Original Article

Expression of aldehyde dehydrogenase 1A1 in oral squamous cell carcinoma and its correlation with clinicopathological parameters

ABSTRACT

Background: Aldehyde dehydrogenase 1A1 (ALDH1A1) is a key aldehyde dehydrogenase (ALDH) isozyme, related to the cancer stem cells which are responsible for initiating tumor growth, progression, and recurrence. High expression of ALDH1A1 has been reported in several tumor types in humans and its expression is associated with poor prognosis. The aim of this study was to assess the expression of the ALDH1A1 in oral squamous cell carcinoma (SCC) and its correlation with various clinicopathological parameters.

Materials and Methods: ALDH1A1 expression was analyzed by using immunohistochemistry on paraffin blocks of 112 cases of primary oral SCC and their corresponding 68 lymph nodes with metastatic deposits. ALDH1A1 expression was also correlated with various clinicopathological parameters. Statistical analysis was done with statistical analysis software, the Statistical Package for the Social Sciences version 21.0.

Results: High ALDH1A1 expression was observed in 31.2% of cases of primary oral SCC as compared to 73.5% in lymph node metastasis. A statistically significant difference ($P = 0.04$) was observed in high TNM stages (68.6%) of the tumor as compared to low TNM stages (31.4%). However, histopathological grades of tumor showed nonsignificant correlation with ALDH1A1 expression ($P = 0.093$). 40.2% of patients were expired at the end of the study, and the rate of mortality was significantly higher ($P = 0.01$) in patients with high ALDH1A1 expression as compared to low expression (60.0% vs. 31.2%).

Conclusion: High ALDH1A1 expression was associated with higher TNM tumor stage and high nodal stage. It was also associated with high mortality rate which validates it as a marker of invasiveness and poor prognosis in oral SCC.

Keywords: Aldehyde dehydrogenase 1A1 expression, aldehyde dehydrogenase, cancer stem cells, lymph node, metastasis, oral squamous cell carcinoma

INTRODUCTION

Oral carcinoma is the sixth leading cause of death worldwide having 5.75 lakh cases per year.[1] In India, oral cancer is also one of the major causes of death which accounts for 40% of total cancer burden.[2] Although many recent advancements in treatment have been done, still little or no improvement in mortality and morbidity rate was found over the past few decades. Poor prognosis of oral carcinoma is attributed to its aggressive behavior, poor sensitivity to chemotherapeutic agents, and early metastasis.[1,3] Researchers are trying to understand the mechanism through which cancer cells survive, escape immune destruction, and show unlimited self-renewal capacity. Recently, cancer stem-like cells (CSCs)

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were recognized as a subset of cells within the tumor in various tumors such as glioblastoma, breast cancer, colorectal cancer, and head-and-neck cancer.[4-8] CSCs are considered to be a tumor-initiating population which plays an essential role in growth initiation, progression, maintenance, and recurrence.[4,9] Various preclinical studies[4,8-10] suggested that CSCs have a predictive or prognostic role in treatment of oral cancer, thereby targeting the specific subpopulation of the cells (CSC population) in combination with conventional therapies aiming to reduce or eliminate the tumor bulk. It would be a reasonable method to improve therapeutic efficacy and prevention of metastasis or local recurrence. Studies showed that aldehyde dehydrogenase 1 (ALDH1)-positive CSCs own the property of stemness and displayed low expression of adhesion molecules and high expression of motility markers. They have epithelial-mesenchymal transition-related properties which assist the process of invasion and metastasis.[11-14]

ALDHs are an assembly of nicotinamide adenine dinucleotide phosphate positive-dependent enzymes that catalyze the oxidation of both exogenous and endogenous aldehyde substrates to their corresponding carboxylic acids, hence it plays an important role in cellular detoxification.[5,6,15] ALDH1A1 is a member of the ALDH gene superfamily containing multiple isozyme forms (chiefly ALDH1A1, ALDH1A2, and ALDH1A3) which are mostly placed in the cytoplasm of cells of various tissues.[15-17] Clinical studies have shown that ALDH1A1-positive CSCs were detected in head-and-neck cancer, originating from the oropharynx, hypopharynx, oral cavity, and larynx and also in lymph node metastases which are responsible for metastasis and recurrence. Hence, ALDH1A1 can help as a potential target in patients with locally advanced head-and-neck cancer or chemoresistant disease.[13,14,18,19] The aim of this study was to assess the expression of the ALDH1A1 in oral squamous cell carcinoma (SCC) and its corresponding lymph node metastasis cases and to find its correlation with various clinicopathological parameters and also establish its role as a marker of invasiveness and metastasis in the Indian subpopulation.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology in collaboration with surgical oncology, King George's Medical University, Lucknow. The duration of the present study was 1½ years and was approved from our Institutional Ethical Committee. Ethical Clearance was obtained from King George's Medical University U.P., Institutional Ethical Committee with Ref no 529 dated 19-06-2020.

ALDH1A1 immunohistochemistry was done on paraffin blocks of 112 resected specimens and their corresponding 68 lymph nodes having metastatic deposits. ALDH1A1 expression was analyzed and its correlation was done with various clinicopathological parameters.

Case selection and data collection

Formalin-fixed surgical specimens of oral SCC were received for histopathological examination. Clinical history along with details of other investigations was compiled on an Excel sheet, and clinical follow-up data were obtained from the Surgical Oncology Department. After routine histopathology, grading and staging of tumor were confirmed and case was enrolled for the study. Patients were followed up to the end of the study, and mortality and well-being of individual cases were recorded.

Immunohistochemistry

Paraffin blocks of 112 oral tumor cases and corresponding 68 lymph node tissue are collected for application of immunostaining. 3–4 micron meter thin sections were taken and kept at 56°C for overnight fixation. Sections were dewaxed and deparaffinized by keeping them in xylene for 15 min, followed by rehydration by passing through descending grades of alcohol. Antigen retrieval was done at 98°C for 15 min in microwave followed by endogenous peroxidase blocking for 5 min. Primary antibody anti-ALDH1A1 (Abcam, ab131068, USA, 1:200 dilution) was incubated for 1.5 h followed by secondary antibody (poly-horseradish peroxidase) for 30 min. All sections were applied with diaminobenzidine chromogen for 10 min, followed by counterstaining with hematoxylin and mounting in DPX. Normal human kidney tissue section was used as a positive control.

Evaluation of staining

To evaluate the ALDH1A1 expression, we followed the scoring system used by Ortiz *et al*.[20] We noticed a brown cytoplasmic and membranous staining of ALDH1A1 in tumor cells which appeared as patchy or diffuse pattern. Minor salivary gland acini were taken as internal control whenever they present in the sample. Immunostaining was evaluated at invasive tumor front in primary tumor and lymph node as whole in metastatic lymph node under 400x magnifications. Staining was measured by multiplying intensity and proportion of immunopositive tumor cells, ranging from 0 to 9. For score of intensity, we considered 0 – no staining, 1 – weak staining, 2 – moderate staining, and 3 – strong staining and for scores of proportions of tumor cells: 0≤5%, 1 = 6%–25%, 2 = 26%–49%, and 3≥50. Based on the above scoring system, ALDH1A1 expression was grouped as low (≤2) or high (>2) immunoexpression. Low expression was considered negative.

Statistical analysis

The statistical analysis was done by statistical analysis software, SPSS version 21.0. The values were represented in number, percentage, and mean ± standard deviation. Combined scores of ALDH1A1 immunoexpression in primary oral SCC and their metastatic lymph nodes were compared by Chi-square test and Mann–Whitney *U*-test. Correlation between clinicopathological parameters and ALDH1A1 protein immunoexpression was measured by Chi-square test and Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

In our study, majority of the oral cancer cases were belonged to the age group of 40–50 years, with a mean age group of 45.07 years, and the male-to-female ratio was 6:1 (96/16). Most of the patients had a history of substance abuse (91.1%), and the most common location was retromolar trigone (34.8%) and tongue (33.9%) followed by alveolar-buccal complex (21.4%) and lip (6.3%) [Table 1].

Weak-to-strong cytoplasmic and membranous expression of ALDH1A1 was observed as patchy and occasionally diffuse pattern and more intensity was present at invasive tumor front [Figures 1 and 2].

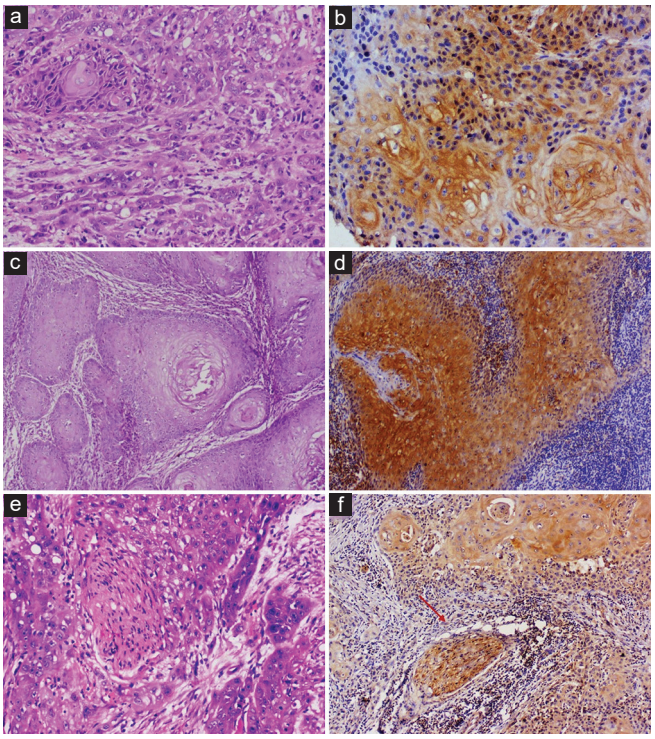

Figure 1: Oral squamous cell carcinoma (primary tumour): Moderately differentiated H&E (a) and Diffuse ALDH1A1 expression (b) x 200X. Well differentiated H&E (c) and Diffuse ALDH1A1 expression (d) x 100X. Perineural invasion H&E x 200X (e). ALDH1A1 expression near invasive front and perineural invasion (arrow) x 100X (f)

High ALDH1A1 expression was observed in 31.2% of primary oral tumor cases while 73.5% in metastatic lymph modes [Table 2]. Among primary oral tumors with high

Table 1: Distribution of study population (oral squamous cell carcinoma)

Tumor characteristics	*n* (%)
Site	
Retromolar trigone	39 (34.8)
Tongue	38 (33.9)
Alveolar-buccal complex	24 (21.4)
Lip	7 (6.3)
Buccal mucosa	4 (3.6)
Histological grade	
Well differentiated	74 (66.1)
Moderately differentiated	36 (32.1)
Poorly differentiated	2 (1.8)
TNM stage	
T1	16 (14.3)
T2	35 (31.3)
T3	48 (42.9)
T4	13 (11.6)
Lymph node metastasis	
N1	24 (35.3)
N2	35 (51.5)
N3	9 (13.2)
Laterality	
Left	58 (51.8)
Right	54 (48.2)
Depth of invasion (mm)	
<10	46 (41.1)
>10	66 (58.9)
Affected area	
Submucosa	24 (21.4)
Muscle	80 (71.4)
Skin and bone	8 (7.1)
Lymphovascular invasion	
Evident	32 (28.6)
Not evident	80 (73.2)
Perineural invasion	
Evident	30 (26.8)
Not evident	82 (73.2)
Necrosis	
Evident	52 (46.4)
Not evident	60 (53.6)
Tumor-infiltrating lymphocytes	
Mild	27 (24.1)
Moderate	65 (58.0)
Dense	20 (17.9)
Tumor advancing edge	
Infiltrating	104 (92.9)
Round	8 (7.1)
Mortality	
Expired	45 (40.2)
Alive	67 (59.8)

TNM: Tumor, node, and metastasis

ALDH1A1 expression, 77.1% of cases (27/35) showed lymph node metastasis as compared to low ALDH1A1 expression cases, where 53.2% of cases (41/77) had lymph node metastasis [Figure 3]. On comparison, the difference was statistically significant ($P = 0.01$) [Table 3].

ALDH1A1 expression was higher in T3 and T4 TNM stages (68.6%) as compared to T1 and T2 stages (31.4%) of the tumor with statistically significant ($P = 0.04$) difference [Table 3 and Figure 3]. 66.1% of cases were well-differentiated SCC followed by moderately differentiated (32.1%) and poorly differentiated (1.8%) tumors. High ALDH1A1 expression was observed in 60.0% of well-differentiated tumors as compared to 34.0% moderate differentiated with statistically nonsignificant ($P = 0.093$) difference.

In all 68 cases of lymph node metastasis, ALDH1A1 expression was evaluated in the infiltrating tumor cells separately. High ALDH1A1 expression was seen in 50 cases (73.5%), while the rest 18 cases (26.5%) showed low expression. Among nodal stage, 51.5% had N2 nodal staging (35/68) followed by 35.3% N1 staging (24/68). High ALDH1A1 expression was observed in N2 68.0% versus N1 24.0% ($P < 0.001$) [Table 4].

In this study, 40.2% of patients were expired at the end of the study. The rate of mortality was significantly higher among patients with high ALDH1A1 expression as compared to low expression (60.0% vs. 31.2%), which was statistically significant ($P = 0.01$) [Table 3 and Figure 4].

On multivariate analysis, ALDH1 expression was dependent on independent variables namely TNM stage, lymph node metastasis, depth of tumor, lymphovascular invasion, tumor advancing edge, and mortality. However, only lymphovascular invasion showed a significant association with ALDH1 expression [Table 5].

DISCUSSION

Oral cancer is an aggressive neoplasm having poor survival rate due to lack of appropriate or advanced treatment options and poor response to chemotherapeutic agents. Recently, CSCs have been identified as a small group of cells within the tumor, which own the properties of self-renewal and play a vital role in tumor progression and recurrence.[4,9,17] ALDHs are a group of enzymes which have proven to be strong CSC markers in various human solid tumors.[4,13,15] They are involved in cell proliferation and differentiation through its product retinoic acid. ALDH inhibition, using specific inhibitors or retinoic acid, has shown to reduction of cell proliferation, invasion, and chemoresistance. Hence, ALDH and retinoic acid are hopeful therapeutic targets in the present scenario.[4,11-14,19] Studies have shown that ALDH1A1 (specific isozyme) is a useful cancer stem cell biomarker which increased in several solid tumors such as glioblastoma, breast cancer, colon

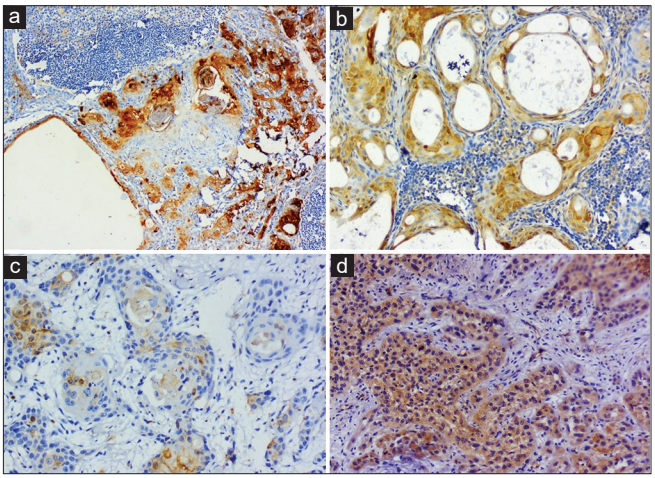

Figure 2: Lymph nodes metastasis: (a) High ALDH1A1 expression in tumour cells with cystic degeneration x 100X. (b) High ALDH1A1 expression in tumour cells showing cystic degeneration x 200X. (c) Oral tumour: Low ALDH1A1 expression in tumor cells x 200X. (d) High ALDH1A1 expression in tumor cells 200X

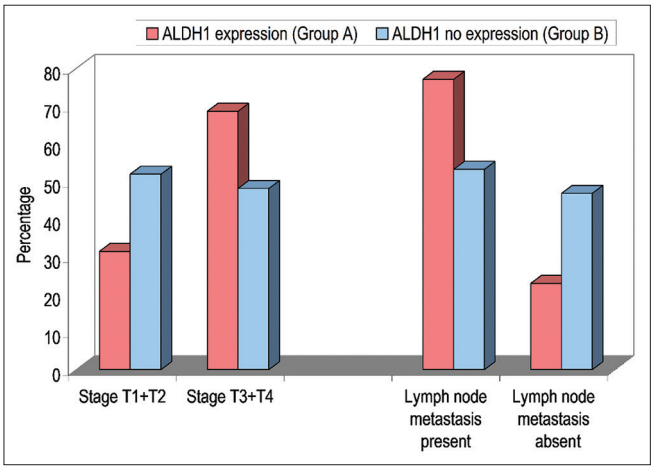

Figure 3: Relationship between ALDH1A1 expression and TNM stage and lymph node metastasis

Table 2: Aldehyde dehydrogenase 1A1 expression in primary (oral) tumor and lymph nodes

Specimen	Total cases	High ALDHIA1 expression, *n* (%)	Low ALDHIA1 expression, *n* (%)
Primary oral tumor cases	112	35 (31.2)	77 (68.8)
Corresponding lymph node metastasis	68	50 (73.5)	18 (26.5)

ALDH1A1: Aldehyde dehydrogenase 1A1

Table 3: Comparison of clinicopathological characteristics of oral squamous cell carcinoma and aldehyde dehydrogenase 1A1 Expression

Variable	ALDH1A1 high expression ($n=35$), n (%)	ALDH1A1 low expression ($n=77$), n (%)	Total ($n=112$), n (%)
Histological grade			
Well differentiated	21 (60.0)	53 (68.8)	74 (66.1)
Moderately differentiated	12 (34.3)	24 (31.2)	36 (32.1)
Poorly differentiated	2 (5.7)	0	2 (1.8)
χ^2, df, P	4.757, 2, 0.093		
TNM stage			
Stage T1+T2	11 (31.4)	40 (51.9)	51 (45.5)
Stage T3+T4	24 (68.6)	37 (48.1)	61 (54.5)
χ^2, df, P	10.593, 2, 0.04		
Lymph node metastasis			
Present	27 (77.1)	41 (53.2)	68 (60.7)
Absent	8 (22.9)	36 (46.8)	44 (39.3)
χ^2, df, P	5.761, 1, 0.016		
Laterality			
Left	20 (57.1)	38 (49.4)	58 (51.8)
Right	15 (42.9)	39 (50.6)	54 (48.2)
χ^2, df, P	0.585, 1, 0.444		
Depth of tumor (mm)			
<10	9 (25.7)	37 (48.1)	46 (41.1)
>10	26 (74.3)	40 (51.9)	66 (58.9)
χ^2, df, P	4.961, 1, 0.026		
Affected area			
Submucosa	4 (11.4)	20 (26.0)	24 (21.4)
Muscle	28 (80.0)	52 (67.5)	80 (71.4)
Skin and bone	3 (8.6)	5 (6.5)	8 (7.1)
χ^2, df, P	3.045, 2, 0.218		
Lymphovascular invasion			
Evident	23 (65.7)	9 (11.7)	32 (28.6)
Not evident	12 (34.3)	68 (88.3)	80 (71.4)
χ^2, df, P	34.415, 1, <0.001		
Perineural invasion			
Evident	12 (34.3)	18 (23.4)	30 (26.8)
Not evident	23 (65.7)	59 (76.6)	82 (73.2)
χ^2, df, P	1.460, 1, 0.227		
Necrosis			
Evident	15 (38.5)	37 (50.7)	52 (46.4)
Not evident	24 (61.5)	36 (49.3)	60 (53.6)
χ^2, df, P	1.527, 1, 0.217		
Tumor-infiltrating lymphocytes			
Mild	7 (20.0)	20 (26.0)	27 (24.1)
Moderate	22 (62.9)	43 (55.8)	65 (58.0)
Dense	6 (17.1)	14 (18.2)	20 (17.9)
χ^2, df, P	0.575, 2, 0.750		
Tumor advancing edge			
Infiltrating	30 (85.7)	74 (96.1)	104 (92.9)
Round	5 (14.3)	3 (3.9)	8 (7.1)
χ^2, df, P	3.916, 1, 0.048		
Mortality			
Expired	21 (60.0)	24 (31.2)	45 (40.2)
Alive	14 (40.0)	53 (68.8)	67 (59.8)
χ^2, df, P	8.322, 1, 0.004		

ALDH1A1: Aldehyde dehydrogenase 1A1, TNM: Tumor, node, and metastasis

carcinoma, gynecologic malignancies, and head-and-neck SCC.[4-7,13,18,21] Chen *et al*.[22] and Qian *et al*.[18] had reported that high expression of ALDH1A1 was strongly associated with aggressive disease and poor prognosis in head-and-neck cancer. In the present study, we found that high ALDH1A1 expression was associated with high tumor stage and nodal stage in oral SCC cases.

The present study was included 96 males and 16 females, and most of the patients were in the age range of 40–50 years (mean age: 45.07 years). Males were more affected (85.7%) than females (14.3%). It may be due to more substance abuse in males. However, no statistically significant correlation was found between ALDH1A1 expression and gender, age, and substance abuse in the oral SCC cases. A similar result was observed in studies done by Ota *et al*.[23] and Herrera Costa *et al*.[24] In our study, the most common location of oral SCC was retromolar trigone (34.8%) and tongue (33.9%). Tandon *et al*.[25] conducted a study on prevalence of oral SCC and found that buccal cavity (31.47%) was the most common site, followed by tongue (19.21%) and lip (2.35%). We could not find any significant correlation between tumor site and ALDH1A1 expression.

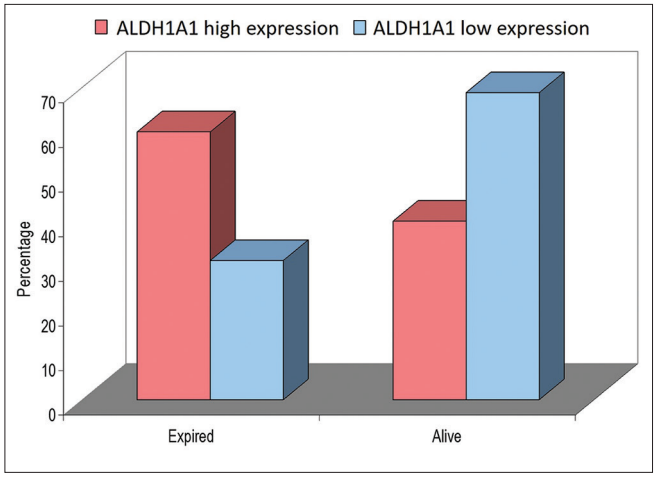

Figure 4: Association of ALDH1A1 expression and outcome

Most of the cases in our study were well-differentiated tumor (67.8%) followed by moderately differentiated (32.14%) and poorly differentiated (1.78%). However, we did not find a statistically significant ($P < 0.09$) correlation between ALDH1A1 expression and histopathological grade of tumor. This finding was concordance with a study of Ortiz *et al*.[20] and Tsai *et al*.[26] whereas Michifuri *et al*.[27] and Tamatani *et al*.[28] reported a statistically significant correlation between ALDH1A1 expression and tumor grade.

The present study showed a linear relationship with high ALDH1A1 expression and TNM tumor staging, where ALDH1A1 expression increases as we move from lower T1 and T2 (31.4%) TNM stages to higher T3 and T4 (68.6%) TNM stages of tumor ($P < 0.04$). This finding was in concordance with previous studies done by Ortiz *et al*.,[20] Szafarowski *et al*.,[29] Wang *et al*.,[30] and Vieira *et al*.,[31] which also suggested that higher stage cancer had high ALDH1A1 expression, hence more aggressive behavior. The above findings implied us to be more cautious about higher stage tumor, containing more tumor budding cells and cancer stem cells as compared to lower stages and have more probability of early metastasis.

Table 4: Comparison of aldehyde dehydrogenase 1A1 expression in nodal stages in oral squamous cell carcinoma

Stage of lymph node	Total cases of lymph node metastasis (68)	High ALDH1A1 expression ($n=50$), *n* (%)	Low ALDH1A1 expression ($n=18$), *n* (%)
N1	24	7 (14.0)	17 (94.4)
N2	35	34 (68.0)	1 (5.6)
N3	9	9 (18.0)	0
χ^2, df, *P*		37.534, 2, <0.001	

ALDH1A1: Aldehyde dehydrogenase 1A1

Table 5: Multivariate analysis to evaluate aldehyde dehydrogenase 1A1 correlation with oral squamous cell carcinoma

Parameters	*B*±SE	Wald	*P*	Exp (B)	95% CI for Exp (B)	
					Lower	Upper
T1T2 or T3T4	−0.233±0.598	0.151	0.697	0.792	0.245	2.558
Lymph node metastasis	0.422±0.573	0.544	0.461	1.525	0.497	4.685
Depth of tumor	−1.087±0.618	3.092	0.079	0.337	0.100	1.133
Lymphovascular invasion	2.499±0.553	20.402	<0.001	12.176	4.116	36.017
Tumor advancing edge (infiltrating/round)	−1.138±1.005	1.283	0.257	0.320	0.045	2.296
Mortality	0.764±0.558	1.879	0.170	2.147	0.720	6.404
Constant	−1.701±2.334	0.531	0.466	0.183		

CI: Confidence interval, SE: Standard error

In the current study, oral SCC cases which possess high ALDH1A1 expression showed positive nodal metastasis in 77.1% of cases as compared to negative metastasis in 22.9% of cases and difference was statistically significant ($P = 0.01$). This finding suggests that high ALDH1A1 expression in primary tumor can predict the chances of nodal metastasis. This finding was in agreement with Michifuri *et al*.,[27] Wang *et al*.,[30] and Tsai *et al*.[26] where they found a similar correlation with nodal metastasis. Whereas, Ota *et al*.[23] found nonsignificant correlation between nodal metastasis and ALDH1A1 expression.

We also analyzed cases from different nodal stages to evaluate the expression of ALDH1A1 and found that higher nodal stages cases (N2) showed high ALDH1A1 expression as compared to low (N1) nodal cases. These results were statistically significant ($P < 0.001$) and were in agreement with the study done by Michifuri *et al*.[27]

In the present study, the rate of mortality was higher in cases with high ALDH1A1 expression as compared to cases with low expression ($P < 0.004$). A similar finding was observed by Götz *et al*.[32] in their study ($P < 0.03$), which implies that high ALDH1A1 expression in primary tumor relates to increase CSCs which impart aggressive nature and poor outcome of the patient.

We observed angiolymphatic invasion in 28.6% of cases of primary tumor; among them, 65.7% of cases showed high ALDH1A1 expression which was statistically significant ($P < 0.001$). A similar finding was reported by Ortiz *et al*.[20] with statistically significant correlation ($P < 0.01$). This result shows that tumor cells showing high ALDH1A1 expression have more invasive properties which enable them to invade early. However, we did not find a significant association between ALDH1A1 expression and laterality, affected area, perineural invasion, amount of necrosis, and tumor-infiltrating lymphocytes. These findings were in agreement with the findings of Junior *et al*.[33] and Ortiz *et al*.[20] with similar result ($P < 0.05$). We also correlated advancing edge with ALDH1A1 expression, where high expression was observed with infiltrating edge as compared to round edge (96.1% vs. 3.9%).

Limitations of study

Limitations of this study were short duration of study and limited number of cases. Further, development of oral SCC is multistep carcinogenesis which is influence by multiple factors at various levels. We evaluated one aspect of this multifactorial process. Hence, further work will be required related to oral cancer.

CONCLUSION

From the above findings, it was concluded that the high ALDH1A1 expression was associated with high TNM tumor stage and high nodal stage of oral SCC cases. High ALDH1A1 expression was also associated with high mortality and poor outcomes of the patients. It implies that ALDH1A1 is a marker of poor prognosis in oral SCC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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