

## Association of Epidermal Growth Factor Receptor (EGFR) with Tumor Location and Clinicopathological Aspect in Head and Neck Squamous Cell Carcinoma

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**Background:** Head and neck squamous cell carcinoma (HNSCC) is the most common type of head and neck cancer. Most patients came with advanced disease which results in suboptimal treatment. EGFR is one of prognostic factors that play important role in HNSCC pathogenesis and progression. Unraveling the expression profile of EGFR in anterior and posterior HNSCC and its comparison would provide physician with better information regarding EGFR based treatment or its prognostic value. **Methods:** An observational cross sectional analytic study was conducted to compare EGFR expression between anterior and posterior HNSCC. The association between EGFR expressions with clinicopathological aspects (tumor stadium and histological grade) of HNSCC which analyzed separately. Baseline characteristic and each variable were first analyzed descriptively. Comparative analysis was conducted using Chi-Square and Fisher exact test with p value less than 0.05 was considered significant. **Result:** 48 samples were used in this study. Most of the subjects were elderly (83.2%), men (72.9%) and came with advanced disease (Clinical Stadium III-IV) (83.3%). Most of the subjects were EGFR (+) (93%). In anterior region group, low expression of EGFR was found in 6 patients (25%) and high expression in 18 patients (75%). Meanwhile in posterior region group we found low expression in 10 patients (41.7%) and 14 patients with high expression of EGFR (58.3%). We found no association between EGFR and HNSCC location (anterior or posterior) ( $p=0.221$ ). Insignificant association also reported between EGFR expression with clinical stage of anterior ( $p=0.625$ ) and posterior ( $p=0.283$ ) HNSCC as well as histopathological grade of both location ( $p=0.33$  for anterior group and  $p=0.371$  for posterior group). **Conclusion:** We concluded that EGFR was diffusely expressed in HNSCC but there was no association between EGFR expression and clinicopathological characteristics and location of HNSCC.

**Keywords:** Epidermal growth factor receptor, Head and Neck Squamous cell carcinoma, Tumor location, Clinical stage, histopathology grade.

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### INTRODUCTION

Head and neck cancer is a broad term for a range of epithelial malignancy of paranasal sinuses, nasal, and oral cavity, as well as for pharyngeal and laryngeal carcinoma. The most common types of head and neck cancer is head and neck squamous cell carcinoma (HNSCC) which has a high incidence, especially among smokers, alcoholism, those with bad oral hygiene as well as unfixed dentures.

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In some cases, viral infection such as *human papillomavirus* (HPV) and *Epstein-Barr Virus* (EBV) are found to be a significant risk factor for HNSCC especially those occurred in the nasopharynx and oropharynx cavities. Based on the etiology, HNSCC can be classified into anterior region HNSCC (oral cancer) and posterior region HNSCC (nasopharynx and oropharynx) with circumvallate papilla as the border. The division of HNSCC anterior and HNSCC posterior are according to *ohngren's line*, the line which is from medial cantus to the mandible angle.

HNSCC is common cancers worldwide with 266.900 cases occurring annually and 128.000 mortality cases in 2008.<sup>1</sup> In the US, the incidence is estimated at 29.800 new cases with the mortality rate at 8.100 cases every year.<sup>2</sup> The relationship

between HNSCC and its risk factors had been well established. It is well known that there is a significant association between HNSCC with the carcinogen such as tobacco, alcohol and (Pinang).<sup>3</sup> There are also evidences that link HNSCC with HPV infection as reveal in demographic data of HNSCC in 2008 which show there were 22.000 HPV (+) cases from 85.000 HNSCC cases worldwide, most of them were not smoking or consuming alcohol.<sup>4</sup> On the other hand, 40-80% of oropharyngeal HNSCC in the US are caused by HPV infection.<sup>5</sup>

Meanwhile, for nasopharyngeal cancer (NPC), it is estimated to occur in 84.400 new cases and 51.600 mortality in 2008<sup>1</sup> with most of the cases occurred in China and Southeastern Asia including Malaysia, Indonesia, and Singapore. There are several risk factors for NPC such as genetic, environmental factors, food, and EBV infection. EBV infection stands as the most prominent risk factors with almost 90% of undifferentiated NPC in England were found with EBV antigen (+).<sup>6</sup>

In Indonesia, HNSCC is one among 10 most prevalent carcinomas in women contributing to nearly 3,03% of all carcinoma cases. Meanwhile, in men, it is the second most common carcinoma (11,27%).<sup>7</sup> According to some pathologic centers in 1998, cancer of oral cavity ranked at no 2 most common cancers in Bali after cervical cancer.<sup>7</sup> Meanwhile, 40 cases of oral cancer and 25 cases of NPC were confirmed in Sanglah General Hospital in 2013-2014. Balinese patients almost always come with advanced disease that can only treat palliative with suboptimal results.

Several literatures state that genetic factors played the significant role as HNSCC risk factor, outcome predictor as well as a prognostic factor that results in changes of HNSCC management. However, despite the changes, there is only minimal improvement in patient survival.<sup>3</sup> This evidence clearly states the need of new biomolecular research of HNSCC in order to detect the alteration responsible for HNSCC cellular behavior as well as to identify the potential prognostic biomarker.<sup>8</sup>

Epidermal growth factor receptor (EGFR) already studied extensively as a therapeutic target for anticancer drugs. The expression of growth factor and its cognate receptors has already known as the key pathogenesis in carcinogenesis as well as tumor progression.<sup>9</sup> In HNSCC, EGFR expression is a strong determinant of tumor prognosis and progression.<sup>10,11</sup> The relationship of EGFR expression with tumor size, lymphatic invasion, and metastasis is already established.<sup>12</sup> High EGFR expression relates to smoking habits, alcoholism, invasive tumor characteristic, and poor cellular differentiation in HNSCC.<sup>13,14</sup> EGFR overexpression is also found in 80% of the

undifferentiated type NPC and associated with poor prognosis. In oropharyngeal cancer, HPV infection is associated with lower EGFR expression but related to more favorable prognosis.<sup>15</sup> in Bali (2008) High EGFR expression was found in 80% of 30 HNSCC patients and associated with tumor size and nodal status.<sup>16</sup>

EGFR was one of the therapeutic targets in HNSCC and can be targeted by anti-EGFR drugs such as Cetuximab.<sup>17</sup> However, despite common application of this type of drugs, the outcome of EGFR based therapy alone is not very satisfied.<sup>18</sup> EGFR drugs are usually combined with chemoradiation therapy to increase the effectiveness of therapy and also to extend the overall survival.<sup>19</sup>

In this research, we evaluate the difference of EGFR expression between anterior HNSCC and posterior HNSCC. We also evaluate the relationship of EGFR expression with clinicopathological characteristic (clinical stadium and histological grade) of the anterior and posterior HNSCC. The aim of this research is to evaluate the difference of EGFR expression between anterior and posterior HNSCC to further validate the EGFR based treatment in HNSCC.

## **MATERIAL AND METHODS**

A cross-sectional analytic study was conducted to evaluate the difference of EGFR expression between anterior and posterior HNSCC. 48 subjects with confirmed HNSCC and complete clinicopathological data were enrolled in this study. The samples were tissue samples obtained from the subjects by biopsy and preserved in the paraffin block. The samples used were obtained from 2013-2014. Patients who's already finished chemotherapy cycles, inadequate specimen from a biopsy and those who refuse to participate were excluded.

Clinicopathological data were obtained from patient's medical records. Paraffinized samples were stained by immunohistochemistry (IHC) technique using a specific EGFR antibody. Clinicopathological data was collected as clinical stadium and histological grades. EGFR staining was examined by a pathologist and classified into 5 values based on the percentage of area stained. 0 stands for no antibody staining, 1 equals  $\leq 10\%$  area stained, 2 if 10-50% area stained, 3 if 50-80% area stained and 4 if more than 80% area was positively stained. The clinical stage was divided into 4 groups based on AJCC 2002. Stadium I and II were classified as an early disease and the others stadiums were classified as an advanced disease. Histological grades were classified as well differentiated, moderately differentiated, poorly differentiated and undifferentiated. Each variable was analyzed by comparing its value in anterior HNSCC and posterior HNSCC. Normality test was

conducted by Shapiro-wilk test before proceeding to bivariate analysis. Then, the association between EGFR expression and clinicopathological variables was analyzed. The analysis of anterior and posterior group was conducted separately. Every analysis in this study was conducted by using SPSS 21.1 with p-value <0.05 was considered significant.

## RESULTS

Total 48 samples were enrolled in this study. Each group (anterior and posterior HNSCC) consists of 24 samples. The youngest subject was 22 years old and the eldest was 83 years old overall mean for age variable was 54±13,72. For the anterior group the lower and upper boundary of age variables was 34-83 years with mean 56,4±13,2. Meanwhile, the average age of posterior HNSCC was 51,58±14 with the youngest subject being 22 years old and 69 years as the upper boundary. Subjects with age ≤ 40 years were considered as young age meanwhile, those with age > 40 years as elder group. According to age classification, we found the majority of samples was elderly with men consist most of our subjects (72,9%) (Table 1).

**Table 1.** Subject Baseline Characteristic of Head and Neck Squamous cell Carcinoma.

Variables	N(%)
Age: ≤ 40 years old (young)	8 (16,7%)
>40 years old (elderly)	<b>40 (83,3%)</b>
Sex : Male	<b>35 (72,9%)</b>
Female	13 (27,9%)
Location : Nasopharynx	<b>21 (48%)</b>
Palatine Tonsils	3 (6,3%)
Tongue	14 (29,2%)
Ginggiva	3 (6,3%)
Buccal	4 (8,3%)
Palate	2 (4,2%)
Lips	1 (2,1%)
Stadium: Stadium I	0 (0%)
Stadium II	8 (16,7%)
Stadium III	<b>31 (64,6%)</b>
Stadium IV	9 (18,8%)
Early Disease (I-II)	8 (16,7%)
Advanced Disease (III-IV)	<b>40 (83,3%)</b>
Grade : Well differentiated	12 (25%)
Moderately Differentiated	13 (27,1%)
Poorly differentiated	2 (4,2%)
Undifferentiated	<b>21 (43,8%)</b>
Low Grade (well differentiated dan moderately differentiated)	<b>25 (52,1%)</b>
High Grade (Poorly differentiated-Undifferentiated)	23 (47,9%)

Nasopharynx (48%) and tongue (29.2%) were the most prevalent tumor location in our subjects. Based on its location, we divided the subjects into 2 groups. Anterior HNSCC (tongue, gingiva, buccal, hard palate, and lips) and posterior HNSCC (nasopharynx, tonsils, soft palate) (Table 2). Most of the cases were at clinical stadium III (64.6%) meanwhile, if further classified into early (clinical stadium I and II) and advanced (clinical stadium III and IV), most of the subjects were at advanced disease (83.3%). More than half of the subjects had large tumor size (T3 [62.5%]) but no nodal involvement (N0 [58.3%]). However, a considerable number of subjects (37.5%) had ipsilateral nodal involvement (N1).

**Table 2.** Comparison of subject's baseline characteristic between anterior HNSCC group and posterior HNSCC group.

	Anterior	Posterior
Age: ≤ 40 years old (young)	2(8,3%)	6(25%)
>40 years old (old)	<b>22(91,7%)</b>	<b>18(75%)</b>
sex : Male	<b>16(66,6%)</b>	<b>19(79,2%)</b>
Female	8(33,3%)	5(20,8%)
Early Disease (I-II)	3(12,5%)	5(20,8%)
Advanced Disease (III-IV)	<b>21(87,5%)</b>	<b>19(79,2%)</b>
Low Grade (well differentiated dan moderate differentiated)	<b>22(91,7%)</b>	3(12,5%)
High Grade (Poorly differentiated-Undifferentiated)	2(8,3%)	<b>21(87,5%)</b>
Low EGFR Expression ( 0, 1, 2)	10(41,7%)	6(25%)
High EGFR Expression ( 3, 4)	<b>14(58,3%)</b>	<b>18(75%)</b>

Based on histological grade, most of our subjects had an undifferentiated type (48.8%) while well differentiated and moderately differentiated type has an equal proportion (25% and 27.1%). Further classification of histological grade into low grade (well differentiated and moderately differentiated) and high grade (poorly differentiated and undifferentiated) showed that both groups had almost equal proportion (52.1% and 47.9%, respectively). Immunohistochemical analysis reveals almost half samples had +4 (>80% area stained) (47.9%) staining score. Further classification into low expression (0, +1, +2) and high expression level (+3 and +4) reveal that most

subjects fell into a high expression level (66.7%). In anterior HNSCC, the proportion of low EGFR expression and high EGFR expression was almost equal. On the other hand, the proportion of high EGFR expression was substantially higher (75%) compared with low EGFR expression (25%) in the posterior HNSCC group.

### The Difference of EGFR Expression between Anterior and Posterior HNSCC

Of 24 subjects in anterior HNSCC group, we found an almost equal proportion of high EGFR expression and low EGFR proportion (58.3% and 41.7%, respectively). Meanwhile, in the posterior group, the proportion of high EGFR proportion was significantly higher (75%) compared with low EGFR expression (25%). However, analyzing the difference between anterior and posterior HNSCC by the chi-square test yield insignificant result ( $p=0,221$  ( $p>0,05$ )) (Table 3).

**Table 3.** Comparison of EGFR Expression between Anterior and Posterior HNSCC

Expression	Anterior	Posterior
High EGFR	14 (58,3%)	18 (75%)
Low EGFR	10 (41,7%)	6 (25%)
<b>Total</b>	<b>24 (100%)</b>	<b>24 (100%)</b>

$p=0,221$  (Chi-Square)

### Relationship of EGFR Expression with Clinical Stadium in anterior HNSCC

**Table 4.** Relationship of EGFR Expression with Clinical Stadium in anterior HNSCC

Expression	Disease	
	Early	Advanced
High EGFR	2 (66,7%)	12 (57,1%)
Low EGFR	1 (33,3%)	9 (42,9%)
<b>Total</b>	<b>3 (100%)</b>	<b>21 (100%)</b>

$p=0,625$  (Fisher's Exact)

Among 24 subjects of anterior HNSCC, we found 3 subjects with early disease (Clinical stadium I and II). Among them, 2 subjects showed high EGFR expression and 1 subject showed low EGFR expression. From the 21 subjects with advanced disease, 12 subjects showed high EGFR expression and 9 subjects with low expression. However, no significant differences or association was detected by Fisher's exact test ( $p=0,625$ ) (Table 4).

### Relationship of EGFR expression with Histological Grade in Anterior HNSCC

Regarding histological grade in the anterior HNSCC group, we found 22 subjects with a low histological grade. Among them, 12 with high EGFR expression and 10 with low EGFR expression. On the other hand, there are only 2

subjects with high histological grade and all of them were with high EGFR expression. However, the fisher exact test showed no significant association between these variables ( $p=0,330$ ) (Table 5).

**Table 5.** Relationship of EGFR expression with Histological Grade in Anterior HNSCC

Expression	Low Grade	High Grade
High EGFR	12 (54,5%)	2 (100%)
Low EGFR	10 (45,5%)	0 (0%)
<b>Total</b>	<b>22 (100%)</b>	<b>2 (100%)</b>

$p=0,330$  (Fisher's Exact)

### Relationship of EGFR expression with Clinical Stadium in Posterior HNSCC.

From 24 posterior HNSCC subjects, we found 4 patients with early disease. Among them, 4 with high EGFR expression and 1 subject with low EGFR expression. Meanwhile, from 19 subjects with advanced disease, there are 10 subjects with high EGFR expression and 9 patients with low EGFR expression. Despite the difference, Fisher's exact test yield insignificant result ( $p=0,283$ ) (Table 6).

**Table 6.** Relationship of EGFR expression with Clinical Stadium in Posterior HNSCC.

Expression	Disease	
	Early	Advanced
High EGFR	4 (80%)	10 (52,3%)
Low EGFR	1 (20%)	9 (47,4%)
<b>Total</b>	<b>5 (100%)</b>	<b>19 (100%)</b>

$p=0,283$  (Fisher's Exact)

### Relationship of EGFR Expression with Histological Grade in Posterior HNSCC

In the posterior HNSCC group, we only found 3 subjects with the low histological grade. Among them, 1 subject had a high EGFR expression and 2 subjects with low EGFR expression. From 21 subjects with high histological grade, there are 13 subjects who had a high EGFR expression and 8 with a low EGFR expression. Fisher's exact test yield non-significant result ( $p=0,371$ ) (Table 7).

**Table 7.** Relationship of EGFR Expression with Histological Grade in Posterior HNSCC

Expression	Low Grade	High Grade
High EGFR	1 (33,3%)	13 (61,9%)
Low EGFR	2 (66,7%)	8 (38,1%)
<b>Total</b>	<b>3 (100%)</b>	<b>21 (100%)</b>

$P=0,371$  (Fisher's Exact)

## DISCUSSION

In this study, we found that the majority of subjects were men (72.9%) with the mean age was  $54 \pm 13,72$ . There were no significant differences in

age among subjects from both anterior HNSCC and posterior HNSCC group. In more than half of anterior HNSCC cases, tumor occurs on the tongue. Meanwhile, nasopharynx is the region preferred by the majority of posterior HNSCC (87.5%). The majority of subjects had advanced disease and low histological grade and overall had high EGFR expression.

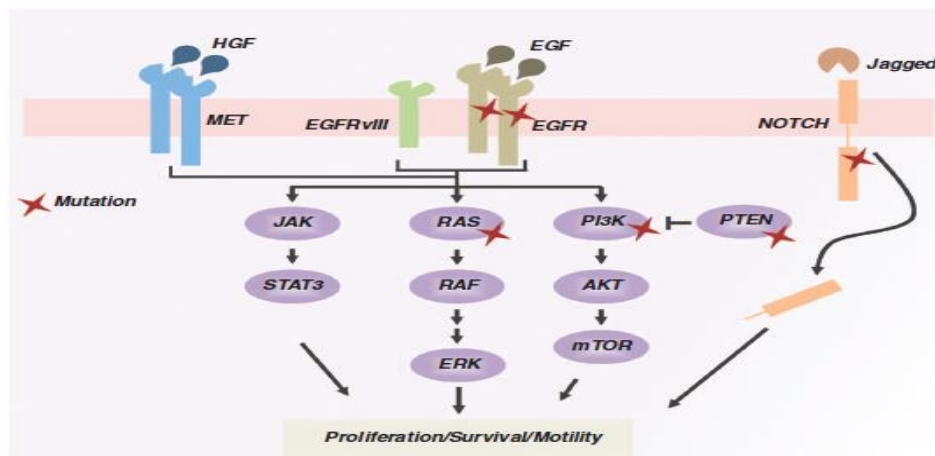
Epidemiological data show that men have a higher risk acquiring HNSCC compare with women with ratio approximately 3:1 and tend to be in older age groups. The main causes of this phenomenon are smoking habits and alcoholism that more often be found in men than women.<sup>1</sup> In addition, Curado and Hashibe reveal that smoking and alcoholism had a supra-additive effect as risk factors of HNSCC.<sup>20</sup> However, in the last 10 years, HPV emerges as new risk factors for HNSCC, independent from smoking habits and alcohol consumption. The patients tend to be younger, not always smoking or consuming alcohol, and has a predilection to occur at oropharynx. The presence of HPV antigen within tumor tissue is associated with oral sex and more favorable prognosis.<sup>21</sup> However, the prognostic value of HPV infection is

still controversial. In this study, we found a wide range of age (22-83 years old). Sociocultural factors (tobacco, alcohol consumption, educational level, oral sex, and HPV infection) that were not evaluated seems to contribute to this phenomenon.

In this study, almost all subjects have a high EGFR expression (93.75%). Despite EGFR expression appeared to be higher in posterior HNSCC compared with anterior one, the difference was not statistically significant. So far, there is no study that compares EGFR expression between anterior and posterior HNSCC. However, most of the studies clearly show high EGFR expression in oral cancer (>80%), nasopharynx carcinoma, and oropharynx carcinoma with HPV (-).<sup>14,22,23,24</sup>

Our study also found no association between EGFR expression with the clinical stadium and histopathological grade, both in the anterior and posterior group. Based on several studies, the relationship between EGFR and clinicopathological status is still controversial. Some of them found a significant relationship between HNSCC and some features of clinicopathological status, such as tumor stadium, mitotic index, nodal status and histological grade.<sup>12,14,25,26,27,28</sup>

**Figure 1.** Interconnection of pathways responsible for HNSCC pathogenesis.<sup>10</sup>



However, other studies did not arrive at the same conclusion which all clearly state no association between EGFR expression and clinicopathological features mainly the clinical stadium and histological grade.<sup>13,15,29,30,31,32</sup> Similar results were found in *EXTREME* and *CRYSTAL* studies in 2012 which involved 411 subjects.<sup>33</sup> Study from Bali (2008) also yielded no association with clinical stadium and histological grade, although there was a significant association with nodal status.<sup>16</sup>

Because of conflicting reports from our study as well as several other studies, we try to construct the theoretical basis of this phenomenon. In multistep carcinogenesis, EGFR plays important role in dysplastic phase and precancerous lesion.<sup>3,34</sup>

A report from Mahendra et.al found that

EGFR was highly expressed in oral leukoplakia, which is in accordance with the multistep theory.<sup>13</sup> However, EGFR and its corresponding signaling pathway are just one of several pro-carcinogenic pathways important for HNSCC progression. Among them are Ras/Raf, JAK/STAT, PI3K/AKT, Met, and NOTCH signaling pathway. In fact, the mutation in Ras isoform (HRAS, NRAS, and KRAS) contributes to 10-15% of HNSCC and HGF-Met contributes to nearly 80% of HNSCC cases. **Figure 1** resumes the interaction of the aforementioned pathways.

Overall, there are several aspects that contribute to the differences among our study and the previous studies described above. The difference in study design, sample collection, EGFR expression examination technique, the

limitation of IHC interpretation, semi-quantitative methods that heavily depend on operators, as well as a diverse oncogenic signaling pathway that contribute to HNSCC progressivity all, contribute to this phenomenon. Further study with more comprehensive patient selection as well as evaluation of HPV or EBV antigen evaluation is needed in order to confirm the exact role of EGFR and its association with corresponding viral infection.

## CONCLUSION

Our study reveals that EGFR was highly expressed in HNSCC and there was no significant difference in EGFR expression between anterior and posterior HNSCC. There was also no significant relationship between HNSCC with the clinical stadium and histological grade either in the anterior or posterior HNSCC. The result of this research suggests that EGFR was diffusely expressed independent of its location, stadium, or histological grades and thus suitable for a prognostic marker and therapeutic target for anterior or posterior HNSCC. However, regarding the conflicting report of an association between HNSCC and clinicopathological features, further study is needed in order to reveal the definitive interaction of EGFR with the clinicopathological aspect of HNSCC either clinically or molecularly.

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