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Novel Correlations among the Histopathological Components of Oral Squamous Cell Carcinoma

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Abstract

BACKGROUND: Oral squamous cell carcinoma (OSCC) is an aggressive tumor with high mortality and morbidity rates.

AIM: In this study, we aimed to probe whether there is association between stromal histopathological parameters with patient data, as well as, the correlation of these parameters with each other.

MATERIALS AND METHODS: This study was performed at College of Dentistry/University of Duhok/Iraq, the practical section was carried out between February 2022 and June 2022 as follow: A group of 86 formalin fixed paraffin embedded (FFPE) samples of OSCC and 20 FFPE samples of healthy gingiva were stained with Hematoxylin and eosin and Masson's trichrome stains. Tumor stroma ratio (TSR), stromal tumor-infiltrating lymphocytes (sTIL), budding activity (BA), cell nest size (CNS), orientation and packing of collagen fibers, and collagen fiber content (CFC) were evaluated. These histomorphological parameters were correlated with clinicopathological characteristics and with each other.

STATISTICAL ANALYSIS USED: Chi-square test, fisher exacts test, and spearman's rank correlation coefficient were used for analyzing our study data.

RESULTS: TSR, BA, CNS, orientation and packing of collagen fibers, and CFC were significantly associated with pT stage of the tumor. Moreover, significant correlations were observed among TSR, BA, CNS, and CFC. In addition, orientation and packing of collagen fibers were significantly correlated with each other.

CONCLUSIONS: Novel correlations were found between collagen fiber features with TSR, BA, and CNS in an easy and cost-effective methods.

Introduction

In spite of crucial advance in developing n e w diagnostic and therapeutic strategies, the incidence of oral squamous cell carcinoma (OSCC) is increasing continuously worldwide and still there is no considerable improvements in the survival rates of the patients [1].

The quality of life in patients that develop this devastating tumor noteworthy impacted by disease progression and by treatment [2]. Therefore, researchers are focusing on identifying sophisticated methods and new biomarkers that aid in stratifying patients regarding their risk or their response to treatment in variable cancers [3]. Unfortunately, great proportion of these biomarkers need highly-cost kits, equipment, and special training that seldom could be implemented in a standard daily practice. Therefore, histopathological assessments of tissue are until now the major prognostic elements routinely utilized in medical practice [4].

Although immense number of researches has been done on the foremost applied histopathological predictors such as the WHO grading systems, vascular, or perineural invasion [5]. Many factors in tumor micro environment including analysis of stromal component of the tumor and local host immune response are still poorly-explored. Moreover, these parameters have been proven to have prognostic value in various tumors in different sites in the body [5].

The existence of the conflicting opinions in determination whether tumor/stroma ration (TSR), stromal tumor-infiltrating lymphocytes (sTIL), budding activity (BA), cell nest size (CNS), orientation and packing of collagen fibers, in addition to collagen fiber content (CFC) is linked with each other in OSCC has led us to perform a detailed histomorphological study, bring them into a single platform and associate them with clinical

characteristics in an attempt to empower the decision of the pertinent clinical management.

Materials and Methods

Samples selection

In this case–control retrospective study, 86 formalin fixed paraffin embedded (FFPE) samples of OSCC and 20 FFPE samples of healthy gingiva were retrieved from the archives of Rizgary Teaching Hospital/Erbil/Iraq. In the period between January 2016 and August 2021, any case did not receive radiotherapy or chemotherapy before the surgical removal was included in this research. The research project was approved by the ministry of health and by research ethics committee at college of dentistry, University of Duhok/Iraq. The acquisition of written informed consent was waived by the institutional review board given the retrospective nature of the study and the anonymization of all data. Selection criteria included localization, availability of clinical data, as well as tissue availability.

Criteria for histological evaluation (Figure 1)

For every hematoxylin and eosin (H&E) slide the invasive front part of the OSCC was selected, four

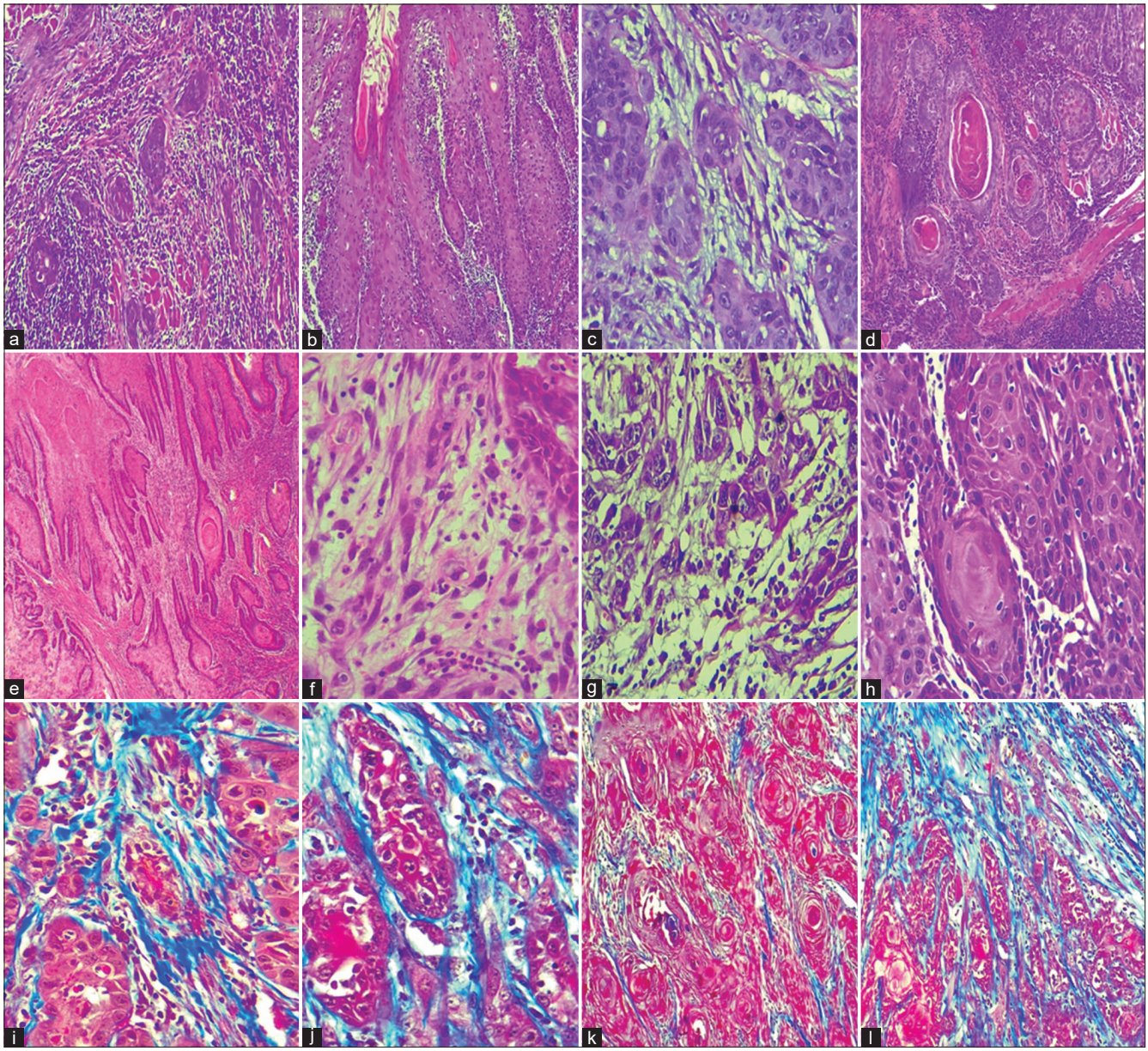

Figure 1: Representative photomicrographs of histopathological evaluation. (a) Stroma rich tumor (H&E 100×). (b) stroma poor tumor (H&E 100×). (c) Low sTIL (H&E 400×). (d) High sTIL (H&E 100×). (e) Low BA (H&E 100×). (f) High BA (H&E 400×). Large CNS (H&E 100×). (g) Intermediate CNS (H&E 100×). (h) Large CNS (H&E 400×). (i) Parallel densely packed collagen fibers (MT 400×). (j) Haphazard loosely packed collagen fibers (MT 400×.) (k) Low CFCs (MT 100×). (l) High CFCs (MT 100×)

parameters were visually assessed by two pathologists as follow: TSR, sTIL, BA, and CNS. The final score for each parameter in a given sample was determined by the calculation of the mean value provided by both pathologists.

TSR was visually evaluated, the invasive front area with stroma surrounded by malignant cells from all borders was identified by the conventional microscopy (Lieca, Germany) at low magnification, then the percentage of the stroma was manually evaluated by two pathologists from 0% to 100% per field, accordingly, each sample was judged as stroma-rich when TSR was <50%, and stroma-poor when TSR was ≥50% [6]. Particularly, the area of TSR evaluation was visualized at high magnification power for confirmation of presence of the malignant cell at all borders.

The sTIL was visually assessed (eyeballing) and manually scored in a single high-power field (HPF) as a percentage of the stromal area that was occupied by lymphocytes, the cases were categorized as high sTIL ≥20% or low sTIL <20% [7]. Tumor budding (small discrete clusters of tumor cells up to five cells) was visually assessed in four HPF, high budding ≥5 buds on average, and low- budding <5 buds [8]. Tumor cell nests were categorized into large >15 malignant cells, intermediate 5–15 malignant cells, and small 1–4 malignant cells [9]. Regarding masson's trichrome (MT) stain, collagen fibers were stained blue, healthy gingiva was considered as control in which collagen fibers appear densely packed with parallel orientation. Based on the appearance of collagen fibers in relation to tumor islands in five fields at low magnification, orientation of collagen fibers was visually judged either as parallel or haphazard and the packing of the collagen fibers was categorized either as dense or loose [10]. The CFC was identified as the percentage area filled by collagen fibers in three HPF, the CFC was categorized into low <23% or high >23% [11]. All H&E and MT stained slides were evaluated and photographed using a light microscope with attached camera (Lieca, Germany) and personal computer.

Statistical analysis

Analyzation of data was done using IBM SPSS Statistics (Version: 28.0.1.1). Associations of morphological characteristics with clinicopathological variables were tested with Chi-square test and Fisher's exact test. Correlations of morphological characteristics with each other were tested with Spearman's rank order correlation (denoted by r). Values of r < 0.20 were judged as "weak ", r between merge.

0.21 and 0.40 as "fair", "moderate "when r was 0.41–0.60, 0.61–0.80 as "high" and r > 0.80 as "very high." p < 0.05 was regarded as statistically significant.

Results

The distribution and association between H&E and MT investigated parameters and clinicopathological characteristics in OSCC cases are listed in (Tables 1-3). TSR, BA, CNS, and CFC were significantly associated with pT stage. Moreover, orientation and packing of fibers were significantly associated with both grade and pT stage of the tumor.

Table 1: Association between TSR and sTIL with clinicopathological characteristics of OSCC cases

Variables	OSCC cases	TSR		sTIL	
	n	< 50% n	≥ 50% n	low n	High n
Total	86	38	48	16	70
Age					
< 60	37	14	23	9	28
≥ 60	49	24	25	7	42
p		0.209			0.272
Gender					
Male	58	24	34	12	46
Female	28	14	14	4	24
p		0.493			0.565
Site					
Lip	44	20	24	11	33
Tongue	20	9	11	1	19
Palate	16	6	10	2	14
Others	6	3	3	2	4
p		0.958			0.144*
Grade					
G1	26	12	14	4	22
G2	56	25	31	11	45
G3	4	1	3	1	3
p		0.863			0.802*
pT stage					
T1+T2	58	15	43	10	48
T3+T4	28	23	5	6	22
p		< 0.001	**		0.769

n: Number, p: Level of significance *: Fisher exact test, **: Significant correlation, sTIL: Stromal tumor infiltrating lymphocytes, TSR: Tumor/stroma ratio.

Correlations of investigated parameters with each other

TSR has strong correlation with BA (r = –0.653, p < 0.001), intermediate with CNS (r = 0.584, p < 0.001) and fair correlation with CFC (r = 0.454, p = 0.000). BA has intermediate correlation with CNS (r = –0.563, p < 0.001) and fair with CFC (r = –0.398, p = 000) and

Table 2: Association between BA and CNS with clinicopathological characteristics of OSCC cases

Variables	BA		CNS		
	Low (n)	High (n)	Small (n)	Int. (n)	Large (n)
Total	57	29	52	9	25
Age					
< 60	24	13	22	4	11
≥ 60	33	16	30	5	14
p	0.882			1.000	
Gender					
Male	40	18	36	6	16
Female	17	11	16	3	9
p	0.474			0.941	
Site					
Lip	31	13	27	5	12
Tongue	11	9	12	3	5
Palate	13	3	9	1	6
Others	2	4	4	0	2
p	0.109*			0.965*	
Grade					
G1	16	10	16	2	8
G2	38	18	34	6	16
G3	3	1	2	1	1
p	0.849*			0.819*	
pT stage					
T1+T2	54	4	25	9	24
T3+T4	3	25	27	0	1
p	< 0.001**			< 0.001**	

BA: Budding activity, CNS: Cell nest size, n: Number, p: Level of significance *: Fisher exact test, **: Significant correlation.

Table 3: Association between MT studied parameters and clinicopathological characteristics of OSCC cases

Variables	Orientation		Packing		CFC	
	Parallel (n)	Haphazard (n)	Dense (n)	Loose (n)	High (n)	Low (n)
Total	38	48	35	51	33	53
Age						
< 60	16	21	13	24	13	24
≥ 60	22	27	22	27	20	29
p	0.878		0.362		0.658	
Gender						
Male	27	31	24	34	20	38
Female	11	17	11	17	13	15
p	0.525		0.853		0.347	
Site						
Lip	22	22	21	23	19	25
Tongue	6	14	10	10	9	11
Palate	7	9	4	12	2	14
Others	3	3	0	6	3	3
p	0.489*		0.031**		0.101*	
Grade						
G1	22	4	21	5	13	13
G2	15	41	14	42	20	36
G3	1	3	0	4	0	4
p	< 0.001*		< 0.001*		0.120*	
pT						
Stage	32	26	32	26	16	42
T1+T2						
T3+T4	6	22	3	25	17	11
p	0.003**		< 0.001**		0.004**	

CFC: Collagen fiber content, n: Number, p: Level of significance *: Fisher exact test, **: Significant correlation.

Packing of fibers (r = –0.240 p = 0.026). In addition, collagen fibers orientation fairly correlated with packing of fibers (r = 0.407, p < 0.001).

Discussion

The clinical and prognostic significance of the tumor stroma has been documented in oncologic literature, certainly, it is of high diagnostic and clinical relevance to fill the gap presented in the studies of literature, whether there is correlation between tumor stromal parameters, which could add valuable information to the daily pathology practice that result in deeper understanding and proper stratifications of carcinoma patients. In an attempt to answer these open questions, we evaluated the correlation of TSR, sTIL, BA, CNS, orientation, and packing of collagen fibers in addition to collagen fibers contents to each other's, as well as their association with clinicopathological features of the studied OSCC patients.

The results of our study found that stroma poor tumors are significantly correlated with advanced pT stage of the disease, but such a robust relationship was lacking between TSR and age, gender, site and grade of the tumor. Our results revealed partial agreement with what was revealed by Caruntu *et al*. in their work, where they reported that TSR does not correlate with age, gender, grade, and even pT stage of the tumor [4]. TSR cannot be utilized in carcinoma patients that received pre-operative chemotherapy and/or radiotherapy because these therapies could alter the formation of stroma in the tumor microenvironment [11]. However, our findings did not affect since all patients with the pervious radiotherapy or chemotherapy were excluded from this work. Tumor budding is a reliable, reproducible marker; it has been established as independent prognostic predictor in various tumors such as oral tongue squamous cell carcinoma [12]. Our findings were in concordance with Nakashima *et al*., where BA in their carcinoma cases was significantly higher in advanced stage as compared to early stages of the disease [13]. Mounting data proposed that tumor buds may adopt properties of cells that undergoing epithelial to mesenchymal transition (EMT), such cells carry higher potential of invasiveness and migration thus associated with poor clinical outcome [14].

CNS is a parameter of tumor aggressiveness in diverse solid tumors, it represents the loss of adhesion among cancer cells and also might represent features of epithelial to mesenchymal transition, which enables the tumor cells to carry a more infiltrative and motile phenotype [15]. Our findings support the notion that small CNS are present in advanced stage of the disease [16].

The interactions between the invading malignant cells and the host extracellular matrix are critical and vital events as they create an ideal environment to tumor cells for growth and metastasis, collagen is a chief protein in the host extracellular matrix which is considered as the essential barrier that cleared away during the process of invasion, providing a room for the infiltrating tumor mass [17].

In our current work, we observed that there are significant differences in both alignment and density of collagen fibers between different grades and stages of the tumor, collagen fibers appeared more parallel and densely packed in well differentiated than in poor differentiated OSCC. In study done by, Manjunatha *et al*. found that collagen fibers were more organized in well than moderately to poorly differentiated OSCC [18].

These results were also reported by Bordoloi *et al*. The collagen fibers presented mainly parallel orientation with dese packing in most of the well differentiated OSCC cases, but it changed gradually to a haphazard and loose pattern in moderate to be totally haphazardly and loosely arranged in poorly differentiated OSCC cases [9]. Importantly, the CFC in the tumor was associated significantly. With P tstage of the tumor, but we did not notice any significant relationship between the CFC with age, gender, site of tumor, and pathological differentiation. Our findings were in consistent with Yu *et al*. (2019). [10] However, these findings were unlike Bordoloi *et al*.(2020), whose samples revealed that CFC reduced significantly as the OSCC cases progressed from well to moderate and poorly differentiation [9].

In our study, stroma poor tumor was found to be correlated with low BA, and both of them were consistently being correlate with larger CNS. Our results were in agreement with findings of Boxberg *et al*. in OSCC sample [19]. Furthermore, TSR, BA, and CNS were for the first time found be correlated with CFC in

OSCC. Furthermore, this study is novel in linking the BA to collagen fiber packing; in other words, we demonstrated that when BA increase the collagen fibers become looser. In addition, the finding that was expected but never evaluated that parallel orientation were correlated with dense packing of collagen fibers in cases of OSCC.

Conclusions

We uncovered the underlying correlations between collagen fiber features with other stromal parameters in an easy and cost-effective methods. However, more cohort studies with larger sample size needed to confirm these findings and compare them with the patient outcome.

Availability of Data and Materials

All essential and relevant data are within this manuscript and its Supporting Information files.

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Author contributions

AAA (Author 1) and JAJ (Author 3) were involved in the conceptualization, methodology, investigations, data analysis and writing. HDM (Author 2) involved in analyzing of control samples, writing of original draft, review, and editing of the manuscript. JAJ and HDM helped in the supervision. All authors read and approved the final manuscript.

Ethical Approval

This study was approved by the scientific committee of College of Dentistry/University of Duhok/ Iraq (approval number: 404). All procedures performed in this work were approved by the research Ethical committee of Duhok Directorate General of Health/ Ministry of Health/Duhok/Iraq (approval number 13072021-7-11 and 13072021-7-11R1) and were in accordance with the 1964 Helsinki declaration.

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