Evaluation of Serum IgA Antibodies to Epstein-Barr Virus Early and Viral Capsid Antigens in Nasopharyngeal Carcinoma

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Evaluation of Serum IgA Antibodies to Epstein-Barr Virus Early and Viral Capsid Antigens in Nasopharyngeal Carcinoma

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Abstract

Nasopharyngeal carcinoma (NPC) has a unique geographic distribution, with endemic areas in southern China and intermediate incidence rates in Southeast Asia including Indonesia. NPC is the most common head and neck cancer in Indonesia. The cause of NPC is suspected to be an interaction of Epstein-Barr virus (EBV) infection, genetic susceptibility and environmental factors. EBV infection has been shown to play an important role in the cause of NPC. EBV DNA and anti-EBV can be detected in most NPC patients in endemic area, making EBV-related antibody as important non-invasive test for nasopharyngeal carcinoma. The objectives of this study were to investigate serum level and prevalence of EBV EA-IgA and VCA-IgA in nasopharyngeal carcinoma patients in West Sumatra, Indonesia. Methods: A total of 15 untreated nasopharyngeal carcinoma patients in Dr. M. Djamil General Hospital Padang were recruited, in parallel with 15 healthy individuals as controls. Serum EA-IgA and VCA-IgA levels were measured by enzyme-linked immunosorbent assay (ELISA). Positive criteria were: EA-IgA > 8 U/ml, VCA-IgA > 8 U/ml. Results: The prevalence of serum EA-IgA and VCA-IgA in NPC patients (66.7% and 80.0%) was significantly higher than in the control groups (0.0% and 0.0%) (p<0.05). Nasopharyngeal carcinoma patients had higher EA-IgA levels (114.705±136.524) compared to healthy controls (1.749±0.498) (P<0.05) and higher VCA-IgA (mean 22.958 ±16.919) compared to healthy controls (1.571±0.572) (P<0.05). Conclusions: The IgA antibodies level to Epstein-Barr EA and VCA were elevated in nasopharyngeal carcinoma patients and were more prevalence in NPC patients than those in the controls.

Keywords: nasopharyngeal, carcinoma, Epstein-Barr virus, IgA antibody

1. Introduction

Nasopharyngeal carcinoma (NPC) is a head and neck squamous cell malignancy that is unique because of its etiology and distribution are influenced by geographic and ethnicity in the world. [1] According to data of the International Agency for Research on

Cancer (IARC), there are around 80,000 new cases of NPC diagnosed throughout the world in 2002. [1] The incidence of NPC is high in South China, especially in Guangdong and Guangxi provinces which reaches 50/100,000 population per year and intermediate incidence rates in Southeast Asia.[1-3]

Indonesia is one of the countries with intermediate prevalence of NPC. [3] The prevalence of NPC in Indonesia is estimated at around 6.2/100,000 populations per year. Based on Hospital-Based Cancer Registry data at the Dharmais National Cancer Center in 2003, NPC ranks first of all primary malignant tumors in men and 8th in women. [4] Nasopharyngeal carcinoma was also the most common head and neck malignancy found in Dr. M. Djamil Padang. In 2006-2008 in West Sumatra based on data from the Pathology Laboratory, the Faculty of Medicine, Andalas University had obtained 45 cases diagnosed with NPC. [3] Rahman S [5] also reported that there were 38 new cases during the period of July 2010 to June 2012 who were treated at the Dr. M. Djamil Hospital in Padang.

The etiology of nasopharyngeal carcinoma is thought to be an interaction of Epstein-Barr virus (EBV) infection, genetic and environmental factors. [6] Epstein-Barr infection has been reported as important factor in the process of NPC. [7] This relationship is evidenced by the presence of antibody against EBV in the majority of NPC patients and the discovery of DNA and RNA or EBV protein in tumor cells taken from biopsies. [7, 8] Although in general most people are infected by EBV, only a small proportion of the population suffer from NPC. This is because many factors can cause EBV activation, such as genetic, carcinogenic, environmental and /or immunodeficiency. [9]

Nasopharyngeal carcinoma is difficult to diagnose early because of its hidden location and in the early stages, there is generally no typical clinical symptoms so patients do not seek for treatment. Most patients with NPC present in advanced stage with enlarged lymph nodes in the neck (regional metastasis). [9] Rahman S [5] reported from 38 cases of NPC diagnosed in July 2010 to June 2012 at Dr. M. Djamil Padang, enlargement of the neck lymph nodes is most commont symptom complained by patients (97.3%).

Some researchers have advocated serology to detect antibody to EBV as early detection to diagnosis of NPC. [10] A number of studies have reported the use of anti-EBV antibodies for early detection and prediction of prognosis in NPC patients. The use of anti-VCA and anti-EA IgA as a tumor marker for NPC patients in endemic areas has been recommended by several researchers.[11-13] The a combination of anti-VCA IgA with anti-EA IgA can be used for screening NPC patients in endemic areas in order to diagnose NPC at an early stage and be useful as a prognostic treatment for NPC.[14,15-18] Wong [16] who conducted serum studies from 164 cases of NPC in

Sarawak concluded that the use of a combination of two serological markers would increase the diagnosis of NPC, where the use of anti-VCA IgA with anti-EA IgA had a sensitivity of 83.6% and specificity of 97.3 %. A similar conclusion was stated by Hsien YC [11] in his study which stated that anti-VCA and anti-EA IgA has high sensitivity and specificity in 38 cases of NPC in Brunei Darussalam. However, a study in Iran by Nikakhlagh [19] found only 3 out of 60 cases of NPC with anti-VCA IgA-positive.

In addition to geographic variations, some ethnic groups also have a tendency to suffer from NPC, Indonesia consists of various ethnicities with different lifestyles and habits, the risk of NPC can vary according to ethnic groups, therefore it is necessary to know the risk factors of NPC for each ethnic, so the role of EBV infection in NPC in Indonesia can be seen in efforts to prevent this disease. West Sumatra is a province in Indonesia with a majority ethnic Minangkabau population with specific habits.

The objective of this study was to investigate serum level and prevalence of EBV EA-IgA and VCA-IgA in nasopharyngeal carcinoma patients in West Sumatra, Indonesia. To achieve this goal, we measured serum EA-IgA and VCA-IgA levels of untreated NPC patients and matched control in Dr. M. Djamil Hospital. Details of the obtained results are discussed in the following sections.

2. Methods

The study was conducted at the Department of Otorhinolaryngology Head and Neck Surgery Dr. M. Djamil Hospital Padang. The case population was nasopharyngeal carcinoma patients who had been diagnosed based on histopathological result. Blood samples were obtained before specific therapy, while the control sample is matched for gender and age ($\pm$ 3 years). The study was approved by the Ethics Committee of Faculty of Medicine Andalas University, Padang, Indonesia (No. 119/KEP/FK/2015).

Serum EA-IgA and VCA-IgA levels were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits (IBL-International, USA). The study was conducted in accordance with the manufacturer's test intructions (IBL-International, USA). Positive criteria were: EA-IgA > 8 U/ml, VCA-IgA > 8 U/ml.

3. Results

A total of 15 untreated nasopharyngeal carcinoma patients in Dr. M. Djamil General Hospital Padang were recruited, in parallel with 15 healthy individuals as controls. The characteristics of the patients were presented in Table 1.

TABLE 1: Clinicopathological characteristic of patients and controls.

Characteristics	Patients (%)	Controls (%)
Total number	15	15
Age in years (range)	44.6 (16-71)	44.5 (17-72)
Gender		
Male	9 (60.0)	9 (60.0)
Female	6 (40.0)	6 (40.0)
Histopathologic subtype		
Keratinizing	0 (0)	-
Differentiated nonkeratinizing	4 (26.7)	-
Undifferentiated nonkeratinizing	11 (73.3)	-

The antibody response to EBV, positive serology (>8 U/ml) anti-VCA IgA was found at 12 NPC (8%) and no positive serology in the control. Positive of Epstein-Barr virus anti-EA IgA was found at 10 NPC respondents (66.7%) and the controls did not reveal any positive serology. Serological results can be seen in Table 2.

TABLE 2: The results of anti-EA IgA and anti-VCA IgA serology.

Antibodies	NPC frequency (%)	Control frequency (%)
Anti-EA IgA		
Positive	10 (66.7)	0 (0)
Negative	5 (33.3)	15 (100)
Anti-VCA IgA		
Positive	12 (80)	0 (0)
Negative	3 (20)	15 (100)

The mean level of anti VCA IgA in NPC was 22.958 ± 16.919 U/ml while the control was 1.571 ± 0.572 U/l. There was a statistically significant difference ($p < 0.05$) the mean value of anti VCA EBV IgA levels between NPC patients and controls, the differences can be seen in Fig. 1.

The mean level of Epstein-Barr virus anti-EA IgA in NPC respondents was 114.705 ± 136.524 U/ml and the control is 1.749 ± 0.498 U/ml. There are statistically significant differences ($p < 0.05$) the mean value of anti- EA EBV IgA levels between NPC and controls.
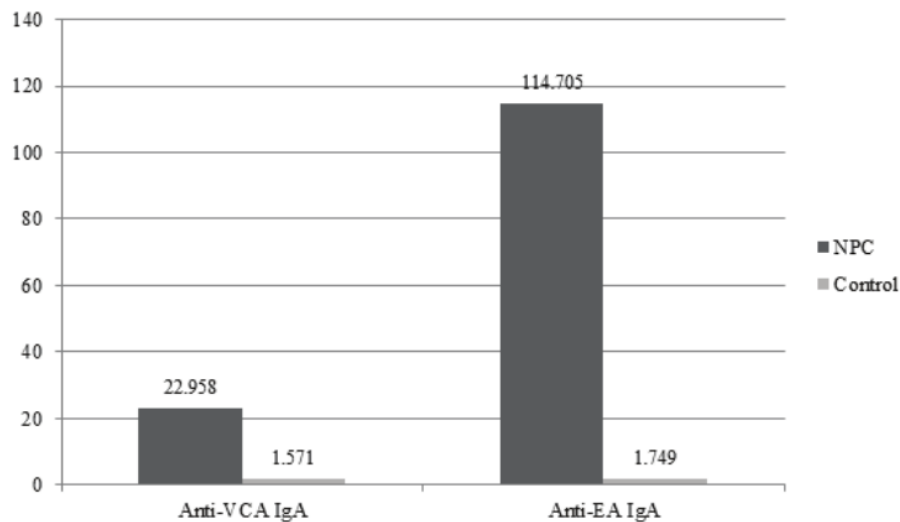
Figure 1: The Average levels of anti VCA-IgA and anti-EA IgA levels (U/ml) in NPC and control.

4. Discussions

4.1. VCA antibodies to Epstein-Barr virus

Anti EBV IgA levels have a wide range in NPC because they can be affected by the stage of the tumor. In this study, the lowest level of anti-VCA IgA in patients with NPC was 3.732 U/ml and the highest was 55.946 U/ml. The same thing was also reported by Widodo et al. [20] who found the lowest levels anti-VCA IgA at 34 cases of NPC were 4.36 U/ml and the highest is 119.4 U/ml and this result is higher compared to the controls (2.6-9.2 U/ml).

In this study, the level of anti-VCA IgA levels was 22.958 ± 16.91 9 U/ml were higher when compared to control, this difference is significant ($p < 0.05$). This is consistent with other studies such as Widodo et al. [20] who examined 34 cases of NPC with anti-VCA IgA in Surabaya's Soetomo Hospital, Cai YL et al. [22] and Shao JY et al. [21]

Lymphocyte cell infiltration in NPC contributes significantly to increased levels of antibodies to EBV lithic antigens such as EA and VCA. [25] Some literature states that an increase in anti-EBV IgA levels in NPC is also influenced by factors such as the duration of clinical onset and tumor stage. [19, 21, 26] Increased levels of IgA anti EBV is expected to begin detected after 3 years of clinical onset. This is also seen at an advanced stage and is associated with the size of the primary tumor due to the large number of lymphocyte infiltration in tumor tissue. [10, 20, 27, 28]

The absence of anti-VCA IgA levels against EBV at the beginning of the process of epithelial change to NPC because in the early stages of nasopharyngeal epithelial changes, environmental factors and habits are more responsible for changes in the nasopharyngeal epithelium which is then followed by EBV activation process. [29] Activation of EBV results in a viral replication process (lytic process) when VCA and EA are expressed. [23, 27] Therefore, morphological changes in epithelium such as metaplasia, insitu carcinoma and micro-invasive carcinomas that can be found in the early stages of NPC usually have not shown an increase of anti-EBV antibodies. [23] In addition, IgA levels are also influenced by the patient's immunological, racial/genetic and geographic conditions. [13]

4.2. EA antibodies to Epstein-Barr virus

In this study, positive anti-EA IgA antibodies were found at 10 NPC (66.7%), while controls did not reveal any positive serology. Elevated levels of anti-EA serum IgA were also reported by Tsang RKY et al. [18] who reported that there were positive anti-EA IgA levels in 67.9% of 215 of NPC.

Increased levels of IgA in patients with NPC vary depending on the stage of the tumor. Increased levels of anti-EA EBV IgA in NPC are caused by the expression of EBV replication products in the lytic phase, where the first EBV product released is EA. [25, 28] Another factor that also affects the increase in anti-EBV IgA levels is the length of clinical onset and tumor stage. [19, 21, 26] Increased anti-EA EBV IgA is seen at an advanced stage because it is associated with the neck lymph nodes enlargement, lymphocyte cell infiltration infected by EBV will increase in large size lymph node.[21,26-28]

Our study reveals that the IgA antibodies level to Epstein-Barr EA and VCA were elevated in nasopharyngeal carcinoma patients and were more prevalence in NPC patients than those in the controls. These results suggest that serum VCA-IgA with EA-IgA can be used for screening of NPC patients.

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