

## Role of Eosinophil in Oral Squamous Cell Carcinoma in Relation to Stage and Grade

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### ABSTRACT

Oral squamous cell carcinoma defined as malignant neoplasm of the oral cavity exhibiting the morphological feature of squamous epithelium and it is the end stage of alteration in the stratified squamous dysplasia when the dysplastic epithelial cell invading the underlying connective tissue and reach the basement membranes.

*Aim:* To evaluate the (Eosinophil cell density) in different grades and stages of oral squamous cell carcinoma (OSCC) using Special stain like Giemsa stain.

*Materials and methods:* Seventeenth intraoral histopathologically proven cases of OSCC were selected (9 cases were well-differentiated squamous cell carcinoma (WDSCC), 6 moderately differentiated squamous cell carcinoma (MDSCC), and 2 poorly differentiated squamous cell carcinoma (PDSCC)). One section of 4  $\mu$ m were taken for each case. All cases were stained using special stain (Giemsa stain) for studying tissue eosinophils. Eosinophil cell density was calculated using the density method.

*Results:* (ECD) was counted & correlated with age, sex, site, grade and stage, a high significant relation between (ECD) was observed with an increasing grade of OSCC from well to poor differentiated oral squamous cell carcinoma. While (ECD) correlation with age, sex, site, and stage were statistically non-significant in all stages. Also a high significant relation bet (ECD) was observed with an increasing grade of OSCC.

*Conclusion:* The findings of the present study highlight the significance of eosinophil counting and that it can be used as an additional morphological parameter in the grading of OSCC which can also be included in the biopsy report.

**Key words:** Eosinophil cell density, Oral squamous cell carcinoma, Giemsa stain

**HOW TO CITE THIS ARTICLE:** Sundus Abdul-Alhussain Jasim, Shorouq M Abass, Effect of Alum Disinfectant Solutions on Some Properties of a Heat-Cured Acrylic Resin, J Res Med Dent Sci, 2021, 9 (5):132-139.

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**Received:** 20/03/2021

**Accepted:** 13/05/2021

### INTRODUCTION

More than 90 % of oral cancers are squamous cell carcinoma. Its biological behavior is influenced by the host immune cells, such as multifaceted eosinophil's, associated with wound healing and tissue damage processes. Their presence within a variety of human cancers raises queries about their role. The infiltrations of tumor stroma by eosinophils are believed to play a significant role in progression of the carcinoma and could be either a potential diagnostic tool for stromal

invasion or as a prognostic indicator. Its role in cancer remains unclear since in the literature, there are very few studies showing improved prognosis and few contradictory studies showing poor prognosis [1].

Oral squamous cell carcinoma made the tumor epithelium and the surrounding connective tissue stroma. The connective tissue stroma creates the tumor microenvironment (TME) within which varying populations of mesenchymal cells, extracellular matrix and inflammatory cells are found. [2]. TME provides the cross-talk between the tumor cells and the stromal elements such as inflammatory cells and cancer-associated fibroblasts, contributing to the development, growth, invasion, and metastasis

of the tumor [3]. Literature suggests six features in the development of cancer. Epidemiological studies, from the beginning of the 19th century, are done to propose the role of inflammation as the seventh feature of cancer. [4].

Inflammation in tumor microenvironment assists in both promotion and growth of tumor. Tumor-associated tissue eosinophilia (TATE) is the term used when eosinophils are detected in a tumor tissue with inflammatory infiltrate. Although carcinogenesis with inflammation is one of the important hallmarks, the exact role of eosinophils remains unclear. Various studies on oral squamous cell carcinoma (OSCC) that focused on eosinophils stated both favorable and unfavorable prognosis in cancer tissue, because of which the exact function of eosinophil's still remains uncertain [5].

Very few studies are conducted to elicit the role of the inflammatory environment in relation to (Oral potentially malignant disorders) and their progression to cancer. However, these studies have provided an evidence that a high level of immune cell infiltration is prognostic of progressing immune reactivity in premalignant lesions and in cancers.

Tissue eosinophils are granulocytes which come under myeloid progenitor series of immune cells system. Eosinophils were first described as "coarse granule cells." The eosinophils are 8  $\mu\text{m}$  in diameter and characterized by its bright red granules. Their nuclei are bilobed usually although three or more lobes observed. An eosinophil is a granular leukocyte which is normally present in the gut lining and the bloodstream [6]. They contain proteins that give the body ability to fight infection of parasitic organisms, such as worms. However, in certain diseases, these proteins can damage the body [7].

The term eosinophilia refers to conditions in which abnormally high levels of eosinophil's are found in either the blood or in body tissues [8], although it is controversial. In this study the counting of eosinophils were correlated with the differences in clinicopathological features of OSCC. The aim of present study was Evaluation of (ECD) in OSCC in relation to age, sex, site, grade and stage by using special stain (Giemsa stain).

## MATERIALS AND METHODS

In this retrospective study the eosinophil cells counting in histopathologic records of 17th OSCC patients who underwent surgery were retrieved from the archives of Oral Pathology laboratory, College of Dentistry Baghdad University, and the histopathological laboratory in Al-Shaheed Ghazi hospital for specialized surgeries which were dated from the period 2014 to 2017. The demographic and histopathologic features of patients were recorded, and descriptive analysis was used for statistical interpretation between them. Evaluation of eosinophil counting cell by using Giemsa staining by counting the eosinophil cells density (ECD).

### Giemsa stain preparation

Giemsa's solution is a mixture of methylene blue, eosin, and Azure B. Giemsa staining Giemsa stain is one of the best-known histological stains, coloring the nuclei dark blue and the cytoplasm blue to pink, according to the acidity of the cytoplasmic contents.

The stain is usually prepared from commercially available Giemsa powder. Generation a thin air-dried samples film of the specimen on a microscope slide is fixed in methanol for 10 min. Air-dry until all methanol has evaporated. Stain in coplin jar containing 5% Giemsa stain (diluted in tap water) for 20 min. Wash sample in large beaker filled with tap water until excess Giemsa stain is removed. Air-dry and examine under microscope [9].

### Counting procedure

Eosinophils were analyzed quantitatively by counting the total number of eosinophils in Giemsa-stained sections. The Giemsa-stained sections were first seen at low power ( $\times 10$ ). Cell counting was then performed under  $\times 40$ . (Four high-density areas) were selected and software grid ( $10 \times 10$ ) was created with an area of  $0.04 \text{ mm}^2$  which was calibrated. The cells were counted throughout each of the tissue sections in four representative and consecutive grid fields ( $\times 40$ ). The mean of four values was calculated and expressed as mean  $\pm$  standard deviation (SD) per  $\text{mm}^2$ . The fields were studied in a step ladder fashion and care was taken to prevent the overlapping of fields. The cells extending over other squares were counted in First Square. (Treville Pereira et al, 2018). And eosinophil cell

density (ECD) was calculated from each 4 fields using formula mentioned below.

Eosinophil cell density (ECD): No. of eosinophil cells in a field/0.04mm (Area of field i.e., 40X magnification=0.04 mm).

**Statistical analysis**

Data were revised, coded, and analyzed using the “Statistical Package of Social Science (SPSS) version 26.0.

For presentation of data using:

Mathematical presentation method (Mean and Stander Deviation).

For analysis of data using:

Independent sample t-test.

Simple Linear regression.

The comparison of significant (p-value) in any test was considered as:

Considering P-Value of less than 0.05 (P<0.05) was statistically significant (S), while P- Value of less than 0.01 (P<0.01) was highly statistically significant (HS).

**RESULTS**

The distribution of study sample according to clinicopathological data was shown in table 1. Patient age was ranging from 18 to 78years

with a (mean ± standard deviation) was (56.29 of ± 15.20) years, the highest proportion of sample was among age group>50years (70.6%) Regarding gender, proportion of males was higher than females (58.8% versus 41.2%) with a male to female ratio of 1.42:1. Regarding the site of the lesion, the most common site of OSCC in our study was the Tongue (29.4%). The most predominant grade is well differentiated grade OSCC was about (52.94%) of lesions, but cases with poorly differentiated OSCC was 11.76%. Concerning the stage of the lesion, the highest proportion is stage IV (70.59%). While N status in this study positive N was (35.3%), while negative N was (64.7%). Regarding T status in the present study (T1-T2) percentage was 29.5% and (T3-T4) percentage was 70.5%.

Table 2 shows the Mean value of ECD according to age group (≤50 year) was (0.50 ± 0.04), while Mean value according to age group (>50 year) was (0.43 ± 0.13). The relationship between ECD and age was non-significant statistically (P=0.276).

Regarding sex, the Mean value according to Male was (0.45 ± 0.11), while Female Mean value was (0.44 ± 0.14), But statistically the relationship between ECD and sex was non-significant (P=0.839).

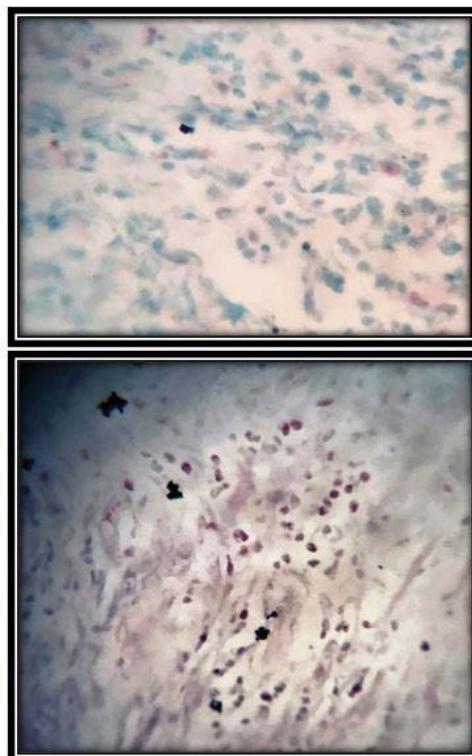
Concerning site, the highest Mean value of ECD according to site in Maxilla with Teeth Mean

**Table 1: Distribution of study group by age (year), Sex and Site, grade & stage.**

		No.(n=17)	(100%)
Age (year)	≤ 50	5	29.4
	>50	12	70.6
Sex	Male	10	58.8
	Female	7	41.2
Site	Anterior part of palate	1	5.9
	Buccal mucosa	4	23.5
	Floor of the mouth	4	23.5
	Lower lip	1	5.9
	Mandible	1	5.9
	Maxilla with Teeth	1	5.9
	Tongue	5	29.4
Grade	Well	9	52.9
	Moderately	6	35.3
	Poorly	2	11.8
Stage	I (1st)	2	11.8
	II (2nd)	1	5.9
	III (3rd)	2	11.8
	IV (4th)	12	70.6
N Status	Positive	6	35.3
	Negative	11	64.7
T Status	T1-T2	5	29.4
	T3 –T4	12	70.6

**Table 2: Comparison between eosinophil cell density (ECD) and age, sex, site, grade & stage.**

		N	Mean ±Sd.	Sig. Test
Age (year)	≤50	5	0.50±0.04	P=0.276 P>0.05 (Non-Significant)
	>50	12	0.43±0.13	
Sex	Male	10	0.45±0.11	P=0.839 P>0.05 (Non-Significant)
	Female	7	0.44±0.14	
Site	Anterior part of palate	1	0.56±0.00	P=0.643 P>0.05 (Non-Significant)
	Buccal mucosa	4	0.47±0.06	
	Floor of the mouth	4	0.45±0.08	
	Lower lip	1	0.40±0.00	
	Mandible	1	0.16±0.00	
Grade	Well	9	0.22±0.09	P=0.001 P<0.01 (High-Significant)
	Moderately	6	0.40±0.03	
	Poorly	2	0.52±0.06	
Stage	I (1st)	2	0.52±0.11	P=0.518 P>0.05 (Non-Significant)
	II (2nd)	1	0.40±0.00	
	III (3rd)	2	0.46 ±0.09	
	IV (4th)	12	0.44±0.13	
T Status	(T1-T2)	5	0.42±0.05	P= 0.607 [P>0.05 (Non-significant)]
	(T3-T4)	12	0.46±0.03	
N Status	Positive	5	0.47±0.05	P= 0.578 [P>0.05 (Non-significant)]
	Negative	12	0.43±0.03	



**Figure 1: Eosinophil's giemsa stain (a) Well differentiated (b) Poor differentiated.**

value was (0.60 ± 0.00), while the lowest mean value in Lower lip Mean value was (0.40 ± 0.00). The relationship between ECD and site was non-significant statistically (P=0.643).

The Mean value of ECD according to grade (WDSCC) was (0.22 ± 0.06), while in Moderate grade Mean value was (0.40 ± 0.03), and (Poor)

grade Mean value was (0.52 ± 0.09). The relationship between ECD and grades were high significant statistically (P=0.001).

According to stage, the Mean value of I (1st) stage was (0.52 ± 0.11), while II (2nd) stage Mean value was (0.40 ± 0.00), while Mean value of III (3rd) was (0.46 ± 0.09), otherwise Mean value of



IV (4th) stage was ( $0.44 \pm 0.13$ ). The relationship between ECD and all stages was non-significant statistically ( $P=0.518$ ).

Regarding T status, the Mean value of (T1-T2) was ( $0.42 \pm 0.05$ ), while Mean of (T3-T4) was ( $0.46 \pm 0.03$ ). The relationship between ECD and T status was statistically non-significant ( $P=0.607$ ). While N status in this study, Mean Value of (ECD) higher in positive case ( $0.47 \pm 0.05$ ) than negative was ( $0.43 \pm 0.03$ ), but the relationship was statistically non-significant ( $P=0.578$ ) (Figure 1).

### DISCUSSION

The pathogenesis of invasive oral cancer is not only based on the genetic changes in the tumor cells but also based on the absolute communication between the tumor cells, inflammatory cells, endothelial cells, fibroblasts, and other stromal cells [10]. In view of inflammation-associated carcinogenesis, the role of eosinophils in tumor cytotoxicity is not well assumed but has been characterized as more potent than other inflammatory cells in tumor-associated cytotoxic reaction [11,12].

Based on the above facts, this study is based on the ambiguity in functional role of eosinophils in oral squamous cell carcinoma as they are hypothesized as being immunologically directed against tumor cells as well as in lowering the immune response facilitating the tumor growth. Oral squamous cell carcinoma has considered for a long time as a tumor of elderly patient and that may be explained by the prolonged exposure to environmental carcinogens [13] or impaired immune system [14]. In this study, the age of patients was mostly above 50 years of age with mean age of 57.1 years old and the most affected age group was (60-70) years and that analogy to the results were reported by many Iraqi studies [15-20], as well as foreign studies that founded more than 90% of OSCC were above 40 years [21-23]. However, 29.41 % (5 cases) of patient younger than 50 years old and that agreement with some studies which showed the increase of incidence among the younger population [24-26]. Among these young cases (under 50 years) 2 cases affect females and this agreed with other studies [27,28].

The male to female ratio in this study was 1.42:1, and that identical to most studies which

reported that the OSCC affect males more than females [29-31]. The global OSCC M:F ratio is about 5.5:2.5, ranging from 1.2:1 [32] to 3.02:1 [33], such range is similar to most arab nations [34].

Concerning the site distribution, in the current study, the tongue comprised most of the cases (29.41%), followed by the floor of the Mouth and buccal mucosa that represent. (23.53%) of cases, this finding is in accordance with most of published literature, where the tongue was the most common [34-38], nevertheless in contrast with other studies where buccal mucosa was the most common location, especially in south Asian [33].

While in this study we show that stage IV is the highest stage which was agreement with other studies [39,40]. It was obvious that almost all the cases were seen in an advanced stage, the delay was found to be mainly due to ignorance of the patients, and delay in diagnosis and referral by dental practitioners. More than half of the study sample was revealed to be of the advanced stage IV, which was in accordance with the study of [39,41] denoting an aggressive behavior of the tumor and delay in the diagnosis. Regarding the histopathological grading, the well differentiated OSCC is the most predominance one in this study (70.6%) which agree with many previous studies [39,40]. While disagree with several studies [17,19,42,43] who reported (moderately differentiated) grade as a most common. Moreover [44] reported that (poor differentiated) grade was most common.

Differences among these studies may be due to the sample size and to criteria of analysis. Regarding to Distribution of Eosinophil cell density (ECD) by clinicopathological parameters there is no significant relation between ECD and age >50 and <50 also. And non-significant with sex and site. Distribution of Eosinophil cell density (ECD) by Grade: High significant between ECD and all Grades of OSCC. Some researchers employ grade as a part of the risk-assessment to predict prognosis and survival [45].

And this result in accordance with Kargahi et al. [46] reported that the number of eosinophil's progressively increased from mild to severe at different levels of dysplastic mucosa and from well differentiation to poor differentiation in squamous cell carcinoma. Also, this study is in

agreement with [5] suggesting a statistically significant increase in the mean value of tumor eosinophil count from well differentiated to poor differentiated.

Our result was disagreeing with [47] was showed that no correlation was noted between the eosinophilic infiltration and the histologic grades of OSCC. However, this may be because the distribution of OSCC cases according to histologic grades was unequal and not consistent. A conceivable explanation for the disparity in the results of various studies could be due to the lack of a standard criteria for grading TATE that is universally followed and use of biopsy specimens that run the risk of being unrepresentative. This study disagrees with other study [1].

Distribution of Eosinophil cell density (ECD) by Stage: non-significant. This study show increase in all stages but non-significant in the eosinophil count. Others showed absent/mild eosinophils in early clinical stages, i.e., stages I and stage II while significantly higher eosinophilia was seen in stages III and IV, suggesting increased eosinophilia with increasing clinical stages. In the present study, eosinophils were found in most cases of OSCC. There was elevated but not significant Eosinophil count in stages III and IV, so increased eosinophilia was associated with T3 and T4, suggestive of increased eosinophil count with increased primary tumor size [48] have also reported an increase in eosinophil count with an increase in the tumor size. Accordingly, eosinophil's can be regarded as an indicator of a developing malignancy along with other indicators, and they possibly can be used to develop the prognosis for the disease [46].

#### FINANCIAL DISCLOSURE

There is no financial disclosure.

#### CONFLICT OF INTEREST

None to declare.

#### ETHICAL CLEARANCE

All experimental protocols were approved under the College of Dentistry University of Baghdad and all experiments were carried out in accordance with approved guidelines.

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