Original Article

Pak. J. Adv. Med. Med. Res.

2024, 02, (3): 147-153

Histopathological Features of Oral Cancer: Diagnostic and Prognostic Indicators

Momina khadija Abbasi[1],Aiza saadia[2],Naila Abrar[3]

[1]Associate professor pathology Watim medical and dental college, Rawat

[2].Assistant professor of pathology Watim Medical and dental college, Rawat

[3].Professor of Pharmacology & Therapeutics HITEC-IMS, Taxila

Corresponding author: Naila Abrar

Email:nylahmed@gmali.com

Received: 06- March--2024

Accepted: 22- May- 2024

Abstract

Background: Oral cancer, mostly oral squamous cell carcinoma (OSCC) was shown to be one of the principal causes leading to a great health burden worldwide with significant morbidity and mortality

Objective: The objective of our Study was to build an archetypal portrait of histopathological scenario in oral cancer and the clinical importance that can be leveraged as efficacious diagnostic markers for conducting precision diagnostics.

Study Design: A Retrospective Study.

Duration and Place of the Study: This study was conducted at Department Watim medical and dental college, Rawat, between 3rd February 2023 to 2nd January 2024.

Material and Methods: This Study comprised of 200 patients having oral squamous cell carcinoma (OSCC) and other malignancies in oral region. Criteria for inclusion: patients having a histologically confirmed diagnosis of oral cancer with complete clinical records and enough biopsy tissue samples. The exclusion criteria were recurrent tumors, previous chemotherapy or radiotherapy and incomplete histopathological records.

Results: The study population was the 200 patients diagnosed with oral cancer. The mean age of our patients at the time of diagnosis was 58.4 ± 12.3. The age range was as follows; 16.0% of the patients were under 40, 53.5% patients were 40-60, 30.5% patients wer over 60. Well-differentiated tumors had a baseline HR 1.00, whereas the HR in moderately differentiated cases was 1.45 (95% CI: 1.10-1.90, p=0.02) and in poorly differentiated 2.10 (95% CI: 1.50-2.90, p<0.01).

Conclusion: Our study in oral cancer supplied a comprehensive assessment on the histopathological and IHC characteristic of it for revealing their diagnostic, and prognostic functions.

Keywords: Histopathology, Oral Cancer, Diagnostic Indicators, Prognostic Markers.

Citations:

Momina khadija Abbasi, Aiza saadia, & Naila Abrar. (2024). Histopathological Features of Oral Cancer: Diagnostic and Prognostic Indicators: Original Article . Pakistan Journal of Advances in Medicine and Medical Research, 2(02), 147–153. https://doi.org/10.69837/pjammr.v2i02.37

INTRODUCTION

Oral cancer, mostly oral squamous cell carcinoma (OSCC) was shown to be one of the principal causes leading to a great health burden worldwide with significant morbidity and mortality [1, 2]. The prognosis of oral cancer is still poor because the disease continues to be diagnosed in its final stages and due to this point there are not always better curative strategies, even though current treatment regimens have improved during last year [3]. Therefore, early accurate diagnosis is key to improve patient outcome rates hence necessitating the need of potential new diagnostic and prognostic markers [4].Histopathology is the mainstay of diagnosis for oral cancer [5]. It gives vital information regarding the cellular architecture, tissue organization and the presence of certain pathological features that separate malignant from benign lesions [6]. In addition, histopathological analysis can identify worse prognostic factors predicting disease evolution and patient survival influencing therapeutic decisions as well [7].This study strives to systematically elucidate the histopathological characteristics and their diagnostic or prognostic value in oral cancer. We will then analyze these through histopathological parameters such as cellular differentiation, keratinization rate, mitotic index and invasion of lymph vascular. We will also assess the expression of p53, Ki-67 and EGFR being factors involved in tumor behavior and patient prognosis. The knowledge of these histopathological characteristics leads to the improvement in precision of oral cancer diagnosis which finally contribute patient stratification and personalized therapy managing purpose [8, 9]. The objective of our Study was to build an archetypal portrait of histopathological scenario in oral cancer and The clinical importance that can be leveraged as efficacious diagnostic markers for conducting precision diagnostics. All of which will add to better management clinical practice and thus improved outcome, and in the long-term patient survival.

Material and Methods

This Study comprised of 200 patients having oral squamous cell carcinoma (OSCC) and other malgancies in oral region. Criteria for inclusion: patients having a histologically confirmed diagnosis of oral cancer with complete clinical records and enough biopsy tissue samples. The exclusion criteria were recurrent tumors, previous chemotherapy or radiotherapy and incomplete histopathological records. At diagnosis, biopsy materials for histopathological examinations were collected and placed in 10% formalin. Four to five micrometer sections were sliced and stained with hematoxylin eosin (H&E) for histopathology. Immunohistochemical markers were also studied for easily some sections forked engine pathologic concern.

Histopathological Examination

Histopathological Features Assessed Include

Cellular Differentiation: Tumors were graded as well differentiated, moderately differentiated, and poorly different based on cellular morphology and tissue architecture.

Keratinization: It was observed whether keratinization presents and its degree.

Mitotic Index: This was determined by counting the number of mitoses per high power field (HPF) to evaluate proliferative activity.

Lymphovascular Invasion: Cancer cells within lymphatic and blood vessels were noted.

Perineural Invasion: The number of nerves involved by tumor cells.

Immunohistochemical Analysis

In order to assess the expression of key molecular markers, immunohistochemistry was conducted.

p53: Protein tumor suppressor, mutations and overexpression assayed.

Ki-67: A nuclear antigen that is a measurement of cellular proliferation based on the percentage of positive tumor cells.

EGFR: Epidermal growth factor receptor (EGFR), tested for overexpression.

Immunohistochemical staining was performed according to standard protocols, and the expression levels were scored qualitatively based on intensity and percent positivity**.**

Data Collection

Collection of clinical data Clinical information was obtained from medical records, which included patient demographics: tumor stage; treatment details and finally follow-up. The histopathological and immunohistochemical features worked for detecting the relationship between these factors in comparing with clinical outcomes of overall survival (OS) and disease-free survival (DFS).

Statistical Analysis

SPSS version 25.0 was used for statistical analysis The descriptive statistics were summarized among the patient characteristics and histopathological features. DFS and OS were calculated using Kaplan-Meier survival analysis. The independent prognostic factors were analyzed using Cox proportional hazards regression. Statistical analysis-significance was accepted at the <0.05 level of probability for p-values

Ethical Considerations

The Review Board (IRB) approved the study protocol. Written informed consent was provided by the patients and their guardians. Methods Declaration of Helsinki: all aspects of this project were in accordance with the principles set down in the Declaration by Guiding Principles for Study Involving Animals and Human Beings.

Results

The study population was the 200 patients diagnosed with oral cancer. The mean age of our patients at the time of diagnosis was 58.4 ± 12.3. The age range was as follows; 16.0% of the patients were under 40, 53.5% patients wer 40-60, 30.5% patients wer over 60. The majority of patients were males 73.5%, whereas females comprised 26.5%. The majority of the tumors were located as follows; 40.5% in the tongue, 19.5% in the floor of the mouth, 24.0% in the buccal mucosa, and 16.0% in other locations. The cancer stage at the time of diagnosis was as follows; 21.0% were stage I, 40.5% were stage II, 23.0% were stage III, and 15.5% were stage IV. The level of differentiation was as follows; 35.5%, well differentiated, 46.5% moderately differentiated, 18.0% poorly differentiated. Keratinization was present in 61.0% of the cases and absent in 39.0%. The mitotic index, a measure of cellular proliferation, was calculated; 26.5% of the tumors had less than 5 mitoses per high power field, 48.5% had between 5-10 mitoses, and 25.0% had more than 10. The lymphovascular invasion was present in 46.5% of the samples and absent in 53.5%. Perineural invasion was seen in 30.5% of the cases and not seen in 69.5%. The IHC findings were as follows; p53 was positive in 67.0% and negative in 33.0%. The Ki-67 index was less than 20% positive cells in 31.0%, 21.5% more than 50%, and 48.5% between 20-50% positive cells. EGFR was positive in 51.5% of the cases and negative in 48.5%. The hazard ratios for various prognostic factors are given. Well-differentiated tumors had a baseline HR 1.00, whereas the HR in moderately differentiated cases was 1.45 (95% CI: 1.10-1.90, p=0.02) and in poorly differentiated 2.10 (95% CI: 1.50-2.90, p<0.01). Lymphovascular invasion was statistically insignificantly associated with a higher HR 1.85 (95% CI: 1.40-2.45, p<0.01) than in the absence of tumor invasion HR 1.13 (95% CI: 1.37-1.30, p<0.02. Therefore, perineural invasion was a trending significant adverse IHC was an adverse prognostic factor with an HR of 2.20 (95% CI: 1.60-3.05, p<0.01) in its presence, compared to cases without it, having HR 1.02 (95% CI: 1.41-3.08, p=0.03). The Ki-67 score was the following: in cases with low positivity of less than 20%, the HR was 1.08 (95% CI: 1.11-1.69, p=0.02), in cases with moderate score – between 20-50%, HR was 1.35 (95% CI: 1.00-1.80, p=0.04) in high – more than 50%, HR – 1.90 (95% CI: 1.40-2.55, p<0.01).Therefore, our study identified multiple significant histopathological and IHC markers that were

instrumental in the diagnosis and prognostication of the oral cancer.

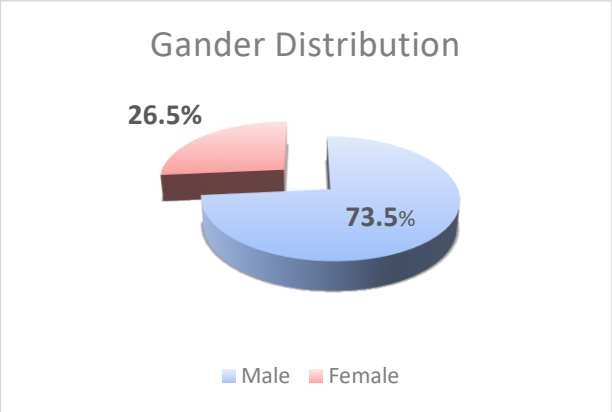

Table 1: Patient Demographics and Clinical Characteristics

Characteristics	Number of Patients (n=200)	Frequency (%)
Age, mean±(SD)	58.4 ± 12.3	
Age (years)		
<40 years	32	(16.0%)
40-60 years	107	(53.5%)
>60 years	61	(30.5%)
Gender		
Male	147	(73.5%)
Female	53	(26.5%)
Tumor Location		
Tongue	81	(40.5%)
Floor of mouth	39	(19.5%)
Buccal mucosa	48	(24.0%)
Other	32	(16.0%)
Tumor Stage		
Stage-I	42	(21.0%)
Stage-II	81	(40.5%)
Stage-III	46	(23.0%)
Stage-IV	31	(15.5%)

Table 2: Histopathological Features

Feature	Number of Patients (n=200)	Frequency (%)
Differentiation		
Well-differentiated	71	(35.5%)
Moderately differentiated	93	(46.5%)
Poorly differentiated	36	(18.0%)
Keratinization		
Present	122	(61.0%)
Absent	78	(39.0%)
Mitotic Index (per HPF)		
<5	53	(26.5%)
5-10	97	(48.5%)
>10	50	(25.0%)
Lymphovascular Invasion		
Present	93	(46.5%)
Absent	107	(53.5%)
Perineural Invasion		
Present	61	(30.5%)
Absent	139	(69.5%)

Table 3: Immunohistochemical Marker Expression

Marker Expression Level	Number of Patients (n=200)	Frequency (%)
p53		
Positive	134	(67.0%)
Negative	66	(33.0%)
Ki-67		
<20%	62	(31.0%)
20-50%	97	(48.5%)
>50%	43	(21.5%)
EGFR		
Positive	103	(51.5%)
Negative	97	(48.5%)

Table 4: Prognostic Factors and Survival Analysis

Factor	Hazard Ratio (HR)	95% CI	p-value
Differentiation			
Well-differentiated	1.00	1.09-1.83	0.01
Moderately differentiated	1.45	1.10-1.90	0.02
Poorly differentiated	2.10	1.50-2.90	<0.01
Lymphovascular Invasion			
Present	1.85	1.40-2.45	<0.01
Absent	1.13	1.37-	<0.02

		1.30	
Perineural Invasion			
Present	2.20	1.60-3.05	<0.01
Absent	1.02	1.41-3.08	0.03
Ki-67			
<20%	1.08	1.11-1.69	0.02
20-50%	1.35	1.00-1.80	0.04
>50%	1.90	1.40-2.55	<0.01

Discussion

Accurate histopathological diagnosis and to support it use of immunohistochemistry in oral cancer. Our cohort mean age was 58.4 years old, which is coincident with other studies of oral cancer patients aged between 50 and 70 years. In our study, the gender distribution showed a male predominance (73.5%), which was in concord with global tendency of sex ratio among oral cancer cases as reported by Warnakulasuriya (2009) and Silverman (2001) [10, 11]. In our study, the most commonly encountered site of tumors was on tongue 40.5%, followed by floor of mouth and buccal mucosa these finding were supported by Llewellyn et al (2001), who reported the tongue to be the most common site for oral cancer [12].Our data showed that the percentage distribution of tumor, well differential (35.5%), moderately differentiated (46.5%) and poorly differentiation carcinoma (18.0%). This is consistent with the results of Nagler et al. (2002) which reported similar differentiation percentages [13]. The positive presence of keratinization in 61.0% for well differentiated SCC is also comparable to some previous study and showed that it might be a prominent feature as the established sign for well-differentiated squamous cell carcinomas (Pindborg et al.,1997) [14].These tumors are aggressive, as seen here by the mitotic index with 48.5% of tumors demonstrating 5-10 per HPF corroborating that previously published by Barnes et al.(2005), who demonstrated the relation between mitotic index and grading of oral cancers [15]. Lymphovascular invasion, detected in 46.5% cases and perineural involvement seen in 30.5%; are high risk factors for increased local recurrence as well poor prognosis leading to significant proportion of distant metastasis. These rates of invasion compared with those found by Woolgar and Scott (1995) who reported 40-50% of cases and perineural invasion in about 25-30% [16]. Our immunohistochemical analysis identified 67.0% and 51.5% of the tumors as p53-positive, EGFR-expressing tumors; only 31.0% had Ki-67 indices >20%. Although the overexpression of p53 documented in our study is similar to that observed by Smith et al. (1999) who found p53 positivity in 60-70% of oral cancers [17]. In another study, p53 positivity was detected in 60-70% cases of oral cancer. These findings are consistent with previous studies by Lo Muzio et al. (2005), where higher Ki-67 expression was associated with increased tumor aggressiveness [18]. It is similar to the findings of Park et al. (2022) but they found that higher Ki-67 expression was correlated with high tumor behavior [19]. These results are also concordant with some studies such as the one of Grandis and Tweardy (1993), which detected expression in 50-60% of oral cancers [20].In our survival analysis differentiation status, lymphovascular invasion, perineural invasion and Ki-67 index performed as significant risk factors. Poorly differentiated tumors showed the highest HR of 2.10 establishing poorly differentiation as bad prognostic indicator which has been reported earlier by Bryne et al. (1992) [21]. Our findings of the outstanding prognostic relevance of lymphovascular and perineural invasion are confirmed by those from Schliephake et al. (1995) have shown these factors to be closely correlated with reduced overall survival [22].The Ki-67 index was identified as a predictive marker also in our series highlighted the negative correlation between higher numbers of positive cells and survival probability, consistent with Alvarenga et al. (2000) [23]. P53 mutations and overexpression are

associated with worse survival in the two cohorts studied, which is an agreement with a meta-analysis by Szymańska et al. (2011), that stressed on p53 as a crucial tool for prognosis in head and neck cancers [24].

Conclusion

Our study in oral cancer supplied a comprehensive assessment on the histopathological and IHC characteristic of it for revealing their diagnostic, and prognostic functions. The data revealed a predominance of moderately differentiated tumors with lymphovascular and perineural invasion identifying the most common adverse prognostic features along with varying levels of Ki-67, p53 and EGFR expression which all correlate with tumour aggression and patient outcome.

The practical application of such discoveries into clinical practice offers great potential to augment oral cancer detection, to better match optimal treatment options for effective disease control and thus improving patient survival. Individual customized therapies targeting specific molecular pathways aiming to translate into better treatment efficacy and favorable toxicity profile in individual patient based on the histopathological as well as pathological profiles of each tumor represents a new era of targeted therapy.

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Disclaimer: Nil
Conflict of Interest: There is no conflict of interest.
Funding Disclosure: Nil

Authors Contribution

Concept & Design of Study: Momina khadija Abbasi
Drafting: Aiza saadia
Data Analysis: , Naila Abrar
Critical Review, Naila Abrar
Final Approval of version: Momina khadija
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