Determination of Isocitrate Dehydrogenase (IDH1) mutation in laryngeal squamous cell carcinoma specimens

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Abstract

Introduction: Laryngeal squamous cell carcinoma (LSCC) is a significant cause of mortality and morbidity among cancers. To date, no tools have been identified to predict the evolution of this cancer. There is an interest in recognizing new biomarkers that can help in the early detection and prediction of the clinical outcome of this cancer. Therefore, the present study evaluates the Isocitrate Dehydrogenase 1 (IDH1) mutation in LSCC patients.

Methods: In this study, 50 patients with LSCC were studied. Immunohistochemistry was used to assess the tissue expression of IDH1. In samples reported negative or poor positive in IHC staining, gene sequencing using the PCR technique was used to confirm the diagnosis. The relationship between IDH1 mutation and tumor differentiation, pathological tumor stage, lymph node involvement, lymphovascular and perineural involvement, tumor size, age, and sex of the patient were evaluated.

Results: The mean age of patients was 61 ± 8.46 years, of which 98% were male. The frequency of IDH1 mutation was 78%. The relationship between the pathological stage and lymphovascular invasion status of the tumor and IDH1 status was not statistically significant. In contrast, the relationship between the degree of differentiation, lymphatic involvement, perineural invasion status, and IDH1 status showed a statistically significant correlation ($p < 0.05$).

Conclusion: This study showed a high frequency of IDH1 mutation in patients with LSCC. The IDH1 mutation was also associated with more aggressive tumor behavior and perineural involvement. These findings can help refine potential treatment and monitoring strategies in LSCC patients.

1. Introduction

Laryngeal squamous cell carcinoma (LSCC) is an important cause of mortality and morbidity among different types of cancer. It was established that about 4,000 deaths annually in the United States alone (1, 2). Radiotherapy of organs and chemoradiotherapy are the only known standard treatment for LSCC patients (3). However, relapse is high for a variety of reasons (4). After treatments, the five-year survival has varied between 40 and 70 percent (5). Especially in patients with high treatment resistance, a highly weak prognosis will be expected for treatment (6–8).

Several genetic mutations have been evaluated and finally confirmed. IDH1 plays an important role in the metabolism of various substances such as glucose, fatty acids, and glutamine and has an important role in maintaining cellular structure (9).

Recent studies on IDH1 in different types of cancers identify different types of mutations. The gene has become an IDH1 code-holder. In this regard, such mutations have been identified in different kinds of cancers, including glioblastoma, acute myeloid leukemia, chondroblastoma, intrahepatic cholangiocarcinoma, melanoma, and esophageal carcinoma (10–12). These studies have well described the relationship between mutations in the IDH1 gene, tumorigenesis, and tumor progression. Also, it has

been shown that the wild form of this gene has been associated with the growth and proliferation of tumoral cells (13). The relationship between protein biomarkers associated with this gene mutation and clinical manifestations, as well as the consequences for patients with a tumor, has been confirmed clinically and molecularly (14).

Mutations in the IDH1 gene and changes expression of this gene as a prognostic factor for predicting progression, recurrence, and tumor-related outcomes and target therapy has been used. It has been shown that increased gene expression in patients with squamous cell carcinoma had a higher increase than in healthy people (15, 16). To date, there has been a study of the behavior of the IDH1 gene or its mutations with the laryngeal squamous cell carcinoma is not performed. Therefore, the present study evaluated IDH1 gene mutation in patients with laryngeal squamous cell carcinoma.

2. Methods

2.1. Patient characteristics and tumor samples

A total of 50 formalin-fixed paraffin-embedded tissues from LSCC samples (signet ring cell and intestinal subtypes) were collected from the pathology lab of Rasoule-e-Akram hospital, Tehran, Iran, from 2019 to 2018. The patients with primary LSCC who had undergone laryngectomy surgery but had not received either chemotherapy or radiotherapy were included. Besides, the hematoxylin and eosin (H & E) stained slides and medical records of enrolled patients were collected to acquire clinicopathological data including gender, age, tumor size, differentiation, TNM stage, lymph node involvement, perineural invasion, and lymphovascular invasion.

2.2. Tissue microarray (TMA) construction

Concisely, the most representative regions of tumor and normal were selected by an expert pathologist (N.S.) through assessing hematoxylin and eosin slides. Then, using precision arraying equipment (Tissue Arrayer Mini core; ALPHELYS, Plaisir, France), a core of 0.6 mm diameter was removed from the selected areas in each donor block and transferred to a new recipient paraffin block. In the current study, three cores were punched and assessed from each tumor and scored separately. TMA slides were obtained by cutting sections of TMA blocks to a thickness of about 4 micrometers, which were transferred to an adhesive slides system (SuperFrost Plus, Termo Scientifc™, Germany). Next, TMA blocks were constructed in three copies for each specimen. In order to increase the accuracy and validity, the mean overall histochemical score (H score) value of three cores was calculated as final scores.

2.3. Immunohistochemistry staining

The expression of IDH1 at the protein level was assessed using TMA-based IHC. There have been multiple processes to do IHC. In the first step, all the slides were deparaffinized after 30 min at 60°C and then rehydrated in xylene and serial ethanol dilutions. In order to blockage of endogenous peroxidase activity, H202 0.3% was used for 20 min at room temperature. After washing the tissue sections three

times in Tris Buffered Saline (TBS), antigen retrieval was executed by covering the tissues in Tris/EDTA (pH 9.0) for 10 min in an autoclave.

Consequently, all tissue sections were incubated with the primary antibody specific to IDH1 (1:500 dilution, Abcam: ab109215, Cambridge, UK) for one overnight at 4°C. The next day, after three times washing in TBS for visualizing bound antibody-antigen, TMA slides were incubated with the secondary antibody (Envision, standard Envision HRP kit, Biopharmadx) for 30 min and then 3, 30-diaminobenzidine as chromogen (DAB, Dako, Glostrup, Denmark). Then, for visualization of the antigen, TMA sections were incubated with hematoxylin (Dako, Glostrup, Denmark). Finally, the tissues slides were dehydrated in alcohol, washed in xylenes, and prepared for scoring by pathologists. The normal liver tissue was applied as the positive control while, for the negative control, TBS was utilized in place of the primary antibody. Besides, rabbit immunoglobulin IgG (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was used as an isotype control test.

2.4. Evaluation of immunostaining

The staining of IDH1 on TMA LSCC sections were assessed by the pathologist (N.S.) by applying a semi-quantitative scoring system that is blinded to the clinicopathological characteristics of the patients. There are three scoring systems that are utilized for the expression of IDH1, namely the intensity of staining, the percentage of positive tumor cells, and the H-score. The intensity of staining ranging from 0 to 3 scores as follows: 0 = negative or non-staining, 1 = weak, 2 = moderate, and 3 = strong. The percentage of positive cells was classified into four groups comprising Group 1:<25% positive cells, Group 2: 25–50% positive cells, Group 3: 51–75% positive cells, and Group 4:>75% positive cells. Finally, the histochemical score (H score) was attained by multiplying the staining intensity in the percentage of positive tumor cells, ranging from 0 to 300. In this study, the H-scores were categorized into two groups, including 0-200 as the low expression group and 201–300 as the high expression group.

2.5. Statistical analysis

The analysis of all data were performed by the “statistical software SPSS, version 25.0 (SPSS, Inc., IBM Corp, USA). The categorical and quantitative data were reported by N (%) as valid percent and mean (SD) or median (Q1, Q3), respectively. Pearson’s $\chi 2$ test was applied to analyze the association and correlation between IDH1 protein expression and clinicopathological parameters. Furthermore, the pairwise comparison between the groups of the study was executed by Kruskal–Wallis, and Mann–Whitney U tests. The statistically significant difference in all sections was considered as $p < 0.05$.

3. Results

Patients’ characteristics

In this cross-sectional study, the sample population comprised a total of 50 LSCC patients with mean age 61 years. Of them, 49 cases are men (98.0%) and 1 case is woman (2.0%). The clinicopathological characteristics of our samples are summarized in Table 1.

Table 1. Patients and tumor clinicopathological characteristic of laryngeal squamous cell carcinoma

Patients and tumor characteristics	SCC laryngeal, N (%)
Number of samples	50
Mean age, years (range)	61 (44-78)
Sex	
Male	49 (98.0)
Female	1 (2.0)
Mean tumor size (cm) (rang)	3.98 (1.5-7)
Histological grade	
I (well differentiated)	30 (60.0)
II (moderate differentiated)	18 (36.0)
III (poor differentiated)	1 (2.0)
Primary tumor (PT) stage	
pT1	0 (0.0)
pT2	10 (20.0)
pT3	21 (42.0)
pT4	15 (30.0)
pT4a	3 (6.0)
Lymph node involvement	
Yes	15 (30.0)
No	35 (70.0)
Lymphovascular invasion	
Present	2 (4.0)
Absent	48 (96.0)
Perineural invasion	
Present	11 (22.0)
Absent	39 (78.0)
Intensity of staining of IHC	
Negative (0) or Weak (+ 1)	12 (24.0)
Positive (+ 2)	15 (30.0)

Strong positive (+ 3)	23 (46.0)
PCR	
Positive	1 (2.0)
Negative	11 (22.0)

Evaluation of IDH1 protein expression of LSCC

The expression level of IDH1 protein was appraised using the IHC technique on TMA sections by three scoring methods as follows: intensity of staining, percentage of positive tumor cells, and H-score (Fig.1). Our findings showed that 39 LSCC patients have high expression of IDH1 and 11 patients have Low expression of IDH1.

Associations between IDH1 protein expression and clinicopathological characteristics in LSCC

Pearson's χ2 demonstrated a significant association between IDH1 protein expression and histological grade (*P <0.001*) and lymph node invovlement (*P = 0.014*). Furthermore, our analysis showed a significant correlation between high expression of IDH1 and perineural invasion (*P = 0.046*). The association of expression of IDH1 protein and all clinicopathological characteristics of patients with LSCC were described in Table 2.

Table 2. The association between low and high IDH1 protein expression and clinicopathological characteristic of laryngeal squamous cell carcinoma samples

Characteristics of tumor	Expression of IDH1 N (%)		*P value*
	High = 39	**Low = 11**	
Mean age, years (SD)	61 (9)	60 (6)	0.624
Sex			
Male	38 (78.6)	11 (22.4)	1.000*
Female	1 (100.0)	0 (0.0)	
Mean tumor size (cm) (SD)	3.90 (1.46)	4.24 (1.83)	0.621
Histological grade			
Well differentiated	30 (100.0)	0 (0.0)	***<0.001***
Moderate differentiated	8 (44.4)	10 (55.6)	
Poor differentiated	0 (0.0)	1 (100.0)	
Primary tumor (PT) stage			
pT1	0 (0.0)	0 (0.0)	0.460
pT2	8 (80.0)	2 (20.0)	
pT3	15 (71.4)	6 (28.6)	
pT4	13 (86.7)	2 (13.3)	
pT4a	2 (66.7)	1 (33.3)	
Lymph node involvement			
Yes	15 (100.0)	24 (68.6)	***0.014***
No	0 (0.0)	11 (31.4)	
Lymphovascular invasion			
Present	2 (100.0)	37 (77.1)	1.000
Absent	0 (0.0)	11 (22.9)	
Perineural invasion			
Present	11 (100.0)	28 (71.8)	***0.046***
Absent	0 (0.0)	11 (28.2)	

*Fisher's Exact test

4. Discussion

The effect of IDH1/2 mutations in IDH1 (R132) or IDH2 (R140, R172), for the first time recognized in colorectal cancer (17). These mutations were detected in a wide range of cancers, including 80% of gliomas and glioblastomas (10, 18, 19), 60% of chondrosarcoma (20), 20% of

Intrahepatic chloangiocarcinoma (21), and about 10% of AMLs (22, 23, 24). IDH1 mutation is most common (21, 25–36) and IDH2 mutation exists in 6–11% of cases (21, 37, 38). As far as we know, this is the first study to determine the frequency of IDH1 mutation in LSCC tumor samples. Based on the results of our study, the prevalence of IDH1 mutation in the sample was reported to be 78%, which is similar to the prevalence of this mutation in glioma and glioblastoma (10, 18, 19). There have not shown any significance difference in age and tumor size between samples with IDH1 mutation and non-mutant samples. Among the factors affecting LSCC prognosis, pathological tumor stage was not related to IDH1 mutation. In contrast, in tumor samples with well differentiation, IDH1 mutation were significantly high.

Approximately 90 percent of laryngeal carcinomas are squamous cell carcinoma and are classified into three as good, moderate, and poor differentiation, good, moderate (39). Cancers with poor differentiation usually compared to cancers that have a good differentiation are more metastatic (40).

5. Conclusion

Our study showed that IDH1 expressed high in patients with laryngeal squamous cell carcinoma. Also, IDH1 mutation in these patients was associated with more aggressive tumor behavior and perineural involvement. This could be useful in prognosis and treatment of LSCC.

Declarations

Ethics approval and consent to participate

All procedures performed in this study were in line with the ethical standards of the institution at which this study was conducted. Informed consent was individually obtained from all participants. The Research Ethics Committee of Iran University of Medical Sciences issued IR.IUMS.FMD.REC.1399.276 for this study.

Informed consent was obtained from all individual participants, parents or legally authorized representatives of participants under legal age years old at the time of sample collection with routine consent forms.

Availability of data and materials

The analyzed data during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interests.

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Authors' contributions

N.S, N.E, and F.T. designed and supervised the project, rechecked and approved all parts of the manuscript and data analysis; N.E, and F.T. wrote the manuscript. M.M., E.J. and A.M. collected the paraffin embedded tissues, collected the patient data, and performed IHC experiment. A.Z.M. analyzed and interpreted the SPSS data, helped to prepare the tables. N.S. marked the most representative areas in different parts of the tumor for the construction of TMAs blocks and scored TMAs slides after IHC staining and helped to prepare the figures. All authors read and approved the final manuscript.

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Figures

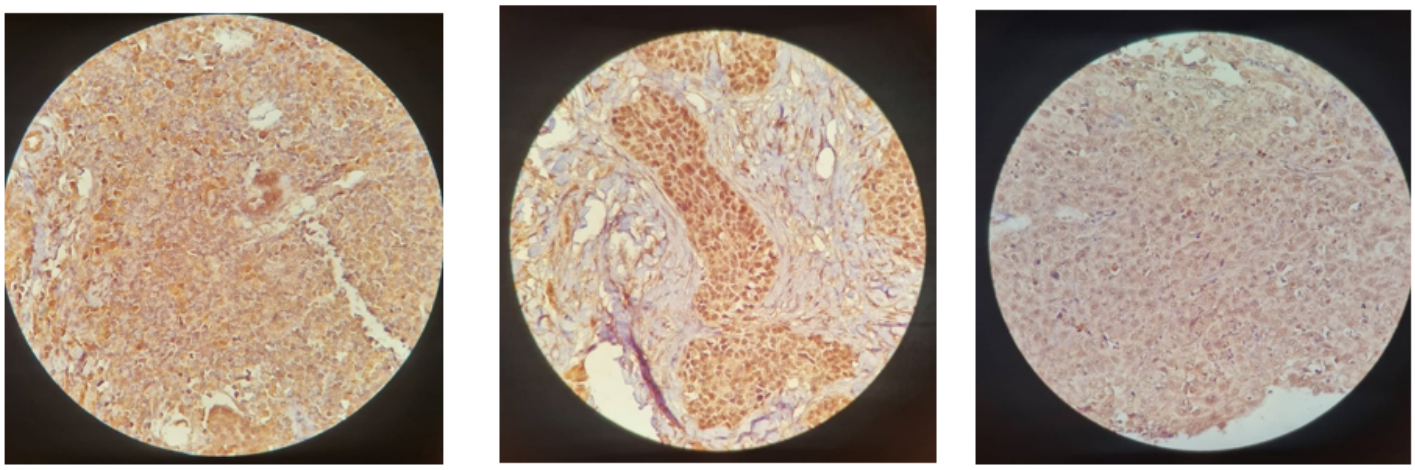

Figure 1

Immunohistochemical expression patterns of TSG101 in laryngeal squamous cell carcinoma (LSCC) tissues