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Differential IDH1 Immunohistochemical Expression Across the Pathologically Stratified Spectrum of Intraepithelial and Invasive Laryngeal Squamous Cell Lesions

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ABSTRACT

Background & Objective: Laryngeal carcinoma is a common head and neck cancer with high morbidity and mortality. This study evaluates IDH1 immunohistochemical expression in pre-invasive and invasive laryngeal squamous cell lesions, its correlation with clinicopathological features, and its prognostic and therapeutic potential.

Methods: A retrospective analysis of 65 laryngeal squamous cell lesion cases was conducted using IDH1 immunohistochemistry.

Results: IDH1 expression significantly correlated with invasion status (intensity and H-score; p = 0.026, 0.025) and histopathological progression and grade (p = 0.033, 0.036). Two-year DFS and OS were 81.5% and 69%, respectively, with no significant association between survival outcomes and IDH1 H-score (p = 0.851, 0.225).

Conclusion: According to our findings, increased IDH1 expression may be associated with features of aggressive tumor behavior and could hold clinical significance in laryngeal squamous cell lesions, potentially serving as a future therapeutic target.

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Introduction

Laryngeal carcinoma, one of the most common head and neck cancers (1,2), has a high global burden of morbidity and mortality (3,4). Every year, over 90,000 deaths are attributed to laryngeal squamous cell carcinoma (LSCC), which accounts for approximately 1.7 million cases worldwide (5,6).

Isocitrate dehydrogenase (IDH) enzymes play a key role in cellular metabolism by catalyzing the oxidative decarboxylation of isocitrate to α-ketoglutarate (7-10). IDH exists in three isoforms: IDH1 (cytoplasm and peroxisomes), IDH2, and IDH3 (mitochondrial matrix) (11,12).

Somatic mutations in the catalytic domains of IDH1 and IDH2 disrupt this process, leading to reduced α-ketoglutarate levels and accumulation of 2-hydroxyglutarate, a metabolite with oncogenic potential (7-10).

Mutations in these genes are prevalent in several cancers, notably in over 80% of WHO-classified grade II/III gliomas (11,12), as well as acute myeloid leukemia, nonepithelial melanoma, chondrosarcoma, prostate cancer, and intrahepatic cholangiocarcinoma (13). Several tumor types, such as osteosarcoma or gastric cancer, exhibit higher levels of its expression than normal tissues (14-17). Huang et al. (2021) reported that IDH1/2 mutations in colorectal carcinoma may be a main driver that occurs as the cancer progresses from adenoma to adenocarcinoma in colorectal carcinoma (18).

IDH1 gene mutations and changes in its expression have been used as prognostic indicators to guide targeted therapy and predict tumor recurrence and tumor-related outcomes (19,20). However, their relevance in laryngeal squamous cell carcinoma (LSCC) remains underexplored (13).

IDH1 has clinical significance in LSCC cases and is associated with increased invasiveness, features of aggressive tumor behavior, and poorer outcomes. Its expression is considered a useful predictive marker of cancer progression in tumor biopsies from LSCC patients (13,20).

Therapeutically, IDH1 inhibitors such as ivosidenib (AG-120) have shown promise in clinical trials for leukemia and cholangiocarcinoma, further underscoring the potential of IDH1 as a target in LSCC management (17).

Materials and Methods

After approval by the Kasr Alainy Research Ethics Committee (REC) (code: N-184-2024) in 2024, 65 patients with laryngeal pre-invasive and invasive squamous cell lesions who were diagnosed either by laryngoscopic biopsy or radical laryngectomy were collected for this retrospective study from the Anatomic Pathology Department at Kasr Alainy Hospital during the period from January to December 2022. Only cases with at least 2 years of follow-up from diagnosis, or until the occurrence of recurrence or death, were included. The date of the first (diagnostic) operation was chosen as the date of diagnosis, and survival was evaluated from this date.

Exclusion Criteria: Patients with poorly fixed or inadequately preserved tumors, those whose tumors had significant necrosis or cautery artifacts, and cases with unavailable paraffin blocks or incomplete case data were excluded from this study.

Case Parameters

All available clinicopathological data present in the patients' request sheets were registered, including age and sex of the patients, history of smoking, nature of the specimen, lesion site and size, histopathological diagnosis and grading, presence of metastasis, lymphovascular emboli, perineural invasion, thyroid cartilage invasion, necrosis, keratosis, koilocytosis, papillomatous lesions, resection margin involvement, and WHO pT and pN stages.

Histopathological Evaluation

Each paraffin block was re-cut by microtome at 4 μm thickness, then mounted on glass slides and stained with hematoxylin and eosin for re-evaluation under a light microscope (Zeiss, Germany) by 2 pathologists who confirmed the diagnosis and the histopathologic grades. The WHO grade was assigned to each tumor according to the criteria of the World Health Organization (WHO) classification of tumors of the head and neck (21). Histological tumor staging and grading were confirmed according to AJCC Cancer Staging 2017 (22).

Immunohistochemical Procedure

Paraffin blocks were serially sectioned at 4 μm thickness, mounted on positively charged slides, and immunostained with IDH1 (R132H) mouse monoclonal antibody (clone H09), manufactured by Dianova, Germany. A fully automated immunohistochemical staining protocol was applied. A Dako Autostainer Link 48 was used, and positive controls for each antibody were applied according to the manufacturer's protocol. The primary antibody was omitted as a negative control in the same tumor sections.

We analyzed IDH1 expression in different lesions and its correlation with clinicopathological features and patients' survival. Predictors of both mortality and disease-free survival (DFS) were also assessed.

Immunohistochemical Interpretation

Three scoring systems were used to analyze IDH1 immunohistochemical staining: the H-score, the staining intensity, and the proportion of positive lesional cells. There were 4 possible staining intensity scores: 0 for negative, 1 for weak, 2 for moderate, and 3 for strong. The following rating was applied to the percentage of positive lesional cells: 1, less than 25%; 2, 25%–50%; 3, 51%–75%; and 4, greater than 75%. The histochemical score (H-score), which ranges from 0 to 300, was then calculated by multiplying the staining intensity by the proportion score of positive lesional cells. In this investigation, samples were divided into 2 groups based on the median H-score: a low-expression group (H-score 0–40) and a high-expression group (H-score 41–300) (13).

Positive IDH1 immunohistochemical staining was identified as distinct intracellular cytoplasmic staining in lesional cells, while nonspecific or diffuse extracellular background staining was excluded from interpretation. Two independent pathologists scored all IDH1 slides blinded to clinical data. Interobserver agreement was calculated using Cohen's kappa (κ). Discrepant cases were reviewed jointly to reach consensus.

Statistical Methods

All results of the present study were entered into the SPSS statistical software program, version 26. Simple descriptive statistics were used (arithmetic mean and standard deviation) to summarize quantitative data, and frequencies were used for qualitative data. The bivariate relationship was displayed in cross-tabulations, and comparison of proportions was performed using the chi-square test. The independent t test was used to compare normally distributed quantitative data. The main outcome was time-to-event analysis. Survival rates were calculated using the Kaplan–Meier method. The log-rank test was used to compare significance between groups. The results were evaluated at a significance level of $p \leq 0.05$. Microscopic photographs were captured using a digital camera attached to an Olympus microscope model BX 53.

Results

This retrospective study included 65 patients with laryngeal pre-invasive and invasive squamous cell lesions (Table 1) who were diagnosed either by laryngoscopic biopsy (18 cases; 27.7%) or laryngectomy (47 cases; 72.3%), with a mean age of 59 years (SD = 11.27; range, 33–80 years). Of them, 63

cases were males (96.9%), and only 2 were females (3.1%). The laryngeal lesions of the investigated cases ranged from 0.5 to 12 cm in maximal diameter (mean = 3.4 cm). Most cases had glottic lesions (30 cases; 46.2%), followed by 17 cases with supraglottic lesions (26.2%). Invasion was identified in 78.5% of the enrolled cases (51 of 65 cases). The studied cases of laryngeal squamous lesions were classified according to histopathological graded progression into 5 groups as follows: mucosal dysplasia (8 cases; 12.3%), squamous cell carcinoma in situ (6 cases; 9.2%), microinvasive squamous cell carcinoma (4 cases; 6.2%), invasive moderately differentiated squamous cell carcinoma (35 cases; 53.8%), and invasive poorly differentiated squamous cell carcinoma (12 cases; 18.5%). At the time of diagnosis, after exclusion of cases with mucosal dysplasia, primary tumor (pT) stages were as follows: 6 cases (10.5%) Tis, 4 (7%) pT1, 16 (28.1%) pT2, 21 (36.8%) pT3, and 10 (17.5%) pT4a. Among patients who underwent radical laryngectomy, 23 (48.9%) had lymph node tumor deposits. Lymphovascular invasion and perineural invasion were identified in 16 (34%) and 2 (4.3%) of laryngectomy specimens, respectively. The thyroid cartilage was infiltrated in more than 60% (30 cases) of laryngectomy specimens, and the surgical resection margin was involved in about 13% (6 cases) of them. Recurrence was recognized in 18.5% (12 cases), while only 4 cases (7.8% of squamous cell carcinomas) presented with distant metastasis.

Approximately 65% of our patients received chemotherapy and/or radiotherapy. Lesion recurrence occurred in 15.4% of these patients (10 cases), compared with 3% (2 cases) among those who did not receive such treatment. There was no statistically significant association between lesion recurrence and receiving chemotherapy and/or radiotherapy ($p = 0.133$).

The degree of concordance between 2 independent pathologists in interpreting immunohistochemical staining of IDH1 was evaluated using Cohen κ statistics. For IDH1, the agreement was substantial ($\kappa = 0.68$, $p = 0.014$), with an observed concordance of 82%. These results demonstrate good interobserver reliability, suggesting that the immunohistochemical evaluation was reproducible and consistent across both pathologists.

Table 1. Clinical data and characteristics of the studied cases of laryngeal squamous cell lesions

Clinico-Pathological Features	n (%)
Age (Mean ± SD)	58.74 ± 11.274
Sex	
Male	63 (96.9)
Female	2 (3.1)
Smoking	
No	15 (23.1)
Yes	50 (76.9)
Lesion Size (Mean ± SD)	3.4 ± 199
Site	
Glottic	30 (46.2)
Glottic-subglottic	4 (6.2)
Glottic-supraglottic	5 (7.7)
Subglottic	4 (6.2)
Supraglottic	17 (26.2)
Trans-glottic	5 (7.7)
Type of Specimen	
Laryngoscopic biopsy	18 (27.7)
Radical laryngectomy	47 (72.3)
Invasion Status	
No	14 (21.5)
Yes	51 (78.5)
Histopathological Progression and Grade	
Dysplasia	8 (12.3)
Squamous cell carcinoma in situ	6 (9.2)
Microinvasive SCC	4 (6.2)
Moderately differentiated SCC	35 (53.8)

Clinico-Pathological Features	n (%)
Poorly differentiated SCC	12 (18.5)
Necrosis (in the radical laryngectomy specimens)	
Absent	33 (70.2)
Present	14 (29.8)
Lympho-Vascular Emboli (in the radical laryngectomy specimens)	
Absent	31 (66)
Present	16 (34)
Peri-Neural Invasion (in the radical laryngectomy specimens)	
Absent	45 (95.7)
Present	2 (4.3)
Thyroid Cartilage Infiltration (in the radical laryngectomy specimens)	
Free	17 (36.2)
Infiltrated	30 (63.8)
Margin infiltration (in	
Free	41 (87.2)
Infiltrated	6 (12.8)
pT Stage	
Tis	6 (10.5)
T1	4 (7.1)
T2	16 (28.1)
T3	21 (36.8)
T4a	10 (17.5)
pN Stage (in the radical laryngectomy specimens)	
Nx	8 (17)
N0	24 (51.1)
N1	4 (8.5)
N2a	1 (2.1)
N2b	3 (6.4)
N2c	2 (4.3)
N3b	5 (10.6)
Metastasis (in the invasive squamous cell carcinoma cases)	
No	47 (92.2)
Yes	4 (7.8)
Chemo and/or Radiotherapy	
No	23 (35.4)
Yes	42 (64.6)
Recurrence	
No	53 (81.5)
Yes	12 (18.5)

About 86% (56 cases) of the studied cases expressed IDH1 with variable staining intensities, including 9 (13.8%) negative, 21 (32.3%) weak, 26 (40%) moderate, and 9 (13.8%) strong (Figures 1, 2, 3, 4, and 5). Twenty-five (38.5%) samples had low IDH1 expression, while 40 (61.5%) samples had high IDH1 expression.

Statistically, IDH1 expression (Tables 2 and 3) was significantly correlated with thyroid cartilage infiltration regarding intensity ($p = 0.01$) as well as with lesion site regarding H-score ($p = 0.016$).

Moreover, regarding both IDH1 expression intensity and H-score, our analysis showed a significant association with type of specimen (p = 0.009 and 0.004, respectively), lesion size (p = 0.03 and 0.02, respectively), invasion status (p = 0.026 and 0.025, respectively), as well as histopathological progression and grade of the lesion (p = 0.033 and 0.036, respectively), whereas there was no apparent relationship between IDH1 expression and other clinicopathological factors.

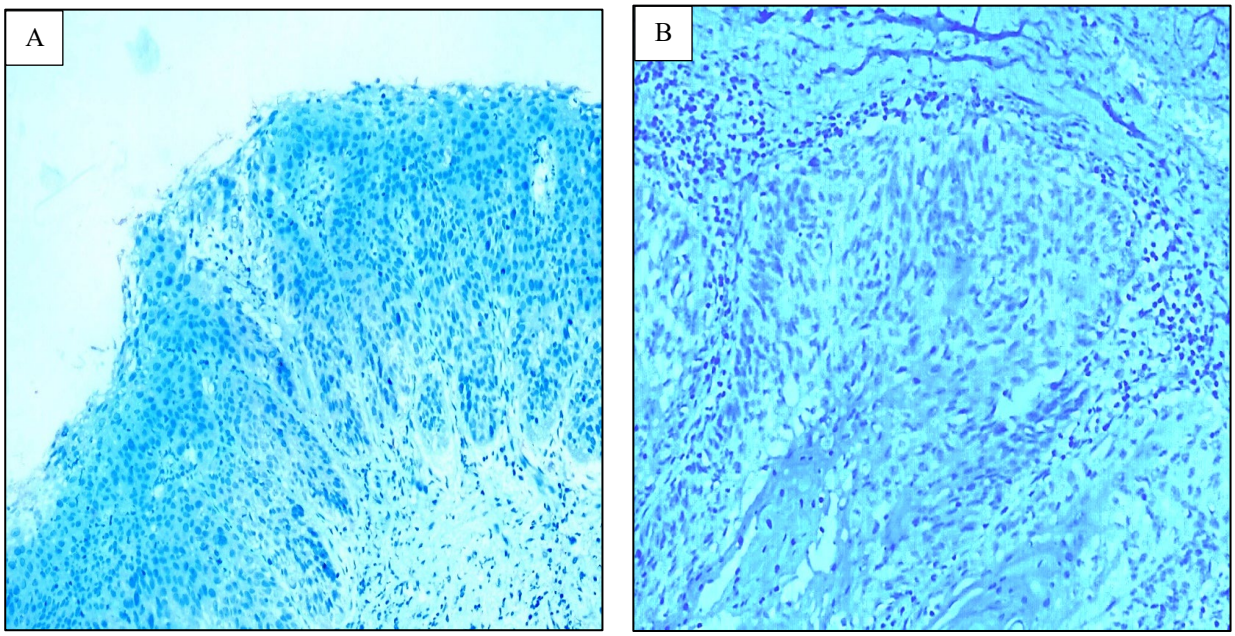


Fig.1. Negative cytoplasmic immunohistochemical expression of IDH1; (A) in squamous CIS (x200 original magnification) & (B) in moderately differentiated SCC (x200 original magnification).

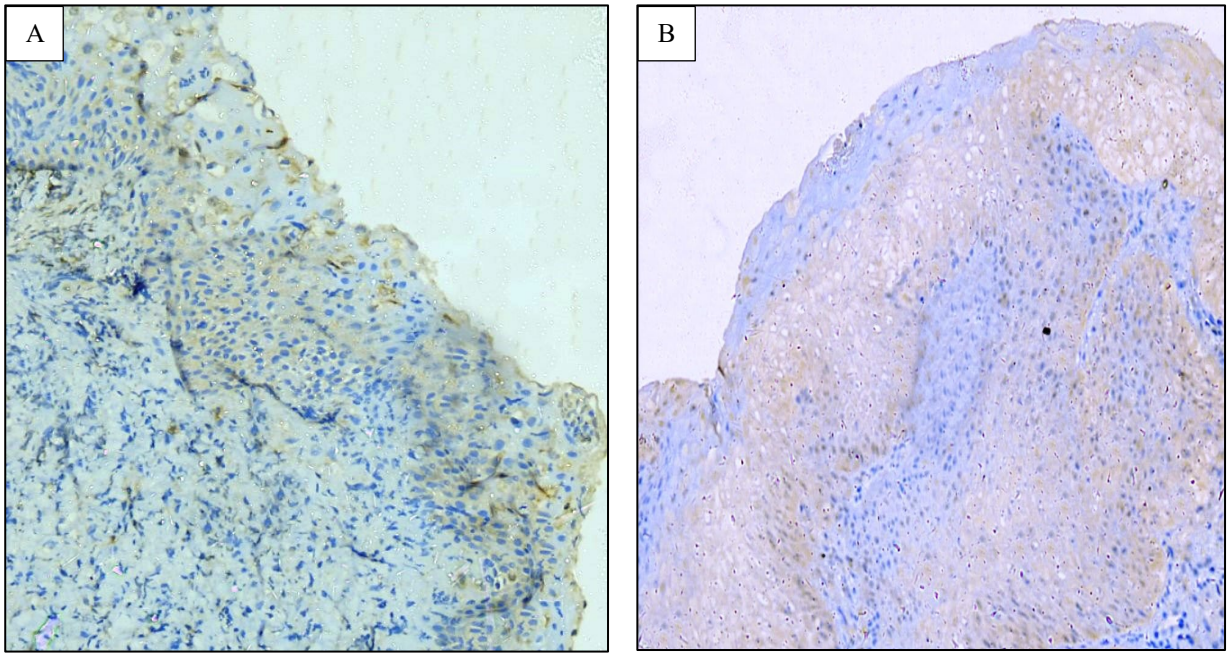


Fig.2. Immunohistochemical expression of IDH1 in dysplasia; (A) Weak cytoplasmic staining (x200 original magnification) & (B) Moderate cytoplasmic staining (x200 original magnification).

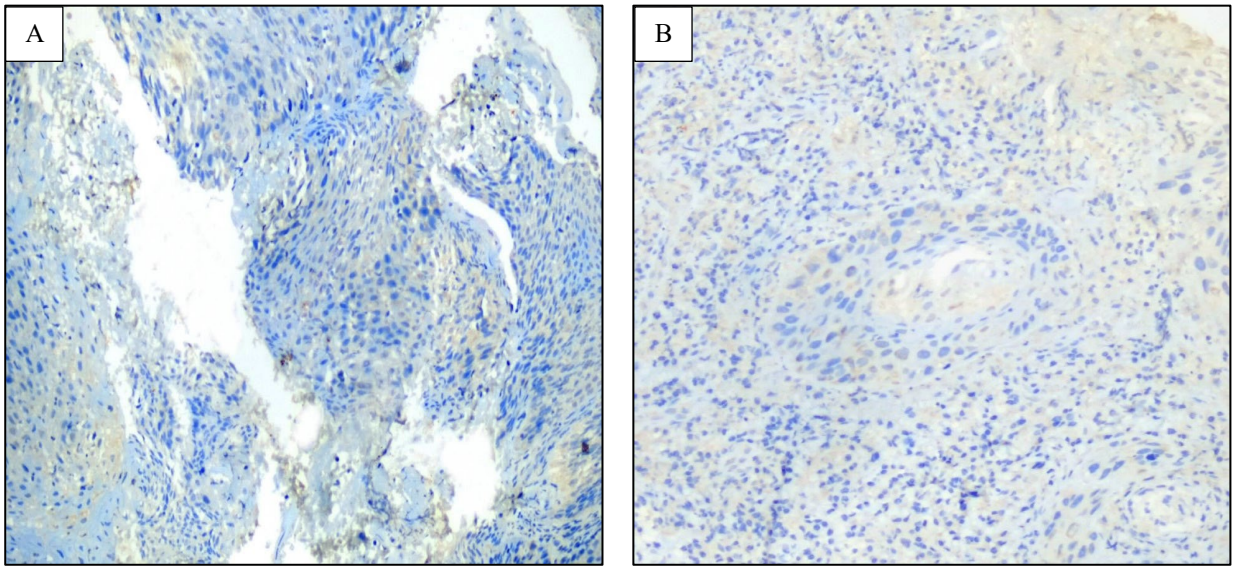


Fig.3. Immunohistochemical expression of IDH1; (A) Weak cytoplasmic staining in squamous CIS (x200 original magnification) & (B) Weak cytoplasmic staining in microinvasive SCC (x200 original magnification).

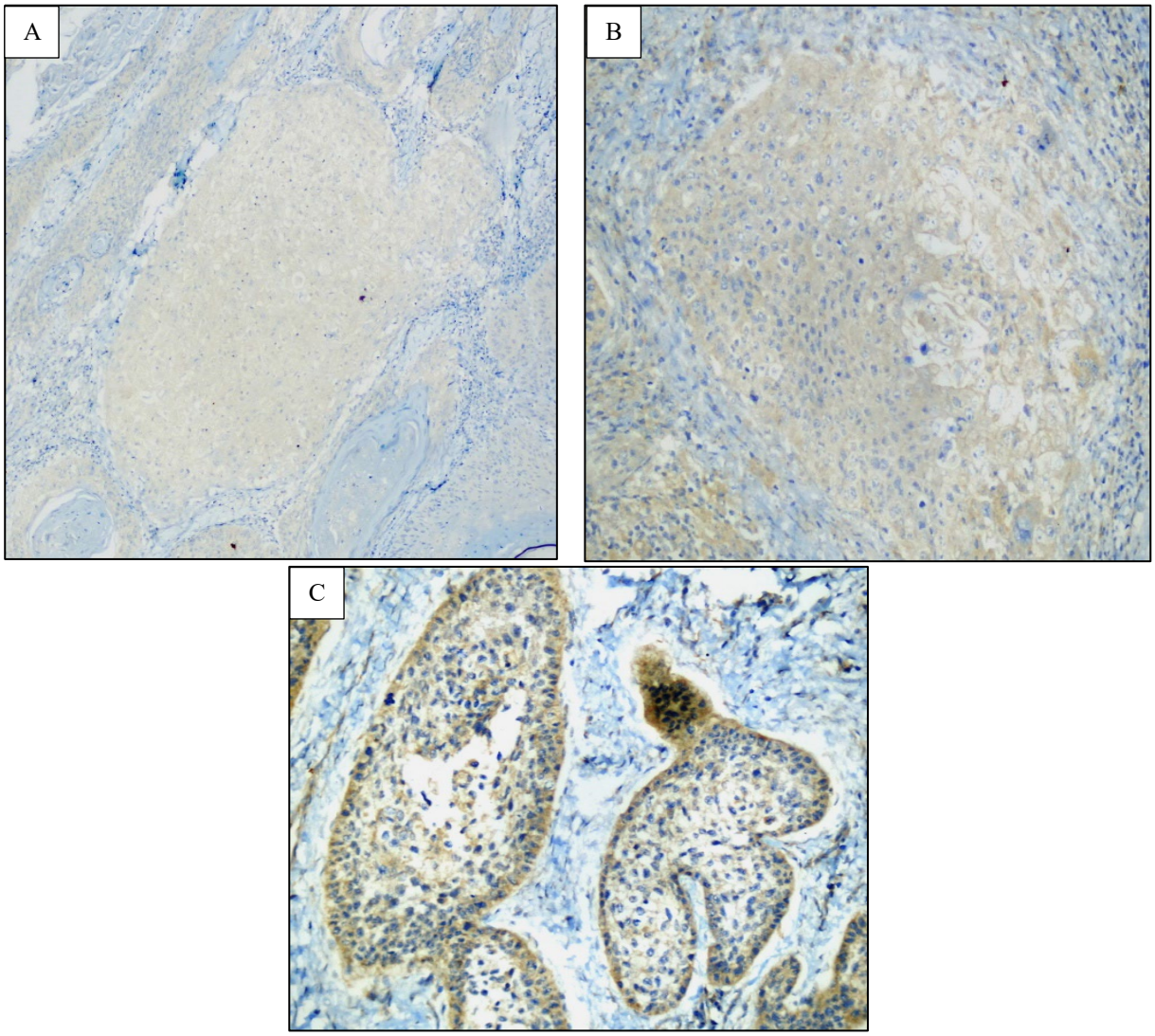


Fig.4: Immunohistochemical expression of IDH1 in moderately differentiated SCC; (A) Weak cytoplasmic staining (x100 original magnification), (B) Moderate cytoplasmic staining (x200 original magnification) & (C) Strong cytoplasmic staining (x200 original magnification).

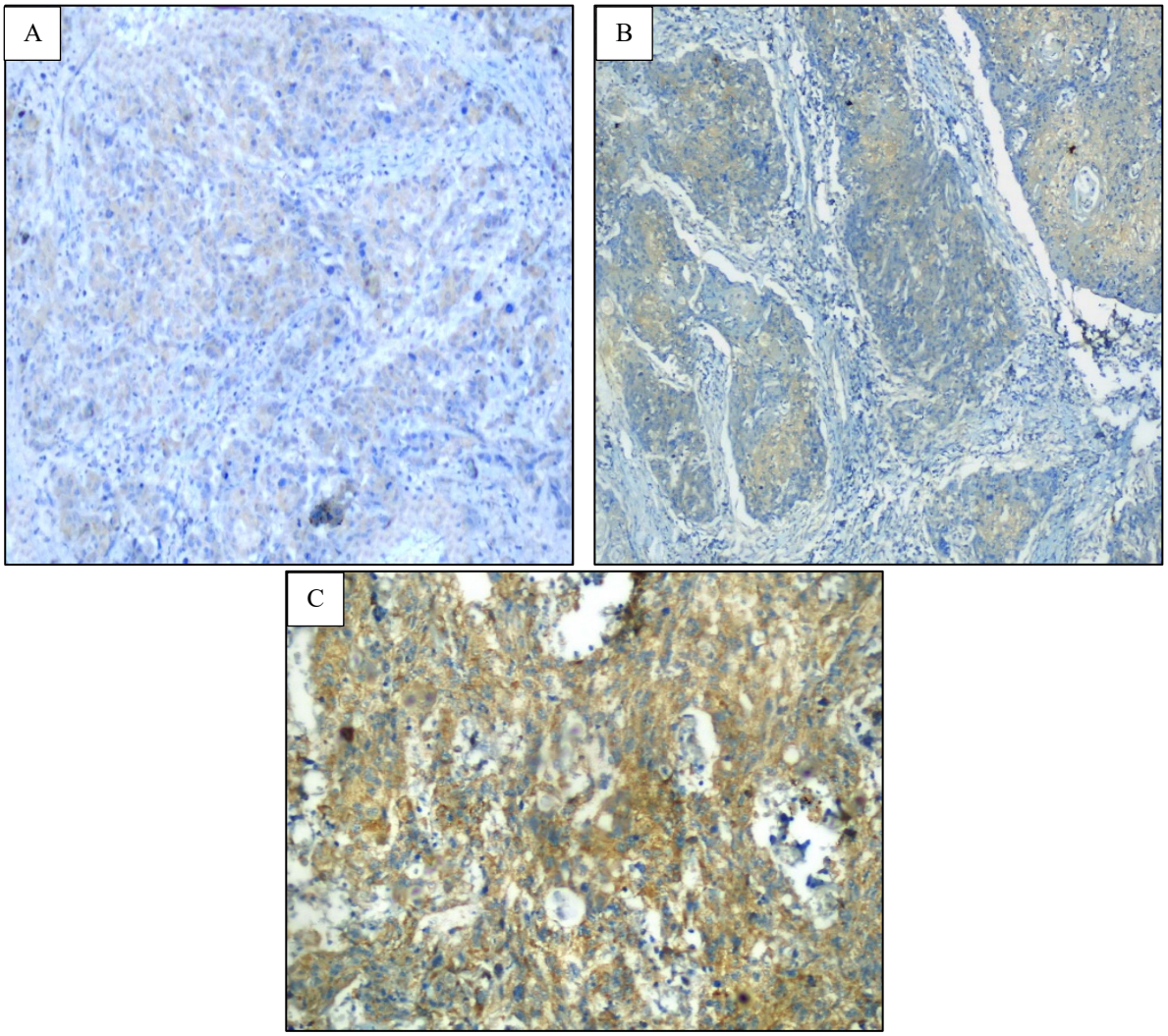


Fig.5: Immunohistochemical expression of IDH1 in poorly differentiated SCC; (A) Weak cytoplasmic staining (x200 original magnification), (B) Moderate cytoplasmic staining (x100 original magnification) & (C) Strong cytoplasmic staining (x200 original magnification).

Table 2. Association of IDH1 immunostaining intensity and patients' clinicopathologic variables

Clinico-Pathological Features	IDH1 Intensity					P- value
	Negative n (%)	Weak n (%)	Moderate n (%)	Strong n (%)	Total n (%)	
Age						
<59	5 (18.5)	9 (33.3)	11 (40.7)	2 (7.4)	27 (41.5)	0.06
≥59	4 (10.5)	12 (31.6)	15 (39.5)	7 (18.4)	38 (58.5)	
Sex						
Male	9 (14.3)	20(31.8)	25 (39.7)	9 (14.3)	63 (96.9)	0.844
Female	0 (0)	1(50)	1 (50)	0 (0)	2 (3.1)	
Smoking						
No	2 (13.3)	7 (46.7)	5 (33.3)	1 (6.7)	15 (23.1)	0.534
Yes	7 (14)	14 (28)	21 (42)	8 (16)	50 (76.9)	
Lesion size						
< 3.4cm	6 (27.3)	7 (31.8)	7 (31.8)	2 (9.1)	22 (33.8)	0.07
≥ 3.4cm	3 (6.9)	14 (32.6)	19 (44.2)	7 (16.3)	43 (66.2)	

Clinico-Pathological Features	IDH1 Intensity					P- value
Site						
Glottic	6 (20)	7 (23.3)	11 (3.7)	6 (20)	30 (46.2)	
Glottic-subglottic	0 (0)	1 (25)	2 (50)	1 (25)	4 (6.2)	
Glottic-supraglottic	0 (0)	1 (20)	4 (80)	0 (0)	5 (7.7)	0.291
Subglottic	0 (0)	3 (75)	1 (25)	0 (0)	4 (6.2)	
Supraglottic	2 (11.8)	5 (29.4)	8 (47.1)	2 (1108)	17 (26.2)	
Trans-glottic	1 (20)	4 (80)	0 (0)	0 (0)	5 (7.7)	
Type of Specimen						
Laryngoscopic biopsy	6 (33.3)	7 (38.9)	5 (27.8)	0 (0)	18 (27.7)	0.009*
Radical laryngectomy	3 (6.4)	14(29.8)	21 (44.7)	9 (19.1)	47 (72.3)	
Invasion Status						
No	5 (35.7)	5 (35.7)	4 (28.6)	0 (0)	14 (21.5)	0.026*
Yes	4 (7.8)	16(31.4)	22 (43.1)	9 (17.6)	51 (78.5)	
Histopathological Progression and Grade						
Dysplasia	3 (37.5)	4 (50)	1 (12.5)	0 (0)	8 (12.3)	
Squamous cell carcinoma in situ	2 (33.3)	1 (16.7)	3 (50)	0 (0)	6 (9.2)	
Microinvasive SCC	1(25)	2 (50)	1 (25)	0 (0)	4 (6.2)	0.033*
Moderately differentiated SCC	3 (8.6)	10(28.6)	18 (51.4)	4 (11.4)	35 (53.8)	
Poorly differentiated SCC	0 (0)	4 (33.3)	3 (25)	5 (41.7)	12 (18.5)	
Necrosis (in the radical laryngectomy specimens)						
Absent	2 (6.1)	10(30.3)	15 (45.6)	6 (18.2)	33 (70.2)	0.992
Present	1 (7.1)	4 (28.6)	6 (42.9)	3 (21.4)	14 (29.8)	
Lympho-Vascular Emboli (in the radical laryngectomy specimens)						
Absent	2 (6.5)	11(35.5)	12 (38.7)	6 (19.4)	31 (66)	0.632
Present	1 (6.3)	3 (18.8)	9 (56.3)	3 (18.8)	16 (34)	
Peri-Neural Invasion (in the radical laryngectomy specimens)						
Absent	2 (4.4)	14(31.1)	20 (44.4)	9 (20.1)	45 (95.7)	0.64
Present	1 (50)	0 (0)	1 (50)	0 (0)	2 (4.3)	
Thyroid Cartilage Infiltration (in the radical laryngectomy specimens)						
Free	1 (5.9)	10(58.8)	5 (29.4)	1 (5.9)	17 (36.2)	0.01*
Infiltrated	2 (6.7)	4 (13.3)	16 (53.3)	8 (26.7)	30 (63.8)	
Margin infiltration (in the radical laryngectomy specimens)						
Free	2 (4.9)	13(31.7)	18 (43.9)	8 (19.5)	41 (87.2)	0.659
Infiltrated	1 (16.7)	1 (16.7)	3 (50)	1 (16.7)	6 (12.8)	
pT Stage						
Tis	2 (33.3)	1 (16.7)	3 (50)	0 (0)	6 (10.5)	
T1	1 (25)	2 (50)	1 (25)	0 (0)	4 (7.1)	
T2	1 (6.3)	8 (50)	6 (37.5)	1 (6.3)	16 (28.1)	0.165
T3	1 (4.8)	5 (23.8)	11 (52.4)	4 (19)	21 (36.8)	
T4a	1 (10)	1 (10)	4 (40)	4 (40)	10 (17.5)	
pN Stage (in the radical laryngectomy specimens)						
Nx	1 (12.5)	2 (25)	4 (50)	1 (12.5)	8 (17)	
N0	1 (4.2)	9 (37.5)	8 (33.3)	6 (25)	24 (51.1)	
N1	1 (25)	0 (0)	2 (50)	1 (25)	4 (8.5)	
N2a	0 (0)	1 (100)	0 (0)	0 (0)	1 (2.1)	0.459

Clinico-Pathological Features	IDH1 Intensity					P- value
N2b	0 (0)	1 (33.3)	2 (66.6)	0 (0)	3 (6.4)	
N2c	0 (0)	1 (50)	0 (0)	1 (50)	2 (4.3)	
N3b	0 (0)	0 (0)	5 (100)	0 (0)	5 (10.6)	
Metastasis (in the invasive squamous cell carcinoma cases)						
No	4 (8.5)	15(31.9)	20 (42.6)	8 (17)	47 (92.2)	0.902
Yes	0 (0)	1 (25)	2 (50)	1 (25)	4 (7.8)	
Chemo and/or Radiotherapy						
No	5 (21.7)	10(43.5)	7 (30.4)	1 (4.3)	23 (35.4)	0.106
Yes	4 (9.5)	11(26.2)	19 (45.2)	8 (19.1)	42 (64.6)	
Recurrence						
No	8 (15.1)	20(37.7)	17 (32.1)	8 (15.1)	53 (81.5)	0.05*
Yes	1 (8.3)	1 (8.3)	9 (75)	1 (8.3)	12 (18.5)	

Table 3. Association of IDH1 immunostaining H-score and patients' clinicopathologic variables

Clinico-Pathological Features	IDH1 H-score			P- value
	Low n (%)	High n (%)	Total n (%)	
Age				
<59	12 (44.4)	15(55.6)	27 (41.5)	0.099
≥59	13 (34.2)	25(65.8)	38 (58.5)	
Sex				
Male	24 (38.1)	39(61.9)	63 (96.9)	0.733
Female	1 (50)	1 (50)	2 (3.1)	
Smoking				
No	7 (46.7)	8 (53.3)	15 (23.1)	0.456
Yes	18 (36)	32 (64)	50 (76.9)	
Lesion size				
< 3.4cm	12 (54.5)	10(45.5)	22 (33.8)	0.362
≥ 3.4cm	13 (30.2)	30(69.8)	43 (66.2)	
Site				
Glottic	12 (40)	18 (60)	30 (46.2)	
Glottic-subglottic	0 (0)	4 (100)	4 (6.2)	
Glottic-supraglottic	0 (0)	5 (100)	5 (7.7)	0.016*
Subglottic	2 (50)	2 (50)	4 (6.2)	
Supraglottic	6 (35.3)	11(64.7)	17 (26.2)	
Trans-glottic	5 (100)	0 (0)	5 (7.7)	
Type of Specimen				
Laryngoscopic biopsy	12 (66.6)	6 (33.3)	18 (27.7)	0.004*
Radical laryngectomy	13 (27.7)	34(72.3)	47 (72.3)	
Invasion Status				
No	9 (64.3)	5 (35.7)	14 (21.5)	0.025*
Yes	16 (31.4)	35(68.6)	51 (78.5)	
Histopathological Progression and Grade				
Dysplasia	6 (75)	2 (25)	8 (12.3)	
Squamous cell carcinoma in situ	3 (50)	3 (50)	6 (9.2)	

Clinico-Pathological Features	IDH1 H-score			P- value
Microinvasive SCC	3 (75)	1 (25)	4 (6.2)	0.036*
Moderately differentiated SCC	11 (31.4)	24(68.6)	35 (53.8)	
Poorly differentiated SCC	2 (16.7)	10(83.3)	12 (18.5)	
Necrosis (in the radical laryngectomy specimens)				
Absent	9 (27.3)	24(72.7)	33 (70.2)	0.927
Present	4 (28.6)	10(71.4)	14 (29.8)	
Lympho-Vascular Emboli (in the radical laryngectomy specimens)				
Absent	10 (32.3)	21(67.7)	31 (66)	0.327
Present	3 (18.7)	13 (81.3)	16 (34)	
Peri-Neural Invasion (in the radical laryngectomy specimens)				
Absent	12 (26.7)	33(73.3)	45 (95.7)	0.47
Present	1 (50)	1 (50)	2 (4.3)	
Thyroid Cartilage Infiltration (in the radical laryngectomy specimens)				
Free	7 (41.2)	10(58.8)	17 (36.2)	0.119
Infiltrated	6 (20)	24 (80)	30 (63.8)	
Margin infiltration (in the radical laryngectomy specimens)				
Free	11 (26.8)	30(73.2)	41 (87.2)	0.739
Infiltrated	2 (33.3)	4 (66.6)	6 (12.8)	
pT Stage				
Tis	3 (50)	3 (50)	6 (10.5)	
T1	3 (75)	1 (25)	4 (7.1)	
T2	5 (31.3)	11(68.7)	16 (28.1)	0.296
T3	6 (28.6)	15(71.4)	21 (36.8)	
T4a	2 (20)	8 (80)	10 (17.5)	
pN Stage (in the radical laryngectomy specimens)				
Nx	3 (37.5)	5 (62.5)	8 (17)	
N0	7 (29.2)	17(70.8)	24 (51.1)	
N1	1 (25)	3 (75)	4 (8.5)	
N2a	0 (0)	1 (100)	1 (2.1)	0.774
N2b	1(33.3)	2 (66.6)	3 (6.4)	
N2c	1 (50)	1 (50)	2 (4.3)	
N3b	0 (0)	5 (100)	5 (10.6)	
Metastasis (in the invasive squamous cell carcinoma cases)				
No	15 (31.9)	32(68.1)	47 (92.2)	0.775
Yes	1 (25)	3 (75)	4 (7.8)	
Chemo and/or Radiotherapy				
No	13 (56.5)	10(43.5)	23 (35.4)	0.027*
Yes	12 (28.6)	30(71.4)	42 (64.6)	
Recurrence				
No	23 (43.4)	30(56.6)	53 (81.5)	0.086
Yes	2 (16.7)	10(83.3)	12 (18.5)	

Among the 12 patients with recurrence and the 53 patients without recurrence, moderate (75%; 9 cases) and weak (38%; 20 cases) IDH1 expression were the most frequent, respectively. In addition, high IDH1 H-scores were most frequently observed in both patients with recurrence (83%; 10 cases) and without recurrence (57%; 30 cases). A significant correlation was observed between disease recurrence and IDH1 expression intensity, but not with H-score ($p = 0.05$ and $p = 0.086$, respectively).

Moreover, patients who received chemotherapy and/or radiotherapy exhibited longer survival, with the maximum time to death being 24 months, compared with 18 months among those who did not receive treatment.

Among patients who received and those who did not receive chemotherapy and/or radiotherapy, moderate (45%; 19 cases) and weak (43%; 10 cases) IDH1 expression levels were most frequently exhibited, respectively. Furthermore, a high IDH1 H-score was predominantly observed in patients who underwent chemotherapy and/or radiotherapy (71%; 30 cases), whereas a low IDH1 H-score was most frequently identified among patients who did not receive such treatment (57%; 13 cases). Statistical analysis revealed a significant association between receiving chemotherapy and/or radiotherapy and IDH1 expression H-score, but not intensity ($p = 0.027$ and 0.106, respectively).

Twenty patients had died by the end of follow-up in December 2024; according to Kaplan–Meier survival analysis, the 2-year OS and DFS rates were 69% and 81.5%, respectively.

Our analysis demonstrated that the maximum disease-free survival duration was 24 months among patients who received chemotherapy and/or radiotherapy, compared with 10 months in those who did not receive these treatments, indicating a potential survival benefit (Figure 6).

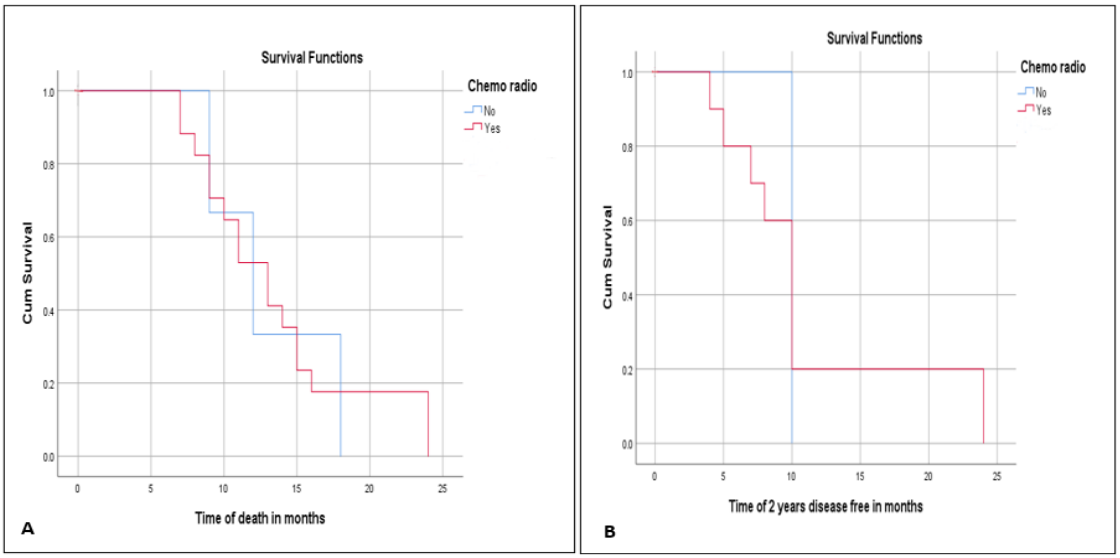


Fig 6. Kaplan–Meier curve shows; (A) Patients who received chemotherapy and/or radiotherapy had a longer survival duration, with the longest time to death occurring after 24 months, compared to 18 months in those who did not receive such treatment & (B) The maximum disease-free survival duration was 24 months among patients who received chemotherapy and/or radiotherapy, compared to 10 months in those who did not receive these treatments.

Patients who received chemotherapy and/or radiotherapy had a longer survival duration, with the longest time to death occurring after 24 months, compared with 18 months in those who did not receive such treatment (Figure 6).

Data analysis revealed that the 2-year overall survival and disease-free survival rates were higher in IDH1-positive cases. Half of the patients who died in this study moderately expressed IDH1, and 65% of them exhibited a high IDH1 H-score. Despite this, no significant association was identified between overall survival and either IDH1 expression intensity (log-rank $\chi^2 = 2.253$, $p = 0.522$) or H-score (log-rank $\chi^2 = 0.035$, $p = 0.851$) (Figure 7). Likewise, IDH1 expression intensity and H-score were not significantly associated with disease-free survival (log-rank $\chi^2 = 4.132$, $p = 0.248$; and log-rank $\chi^2 = 1.473$, $p = 0.225$, respectively) (Figure 8).

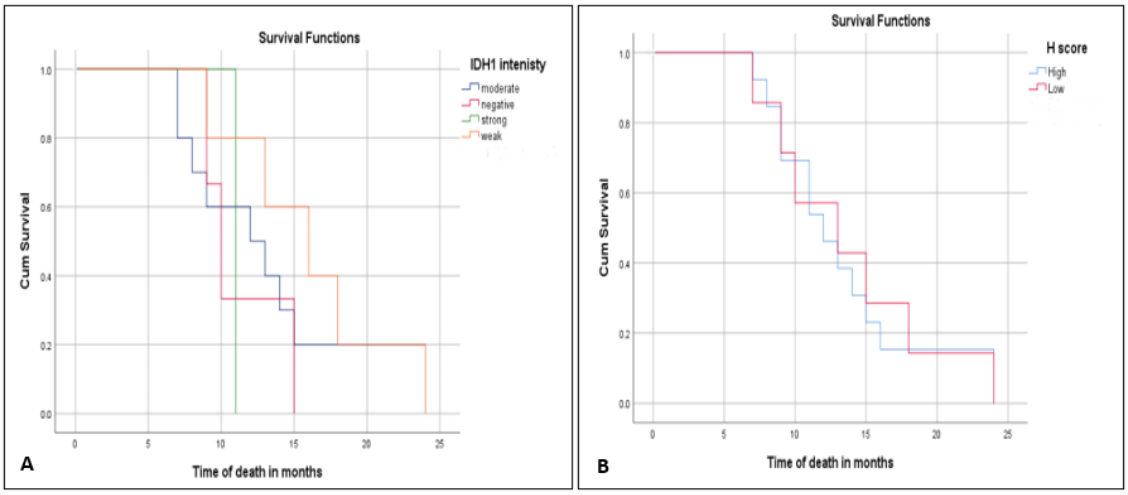


Fig.7: Kaplan–Meier curve shows 2 years survival estimate categorized by (A) IDH1 intensity (log-rank x2: 2.253, p value=0.522) & (B) H score (log-rank x2: 0.035, p value=0.851).

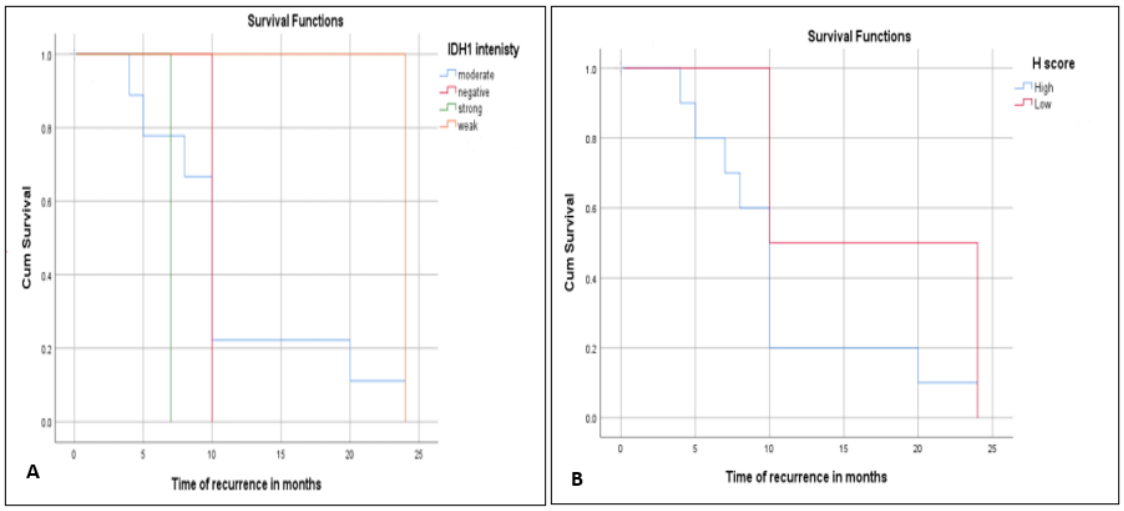


Fig.8: Kaplan–Meier curve shows 2 years disease free estimate categorized by (A) IDH1 intensity (log-rank x2: 4.132, p value=0.248) & (B) H score (log-rank x2: 1.473, p value=0.225).

Discussion

About 90%–95% of all laryngeal malignancies are laryngeal squamous cell carcinomas (LSCCs), making them the most common type of head and neck cancer (23). Considering the limitations of currently available biomarkers, there is a critical need to identify more effective biomarkers to better guide treatment decisions and enable accurate monitoring of recurrence and metastasis (13).

The present study involved 65 patients with laryngeal pre-invasive and invasive squamous cell lesions that were diagnosed either by laryngoscopic biopsy or laryngectomy specimens, representing various histopathological diagnoses, and were assessed for IDH1 expression using immunohistochemistry. Furthermore, the study examined the relationship between the expression of this biomarker and available clinicopathological variables. In this work, most of the cases (65.2%) expressed IDH1. This aligns with findings by Shayanfar et al., 2022, and Shayanfar et al., 2023, who observed that 76% and 90% of their studied LSCC cases showed IDH1 immunohistochemical expression, respectively (21,13).

Moreover, we found that 13.8%, 40%, and 32.3% of the studied cases showed strong, moderate, and weak IDH1 cytoplasmic immunostaining, respectively. This is somewhat different from the figures reported by Shayanfar et al., 2022, and Shayanfar et al., 2023, who found that 46%, 30%, and 24%, and 42%, 34%, and 14% of the enrolled cases showed strong, moderate, and weak IDH1 cytoplasmic immunostaining,

respectively (21,13). Most of our studied cases showed high expression of IDH1 (61.5%), whereas lower IDH1 expression was found in 38.5% of cases. This coincides with what was observed by Shayanfar et al., 2022, and Wang et al., 2025, who stated that 78% and 55% of enrolled LSCC cases showed high IDH1 expression (13,25). In contrast, most of the cases studied by Shayanfar et al., 2023 (58%) exhibited low IDH1 expression, and 42% exhibited high IDH1 expression (13).

This study was not completely equivalent to previous studies, which included invasive LSCC patients only without pre-invasive squamous lesions (mucosal dysplasia and squamous cell carcinoma in situ), whereas our investigation revealed a statistically significant relation between histopathological progression and grade of the studied laryngeal squamous lesions and both IDH1 expression intensity and H-score ($p = 0.033$ and 0.036).

Similarly, the studies by Shayanfar et al., 2022, and Shayanfar et al., 2023 highlighted a significant association between histological grade and intensity of IDH1 expression ($p < 0.001$ for both), with no significant relationship between histological grade and H-score of IDH1 expression. Notably, Shayanfar et al., 2023 found that lower cytoplasmic IDH1 expression was associated with higher histological grades, which contrasts with our findings. In alignment with Shayanfar et al., 2022, our study demonstrated that increased IDH1 expression correlated with higher histological grades (21,13). This discrepancy may be attributed to several factors, including the unique inclusion of pre-invasive lesions in our study, differences in antibody interpretation methodologies, or underlying biological heterogeneity of IDH1 expression across tumor grades. Wang et al., 2025 found that IDH1 expression level and histologic grade were not significantly related ($p = 0.445$) (25).

Moreover, our analysis did not find a significant association between either IDH1 expression intensity or H-score and perineural invasion ($p = 0.64$ and 0.47, respectively). However, the findings of the studies conducted by Shayanfar et al. in 2022 and 2023, and Wang et al. in 2025 showed a significant relationship between perineural invasion and level of IDH1 expression ($p = 0.046$, 0.019, and 0.045, respectively) (21,13,25).

Considering type of specimen, invasion status, and thyroid cartilage infiltration of the enrolled cases, a significant relationship was demonstrated between these factors and both IDH1 expression intensity ($p = 0.009$, 0.026, and 0.033, respectively) and H-score ($p = 0.004$, 0.025, and 0.01, respectively). To our knowledge, no prior research has demonstrated these associations.

In addition, lesion site and recurrence were significantly related to IDH1 expression H-score ($p = 0.016$) and intensity ($p = 0.05$), respectively. Moreover, IDH1 expression H-score was linked to planning of chemotherapy and/or radiotherapy ($p = 0.027$). Nearly identical results were reported by Wang et al. in 2025, who observed no correlation with receiving chemotherapy ($p = 0.659$) but a strong correlation between IDH1 expression levels and lesion site and receiving radiation ($p < 0.001$ and 0.023, respectively) (25).

The findings of the investigations conducted by Shayanfar et al. in 2022 and 2023 demonstrated a substantial correlation between IDH1 expression and lymph node involvement ($p = 0.014$ and < 0.001, respectively) (20,13); however, we failed to find any significant correlation regarding this association. This might be explained by the fact that not all our examined samples were radical laryngectomy specimens, compared with the aforementioned studies, in which all examined specimens were radical laryngectomies that included lymph nodes.

Among the factors affecting laryngeal squamous lesion prognosis, pathological tumor stage and lymphovascular invasion were not related to either IDH1 expression intensity or H-score, which agrees with what was stated by Shayanfar et al., 2022, Shayanfar et al., 2023, and Wang et al., 2025 (20,13,24).

Data analysis revealed that the 2-year overall survival and disease-free survival rates were higher in IDH1-positive cases. The disproportionately small number of IDH1-negative individuals in our group compared with IDH1-positive cases may help explain this finding. IDH1 expression intensity and H-score did not predict either patient disease-free survival ($p = 0.248$ and 0.225, respectively) or overall survival ($p = 0.522$ and 0.851, respectively) in our cohort.

Our findings demonstrate that elevated IDH1 expression is significantly associated with tumor invasion but not with overall survival. This suggests that IDH1 may contribute primarily to local invasive behavior rather than long-term disease progression. One possible explanation is that IDH1-mediated metabolic reprogramming enhances cellular motility and matrix degradation without directly influencing resistance to therapy or metastatic potential.

While this correlation has not been assessed in other comparative studies on laryngeal squamous cell lesions, it has been investigated in other tumor types, such as gliomas, leukemia, chondrosarcoma, and intrahepatic cholangiocarcinoma. Although some studies align with our findings, variations do exist. For instance, Rimini et al., 2022 reported no statistically significant correlation between IDH1 mutation in cholangiocarcinoma and overall survival (OS) or disease-free survival (DFS) ($p = 0.26$ and 0.61, respectively) (25). Cho et al., 2012 also did not find a significant relation between IDH1 mutation and overall survival in glioblastoma cases (26). Wang et al., 2025 stated that, by Kaplan–Meier curve analysis, in head and neck squamous cell carcinoma, higher IDH1 expression levels were associated with a lower overall survival rate (24). Similarly, Tlemsani et al., 2024 found a negative impact of IDH1 mutation in

chondrosarcoma on patient overall survival (p = 0.03) (27).

On the other hand, Cho et al., 2012, Houillier et al., 2010, Myung et al., 2012, and Husain et al., 2023 stated that IDH1 mutation was associated with prolonged overall survival in low-grade gliomas (p = 0.016, 0.002, < 0.05, and 0.00, respectively) (26,28-30).

The discrepancy between invasion and survival associations may reflect the multifactorial nature of tumor progression, where other genetic or microenvironmental factors dominate prognosis.

Future research should focus on elucidating the molecular mechanisms by which IDH1 promotes invasion and on assessing whether targeting IDH1 can limit local spread without adversely affecting normal cellular metabolism. Larger, multicenter studies are also needed to confirm these observations and to evaluate IDH1 potential as a biomarker for invasive behavior.

Conclusion

Our study demonstrated that higher IDH1 expression was associated with invasion, increased histological progression, higher grades of laryngeal squamous cell lesions, recurrence, as well as patients who were planned for chemotherapy and/or radiotherapy. However, IDH1 expression did not predict either disease-free survival (DFS) or overall survival (OS) in our cohort. These findings offer hypothesis-generating insights into the complex biological role of IDH1 in LSCC. While not prognostic in terms of survival outcomes, increased IDH1 expression may be associated with features of aggressive tumor behavior and could hold clinical significance, potentially serving as a future therapeutic target in laryngeal squamous cell lesions.

Nevertheless, the study has some limitations, including the relatively small sample size and the current lack of validated biomarkers to fully assess the predictive significance of IDH1 in LSCC. To address these gaps, we recommend extending the clinical follow-up period and investigating IDH1 molecular pathways, testing inhibitors, validating results in larger cohorts, and investigating additional biomarkers. This approach may better inform treatment decisions and improve monitoring for recurrence and metastasis.

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Authors' Contributors

All authors contributed significantly to the study: Nora Tawhid Abdelfattah participated in reading the slides, interpreting results, analyzing data, and revising the manuscript. Abeer Mohammad Amal contributed to the study design, data collection, result interpretation, and data analysis and participated in writing the manuscript. Sara Mohamad Abou-fandoud contributed to the research concept, data collection, and result interpretation and participated in writing and revising the manuscript. Asmaa Mohamed Saad Zaghloul Attaalla contributed to the follow-up of cases & their neoadjuvant treatment.

Data Availability

The datasets generated and analyzed during the current study are not publicly available; however, the data can be shared for research and authentication purposes upon reasonable request.

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Ethics Approval

This study protocol was approved by the Kasr Alainy Research Ethics Committee (REC) of the Faculty of Medicine, Cairo University, and conducted following ICH GCP standards, as well as relevant local and institutional regulations and guiding principles governing REC operations (code: N-184-2024, approved on 2024). A waiver of consent was obtained from the Kasr Alainy Research Ethics Committee (REC) as this study of stored existing pathology specimens.

Conflict of Interest

The authors declared no conflict of interest.

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