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Non-expression of Epithelial Membrane Antigen Protein is Diagnostic for Poorly Differentiated Oral Squamous Cell Carcinoma from Ibadan, Nigeria

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Abstract

Objective: The high morbidity rate and poor prognosis associated with oral squamous cell carcinoma (OSCC) in developing countries is attributed to late diagnosis. In this study, we sought to determine the value of age, gender, tumor location, immunohistochemical expression of epithelial membrane antigen (EMA) and cytokeratin (CkAE1/AE3) proteins in OSCC diagnosis.

Methods: Cases of OSCC at the Dental Clinic, University College Hospital, Ibadan, Nigeria between January 2004 and December 2015 were profiled for gender, age, tumor location and histologic class. The pattern of immunoreactivity was also determined. Descriptive statistics were used to analyze patients' demographic data which were then presented as frequencies and percentages, while Pearson's χ^2 test was used to assess the association between demographic variables and OSCC.

Results: Out of 1527 tumor cases accessioned 100 (6.5%) were OSCC with a male to female ratio of 1.4:1. Peak prevalence was observed at the 7th decade age group. The moderately differentiated class which was observed to be associated with patients in the 1st, 2nd and 4th decade age groups was the most preponderant constituting 65% of cases. The palate was the most commonly affected site. While the floor of the mouth, parotid, buccal mucosa and commissure were associated with the moderately differentiated class, the oropharynx was associated with the poorly differentiated class. Cytokeratin was expressed by all the histologic classes. However, only the poorly differentiated class failed to express EMA protein.

Conclusions: In conclusion gender ($P > 0.05$), age ($P > 0.05$) and tumor location ($P > 0.05$) do not seem to be critical diagnostic factors associated with OSCC. However, the EMA seems to be a valuable predictive diagnostic marker for the poorly differentiated oral squamous cell carcinoma.

Keywords: Epithelial proteins, Demography, Histology, Immunohistochemistry, Carcinoma

1. Introduction

Head and neck cancers are malignancies with a large array of histological types and sub-types occurring in the nasal cavities, paranasal sinuses, nasopharynx, hypopharynx, oropharynx, ear, scalp, oral cavity and salivary glands. About 70 - 90% of them originate in the epithelium; 47.8% are squamous cell carcinoma which constitutes 66.7% of all carcinomas [1,2]. Oral squamous cell carcinoma is thus a relatively common human cancer characterized by high morbidity, high mortality and a few therapeutic options outside of surgery, standard cytotoxic chemotherapy and radiation [3,4].

It is by far the most common type of cancer affecting the oral cavity, accounting for more than 90% of all oral cancers with a resultant poor prognosis for patients despite advancement in surgical techniques and adjuvant therapies [5,4] which depends on several clinicopathologic and demographic factors such as histologic class, degree of histologic differentiation of the tumor cells, clinical staging, primary site of tumor, age of patient, co-morbid conditions, neuro-vascular invasion, occupation of patients and late presentation at the clinic [1,6,4].

Although the most important risk factors are tobacco use and alcohol consumption, the disease is also linked to infection with high-risk types of human papilloma viruses [7,8,9]. However, for the purpose of this paper, we present our findings on the diagnostic value of gender, age, tumor location and pattern of EMA and CKAE1/AE3 expression in OSCC cases accessioned at the Dental Clinic, University College Hospital, Ibadan, Nigeria over a twelve-year period (2004-2015) and histologically classified by the Broder's grading system [10].

2. Materials and Methods

Study Area:

This was a hospital based study using archived formalin-fixedparaffin-embedded (FFPE) specimens at the Department of Oral Pathology, Dental Clinic, University College Hospital, Ibadan, Nigeria.

Selection Criteria:

The histology records of 1527 patients who were biopsied or had excision surgery and subsequent histopathology procedures, irrespective of final diagnosis between the year 2004 and 2015 were retrieved, reviewed and used for this study. Data for OSCC were thereafter extracted and analyzed for gender, age, tumor location and histology.

Immunohistochemistry of Tumour Samples:

Immunohistochemical analysis was conducted at the Breast Cancer Laboratory, Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The primary antibodies used were EMA and CkAE1/AE3 (DAKO A/S, Denmark) based on availability and specificity for squamous epithelial oral lesions [11]. Tissue blocks were cut into 5-µm thick sections, deparaffinized in xylene and thereafter rehydrated in graded ethanol. Antigen retrieval was done by heating the sections in a microwave oven in 10mmol/L citrate buffer, pH 6.0 for 10 minutes. Endogenous peroxidase activity was quenched in 3.0% hydrogen peroxide diluted in H_2O.

The sections were thereafter incubated with the primary antibodies for 60 minutes at room temperature and then overlaid with a biotinylated secondary antibody for antibody detection. Color was developed in diaminobenzidine solution (DAKO A/S Denmark). Counterstaining was done with Mayer's hematoxylin and the slides were dehydrated and mounted. Benign epithelial oral lesions were used as negative controls. All steps were accompanied with washes in phosphate buffered saline (PBS). Slides were thereafter viewed microscopically to determine immunostaining characteristics.

Statistical Analysis:

Data for gender, age, tumour location and histology were presented as frequencies and percentages using descriptive statistic. Pearson's χ^2 test was used to assess association between gender, age, tumor location and OSCC. For all analysis, $P \leqslant 0.05$ was considered significant.

3. Results

Table 1: Annual occurrence of OSCC

Year	Male			Female			Total	
	All cases	Sq.cell ca.	P-value	All cases	Sq. cell ca.	P-value	All cases	Sq.cell ca.
2004	40	1	0.371	51	5	0.482	91	6
2005	45	2		39	0		84	2
2006	38	2		63	6		101	8
2007	50	5		57	3		107	8
2008	57	1		60	1		117	2
2009	61	6		51	2		112	8
2010	75	6		80	3		155	9
2011	97	6		89	4		186	10
2012	69	6		87	5		156	11
2013	73	2		75	3		148	5
2014	56	9		69	9		125	18
2015	68	12		77	11		45	13
Total	729(47.7%)	58(3.8%)		798(52.3%)	42 (2.7%)		1527	100 (6.5%)

Key: Sq. cell ca (squamous cell carcinoma).

In the 12-year period covered by this study, a total of 1527 tumor cases were accessioned. Table 1 shows the comparative prevalence of patients with OSCC relative to the total number of cases accessioned. We observed that of the 1527 patients that underwent biopsy or excision surgery, 729 (47.7%) were males and 798 (52.3%) were females. Furthermore, of the 1527 patients, 100 (6.5%) were histologically diagnosed as OSCC of which 58 (3.8%) were males and 42 (2.7%) were females. It was also observed that at the inception of the study (January, 2004), 6 cases of OSCC were accessioned with a drastic increase to 13 cases when the study was terminated (December, 2015).

Table 2: OSCC distribution by histology

Year	Histologic class						
	Well diff. sq. cell ca.	P- value	Mod. diff. sq. cell ca.	P-value	Poorly diff. sq. cell ca.	P-value	Total
2004	5	0.426	1	0.098	0	0.206	6
2005	0		2		0		2
2006	5		3		0		8
2007	4		3		1		8
2008	1		1		0		2
2009	2		6		0		8
2010	4		5		0		9
2011	2		6		2		10
2012	2		8		1		11
2013	0		4		1		5
2014	2		16		0		18
2015	3		10		0		13
Total (%)	30 (30%)		65 (65%)		5 (5%)		100

Key: Well diff. sq. cell ca. (well differentiated squamous cell carcinoma); Mod. Diff. sq. cell ca. (moderately differentiated squamous cell carcinoma); Poorly diff. sq. cell ca. (poorly differentiated squamous cell carcinoma)

With respect to histological class, Table 2 shows that of the 100 cases of OSCC recorded during the study period, the well differentiated class accounted for 30%, moderately differentiated class accounted for 65% and poorly differentiated class accounted for 5%.

Table 3: OSCC distribution by age

Age group	Histologic class						
	Well diff. sq. cell ca.	P-value	Mod. diff. sq. cell ca.	P-value	Poorly diff. sq. cell ca.	P-value	Total
0-10	0	0.291	1	0.253	0	0.341	1
11-20	0		2		0		2
21-30	2		4		0		6
31-40	0		8		0		8
41-50	6		6		2		14
51-60	4		10		1		15
61-70	5		15		0		20
71-80	6		12		2		20
81-90	1		3		0		4
91-100	1		1		0		2
Not indicated	5		3		0		8
Total	30		65		5		100

Key: As in Table 2

The distribution of OSCC among the different age groups is shown in Table 3. It was observed that the moderately differentiated class occurred selectively in patients who are in their 1st, 2nd and 4th decades of life. Patient's with the well differentiated and poorly differentiated class exhibited two peak prevalence's at the 5th and 8th decades of life, while patients with the moderately differentiated class exhibited peak prevalence at the 7th decade of life. However, a general peak irrespective of histological class was observed at the 7th decade of life.

Table 4: OSCC distribution by tumour location

Tumor location	Histologic class						
	Well diff. sq. cell ca	P-value	Mod. diff. sq. cell ca.	P-value	Poorly diff. sq. cell ca.	P-value	Total
Floor of mouth	0	0.336	2	0.282	0	0.353	2
Parotid	0		1		0		1
Oropharynx	0		0		1		1
Antrum	0		1		2		3
Facial mass	2		1		0		3
Buccal mucosa	0		4		0		4
Lip	2		5		0		7
Tongue	8		5		0		13
Maxilla	2		13		2		17
Mandible	7		12		0		19
Palate	7		16		0		23
Commissure	0		1		0		1
Not indicated	2		4		0		6
Total	30		65		5		100

Key: As in Table 2.

The distribution of OSCC among the different anatomic sites is shown in Table 4. This study observed that the moderately differentiated class exhibited selective predilection for the floor of the mouth, parotid, buccal mucosa and commissure respectively, while the poorly differentiated class exhibited selective predilection for the oropharynx. It was also observed that the palate was the most commonly affected site. Other commonly affected sites were the mandible, maxilla and tongue in descending order of preponderance.

Table 5: Relative distribution of OSCC by histology and gender

Age	Histologic class/Age							
	Well diff. sq. cell ca.		Mod. diff. sq. cell ca.		Poorly diff. sq. cell ca.		Total	
	M	F (M:F)	M	F (M:F)	M	F (M:F)	M	F (M:F)
0-10	0	0	0	1	0	0	0	1
11-20	0	0	2	0	0	0	2	0
21-30	1	1	3	1	0	0	4	2
31-40	0	0	5	3	0	0	5	3
41-50	3	3	4	2	1	1	8	6
51-60	2	2	7	3	1	0	10	5
61-70	2	3	10	5	0	0	12	8
71-80	3	3	7	5	1	1	11	9
81-90	1	0	1	2	0	0	2	2
91-100	1	0	0	1	0	0	1	1
Not indicated	2	3	1	2	0	0	3	5
Sub-total	15	15 (1:1)	40	25 (1.6:1)	3	2 (1.5:1)	58	42 (1.4:1)
Total	30		65		5		100	

Key: As in Table 2

The relationship between histology, age and gender is shown in Table 5. Of the 100 OSCC patients, a male to female ratio of 1.4:1 was observed. Within the well differentiated class, a male to female ratio of 1:1 was observed. Furthermore, a male to female ratio of 1.6:1 was observed for the moderately differentiated class while a male to female ratio of 1.5:1 was observed for the poorly differentiated class.

Table 6: Pattern of CK (AE1/AE3) and EMA proteins expression in OSCC

Histologic class	EMA	CK
Well differentiated		
epithelium	+	+
connective tissue	+	+
Moderately differentiated		
epithelium	+	+
connective tissue	+	+
Poorly differentiated		
epithelium	-	+
connective tissue	-	+

EMA = Epithelial membrane antigen; CK = Cytokeratin (AE1/AE3)

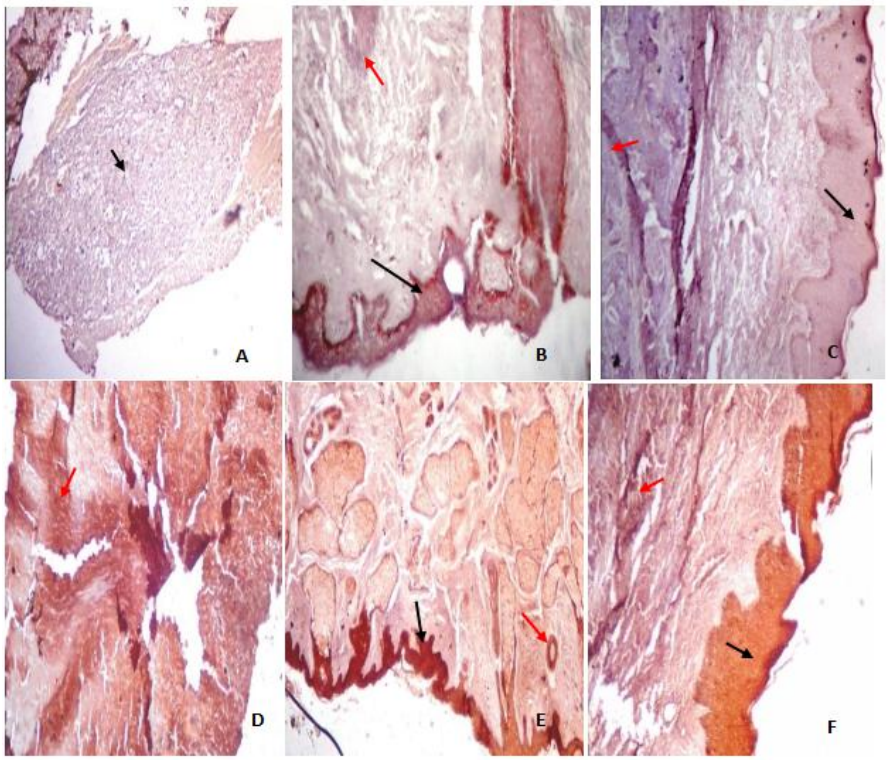

Figure 1: Photomicrographs of OSCC Tissues Stained with EMA and CkAE1/AE3 Antibodies

(A) Poorly differentiated squamous cell carcinoma showing negative EMA reaction (black arrow); (B) Well differentiated squamous cell carcinoma showing strong positive EMA reaction in the epithelium (black arrow) and weak positive reaction in the connective tissue stroma (red arrow); (C) Moderately differentiated squamous cell carcinoma showing strong positive EMA reaction in the epithelium (black arrow) and weak positive reaction in the connective tissue stroma (red arrow); (D) Poorly differentiated squamous cell carcinoma showing strong positive CkAE1/AE3 reaction (red arrow); (E) Well differentiated squamous cell carcinoma showing strong positive CkAE1/AE3 reaction in the epithelium (black arrow) and strong positive reaction in the epithelial islands and connective tissue stroma (red arrow); (F) Moderately differentiated squamous cell carcinoma showing strong positive CkAE1/AE3 reaction in the epithelium (black arrow) and strong positive reaction in the connective tissue stroma (red arrow).

The pattern of immunoreactivity of the different histological classes of OSCC to EMA and CkAE1/AE3 are shown in Table 6 and Figure 1 (A-F). A positive immunoreactivity of the well differentiated, moderately differentiated and poorly differentiated classes to CkAE1/AE3 was observed. However, with respect to EMA, while the well differentiated and moderately differentiated classes exhibited positive reactivity, the poorly differentiated class exhibited a negative immunoreactivity.

4. Discussion

During the period covered by this study, it was observed that more females (798) than males (729) (with a male to female ratio of 1:1.1) presented with various types of benign and malignant neoplasia of which the number of males diagnosed as OSCC exceeded that of females with a male to female ratio of 1.4:1. The preponderance of male patients compared to female patients was consistent with reports from other parts of the world [12, 13]. However, the gap in the occurrence of OSCC in males and females in our study seem to be closer than in previous studies from other parts of the world. Since tobacco smoking and chewing, alcohol consumption and betel quid chewing have been well documented as major risk factors for OSCC [14]; increased indulgence in these habits on the part of the female gender in our study resulting from Western world influence may possibly explain the trend observed in this study.

We observed that the occurrence of the lesion from the year of inception of the study increased from 6 cases to 13 cases at the terminal year of the study. This trend is consistent with several similar studies from different parts of the world [14, 15, 16, 12]. It was claimed in a study that the availability of facilities for multimodality management of head and neck cancer patients was responsible for the higher number of patients seen at the University College Hospital, Ibadan, Nigeria than in other centers in Nigeria [1]. This, together with increased level of awareness of dental health, could be adduced for the increased occurrence documented in this study.

This present study shows that even though the moderately differentiated class was the commonest, followed by the well differentiated and the poorly differentiated classes in that order. The difference was however not significant ($P > 0.05$). Furthermore, it was observed that for each histologic class, there was a male preponderance, the difference between male and female was not significant ($P > 0.05$). This agrees with reports from Zimbabwe and Kenya where it was documented that the well differentiated type was the commonest although with a similar higher male preponderance [15, 12, 13]. The poorly differentiated type in both reports was nevertheless the least common with a higher male preponderance which agrees with our observations.

We also show in this study that OSCC is particularly associated with the middle-aged and the elderly especially those in their 5th, 6th, 7th and 8th decade of life regardless of histologic class. This is consistent with reports from other parts of the world [15, 14, 16, 17]. However, in this study, fairly high figures for young adults in their 3rd and 4th decades of life (21-40 year age group) were observed, which agrees with reports from Northern Thailand and Nigeria [14, 17].

This was linked to exposure to socio-cultural risk factors such as tobacco use and alcohol consumption at a very young age or other obscure risk factors such as human papilloma virus (HPV) [18] which may play a crucial role in the development of malignancy in this group. Poor dental hygiene at a young age may also account for this unsavory development.

Our data show that OSCC, regardless of histologic class, affects all parts of the oral cavity with the highest predilection for the palate followed by the mandible, maxilla and the tongue in that order. However, mandibular predilection was reported in a Zimbabwean population, laryngeal predilection in an Indian population and predilection for the tongue was reported in a Thai population [15, 16, 14]. This seems to connote the influence of geographic distribution which is directly proportional to level of poverty and in most cases determines the nutritional statuses of individuals and the concomitant ability of the body to fight off cancer development [19, 20].

However, with regard to the relationship between histologic differentiation and anatomic site, there seem not to be a general systematic pattern of relationship even though from our study, the moderately differentiated class exclusively affected the floor of the floor of the mouth, parotid, buccal mucosa and commissure, while, the poorly differentiated class affected only the oropharynx. In a different study, it was reported that the well differentiated class affected the buccal mucosa, while the palate, tongue and the floor of the mouth affected the poorly differentiated class [21].

The cytokeratins are known to be overexpressed in OSCC as compared to normal oral mucosa and epithelial membrane antigen show positive staining of cytoplasmic membranes [22]. In our study, all the histologic classes exhibited strong positive staining for cytokeratin (CkAE1/AE3) throughout the epithelium, islands of malignant epithelial and connective tissue stroma. Similar report of strong positive cytokeratin staining of primary OSCC tumors throughout the epithelium and malignant epithelial islands had been observed in a previous study [23]. EMA had been reported to have 100% specificity and 67% accuracy when used against squamous cell carcinoma of the skin, while a positive expression has also been reported in the well differentiated class [24, 25]. We however report in our study that while the poorly differentiated class failed to express EMA, the other classes exhibited strong positive staining throughout the epithelium but weak positive staining in the connective tissue stroma.

5. Conclusions

While gender, age and tumour location on their own lacks diagnostic value, other emerging facts including the potential of EMA as a differential marker suggests that this could be used for preliminary diagnostic predictions.

Competing Interests

The authors declare that there are no competing interests.

Author's Contributions

Research conceptualization and design; collection, assembly, analysis and interpretation of raw data; statistical analysis and writing of article was done by KCO. Critical revision and final approval of the article was done by KCO, BOE, AOA and CIA.

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Disclosures

Although relatively large biopsy samples were accessioned; oral squamous cell carcinoma samples was limited in number.

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