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The Correlation between Risk Factors and *Epstein-Barr* Virus Serum Antibody with Histopathological Typing of Nasopharyngeal Carcinoma

Awal Prasetyo[1]*, Udadi Sadhana[1], Dewi K. Paramita[2], Sofia Mubarika Haryana[2], Bambang Hariwiyanto[3], Soenarto Sastrowijoto[3], Totok Utoro[4]

[1]Department of Anatomic Pathology, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia; [2]Department of Histology, Molecular Biology Laboratory, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia; [3]Department of Otorhinolaryngology, Head and Neck Surgery, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia; [4]Department of Pathology, Faculty of Medicine Gadjah Mada University, Yogyakarta, Indonesia

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***Correspondence:** Awal Prasetyo, Department of Anatomic Pathology, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia, India. E-mail: awalpras@fk.undip.ac.id
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Abstract

BACKGROUND: The risk-combination of genetic or familial history, environmental risk factors, and EBV infection might cause nasopharyngeal carcinogenesis. The serum antibody for EBV IgA, namely, EBNA1+VCA-p18 has a good sensitivity as an early diagnostic test for nasopharyngeal carcinoma (NPC).

AIM: This study aims to determine the correlation between risk factors and histopathological typing of NPC and also the correlation between the IgA [EBNA-1 + VCA p-18] ELISA and histologic type.

METHODS: A cross-sectional method was used on 108 NPC patients which filled a questionnaire through an in-depth interview on the family condition to cancer, habit/lifestyle, and environmental risks. A total of 47 subjects were willing to donate blood samples for IgA [EBNA1 + VCA p-18] ELISA. Furthermore, Kendall's tau-b (τ) correlation test was performed on NPC keratin type (WHO-1) and non-keratin (WHO-2 and 3).

RESULTS: The results showed that the family history of non-keratinized NPC was associated with NPC WHO-3 as demonstrated by $\tau = 0.473$, as well as salt-eating with $\tau = 0.334$, smoked/grilled fish/meat eating $\tau = 0.205$, instant noodle-eating $\tau = 0.356$, consuming canned/packaged canned foods $\tau = 0.240$, and flavored food eating habits $\tau = 0.364$, along with passive smoking $\tau = 0.377$, and chronic nasopharyngeal infection $\tau = 0.530$. The IgA titers, namely, [EBNA1 + VCA p-18] ELISA for non-keratin type NPC was greater than the keratin type; however, it was not related to WHO-3 NPC as indicated by $\tau = 0.376$, and $p = 0.011$ put this underlying before however.

CONCLUSIONS: The positivity of IgA [EBNA-1 + VCA p-18] ELISA does not correlate with the non-keratin type histologic NPC, family history, as well as salt-eating, instant noodle, and flavored food eating habits, along with passive smoking and nasopharyngeal infection.

Introduction

Nasopharyngeal carcinoma (NPC) is a common malignant epithelium in populations of South, South-east, and East Asia, as well as North Africa [1], with increasing incidences yearly [2], [3]. In Indonesia, the incidence in 2000 ranged between 5 and 15/100,000 population, or 10,000–30,000 cases per year [4]. Furthermore, NPC is the second leading cause of head-and-neck cancer, with Type III being the most common [5].

The increase in incidence is inversely proportional to the rate of survival, NPC survival rate for 18 months is 79.33% [6], but after 24 months, it decreases to 24.2–30.3% [7]. Moreover, the survival rate for keratin NPC is 25%, while that of non-keratin ranges from 55 to 61% [8], [9].

The increasing incidence of NPC and the differences in survival by histopathology type reflects the magnitude of exposure to the etiology and risk. The etiologic and risk factor detection studies showed specific geographic, demographic, and age distribution patterns, as well as confirmed linkages with mutual roles between genetic susceptibility, environmental exposure, and epstein-barr virus (EBV) infections [10], [11]. In nasopharyngeal carcinogenesis, the complexity of the role-related relationship between genetic risk, EBV infection, habit/lifestyle factor, and environmental exposure has been demonstrated [11], [12], [13]. EBV reactivation of the nasopharynx is an important moment of nasopharyngeal carcinogenesis which is detected with immunoglobulin A (IgA) test against viral capsid antigen (VCA), namely, EBV [14]. The increasing IgA titers for early antigen, VCA, and Epstein-Barr nuclear antigen 1 (EBNA1) are characteristic of NPC; hence, the detection of seromarker combinations

is highly potent and useful for early diagnosis and prognosis [15], [16], [17], [18]. The identification of EBV antibody serum with a combination of EBNA1 and VCA-p18 IgA ELISA peptide markers developed in Indonesia has good sensitivity and specificity. It is also an economical and simple early diagnostic method [17], [18]. A positive EBV antibody titer is not always diagnosed with NPC and <5% of positive IgA individuals have NPC [13]. The amount of EBV which indicates the presence of NPC as measured by the EBV/IgA/VCA antibody status is 32.2% [19]. Aside from the viral risk, there is also a non-EBV role in nasopharyngeal carcinogenesis as preliminary studies identified positive IgA [EBNA1 + VCA p-18] in 85% of NPC cases and 15% was reportedly unrelated to EBV. Besides, those study also obtained a positive number in 45.5% of healthy individuals. The amount and the high level of antibody titers of EBV are thought to be associated with increased incidence of NPC. Furthermore, the reactivity of IgA [EBNA1 + VCA p-18] ELISA in the healthy population of Semarang and Makassar is higher than in Yogyakarta, due to ethnic, geographic, and dietary differences which lead to variations in the immune controls against EBV [13]. Aside from the virulence factor, the magnitude of EBV effect on nasopharyngeal carcinogenesis is also influenced by acquired or inherited genetic susceptibility and environmental exposure, this shows that positive reactivity to EBV does not always indicate NPC. Therefore, a quantitative instrument is needed to assess other non-viral risk factors as a seropositive EBV/IgA/VCA population filter in the endemic areas of NPC.

The non-EBV risk is evidenced by the influence of family relations, environmental exposure, and lifestyle on the occurrence of NPC by 2–6 fold [19]. The identification of non-EBV risk patterns in NPC cases among the Indonesian population are limited; hence, there is a need for further investigations. An understanding of the relationship between genetic susceptibility risk to both NPC and EBV immune responses, non-EBV risks including lifestyle and environmental exposures, integrated with the detection of EBV infection risk is expected to reinforce an etiologic and early NPC diagnosis.

This study was conducted on NPC patients by linking etiological identification and NPC risk factors with serological and histopathological parameters, to produce linkages between etiological diagnosis and NPC histopathology type.

Methods

A cross-sectional method was used to determine the correlation between cancer history in the family, as well as lifestyle and environmental exposure risk, with IgA [EBNA1+VCAp-18] ELISA and histopathology of NPC among 108 participants. All participants filled out questionnaires about the risk of habit/lifestyle and environmental exposure risk. The questionnaires were filled by investigators through semi-structured in-depth interviews to explore answers related to the participants' circumstances. The participants were encouraged to answer the open questions and the interviewer can repeat or clarify any question to dig up more answers using a questionnaire guide [20]. Meanwhile, the questionnaire was tested for validity by two ENT experts and two clinical epidemiologists, while a reliability test was conducted by several volunteers. A total of 47 subjects were willing to donate blood samples for ELISA examination.

Study samples

The samples were obtained consecutively, missing affiliation of the study and diagnosis, these include individuals diagnosed with histopathologic nasopharyngeal epidermoid carcinoma with histopathologic type determined according to the WHO standard or diagnostic criteria published in 2005. The examination was blindly performed by two anatomical pathologists. In addition, NPC histopathological type was divided into two groups, namely, NPC type WHO 1 (keratin) and NPC type WHO 2 and 3 (non-keratin).

Procedures

This study used paraffin blocks for NPC patients who performed rediagnosis of a double-blind PA examination by two anatomical pathologists. A sample is excluded when there is an error with the initial diagnosis, while the diagnostic agreement between the two observers was calculated using the kappa test. The subjects' medical records were tracked by first asking for permission, then only the necessary secondary data were recorded. Each subject's address was located, then an in-depth interview was conducted with the questionnaire material that has been approved by the Ethics Commission.

Questionnaire

The questionnaires of NPC's etiology and risk factors were developed with three main constructs, namely; genetic, lifestyle, and environmental exposure. The filled-questionnaires were tested by correlation analysis of product moment for the validity, and the reliability tests conducted with Cronbach's Alpha.

Family cancer risk history

Family history is a condition of subjects with a record of cancer in any of the family relations including

grandparents, parents, siblings, especially with head-and-neck, or nasopharyngeal cancer. This risk consists of not-at-risk/low-risk with scores ranging from 1 to 6 and medium-high risk between 7 and 13.clarify how scores be calculated and according to what?

Habit/lifestyle risk

The risk of habit/lifestyle was obtained from an in-depth interview using a questionnaire, these risks include active smoking, consumption of salted fish, pickles, alcohol, and nibbling, as well as instant noodle and preserved/flavored food eating habits. This each variable was graded as follows, not at risk-low risk with scores ranging from 1 to 86, and medium to high risk between 87 and 159. Clarify how scores be calculated.

Environmental exposure risk

The environmental exposure risk was obtained from an in-depth interview with a questionnaire on various sub-variable of NPC risk factors, such as passive exposure to cigarette smoke, exposure in the home environment to firewood smoke, and anti-mosquito repellent, as well as occupational exposure to wood dust, and solar smoke; and chronic infection of the nasopharynx. The unit of measurement includes not at risk-low-risk with scores ranging from 1 to 58, and low-high risk between 59 and 94. Clarify how scores be calculated.

Blood samples

Venous blood of 5 cc was used for the IgA [EBNA-1 + VCAp-18] ELISA examination. The blood collection protocol was carried out according to the laboratory standard procedure of PRODIA Laboratory Semarang to avoid the danger of infection and side effects due to the non-sterile actions performed. The blood sampling procedures, including plasma isolation and transportation, was carried out as follows; (a) blood was taken from the vein up to 5 ml, (b) it was inserted into a heparin tube or heparinized vacutainer, (c) the blood was mixed homogeneously with heparin 10 times, (d) the sample was centrifuged at 300 rpm or allowed to stand for several hours until the plasma separated, (e) the supernatant was isolated and then transferred into the microfuge tube, and (f) the plasma was stored at –20°C in a freezer, while the serum was sent to the Biomolecular laboratory of UGM Medical Faculty for examination of IgA antibody titers [EBNA-1 + VCAp-18] ELISA.

Titer IgA [EBNA1+VCA p-18] with two steps sandwich indirect ELISA

IgA titer [EBNA1 + VCA p-18] is a measurement of antibody response to EBV in blood serum examined using the two-step sandwich indirect ELISA method according to Fachiroh [17]. The examination was conducted at the Biomolecular Laboratory of UGM, Faculty of Medicine, and the result was obtained using ELISA reader OD spectrophotometer at 450 nm in duplo, the result is the average of two measurements. Furthermore, the NPC risk limits were determined using the value of cut ov value (CoV) obtained from the mean titer of three normal individuals plus the standard deviation [21], namely, 0.352. The variable measurement unit consists of two groups, with a cut limit value of 0.352.

The Two Steps Sandwich Indirect ELISA Procedures are as follows; combi coating well with EBNA1 consisting of 1μg/ml in 0.05 M Na_2Co_3 with pH 9.6 and 135–150 1μl/well, incubated for 2 h in 37°C. Combi coating discharge solution was coated with p18 consisting of 0.5 or 1μg/ml in 0.05 M Na_2Co_3 with pH 9.6 and 135–150 1μl/well, incubated at 4°C. Furthermore, 3% BSA was diluted in PBS which contain 8.2 g NaCl; 1.9 g $Na_2HPO_4.2H_2O$; 0.3 g $NaH_2PO_4.2H_2O$, with pH 7.3 and PBS-twen 20 (0.05%); Solution A consists of: PBS-tween 0.05%, 1% BSA; Na_2CO3 0.05 M pH 9,6 (HCl 1 M); TMB: TMB*2HCl stock in 6 mg/ml in DMSO (4°C); Working solution: 200 μl TMB stock, 12 ml 0.1 M Na acetate with pH 5.5; adjusted with 1 m HCl, as well as 12 μl 30% H_2O_2 freshly made and 1 M of H_2SO_4 9.26% in aquadest.

Statistical analysis

The data collected included *cleaning, coding,* and tabulation followed by processing, then they were tested for normality using Kolmogorov–Smirnov and grouped by their variables. Furthermore, the data were presented descriptively in tables, histograms, and line charts. Inferential analysis for the age and sex of the samples used a *two-sample t-test, Independent Samples Test,* and *Chi-square continuity* correction type, while NPC risk factor analysis used *Pearson* or *Continuity correction Chi-square*, with odds ratio calculation. Cross-tabulation of study group variables including age, gender, family history of cancer, lifestyle, and environmental exposure risk, as well as IgA [EBNA-1 + VCA-p18] ELISA titers against NPC histopathologic type was carried out using SPSS for Windows version 17.0.

Research ethics

This study obtained ethical approval from the Health Research Ethics Committee (KEPK) Faculty of Medicine, University of Diponegoro, and Kariadi Hospital, Semarang with the number (012/EC/FK/RSDK/2012).

Results

Characteristics of patients based on NPC histopathology type

One hundred and eight NPC patients who performed histopathology type rediagnosis consisting of five keratinized (WHO-1), 22 non-keratinized well-differentiated (WHO-2), and 81 non-keratinized poorly-differentiated (WHO-3). The ratio of men to women was 1.5: 1, 1.2: 1, and 2.4: 1 for NPC WHO-1, WHO-2, and WHO-3 with values of 4.6%, 20.4%, and 75% of total cases, respectively. Furthermore, the age range for WHO-1 NPC was between 30 and 60 years, while WHO-2 and WHO-3 are spread over all ages, with the highest frequency being 40–49 years. The Pearson Chi-square test showed no difference between gender and histopathology type with p = 0.358, as well as for age group p = 0.566, indicating that gender and age group do not correlate with the determination of NPC histopathology type as shown in Table 1.

Table 1: Frequency distribution characteristic of NPC patient (n = 108)

Characteristic	Histopathology Type NPC			Total f (%)	Different test/p-value
	WHO-1 f (%)	WHO-2 f (%)	WHO-3 f (%)		
Gender					
Male	3 (2.8)	12 (11.1)	57 (52.8)	72 (66.7)	X^2 = 2.055
Female	2 (1.9)	10 (9.3)	24 (22.2)	36 (33.3)	p = 0.358**
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	
Ages group					
<20th	0 (0.0)	2 (1.9)	1 (0.9)	3 (2.8)	X^2 = 8.646
20–29th	0 (0.0)	0 (0.0)	5 (4.6)	5 (4.6)	p = 0.566**
30–39th	1 (0.9)	5 (4.6)	15 (13.9)	21 (19.4)	
40–49th	1 (0.9)	8 (7.4)	27 (25.0)	36 (33.3)	
50–59th	1 (0.9)	3 (2.8)	20 (18.5)	24 (22.2)	
≥60th	2 (1.9)	4 (3.7)	13 (12.0)	19 (17.6)	
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	

**Pearson Chi-square (X^2), p > 0.05 (not significant).

The relationship between npc risk factors and histopathologic types

A total of 108 subjects diagnosed with NPC consisted of WHO-1 with 4.6%, WHO-2 of 20.4%, and WHO-3 with 75.0%. Given that the proportion of keratinized type NPC (WHO-1) to non-keratinized (WHO-2 and WHO-3) was not comparable, the analysis was performed on all three types. Various risk factors were significantly tested for correlation with NPC histopathology type. These include the family history of cancer, smoked/grilled fish/meat, instant noodle, preserved/canned food, and flavored food eating habits, as well as passive smoke exposure and risk of chronic nasopharyngeal infection. The relationship between NPC risk factors and histopathology type was analyzed with Kendall's tau-b (τ) correlation coefficient. Based on the results, NPC histopathological type correlated with family history of cancer with τ = –0.473, salted fish τ = –0.334, bacon/roasted meat τ = –0.205, instant noodle τ = –0.356, and flavored food eating habits τ = –0,364, as well as passive smoking exposure τ = –0.377, and risk of nasopharyngeal chronic infection with τ = –0,530 as shown in Table 2. The association was by negative what does this mean? The most of patients have low risk even with the WHO 3. The risk of above-mentioned variables ranged from negatively weak to strong, according to cut ov value (CoV) 0.352, with NPC histopathology type, and any increased risk might potentially lead to the WHO-3.

Table 2: The connection between risk factors and NPC histopathology type (n = 108)

Variable	Histopathology tipe			Total f (%)	Correlation test/p-value
	WHO-1 f (%)	WHO-2 f (%)	WHO-3 f (%)		
Family cancer history					
No risk-low risk	4 (3.7)	19 (17.6)	71 (65.7)	94 (87.0)	τ = –0.473*
Mid-high risk	1 (0.9)	3 (2.8)	10 (9.3)	14 (13.0)	p = 0.00
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	
Salted Fish eating habit					
No risk-low risk	4 (3.7)	19 (17.6)	65 (60.2)	88 (81.5)	τ = –0.334*
Mid-high risk	1 (3.0)	3 (2.8)	16 (14.8)	20 (18.5)	p = 0.00
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	
Smoked/grilled fish/meat eating habit					
No risk-low risk	4 (3.7)	21 (19.4)	64 (59.3)	89 (82.4)	τ = –0.205*
Mid-high risk	1 (3.8)	1 (3.8)	17 (15.7)	19 (17.6)	p = 0.008
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	
Instans noodle eating habit					
No risk-low risk	4 (3.7)	21 (19.4)	61 (56.5)	86 (79.6)	τ = –0.356*
Mid-high risk	1 (0.9)	1 (0.9)	20 (18.5)	22 (20.4)	p = 0.00
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	
Preserved/canned food eating habit					
No risk-low risk	4 (3.7)	20 (18.5)	74 (68.5)	98 (90.7)	τ = –0.240*
Mid-high risk	1 (0.9)	2 (1.9)	7 (6.5)	10 (9.3)	p = 0.002
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	
Flavored food eating habit					
No risk-low risk	4 (3.7)	20 (18.5)	65 (60.2)	89 (82.4)	τ = –0.364*
Mid-high risk	1 (0.9)	2 (1.9)	16 (14.8)	19 (17.6)	p = 0.00
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	
Drinking alcohol					
No risk-low risk	2 (1.8)	16 (14.8)	74 (68.5)	92 (85.2)	τ = –0.366*
Mid-high risk	1 (0.9)	1 (0.9)	14 (12.9)	16 (14.8)	p = 0.00
Total	3 (2.7)	17 (15.7)	88 (81.5)	108 (100.0)	
Passive cigarette smoke exposure					
No risk-low risk	3 (2.8)	19 (17.6)	65 (60.2)	87 (80.6)	τ = –0.377*
Mid-high risk	2 (1.9)	3 (2.8)	16 (14.8)	21 (19.4)	p = 0.00
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	
Nasopharyngitis chronic					
No risk-low risk	3 (2.8)	19 (17.6)	64 (59.3)	86 (79.6)	τ = –0.530*
Mid-high risk	2 (1.9)	3 (2.8)	17 (15.7)	22 (20.4)	p = 0.00
Total	5 (4.6)	22 (20.4)	81 (75.0)	108 (100.0)	

*Kendall's tau-b (τ) Coefficient, significant corelation on level 0.01 (two-tailed).

Differences of IgA titers [EBNA-1 + VCA p-18] ELISA on NPC

A total of 47 NPC patients were examined for IgA [EBNA-1 + VCA p-18] ELISA titers as shown in Table 3. Based on the results, the IgA titer positive ELISA with ≥0.352 was more distributed in males, namely, 72.3% than in females with 23.4%, in other words, the male to female ratio was 3:1. Furthermore, the number of sufferers aged <40 years who expressed

Table 3: The different of titer IgA [EBNA-1+VCA p-18] ELISA on NPC (n = 47)

Characteristic	IgA [EBNA-1+VCA p-18] ELISA		Total f (%)	Different test/p-value
	Negative (<0.352)	Positive (≥0.352)		
Gender				
Male	1 (2.1)	34 (72.3)	35 (74.5)	X^2 = 0.00
Female	1 (2.1)	11 (23.4)	12 (25.5)	p = 1.00*
Total	2 (4.3)	45 (95,7)	47 (100.0)	
Ages group				
<20th	0 (0)	1 (2.1)	1 (2.1)	X^2 = 11.937
20–29th	0 (0)	3 (6.4)	3 (6.4)	p = 0.0360**
30–39th	0 (0)	10 (21.3)	10 (21.3)	
40–49th	0 (0)	19 (40.4)	19 (40.4)	
50–59th	0 (0)	7 (14.9)	7 (14.9)	
≥60th	2 (4.3)	5 (10.6)	7 (14.9)	
Total	2 (4.3)	45 (95.7)	47 (100.0)	

*Continuity correction Chi-square test (X^2_{Yates}), p > 0.05 (not significant), **Pearson Chi-square (X^2), p < 0.05 (significant).

positive ELISA count was 14 or 29.8%, while 31 or 57.4% were ≥40 years. The age group between the IgA positive and negative ELISA was significantly different (Pearson Chi-square, X2 = 11,937, and p = 0.036). The mean age group influenced IgA positivity, the older the age, the higher the tendency to get a positive titer. How and the only two negative results were <60 years? The results also showed 45 cases of NPC with IgA positive ELISA, of which 35 (74.5%) were WHO-3, or had no correlation as indicated by Kendall's tau-b = 0.170, and p = 0.239 (Table 4). The relationship between IgA ELISA with NPC keratin and non-keratin was obtained by Kendall's tau-b correlation coefficient namely 0.376 and p = 0.011, indicating that IgA [EBNA-1 + VCA p-18] ELISA positivity did not correlate with the determination of non-keratin NPC histologic type (Table 5).

Table 4: The correlation of IgA [EBNA-1+VCA p-18] ELISA and NPC histopathology type (n = 47)

IgA [EBNA-1+VCA p-18] ELISA	NPC histopathology type			Total f (%)	Correlation test/p-value
	WHO-1 f (%)	WHO-2 f (%)	WHO-3 f (%)		
Negative (<0.352)	1 (2.1)	0 (0.0)	1 (2.1)	2 (4.3)	
Positive (≥0.352)	2 (4.3)	8 (17.0)	35 (74.5)	45 (95.7)	τ = –0.170
Total	3 (6.4)	8 (17.0)	39 (76.6)	47 (100.0)	p = 0.239

Kendall's tau-b (τ) Coefficient, significant corelation on level 0.01 (two-tailed) (p > 0.05, not significant).

Table 5: The correlation of IgA [EBNA-1 + VCA p-18] ELISA and keratinized NPC and non keratinized NPC (n = 47)

IgA [EBNA-1 + VCA p-18] ELISA	NPC histopathology type		Total f (%)	Correlation test/p-value
	Keratin WHO-1 f (%)	Non keratin WHO-2 and WHO-3 f (%)		
Negative (<0.352)	1 (2.1)	1 (2.1)	2 (4.3)	
Positive (≥0.352)	2 (4.3)	43 (91.5)	45 (95.7)	τ = –0.376
Total	3 (6.4)	44 (93.6)	47 (100.0)	p = 0.011*

Kendall's tau-b (τ) Coefficient, significant corelation on level 0.01 *(two-tailed)* p < 0.05, significant).

Discussion

Ethnic and geographic

NPC histology type is influenced by ethnicity and geographic variation, but the ethnic aspect cannot be proven in this study as the majority of subjects, namely, 99% were Javanese. The geographical location particularly the subjects' residence was majorly in the lowlands with 52%, followed by highlands 28.9%, and the coast 15.6%. This variable is presumably related to the geographic distribution of EBV infection and risk factors supporting NPC etiology [22]. The previous studies have proven the association between squamous cell NPC with EBV, which is influenced by geographic variation [23]. In the United States, where WHO-1 NPC cases (39.4%) were higher than WHO-3 (25%), the incidence of NPC in ethnic Chinese was mostly WHO-3 (58%) and WHO-2 (55.9 %). Moreover, NPC WHO-1 is more predominant among the whites (Caucasian) with 55.4%, African-American 44.4%, and Hispanic 41.3% [24] old study since 2007. Try to update.

Family history

Based on the results, undifferentiated WHO-3 was the most common nasopharyngeal mucosal malignancy with 75%; hence, it was concluded that medium-high risk related to the family history of cancer can lead to this histologic type NPC with τ = 0.473. Furthermore, a significant association between familial risk and histologic type NPC WHO-3 adds suitable punctuation, improper syntax was more prevalent in men <40 years of age. Similar to other types of cancers, for example, breast cancer, familial risk of NPC also determines its histologic character. Meanwhile, an increased risk of cancer in NPC families is also associated with other comorbidities associated with viral infections [25]. Among the whites, familial NPC cases are often presented histologically as WHO-2 or WHO-3, while the WHO-1 type is more dominant in non-familial NPC [26].

The proportion of males to females with WHO-3 NPC was 2.4: 1, this result is similar to Zheng *et al*., (1994) [27], which reported that the ratio of men to women with WHO-3 was 2.7: 1. Furthermore, the WHO-3 NPC frequency distribution in the <40 years age group, namely, 25.9% was significantly different compared to 48.8% obtained by Zheng *et al*., (1994) [27]. Syntax error. reformulate In this study, WHO-3 NPC cases were more widely distributed in the >40 years age group indicating a long or slow process of carcinogenesis. This result shows that family history of cancer is strongly associated with NPC WHO-3, suggesting that the low incidence in this study compared to Zheng's was due to late diagnosis. What the relation between family history and late diagnosis?

Based on the results, it was concluded that the family history of cancer correlates positively with NPC WHO-3 (τ = 0.473) ADD in comparison to WHO 1 and 2, AS 65% are associated with low risk. The familial risk pattern that determines the differentiation trend of nasopharyngeal malignancy in the non-keratinized histologic type of NPC has not been inferred in previous publications. However, it has been proven that the family history of cancer can determine the histologic character of a malignancy. In addition, there is a link between histologic types of mammary carcinoma and family history of cancer [28].

Habit/lifestyle risk

The high incidence of WHO-3 NPC in the study by Zheng (Zheng *et al*., 1994) [27], was due to the habitual consumption of salted fish, especially since the age of five. Similarly, this study also proves that the consumption of salted fish in childhood specifically <10 years is more likely to cause NPC. Moreover, the risk pattern of salted-fish eating habits tends to produce WHO-3 as indicated by τ = 0.334. The result also showed that 31 out of 37 NPC patients had started the

habit of eating salted fish since they were <10 years old. Additional data from other studies in China found that early salted fish exposure in under-five children and inherited susceptibility is more prevalent in cases of NPC among the <50 years age group [29]. These facts further reinforce the hypothesis of the age shift in NPC incidence toward the younger age groups, due to potential synergies between at least two risk factors, namely, inherited susceptibility and habitual eating of salted fish.

The large proportion of undifferentiated and non-keratin NPC cases, namely, 95.4% which is similar to the results of the previous studies conducted in South Asia and other high-risk areas [30], improper syntax. Reformulate raises the question of a potential link between NPC histologic type with alcohol consumption and smoking habits [31], [32]. The results also prove that the passive exposure of cigarette smoke is strongly associated with the occurrence of NPC WHO-3 as demonstrated by $\tau = 0.377$. Meanwhile, Yu in [33] reported that increased NPC risk is twofold in connection to cigarette smoke exposure from a family member compared to smoking from birth until the age of <45, although this result was not supported by other studies [33]. In further developments, Zhu [34], [35] suggest a different profile of NPC risk factors based on histologic type, mainly due to the risk of smoking and excessive alcohol consumption. Both studies concluded that the two habits were more related to WHO-1 NPC, especially in individuals >50 years which account for 70% of all cases. Smoking habits and alcohol consumption potentially lead to WHO-1 type NPC in nearly two-thirds of cases in non-endemic areas; hence, the current trend in its incidence is probably due to the decreased smoking habits [36]. Several previous studies found a significant association between smokers and the incidence of well-differentiated NPC type [36].

The results showed significant differences with $p = 0.011$ in alcoholic drinking habits, between the case and control group. The pattern of increased risk associated with alcohol consumption was insignificant, although this can also be a confounding effect of cigarette smoke, since alcohol and smoking habits are often inseparable. The weak association between smoking and drinking habits indicated by the low risk of WHO-1 type NPC is consistent with the likelihood of a greater role of EBV infection. This implies that the WHO-1 NPC that occurs at a young age might have a very different etiology compared to those in older individuals. Based on this evidence, it was concluded that alcohol consumption habits are not a risk factor for NPC in some areas, especially for the undifferentiated type [Table 2].

The differences in the association between exposure risk and drinking habits with NPC histology type which is consistent with the previous studies further confirm the absence of consensus on the relationship [13], [38]. The absence of a reference to the relationship between age factor, as well as the cessation of smoking and drinking habits with decreased incidence of NPC, also makes this issue unsolved [13].

The results showed a slight relationship between increased NPC risk and the diagnosis of nasal polyps, sinusitis, or recurrent epistaxis, as well as chronic nasal and ear diseases [33]. The inflammatory process does not explain the association with EBV infection, especially for the determination of histologic NPC type. EBV infection at a younger age can modify the virus thereby causing carcinogenic effects, while in old age, it is associated with decreased risk of NPC [39], [40]. Furthermore, EBV infection is consistent with an increased risk of NPC in low-educated individuals, or low socioeconomic status [29], but non-keratin and undifferentiated NPC linkages with EBV infection are inferred inconsistently. Based on the results, the risk of chronic nasopharyngeal infection is strongly associated with WHO-3 type NPC as demonstrated by $\tau = 0.530$. According to several reports, nearly 90% of the world's population have EBV infection, this indicates that the infection is closely related to the determination of WHO-3 NPC histologic differentiation patterns. In this study, it was demonstrated that the risk of chronic nasopharyngeal infection correlates strongly ($\tau = 0.680$, $p = 0.00$) with serological markers of latent EBV infection, namely, IgA [EBNA-1 + VCA p-18] ELISA. Meanwhile, the positivity of IgA titers determines the histological type of non-keratin NPC with $\tau = 0.376$, $p = 0.011$.

It was also concluded that NPC WHO-3 is associated with habits such as eating smoke/grilled fish or meat with $\tau = 0.205$, instant noodles $\tau = 0.356$, preserved/canned food $\tau = 0.240$, and flavored food with $\tau = 0.364$. At present, no publication specifically discloses the association between these risk variables and the incidence or type of NPC histology. However, the prevalent use of formalin (formaldehyde solution) as a preservative of various foods including meatballs, tofu, noodles, salted fish, etc. in Indonesia justifies the presence of a linkage with these risks. The observed trend between the above-mentioned risk variables and NPC WHO-3 is different from that of Vaughan [35] who stated that exposure to formaldehyde in the work environment is associated with an increased risk of developing squamous type NPC (WHO-1). Therefore, it was concluded that EBV infection factors are also associated with the distribution of viruses in certain geographical areas, ethnicities including China, and Java, family history of cancer, men >40 years old, as well as NPC risk factors, namely, consumption of Salted fish since age <10 years, passive smoke exposure, and non-keratin NPC histologic type.

IgA titers [EBNA1 + VCA p-18] are positively associated with NPC

Specific reactive antibody detection techniques for EBV were developed from a combination of two synthetic peptides, namely, EBNA1 and VCA-p18 using the ELISA method by Fachiroh

et al. (2006) [17]. In the Yogyakarta population, it had a sensitivity of 90.1% and specificity of 85.4%, as well as positive and negative predictive values of 78.7% and 93.9%, respectively. The previous studies conducted in several Indonesian cities concluded that there was a difference in the reactivity of IgA [EBNA1 + VCA-p18] between the sample population with a mean of 91.5% sensitivity and 76.3% specificity in the combined panel. This study obtained similar results with a sensitivity of 95.7%, specificity of 77.1%, and AUC on the ROC curve of 86.4%, indicating that the IgA [EBNA-1 + VCA p -18] ELISA correlates positively with NPC diagnosis as demonstrated by X2 = 53.86, and p = 0.00. Furthermore, IgA ELISA examination provides non-invasive diagnostic support information, to complement the history and clinical test of suspected NPC cases. The combination of targeted risk-based history, physical examination, and non-invasive investigation by measuring IgA titers of ELISA can further enhance the accuracy of early diagnosis of NPC. In this study, 95.7% of NPC cases compared to 22.9% healthy individuals showed positive results for IgA [EBNA-1 + VCA p-18] ELISA titers, with Continuity correction Chi-square test (X2Yates) = 53,855, and p = 0.00, which implies that EBV infection is closely related to the occurrence of NPC. These results are similar to those of Wei-Hua Jia [41] who obtained 96.3% of NPC cases compared to 17% healthy controls that were IgA VCA positive.

Studies in Tunisia reported that in younger NPC patients, the antibody response of IgG and IgA anti-EBV was lower compared to that of the adults [16]. The study found that IgA [EBNA-1 + VCA p-18] ELISA titer tends to be higher in older age as demonstrated by X2 = 9.176, and p = 0.0020; however, there was no difference between the men and women ratio. A previous study also showed that patients with the NPC remission phase often experience a decrease in the reactivity of the antibody response compared to patients who are persistent or recurrent [16]. Consequently, it was concluded that immunoserology IgA [EBNA-1 + VCA p-18] ELISA is potentially reliable as a routine diagnostic marker of NPC, especially due to its ability to exclude negative outcomes.

As a serological marker of EBV infection, IgA [EBNA1 + VCA p-18] ELISA titers showed no difference between males and females but tended to be higher in older age. This study is in line with Dardari in [42], which stated that EBV-associated NPC generally occurs in adulthood, especially in areas with high incidence. However, other studies suggest that there is no association between EBV latency and gender, age, and ethnic origin, as well as stage or survival [43], [44]. EBV infection plays a significant role in the non-keratin type NPC pathogenesis (WHO-2 and WHO-3), while keratinized NPC squamous type is less associated (Niedobitek, Herbst and Young, 1991).put it in proper form of citation This hypothesis is supported by the results obtained in this study, which demonstrated a positive relationship between IgA [EBNA-1 + VCA p-18] ELISA titers and histologic NPC non-keratin type with τ = 0.376, and p = 0.011, in other words, the positivity of IgA ELISA is associated with histologic NPC non-keratin type.

Conclusion

Based on the results, the moderate-high risk of family history, along with eating habits such salty food, smoked/grilled fish/meat, instant noodles, canned/packaged foods, and flavored food, as well as passive smoke, and exposure to chronic nasopharyngeal infection for non-keratinized NPC types was greater than the keratinized and was associated with WHO-3 NPC. Moreover, IgA titers [EBNA1 + VCA p-18] positive ELISA for non-keratin type NPC was greater than the keratin; however, it was not related to WHO-3 NPC.

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