547–553.

Chen CS, Pignatelli B, Malaveille C, Bouvier G, Shuker D, Hautefeuille A, Zhang RF, Bartsch H. 1992. Levels of direct-acting mutagens, total N-nitroso compounds in nitrosated fermented fish products, consumed in a high-risk area for gastric cancer in southern China. Mutat Res 265:211–221.

Chen L, Gallicchio L, Boyd-Lindsley K, Tao XG, Robinson KA, Lam TK, Herman JG, Caulfield LE, Guallar E, Alberg AJ. 2009. Alcohol consumption and the risk of nasopharyngeal carcinoma: A systematic review. Nutr Cancer 61:1–15.

Cheng YC, Chen JY, Glaser R, Henle W. 1980. Frequency and levels of antibodies to Epstein–Barr virus-specific DNase are elevated in patients with nasopharyngeal carcinoma. Proc Natl Acad Sci USA 77:6162–6165.

Cheng YJ, Hildesheim A, Hsu MM, Chen IH, Brinton LA, Levine PH, Chen CJ, Yang CS. 1999. Cigarette smoking, alcohol consumption and risk of nasopharyngeal carcinoma in Taiwan. Cancer Causes Control 10:201–207.

Cheng J, Cai Y, Zheng Y, Li J, Mo Y. 2009. Analysis of serum levels of IgA antibodies to Epstein–Barr virus capsid antigens in the spouses and the children of patients with nasopharyngeal carcinoma. Clin Oncol Cancer Res 6:267–270.

Chien YC, Chen JY, Liu MY, Yang HI, Hsu MM, Chen CJ, Yang CS. 2001. Serologic markers of Epstein–Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. N Engl J Med 345:1877–1882.

Chow WH, McLaughlin JK, Hrubec Z, Nam JM, Blot WJ. 1993. Tobacco use and nasopharyngeal carcinoma in a cohort of US veterans. Int J Cancer 55:538–540.

Coskun O, Sener K, Kilic S, Erdem H, Yaman H, Besirbellioglu AB, Gul HC, Eyigun EP. 2010. Stress-related Epstein–Barr virus reactivation. Clin Exp Med 10:15–20.

Dolken G, Weitzmann U, Boldt C, Bitzer M, Brugger W, Lohr GW. 1984. Detection of IgA antibodies to Epstein–Barr virus-associated antigens by ELISA. J Immunol Meth 68:331–339.

Fachiroh J, Paramita DK, Hariwiyanto B, Harijadi A, Dahlia HL, Indrasari SR, Kusumo H, Zeng YS, Schouten T, Mubarika S, Middeldorp JM. 2006. Single-assay combination of Epstein–Barr Virus (EBV) EBNA1- and viral capsid antigen-p18-derived synthetic peptides for measuring anti-EBV immunoglobulin G (IgG) and IgA antibody levels in sera from nasopharyngeal carcinoma patients: Options for field screening. J Clin Microbiol 44:1459–1467.

Fachiroh J, Prasetyanti PR, Paramita DK, Prasetyawati AT, Anggrahini DW, Haryana SM, Middeldorp JM. 2008. Dried-blood sampling for Epstein–Barr virus immunoglobulin G (IgG) and IgA serology in nasopharyngeal carcinoma screening. J Clin Microbiol 46:1374–1380.

Feng BJ, Khyatti M, Ben-Ayoub W, Dahmoul S, Ayad M, Maachi F, Bedadra W, Abdoun M, Mesli S, Bakkali H, Jalbout M, Hamdi-Cherif M, Boualga K, Bouaouina N, Chouchane L, Benider A, Ben-Ayed F, Goldgar DE, Corbex M. 2009. Cannabis, tobacco and domestic fumes intake are associated with nasopharyngeal carcinoma in North Africa. Br J Cancer 101:1207–1212.

Friborg J, Wohlfahrt J, Koch A, Storm H, Olsen OR, Melbye M. 2005. Cancer susceptibility in nasopharyngeal carcinoma families—A population-based cohort study. Cancer Res 65:8567–8572.

Friborg J, Jarrett RF, Liu MY, Falk KI, Koch A, Olsen OR, Duncan P, Wohlfarht J, Chen JY, Melbye M. 2007. Epstein–Barr virus immune response in high-risk nasopharyngeal carcinoma families in Greenland. J Med Virol 79:1877–1881.

Gallicchio L, Matanoski G, Tao XG, Chen L, Lam TK, Boyd K, Robinson KA, Balick L, Mickelson S, Caulfield LE, Herman JG, Guallar E, Alberg AJ. 2006. Adulthood consumption of preserved and nonpreserved vegetables and the risk of nasopharyngeal carcinoma: A systematic review. Int J Cancer 119:1125–1135.

Glaser R, Padgett DA, Litsky ML, Baiocchi RA, Yang EV, Chen M, Yeh PE, Klimas NG, Marshall GD, Whiteside T, Herberman R, Kiecolt-Glaser J, Williams MV. 2005. Stress-associated changes in the steady-state expression of latent Epstein–Barr virus: Implications for chronic fatigue syndrome and cancer. Brain Behav Immun 19:91–103.

Goldgar DE, Easton DF, Cannon-Albright LA, Skolnick MH. 1994. Systematic population-based assessment of cancer risk in first-degree relatives of cancer probands. J Natl Cancer Inst 86:1600–1608.

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

Hildesheim A, Levine PH. 1993. Etiology of nasopharyngeal carcinoma: A review. Epidemiol Rev 15:466–485.

Hildesheim A, West S, DeVeyra E, De Guzman MF, Jurado A, Jones C, Imai J, Hinuma Y. 1992. Herbal medicine use, Epstein–Barr virus, and risk of nasopharyngeal carcinoma. Cancer Res 52:3048–3051.

Hildesheim A, Dosemeci M, Chan CC, Chen CJ, Cheng YJ, Hsu MM, Chen IH, Mittl BF, Sun B, Levine PH, Chen JY, Brinton LA, Yang CS. 2001. Occupational exposure to wood, formaldehyde, and solvents and risk of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 10:1145–1153.

Ho JH. 1972. Nasopharyngeal carcinoma (NPC). Adv Cancer Res 15:57–92.

Ho HC, Ng MH, Kwan HC. 1978. Factors affecting serum IgA antibody to Epstein–Barr viral capsid antigens in nasopharyngeal carcinoma. Br J Cancer 37:356–362.

Hsu WL, Yu KJ, Chien YC, Chiang CJ, Cheng YJ, Chen JY, Liu MY, Chou SP, You SL, Hsu MM, Lou PJ, Wang CP, Hong JH, Leu YS, Tsai MH, Su MC, Tsai ST, Chao WY, Ger LP, Chen PR, Yang CS, Hildesheim A, Diehl SR, Chen CJ. 2011. Familial tendency and risk of nasopharyngeal carcinoma in taiwan: Effects of covariates on risk. Am J Epidemiol 173:292–299.

Ireland B, Lanier AP, Knutson L, Clift SE, Harpster A. 1988. Increased risk of cancer in siblings of Alaskan native patients with nasopharyngeal carcinoma. Int J Epidemiol 17:509–511.

Ji MF, Wang DK, Yu YL, Guo YQ, Liang JS, Cheng WM, Zong YS, Chan KH, Ng SP, Wei WI, Chua DT, Sham JS, Ng MH. 2007. Sustained elevation of Epstein–Barr virus antibody levels preceding clinical onset of nasopharyngeal carcinoma. Br J Cancer 96:623–630.

Ji X, Zhang W, Xie C, Wang B, Zhang G, Zhou F. 2011. Nasopharyngeal carcinoma risk by histologic type in central China: Impact of smoking, alcohol and family history. Int J Cancer 129:724–732.

Jia WH, Luo XY, Feng BJ, Ruan HL, Bei JX, Liu WS, Qin HD, Feng QS, Chen LZ, Yao SY, Zeng YX. 2010. Traditional Cantonese

diet and nasopharyngeal carcinoma risk: A large-scale case–control study in Guangdong, China. BMC Cancer 10:446.

Lee AW, Sze WM, Au JS, Leung SF, Leung TW, Chua DT, Zee BC, Law SC, Teo PM, Tung SY, Kwong DL, Lau WH. 2005. Treatment results for nasopharyngeal carcinoma in the modern era: The Hong Kong experience. Int J Radiat Oncol Biol Phys 61:1107–1116.

Lee YC, Boffetta P, Sturgis EM, Wei Q, Zhang ZF, Muscat J, Lazarus P, Matos E, Hayes RB, Winn DM, Zaridze D, Wunsch-Filho V, Eluf-Neto J, Koifman S, Mates D, Curado MP, Menezes A, Fernandez L, Daudt AW, Szeszenia-Dabrowska N, Fabianova E, Rudnai P, Ferro G, Berthiller J, Brennan P, Hashibe M. 2008. Involuntary smoking and head and neck cancer risk: Pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev 17:1974–1981.

Li S, Deng Y, Li X, Chen QP, Liao XC, Qin X. 2010. Diagnostic value of Epstein–Barr virus capsid antigen-IgA in nasopharyngeal carcinoma: A meta-analysis. Chin Med J (Engl) 123:1201–1205.

Middeldorp JM, Brink AA, van den Brule AJ, Meijer CJ. 2003. Pathogenic roles for Epstein–Barr virus (EBV) gene products in EBV-associated proliferative disorders. Crit Rev Oncol Hematol 45: 1–36.

Nam JM, McLaughlin JK, Blot WJ. 1992. Cigarette smoking, alcohol, and nasopharyngeal carcinoma: A case–control study among U.S. whites. J Natl Cancer Inst 84:619–622.

Nesic V, Sipetic S, Vlajinac H, Stosic-Divjak S, Jesic S. 2010. Risk factors for the occurrence of undifferentiated carcinoma of nasopharyngeal type: A case–control study. Srp Arh Celok Lek 138:6–10.

Ng WT, Yau TK, Yung RW, Sze WM, Tsang AH, Law AL, Lee AW. 2005. Screening for family members of patients with nasopharyngeal carcinoma. Int J Cancer 113:998–1001.

Ng WT, Choi CW, Lee MC, Law LY, Yau TK, Lee AW. 2009. Outcomes of nasopharyngeal carcinoma screening for high risk family members in Hong Kong. Fam Cancer 9:221–228.

Ning JP, Yu MC, Wang QS, Henderson BE. 1990. Consumption of salted fish and other risk factors for nasopharyngeal carcinoma (NPC) in Tianjin, a low-risk region for NPC in the People's Republic of China. J Natl Cancer Inst 82:291–296.

Paramita DK, Fachiroh J, Haryana SM, Middeldorp JM. 2009. Two-step Epstein–Barr virus immunoglobulin A enzyme-linked immunosorbent assay system for serological screening and confirmation of nasopharyngeal carcinoma. Clin Vaccine Immunol 16:706–711.

Parkin DM, Bray F, Ferlay J, Pisani P. 2005. Global cancer statistics, 2002. CA Cancer J Clin 55:74–108.

Pickard A, Chen CJ, Diehl SR, Liu MY, Cheng YJ, Hsu WL, Sun B, Hsu MM, Chen IH, Chen JY, Yang CS, Mittl BL, Chou SP, Ruggles DD, Goldstein AM, Hildesheim A. 2004. Epstein–Barr virus seroreactivity among unaffected individuals within high-risk nasopharyngeal carcinoma families in Taiwan. Int J Cancer 111: 117–123.

Poirier S, Ohshima H, de-The G, Hubert A, Bourgade MC, Bartsch H. 1987. Volatile nitrosamine levels in common foods from Tunisia, south China and Greenland, high-risk areas for nasopharyngeal carcinoma (NPC). Int J Cancer 39:293–296.

Poirier S, Bouvier G, Malaveille C, Ohshima H, Shao YM, Hubert A, Zeng Y, de The G, Bartsch H. 1989. Volatile nitrosamine levels and genotoxicity of food samples from high-risk areas for nasopharyngeal carcinoma before and after nitrosation. Int J Cancer 44:1088–1094.

Qin HD, Jia WH, Zhang LL, Liu N, Zhou XX, Wang MH, Feng QS, Chen LZ, Zhang Y, Jorgensen TJ, Zeng YX, Shugart YY. 2011. Elevated Epstein–Barr virus seroreactivity among unaffected members of families with nasopharyngeal carcinoma. J Med Virol 83:1792–1798.

Raab-Traub N. 1992. Epstein–Barr virus infection in nasopharyngeal carcinoma. Infect Agents Dis 1:173–184.

Schuurs AH, Verheul HA. 1990. Effects of gender and sex steroids on the immune response. J Steroid Biochem 35:157–172.

Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Talaa L, Guha N, Freeman C, Galichet L, Cogliano V. 2009. A review of human carcinogens-Part E: Tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol 10:1033–1034.

Shao YM, Poirier S, Ohshima H, Malaveille C, Zeng Y, de The G, Bartsch H. 1988. Epstein–Barr virus activation in Raji cells by extracts of preserved food from high risk areas for nasopharyngeal carcinoma. Carcinogenesis 9:1455–1457.

Soeripto. 1998. Epidemiology of nasopharyngeal carcinoma. Berita Kedokt Masyarakat (Ina) XIII:207–211.

Sriamporn S, Vatanasapt V, Pisani P, Yongchaiyudha S, Rungpitarangsri V. 1992. Environmental risk factors for nasopharyngeal carcinoma: A case–control study in northeastern Thailand. Cancer Epidemiol Biomarkers Prev 1:345–348.

Ung A, Chen CJ, Levine PH, Cheng YJ, Brinton LA, Chen IH, Goldstein AM, Hsu MM, Chhabra SK, Chen JY, Apple RJ, Yang CS, Hildesheim A. 1999. Familial and sporadic cases of nasopharyngeal carcinoma in Taiwan. Anticancer Res 19:661–665.

Wagner HJ, Hornef M, Teichert HM, Kirchner H. 1994. Sex difference in the serostatus of adults to the Epstein–Barr virus. Immunobiology 190:424–429.

West S, Hildesheim A, Dosemeci M. 1993. Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: Results from a case–control study. Int J Cancer 55:722–727.

Yang XR, Diehl S, Pfeiffer R, Chen CJ, Hsu WL, Dosemeci M, Cheng YJ, Sun B, Goldstein AM, Hildesheim A. 2005. Evaluation of risk factors for nasopharyngeal carcinoma in high-risk nasopharyngeal carcinoma families in Taiwan. Cancer Epidemiol Biomarkers Prev 14:900–905.

Yu MC, Ho JH, Lai SH, Henderson BE. 1986. Cantonese-style salted fish as a cause of nasopharyngeal carcinoma: Report of a case–control study in Hong Kong. Cancer Res 46:956–961.

Yu MC, Huang TB, Henderson BE. 1989a. Diet and nasopharyngeal carcinoma: A case–control study in Guangzhou, China. Int J Cancer 43:1077–1082.

Yu MC, Nichols PW, Zou XN, Estes J, Henderson BE. 1989b. Induction of malignant nasal cavity tumours in Wistar rats fed Chinese salted fish. Br J Cancer 60:198–201.

Yu MC, Garabrant DH, Huang TB, Henderson BE. 1990. Occupational and other non-dietary risk factors for nasopharyngeal carcinoma in Guangzhou, China. Int J Cancer 45:1033–1039.

Yuan JM, Wang XL, Xiang YB, Gao YT, Ross RK, Yu MC. 2000a. Non-dietary risk factors for nasopharyngeal carcinoma in Shanghai, China. Int J Cancer 85:364–369.

Yuan JM, Wang XL, Xiang YB, Gao YT, Ross RK, Yu MC. 2000b. Preserved foods in relation to risk of nasopharyngeal carcinoma in Shanghai, China. Int J Cancer 85:358–363.

Zeng Y, Zhang LG, Li HY, Jan MG, Zhang Q, Wu YC, Wang YS, Su GR. 1982. Serological mass survey for early detection of nasopharyngeal carcinoma in Wuzhou City, China. Int J Cancer 29:139–141.

Zeng J, Gong CH, Jan MG, Fun Z, Zhang LG, Li HY. 1983. 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, Huang YS, Huang NQ, Li JY, Wang YB, Jiang MK, Fang Z, Meng NN. 1985. Prospective studies on nasopharyngeal carcinoma in Epstein–Barr virus IgA/VCA antibody-positive persons in Wuzhou City, China. Int J Cancer 36:545–547.

Zheng X, Yan L, Nilsson B, Eklund G, Drettner B. 1994a. Epstein–Barr virus infection, salted fish and nasopharyngeal carcinoma. A case–control study in southern China. Acta Oncol 33:867–872.

Zheng YM, Tuppin P, Hubert A, Jeannel D, Pan YJ, Zeng Y, de The G. 1994b. Environmental and dietary risk factors for nasopharyngeal carcinoma: A case–control study in Zangwu County, Guangxi, China. Br J Cancer 69:508–514.

Zou J, Sun Q, Akiba S, Yuan Y, Zha Y, Tao Z, Wei L, Sugahara T. 2000. A case–control study of nasopharyngeal carcinoma in the high background radiation areas of Yangjiang, China. J Radiat Res (Tokyo) 41:53–62.

zur Hausen H, Schulte-Holthausen H, Klein G, Henle W, Henle G, Clifford P, Santesson L. 1970. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. Nature 228:1056–1058.