EPIDERMAL GROWTH FACTOR RECEPTOR EXPRESSION IN NON-KERATINIZED NASOPHARYNGEAL CARCINOMA SUBTYPE AT PADANG

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Abstract

Introduction: Patients with nasopharyngeal carcinoma have a poor prognosis, there are several factors that cause it to happen, one of the existing therapeutic response has been inadequate. Expression of Epidermal Growth Factor Receptor (EGFR) has been used as a biological marker targeted therapy in nasopharyngeal carcinoma. Histopathologic subtype tumors also determine the prognosis of patients with nasopharyngeal carcinoma.

Objective: The aim of the study to determine between the expression of epidermal growth factor receptor between non-keratinized differentiated and undifferentiated subtypes in nasopharyngeal carcinoma and correlation with their clinical stage. Study design, Cross-sectional comparative study. Place and duration study, Department of Otorhinolaryngology, Department of Pathology Anatomy in Dr. M. Djamil Hospital, Padang and Department of Pathology Anatomy in Gajah Mada University, between May 2015 until October 2015

Method: There were 36 samples paraffin blocks of nasopharyngeal carcinoma biopsy, respectively 18 paraffin blocks are non-keratinized differentiated and 18 non-keratinized undifferentiated nasopharyngeal carcinoma subtypes. Each sample examined EGFR expression by immunohistochemical staining methods.

Result: There were positive EGFR expression results in all sample as 69.4%. Expression of EGFR positive non-keratinized differentiated subtypes in nasopharyngeal carcinoma as 77.8% and undifferentiated subtype as 61.6%. There were no significant differences of EGFR expression between non keratinized differentiated and undifferentiated subtypes nasopharyngeal carcinoma (p>0.05). There are no significant differences of EGFR expression between new and advanced stage nasopharyngeal carcinoma (p>0.05).

Conclusion: There were no significant differences of EGFR expression between non-keratinized differentiated and non-keratinized undifferentiated subtypes in nasopharyngeal carcinoma. Analysis of the study also showed no significant differences of EGFR expression based on the clinical stage nasopharyngeal carcinoma.

1. INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a malignant tumor from nasopharyngeal epithelium. The etiology of NPC is multifactorial, ethnic and geographical factors influence the risk of disease [1]. The etiology of nasopharyngeal carcinoma associated with the Epstein Barr virus infection factors, genetic and environmental [2,3].

Epidemiology of NPC can be found in all countries where the incidence is highest in southern China, especially in the province of Guangdong [4]. Incidence in Guangdong province in men reach 20-50 per 100,000 population/year [1]. NPC is found in other countries and certain in ethnic groups such as Chinese, Southeast Asia, and North Africa [5].

In 2005, WHO classified the NPC into 3 subtypes; 1) Keratinized squamous cell carcinoma, 2) Non-keratinized differentiated and 3) Basaloid squamous cell carcinoma [6]. Keratinized squamous cell carcinoma subtypes rare in endemic areas, otherwise, non-keratinized squamous cell carcinoma subtype frequent in endemic areas and is closely linked with infections of Epstein Barr Virus (EBV) [7-9].

One of the most important elements factors to study the behavior of biological NPC is to understand the signaling pathways that are formed at the level of intracellular. There are several signaling pathways of NPC, one is the Epidermal Growth Factor Receptor (EGFR). EGFR is a receptor tyrosine kinase that is frequently expressed in the epithelial tumor. Activation of EGFR (phosphorylated EGFR (p-EGFR)) is stimulated by several different signal transduction pathways such as Ras signaling (Ras/mitogen activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)/Akt pathway, phospholipase-Cγ (PLCγ)/PLC protein kinase C pathway, Ras/PI3K/Akt, and Src/Src kinase Pathway). Transduction pathways activated by pEGFR play an important role in several cell processes, such as cell proliferation, cell differentiation, adhesion, migration and apoptosis[7].

EGFR expression was also associated with clinical stage of the tumor such as tumor size, lymph node involvement and distant metastasis that readvanced with prognosis [10]. In the last two decades, EGFR receptors as therapeutic targets in cancer therapy with several forms of anti-EGFR. Some studies try to learn and understand the mechanisms of activation and function of this receptor, which can be used as an anti-EGFR targeted therapy in NPC [10].

2. MATERIAL AND METHODS

The study included 36 samples blocks paraffin of nasopharyngeal carcinoma biopsy that examined EGFR expression by immunohistochemical staining method, consisting of 18 samples with non-keratinized differentiated and 18 samples undifferentiated NPC subtypes. Methods study is a cross-sectional comparative study. Place and duration study is Department of Otorhinolaryngology and Pathology Anatomy at Dr. M. Djamil Hospital, Padang and Department of Pathology Anatomy at Gajah Mada University, Yogyakarta between May 2015 and October 2015.

Immunohistochemical staining: Paraffin blocks were cut with a thickness of 4-6 microns then placed on a slide. The slide was carried out deparaffinization and rehydration process and antigen retrieval. The slide was giving 0.3% H2O2 (in distilled water) for 30 minutes at room temperature. Wash slides with distilled water and PBS, incubation in 1% normal serum for 30 minutes at room temperature. Incubation with anti-EGFR antibody dilution of 1:50 at room temperature for 120 minutes. Wash slides with PBS 3 times. Incubation in biotin-labeled secondary antibody for 30-60 minutes. Incubate slides in streptavidin complex for 60 minutes. Wash slides with distilled water+2 drops of buffer DAB DAB+1 drop of liquid 20 minutes before use. Slide gives a drop of fresh solution for 5-10 minutes. Counterstain with hematoxylin, dehydration in alcohol and enter into xylene 2 times, each 10 minutes, slide cover with a glass deck.
Expression of EGFR with immunohistochemical staining methods was read an analysis by one person from the Department of Pathology Anatomy, Gajah Mada University, Yogyakarta. Data were analyzed using the Chi-Square test and considered statistically significant if p<0.05.

3. RESULT

Table 1. Differences frequency based on EGFR expression between non-keratinized differentiated and undifferentiated subtypes in nasopharyngeal carcinoma

<table>
<thead>
<tr>
<th>Non-Keratinized Subtype</th>
<th>EGFR</th>
<th>Total f (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differentiated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative f (%)</td>
<td>4 (62.3)</td>
<td>14 (17.8)</td>
<td>18 (100.0)</td>
</tr>
<tr>
<td>Positive f (%)</td>
<td>7 (38.9)</td>
<td>11 (61.1)</td>
<td>18 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>11 (30.6)</td>
<td>25 (69.4)</td>
<td>36 (100.0)</td>
</tr>
</tbody>
</table>

Table 2. Differences frequency based on EGFR expression between newly and advanced stage nasopharyngeal carcinoma

<table>
<thead>
<tr>
<th>Stage EGFR</th>
<th>Total f (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly Stage (I-II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative f (%)</td>
<td>0 (0.0)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>Positive f (%)</td>
<td>8 (88.9)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Total</td>
<td>11 (30.6)</td>
<td>25 (69.4)</td>
</tr>
</tbody>
</table>

4. DISCUSSION

The expression of EGFR in non-keratinized differentiated subtypes as 77.8% compared with undifferentiated as 61.6%. There was no significant difference between non-keratinized differentiated and undifferentiated NPC subtypes with a value of p=0.469 (table 1) Huang et al [12] did not found a significant correlation between these subtypes. Huang et al. found in non-keratinized differentiated 45% and undifferentiated 55%. On immunohistochemical examination, there are two factors that affect the EGFR expression that is a pramalytic and analytic factor. The difference in sample tissue tumor (frozen sample tissue tumor or sample tissue tumor in blocks paraffin), type of fixation, large and tissue cutting techniques and time fixation will affect the results of the examination [13].

In this study, the long duration of sample tissue NPC has no effect on the results of EGFR expression. Zhang et al., in the retrospective study period 2005-2009, found 10 samples of a total of 96 samples with negative EGFR expression [14]. Zhang’s study concludes the long duration of sample tissue tumor does not affect the results of EGFR expression. Same with Huang et al study which collecting 170 paraffin blocks of NPC sample biopsy began the period 1996-1999 [10].

Negative false of EGFR expression could be caused by the type of fixative solution. The recommended fixation solution is 10% buffered formalin solution, while fixation in this study is a usual formalin solution. Late fixation and excessive fixation may also cause loss of staining reaction [13, 15]. Analytic factor is sample tissue processed to be ready preparation and interpreted by pathological experts. Methods dilution/dilution, temperature, time of incubation, the use of primary antibodies and others will affect the EGFR expression.

The expression of EGFR in newly stage as 100%, while in the advanced stage as 42% and stage III-IV as 58% with p=0.10. Huang et al. also found no significant difference between size of the T (Tumor) and N (Node) with a p-value of 0.18 and 0.15 respectively.

In contrast with Zhang et al [14], there were significant differences in EGFR expression (p<0.01) between the newly stage 66% and advanced stage 96%. Zhang also found significant differences in EGFR expression base on the tumor size (p<0.005), although there are no significant differences (p=0.28) based on lymph node metastasis.

5. CONCLUSION

In conclusion, a number of EGFR positive expression in non-keratinized differentiated subtype nasopharyngeal carcinoma greater than undifferentiated subtypes. There was no significant differential expression of EGFR between non-keratinized differentiated and undifferentiated NPC subtypes. Statistic analysis of the study also showed no significant differences of EGFR expression based on the clinical stage nasopharyngeal carcinoma.

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