Corelation Between Epstein Barr Virus Epstein Barr Nuclear Antigen 1 DNA with Nasopharyngeal Carcinoma in Minangkabau Ethnic

Desy Ambriani[1,2,*], Sukri Rahman[1,2], Al Hafiz[1,2], Hirowati Ali[3], and Hafni Bachtiar[4]

[1]*Department of Otorhinolaryngology Head & Neck Surgery of Faculty Medicine Andalas University, Padang, Indonesia*
[2]*Dr. M. Djamil General Hospital, Padang, Indonesia*
[3]*Department of Biochemical Science of Faculty Medicine Andalas University, Padang, Indonesia*
[4]*Department of Public Health of Faculty Medicine Andalas University, Padang, Indonesia*
[*]*Corresponding author. Email: dr.DesyAmbriani@gmail.com*

ABSTRACT
Nasopharyngeal carcinoma (NFC) is a malignancy that come from the nasopharyngeal epithelial cell which is that distribution has been affected by geographic and ethnicity. NFC is caused by multifactorial causes, one of the is Epstein Barr Nuclear Antigen-1 (EBNA-1). EBNA-1 affects some of the signalling pathways, including cell proliferation, invasion, survival, and DNA Repair. This study aimed to know the correlation between EBV (EBNA-1) DNA with NFC incidence in Minangkabau Ethnicity. This study is analytic observational with a cross-sectional comparative study. Twenty-two plasmas of the NFC patients with Minangkabau ethnicity compared with 22 plasma of NPC patients with non-Minangkabau ethnicity as control, which matched their sex and age (±3 years). EBNA-1 level expression was checked with quantitative real-time PCR (qPCR) methods to check the DNA targeting EBNA-1 from the EBV genome. The data was then analyzed with Saphiro Wilk for normality and continued with the LG10 transformation test. They are then finalized with Mann-Whitney for the two groups. The average expression level of the EBNA-1 in NFC patients is higher than the control's plasma in Minangkabau ethnicity but not statistically significant.

Keywords: *epstein barr nuclear antigen-1, nasopharyngeal carcinoma, Minangkabau.*

1. INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a malignancy originating from the nasopharyngeal epithelium [1]. Nasopharyngeal carcinoma spread varies according to geographic and ethnic distribution worldwide [2]. In Indonesia, NPC is the fourth after cervical, breast and skin malignancies and the first most common malignancy in the head and neck [3]. Incidence rate of NPC is around 6.2/100,000 population with an estimated 12,000 cases per year [3]. In Jakarta, based on the data at the RSUPN Dr. Cipto Mangunkusumo reported that NPC was the most common malignancy in the head and neck region [3]. While in West Sumatra, based on data collected at Dr.M. Djamil also reported that NPC is the most common malignancy in the head and neck area [4].

Nasopharyngeal carcinoma is a multifactorial disease, and the exact cause is unknown [5]. Several risk factors of Epstein Barr Virus (EBV) infection are investigated, including genetic and environmental factors [6]. Some researchers have stated that the incidence of NPC is closely related to EBV infection [7]. This relationship is evidenced by the presence of antibodies titres to EBV antigen in most patients with NPC and the detection of deoxyribonucleic (DNA) and ribonucleicacid (RNA) of EBV in tumor cells from biopsies in patients with NPC [8]. The transformation of tumorigenesis by EBV involving the interaction of an impaired host immune response and the chronic inflammatory environment may play a major role [9].

Primary EBV infection generally occurs early in life and is asymptomatic [9]. After being infected, the virus remains in circulation and is transmitted through saliva. The infection could be permanent (persistent), hidden (latent) and lifelong [10]. Although infection is lifelong. It is usually harmless unless the balance between host and virus changes [11]. Epstein Barr Virus is dual tropism because EBV can infect B cells and epithelial cells.

In the process of primary infection, EBV infects and replicates in the oropharynx squamous epithelial cells and cause lytic infection and viral replication to produce virions that coincide with latent infection in B lymphocytes [12]. EBV-infected B lymphocytes are thought to be present in oropharyngeal lymphoid organs and this virus will remain in memory B lymphocyte cells, thus making these cells immortal [10]. In healthy individuals, approximately 50 million circulating memory B cells have been infected with EBV and will remain stable for many years [9].

About 90% of the world's population has been infected with EBV, but only a small percentage of the EBV-infected population has NPC [9, 13]. This is due to many factors that can cause EBV reactivation which is triggered by genetic, carcinogenic and environmental factors [4, 8]. This study aimed to know the correlation between EBV (EBNA-1) DNA with NFC incidence in Minangkabau Ethnicity. This study is analytic observational with a cross-sectional comparative study. Twenty-two plasma of the NFC patients with Minangkabau ethnicity compared with 22 plasma of NPC patients with non-Minangkabau ethnicity as control.

2. METHOD

The study was conducted on 22 plasma of Minangkabau ethnic patients diagnosed with NPC based on anatomical pathology examination of the nasopharyngeal biopsy results who came to Dr. M. Djamil Hospital Padang and have not received chemotherapy or radiotherapy treatment. The control group were the 22 plasma of NPC patients with non-Minangkabau ethnicity, matched on age (±3 years) and sex.

The plasma was examined for EBV DNA isolation and followed with the real-time polymerase chain reaction or quantitative real-time PCR (qPCR) method to detect DNA by targeting EBNA-1 from the EBV genome. The data obtained were statistically processed by SPSS. To assess the expression of the EBNA-1 gene from the EBV genome in the NPC group and the non-NPC group, the Shapiro-Wilk normality test was first performed. Data was not normally distributed ($p<0.05$), then the transformation test was continued with Log10 and the distribution was obtained. If the data were not normally distributed ($p<0.05$), so the *Mann-Whitney Non-Parametric* test was performed.

3. RESULT AND DISCUSSION

3.1. Result

Based on the characteristics of the respondents, NPC cases were more common in male (63.6%) than in female (36.4%) with a ratio of 1.7:1. The most common age group of NPC respondents was 40-49 years (36.4%), with a mean age of 46.68 ± 13.19. The youngest respondent who suffered from NPC was 20 years old, while the oldest was 69 years old. Based on the results of histopathological examination, the most common type was undifferentiated squamous cell carcinoma (WHO III) (63.6%) followed by undifferentiated non-keratinizing carcinoma (WHO II) (36.4%) and no keratinized squamous cell type (WHO I). Most of the NPC patients came in stage IV (77.3%), followed by stage III (13.6%), stage II (9.1%) and no patient was diagnosed with stage I.

The expression of the EBV gene (EBNA-1) based on the history of salted fish consumption habits between NPC patients and non-NPC plasma from three-generation of Minangkabau ethnicity in the Minangkabau ethnic group showed a significant difference in the level of EBNA-1 expression between the NPC and healthy control ($p < 0.05$). There were no significant differences in expression of the EBV gene (EBNA-1) based on a history of alcohol consumption and smoking habits between NPC patients and non-NPC plasma from three-generation of Minangkabau ethnicity ($p > 0.05$).

The mean expression levels of EBV DNA (EBNA-1) in the NPC patient was 4.497 ± 3.483 that was higher than the control group of 3.328 ± 4.095, but not significantly different ($p > 0.05$). The mean level of gene expression of EBV (EBNA-1) in the age group of >40 years (5,393 ± 3.618) was higher than in the age group of δ 40 (2.109 ± 1.510). After the independent t-test statistical analysis was performed, there was a significant difference in the level of EBNA-1 expression between the two research groups with a p value of 0.046 ($p <0.05$). The mean level of gene expression of EBV (EBNA-1) in the anatomic pathology groups was higher in a patient with WHO type III (4.766 ± 3.353) than the WHO type II (4.028 ± 3.889). After statistical analysis of the independent t-test in both groups, no significant difference in the level of EBNA-1 expression between the WHO type II and WHO type III groups were found, with a p-value of 0.267.

The mean EBNA-1 expression level was highest at the advanced stage (4.514 ± 3.654) compared to the initial stage (4.329 ± 0.988). After the independent t-test was performed in the two groups, it was seen that there was no significant difference in the level of EBNA-1 expression between the initial and advanced stages with a p-value of 0.945.

3.2. Discussion

In this study, gene expression of EBV (EBNA-1) in plasma was detected in both groups with variable EBV

DNA (EBNA-1) expression, but the mean gene expression of EBV (EBNA-1) in NPC was higher than the control group. From this study, there was no significant difference between the NPC and healthy controls group targeting EBNA-1. This explains that EBNA-1 expression levels are not always positively correlated with the incidence of NPC. Sugiyanto in 2018 showed that many factors and parameters influence the correlation between EBV and NPC. Including the rate of interaction between dysplastic cells, the interaction between EBV-infected cells, the rate of invasive carcinoma cell proliferation, the apoptosis rate of dysplastic cells, and immune response to viruses [14,15].

In this study, all healthy controls showed the variable expression level of the EBV gene (EBNA-1), showing that in the healthy population, there was also EBV infection but still in the early stages or in the asymptomatic phase. Primary EBV infection generally occurs early in life and is asymptomatic [9]. This can also be due to the limited number of samples so that the entire sample in the healthy population involved can be found to have EBV infection.

In healthy individuals, EBV DNA can be detected in infected memory B cells at about 1-50 B cells/million and subsequently stabilizes over years so that EBV DNA can be detected in this population, even though it is asymptomatic [12]. Lifelong infection is usually harmless because in healthy individuals EBV carriers are only in a few memory B cells and are under the control of the immune system [16]. However, imbalance between host immune system and virus activates latent phase II EBV and cell proliferation that causes these cells to become immortal [17].

In this study, the mean expression of EBV DNA (EBNA-1) in the NPC group was higher compared to the control group. Various studies also reported that the expression value of EBV DNA in the NPC group was higher than in the healthy control group. Research conducted by Lo [18] in Hong Kong, the qPCR examination found that DNA EBNA-1 level in the NPC was higher compared to the healthy control group with a median of 21058 copies/ml *and* 0 copies/ml, respectively. This is because the EBV viral load of healthy individuals is measured from the circulating blood whereas in NPC patients the EBV viral load is derived from circulating epithelial malignant cells and B cells so that DNA from the epithelium and B cells may be responsible for the high viral load in NPC patients when compared to healthy controls [19].

The differences in Human Leukocyte Antigen (HLA) alleles based on ethnicity, resulting in variable individual responses to viral infections based on ethnic groups. This causes different incidences of NPC in different ethnic even though they live in the same geographic area [20]. This is supported by Tsao [21] that found the high incidence of NPC population in South China, although the second and third generation descendants have migrated to areas with low NPC incidence (such as in America) compared to the native population, this explains that ethnic differences in NPC cases indicate a contribution of genetic susceptibility in the pathogenesis of NPC.

In this study, there was no significant difference between the expression of the EBNA-1 gene and NPC in the Minangkabau ethnic group since the expression of the EBV gene was detected in the entire control group and the population with NPC. In addition, from research conducted by Smatti [22], it was stated that sample size is very important to determine the relationship between the variables studied, both the independent (age, gender, ethnicity) and the dependent variable (incidence of NPC). The study conducted using cross sectional design, so the number of subjects required is generally very large to be able to accurately determine the relationship between variables. The study conducted by Smatti involved 673 populations in Qatar, but researchers still considered the number of samples to be inadequate, so further studies were still needed [22].

4. CONCLUSION

The mean value of EBV DNA (EBNA-1) expression in the NPC group was higher compared to control group and there was no significant difference between EBV DNA (EBNA-1) and the incidence of nasopharyngeal carcinoma in the Minangkabau ethnic group.

REFERENCES

[1] [1] YY Yap, S Hassan, M Chan, PK RM Choo. Epstein Barr Virus DNA Detection in the Diagnosis of Nasopharyngeal Carcinoma. Am Acad Otolaryngol Neck Surg Found. 136(6):986-91, 2007.

[2] MC Yu, JM Yuan, “Epidemiology of Nasopharyngeal Carcinoma,” Cancer Biol, vol 12 no 2 pp. 421-9, 2002.

[3] M Adham, AN Kurniawan, AI Muhtadi, A Roezin, S Hermani, Gondhowiardjo et al., “Nasopharyngeal Carcinoma in Indonesia: Epidemiology, Incidence, Signs, and Symptoms at Presentation,” Chin J Cancer, 31(4):185-96, 2012.

[4] S Rahman, H Kurniawan, BJ Budiman, E Yerizel, H Bachtiar, “Evaluation of Serum IgA Antibodies to Epstein-Barr Virus Early and Viral Capsid Antigens in Nasopharyngeal Carcinoma,” KnE Eng. 1(2):275-83, 2019.

[5] WL Hsu, KJ Yu, YC Chien, CJ Chiang, YJ Cheng, JY Chen et al. "Familial Tendency and Risk of Nasopharyngeal Carcinoma in Taiwan: Effects of Covariates on Risk,". Am J Epidemiol. 173(3):292-9, 2011.

[6] Q Tao, ATC Chan. "Nasopharyngeal Carcinoma : Molecular Pathogenesis and Therapeutic Development," Expert Rev Mol Med. 9(12):1-24, 2007.

[7] W Wen, SJ Mai, HX Lin, MY Zhang, JL Huang, X Hua et al. "Identification of Two MicroRNA Signatures in Whole Blood as Novel Biomarkers For Diagnosis of Nasopharyngeal Carcinoma," J Transl Med. 17(1):1-13, 2019.

[8] SH Hutajulu, J Kurnianda, IB Tan, JM Middeldorp. "Therapeutic Implications of Epstein – Barr Virus Infection For The Treatment Of Nasopharyngeal Carcinoma," Dove Press J. 10(9):721-36, 2014.

[9] CM Tsang, SW Tsao. "The Role of Epstein-Barr virus Infection in The Pathogenesis of Nasopharyngeal Carcinoma," Virol Sin. 30(2):107-21, 2015.

[10] MP Thompson, R Kurzrock. "Epstein-Barr Virus and Cancer," Clinical Cancer Research. 10(713):803-21, 2004.

[11] J Chou, YC Lin, J Kim, L You, Z Xu, B He et al. Nasopharyngeal Carcinoma - Review of the Molecular Mechanisms of Tumorigenesis. Head & Neck. 2008;1(0):946-63.

[12] Korcum AF, Ozyar E, Ayhan A. Epstein-Barr Virus Genes and Nasopharyngeal Cancer. Turkish J Cancer. 2006;36(3):97-107.

[13] Traub NR. Epstein – Barr Virus in The Pathogenesis of NPC. Elsevier Sci. 2002;12(02):431-41.

[14] Lung ML, Cheung AKL, Ko JMY, Lung HL, Cheng Y, Dai W. The Interplay of Host Genetic Factors and Epstein-Barr Virus in The Development of Nasopharyngeal Carcinoma. Chin J Cancer. 2014;33(11):566-8.

[15] Sugiyanto S, Aryati L, Kusumo FA, Hardianti MS. Link of Nasopharyngeal Carcinoma and Epstein-Barr Virus. Biol Med Nat Prod Chem. 2018;7(2):51-5.

[16] Hau PM, Lung HL, Wu M, Tsang CM, Wong KL, Mak NK, et al. Targeting Epstein-Barr Virus in Nasopharyngeal Carcinoma. Front Oncol. 2020;10(5):1-18.

[17] Hausen HZ. Gammaherpesvirinae (Lymphocryptoviruses). Dalam: Hausen HZ. Infection Causing Human Cancer. Germany: Wiley-VCH; 2006. p. 65-117.

[18] Lo YMD, Chan LYS, Lo KW, Leung SF, Zhang J, Chan ATC et al. Quantitative Analysis of Cell-Free Epstein-Barr Virus DNA in Plasma of Patients With Nasopharyngeal Carcinoma. Cancer Res. 1999;59(6):1188-91.

[19] Ayee R, Ofori MEO, Tagoe EA, Languon S, Searyoh K, Armooh L et al. Genotypic Characterization of Epstein Barr Virus in Blood of Patients with Suspected Nasopharyngeal Carcinoma in Ghana. MDPI. 2020;12(5):1-9.

[20] Cao SM, Simons MJ, Qian C. The Prevalence and Prevention of Nasopharyngeal Carcinoma in China. Chin J Cancer. 2011;30(2):114-9.

[21] Tsao SW, Yip YL, Tsang CM, Lau VMY, Zhang G, Lo KW. Etiological Factors of Nasopharyngeal Carcinoma. Oral Oncol. 2014;50(5):330-8.

[22] Smatti MK, Yassine HM, AbuOdeh R, Almarawani A, Taleb SA,bAlthani AA, et al. Prevalence and molecular profiling of Epstein Barr virus (EBV) among healthy blood donors from different nationalities in Qatar. PLoS One. 2017;12(12):1-20.