Correlation of CD10 immunoexpression and eosinophil count in stromal tissue as predictors of prognosis of squamous cell carcinoma of the oral cavity

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ABSTRACT

Squamous cell carcinoma is the most common cancer in oral cavity and it often metastases to regional lymph nodes. The treatment planning and prognosis of oral squamous carcinoma which is based on histopathology grading and clinical stage still could not optimally given information relating to cancer biological characteristics of the tumor that is why examination of immunohistochemical is needed and see whether there is invasion and metastases, by using CD10 and by counting the amount of eosinophil with the discoloration of Hematoxylin Eosin (HE). These research was a retrospective cross sectional analysis, using immunohistochemical at the Department of Pathology Hasan Sadikin Hospital with secondary data drawn from the Medical Records Hasan Sadikin Hospital. Correlation immunoexpression CD10, the number of eosinophils with regional lymph nodes enlargement and histopathological grade of oral squamous cell carcinoma analyzed using the Spearman rank correlation (with 95% confidence level). The results showed no significant correlation between immunoespression CD10 with regional lymph node enlargement (r=-0.236), there was no significant correlation between immunoexpression CD10 with histopathologic grading (r=0.033), there was significant correlation between the number of eosinophils with regional lymph node enlargement (r=0.372) and there was no significant correlation between the number of eosinophils with histopathologic grading (r=-0.313) Conclusion this study showed that increased immunoexpression CD10 not significantly with regional lymph node enlargement, immunoexpression CD10 not significantly with histopathologic grading, the number of eosinophils increased significantly in the regional lymph node enlargement, and increased the number of eosinophils not significantly with histopathologic grading.

Key words: CD10, eosinophil, immunohistochemical, regional lymph node enlargment, histopathologic grades, oral squamous cell carcinoma

INTRODUCTION

Oral cavity tumors are a severe health problem with more than 200,000 new cases occur each year worldwide. Dad head and neck tumors, 40% are squamous cell carcinomas of the oral cavity. In 2008, in Canada, there were 3,400 new cases of oral cavity tumors, and 1,150 of them died. In

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Southeast Asia alone, oral cavity tumors account for 40% of all malignant diseases. At Hasan Sadikin Hospital Bandung and 1996-2000, the incidence of squamous cell carcinoma of the oral cavity is 35.85% and is the most tumor of all head and neck tumors. Lips are the most commonly affected area, i.e., as many as 12% of all head and neck tumors and 98% grow on the lower lip. Other areas that are often exposed after lips are the tongue, the base of the mouth, the mandible gingiva, the buccal mucosa, the durum palate and the maxillary gingiva.1,2 Although the medic services are so advanced, the mortality rate from oral tumors is still about 50% the disease is diagnosed already at an advanced stage. Morbidity and mortality of squamous cell carcinoma of the oral cavity are mainly due to local and regional tumor invasion as well as distant metastasis.

The decrease in survival rates is mainly due to the extra-capsular spread of the tumor, i.e., to lymph nodes, perineural invasion, and bone invasion.1,3 One of the essential properties of cancer is its ability to grow infiltratively into the surrounding tissue. This ability to make tumor cells can penetrate the lymphatic vessels or blood vessels and taken to other organs, and if the tumor cells can form a mass within the body of destination, called metastasis. Fidler and colleagues have described a metastatic process consisting of a following, particular and interdependent sequence, transformation involvement, angiogenesis and local invasion, release and embolization, attachment and ultimately proliferation in distant places. Tumor invasion and metastasis are active processes and loss of normal cell adhesion, breakdown of cell membranes and extracellular matrix, stimulation for tumor migration and angiogenesis. Clinically, enlarged lymph nodes (KGB) can be known by palpation or radiography, and the detection of KGB enlargement in squamous cell carcinoma of the oral cavity requires aggressive treatment of involved or likely lymph nodes. Metastasis to lymph nodes is the most critical factor in determining the prognosis of head and neck carcinoma, including oral carcinoma.3,4

At present, the focus is on identifying molecular factors that can influence clinical outcomes by using molecular markers. This molecular marker not only provides useful prognostic information, but it can also be a target of pharmacotherapy by disrupting specific cellular processes so that cell proliferation is inhibited. In essence, various protein molecules are produced by abnormally abnormal tumor cells or unusual conditions that are useful as tumor markers.4,5 Cluster of Differentiation 10 (CD10) known by immunologists as CALLA (common acute lymphoblastic leukemia antigen) is the first marker used to identify leukemia cells in children with acute lymphoblastic leukemia. CD10 also has a vital role in the development of malignant melanoma. CD10 is an indicator of tumor malignancy when exposed to stromal cells. In a study of 116 cases of gastrointestinal tumors analyzed immunohistochemically, CD10 was expressed in stromal cells that seemed to trigger invasion and metastasis from gastrointestinal tumors. The likelihood of this CD10 expression plays an essential role in tumor invasion, thereby facilitating the occurrence of metastasis.5-7

Eosinophils granulocytes, commonly referred to as eosinophils, are white blood cells that are responsible for fighting parasitic infections in the body, as well as various immunological functions, including allergies and asthma. Frequently eosinophils are found in the thymus, lower digestive tract, uterus, spleen and lymph nodes. Eosinophils are located in the lungs, skin, and esophagus or other internal organs associated with the disease process. Since the discovery of eosinophils in blood in malignant disease by Rheinbach in 1893, eosinophilia has been found in various human bodies with tumors. The presence of eosinophilia is associated with a reduced prognosis, but other researchers mention a good prognosis. Encouraged by the uncertain role of CD10 and eosinophils in the process of invasion and metastasis and the absence of studies assessing the role of eosinophils in histopathologic gradations, the authors have investigated this to assist in determining the prognosis of squamous cell carcinoma of the oral cavity. "

Based on the above background, the following problems can be formulated:

1. Whether there is a CD10 immunoexpression correlation in stromal tissue in the presence of enlarged regional lymph nodes of squamous cell carcinoma of the oral cavity.
2. Whether there is a CD10 immunoexpression correlation in stromal tissue with histopathologic gradations of squamous cell carcinoma of the oral cavity.

3. Is there a correlation of the number of eosinophils in stromal tissue in the presence of regional lymph node enlargement of squamous cell carcinoma of the oral cavity.
4. Is there a correlation between the number of eosinophils in stromal tissue with histopathologic gradations of squamous cell carcinoma of the oral cavity.

Research purposes

1. Analyze the CD10 immunoexpression correlation with enlarged regional lymph nodes as a predictor of the prognosis of squamous cell carcinoma of the oral cavity.
2. Analyzes the correlation between immunoexpression of CD 10 with histopathologic gradation as a predictor of prognosis of squamous cell carcinoma of the oral cavity.
3. Analyze the association of the number of eosinophils in stromal tissue with enlarged regional lymph nodes as a predictor of the prognosis of squamous cell carcinoma of the oral cavity.
4. Analyze the correlation between the number of eosinophils with histopathologic gradation as a predictor of the prediction of squamous cell carcinoma of the oral cavity.

Usefulness Research

1. Theoretical Aspect: To analyze the role of CD10 and eosinophils molecularly in the process of invasion and metastasis of squamous cell carcinoma of the oral cavity.
2. Practical Aspects: Helps the clinician in early detection of a metastatic tendency in a squamous cell carcinoma of the oral cavity, to determine subsequent therapy appropriately, to help identify prognosis.

METHODS

This study was preceded by data collection of patients with squamous cell carcinoma of the oral cavity in the medical record and then sought the current paraffin block in the Anatomical Pathology section of 15 samples with enlargement of regional lymph nodes and 15 samples without amplification of local lymph nodes. Two preparations were made, the first development was stained with Hematoxylin-Eosin (HE) to establish the diagnosis and determine the histopathologic gradation, then count the eosinophil count, and the other preparation at the same time with CD10. Immunohistochemical reactions use the Labelled streptavidin-biotin immunoperoxidase complex (LSAB) method using Starr Trek Universal HRP Detection system (Biocare Medical, USA). The primary antibodies used were CD10 (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) with 1:25 dilution - 1:50.

The paraffin block specimens of all the preparations selected as the study population were cut in 4 microns by microtome rotary, each of 2 stocks. One development for staining with HE, a preparation to be immersed with immunohistochemistry CD10.

Immunohistochemical immunity assessment of CD10 antibody was performed on brown stromal 5-place tissue using evaluation as follows: 7

1) Negative (-): Indicates an immunoreactive CDE <10% stromal tissue.
2) Positive (+): Shows ≥10% immunoreactive stroma CD10.

The assessment of the distribution of eosinophils in stromal tissue was calculated based on 10X10 magnification microscopy. Eosinophils are counted on ten large fields of view and written as eos / 10hpf. The number of eosinophils is determined by category: 9

Negative: 0-4 eosinophils / 10hpf is referred to as the baseline,
Positive 1 (+): 5-9 eos / 10hpf
Positive 2 (++): 10-14 eos / 10hpf
Positive 3 (+++): 15-19 eos / 10hpf
Positive 4 (++++):> 20eos / 10hpf

Data obtained on the ordinal scale were grouped according to the distribution of CD10 and eosinophils in squamous cell carcinoma with and without enlargement of regional lymph nodes with their histopathologic gradations. To see the relation of one variable with other variables used Rank Spearman correlation (rs) which tested with t statistic student.Analisis data is done using program Mega Stat Excel, the significance of test result determined according to value p <0,05.

RESULTS

Table 1 CD10 distribution based on gradation, regional KGB enlargement, stage, tumor size, and tumor location in squamous cell carcinoma of the oral cavity

Variable	CD10(+)	CD10(-)	p value
Histological Gradation			
Gradation I	15	5	
Gradation II	6	1	p = 0,86
Gradation III	2	1	
KGB Enlargement			
Positive (+)	10	5	
Negative (-)	13	2	p = 0,209

Tabel 2 Distribution of eosinophil count based on gradation, KGB enlargement, tumor size and location of squamous cell carcinoma of oral cavity

Variable	Eosinofil				
	(-)	+1	+2	+3	+4	p Value
Histological Gradation						
Gradation I	4	2	1	5	8	
Gradation II	0	4	0	2	1	p=0,092
Gradation III	1	0	2	0	0	
KGB Enlargement						
Positive	1	2	2	3	7	p = 0,043
Negative	4	4	1	4	2	(with meaning)

DISCUSSION

According to a Piatteli7 study on squamous cell carcinoma in the oral cavity, positive CD10 is an indication of a poor prognosis; because in reality, the presence of CD10-positive cells is associated with metastasis. Huang BW et al.'s study showed that CD10 overexpressed in patients with primary gastric tumors when compared with typical dysplasia and gastric mucosal conditions.

In this study found CD10 (+) in 23 patients, and CD10 (-) in 7 patients. In cases of squamous cell carcinoma of the oral cavity with regional KGB enlargement, CD10 (+) was found in 10 of 15 patients (43.5%), and CD10 (-) was found in 5 patients. In the case of squamous cell carcinoma of the oral cavity without regional KGB enlargement, CD10 (+) was found to be 13 patients (56.5%), and CD10 (-) was found in 2 patients, but statistically there was no significant correlation between CD10 and KGB enlargement regionally tested using Spearman rank test rs = -0.236, meaning that increased CD10 immunoexpression was inversely proportional to regional KGB enlargement, but the correlation was not statistically significant.

The amount of CD10 (+) in cases with regional KGB enlargement or without territorial KGB enlargement may be due to squamous cell carcinoma of the oral cavity is a malignant tumor with microinvasive capability, biologically capable of achieving access to the lymphatic or vascular pathways in the lamina propria metastasis, and squamous cell carcinoma of the oral cavity studied are on average more than 2 cm in size, so it is likely that there has been an invasion of surrounding structural structures and may even occur both regional metastases and distant metastases, as well as the enlarged KGB, examined in this study based in KGB abscess during clinical palpation, not metastasis to KGB based on histopathologic examination, so that clinically implantable KGB is not a free assurance of metastasis during histopathologic examination, and infection or histiocytosis sinus may also cause clinically sensitive KGBs.

According to Piattelli A et al., histopathologic levels have a significant correlation with CD10 (+), ie in gradation I, 6 of 28 cases found CD10 (+), gradation II only 9 of 23 cases with CD10 (+), while in gradation III, 10 of 17 cases found CD10 (+), 6.7 In this study different results were observed with Piattelli et al., CD10 (+) found in 23 cases and CD10 (-) in 7 cases with various histopathologic gradations. In the first gradient, CD10 was overexpressed in 15 of the 20 cases. CD10 overexpression in this examination is likely because most of the tumors examined are above 2 cm in size and are in advanced stage (stage III and IV), although the histopathologic gradation is level I.

In this research, we found that eosinophil distribution in squamous cell carcinoma of oral cavity that was palpable with regional KGB enlargement obtained negative value (-) in 1 case (20%), positive 1 (+) 2 cases (33%), positive 2 (++) as many as 2 cases (67%), positive 3 (+++) as many as 3 cases (43%), and positive 4 (++++) 7 cases (78%), while the eosinophil distribution in squamous cell carcinoma of the oral cavity without (4%), positive 1 (+) of 4 cases (67%), positive 2 (++), 1 case (33%), positive 3 (+++) as many as 4 cases (57%), and pos-

itive 4 (++++) in 2 cases (22%). The result of analysis using Spearman rank test found significance p = 0,043 (meaning) with correlation strength equal to 37,2%. In this study it was revealed that the increasing number of eosinophils correlated with high regional KGB enlargement rates with correlation strength of 19.8% (rs = 0.033 and p <0.05).

Based on the above data it can be seen that the number of eosinophils is increased in squamous cell carcinoma of the oral cavity accompanied by regional KGB enlargement, and in squamous cell carcinoma of the oral cavity without territorial KGB enlargement the number of eosinophils decreases. This is following research conducted by Said Mahmoud et al on laryngeal carcinoma that the eosinophil infiltration> 10 / field of view or> 20/10 field of view is an indicator for the possibility of tumor invasion. This is also consistent with the study by Olivera DT et al investigating the presence of TATE (tumor-associated tissue eosinophilia) in patients with squamous cell carcinoma of the oral cavity and suggesting that patients with advanced-stage squamous cell carcinoma with tumor-to-muscular infiltration, TATE increases in advanced stages (III and IV) compared with early stages (I and II). The mechanism of accumulation of eosinophils in the stroma in invasive carcinoma cases is probably caused by the activation of 92-kd gelatinase (MMP-9) that destroys the basement membrane of the extracellular matrix, in addition, the MMP-9 produced by this eosinophil can promote tumor angiogenesis, thus facilitating invasion and tumor metastasis.8-10

Distribution of negative eosinophils (-) on gradation I squamous cell carcinoma of the oral cavity in 4 cases, II gradation as much as 0 cases, and gradation III by 1 case. Positive eosinophil distribution 1 (+) on squamous cell carcinoma of oral cavity with gradation I as much as 2 cases, gradation II as many as 4 cases, and III gradation as many as 0 cases. Positive eosinophil distribution 2 (++) in squamous cell carcinoma of oral cavity with 1 gradation of I case, II gradation as much as 0 cases, and gradation III 2 cases. Positive eosinophil distribution 3 (+++) on squamous cell carcinoma of oral cavity of gradation I in 5 cases, 2 gradation II as much as 2 cases, and III gradation of 0 cases. Positive eosinophil distribution 4 (++++) in squamous cell carcinoma of oral cavity with gradation I of 8 cases, gradation II as much as 1 case, and III gradation as many as 0 cases. The result of analysis using Spearman rank test found no significant correlation with rs = -0.313 (p <0,05) which means that eosinophil count increased in squamous cell carcinoma of oral cavity of gradation I (well differentiated) with 90% significance level. This study shows that the number of eosinophils is not correlated with histopathologic gradation, because in this study the number of gradations I is more than the gradations II and III and generally KSSRM is gradation I. In addition, perhaps because the role of eosinophils is more involved in extracellular matrix damage because most eosinophils +4 whose histopathologic gradation I tumor size> 2 cm. In addition, the amount of eosinophils around the tumor is also determined by many factors, among others, depending on the damage around the tumor, tumor differentiation, and immunity.

CONCLUSION

Conclusions General of this study is CD10 immunoexpression not correlated with regional KGB enlargement of squamous cell carcinoma of the oral cavity, CD10 immunoexpression is not correlated with gradation of squamous cell carcinoma of the oral cavity, the number of eosinophils associated with regional KGB enlargement of squamous cell carcinoma of the oral cavity, the number of eosinophils is not correlated with gradation histopathologic squamous cell carcinoma of the oral cavity. And as a specific conclusion, increased CD10 immunoexpression in stromal tissue is not followed by regional KGB enlargement of squamous cell carcinoma of the oral cavity, increased CD10 immunoexpression in stromal tissue is not supported by increased histopathologic carcinoma of squamous cell carcinoma of the oral cavity, increased eosinophils in stromal tissue accompanied by increased regional KGB enlargement by 19.8%. ((p <0.05), and the increasing number of eosinophils in stromal tissue was not supported by increased histopathologic gradations of squamous cell carcinoma of the oral cavity.

REFERENCES

11. Priebe SL, Aleksejuniene J, Dharamsi S, Zed C, Oral Cancer and Cultural Factors in Asia, Canadian J Dental Hygiene 2008[diunduh 18 Januari 2009]42(6):291-4. Tersedia dari: http://www.cih.ubc.ca/media/OraleY020 cancer%20and%20

cultural%20factors%20in%20Asia.pdf

2. Awalieyah N. 2002 Insidensi Tumor Ganas Rongga Mulut Ditinjau dari Segi Umur, Jenis Kelamin, Lokasi dan Jenis Histopatologi di Bagian Patologi Anatomi FKUP/RSHS Bandung periode Lima Tahun (1 Januari 1996 —31 Desember 2000), Universitas Padjadjaran Bandung, Skripsi.

3. Holsinger FC, Lee JE, Lentsch EJ, Myers JN. Invasion and Metastases in Head and Neck Cancer. Dalam: Harrison LB, Sessions RB, Hong WK„ editor. Head and Neck Cancer. edisi ke 2, Philadelphia: Lippincott Williams & Wilkins; 2004, h. 949-72.

4. Weinberg RA, Hanahan D. The Molecular Pathogenesis of Cancer. Dalam Bishop JM, Weinberg RA, editor. Molecular Oncology. New York: Scientific America; 1996

5. Huang BW, Zhou Xi, Zhang LH. CD10-positive Stromal Cell in Gastric Carcinoma : Correlation with invasion and Metastasis, Japan J Clinical Oncology 2005[diunduh 14 Januari 2009] 35(5):245-50.Tersedia dari: http://wwv, n at u re.com/modpathol/j ournal/v.20/nl/fu 1 1/3800713a. html

6. Bilavolic N, Sandstad B, Golouh R. CD10 protein expression in tumor and Stromal cells of Malignant Melanoma in Associated with tumor Progression, J Modern Pathology 2004[diunduh 5 September 2009] ;17:1251-58. Tersedia dari : http://www .nature.com/modpatho l /journal/v.17/n10/ful1/3800174a.htm l

7. Piattelli A, Fioroni M, Iezz G, Stellini E, Piattelli M, Rubini C. CD10 Expression in Stromal Cells of Oral Cavity Squamous Cell Carcinoma: a Clinic and Pathologic Correlation. J Oral Diseases 2006.[diunduh 20 Oktober 2008]12:301-4. Tersedia dari http://www.blackwellmunks gaard.com

8. Said M, Wiseman S, Yang J, Alrawi S, Douglas W. Tissue Eosinophilia : a Morphologic Marker for Asssesing Stromal Invasion in Laryngeal Squamous Neoplasms. Biomed Central Clinical Pathology 2005.[diunduh 1 September 2009]:5:(1):1-6 Tersedia dari http://www. biomedcentral. com/1472-6890/5/1.

9. Olivera DT, Tjioe KC, Assao A, Sita Faustino SE, Carvalho AL, Landman et al. Tissue Eosinophilia and Its Association with tumoral Invasion of Oral Cancer, International J Surgical Pathology.2009.[Diunduh 30 Oktober] 17:3:244-249. Tersedia dari: http://www.ijs.sagepub.com/cgi/reprint/17/3/244.pdf

10. Backdahl MS, Parks WC. 92-kd Gelatinase is actively expressed by eosinophil and stored by neutrophils in squamous cell carcinoma. American Journal of Pathology.1993 [Diunduh 24 Nopember 2009] 142:995-9

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