International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Cyclooxygenase-2 Expression Associated with the Degree of Differentiation of Oral Cavity Squamous Cell Carcinoma at Sanglah Hospital Denpasar

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Abstract: ***Cancer of the oral cavity is a serious problem and developed in various parts of the world with high morbidity and mortality. Cyclooxygenase-2 expression and the degree of differentiation of oral cavity SCC are prognostic factors, but the relationship between them still cause difference of opinions. The aim of this study was to prove the expression of COX-2 associated with the degree of differentiation of oral cavity SCC. This study used cross sectional analytical method in 45 histopathology samples of patients with oral cavity SCC which divided into well, moderately, and poorly differentiated. The results were analyzed by Kolmogorov Smirnov, One Way Anova and Post Hoc. P value less than 0.05 was considered significant. The results showed there was a significant association between COX-2 expression and degree of differentiation (p=0,036). There was a significant difference in COX-2 expression score mean in the three differentiation-degree groups on well and poorly differentiated (p=0,000) as well as moderately and poorly differentiated (p=0,001). In Conclusion there was association between COX-2 expression and degree of differentiation. Cyclooxygenase-2 is expected to be a marker of progressivity and indirect prognostic factors that may later be useful in oral cavity SCC therapy.***

Keywords: Cyclooxygenase-2, degree of differentiation, squamous cell carcinoma of the oral cavity

1. Introduction

The majority of oral and oropharyngeal cancers consist of heterogeneous neoplasm groups consisting of malignant surface epithelial tumors, soft tissue tumors, oral mucosa melanomas, salivary type tumors and haematolymphoid tumors. Over 90% of oral cavity malignancies are SCC [1]. Oral cancer and oral cavity SCC terms are used interchangeably [2], [3].

The prevalence of oral cancer is high in Asian countries, especially South Asia and Southeast Asia. Oral cavity cancer is included in the 6 most frequent malignancies in Asia. Nearly 247.300 new cases of oral cancer occur every year [4]. The mortality rate of oral cancer patients worldwide is estimated at 3-4 per 100.000 men and 1.5-2 per 100.000 women. In most countries, the 5-year survival rate for tongue and oral cancer averages about 50% [5], [6], [7]. Based on the registration of all pathology centers in Indonesia in 2012, the prevalence of SCC in the oral cavity was 1.83% of all cancers in Indonesia while in Bali prevalence of cancers of the oral cavity was 2.16% of all cancers in Bali [8].

Squamous cell carcinoma of the oral cavity is a carcinoma characterized by invasion and differentiation of the squamous epithelium derived from oral mucosal epithelial [1]. There was consistent evidence of the degree of differentiation in determining prognosis. The higher degree of differentiation is similar to the worse prognosis [6]. According to World Health Organization (WHO), the degree of differentiation of SCC is divided into well, moderately and poorly differentiated based on squamous differentiation, cell and nucleus pleomorphia and mitotic activity [1].

Many molecular studies have been done to find pathways of gene expression and modified protein levels in oral cavity SCC, with particular emphasis on their prognostic significance including COX-2 [6]. Cyclooxygenase-2, one of the COX isoforms, is a triggering enzyme that is considered to be one of the major mediators in the inflammatory process. Cyclooxygenase-2 has received more attention because it can play an important role in the initiation and development of various organ carcinomas and is associated with poor prognosis [9].

Initially in the cyclooxygenase reaction, the COX enzyme catalyzes Arachidonic Acid to form an unstable intermediate prostaglandin G2, which is then converted to prostaglandin H2 by COX peroxidase activity. Prostaglandin H2 is a precursor for some prostaglandin-related structures formed by the action of special prostaglandin synthase [10]. The most important prostaglandins in inflammation are PGE2, PGD2, PGF2α, PGI2 (prostacyclin) and thromboxane A2 (TxA2) [7]. Prostaglandin E2 has many diverse functions and is mainly involved in inflammation, infection and cancer. Prostaglandin E2 works by binding of E-prostanoid receptor (EP) ie EP1, EP2, EP3 and EP4. After binding of the ligand, the EP receptor induces the activation of the MAP kinase pathway through cAMP upregulation (EP2, EP3 and EP4) or through the activation of phospholipase C (EP1) and promotes carcinogenesis: increased neoplastic cell proliferative activity, increased angiogenesis, inhibition of immune surveillance, inhibition of apoptosis and enhancement of invasion [9], [11], [12].

Several studies on the association of COX-2 expression with the degree of differentiation of oral cavity SCC have been performed and the results still debated. Numerous studies have shown that COX-2 overexpression was significantly associated with tumor degree of differentiations [9], [13],

[14]. However, there were several studies that showed the opposite result that there was no significant difference between COX-2 overexpression with the degree of differentiation of oral cavity SCC (15], [16]. The aims of this study was to prove that COX-2 expression associated with the degree of differentiation of oral cavity SCC.

2. Material and Methods

2.1 Spesimens

Slides and paraffin embedded tissue blocks from 45 patients of oral cavity SCC were retrieved from the histopathology archives in Anatomic Pathology Laboratory of Sanglah Hospital, Bali in the year 2014-2017. Clinical data were collected from the medical report and cancer registry.

2.2 Histopathologic evaluation

The slides from these cases were reviewed and histopathologic diagnoses in the histopathologic reports were confirmed independently by two pathologists and one resident.

2.3 Immunohistochemistry and interpretation

Tissue section at 4 µm thickness from each case were prepared for immunostaining. After 30 minutes incubation in a 60ºC oven, deparaffinization, and rehidration tissue sections were treated with 3% hydrogen peroxide for 10 minutes. Following incubation in blocking buffer for 30 minutes in room temperature, the slides were incubated with one of the following primary antibodies COX-2 *monoclonal Rabbit* 1:200 dilution. The colour was visualized by DAB as chromogen.

Immunostaining were interpreted independently by two pathologists and one resident. The immunohistochemical score (IHS) was calculated by combining the proportion score (percentage of positive stained cells) with the staining intensity score. The proportion score ranged from 0–4, as follows: 0 (<5%), 1 (5–24%), 2 (25–49%), 3 (50–74%), and 4 (≥75%). Staining intensity was scored as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The proportion and staining intensity scores were then multiplied to generate the IHS for each case. A case with IHS ≥4 was considered high expression [14].

2.4 Statistical analysis

Descriptive statistics were calculated. The results were analyzed by Kolmogorov Smirnov to determine the association between COX-2 expression and degree of differentiation of the oral cavity SCC, and One Way Anova and Post Hoc to know the mean difference of COX-2 expression score on degree of differentiation. P value less than 0.05 was considered significant. All statistical analyses were performed using SPSS 16.0.

3. Result

3.1 Characteristic of Study Sample

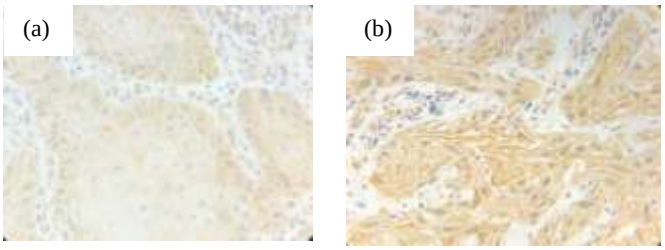
In the study period (2014-2017) there were 45 samples met the study criteria, consisted of 10 samples well differentiated SCC, 27 samples moderately differentiated SCC, and 8 samples poorly differentiated SCC.

Table 1: Characteristic of study sample

Characteristic	*n*	*%*
Gender		
Man	30	66,7
Woman	15	33,3
Degree of differentiation		
Well differentiated	10	22,2
Moderately differentiated	27	60,0
Poorly differentiated	8	17,8
Location		
Buccal	5	11,1
Ginggiva	10	22,2
Lip mucosa	4	8,90
Tongue	23	51,1
Hard palate	3	6,70
Percentage of COX-2 expression		
0 (<5%)	0	0
1 (5-24%)	1	2,20
2 (25-49%)	8	17,8
3 (50-74%)	14	31,1
4 (≥75%)	22	48,9
Staining Intensity		
0 (negative)	0	0
1 (weak)	14	31,1
2 (moderate)	19	42,2
3 (strong)	12	26,7

45 samples in this study consisted of 30 men and 15 women with varying age ranging from age 23 years to age 87 years. The mean age was 58.71 ± 13.6 years. The Most sites of oral cavity SCC were tongue 23 samples (51,1%), ginggiva 10 samples (22,2%) and buccal mucosa 5 samples (11,1%).

In this study, the percentage of stained cells > 75% of the tumor cells were 22 samples (48.9%), 50-74% of the tumor cells were 14 samples (31.1%), 25-49% of the tumor cells were 8 samples (17.8%), and 5-24% of the tumor cells as many as 1 samples (2.2%). The intensity of COX-2 was strong in 12 samples (26.7%), moderate in 19 samples (42.2%) and weak in 14 samples (31.1%).



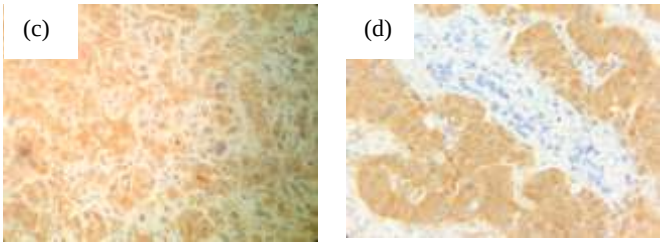


Figure 1: COX-2 staining. (a) weak (1+), (b) moderate (2+), (c) strong (3+), (d) Adenocarcinoma colon as positive control

3.2 Mean Difference of COX-2 Expression Scores In well, moderately, and poorly differentiated oral cavity SCC

Shapiro-Wilk normality test showed the COX-2 expression score was normally distributed and the homogeneity test concluded that the variants of the three degree of differentiations were equal or homogeneous.

The mean score of COX-2 in oral cavity SCC in well differentiated was 4.50 ± 2.32, moderately differentiated was 6.00 ± 2.34, and poorly differentiated was 9.25 ± 2.55. Based on the One Way Anova test, the significance value p = 0,000 (p <0,05), can be concluded that the mean score of the three groups of degree of differentiation differ significantly.

Table 2 : One Way Anova Analysis

Degree of differentiation	n	COX-2 expression mean	*p value*
Well	10	4.50±2,32	0.000
Moderate	27	6.00±2,34	
Poorly	8	9.25±2,55	

Post-Hoc analysis was found to have a significant difference in COX-2 score mean (p <0.05) between poorly differentiated and well differentiated oral cavity SCC and between moderately differentiated and poorly differentiated oral cavity SCC. Only between well differentiated and moderately differentiated were not significantly different.

Table 3: Post Hoc Analysis

Degree of differentiation(I)	Degree of differentiation(J)	Mean Difference (I-J)	p value
Well	Moderately	1,50	0,095
Moderately	Poorly	3,25	0,001
Poorly	Well	4,75	0,000

3.3 Association between COX-2 Expression and Degree of differentiation of Study Sample

Association between COX-2 expression and degree of differentiation in this study analized by Kolmogorov-Smirnov test showed a statistically significant relationship (p = 0.036). The proportion of samples with COX-2 expression score (≥4) was greater in the oral cavity group of moderately and poorly differentiated as many as 65.8% and 21.1% respectively than in the well differentiated group as many as 13.2%.

Table 4 : Association between COX-2 expression and degree of differentiation

COX-2 Expression	Degree of differentiation						p value
	Well		*Moderately*		*Poorly*		
	n	*%*	*N*	*%*	*n*	*%*	
Low	5	71.4	2	28.6	0	0	0.036
High	5	13.2	25	65.8	8	21.1	

4. Discussion

The mean age of oral cavity SCC sample in this study was 58.71 ± 13.6 years with an age range that varied between 23 to 87 years. The results of this study were similar to those of Aruldoss et al. (2016) in Saudi Arabia showing an average sample age of 55.75 ± 13.21 years [17].

The results of this study showed the predominance of cases of oral cavity in men (30 patient) with a ratio of 2 : 1. This is in accordance with study conducted by Pires et al. (2013) showing the overall incidence rate of men compared to women was 2 : 1 [18]. The prevalence of cases of oral cavity in men are related to habits such as smoking, chewing quid and drinking alcohol which are the three main risk factors of carcinogenesis.

Based on the oral cavity location in this study, the most SCC location was on tongue 23 samples (51.1%), ginggiva 10 samples (22.2%), buccal mucosa 5 samples (11.1%), lip mucosa 4 samples (8.9%), and hard palate 3 samples (6.7%). This is also in accordance with study conducted by Damayanti and Setiawan (2018), which was the most oral cavity cancer in Bali was on tongue, ginggiva, and buccal mucosa respectively [19].

Most cancers of the oral cavity and tongue have well or moderately differentiated, whereas poorly differentiated SSC is less commonly seen [1]. In this study, as many as 37 cases were well and moderately differentiated oral cavity SCC whereas poorly differentiated case only 8 cases. This is also in accordance with study conducted by Aruldoss et al. (2016) where the well or moderately differentiated number of SCC cases as many as 19 cases in comparison with the poorly differentiated that was only 1 case [17].

In this study, COX-2 expression was present in 100% of oral cavity SCC, 15.6% for low expression and 84.4% for high expression. Cyclooxygenase-2 is only expressed constitutively in the central nervous system and seminal vesicles, in contrast to COX-1 expressed in most tissues. Study conducted by Byatnal et al. (2015) get 100% oral cavity SCC with COX-2 expression of low expression 22.67%, moderate expression 29.33%, and 48% high expression [9]. Another study conducted by Aruldoss et al. (2016) get 80% of cases of oral cavity SCC with positive COX-2 expression [17].

The results of statistical analysis comparing COX-2 expression in the three differentiation-degree groups in this study showed statistically significant differences p = 0.036 (p <0.05) analyzed by Kolmogorov-Smirnov test, where it was found that COX-2 expression was associated with tumor

biological behavior. The proportion of high COX-2 expression cases, more common in poorly differentiated (21.1%) and moderately differentiated SCC (65.8%) compared with well differentiated SCC (13.2%).

This was in accordance with Mohammad et al. (2011) in 44 cases of oral cavity SCC which had a negative proportion of COX-2 expression was high on well differentiated SCC and positive expression was higher in the moderately differentiated [13]. Similar results were also obtained by Byatnal et al. (2015) in their study consist of 75 oral cavity SCC cases showed that COX-2 expression significantly associated with the degree of differentiation ($p = 0.006$) [9]. Study conducted by Aruldoss et al. (2016) also found a significant association between COX-2 expression and degree of differentiation of oral cavity SCC in 20 cases ($p = 0.000$) [17].

The difference in COX-2 scores mean at different degrees of differentiation showed significant results ($p = 0.000$). In Post Hoc analysis only between well differentiated and moderately differentiated were not significantly different ($p = 0.095$). This could be due to the lack of sample size in this study.

High COX-2 expression in oral cavity SCC is associated with disease progression, distant metastatic tendency, resistance to therapy, and low survival. This shows the role of COX-2 as a predictor and prognostic factor [9], [13], [17].

This study showed that there was a significant association between COX-2 expression with degree of differentiation of oral cavity SCC where high COX-2 expression was more common in moderately and poorly differentiated oral cavity SCC. Cyclooxygenase-2 plays a role in the disease progressivity so can help to determine the prognosis of patients with oral cavity SCC as well as its possibility to become target therapy for oral cavity SCC.

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