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CASE REPORT:

CD44+ Cells Influence Oral Squamous Cell Carcinomas in Two Comparative Age Groups

Influencia de Células CD44+ en carcinomas orales de células escamosas en dos grupos comparativos de edad

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ABSTRACT: Head and neck cancer (HNC) is the sixth most common malignancy worldwide, with high morbidity and mortality rates mainly due to delayed diagnosis. Cancer stem cells (CSCs) are involved in HNC progression and treatment response, carrying important therapeutic and prognostic implications. CD44, a transmembrane glycoprotein linked to cartilage, has been proposed as a CSC biomarker and is associated with cancer progression and poor survival. CSCs may also play a role in oral carcinogenesis among young individuals. This retrospective cross-sectional study aimed to identify CD44+ cells in oral squamous cell carcinoma (OSCC) across two age groups. Thirty-six OSCC cases from a teaching service for oral histopathological diagnosis were identified between January 2005 and December 2019. Cases were divided into two groups: young group (17 patients aged ≤ 40 years) and adult/elderly group (19 patients aged ≥ 41 years). Histological slides were obtained and stained using the hematoxylin and eosin technique and the immunohistochemical technique with anti-CD44 antibodies. In the young group, males predominated (70%) with the gingiva as the main site (9 cases), while the adult/elderly group had more females (52.5%), with eight cases on the tongue. Both groups primarily showed well-differentiated OSCC. In both groups, islands of CD44+ cells, characterized by large, basophilic nuclei and scant cytoplasm, were identified in the stroma. No significant association was found between CD44 immunoreactivity and age, sex, location, or differentiation degree ($P > 0.05$). The identification of CD44+ could contribute to improving the diagnosis and prognosis of oral cancer, and eventually offer targeted therapies.

KEYWORDS: Cancer stem cells; CD44+ cells; Oral cancer; Oral squamous cell carcinoma; Invasive tumor front.

RESUMEN: El cáncer de cabeza y cuello, sexta neoplasia maligna más frecuente mundialmente, presenta tasas elevadas de morbi-mortalidad, asociadas al retraso en el diagnóstico. Células madre cancerosas (CSCs) participan en progresión tumoral y en respuesta al tratamiento con implicaciones terapéuticas y pronósticas. CD44, una glicoproteína transmembrana ligada al cartílago, es un biomarcador de CSCs asociado a progresión tumoral y disminución en sobrevida. CSCs están involucradas en carcinogénesis oral de jóvenes. El objetivo de este estudio transversal retrospectivo fue identificar células $CD44^+$ en casos de carcinoma oral de células escamosas (CCEO) en dos grupos de edad. Se identificaron 36 casos de OSCC en un servicio de enseñanza de diagnóstico histopatológico oral entre enero de 2005 y diciembre de 2019. Los casos se dividieron en: grupo joven (17 pacientes; ≤ 40 años) y grupo adulto/anciano (19 pacientes; ≥ 41 años). Se obtuvieron cortes histológicos para teñirse con hematoxilina-eosina y con inmunohistoquímica utilizando anticuerpo anti-CD44. En el grupo joven predominaron los varones (70%), siendo la encía la localización principal (9 casos). El grupo adultos/ancianos había más mujeres (52.5%), con 8 casos en la lengua. En ambos grupos predominaron COCEs bien diferenciado. En ambos grupos se identificaron en el estroma islas de células $CD44^+$, caracterizadas por núcleos grandes basófilos con escaso citoplasma. No se encontró asociación significativa entre inmunoreactividad y edad, género, localización o grado de diferenciación ($P > 0.05$). La identificación de $CD44^+$ puede contribuir a mejorar el diagnóstico y pronóstico del cáncer oral y eventualmente ser un blanco de terapia.

PALABRAS CLAVE: Células madre cancerosas; Células $CD44^+$; Cáncer oral; Carcinoma oral de células escamosas; Frente de invasión tumoral.

INTRODUCTION

Head and neck cancer (HNC) is the sixth most common cancer worldwide, with squamous cell carcinoma being the most prevalent (1). Oral squamous cell carcinoma (OSCC) is linked to high morbidity and mortality and significantly affects patients' quality of life. The poor prognosis and high mortality rate have been associated with delayed diagnosis, even though its anatomical location allows for easy visual identification (2, 3). Conversely, a concerning increase in OSCC cases among patients under 40-45 years old has been observed in recent decades (4-6), showing rapid progression and treatment failure (5-7). The causes of OSCC in young adults include early exposure to carcinogens, genetic predisposition, human papillomavirus infection, and the presence of cancer stem cells (CSCs) (4-6). The hypothesis that malignant tumors, including OSCC, originate from CSCs is widely accepted (8). According to the CSCs model, stem cells in the basal layer become malignant. A common way to isolate CSCs is by sorting them based on surface marker expression (1).

CSCs are part of the diverse cellular population found in malignant tumors. CSCs have the ability to self-renew, differentiate, and exhibit a high rate of proliferation (9). They are resistant to chemotherapy and radiation therapy, remain quiescent, and can evade immune responses, which can lead to the development of distant metastasis even after treatment (10). CSCs play a vital role in OSCC progression (11, 12) and are associated with oral cancers that are resistant to treatment. These characteristics are observed regardless of the tumor's histological type. Therefore, CSCs are promising targets for anticancer therapy in oral

cancer (10). Identifying CSCs is crucial, with the most promising biomarkers including ALDH, CD133, Cyclin D1, VEGF-A, GD15, SOX, and, of special importance to the present study, CD44 (13).

CD44, a transmembrane glycoprotein linked to cartilage in mammalian cells, has been suggested as a CSCs biomarker. CD44 is involved in cell-cell interactions, adhesion, migration (14), motility, and differentiation (11). It is overexpressed in CSCs, including both the standard and spliced variant isoforms (CD44v). These isoforms interact with ligands such as hyaluronic acid, osteopontin, and matrix metalloproteinases, which promote various cancer-related signals (15, 16). Due to its involvement in immune functions such as lymphocyte activation, recirculation, and homing, CD44 is linked to immune responses in several cancer types, potentially facilitating tumor metastasis (17). Although the prognostic significance of CSCs in OSCC remains debated, high CD44 expression in cancer cells has been linked to disease progression, including local recurrence, regional or distant metastasis, perineural invasion, poor survival, and unfavorable outcomes, making it a potential risk factor and indicator of poor prognosis in most cancers, including HNSC (13l). CD44 is associated with resistance to radiotherapy and chemotherapy; therefore, it has been proposed as a target for cancer therapy (18). Although it remains unclear whether high or low CD44 expression correlates with worse clinicopathological features and survival rates (19), CD44 may serve as a promising biomarker for therapeutic targets in various types of cancer, including metastatic cancers. Potential treatment strategies include CD44 neutralizing antibodies, pharmacological inhibitors, peptide mimetics, HA oligomers, and aptamers, which are currently in preclinical and clinical development (20).

Scientific information on the presence and potential role of CSCs in OSCC among young patients needs to be more comprehensive and conclusive (14,15,21). Therefore, the main goal of this study was to identify the presence of CSCs using anti-CD44 positivity in both young and adult/elderly OSCC cases from an oral histopathological teaching service, based on the hypothesis that OSCC in young patients exhibits higher immunoreactivity to anti-CD44 antibody than in adults/elderly patients, and to compare their clinical and histopathological features.

MATERIALS AND METHODS

This retrospective cross-sectional study was conducted at the Histopathological Diagnostic Service of the Dental School, National Autonomous University of Mexico, Mexico City, Mexico. From January 2014 to December 2019, all cases of OSCC diagnosed in subjects younger than 40 years (young group) at the time of diagnosis were identified and isolated. For comparison purposes, from the same archive and frame time, all cases diagnosed with OCSC in patients older than 40 years (adult/elderly group) were identified and isolated. To achieve the same number of cases in the young group, adult/elderly cases were randomly selected using a random number table, considering a 10% loss.

Although the criteria for defining the “young” and "old” groups are not always consistent, most cancer studies use 40 years as the cutoff age (16,22-24). Therefore, it is recommended that studies adopt 40 years as a standard age cutoff to promote more uniform data, as suggested by Mahajan *et al*. (25). In the current study, 40 years was used as the dividing line: the young group included patients aged 40 years or younger, while the adult/elderly group included patients aged 41 years or older. Two experts in oral pathology (FGVC/LAGC) reviewed all cases to confirm the diagnosis and classified them according to the Broders' criteria (26). Patients diagnosed with carcinoma in situ or presenting a diagnostic conflict were excluded, as were those with incomplete clinical or demographic data. Cases with insufficient

biological material embedded in paraffin to obtain histological slides for staining with the Hmatoxiline and Eosine technique or to be processed with immunohistochemistry were eliminated. All patients included in this study were codified to guarantee patient anonymity.

Additional histological slides were subjected to conventional immunohistochemistry using anti-CD44 monoclonal antibody (Santa Cruz Biotechnology, Inc. Dallas, Texas, USA, Sc-53298 clone p2a1; dilution 1:100). Briefly, after deparaffinization, hydration, microwave antigen retrieval (citrate buffer, ph6; ImmunoDNA Retriever Citrate 20X, BioSB, Santa Barbara Ca, USA) and endogenous peroxidase blocking, histological slides were incubated overnight at 4°C in a humid chamber. Staining was performed using the Mouse/Rabbit ImmunoDetector System BIO-SB (BioSB 003 HL, Santa Barbara, CA, USA) following the manufacturer's instructions, using the chromogen diaminobenzidine (ImmunoDNA Retriever Citrate 20X, BioSB, Santa Barbara Ca, USA) as the substrate. Finally, histological slides were counterstained with Mayer's hematoxylin (Sigma-Aldrich, Saint Louis, Mo, USA). Tonsillar tissue was used as a positive control and the primary antibody was omitted as a negative control. The adjacent oral mucosa was used as an internal control. CD44 immunoreactivity was scored as follows: quantitative scores from 0 to 5 were assigned to 0%, 1% to 10%, 11% to 30%, 31% to 50%, 51% to 80%, and 81% to 100% of the tumor cells that were positive. Antibody immunostaining intensity was graded on a scale of 0 to 3 (0=negative, 1=weak, 2=moderate, and 3=strong). Finally, the raw data were converted to an immunoreactivity score by multiplying the staining amount and intensity scores. Thus, the minimum possible score was 0, and the maximum score was 15. Scores ≥ 7 were considered to indicate high reactivity, whereas scores ≤6 were considered weak. An ex-professo database was constructed using the obtained data. To establish a possible association between demographic, clinical, and histopathological characteristics, bivariate analysis was performed using Fisher's exact test (p 0.05, 95% CI) (EPIINfo, CDC-Atlanta, Go, USA). The study was conducted following the Declaration of Helsinki, and according to the Reglamento de la Ley General de Salud en Materia de Investigación para la Salud, Título Dos, De los Aspectos Éticos de la Investigación en Seres Humanos, artículo 17, sección I (Mexican General Health Law , article 17, fraction I) (27); this research is classified as non-risk research. Informed consent was obtained from all patients.

RESULTS

YOUNG GROUP

The young group consisted of 17 patients: 12 (70.6 %) males and five (29.4%) females. The average age was 29.05 ± 6.9. Regarding location, the gingiva was the most frequent site, with nine cases (52.9%), followed by the tongue, buccal mucosa, and palate, with two cases (11.7%) each. Regarding differentiation degree, 14 (82.3%) cases were classified as “well-differentiated.” Histopathologically, neoplastic epithelial cells showed nuclear pleomorphism, cellular pleomorphism, individual and grouped keratinization, and abnormal mitosis. A tumor invasive front was seen near the capillary blood vessels. Most cases had a strong inflammatory infiltrate in the stroma. In three cases, a higher number of mitoses per optical field was observed, and in tumor invasion, neoplastic cells infiltrating blood vessels and exhibiting perineural invasion were noted. The last three cases were classified as moderately differentiated. All these criteria were evaluated in two random fields by each pathologist. All cases exhibited anti-CD44 immunoreactivity primarily in a membranous staining pattern (Figure 1. A-D). However, only a

few cases showed cytoplasmic immunoreactivity (Figure 2. A-D). Regarding the immunoreactivity score, three cases showed high immunoreactivity, whereas 14 (82.3%) cases showed low immunoreactivity (Figure 2. B).

ADULT/ELDERLY GROUP

The adult/elderly group consisted of 19 patients: 9 (47.4%) were male, and 10 (52.6%) were female. The average age was 72.7 years (SD ± 11.5). The most common location was the lateral border of the tongue (8 cases, 42.1%), followed by the gingiva (5 cases, 26.3%). In terms of differentiation degree, 13 cases (68.4%) were classified as "well-differentiated," while 6 (31.6%) were "moderately differentiated." In the study group, 13 patients exhibited cellular and nuclear pleomorphism, increased mitosis, abnormal mitosis, and keratinization, both individually and in clusters. The invasive tumor front pattern was near blood vessels, with strong inflammatory infiltrate, and was classified as "well-differentiated." In contrast, 6 cases showed increased mitoses and had invasive tumor fronts closely associated with blood vessels, with perineural invasion and mild stromal inflammatory infiltrate, classifying them as "moderately differentiated."

Bivariate analysis did not show a statistically significant association between age and localization (Table 1). Similar to that in the young group, all cases in the adult/elderly group exhibited a membranous pattern of anti-CD44 immunoreactivity. Regarding the immunoreactivity score, seven cases showed high immunoreactivity, whereas 12 (63.1%) cases showed low immunoreactivity (Figure 2. B).

Statistical analysis did not reveal an association between CD44 immunoreactivity and age group, sex, location, or degree of differentiation (Table 2). Notably, in 12 cases, five patients from the young group (Figure 3. A), and seven in the adult/elderly group (Figure 3. B), islands or nests of CD44+ neoplastic epithelial cells were identified. Many of these islands comprised fewer than five cancer cells in the stroma adjacent to the invasive tumor front. These cells were small, with intense basophilic, large oval nuclei and scant cytoplasm. These islets were typically located near the blood vessels (Figure 3. C-D) and was associated with inflammatory cells (Figure 3. E-F). Their morphology and strong CD44+ immunoreactivity suggested the presence of CSCs. Additionally, we observed single long and splinde-shaped CD44+ neoplastic.

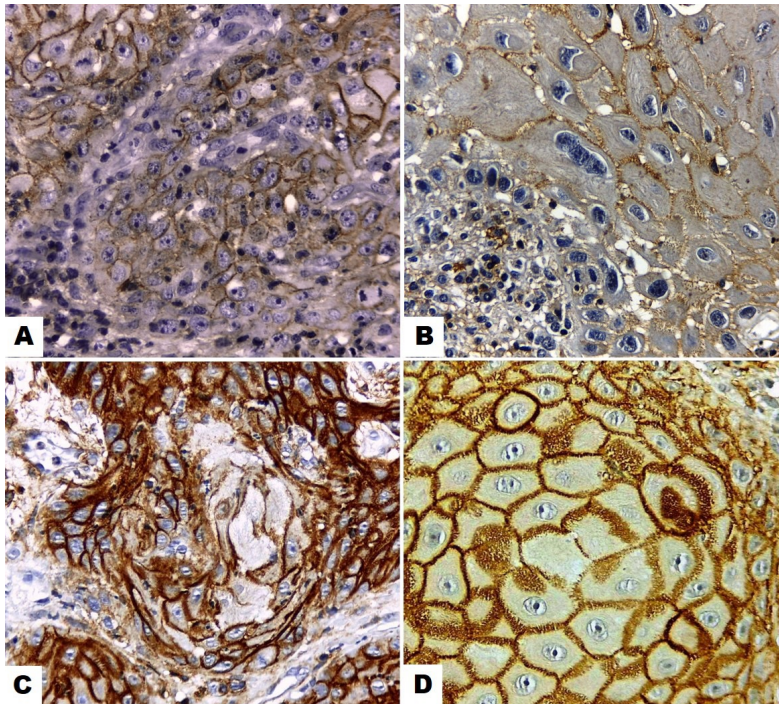

Figure 1. Representative cases of membranous CD44 immunoexpression in OSCC in two different age groups. A. Low immunoreactivity score (young group). Thirty-four-year-old male; OSCC on the lateral border of the tongue (X400). B. Low immunoreactivity score (adult/elderly group). Fifty-four-year-old female; OSCC on the lateral border of the tongue (X400). C. High immunoreactivity score (young group). Nineteen-year-old male; OSCC on the palate (X400). D. High immunoreactivity score (adult/elderly group). Seventy-eight-year-old female; OSCC on the floor of the mouth (X400).

Table 1. Demographical data, topographical distribution and differentiation degree of OSCC in two different age groups.

		Young (n=17)	Elderly (n=19)	p
Gender	Female	5 (29.4%)	10 (52.5%)	
	Male	12 (70.6%)	9 (47.3%)	NS
Topographical distribution	Gum	9 (52.9%)	5 (26.3%)	
	Tongue	2 (11.7%)	8 (42.1%)	
	Buccal mucosa	2 (11.7%)	2 (10.5%)	NS
	Palate	2 (11.7%)	2 (10.5%)	
	Floor of the mouth	1 (5.8%)	1 (5.2%)	
	Intraoseous	1 (5.8%)	1 (5.2%)	
Differentiation degree	Well	14 (82.3%)	13 (68.4%)	NS
	Moderately	1 (5.8%)	6 (31.5%)	
	Poorly	2 (11.7%)	0	

n= number of cases; % = percentage; NS: no statistically significant; Fisher's exact.

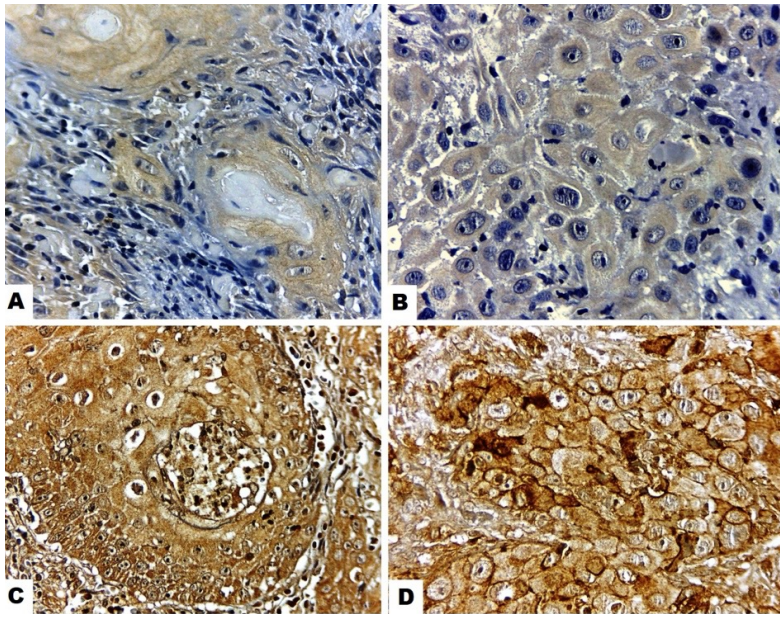

Figure 2. Representative cases of cytoplasmic CD44 immunoexpression in OSCC in two different age groups. A. Low immunoreactivity score (young group). Twenty-six-year-old female with OSCC on the palate (X400). B. Low immunoreactivity score (adult/elderly group). Seventy-three-year-old female with OSCC on alveolar ridge mucosa (X400). C. High immunoreactivity score (young group). CD44 expression is seen in the cytoplasm, nucleus, and stroma of the tumor cells. Twenty-five-year-old female with OSCC on the gingiva (X400). D. High immunoreactivity score (adult/elderly group). Eighty-one-year-old female with OSCC on the lateral border of the tongue (X400).

Table 2. Anti-CD44 Immunoreactivity score of OSCC in two different age groups.

		Young (n=17)		Elderly (n=19)		p
		Weak (n=14)	High (n=3)	Weak (n=12)	High (n=7)	
Gender	Female	5 (35.7%)	0	5 (41.6%)	5 (71.4%)	NS
	Male	9 (64.2%)	3 (100%)	7 (58.3%)	2 (28.5%)	
Topographical distribution	Gum	8 (57.1%)	1 (33.3%)	3 (25%)	2 (28.6%)	
	Tongue	1 (7.1%)	1 (33.3%)	4 (33.3%)	4 (57.1%)	
	Buccal mucosa	2 (14.3%)	0	2 (16.7%)	0	NS
	Palate	1 (7.1%)	1 (33.3%)	1 (8.3%)	1 (14.3%)	
	Floor of the mouth	1 (7.1%)	0	1 (8.3%)	0	
	Intraosseous	1 (7.1%)	0	1 (8.3%)	0	
Differentiation degree	Well	11 (78.6%)	3 (100%)	10 (83.3%)	3 (42.9%)	NS
	Moderately	1 (7.1%)	0	2 (16.7%)	4 (57.1%)	
	Poorly	2 (14.3%)	0	0	0	

n= number of cases; % = percentage; NS: no statistically significant, Fisher's exact.

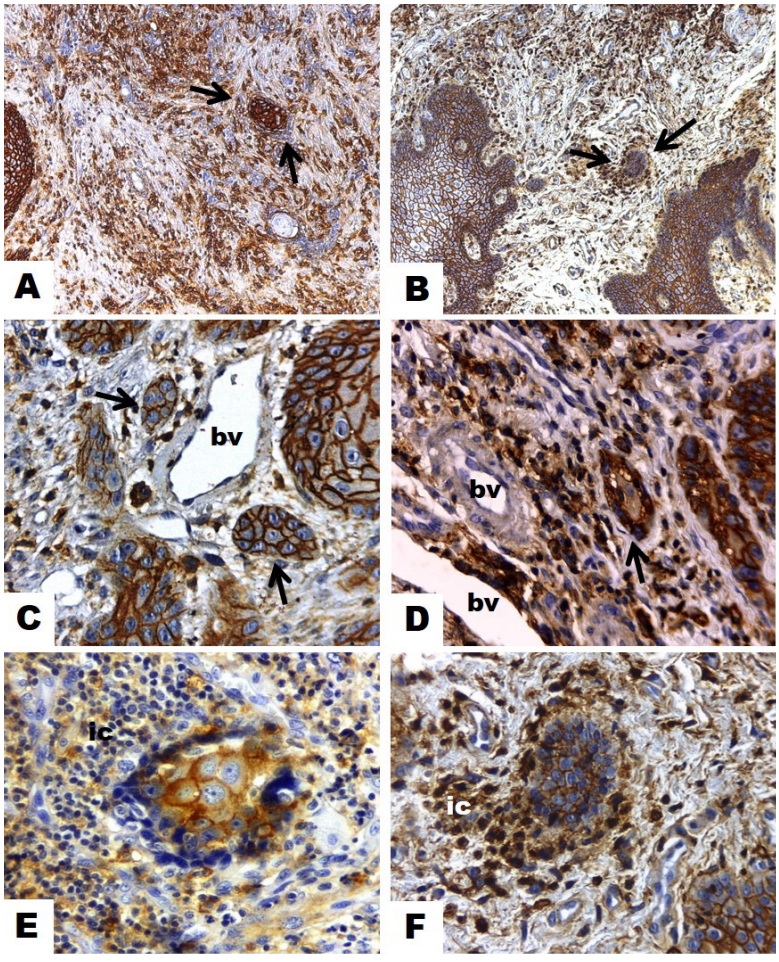

Figure 3. Representative cases of CD44+ cells at the invasive tumor front of OSCC in two different age groups. A. Island of CD44+ neoplastic epithelial cells (arrows) immersed in the stroma of connective tissue (young group) and single cancer cells or clusters with less than five cancer cells at the invasive front were positive for CD44. Thirty-four-years-old males with OSCC on the buccal mucosa (X100), B. Island of CD44+ neoplastic epithelial cells (arrows) immersed in the connective tissue stroma. In the present case, the staining is exclusively membranous (adult/elderly group)—fifty-nine-years-old male with OSCC on the lateral border of the tongue (X100). C. Island of CD44+ neoplastic epithelial cells (arrows) near the blood vessel (bv); the morphological characteristics of the area are suggestive of the process of epithelial-mesenchymal transition. Notice their positive anti-CD44 membranous immunoreactivity (young group). Nineteen-year-old male with OSCC on the palate (X200). D. Island of CD44+ neoplastic epithelial cells (arrows) near the blood vessel (bv), showing positive anti-CD44 membranous immunoreactivity (adult/elderly group). Fifty-two-year-old male with OSCC on the lateral border of the tongue (X200). E. Island of CD44+ neoplastic epithelial cells. Observe their small size, scarce cytoplasm, and large basophilic nuclei. The islands of CD44+ cells are surrounded by inflammatory cells (ic) (young group). Twenty-five-year-old male; OSCC on the lateral border of the tongue (X400). F. Island of CD44+ neoplastic epithelial cells. Note their close relationship to inflammatory cells (ic) (adult/elderly group). Fifty-nine-year-old male; OSCC on the lateral border of the tongue (X400).

DISCUSSION

The present study aimed to identify CD44 + neoplastic cells in OSCC patients from two age groups. The mean age for the young group was 29.05 years, similar to what was reported in Thais, 33.5 years (16), 38.3 years in Brazilians (19), 34.2 years in Germans (22), 34 years in Chinese (23), and 29 years in Pakistanis (24). These diverse populations indicate that OSCC development in young people is independent of ethnicity. Concerning sex, a strong link between male patients aged ≥ 40 years and OSCC is well documented (28). However, our results did not show this link; the male-to-female ratio in the adult/older age group was 1:1.1. Our research group previously reported an increasing trend of OSCC cases among Mexican women (29). In contrast, a slight male predominance has been observed in young OSCC patients (24, 30), with a male-to-female ratio of 1.8:1 (16), which matches our finding of 2:1.1. The most common location of OSCC is the lateral border of the tongue (23, 24, 29-31), consistent with the adult/elderly group. However, data on preferred sites in young patients is inconclusive. Some suggest that the tongue and floor of the mouth are the most frequent sites (5, 7, 16, 19, 22-24), whereas in our study, the gingiva was the most common site. We lack an explanation for these differences, and causality cannot be confirmed due to the small sample size. OSCC in young individuals exhibits aggressive biological behavior with poor prognosis (5-7, 32). Therefore, it would be expected that in most cases young people involve undifferentiated histologic types; however, the link between differentiation grade and clinical progression remains unproven (19, 22). Interestingly, most OSCC cases reported, including in this study, in young patients are well- differentiated tumors (16, 23, 28, 33). A recent study indicated that increasing treatment intensity in younger OSCC patients could lead to a significant rise in morbidity, treatment costs, and the burden on treatment centers without a clear oncological benefit. Furthermore, this study shows that younger and older patients with SCC of the tongue have comparable LRC, DMFS, and OS (25).

Our study contributes to the understanding of cancer stem cells (CSCs) in oral squamous cell carcinoma (OSCC) and has the potential to shape future research in this area. CD44+ cells are isolated from samples of HNSCC from many sources, and high CD44 expression is a marker of CSCs. Some observations have raised questions about how CD44 can function as a CSC marker in HNSCC. For example, the number of CD44+ cells stained histochemically is too large and too widely distributed to be considered stem cells (11). We observed a very broad pattern of immunostaining in the epithelial tumor cells (Figure 1. C, D). CSC subpopulations exist as at least two distinct phenotypes: 1) CSCs with an epithelial phenotype, which are proliferative and non-migratory, with high expression levels of both CD44 and ESA; and 2) CSCs with a mesenchymal phenotype, which are migratory and have high CD44 expression but low ESA expression (11). In this way, we could suggest that CD44+ cells identified by us in the tumoral stroma might be CSCs with a mesenchymal phenotype.

Two subpopulations of CSCs in OSCC have been suggested: epithelial CSCs, responsible for chemotherapy and radiotherapy resistance, and large mesenchymal CSCs, implicated in metastasis through epithelial-mesenchymal transition (EMT). The spindle-shaped morphology of single CD44+ cells near the invasive tumor front and blood vessels identified in our study could be a key indicator of EMT. The identified putative CSCs in the transition zone without tumor contact could be CSCs before EMT, representing an essential step in the progression of metastasis. CD44 overexpression, which correlates with high tumor proliferation activity, small cell nests, and low OSCC differentiation (34, 35), has significant implications for understanding CSCs in OSCC and could inspire and guide future research in this area. This

study demonstrated membranous and cytoplasmatic overexpression of the CD44 in OSCC cases, consistent with previous reports (14, 15, 34, 35). Cytoplasmic staining with CD44 antibody suggested that the extracellular domain of the membrane-anchored protein CD44 was released from the cell surface as a soluble protein through a regulated proteolytic mechanism. However, the putative mechanism remains to be elucidated. The clinical significance of this possible mechanism must be interpreted by other research groups (12,14,34). CD44 undergoes alternative splicing, resulting in the production of different isoforms, including CD44s, CD44v3, and CD44v6. The antibody used in this study targeted the standard isoform and showed strong expression in well-differentiated carcinomas in the elderly group. The expression of CD44s and CD44v6 varied according to the degree of differentiation (36). Moderately differentiated carcinomas showed a slight increase, while poorly differentiated carcinomas exhibited decreased CD44 expression (34, 35). Differences in the expression and staining patterns could be attributed to the use of different antibodies and dilutions. In the tumor stroma, CSCs reside within a microenvironment known as the "niche," which comprises stromal cells, inflammatory cells, vasculature, soluble factors, and hyaluronan (8). Hyaluronan influences the stemness properties of CSCs by regulating proliferative signals for renewal and self-renewal (37). In the present study, $CD44^+$ cells closely associated with blood vessels and surrounded by inflammatory cells were observed, suggesting local recurrence, increased invasiveness, and metastasis (38). Further research using an experimental model is needed to clarify the contribution of these CSCs to the proliferation and invasion of OSCC.

The present study contributes to understanding the potential role of CD44 in OSCC. It has been suggested that the expression of CD44 in CSCs is essential for their function. Overexpression of CD44 has been related to tumor growth and survival, as well as resistance to apoptosis and therapy, specifically chemotherapy. Hyaluronan appears to play a significant role, as the interaction between hyaluronan and CD44 increases resistance to cisplatin, methotrexate, and doxorubicin. Knowledge of CD44 function on CSCs suggests that CD44 protein could be a promising therapeutic target. In vivo experiments and mouse models suggest that reduced CD44 levels decrease population expansion, lower stem cell colony formation, reduce tumor sphere formation, and promote cell differentiation (11; 39).

To date, prediction of OSCC behavior based on clinical features or histopathological examinations has been limited. It has been suggested that young patients with OSCC have worse prognosis. Thus, our hypothesis proposes that OSCC originating in young patients should show higher immunostaining of CD44 than OSCC cases from adult or elderly patients. Our results did not reveal any differences in immunostaining between the two study groups. The prognostic significance of the CD44 expression remains unclear and not fully understood. Immunohistochemical analyses of OSCC have shown conflicting results, with reports of both increased and decreased CD44 expression. Increased CD44 expression has been linked to advanced T stage, regional and distant metastasis, lymph node involvement, perineural invasion, radiation failure, and shorter disease-free survival (11; 39). Conversely, decreased CD44v6 expression has been observed in cases of higher dysplasia, poorly differentiated OSCC, or primary tumors with cervical lymph node metastases. Other studies have reported unchanged CD44 expression patterns in malignant tissues compared with healthy tissues (1, 11). Most studies have used immunohistochemistry to identify CD44 expression, and differences in technique and interpretation may have contributed to these conflicting findings (39).

This study has some limitations. First, the cross-sectional methodological design precludes inferences of causality. Second, the study was conducted using data from an archive of a teaching oral histopathological diagnostic service. As a result, the cases included came from two sources: patients attending the same institution and surgical samples referred by public and private health services for diagnosis. The oral histopathological diagnostic service at the Dental School of the National Autonomous University of Mexico is a national reference center for oral pathology. One major limitation is that the referrer only provided the patient's clinical information, which left out important data such as predisposing factors or OSCC risk factors; consequently, potential confounding factors cannot be ruled out. The results should be confirmed using larger case series studies. Another limitation was that most samples were from incisional biopsies, so tumor cell heterogeneity cannot be ruled out. Additionally, CD44 is expressed in the upper layers of normal oral epithelia (18). Therefore, confirming the presence of CSCs would have been helpful through double immunohistochemical labeling with ALDH1. Technical limitations prevented their use in this study.

We observed no difference in CD44 expression in cancer cells based on age at diagnosis, indicating that CSCs remain viable throughout life and can contribute to tumor development at any age. The presence of CSCs in young oral cancer patients raises questions regarding the factors that activate these cells in young individuals with minimal exposure to carcinogens.

CONCLUSIONS

In oral squamous cell carcinoma, immunoreaction with anti-CD44 antibody showed similar results in both young and adult patients. Demonstrating the presence of CD44 in both age groups helps expand our understanding of the potential role of the CD44 protein in tumor biology, which could eventually contribute to the development of oncological therapies. Furthermore, studying the role of the CD44 protein in supporting tumor growth and in factors closely related to patient survival in OSCC across populations of different demographics will provide vital knowledge for designing specifically targeted anti-tumor therapies. This could be a crucial step toward developing personalized cancer treatments.

AUTHOR CONTRIBUTION STATEMENT: Conceptualization and design: L.A.G.C. and F.G.V.S.; Literature review: F.G.V.S. and D.I.R.R.; Methodology and validation: L.A.G.C., P.G.A. and F.G.V.S.; Formal analysis: L.A.G.C., P.G.A. and F.G.V.S.; Investigation and data collection: L.A.G.C., P.G.A. and F.G.V.S.; Resources: L.A.G.C. and P.G.A.; Data analysis and interpretation: L.A.G.C., P.G.A. and F.G.V.S.; Writing-original draft preparation: F.G.V.S. and L.A.G.C.; Writing-review & editing: L.A.G.C., P.G.A., F.G.V.S. and D.I.R.R.; Supervision: L.A.G.C. and P.G.A.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest.

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