The Serological Examination of Immunoglobulin A Anti-Early Antigen of Epstein–Barr Virus (Anti-EA EBV IgA) in the Nias Tribe Nasopharyngeal Cancer Patients

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Abstract

BACKGROUND: Nasopharyngeal cancer (NPC) is a health problem associated with malignancies of the ear, nose, and throat. The main pathogenesis of NPC is Epstein–Barr virus (EBV) infection. The signs and symptoms of NPC are non-specific, causing the occurrence of delay in diagnosis leading to treatment failure. The early detection based on the NPC profile using immunoglobulin A (IgA) antibodies against the early antigen (EA) can be done, but many factors affect anti-EA EBV IgA levels, one of which is ethnicity.

AIM: This study aimed to compare levels of anti-EA EBV IgA in NPC patients and non-NPC in the Nias tribe.

MATERIALS AND METHODS: This study was cross-sectional involving 29 NPC patients and 29 non-NPC in the Nias tribe. The subjects of the study were blood tests to measure the levels of anti-EA EBV IgA by a serological test. Data were analyzed by Chi-square test.

RESULTS: The results showed that the mean value of anti-EA EBV IgA levels is 246.22 ± 320.05 U/mL and the proportion of positive serology tests is 10.3% in non-NPC. The comparison of mean anti-EA EBV IgA in blood samples (dry) can be a measure of detection of serum EBV immunoglobulin A (IgA). The results of different EBV antibody profile between NPC patients and non-NPC was significant (p < 0.001). The comparison of positive and negative serology tests of anti-EA EBV IgA between NPC patients and non-NPC was significant (p < 0.05).

CONCLUSION: The serology tests of anti-EA EBV IgA play a role in differentiating NPC patients from non-NPC, so it can be useful as a screening tool for NPC in the Nias tribe.

Introduction

Nasopharyngeal cancer (NPC) is one of the health problems associated with malignancies of the ear, nose, and throat (ENT). NPC is a throat cancer that occurs in the outer layer of the nasopharynx with a predilection for Rosenmuller fossa in the nasopharynx which is a transitional area, where the cuboidal epithelium changes to squamous epithelium [1], [2]. Globally, NPC is uncommon cancer and the incidence of NPC is around 80,000 new cases reported per year, which is 0.7% of all cancers that occur globally in 2014 [3]. In 2018, the highest incidence of NPC occurred in Southeast China at 40–50 cases per 100,000 population [1]. The incidence of NPC ranks eighth of the types of cancer that occurs in Indonesia, namely, 6.6 cases per 100,000 population in 2018. In Indonesia, NPC occurred as many as 17,992 new cases, namely, 5.2% in 2018. Over 5 years, lastly, the incidence of NPC was 48,401 cases [4].

Risk factors of NPC are genetic, environmental, and lifestyle [5], [6]. One of the environmental factors is EBV infection [7], [8]. EBV infection is a significant factor involved in the pathogenesis of NPC, especially in undifferentiated nasopharyngeal carcinoma [9]. The increased risk of NPC that occurs due to EBV infection indicates an increased frequency of viral reactivation. Aberrant EBV reactivation was demonstrated by the detection of serum EBV immunoglobulin A (IgA). The results of different EBV antibody profile between healthy individuals and individuals with NPC so that EBV IgA in blood samples (dry) can be a measure of detection of NPC patients. Individuals suffering from NPC have a higher level of IgA EBV seroactivity than normal individuals [10].

Non-specific signs and symptoms of NPC and lack of awareness of NPC symptoms make NPC diagnosis late or fail. Delay in diagnosis causes treatment failure, the cure rate is low, and the recurrence rate is high. This condition makes the need for prevention and early detection efforts based on the NPC profile [11]. Therefore, it is necessary to develop a non-invasive NPC diagnostic tool so that the tool can be developed into an early detection tool for NPC.
Materials and Methods

The study was conducted at the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra, Medan in 2020. It was a prospective cross-sectional study involving 29 NPC patients and 29 non-NPC who met the inclusion and exclusion criteria. The inclusion criteria for the NPC group were patients diagnosed with NPC, Nias ethnic patients, willing to sign the informed consent. The inclusion criteria for the control (non-NPC) group were individuals from the Nias tribe, had good physical or psychological conditions and awareness, and were willing to sign an informed consent. The exclusion criteria had other malignancies and pregnant and lactating women.

The study has received approval from the Health Research Ethics Commission, Faculty of Medicine, University of North Sumatra/H. Adam Malik Hospital Medan (No. 221/TGL/KEPK FK USU-RSUP HAM/2020). The study was conducted by taking blood samples and IgA anti-EA EBV measured by serological test using an enzyme-linked immunosorbent assay kit. Positive and negative serology were based on the criteria, namely, negative serology <cut off and positive serology cut off. The cutoff value is 0.10 + the average negative control absorbance if the negative control absorbance is <0.05. The cutoff value is the average negative control if the absorbance of the negative control is 0.05.

All data were collected and recorded using the Statistical Product and Service Solution (SPSS) version 23 program. Values are expressed as mean ± SD or the appropriate percentage. Comparative analysis of IgA anti-EA EBV between NPC patients and non-NPC patients was calculated statistically using the Chi-square test for categorical data and using the independent sample t-test for normal data and using the Mann–Whitney U-test for abnormal data. A difference was considered significant at p < 0.05.

Results

The research subjects consisted of 58 patients consisting of 29 NPC patients and 29 non-NPC individuals. Most of the study subjects were <60 years old in both groups. Subjects aged more than or equal to 60 years were found in the group of NPC patients (6.9%). The subjects of this study, both in the group of NPC patients and non-NPC, were mostly male.

Hypothesis test results in Table 1 showed that there is no significant difference in the age between NPC patients and non-NPC with p = 0.491 (>0.05). The results of the hypothesis test showed that the sex did not differ significantly between the Nias ethnic group between NPC patients and non-NPC with p = 1.000 (>0.05). Table 2 showed that the mean level of IgA anti-EA EBV in NPC patients was greater (246.22 ± 320.05 U/mL) than in non-NPC (51.79 ± 1.55 U/mL). Comparison of the mean levels of IgA anti-EA EBV between NPC patients and non-NPC is shown in Figure 1. The results of the Shapiro–Wilk normality test obtained p = 0.000 (<0.05) for data on anti-EA EBV IgA levels in the NPC patients, which showed that the data were not normally distributed. The results of the Shapiro–Wilk normality test obtained p = 0.845 (>0.05) for data on IgA levels of anti-EA EBV in the control group, which showed that the data were normally distributed. Hence, the test of the difference in the mean of anti-EA EBV IgA levels of the two groups was carried out using
the Mann–Whitney U-test. The test results showed that there was a significant difference in the mean of anti-EA EBV IgA levels between NPC patients and non-NPC with p = 0.000 (<0.05).

Table 2: Differences in IgA levels of anti-EA EBV in NPC patients and non-NPC

| Group   | IgA anti-EA EBV (U/mL) | p-value | p-value# |
|---------|------------------------|---------|----------|
| NPC     | n=16                    | 51.00   | 124.00   | 246.22 ± 330.05 | 0.000 | 0.000** |
| Non-NPC | n=13                   | 48.00   | 55.00    | 51.79 ± 1.55    | 0.845 |        |

Table 3 showed that the IgA anti-EA EBV serology test obtained positive results in 16 (55.2%) in NPC patients, and 3 (10.3%) in non-NPC. The results of the Chi-square test showed that there was a significant difference of IgA anti-EA EBV serological tests between NPC patients and non-NPC with p = 0.001 (<0.05). The positive results of IgA anti-EA EBV serology were more commonly found in NPC patients than in non-NPC.

Table 3: Comparison of IgA anti-EA EBV serology tests between NPC patients and ncri-NPC in the Nias tribe

| IgA anti-EA EBV | NPC | Non-NPC | Total | p-value |
|-----------------|-----|---------|-------|---------|
| EBV             | F   | %       | F     | %       | F      | %     |        |
| Positive        | 16  | 55.2    | 3     | 10.3    | 19     | 32.8  |        |
| Negative        | 13  | 44.8    | 26    | 89.7    | 39     | 67.2  | 0.001**|
| Total           | 29  | 100     | 29    | 100     | 58     | 100   |        |

Table 4 showed that based on age, the IgA anti-EA EBV serology test results were positive, that mostly at the age of fewer than 60 years. The same results for the serological negative. The Chi-square test results showed that the age is not significantly associated with IgA anti-EA EBV serology test results in NPC patients with p = 1.000 (>0.05). Based on sex, the results of the IgA anti-EA EBV serology test were positive, mostly in the male (56.3%). The same results for negative results 61.5%). The Chi-square test results showed that sex is not significantly associated with IgA anti-EA EBV serological test results in NPC patients with p = 1.000 (>0.05).

Discussion

Nias tribe NPC patients in this study was 44.86 ± 12.70 years old. Most NPC patients were <60 years old (93.1%) and most of them were male (58.6%). In line with this study, Rahman et al. reported that NPC patients in Padang were found at an average age of 44.6 years and mostly occurred in men (60%) [13]. The results are in line with research in Denpasar Bali that the incidence of NPC is mostly found in patients aged more than 50 years [16]. Research in North Sumatra also reported that 58.6% of NPC patients were male and most of them occurred at the age of fewer than 60 years (93.1%), especially at the age of 41–60 years (55.2%) [17]. The age distribution of patients with NPC is different. In areas with a low incidence of NPC, the incidence of NPC increases with increasing age, in areas with a high incidence of NPC that increases after 30 years old [18].

Serological results obtained Ig A anti-EA EBV levels in NPC Nias patients with a mean of 246.22 ± 320.05 U/mL. This result is higher than the level of IgA anti-EA EBV in NPC patients in the hospital Hasan Sadikin of 52.18 ± 8.93 U/mL and in NPC patients at Dr. M. Djamil Padang of 114.705 ± 136.524 U/mL [13], [19].

Serology tests of anti-EA EBV IgA in Nias tribal NPC patients found positive in 16 (55.2%). This result is smaller than previous studies that were found in 10 NPC patients in North Sumatra (66.7%) and 212 NPC patients in Yogyakarta (41.7%) [13], [15]. Serology tests Ig A anti-EA EBV of non-NPC Nias tribe found positive in 3 (10.3%). The results differ in individuals in Padang, there were no positive results [13].

Serological results showed that there was a significant difference in IgA anti-EA EBV levels in NPC patients compared to non-NPC individuals with p < 0.05. The mean levels of IgA anti-EA EBV in NPC patients (246.22 ± 320.05 U/mL) were greater than in non-NPC (51.79 ± 1.55 U/mL) in the Nias tribe. These results are in line with Rahman et al. that NPC patients had higher levels of EA-IgA (114.705 ± 136.524 U/mL) compared to healthy controls (1.749 ± 0.498 U/mL) with p < 0.05 [13]. Anugrahani et al. also reported an increase in the levels of IgA anti-EA EBV in NPC patients compared to non-Hodgkin lymphoma [19].

These results showed that there had a significant difference in the serological tests of IgA anti-EA EBV between NPC patients and non-NPC with p = 0.002 (<0.05). Positive results of IgA anti-EA EBV serology were more commonly found in NPC patients than non-NPC. Thus, the serological tests of IgA anti-EA EBV play a role in screening of NPC patients of the Nias tribe. Positive results serological tests of IgA anti-EA EBV in patients with NPC Nias tribe is 16 (55.2%). Thus, serological tests can be used to distinguish NPC patients from non-NPC based on IgA anti-EA EBV values. A serological test by measuring IgA anti-EA EBV can serve as a diagnostic tool for NPC patients of the Nias tribe.

In line with this research, Rahman et al. reported that NPC patients had higher levels of EA-IgA compared to healthy controls. Antibody levels of IgA anti-EA EBV increased in NPC patients and were more common in NPC patients than in control [13]. Sun et al. also reported similar results in a study in Shandong, China, that there was an increase in serum IgA anti-EA EBV in NPC patients [20].

EBV infects two sites in the human body, including lymphocytes and salivary gland epithelial cells. The incoming virus replicates in epithelial cells which, then, become latent in B lymphocytes. This viral infection occurs by binding to complement C3d (CD21 or CR2). Several mechanisms of EBV infection
may result from direct contact between infected lymphocytes and cells in the apical membrane, through the interaction of integrins 1 or 5B1 with EBV on the basolateral membrane, and through the direct effect of viral infection on the lateral membrane [21, 22].

The surface of B lymphocytes consists of the CD21 protein which is a target for EBV infection, because this protein can bind to the membrane glycoprotein receptor gp 350/220 on the EBV capsule. EBV enters the host cell cytoplasm after 1–2 h of binding. Terminal repeat fuse to form a circle of the epitope. EBV particles decompose and latent EBV infection occurs after the EBV genome enters the nucleus. The latent infection is characterized by EBV activation in B lymphocyte cells as a result of cell proliferation and activation processes [21, 22].

Differences between normal and malignant EBV infection have been described. In normal conditions, a few B cells are infected, because the EBV infection can be controlled. In malignant conditions, a latent epitome is formed as a result of the presence of the EBV genome in each tumor cell. During cell division, genome replication occurs. The DNA expression in EBV in the latent episomal form was identified, so it was useful as a marker of the development of NPC [23].

The occurrence of NPC is closely related to Epstein–Barr virus (EBV) infection. EBV antibodies are widely used as markers in NPC screening. Several studies have shown that screening for NPC using EBV antibodies is an effective measure to increase the survival rate of NPC patients [24].

Positive results of the IgA anti-EA EBV serology test were mostly found at the age of fewer than 60 years and in males. The age was not significantly related to the IgA anti-EA EBV serological test results with p = 1.000 (>0.05). Sex was not significantly related to the IgA anti-EA EBV serological test results with p = 1.000 (>0.05). These results are in line with research by Zhou et al. that no significant association was observed between sex, education, BMI, and family history of tumors or NPC and IgA anti-EA EBV [25].

However, several studies report different results. The age was found to be linearly correlated with antibody levels IgA anti-EA EBV [25]. Older age and females increase positive results of IgA anti-EA EBV. This is assuming that older patients have more exposure to EBV triggers along with a weakened immune response that could facilitate EBV reactivation, leading to increased anti-EBV IgA [26]. Seroreactivity to EBV in females is stronger in men, probably due to hormonal effects on the immunologic response [15].

The others studies have reported various factors associated with antibody level IgA anti-EA EBV in NPC patients. Januardi et al. reported that there was an association between increased titration of IgA anti-EA EBV in type III NPC with risk factors for salted fish and preserved food, smoking, and the use of smoked mosquito coils [27]. Serology IgA VCA/IgA EA as a tumor marker (tumor marker) does not play a role in establishing the diagnosis but is used as screening and basic data for treatment evaluation. This serological examination is performed before a series of diagnostic tests for NPC [1].

| Characteristics | Positive n = 16 | Negative n = 13 | Total | p-value |
|-----------------|----------------|----------------|-------|---------|
| Age             |                |                |       |         |
| <60 years       | 15             | 12             | 27    | 1,000'  |
| 60 years        | 7              | 7              | 14    | 0.62    |
| Sex             |                |                |       |         |
| Male            | 9              | 8              | 17    | 1,000'  |
| Female          | 7              | 5              | 12    | 41.4    |

Conclusion

Serology results antibody IgA anti-EA EBV plays a role in differentiating NPC patients from healthy individuals with the result that there was a significant difference in serological results antibody IgA anti-EA EBV between NPC patients and non-NPC in the Nias tribe. Thus, serology antibody IgA anti-EA EBV can be useful as a biomarker for screening NPC in the Nias tribe. This study implies that serological tests can be applied in clinical practice in screening and early diagnosing NPC. Age and sex were not significantly associated with IgA anti-EA EBV so that screening tests with serological tests could be applied with the same cutoff between overall age and sex. Research only studies differences of IgA anti-EA EBV between NPC patients and non-NPC with small sample size and homogenous, only in the Nias tribe. The study also did not measure EBV anti-EA IgA levels before and after the diagnosis of NPC and only after NPC and not NPC. This research can be carried out by involving various tribes and ethnicities in Indonesia to examine the influence of tribes and ethnicities on levels of IgA anti-EA EBV in NPC patients supports these results so that they can be practiced clinically for various ethnic groups. The results of this study only imply IgA anti-EA EBV use as a screening of NPC. Its use as a diagnostic tool needs further research.

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