THE COMPARISON OF ELEVATED LEVELS OF EBV IMMUNOGLOBULIN A EARLY ANTIGEN BETWEEN NASOPHARYNGEAL CARCINOMA WHO TYPE 3 WITH MALIGNANT NON-HODGKIN LYMPHOMA

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Abstract

Introduction: Epstein Barr Virus (EBV) replicate in the epithelial cells and becomes latent in lymphocytes B. This virus has also been isolated and reached its peak in tumor tissue. The expression product of EBV acute infections can interact with genes in the epithelial cells to start the molecular level that leads to malignancy so that an increase in levels of IgA antibodies EA can indicate high levels of EBV infection.

Objective: Comparing the level of IgA EA antibody for EBV between NPC WHO type III and NHL.

Method: Observational comparative analytic method with cross-sectional study design. The subject of this study are 16 NPC patients and 16 NHL patients, consists of 9 males and 7 females. Numeric data of IgA EA calculated by Mann Whitney and the categoric data is calculated by Kolmogorov Smirnov. The examination of IgA EA level in EBV using ELISA method, with p-value <0.05.

Result: This study reveals that there were increasing IgA EA levels in NPC patients (3.71±2.36) compared to NHL (0.27±0.20). There is a difference value of IgA EA in study groups both numerical and categorical.

Conclusion: The level of IgA EA EBV antibody in NPC is higher than NHL.

1. INTRODUCTION

Nasopharyngeal Carcinoma (NPC) is an epithelial neoplasm arising from the fossa of Rosenmuller of the postnasal space [1]. Epstein-Barr virus (EBV) or human herpesvirus 4 is ubiquitous, and about 90% of adults throughout the world have antibodies against it. Acute infection is usually asymptomatic in immunocompetent children and manifests itself as mononucleosis in 30%-50% of immunocompetent adolescents and adults [2, 3]. This is a rare tumor in the most part of the world, but it occurs endemically in Southern China, Hong Kong, Singapore and some other part of Southeast Asia with high incidence [1, 2]. Epstein-Barr Virus (EBV) has been implicated as an important etiological factor of NPC with histological evidence, indicating the consistent presence of the viral DNA and proteins in malignant tissue. The EBV is clonally present in virtually all NPC patients [4]. This virus is also associated with NPC, NHL, infectious mononucleosis and Burkitt’s lymphoma. The expression of EBV early antigen (EA) antibodies directed against EBV viral capsid antigen (VCA/IgA) antibodies have been used widely to screen for NPC in the areas of China in which it is endemic [5].

Especially in immunocompromised patients, EBV is associated with various lymphoproliferative disorders and some neoplastic diseases, including Burkitt’s lymphoma and nasopharyngeal carcinoma. Like other herpes viruses, EBV has a productive lytic cycle and a latent phase. B lymphocytes are infected after the viral envelope glycoprotein gp350/220 has bound to the CD21 cell receptor, which is also the receptor for the C3d component of complement [6]. During the lytic cycle, regulatory proteins belonging to the immediately early antigen (IEA) can cause reactivation and replication of EBV. Reacting EBV itself can be a risk factor for NHL. Non-Hodgkin's lymphoma is a lymphocyte cell malignancy, associated with immune dysregulation, a decrease in immunity resulting in immune response and lytic reactivation of herpesviruses such as EBV in mucous tissue. Immune dysregulation occurs due to loss of immune control due to reactivation of the herpes viruses and the production of infectious viral particles, which triggers the serum IgA profile to reactivate herpes viruses such as EBV [11]. Pathogenesis of Non-Hodgkin Lymphoma (NHL) with EBV is the most common hematopoetic cancers, representing most common cancer, in terms of incidence rate, and the sixth in terms of cancer deaths, in both men and women in the United States. 3 Lymphomas represent diverse and heterogeneous groups of cancers, consisting of Hodgkin lymphoma, T cell and natural killer cell lymphoma, and various types of B cell non-Hodgkin lymphoma [7, 8]. Most types of B cell NHL correspond to B cells that have undergone various

Copyright © International Journal of Nasopharyngeal Carcinoma, Published by Talenta Publisher, ISSN: 2656-9027 e-ISSN: 2656-9035, DOI: 10.32734/ijnpc.v1i2.1137

41
molecular changes that occur after an initial encounter with antigen and exposure to helper T cells [5]. As these activation-promoting interactions occur in the germinal centers of lymph nodes and other lymphoid organs, these cells are referred to as post-germinal center B cells. The germinal center reaction involves rapid B cell proliferation and somatic DNA changes that result in both changes in the isotype of the Ig that is produced by these cells (from IgM to IgG or other isotypes), as well as enhanced antigen-binding affinity [9].

The standard method of diagnosis of EBV is Immunofluorescence Assay (IFA), but because this method is not practical, subjective and relatively expensive, a method of enzyme-linked immunosorbert assay (ELISA) is developed. The ELISA method has better sensitivity and specificity than IFA, is quantitative, objective, costs are relatively cheap, and is suitable for population detection on a large scale [12].

2. MATERIAL AND METHODS

This study is a comparative analytic observational study with cross-sectional design. The research subjects were patients diagnosed with NPC and NHL in Hasan Sadikin Hospital from June 2016 to July 2017 who were in accordance with inclusion and exclusion criteria and were willing to participate in the study and sign an informed consent.

Inclusion criteria: 1. WHO type III NPC patients from histopathological examination who have not undergone radiotherapy, chemotherapy, or combination therapy. 2. NHL patients from histopathological results who have not undergone chemotherapy. Exclusion criteria: 1. Patients with multiple carcinomas. 2. NPC and NHL residual/recurrent patients.

The sample selection technique used in this study is nonprobability sampling with consecutive sample matching. There are 16 samples each for NPC and NHL samples. Every NPC and NHL patients who come to the hospital who has not received treatment therapy, checks starting from the history (history of patient complaints, symptoms, duration, and onset of disease and previous history), then a general physical examination. Nasopharyngoscopy, biopsy and immunohistochemical examination, and investigations (hematological ultrasound, chest X-ray, nasopharyngeal CT scan) in accordance with the applicable standard operating procedure. The examination was carried out using the ELISA method to obtain IgA EA EBV levels found in both NPC and NHL patient.

3. RESULT

Table 1. Comparison of the Characteristics of the Two Groups of Research Subjects by Age

<table>
<thead>
<tr>
<th>Variable</th>
<th>NPH n=16</th>
<th>NPC n=16</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>52.18±8.93</td>
<td>46.81±16.42</td>
<td>.262</td>
</tr>
<tr>
<td>Mean</td>
<td>52.30</td>
<td>52.00</td>
<td>.000</td>
</tr>
<tr>
<td>Median</td>
<td>37.00–65.00</td>
<td>18.00–74.00</td>
<td>.000</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Characteristics of Both Study Subject Groups by Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>NPH n=16</th>
<th>NPC n=16</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>9 (56.2%)</td>
<td>9 (56.2%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Female</td>
<td>7 (43.8%)</td>
<td>7 (43.8%)</td>
<td>.000</td>
</tr>
</tbody>
</table>

In the table, 1 and table 2 of the NHL group found at an average age of 52.18±8.93, male sex as many as 9 people or equal to 56.2%, and female sex at 7 people by or 43.8%. In the NPC group, the average age was 46.81±16.42, male sex was 9 people or 56.2%, and female sex was 7 people or 43.8%.

Statistical tests to compare the average numerical data with two unpaired groups, the analysis carried out to test this numerical data by using the Mann Whitney analysis test because the data are not normally distributed, namely the numerical EA IgA variable. The results of the statistical test on the IgA EA variable obtained a value of p-value of 0.001 where the p-value was smaller than 0.05 (p<0.05). This shows significant and statistically significant. Thus it can be said that there are differences in the mean between the IgA EA variables in the two study groups. In the NPC group, the IgA EA value of 0.27±0.20 did not increase compared to the NPC group which

4. DISCUSSION

In this study, the results of statistical tests obtained on the age variable obtained p-value of 0.262 where the p-value is greater than 0.05 (p>0.05). So this shows not significant or not statistically significant. Thus it can be said that there is no difference in the average between age variables in the two study groups. In this study, the highest age was obtained in patients with NPC and NHL in Hasan Sadikin Hospital between 40 to 50 years with an average age at NPC of 52.18±8.93 and NHL at the age of 46.81±16.42. This is in accordance with Adham M et al. Getting the most age of NPC patients at the age of 40-50 years as much as 32.4% 2 and Cao SM et al in China getting the most age is decades 4-6 [3, 13]. Ahmad A et al. in research at Kermanshah, Iran, found patients with non-Hodgkin's lymphoma associated with EBV aged 45±13 years with a range of 28-73 years. Of the 12 patients (60%) male and 8 patients (40%) women [14].

The occurrence of NPC in the age range of 4-6 decades is caused by exposure to risk factors from the environment such as consumption of preserved and salted foods and exposure to carcinogenic substances that are long enough good so that it takes more time for the carcinogenesis process to occur [1-3, 6]. Nasopharyngeal carcinoma increases in humans according to age. There are differences in age-related tumor distribution patterns in various organs and tissues. Aging can increase or decrease the susceptibility of various tissues to the initiation of carcinogenesis and usually facilitates the promotion and progression of carcinogenesis. Aging can predispose to cancer through several mechanisms, namely the accumulation of tissue from cells in the later stages of carcinogenesis, changes in homeostasis, specifically changes in the immune system and endocrine system, telomere instability related to aging and increased risk of cancer [15].

The prevalence of EBV in NHL in adults is >90%, and EBV establishes a lifetime of latent infection in the memory B cells. Mechanisms Immunocompromised individuals have a higher risk for NHL due to immunosuppression which causes increased replication of EBV and other carcinogenic viruses. Decreased immune function can cause reactivation and...
replacation of EBV. Psychological stress and aging can reduce the function of immune regulation and are involved in subclinical reactivation of EBV. Reactivation of EBV itself can be a risk factor for NHL. Besides, external factors such as EBV may cause reactivation and an increased risk of NHL [16].

Most NHL are men. This result is in accordance with Sudrajat's research which states that there are 14 male NPC patients and 9 female patients with a ratio of 1.5:1.35 [17]. The same results were also mentioned by Cahyadi et al. 's study of male and female NPC patients with a ratio of 1:9.1.3 [18]. Madani et al. In 2015 mentioned patients with NPC in the Department of ORL-HNS Hasan Sadikin hospital in the period 2010-2014, which showed more male sufferers with a ratio of men to women 2:3:1 [19]. Xiao et al. Reported men are diagnosed with NPC more than women, with 213 people (71.2%) and 86 people (28.8%) respectively [20]. Tahyouni et al. In Morocco getting a ratio between men and women is 2:2.8:1. Boffetta, 2011, states that there are 356,000 new cases of NHL and 192,000 cases with mortality from NHL spread in 2008. Non-Hodgkin's malignant lymphoma or non-Hodgkin's lymphoma is a primary malignant lymphoid tissue. More than 45,000 patients are diagnosed as non-Hodgkin's lymphoma (LNH) every year in the United States. Non-Hodgkin's lymphoma, especially central nervous system lymphoma is commonly found in patients with immune deficiencies and who get immunosuppressive drugs. Non-Hodgkin's Malignant lymphoma is the eighth-ranked malignancy found in men and ranks 11th in women. Countries with the highest incidence are in North America, Europe, Oceania and Africa. The incidence of NPC is more prevalent among men than women. This is because men are more exposed to environmental risk factors such as carcinogens, alcohol, and cigarette smoke. Also, exposure caused by work such as smoke, dust, steam, and chemicals as a risk factor for the occurrence of NPC is more common in men. In individual factors, there is a role for estrogen in women which has an impact on the latency of EBV. This viral infection occurs by means of a direct immune response to the host cell then fusion occurs TR, which causes the epitope to be exposed to the immune system. EBV particles will adsorb to the EBV genome will enter the nucleus, which is a form of latent EBV infection, which is characterized by process of cell activation and proliferation called EBV activation on B lymphocyte cells [7, 8, 23, 24].

Under normal conditions, EBV infection can be controlled and enter the latent phase, where only a few B cells are infected. The lytic phase can occur both in the epithelium of the oral cavity and in B cells which are located adjacent to the epithelium of the oral cavity, which causes many infectious EBV in the oral cavity so that it can spread to other people. In malignancies associated with EBV, the EBV genome appears in each tumor cell in the form of a latent epicone, and the genome will replicate during cell division. The expression of DNA in EBV in the form of latent episomal can be used as a basis in detecting viral infection in the development of NPC [4].

The initial steps of EBV lytic infection are characterized by ZEBRA protein activity encoded by the BZLF1 gene found in epithelial cells and B lymphocytes. Several different products from genes that have correlations with lytic replication cycle stages can be identified and categorized into EMA, EA, VCA, LMA. In latent infections, expression of several proteins occurs: Epstein Barr Nucleus Antigen 2 & 5 (EBNA 2 & 5) which can be detected 2-5 hours after infection, Latent Membrane Protein 1 & 2 (LMP 1 & 2) which can be detected 5-7 hours after infection. Latent infections that are silent and do not produce new viral particles are associated with one of them with NPC. The latent form of EBV infection in NPC includes type II with the expression characteristics of LMP proteins in addition to EBER and EBNA proteins [1, 10, 25, 26].

The exact mechanism by which EBV can induce cancer remains uncertain. However, further research on the expression of the LMP gene shows that it can alter nasopharyngeal epithelial cells in vitro, and it is estimated that LMP in cells infected with EBV protects these cells from a program of cell death or apoptosis. Whereas in other studies it was also found that the LMP gene was found in 65% of people with NPC [10].

The etiology of non-Hodgkin's lymphoma is oncogenes, EBV infections, Human T-leukemia Virus-I (HTLV-I), autoimmune diseases and immune deficiency. More than 90% of non-Hodgkin's lymphoma is Mature B-cell neoplasm. In North America and Europe, B-cell lymphoma is Follicular Lymphoma. Whereas, in Asia, 80-90% form of diffuse lymphoma and T-cell lymphoma is more common. Lymphoma is differentiated based on cell type description. Growth small cells, intermediate and large cells, and the core form (cleaved and non-cleaved). In normal cell follicles polyclonal, whereas in monoclonal lymphoma cells with uniform morphological form and express the same immunoglobulin cells, namely light chain. Lymphoma originating from B cells can be identified with monoclonal antibodies that are specific to B-cells such as CD19, CD20, CD22, and CD23. B-cell lymphoma cells also have their immunophenotypic component which requires parameter analysis. Small B-cell lymphoma consists of B-CLL/SLL (B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma), LPL (Lymphoplasmacytic lymphoma), MCL (Mantle cell lymphoma), FL (Follicular lymphoma), MZL (nodal marginal zone B-cell lymphoma) identified with monoclonal antibodies CD5, CD10, CD23, CD43, slg, cytoly, bcl-1 and bcl-6. But not one antigen is specific to one type of lymphoma, so an antibody panel is needed. The type of B lymphoma is important for prognosis and treatment [9, 11, 14, 16].

In the WHO classification in 2008, the age of the patient was considered as a feature of some newly entered disease entities. For example, in the FL category and nodal MZLs there are distinctive variants that are almost exclusively in the child's age group and are different from clinically and biologically mature. FL pediatric variants are usually local and high histological classes. This lymphoma lacks BCL2/IGH translocation and does not express BCL-2 protein. They may involve nodal or extranodal sites (testis, digestive tract, Waldeyer's ring). Pediatric FL has a good prognosis, with optimal management better results. The difficulty of diagnosis is a rare case of reactive follicular hyperplasia in children who have been reported to contain B-cell clonal CD10* germinal center populations but have not progressed to lymphoma. Nodal MZL in children seems to have a low risk of progression. Most patients present with stage I disease and have a low risk of relapse after conservative therapy [10]. In contrast, some diseases appear to occur most frequently in the elderly, such as EBV+DLBCL from parents, which may arise due to decreased surveillance of immunology. This lymphoma is clinically aggressive and is more common in extranodal than
nadal sites. Neoplastic cells can resemble Hodgkin/Reed-Sternberg cells and show marked pleomorphism, with wider morphology than is usually seen in CHL. Necrosis and general inflammatory background. EBV+DLBCL parents must be distinguished from reactive hyperplasia associated with EBV, which is also found in older people, who usually have benign results, most of which experience spontaneous regression [14, 27]. The antigen chosen for analysis represents a different phase of infection (ie primary, latent and reactive infection), and was chosen to increase the sensitivity of detection of EBV infection and potentially allow differentiation of the stages of infection. Immunoglobulin G (IgG) antibodies to viral capsid antigen (VCA) are produced within a few days of primary EBV infection and peak after 3-4 weeks.

Furthermore, these antibodies decrease slowly but will remain throughout life. Antibodies for EBV nuclear antigen (EBNA) released in the subacute stage of the disease, and like VCA antibodies, persist indefinitely. BZLF1 encoded replicator activator (ZEBRA) is released during the lytic cycle in EBV permissive cells and antibodies are produced during primary EBV infection. ZEBRA is a key mediator of transition from latent to productive cycles on EBV permissive cells and also as a virus reactivation marker. Antibodies to the initial antigen (EA) appear temporarily for up to 3 months during the acute phase of mononucleosis.

During EBV reactivation due to immunosuppression, antibodies for EA increase quite high and persist in chronic infections [15, 23, 28, 29]. The limitation of this study is that it cannot determine the sensitivity and specificity of EA EBV IgA levels ELISA semiquantitatively because the results are divided according to positive, borderline, and negative values.

5. CONCLUSION

The EA EBV IgA antibody levels in WHO type III NPC were higher when compared to NHL. People with NPC and NHL are more likely to have male sex. The age of patients with NPC and NHL is highest in decades 4-6. NHL can give negative results on EA EBV IgA antibody examination and IgA EA cannot be used as early detection for NHL.

REFERENCE