Original Article

Expression of Hypoxia Inducible Factor-1α in Esophageal Squamous Cell Carcinoma and its correlation with clinicopathological parameters

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ABSTRACT

Background: Esophageal cancer is an aggressive tumour carrying a very poor prognosis. HIF-1α (Hypoxia induced factor) is an oxygen sensitive transcription factor which activates transcription of proangiogenic cytokines like VEGF (Vascular Endothelial Growth Factor) responsible for angiogenesis. In the present study we examined the HIF-1α expression in esophageal carcinoma cases and its correlation with various clinicopathological parameters. We also determined the clinical outcomes in such patients with chemoradiotherapy.

Methods: The study was conducted on 50 cases of Esophageal Squamous Cell Carcinomas diagnosed in SGRDIMSAR, Amritsar. Detailed clinical data of the patient was recorded as per proforma attached. Tissue were formalin fixed, paraffin embedded and were studied for histopathological grading after staining with haematoxylin-eosin. All cases were subjected to immunohistochemistry for HIF-1α expression.

Results: The maximum incidence was seen in age group of 41-60 years. Most of the cases were moderately differentiated 34 cases (68%) followed by poorly differentiated and well differentiated consisting of 20% and 12% respectively. The HIF-1α positivity was observed in 34 cases. 16/34 cases were classified as high score group. High HIF-1α expression significantly correlated with poor degree of differentiation (P=0.005), with the lymph node metastasis(P=0.015), lymhpatics invasion(P=0.018) and depth of invasion. Out of 20 patients resistant to therapy, 12 showed high HIF-1α score.

Conclusion: The present study concluded that expression of HIF-1α was significantly correlated with poorer clinicopathological variables including higher grade, lymph node metastasis, lymphatics invasion and increased depth of tumour invasion. It was also determined that HIF-1α expression significantly correlates with an unfavourable prognosis in such patients treated with adjuvant chemoradiotherapy.

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Introduction

Esophageal cancer is an aggressive tumour carrying a very poor prognosis. Esophageal cancer is the eighth most common cancer around the world. In 2008, an estimated 4,82,000 new esophageal cancer cases were diagnosed and 4,07,000 related deaths occurred globally.[1] Esophageal cancers have many associations and risk factors. In Indian studies the role of nitrosamines and nitroso compounds, tobacco, alcohol and dietary/nutritional factors in the causation of esophageal cancer have been evaluated by various researchers.[2,3]

Hypoxia in tumour tissues induces serial changes that promote tumor growth, invasion and metastasis among which the over-expression of HIF-1α (Hypoxia induced factor) is the most predominant regulator of oxygen homeostasis. HIF-1α is an oxygen sensitive transcription factor which activates transcription of various proangiogenic cytokines like VEGF (Vascular Endothelial Growth Factor) responsible for angiogenesis. Evidence has been provided that over-expression of HIF-1α is associated with a malignant phenotype. It has been shown that HIF-1α is a key player in the cancer cells response to low-oxygen tension in a variety of physiologic processes including angiogenesis, tumorigenesis and metastasis. Moreover, hypoxic regions have been shown to be both chemo- and radiation resistant.[4,5,6] HIF-1α expression has been found to be associated with poor clinicopathological factors in various malignancies. HIF-1α positivity can predict lymph node metastasis thus can be useful in the choice of therapeutic strategy in esophageal cancers as lymph node involvement is the most important determinant in such cases.[7] It is seen that the pretreatment evaluation of HIF-1α is a useful and sensitive indicator of response to radiation and chemotherapy in esophageal cancer thus targeting HIF-1α could represent a novel approach to cancer therapy.[7,8]

In the present study we examined the HIF-1α expression in esophageal carcinoma cases and its correlation with various clinicopathological parameters. We also determined the clinical outcomes in such patients with chemoradiotherapy.

Material and Methods

The study was conducted on 50 cases of Esophageal Squamous Cell Carcinomas diagnosed in Sri Guru Ramdas Institute of Medical Sciences And Research (SGRDIMSAR), Amritsar. Detailed clinical data of the patient was recorded as per proforma attached.

Histopathological examination of the tissues obtained was done after processing them to prepare paraffin blocks. Blocks were cut and stained with Haematoxylin and Eosin stain and studied under light microscope for histopathological grading. All the cases were subjected to immunohistochemistry for HIF-1α expression. Immunohistochemistry was performed by using antibodies against HIF -1α (Diagnostic Biosystem). The sections were taken on Poly – L – lysine hydrobromide (0.1%) pre coated slides. The antigen retrieval was done by using pressure cooker method with 10 mmol citrate buffer at ph 6.0 . Tris buffer was used as wash buffer . Endogenous activity was blocked by using hydrogen peroxide. After Protien blocking slides were incubated overnight with anti HIF-1α antibodies and were conjugated with Streptavidin Horse Radish Peroxidase (HRP). DAB (Diaminobenxene tetra hydrochloride) was used as the chromogen. The slides were counterstained with hematoxylin and were examined by light microscopy. For HIF-1α brown nuclei and cytoplasmic staining were taken positive.

1 = Nuclear staining < 1% of cells

2 = Nuclear staining 1-10% of cells and /or with weak cytoplasmic staining

3 = Nuclear staining 10-50% of cells and/or with distinct cytolasmic staining

4 = Nuclear staining >50 % of cells and /or with distinct cytoplasmic staining

HIF-1α 3+ and 4+ were considered high expression and while the others were taken as HIF-1α low expression.

Results

The maximum incidence was seen in age group of 41-60 years. The youngest patient was 28 years old and the oldest was 89 years old. The incidence of esophageal cancer in females was found to be slightly higher than the males. Most of the patients presented with complaints of 3-6 months duration. The lower segment of esophagus was involved in 56% of cases followed by middle segment with upper segment showing least involvement. The most commonly observed growth was of polypoidal type which was seen in 28 cases (56%) with proliferative and ulcerative showing equal frequency. Most of the cases were found to show moderate differentiation 34 cases (68%) (Fig A) followed by poorly differentiated (Fig B) and well differentiated consisting of 20% and 12% respectively. Out of 50 cases 33 were esophagectomy specimens while remaining 17 were endoscopic biopsies. Among the 33 cases in which lymph nodes were recovered the metastasis was seen only in 21 cases (63.7%) while 12 cases (36.3%) showed reactive pathology. All the tumours from these 33 cases of esophagectomy specimen were divided into four stages depending upon the depth

of invasion. There were 15 cases (45.5%) of T4 (tumour invading adjacent structures), 8 cases (24.5%) of T3 (tumour invading the adventitia), 7 cases (21%) of T2 (tumour invading the muscularis propria), 3 cases (9%) of T1 (tumour invading the lamina propria or submucosa). Mean follow up of the patients were 18 months (median 12 months; range 1-36 months).

The HIF-1α positivity was observed in 34 cases comprising 68% of the total cases. 16 out of these 34 cases were classified as high score group- HIF-1α 3+ and 4+ (Fig C). Rest of 18 cases were of low score group for HIF-1α expression- HIF-1α 1+ and 2+ (Fig D).

Correlating the HIF-1α expression with the degree of differentiation it was observed that 8/10 (80%) cases of poorly differentiated squamous cell carcinoma showed high HIF-1α expression as compared to 8/34 (23.5%) cases of moderate differentiation showing high HIF-1α positivity. It was concluded that high HIF-1α expression was significantly correlated with the higher grade (poor differentiation) with P=0.005 (Table 1). High HIF-1α expression correlated with the lymph node metastasis as compared to cases showing no metastasis ((12/21- 57.2% vs 02/12-16.5% respectively) with significant P=0.015 (Table 1). Similarly high HIF-1α expression correlated with lymphatic invasion with 12/23-52.2% cases positive for lymphatic invasion showed high HIF-1α expression as compared to 2/10-20% cases without lymphatic invasion (P=0.018) (Table1). The frequency of high HIF-1α expression increased with increased depth of invasion. No case of T1, 14.2% of T2 (one of seven cases), 50% of T3 (4 of 8 cases) and 60% of T4 (9 of 15 cases) had high HIF-1α.

On comparing the response of the patients to chemoradiotherapy it was observed that out of 20 patients who were resistant to therapy and had reccurence within six months after therapy 12 showed high HIF-1α as compared to eight patients of low HIF-1α score and negative cases.

Discussion

Nationally, the incidence of esophageal cancer in western, southern and northern India is 4.48, 3.50 and 2.36 per 1,00,000 respectively with south and western India contributing 55% of all the cases.[2] Despite advances in screening and multimodal management of this disease, overall survival for esophageal cancer remains poor with median survival of being less than one year after diagnosis. [9] Various factors thought to influence survival are stage of the tumour, lymph node metastasis, tumour length, grade of the tumour, lymphatic invasion, tumour necrosis, involvement of the surgical margins, response to therapy and various molecular markers like Ki-67 proliferation index, p53, growth factors and their receptors, cell cycle regulator, heat shock proteins and HIF-1α expression.[10.11]

In the current study most common age group was 41-60 years with incidence being slightly higher in females. The findings concur with other studies where most of carcinoma esophagus patients were in their 50s with mean age of esophageal resection at an age of 64 years (range, 42–79 years).[12] Indian data reveals a low sex ratio as compared to worldwide ratio, all major registries with a national average of 1.2:1. The possible explanation for this could be a high prevalence of betel quid chewing habit amongst Indian women.[13,14] The lower segment of esophagus was found to be affected in majority of the cases (constituting about 56 % of the total cases) followed by middle and

Tasble 1:Correlation of HIF 1- α expression with various clinicopathological features of Esophageal carcinoma.

Parameter	HIF 1- α High score	HIF 1- α low score	HIF 1- α negative
Differentiation			
a)Well differentiated	00	01	05
b)Moderately differentiated	08	16	10
c)Poorly differentiated	08	01	01
2. Lymph node involvement			
a)Positive	12	06	03
b)Negative	02	04	06
c) Not recovered	02	08	07
3. Lymphatics/ blood vessel involvement	12	08	03
a)Positive b)Negative	02	04	04
c)Not identified in biopsy	02	06	09
Invasion depth			
T1	00	01	02
T2	01	01	05
T3	04	03	01
T4	09	05	01

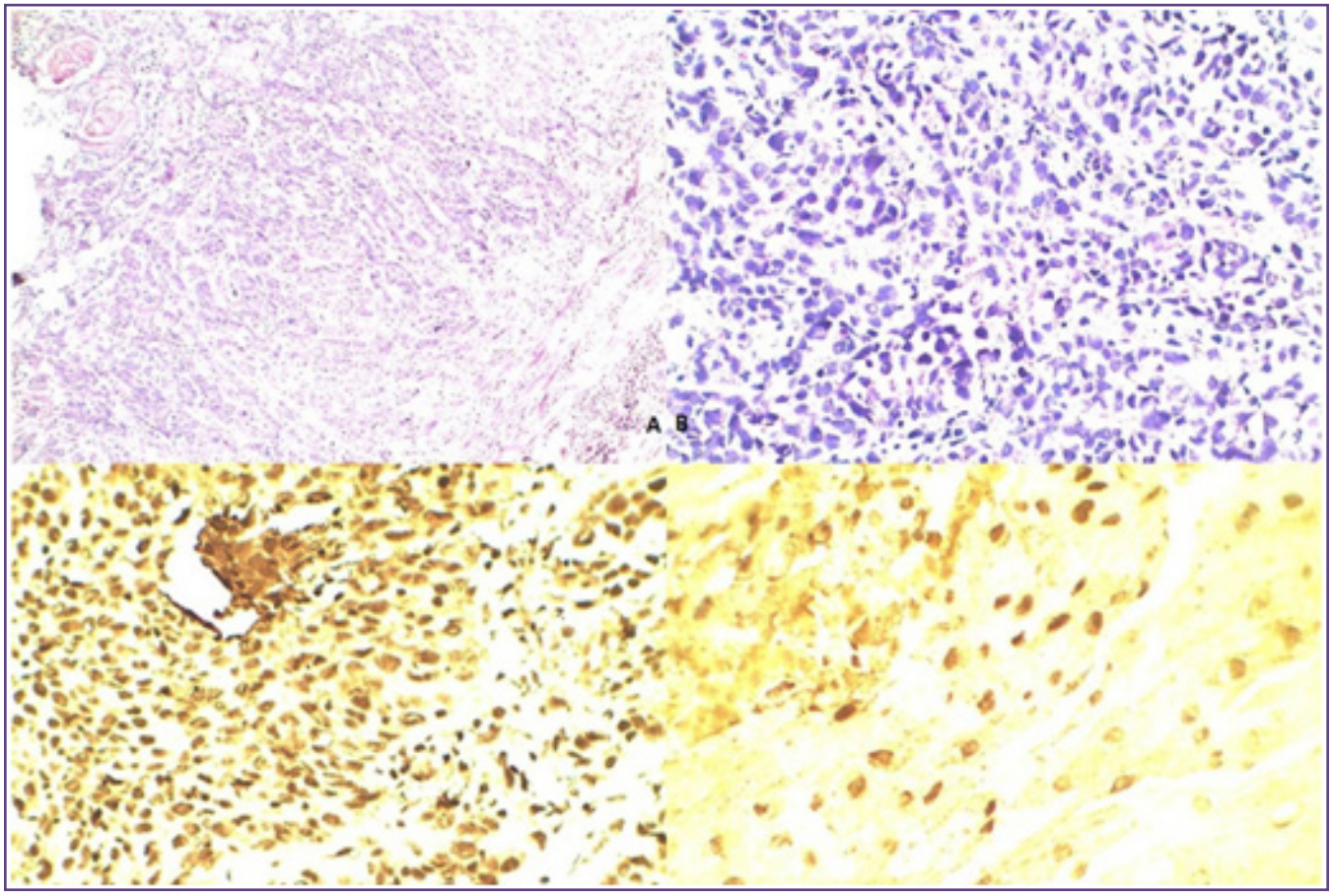

Fig. 1 (A): Squamous cell carcinoma-Moderately differentiated (H&E 100X); (B) : Squamous cell carcinoma-Poorly differentiated (H&E 100X); (C): Immunohistochemistry showing HIF -1α Positivity (Nuclear Strong Intensity) (IHC, 400X); (D): Immunohistochemistry showing HIF -1α Positivity (Nuclear Moderate Intensity) (IHC, 400X).

upper segments. Similar observation was made by other researchers who have reported lower esophageal cancers out numbering the mid esophageal cancers.[15,16] Most of the cases were found to show moderate differentiation 34 cases(68%) corroborating the findings of other studies where this percentage varied from 55-70%.[16] The lymph nodes were recovered from esophagectomy specimens, 21 (63.7%) showed metastasis and remaining 12 cases (36.3%) showed reactive pathology. Other studies had calculated metastatic lymph node percentage varying from 60-96.7%.[8,17]

The HIF-1α positivity was observed in 34 cases comprising 68% of the total cases. Other workers have reported a variable HIF-1α positive expression ranging from 39 to 95% cases.[7,8,18,19] 16 cases (32%) showed high HIF-1α expression which is comparable to 30.8% observed by Kurokawa et al in their study.[8] Correlating the HIF-1α expression with the degree of differentiation it was observed that 8/10 (80%) cases of poorly differentiated squamous cell carcinoma showed high HIF-1α expression as compared to 8/34 (23.5%) cases of moderate differentiation showing high HIF-1α positivity with significant P=0.005. Similar results have been elicited by workers elsewhere who have reported an expression of 53.9% in poorly differentiated carcinomas with a lower incidence of HIF-1α immunoexpression in moderately differentiated tumors (12%).[7] High HIF-1α expression correlated with the lymph node metastasis as compared to cases showing no metastasis ((12/21- 57.2% vs 02/12-16.5% respectively) with significant P=0.015. Same was reflected in the results deduced by other researchers where lymph node metastasis showed significant correlation with high HIF-1α expression.[7,8] HIF-1α expression also correlated with lymphatic invasion with 12/23-52.2% cases positive for lymphatic invasion showed high HIF-1α expression as compared to 2/10-20% cases without lymphatic invasion (P=0.018). Other studies also observed such significant correlation between two with P value ranging from 0.005-0.02.[7,8] The frequency of high HIF-

1α expression increased with increased depth of invasion. No case of T1, 14.2% of T2 (one of seven cases), 50% of T3 (4 of 8 cases) and 60% of T4 (9 of 15 cases) had high HIF-1α. Similar findings were observed by Matsuyama et al in their study where high HIF-1α immunopositivity was seen in T4 and T3 cases as compared to T2 and T1.[7] It was observed that out of 20 patients who were resistant to chemoradiotherapy and had reccurence within six months after therapy 12(60%) showed high HIF-1α as compared to eight patients(40%) of low HIF-1α score and negative cases. Similar results were seen in other studies where patients with HIF-1α expression had significantly poorer local control than those with low expression P=0.0322.[20]

A high expression of HIF-1α has been found associated tumour aggressiveness and unfavourable prognosis in variety of tumours including carcinomas of breast, cervix, colon, lung, ovary and esophagus. Furthermore its expression is associated with poorer response to adjuvant therapy and poorer overall and disease free survival as well.[7,8,20] Hypoxia has been proved to compromise the beneficial effects of radiotherapy and interfere with the response of tumour to chemotherapy.[8]

Conclusion

The present study concluded that expression of HIF-1α was significantly correlated with poorer clinicopathological variables including higher grade, lymph node metastasis, lymphatics invasion and increased depth of tumour invasion. It was also determined that HIF-1α in esophageal carcinoma significantly correlates with an unfavourable prognosis in such patients treated with adjuvant chemoradiotherapy. Preoperative studies on endoscopic biopsies might guide clinicians to design better therapeutic decision in patients with HIF-1α expression. Further studies are required to address the role of introduction of more intensive therapy in such patients with high HIF-1α expression who are poor responders to conventional therapy.

Abbreviations

HIF-1α - Hypoxia induced factor

VEGF - Vascular Endothelial Growth Factor

HRP - Horse Radish Peroxidase

DAB - Diaminobenxene tetra hydrochloride

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None

Competing Interests

None Declared

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