(e) ISSN Online: 2321-9599
(p) ISSN Print: 2348-6805

ORIGINAL ARTICLE

ANALYSIS HIF1 α EXPRESSION IN ESOPHAGEAL CARCINOMA

Harleen Kaur[1],Manjot Kaur[2], Simranjeet Singh[3], Kiranjot Kaur[4], Mridu Manjari[5]

[1]Consultant pathologist Guru NanakAyurvedic Medical College, Muktsar, Punjab, [2]Consultant Department of Pathology IVY Hospital Nawanshahr, Punjab Pathologist, MO Punjab Civil Medical Services, Derabassi[3]Pediatrician Bikaner Hospital, [4]Pathologist, MO Punjab Civil Medical Services[5]Professor and Head, Department of Pathology, SGRDIMSR, Amritsar.

ABSTRACT:
Introduction: Tumour hypoxia is considered as a key factor driving the development of malignancy, and the master regulatory protein in the response of cells to changing oxygen levels is hypoxia inducible factor-1 (HIF-1). Thus, present study was undertaken to evaluate HIF 1 alpha expression in esophageal carcinoma.**Material and Methods:** The study was carried out on 50 cases diagnosed as Esophageal carcinoma. Histopathological examination of the tissues were conducted under light microscope for classification and histopathological grading. Immunohistochemistry of the tumors was carried out for HIF-alpha.Obtained data was arranged accordingly and was expressed as a number and percentage of respondents and were analyzed using the SPSS Version 17 software. **Results**: The HIF-1 alpha positivity was observed in 68% cases with percentage positive cells varied from 1-90% with weak, distinct and strong staining intensity. In HIF-1 alpha positive cases, most of the patients were in the age group 41-60 years. HIF-1 alpha positivity was seen in 70.2% cases of squamous cell carcinoma while adenocarcinomapostivity was seen in 33.1% cases. Since most of the cases included in the study were of moderately differentiated carcinoma, no significance between grading and HIF-1 alpha expression was elicited. HIF-1 alpha expression in metastatic deposit lymph nodes was noted in 41.1% cases. **Conclusion:** The results of the present study concludes that HIF-1alpha is involved in gastric carcinogenesis and disease progression.
Keywords: Hypoxia-inducible factor-1α; Immunohistochemistry; Malignancy

Corresponding Author: Dr. Harleen Kaur, Consultant pathologist Guru Nanak Ayurvedic Medical College, Muktsar, Punjab, India

This article may be cited as: Kaur H, Kaur M, Singh S, KaurK, Manjari M. Analysis HIF1 α expression in esophageal carcinoma. J Adv Med Dent Scie Res 2016;4(6):1-4.

Access this article online	
Quick Response Code	Website: www.jamdsr.com
	DOI: 10.21276/jamdsr.2016.4.6.1

INTRODUCTION

Elevated levels of hypoxia-inducible factor-1α (HIF-1α), a subunit of HIF-1, are noted in various malignant tumours including esophageal carcinomas. Different studies have quoted a positive HIF-1a protein expression (within both the nuclei and/or cytoplasm) in cases of oesophageal carcinoma to be ranging from 58.5 % to 95 %. The positivity was noted in cancer tissue and not in normal epithelia.[1,2]

HIF-1α is a heterodimetric transcriptional factor that regulates O_2 homeostasis and the physiologic response to O_2 deprivation.[3]Tumour hypoxia is considered as a key factor driving the development of malignancy, and the master regulatory protein in the response of cells to changing oxygen levels is hypoxia inducible factor-1 (HIF-1). Hypoxia-inducible factor-1 consists of α and β -subunits which are both members of the helix-loop-helix family of transcription factors. The β -subunit is constitutively expressed and its activity is controlled in an oxygen independent manner. The a-subunit is ubiquitinated and degraded in normoxia, but stabilised in hypoxia. In the hypoxic environment, HIF-1α dimerises with HIF-1β and binds to hypoxia responsive elements (HRE) within the nucleus. A wide variety of genes, including VEGF, Glut-1, CA9, erythropoietin and iNOS are known to have HREs and are activated by HIF-1a.[4]Thus, present study was undertaken to evaluate HIF 1 alpha expression in esophageal carcinoma.

MATERIAL AND METHODS

The study was carried out on 50 cases diagnosed as Esophageal carcinoma. Histopathological examination of the tissues obtained was carried after processing them to prepare paraffin blocks. Blocks were cut and stained with

Haematoxylin and Eosin stain and studied under light microscope for classification and histopathological grading. Immunohistochemistry of the tumors was carried out for HIF-alpha and cases which were positive for HIF 1 alpha were taken as positive control. For IHC,3–5 µm sections were cut and were mounted on poly–l–lysine coated slides. Slides were dried overnight at 37^{o}C and dewaxed in xylene and hydrated. For antigen retrieval, 1500 mL of citrate buffer solution, pH 6.0, was heated unless until boiled in a stainless steel pressure cooker. It was covered but lid was not locked. Slides were positioned into metal staining racks and lowered into pressure cooker ensuring slides were completely immersed in unmasking solution. Lid was locked. When the pressure cooker reached the operating temperature and pressure (after about 5 minutes),1 minute timer was started. When the timer rang, pressure cooker was removed from heat source and was run under cold water with lid on. Endogenous peroxidise was neutralised using Peroxidase Block for 5 minutes. Two washings in Phosphate Buffer Saline/ Tris buffer saline were given each for 5 minutes. Protein Block was incubated for 5 minutes. Then 2 washes in tris buffer were given for 5 minutes each. The primary antibody was put on the sections and sections were kept for 1 hour in the moist chamber. This was followed by 2 washes in tris buffer for 5 minutes each. The post primary block was then applied for 30 minutes at room temperature. Again 2 washings of tris buffer were given for 5 minutes each. Incubation with Polymer was done for 30 minutes. Again 2 washings were given with tris buffer for 5 minutes each with gentle rocking. Slides were then covered with DAB for 2-3 minutes. All the time slides were kept in a moist chamber. Sections were washed in deionised water for 5 minutes. Haematoxylin counterstaining was done for 2-5 minutes and sections were washed under running tap water. Dehydration and clearing of the sections was done in propanol and xylene respectively. Mounting was done by the mounting media DPX. Sections were viewed under the microscope. Obtained data was arranged accordingly and w as expressed as a number and percentage of respondents and were analyzed using the SPSS Version 17 software.

RESULTS

The HIF ALPHA positivity was observed in 34 cases comprising 68% of the total cases (table 1). Percentage positive cells varied from 1-90% with weak, distinct and strong staining intensity.

HIF alpha positive cases had age variation from 28 - 89 years with a mean of 56.74 +_12.189 years with maximum positivity observed in age group of 41-60 yrs (p=0.259;Not Significant , chi square test) (table 2).

HIF alpha positivity was seen in 33 cases of squamous cell carcinoma. In adenocarcinoma, positivity was seen in 1 case.(p=0.184; *Not Significant, chi square test). All the 34 cases which were positive for HIF alpha exhibited moderate differentiation, thus showing 69.4 % positivity in moderately differentiated but was negative in only one poorly differentiated carcinoma present in the study (table 3).

HIF alpha positivity was seen in 13 cases of which 7(53.8%) had metastatic deposits, however positivity was also observed in reactive lymph nodes. Although the cases with lymph node metastasis possibly showed slightly increased HIF-1a positivity also but it was not statistically significant. (p= 0.091; Not significant, chi square test) (table 4, graph 1).

Table 1: Showing HIF alpha expression

HIF alpha expression	No. of cases	% of cases
POSTIVE	34	68
NEGATIVE	16	32
TOTAL	50	100

Table2: Correlation of HIF alpha status with age

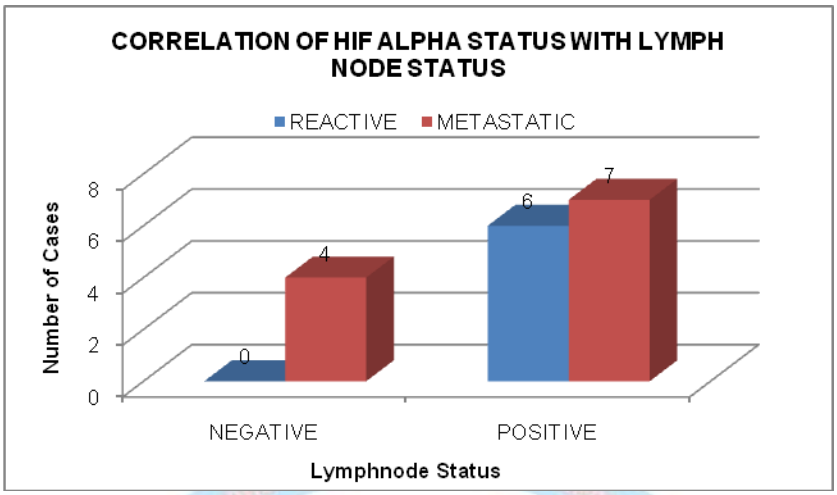
AGE GROUP	HIF ALPHA		Total
	NEGATIVE	POSITIVE	
21-40	2	3	5
41-60	8	22	30
61-80	6	8	14
81-100	0	1	1
TOTAL	16	34	50

Table 3: Correlation of HIF alpha status with type and grade of carcinoma

Correlation of HIF alpha status with type of carcinoma			
CARCINOMA	**HIF**		**Total**
	NEGATIVE	**POSITIVE**	
ADENOCARCINOMA	2(66.7%)	1(33.3%)	3(100.0%)
SQUAMOUS CELL CA	14(29.8%)	33(70.2%)	47(100.0%)
TOTAL	16(32.0%)	34(68.0%)	50(100.0%)
Correlation of HIF alpha status with grade of carcinoma			
DEGREE	**HIF_PN**		**Total**
	NEGATIVE	**POSITIVE**	
MODERATE	15(30.6%)	34(69.4%)	49
POOR	1(100.0%)	0(0.0%)	1
TOTAL	16(32.0%)	34(68.0%)	**50**

Table 4: Correlation of HIF alpha status with lymph node status

LYMPH_STATUS	HIF_PN		Total
	NEGATIVE	POSITIVE	
REACTIVE	0	6	6
METASTATIC	4	7	11
TOTAL	4	13	17



Graph 1: Correlation of HIF alpha status with lymph node status

DISCUSSION

Esophageal cancer is the eighth most common cancer around the world. In 2008, an estimated 482,000 new esophageal cancer cases were diagnosed and 407,000 related deaths occurred globally.[5] Nationally, the incidence of esophageal cancer in western, southern and northern India is 4.48, 3.50 and 2.36 per 100000 respectively[6] with south and western India contributing 55% of all the cases. In Punjab the incidence rates calculated were however lowest in the country.[7]This may be attributed to less intake of tobacco due to religious constraints.

Despite advances in screening and multimodal management of this disease, overall survival for esophageal carcinoma remains poor.[8]The need to identify tumor markers as prognostic indicators and as targets for new therapeutic strategies remains a major challenge in esophageal cancer research.

In the present study 50 histologically proven cases of carcinoma esophagus were studied to find out the expression of HIF alpha and its correlation with other parameters.HIF-1 α positive cases had age variation from 28 - 89 years with maximum positivity observed in age group of 41-60 years (44%).As the maximum cases included were in the same age group; no significant correlation was found between HIF alpha positivity and patients' age and gender. Kurukowa T et al[2] and Matsuyama T et al[1] reported a median patient age to show HIF alpha positivity as 63 years (range, 38–82 years) and 64 years (range, 35-88 years) respectively.

HIF alpha positivity in the study conducted was observed in 34/50 cases thus comprising 68% of the total cases and the percentage positive cells varied from 1-90% with weak, distinct and strong staining intensity.Of the 47 histologically proven cases of ESCC included; HIF alpha positivity was observed in 33 cases (70%). Other workers have reported a variable HIF alpha positive expression in ESCC ranging from 39 to 95% cases.[9-12] Of 3 cases of adenocarcinoma, HIF alpha positivity was observed in only 1 case (33.3%). Extensive literature search failed to yield any substantial study in which HIF 1 alpha expression has been correlated with EAC independently. Hence more research is required in the setting of EAC as prevalence of Barrett'soesophagus and the adeocarcinomas arising from it is on the rise.

Majority of the cases (49 /50) were graded as moderately differentiated and only 1 case was that of poorly differentiated carcinoma. Of these moderately differentiated carcinomas, HIF alpha expression was noted in 34 cases (69.4%). HIF was negative in the single case of poorly differentiated carcinoma. Similar results have been elicited by workers elsewhere with notably Matsuyama et al who reported an expression of 53.9% in moderately differentiated carcinomas with a lower incidence of HIF alpha immunoexpression in poorly differentiated tumours (12%).[1]HIF alpha positivity was seen in 13 cases (76.4%) out of the 17 cases in which lymph nodes were recovered and its expression was 46% and 53.8% in reactive and metastatic lymph nodes respectively. Matsuyama et al[1]

observed HIF alpha positivity in 72% cases showing lymph node positivity.

No significant relationship between metastasis and HIF alpha positivity was noted. Similarly, no association was found between HIF alpha expression and lymph node status by Takala H.[11] However, Matsuyama et al[1] observed a positive correlation and significance between the two and also reflected in the results deduced by Kurokawa et al[2] in ESCC where lymph node metastasis showed significance with HIF 1 alpha.

Increased levels of HIF-1 activity are associated with increased tumor aggressiveness, therapeutic resistance and mortality. HIF-1 can be induced as a result of the high growth rate of tumor cells and intra tumoral hypoxia as well as by O_2-independent genetic alterations that activate a variety of oncogenic signaling pathways or, alternatively, inactivate tumor suppressors.[13]

CONCLUSION

HIF-1 expression is increased in tumor cells by multiple mechanisms and may mediate adaptation to hypoxia that is critical for tumor progression. HIF-1 thus appears to function as a master regulator of O_2 homeostasis that plays essential roles in cellular and systemic physiology, development, and pathophysiology.[14] The results of the present study concludes that HIF-1alpha is involved in gastric carcinogenesis and disease progression.

REFERENCES

1. Matsuyama T, Nakanishi K, Hayashi T, Yoshizumi Y, Aiko S, Sugiura Y, Tanimoto T, Uenoyama M, Ozeki Y, Maehara T. Expression of hypoxia-inducible factor-1α in esophageal squamous cell carcinoma. Cancer Sci 2005;96(3):176-82.
2. Kurokawa T, Miyamoto M, Kato K, Cho Y, Kawarada Y, Hida Y, Shinohara T, Itoh T, Okushiba T, Kondo S, Katoh H. Overexpression of hypoxia-inducible-factor 1a (HIF-1a) in oesophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. Br J Cancer 2003;89:1042–7.
3. Ziello JE, Jovin IS, Huang Y. Hypoxia-Inducible Factor (HIF)-1 Regulatory Pathway and its Potential for Therapeutic Intervention in Malignancy and Ischemia. The Yale Journal of Biology and Medicine 2007;80(2):51-60.
4. Griffiths EA, Pritchard SA, Valentine HR, Whitchelo N, Bishop PW, Ebert MP, Price PM, Welch IM, West CML. Hypoxia-inducible factor-1a expression in the gastriccarcinogenesis sequence and its prognostic role in gastric and gastro-oesophageal adenocarcinomas. British Journal of Cancer (2007) 96, 95–103.
5. Jemal A, Bray F, Center MM. Global cancer statistics. Cancer J Clin 2011;61:69-90.
6. Rao DN, Sanghvi LD, Desai PB. Epidemiology of esophageal cancer. SeminSurgOncol 1989;5:351-4.
7. Rose EF. A review of factors associated with cancer of the esophagus in the Transkei. In: Mettlin C, Murphy G, editors. Cancer among Black populations. New York: Alan R Liss; 1981. p. 67-75.
8. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127(12):2893-917.
9. Kimura S, Kitadai Y, Tanaka S, Kuwai T, Hihara J, Yoshida K, Toge T, Chayama K. Expression of hypoxia-inducible factor (HIF)-1alpha is associated with vascular endothelial growth factor expression and tumour angiogenesis in human oesophageal squamous cell carcinoma. Eur J Cancer 2004;40(12):1904-12.
10. Ginsberg D, Mechta F, Yaniv M, Oren M. Wild-type p53 can down-modulate the activity of various promoters. Proc NatlAcadSciUSA 1991;88:9979–83.
11. Takala H, Saarnio J, Wiik H, Ohtonen P, Soini Y. HIF-1α and VEGF are associated with disease progression in esophageal carcinoma. J Surg Res 2011;167(1):41-8.
12. Koukourakis MI, Giatromanolaki A, Skarlatos J, Corti L, Blandamura S, Piazza M, Gatter KC, Harris AL. Hypoxia inducible factor (HIF-1α and HIF-2α) expression in early esophageal cancer and response to photodynamic therapy and radiotherapy. Cancer Res. 2001;61:1830–2.
13. Mabjeesh NJ, Amir S. Hypoxia-inducible factor (HIF) in human tumorigenesis. HistolHistopathol 2007;22:559-72.
14. Semenza GL. Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol. 1999;15:551-78.

Source of support: Nil **Conflict of interest:** None declared