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# Association of P53 Protein Overexpression with Clinicopathological Features of Oral Squamous Cell Carcinoma Patients in Bali

**[1]Winata, A., [2]Manuaba, I.B.Tjakra .W., [2]Sudarsa, I.W., [1]Mahadewa, Tjok G. B**
*[1]Surgery Department, Faculty of Medicine Udayana University / Sanglah General Hospital*
*[2] Oncological surgery sub department, Surgery Department, Faculty of Medicine Udayana University / Sanglah General Hospital*

**Background**: Oral cancer was a major health problem with a high incidence rate worldwide. Oral Squamous Cell Carcinoma (OSCC) in Bali, ranked as the second most common cancer after cervix carcinoma. Our understanding of OSCC hasn't yielded a satisfactory clinical outcome; therefore, further studies about the role of biomolecular markers in OSCC are still needed. One of the biomolecular markers for prognosis and predictor for OSCC that has been a topic of research to date is p53. **Method**: This is a cross-sectional analytical study of 36 samples to determine the correlation between p53 overexpression with age group, tumor location, tumor stage, and tumor grade in OSCC patients. Data was processed descriptive and analytical using Chi-Square/Fisher 's Exact Test methods with a significance value of p <0.05 was considered significant. **Results**: From the 36 samples collected, the mean age was 55,19±14,712 years, 19 samples (52.8%) were women, 21 samples (58,3%) were ≤60 years, 24 samples (66,7%) had OSCC on the postero-inferior region, 19 samples (52,8%) were in high stage group, 22 samples (61,1%) had high grade OSCC, and 22 samples (61,1%) showed high p53 overexpression. There were significant differences in proportion between OSCC grade with p53 expression (Chi-square test p=0,013; r=0,416; OR=6,12 CI=95%) and between OSCC N stage with p53 expression (Fisher's Exact Test p=0,024; r=0,396; OR=6,33 CI=95%). **Conclusion**: There were significant differences in the proportion between OSCC grade and N stage with p53 expression. Therefore, p53 overexpression can be used as a prognostic indicator and predictor of lymph node involvement in OSCC.

**Keywords**: **Oral Cavity Squamous Cell Carcinoma, p53, Clinicopathology.**

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## BACKGROUND

Oral squamous cell carcinoma (OSCC) had the highest incidence rate among head and neck squamous cell carcinoma (HNSCC). Important risk factors were smoking and alcohol consumption, also the viral infections role and a number of biomolecular markers for OSCC have contributed to our understanding and management of OSCC.

An estimated 263,900 new cases and 128,000 deaths from OSCC occurred worldwide in 2008.[1] In Indonesia, OSCC ranked number 9 (3.03%) out of 10 most common carcinomas in women and ranked 2 (11.27%) out of 10 most common carcinomas in men.[2]

**Corresponding author:**
**Winata A.**
**Address: Department of Surgery, Faculty of Medicine Udayana University / Sanglah General Hospital, Denpasar, Indonesia**

Based on data from 13 pathology centers in Indonesia in 1998, OSCC was the second most common carcinoma in Bali after cervix carcinoma.[2] For the last two decades a number of literatures have found the role of genetic factors as risk, prognostic, and also predictor factor in OSCC. However, these findings have not contributed to better life expectancy nor recurrence rate for OSCC.[3] These advances in the field of head and neck cancer, has led most to believe the importance of molecular genetic studies (EGFR, Cyclin D-1, p53, p16, STATs) to better understand the nature of OSCC.[4]

A high incidence of p53 gene alteration was observed in OSCC, therefore p53 gene mutations was suspected to have an important role in the pathogenesis and progression of OSCC.[5] However, a meta-analysis study conducted by Tandon et al stated that the role of p53 as a prognostic and predictive factor for OSCC has not been established.[6] Nevertheless, p53 has remained a

focus of many researchers in relation to OSCC, particularly as predictor for OSCC.[7, 8]

## MATERIAL AND METHOD

This is a cross-sectional analytical study to determine the correlation between p53 protein overexpression with clinicopathological features of OSCC patients in Bali. A cross-sectional sampling was performed on OSCC cases treated at the surgery department in Udayana University / Sanglah General Hospital in Denpasar Bali from 2009 until 2012. Data were recorded from the medical records, including patient identity, histopathological diagnosis, tumor location, tumor stage and tumor grade.

Based on data obtained from the medical records, data collection and paraffin blocks were obtained from the pathological anatomy department in Udayana University / Sanglah General Hospital. Paraffin blocks were then cut into 3-5 microns thick and immunohistochemistry evaluation was performed to determine p53 protein expression (p53 Tumor Suppressor Protein, *Biocare Medical*) according to immunohistochemistry evaluation protocol in Udayana University / Sanglah General Hospital pathology anatomy department. P53 protein expression was assessed by one pathologist using semi-quantitative method and divided into 2 groups: weak expression and strong expression based on Fisher et al criteria.

Age was determined based from the patient's medical record when the patient was clinically diagnosed with OSCC. Samples were grouped into ≤60 years and >60 years. Tumor location was determined from physical examination of the medical record into: lips, buccal, palatum, tongue, and ginggiva. Tumor location was regrouped into antero-superior location (lips, buccal, and palatum) and postero-inferior location (tongue and gingiva). Tumor stage was obtained from the medical record and based on the AJCC Tumor Staging System (2002). Stage I, II, III were categorized as low stage and stage IVA, IVB and IVC were categorized as high stage. Well differentiated OSCCs were categorized as low grade tumor, while moderately and poorly differentiated OSCCs were categorized as a high grade tumor.

Data from the samples collected was processed descriptively to determine sample characteristics and analyzed to determine the correlation between p53 overexpression with age group, tumor location, tumor stage, and tumor grade in OSCC patients. The data obtained was tested quantitatively using the Shapiro-Wilk test for normality, then the data was processed using univariate analysis Chi-Square Test or Fisher's Exact Test with $p<0,05$ was considered significant. Significant correlations were then analyzed with Pearson's Correlation Test and the Odds Ratio was determined. Analysis was performed using SPSS 21.0 for Windows 7.

## RESULTS

From 36 samples obtained, the average age was 55,19±14,712 years old, 19 samples (52.8%) were women, 21 samples (58,3%) were ≤60 years, 24 samples (66,7%) had OSCC on the postero-inferior region, 19 samples (52,8%) were in High Stage group, 22 samples (61,1%) had high grade OSCC, and 22 samples (61,1%) showed high p53 overexpression. Overall, the characteristics of the sample are presented in Table 1.

**Table 1.** OSCC Sample Characteristics

| Variable | n |
|---|---|
| *Age* | |
| ≤ 60 years | 21 (58,3%) |
| > 60 years | 15 (41,7%) |
| *Sex* | |
| Male | 17 (47,2%) |
| Female | 19 (52,8%) |
| *Tumor Location* | |
| Tongue | 18 (50%) |
| Gingiva | 6 (16,7%) |
| Palatum | 4 (11,1%) |
| Buccal | 6 (16,7%) |
| Lip | 2 (5,6%) |
| *Tumor Location Group* | |
| Antero-superior | 12 (33,3%) |
| Postero-inferior | 24 (66,7%) |
| *Stage* | |
| Stage II | 1 (2,8%) |
| Stage III | 16 (44,4%) |
| Stage $IV_{A-B}$ | 17 (47,2%) |
| Stage $IV_C$ | 2 (5,6%) |
| *T Stage* | |
| $T_{1-3}$ | 20 (55,6%) |
| $T_4$ | 16 (44,4%) |
| *N Stage* | |
| $N_0$ | 10 (27,8%) |
| $N_{1-2}$ | 26 (72,2%) |
| *M Stage* | |
| $M_0$ | 33 (91,7%) |
| $M_1$ | 3 (8,3%) |
| *Tumor Grade* | |
| Well Differentiated | 14 (38,9%) |
| Moderately Differentiated | 18 (50%) |
| Poorly Differentiated | 4 (11,1%) |
| *p53 expression* | |
| 0 | 2 (5,6%) |
| +1 | 12 (33,3%) |
| +2 | 9 (25,0%) |
| +3 | 13 (36,1%) |

**Association of P53 Protein Expression Correlation with OSCC Age Group**

There were 21 samples within the ≤60 years old age group, a total of 10 samples (47,6%) showed weak expression of p53 and 11 samples (52,4%) showed strong expression of p53. Within the >60 years old age group, there were 15 samples (52,4%) in which 4 samples (26,7%) showed weak expression of p53 and 11 samples (73,3%) showed strong expression of p53 (Table 2).
Chi-Square Test result showed p=0,204 (p>0,05), there was no difference in proportions of p53 expression between the ≤60 years old age group and >60 years old age group.

**Table 2.** Association of P53 Protein Expression with OSCC Age Group

| | Age ≤60 years | Age >60 years | p |
|---|---|---|---|
| **Strong p53 expression** | 11 (52,4%) | 11 (73,3%) | 0,204 (*Chi-Square*) |
| **Weak p53 Expression** | 10 (47,6%) | 4 (26,7%) | |
| **Total** | 21 (100%) | 15 (100%) | |

**Association of P53 Protein Expression with OSCC Location**

There were 12 samples with tumors located in the anterior-superior, as many as 4 samples (33,3%) showed weak expression of p53 and 8 samples (66,7%) showed strong expression of p53. The postero-inferior tumor group had 24 samples, 10 samples (41,7%) showed weak expression of p53 and 14 samples (58,3%) showed strong expression of p53 (Table 3).

**Table 3.** Association of P53 Protein Expression with OSCC Location Group

| | Antero-Superior Location | Postero-Inferior Location | p |
|---|---|---|---|
| **Strong p53 expression** | 8 (66,7%) | 14 (58,3%) | 0,456 (*Fisher's Exact*) |
| **Weak p53 expression** | 4 (33,3%) | 10 (41,7%) | |
| **Total** | 12 (100%) | 24 (100%) | |

Fisher's exact test results showed p=0,456 (p>0,05). There was no difference in the proportions of p53 expression between antero-superior tumor location and postero-inferior tumor location.

**Association P53 Protein Expression Correlation with OSCC Stage**

There were 17 (47,2%) samples with early stage, 9 samples (52,9%) showed weak expression of p53 and 8 samples (47,1%) showed strong expression of p53. Within the high stage tumor group, there were 19 samples (52,8%), 5 samples (26,3%) showed weak expression of p53 and 14 samples (73,7%) showed strong expression of p53.

**Table 4**. Association of P53 Protein Expression with OSCC Stage Group

| | Early Stage | Advanced Stage | p |
|---|---|---|---|
| **Strong p53 Expression** | 8 (47,1%) | 14 (73,7%) | 0,102 (*Chi-Square*) |
| **Weak p53 expression** | 9 (52,9%) | 5 (26,3%) | |
| **Total** | 17 (100%) | 19 (100%) | |

Chi-Square Test result showed p=0,102 (p>0,05). There was no difference in the proportions of p53 expression between high stage OSCC and low stage OSCC (Table 4).

**Table 5.** Relationship Between Variables TNM with p53 expression in OSCC

| Variable | | Strong p53 expression | Weak p53 expression | p |
|---|---|---|---|---|
| **T** | $T_{1-3}$ | 10 (50,0%) | 10 (50,0%) | 0,126 (*Chi-Square*) |
| | $T_4$ | 12 (75,0%) | 4 (25,0%) | |
| **N** | $N_0$ | 3 (30,0%) | 7 (70,0%) | 0,024 (*Fisher's exact*) |
| | $N_{1-2}$ | 19 (73,1%) | 7 (26,9%) | |
| **M** | $M_0$ | 19 (57,6%) | 14 (42,4%) | 0,216 (*Fisher's exact*) |
| | $M_1$ | 3 (100%) | 0 (0,0%) | |

The study also found no difference in the proportions of p53 expression between T and M stages, but there was a significant proportional difference in p53 expression between $N_0$ and $N_{1-2}$ stage (p=0,024, Fisher's Exact Test) (Table 5). (OR=6,33; CI=95%). Therefore, OSCC patients with strong expression of p53 will have 6,33 times greater risk for having lymph node involvement compared to OSCC patients with weak expression of p53.

**Table 6.** Relationship between P53 Protein Expression with OSCC Grade

| | High Grade | Low Grade | p |
|---|---|---|---|
| **Strong p53 Expression** | 17 (77,3%) | 5 (35,7%) | 0,013 (*Chi-Square*) |
| **Weak p53 Expression** | 5 (22,7%) | 9 (64,3%) | |
| Total | 22 (100%) | 14 (100%) | |

**Relationship of P53 Protein Expression with OSCC Grade**

There were 14 samples (38,9%) within low-grade tumor grade, as many as 9 samples (64,3%) showed weak expression of p53 and 5 samples (35,7%) showed strong expression of p53. In the group of high-grade tumor, there were 22 samples (61,1%), 5 samples (22,7%) showed weak expression of p53 and 17 samples (77,3%) showed strong expression of p53 (Table 6).

Chi-Square Test result showed p=0,013 (p<0,05). There was a significant correlation between p53 expression with OSCC grade in this study. Pearson's correlation test showed a medium correlation (r=0,416) between p53 expression with OSCC grade. The Odds Ratio was 6,12 (OR=6,12; CI=95%). Therefore, OSCC patients with strong expression of p53 will have 6,12 times greater risk for having a high degree OSCC compared to OSCC patients with weak expression of p53.

**Table 7**. Relationship of p53 with OSCC Characteristics

| Researcher | n | Age | Sex | ♂:♀ |
|---|---|---|---|---|
| Tanuma *et al (2010)* | 150 | Age: 35-80 years Median: 55,5 years | ♂: 80 (53,3%) ♀: 70 (46,7%) | 11,4:10 |
| Tang *et al (2010)* | 30 | Age: 44-75 years *Mean*: 60,2±8,4 years | ♂: 20 (66,7%) ♀: 10 (33,3%) | 2:1 |
| Monteiro *et al (2012)* | 67 | Age: 31-85 years *Mean*: 59±12,6 years | ♂: 52 (77,6%) ♀: 15 (22,4%) | 3,5:1 |
| Winata *et al (2013)* | 36 | Age: 18-80 years *Mean*: 55,19±14,712 years | ♂: 17 (47,2%) ♀: 19 (52,8%) | 8,9:10 |

**DISCUSSION**

In this study, we found relatively younger sample characteristic compared to similar studies. In addition, we found that the ratio of men: women was smaller than other studies (Table 7). This was in contrast with the findings of other studies as well as the general opinion that the ratio of men: women were greater due to risk factor exposure, such as alcohol and tobacco. Leemans et al (2011) along with Curado and Boyle (2013) have found a correlation between smoking and loss of p53 function. However, Westra (2009) stated that there was an OSCC subset which was unrelated to alcohol and tobacco, relatively with younger age, and was associated with Human Papilloma Virus infection, and with good prognosis.[10,11,12] We consider this result should be used as a basis for further research for OSCC in Bali.

This difference in results was likely due to the different characteristics of the population that were used for these studies. Tang et al (2010) found that smoking and alcohol consumption were risk factors for p53 gene mutation that will lead to OSCC. However, their study did not obtain a significant relationship between smoking behavior with p53 gene mutation (p=1,000, p> 0,05). They said one of the difficulties in determining the role of smoking and alcohol consumption as independent risk factors for OSCC was the means to quantify these factors in a population.[13] Nevertheless, Curado and Hashibe (2009) have managed to find a supra-additive effect of smoking and alcohol consumption as OSCC risk factors.[14]

We suspected that the differences in the ratio of men: women, the age range (19-80 years), and the relatively young mean age of the OSCC sample were caused by our retrospective study design, data collection methods, socio-cultural factors (tobacco, alcohol, education, oral sex, HPV infection) were not evaluated in this study, and the specific genetic profile was unknown.

**Association of P53 Protein Expression with OSCC Age Group**

We found no significant association between p53 expression with age group in OSCC patients (Chi-square test p=0.204). This was in accordance with several similar studies on the relationship of p53 with OSCC clinicopathological features.[13,15,16,17] The researchers used the age limit between 55-65 years old, in this study we used the age limit of 60 years. However, among these studies, the age range of patients in our study was the widest (18-80 years) with a mean age of 55,19±14,712 years. The mean age was relatively young compared to other studies. Westra et al

(2009) stated that there was a change in the epidemiological pattern of OSCC in the last 3 decades, where patients tend to be younger (aged between 40-60 years), Caucasian, without history of smoking or alcohol consumption, and was associated with HPV infection.[12] Nonetheless, the findings from Westra et al (2009) did not correspond with other researchers. Waitzberg et al (2004) in a study using 140 samples of OSCC in Brazil found that out of 71 samples that showed overexpression of p53, a total of 35 samples (49,3%) was ≤58 years old and 36 samples (50,7%) was >58 years (p=0, 866).[15] Similar findings were also obtained by other researchers. The author argued that one of the causes of this difference was due to socio-cultural factors between study populations. The weakness of our study was that we did not include the risk factors of alcohol consumption, tobacco, and oral sex. Therefore, we thought that this could be used as a basis for further research on OSCC in Bali.

**Association of P53 Protein Expression with OSCC Location**

Our study found no significant association between p53 expression with tumor location groups in OSCC (p=0,456, p>0,05). This result was also found by other researchers.[13,15,16,17] It was interesting that Al-Swiahb et al (2013) found a significant relationship between the expression of p53, p16, and EGFR with HPV infection in the oropharynx and hypopharynx squamous cell carcinoma (p<0,001 for p53, p16, and EGFR).[18] One of the obstacles for us on this matter was to determine the diagnosis and staging of squamous cell carcinoma in the more posterior region from the oral cavity. We did not have the necessary facility, such as PET-Scan, in our center to determine the diagnosis and stage of tumors in the more posterior region.

**Association P53 Protein Expression with OSCC Stage**

We found 0 sample (0,0%) of OSCC patients in Stage I, 1 sample (2,8%) was in Stage II, 16 samples (44,4%) were in Stage III, 17 samples (47,2 %) were in Stage $IV_{A-B}$, and 2 samples (5,6 %) were in Stage $IV_C$. A total of 19 samples (52,8%) were in the high stage group. This was similar to other studies which found that OSCC patients tend to come on later stages.[13,15,16,17,19,20] We thought that there were a number of factors which contributed in this regard, such as unspecific signs and symptoms, the difficulty of early diagnosis, obscure tumor location, and unavailability of certain investigations (such as PET-Scan). However, these factors still need to be proven statistically in further OSCC research in Bali.

Although in this study, we found no significant correlation between p53 expression with OSCC stage group (Chi-square test p=0,102), we obtained a significant correlation between the expression of p53 with N status of OSCC (Fisher 's Exact Test p=0,024; r=0,396; OR=6,33; CI=95%). Other studies (Waitzberg et al, 2004; Tanuma et al, 2010; Tang et al, 2010; Hoffmann et al, 2011; Kato et al, 2011; Monteiro et al, 2012) obtained different results regarding the correlation of p53 expression with tumor stage. In response to this heterogeneity, we agreed with Perisanidis et al (2012) which stated that the cause of these differences is the vast heterogeneity between the studies.[21]. Several factors have been identified as the cause of this heterogeneity: different study designs, sample collection methods, p53 evaluation methods (Protein vs. gene), limitations in the immunohistochemistry methods used, interpretation techniques, and semi-quantitative method which was highly biased. Regarding the significant correlation between p53 expression with OSCC N stage, Tai et al (2013) found a number of predictive factors of lymph node metastases in tongue and buccal squamous cell carcinoma. They found in their univariate analysis, tumor thickness >6 mm, lymphatic and vascular invasion, and perineural invasion factors were significantly correlated with the occurrence of lymph node metastases of tongue and buccal squamous cell carcinoma (p=0,035, p=0,01, and p<0,001 respectively); while multivariate analysis showed that perineural invasion was an independent predictive factor for lymph node involvement in tongue and buccal squamous cell carcinoma.[22]

**Association of P53 Protein Expression with OSCC Grade**

We obtained 17 samples (77,3%) with strong p53 expression and high grade tumors. There was a significant correlation between p53 expression with OSCC grade in this study (Chi-square test p=0,013; r=0,416; OR=6,12; CI=95%). This was consistent with the results by Monteiro et al (2012) and Kato et al (2011), they found a significant correlation between p53 expression with OSCC grade (p=0,01 and p=0,01, respectively). Both studies were conducted in two different populations, Monteiro et al (2012) used the population in Spain and Kato et al (2011) used the population in Japan. Nonetheless, the two researchers used immunohistochemistry method to evaluate p53 function. Different results were obtained by Hoffmann et al (2011), Tanuma et al (2010), and Waitzberg et al (2004). Waitzberg et al (2004) in their study of 140 samples found no significant correlation between p53 expression with OSCC grade (p=0,845), they used immunohistochemistry method to evaluate p53 function. Tanuma et al (2010) in his study of 150

samples found no significant correlation between p53 expression with OSCC grade (p=0, 101), but they used gene mutation methods to evaluate p53 function. Hoffmann et al (2011) also found no significant correlation between p53 expression with OSCC grade (p>0,05). Hoffmann et al (2011) obtained results that were also insignificant either using immunohistochemistry or gene mutation methods to evaluate p53 function.

Although different studies showed different results on the relationship of p53 expression with OSCC grade, it was generally accepted that the loss of function of p53 will lead to a more aggressive malignancy. In this regard, we concluded that differences in results between researches were caused by the extent of heterogeneity between studies. This heterogeneity includes sample size, study population, study design, p53 evaluation methods, biases that can occur in p53 evaluation, and the lack of consensus regarding the evaluation of p53 (especially on OSCC).

## CONCLUSION

There was a significant difference of proportions in p53 expression with OSCC grade and N stage. The expression of p53 protein in OSCC could be used as a prognostic indicator and predictor lymph node involvement in OSCC.

## REFERENCES

1. Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., dan Forman, D. 2011. *Global cancer statistics*. *CA Cancer J.Clin.*, 61, (2) 69-90. [*cited* 21 Maret 2013]. *Available from*: http://onlinelibrary.wiley.com/doi/10.3322/caac.20107/pdf.
2. Tjindarbumi, D. dan Mangunkusumo, R. 2002. *Cancer in Indonesia, present and future*. *Jpn.J.Clin.Oncol.*, 32 Suppl, S17-S21. [*cited* 23 Maret 2013]. *Available from*: http://jjco.oxfordjournals.org/content/32/suppl_1/S17.full.pdf+html.
3. Argiris, A., Karamouzis, M.V., Raben, D., dan Ferris, R.L. 2008. *Head and neck cancer. Lancet*, 371, (9625) 1695-1709. [*cited* 26 Maret 2013]. *Available from*: http://www.thelancet.com/journals/lancet/article/PIIS0140-6736%2808%2960728-X/fulltext
4. Perez-Ordonez, B., Beauchemin, M., dan Jordan, R.C. 2006. *Molecular biology of squamous cell carcinoma of the head and neck*. *J.Clin.Pathol.*, 59, (5) 445-453. [*cited* 10 Juni 2013]. *Available from*: http://jcp.bmj.com/content/59/5/445.full.pdf+html
5. Lopez-Martinez, M., Anzola, M., Cuevas, N., Aguirre, J.M., dan De-Pancorbo, M. 2002. *Clinical applications of the diagnosis of p53 alterations in squamous cell carcinoma of the head and neck*. *Med.Oral*, 7, (2) 108-120. [*cited* 9 Februari 2013]. *Available from*: http://www.medicinaoral.com/pubmed/medoralv7_i2_p108.pdf.
6. Tandon, S., Tudur-Smith, C., Riley, R.D., Boyd, M.T., dan Jones, T.M. 2010. *A systematic review of p53 as a prognostic factor of survival in squamous cell carcinoma of the four main anatomical subsites of the head and neck*. *Cancer Epidemiol.Biomarkers Prev.*, 19, (2) 574-587. [*cited* 9 Februari 2013]. *Available from*: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3536470/pdf/ukmss-29628.pdf.
7. Roh, J.L., Kang, S.K., Minn, I., Califano, J.A., Sidransky, D., dan Koch, W.M. 2011. *p53-Reactivating small molecules induce apoptosis and enhance chemotherapeutic cytotoxicity in head and neck squamous cell carcinoma*. *Oral Oncol.*, 47, (1) 8-15. [*cited* 2 Februari 2013]. *Available from*: http://www.oraloncology.com/article/S1368-8375%2810%2900327-1/pdf.
8. Wang, W. dan El-Deiry, W.S. 2008. *Restoration of p53 to limit tumor growth*. *Curr.Opin.Oncol.*, 20, (1) 90-96. [*cited* 2 Februari 2013]. *Available from*: http://sfx.ub.rug.nl.proxy-ub.rug.nl/sfx_local?sid=Entrez:PubMed&id=pmid:18043262
9. Fisher, C.J., Gillett, C.E., Vojtesek, B., Barnes, D.M., dan Millis, R.R. 1994. *Problems with p53 immunohistochemistry staining: the effect of fixation and variation in the methods of evaluation. Br.J.Cancer, 69, (1) 26-31*. [*cited* 16 April 2013]. *Available from*: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1968757/
10. Leemans, C.R., Braakhuis, B.J., dan Brakenhoff, R.H. 2011. *The molecular biology of head and neck cancer*. *Nat.Rev.Cancer*, 11, (1) 9-22. [*cited* 24 April 2013]. *Available from*: http://www.nature.com.proxy-ub.rug.nl/nrc/journal/v11/n1/pdf/nrc2982.pdf.
11. Curado, M.P. dan Boyle, P. 2013. *Epidemiology of head and neck squamous cell carcinoma not related to tobacco or alcohol*. *Curr.Opin.Oncol.*. [*cited* 21 Maret 2013]. *Available from*: http://sfx.ub.rug.nl.proxy-ub.rug.nl/sfx_local?sid=Entrez:PubMed&id=pmid:23511666.
12. Westra, W. H. 2009. *The changing face of head and neck cancer in the 21st century: the impact of HPV on the epidemiology and pathology of oral cancer*. *Head Neck Pathol*, 3, 78-81. [*cited* 8 Februari 2013]. *Available from*: http://link.springer.com.proxy-ub.rug.nl/article/10.1007%2Fs12105-009-0100-y
13. Tang, S., Xu, D. dan Zhou, B. 2010. *Analysis of P53 mutation and invasion front grading in*

*oral squamous cell carcinomas*. *J Huazhong Univ Sci Technolog Med Sci*, 30, 525-9. [*cited* 31 Agustus 2013]. *Available from*: http://link.springer.com.proxy-ub.rug.nl/article/10.1007%2Fs11596-010-0462-0.

14. Curado, M.P. dan Hashibe, M. 2009. *Recent changes in the epidemiology of head and neck cancer*. *Curr.Opin.Oncol.*, 21, (3) 194-200. [*cited* 21 Maret 2013]. *Available from*: http://sfx.ub.rug.nl.proxy-ub.rug.nl/sfx_local?sid=Entrez:PubMed&id=pmid:19363341 Agustus 2013]. *Available from*: http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pmc/articles/PMC3721303/
15. Waitzberg, A. F., Nonogaki, S., Nishimoto, I. N., Kowalski, L. P., Miguel, R. E., Brentani, R. R. dan Brentani, M. M. 2004. *Clinical significance of c-myc and p53 expression in head and neck squamous cell carcinomas*. *Cancer Detect Prev*, 28, 178-86. [*cited* 31 Agustus 2013]. *Available from*: http://www.sciencedirect.com.proxy-ub.rug.nl/science/article/pii/S0361090X04000327.
16. Kato, K., Kawashiri, S., Yoshizawa, K., Kitahara, H., Okamune, A., Sugiura, S., Noguchi, N. dan Yamamoto, E. 2011. *Expression form of p53 and PCNA at the invasive front in oral squamous cell carcinoma: correlation with clinicopathological features and prognosis*. *J Oral Pathol Med*, 40, 693-8. [*cited* 31 Agustus 2013]. *Available from*: http://onlinelibrary.wiley.com.proxy-ub.rug.nl/doi/10.1111/j.1600-0714.2011.01032.x/abstract;jsessionid=7B29C01F559BF08578CB0664BE1692F4.d01t03
17. Tanuma, J., Izumo, T., Hirano, M., Oyazato, Y., Hori, F., Umemura, E., Shisa, H., Hiai, H. dan Kitano, M. 2010. *FGFR4 polymorphism, TP53 mutation, and their combinations are prognostic factors for oral squamous cell carcinoma*. *Oncol Rep*, 23, 739-44. [*cited* 31 Agustus 2013]. *Available from*: http://www.spandidos-publications.com.proxy-ub.rug.nl/or/23/3/739
18. Al-Swiahb, J.N., Huang, C.C., Fang, F.M., Chuang, H.C., Huang, H.Y., Luo, S.D., Chen, C.H., Chen, C.M., dan Chien, C.Y. 2010. *Prognostic impact of p16, p53, epidermal growth factor receptor, and human papillomavirus in oropharyngeal cancer in a betel nut-chewing area*. *Arch.Otolaryngol.Head Neck Surg.*, 136, (5) 502-508. [*cited* 9 April 2013]. *Available from*: http://archotol.jamanetwork.com/article.aspx?articleid=496289.
19. Hoffmann, T. K., Trellakis, S., Okulicz, K., Schuler, P., Greve, J., Arnolds, J., Bergmann, C., Bas, M., Lang, S., Lehnerdt, G., Brandau, S., Mattheis, S., Scheckenbach, K., Finn, O. J., Whiteside, T. L. dan Sonkoly, E. 2011. *Cyclin B1 expression and p53 status in squamous cell carcinomas of the head and neck*. *Anticancer Res*, 31, 3151-7. [*cited* 31 Agustus 2013]. *Available from*: http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pmc/articles/PMC3721303/
20. Monteiro, L. S., Diniz-Freitas, M., Garcia-Caballero, T., Warnakulasuriya, S., Forteza, J. dan Fraga, M. 2012. *Combined cytoplasmic and membranous EGFR and p53 overexpression is a poor prognostic marker in early stage oral squamous cell carcinoma*. *J Oral Pathol Med*, 41, 559-67. [*cited* 31 Agustus 2013]. *Available from*: http://onlinelibrary.wiley.com.proxy-ub.rug.nl/doi/10.1111/j.1600-0714.2012.01142.x/abstract
21. Perisanidis, C., Perisanidis, B., Wrba, F., Brandstetter, A., El Gazzar, S., Papadogeorgakis, N., Seemann, R., Ewers, R., Kyzas, P. A. dan Filipits, M. 2012. *Evaluation of immunohistochemistry expression of p53, p21, p27, cyclin D1, and Ki67 in oral and oropharyngeal squamous cell carcinoma*. *J Oral Pathol Med*, 41, 40-6. [*cited* 31 Agustus 2013]. *Available from*: http://onlinelibrary.wiley.com.proxy-ub.rug.nl/doi/10.1111/j.1600-0714.2011.01071.x/abstract
22. Tai, S. K., Li, W. Y., Yang, M. H., Chu, P. Y., Wang, Y. F. dan Chang, P. M. 2013. *Perineural invasion as a major determinant for the aggressiveness associated with increased tumor thickness in t1-2 oral tongue and buccal squamous cell carcinoma*. *Ann Surg Oncol*, 20, 3568-74. [*cited* 9 September 2013]. *Available from*: http://link.springer.com.proxy-ub.rug.nl/article/10.1245%2Fs10434-013-3068-5