P16 and P53 Expression in Esophageal Squamous Cell Carcinoma: A Brief Report From The Experience of South of Iran, and Review of the Literature

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ABSTRACT

BACKGROUND: Iran is one of the high-risk countries for esophageal squamous cell carcinoma (ESCC). Human papillomavirus (HPV) has been reported as one of the etiologic, pathogenetic, and prognostic factors in this tumor, especially in high-risk geographic areas. Previous reports from our geographic area, that is, the South of Iran failed to show any evidence of HPV in the cases of ESCC by molecular methods.

OBJECTIVES: In this study, we evaluated P16 and P53 immunohistochemistry (IHC) expression in the cases of esophageal ESCC from Fars province in the South of Iran to find the presence of any correlation between clinicopathologic findings with P16 and P53 expression by IHC as etiologic and prognostic biomarkers. We also tried to compare the results from other geographic areas of Iran and the world.

RESULTS: P16 and P53 expression were found in 42.9% and 66.12% of ESCCs, respectively. No statistically significant correlation was found between clinicopathologic findings and P16 pr P53 expression.

CONCLUSION: Although P16 and P53 expression in ESCC in the South of Iran is significant, there is no statistically significant correlation between clinicopathologic findings and outcome in ESCC and expression of these 2 proteins to be considered as biomarkers. Results from other geographic areas of Iran and the world are also very controversial and inconsistent.

KEYWORDS: Squamous cell carcinoma, esophagus, P16, HPV

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Introduction

There are several countries in the world which are at high risk regarding esophageal squamous cell carcinoma (ESCC), such as China, Singapore, and Iran.[1] In these countries, infection by human papilloma virus (HPV) is considered as the possible cause of this malignancy.[1] The first report about the association between HPV and ESCC has been more than 30 years ago; however, it seems that this association depends on the geographic region, that is, it is more prevalent in high-risk countries.[2] There are also some studies about the association of HPV infection and clinical outcome of ESCC.[3]

The role of HPV in the cause and prognosis of ESCC is controversial, and some meta-analyses have shown no statistically significant correlation.[4]

There are also controversial reports from different geographic regions of Iran regarding the aforementioned association.[5-16]

Most studies regarding HPV and ESCC have used molecular methods to find HPV genomes in the tumor, and there are very few studies about the protein expression of HPV in the tumor tissue.[17-30] In this study, we tried to evaluate the expression of HPV proteins by immunohistochemical methods to find out the correlation of HPV-associated proteins (as an immunohistochemical biomarker) and clinicopathologic characteristics of ESCC in the largest referral center from the South of Iran. We also tried to perform a thorough search in the literature to compare the results from different geographic areas of Iran and the world.

Patients and Methods

In this cross-sectional study for 5 years (2015-2019), all the cases with the diagnosis of ESCC in the affiliated hospitals of Shiraz University of Medical Sciences were evaluated for the presence of suitable tumoral tissue with no necrosis for staining with immunohistochemical markers. There were 71 cases of ESCC among which, 31 specimens had enough non-necrotic tumoral tissue suitable for immunohistochemistry (IHC). The best paraffin block was selected, and IHC was performed for P16 and P53. The characteristics of antibodies are shown in Table 1. The sections were reported as positive and negative

Table 1. Characteristics of P53 and P16 antibodies which have been used in this study in ESCC.

Antibody	P53	P16
Company	Dako	Biocare
Clone	DO-7	M7001
Dilution	1/50	Predilute
Antigen retrieval	Boiling	Boiling

Abbreviation: ESCC, esophageal squamous cell carcinoma.

according to the documented criteria.[2] P16 staining pattern was qualitatively classified as negative and positive (nuclear-cytoplasmic, and cytoplasmic). Cases with more than 50% positivity were considered as positive. P53 was also scored as <10% (negative), and >10% (positive). Both were also quantitively scored as 0 to 3.

Also, clinicopathologic findings were extracted from the patients' charts and pathology reports.

Chi-square and SPSS 14 were used for the analysis of results and comparison of different prognostic and outcome characteristics. *P* value less than .05 was considered as statistically significant.

Results

There were 71 cases of ESCC during the last 5 years (2015-2019) in the affiliated hospitals of Shiraz University of Medical Sciences. Female-to-male ratio was 1:1.16 (33:38, 53.5%:46.5%).The age range was 44 to 85 (64.92 ± 12.15) years. Only 31 cases had enough suitable tissue for IHC staining for P16 and P53.

Tables 2 and 3 show the correlation of the clinicopathologic cases of ESCC and positivity of P53 and P16.

As Table 2 shows, 41.9% and 90.3% of the cases were positive for P16 and P53, respectively. The significant nuclear and cytoplasmic P16 positivity was seen in 16.1% of the cases with the diagnosis of ESCC.

As the Table 3 shows, there has been no correlation between immunohistochemical positivity of these 2 biomarkers with age, sex, gross findings, grade, and stage of the cases with ESCC.

Discussion

There are controversial reports and studies about the correlation of immunohistochemical positivity of P16 and P53 with the cause, pathogenesis, and outcome of squamous cell carcinoma of upper aerodigestive tract. Most studies have shown that overexpression of P16 can be caused by molecular changes not related to HPV infection and prognosis.[2,17] There are other studies which have shown that staining greater than 50% to 75% have a more correlation with the presence of actively transcribed HPV.[3]

Table 2. The results of P16 and P53 positivity in 31 cases of ESCC.

IMMUNOHISTOCHEMISTRY	N = 31	%
Qualitative P16		
0	3	9.7
1+	15	48.4
2+	5	16.1
3+	8	25.8
P16 positivity	13	41.9
P16 pattern		
Negative	3	9.7
Cytoplasmic	23	74.2
Cytoplasmic and nuclear	5	16.1
Qualitative P53		
0	2	6.5
1+	4	12.9
2+	13	41.9
3+	12	38.7
P53 positivity	28	90.3
	MEAN (%)	±SD
Quantitative P16	42.9	±29
Quantitative P53	66.12	±23.75

Abbreviations: ESCC, esophageal squamous cell carcinoma; SD, standard deviation.

Other studies showed that chromosomal instability is correlated with persistent high-risk HPV infection, and increased expression of viral oncoproteins, that is, E6 and E7 which can interfere with cell cycle regulation and inactivation of p53. These studies claimed that IHC for p16 and p53 can be surrogate markers of HPV infection and good prognosis.[1]

Previous study from our center of the South of Iran and also other studies from Tehran failed to show any evidence of HPV gene in ESCC, by polymerase chain reaction (PCR) method.[10,13] Tables 4 and 5 show the studies from Iran and other geographic areas of the world about the presence of HPV infection in the cases of ESCC and the method which have been used to find the genome. As the tables show the reported incidence of the HPV genome is highly variable, that is, there is no consistent results in different geographic areas, either high or low incidence for ESCC.

Our results also failed to show any correlation between clinicopathologic findings of the cases of ESCC and IHC expression for P16 or P53.

Table 3. Correlation between P16 and P53 characteristics and clinicopathologic findings.

VARIABLES	P16			P53		
	POSITIVE	NEGATIVE	*P* VALUE	POSITIVE	NEGATIVE	*P* VALUE
Age			.718			.281
<60	4	7		9	2	
⩾60	9	11		19	1	
Gender			1			1
Male	8	12		18	2	
Female	5	6		10	1	
Ulcerative mass			.036*			.567
Yes	3	11		12	2	
No	10	7		16	1	
Perforation			.058			.422
Yes	0	5		4	1	
No	13	13		24	2	
Grade			.134			1
Poor diff	4	1		5	0	
Others	9	17		23	3	
Lymphnode involvement			1			.349
Yes	2	2		3	1	
No or unclear	11	16		25	2	
Stage			1			.331
I and II	4	10		13	1	
III and IV	1	2		2	1	

*Statistically significant.

Table 4. Results of HPV studies from different regions of Iran.

AUTHOR	YEAR	PROVINCE	NO. OF CASES	AGE (MEAN ± SD)	NO. OF CONTROLS	METHOD	POSITIVE PCR		POSITIVE IHC	
							CASE	CONTROL	P16	P53
Abbaszadegan et al[5]	2003	Khorasan	45		–	Molecular and IHC	8	–		74%
Abdirad et al[6]	2012	Tehran	93	58.84 ± 11	–	Molecular	8	–		
Emadian et al[7]	2011	Mazandaran	40	37.59 ± 1.33	40	Molecular	15	5		
Far et al[8]	2007	Tehran	140		140	Molecular	33	12		
Farhadi et al[9]	2005	Tehran	38	54.2 ± 13	38	Molecular	14	5		
Haeri et al[10]	2013	Tehran	30	59.6	30	Molecular	0	0		
Mehran[11]	2010	Guilan	45	64	–	Molecular	17	–		
Moradi and Mokhtari-Azad[12]	2006	Golestan	85		31	Molecular	42	18		
Noori et al[13]	2012	Fars	92		20	Molecular	0	–		

(Continued)

Table 4. (Continued)

AUTHOR	YEAR	PROVINCE	NO. OF CASES	AGE (MEAN ± SD)	NO. OF CONTROLS	METHOD	POSITIVE PCR		POSITIVE IHC	
							CASE	CONTROL	P16	P53
Soheili et al[14]	2016	Kermanshah	58	62.63	–	Molecular	7	–		
Yahyapour et al[15]	2013	Mazandaran	177		–	Molecular	49	–		
Yahyapour et al[16]	2016	Mazandaran	51	69.1	45	Molecular	16	20		
This Study	2019	Fars	31	64.92 ± 12.15	–	IHC	28	–	42.9%	66.12%

Abbreviations: HPV, human papilloma virus; IHC, immunohistochemistry; PCR, polymerase chain reaction.

Table 5. Results of HPV studies from different regions of the world.

AUTHOR	YEAR	COUNTRY	NO. OF CASES	AGE, MEAN ± SD	NO. OF CONTROLS	METHOD	POSITIVE PCR		POSITIVE IHC	
							CASE	CONTROL	P16	P53
Cao et al[1]	2014	North China (Shandong)	105	60		In situ Hybridization	29		23.8%	
Pastrez et al[2]	2017	Brazil	87		87	Molecular	12		11.6%	67.5%
Antonsson et al[18]	2010	Australia	222	65.2 ± 9.2		Molecular	8		1.8%	
Castillo et al[19]	2006	South America	73			Molecular	21		16.43%	
		Columbia	47	63.6 ± 12.9			16			
		Chile	26	72.3 ± 8.9			5			
Castillo et al[20]	2011	Pakistan	42	45		Molecular	11		9.63%	
		Columbia	49	64			9			
		Japan	75	64			11			
Ding et al[21]	2010	North China (Henan)	17			Molecular	8		11.70%	
Doxtader and Katzenstein[22]	2012	USA	20	62.1		Insitu Hybridization	1		5.00%	
Herbster et al[23]	2012	Brazil	264			Molecular	34		2.65%	
Koshiol et al[24]	2010	North China (Linxian)	272	60		Molecular	3		0.00%	
Löfdahl et al[25]	2012	Sweden	204			Molecular	20		1.96%	
Malik et al[26]	2011	USA	25	64.3 ± 10.5		Insitu Hybridization	0		0.00%	
Shuyama et al[27]	2007	China	59	61 ± 10		Molecular	19	1	1.00%	
Teng et al[28]	2014	East China (Shanghai)	177			Molecular	6		2.82%	
Vaiphei et al[29]	2013	India	23			Molecular	20			
Sitas et al[30]	2012	Inter SCOPE Study	133			Molecular	10		0.75%	
Astori et al[31]	2001	Italy	14			Molecular	6			
Bellizzi et al[32]	2009	USA	31	63.3		Molecular	8		0	
da Costa et al[17]	2017	Brazil	87	60.9 ± 10.3		Molecular	12		12.2%	66.2%
Katiyar et al[33]	2005	India	101		26	Molecular	19			16.8%
Kawaguchi et al[34]	2000	East Asia	75			Molecular	17			

(Continued)

Table 5. (Continued)

AUTHOR	YEAR	COUNTRY	NO. OF CASES	AGE, MEAN ± SD	NO. OF CONTROLS	METHOD	POSITIVE PCR		POSITIVE IHC	
							CASE	CONTROL	P16	P53
Lu et al[35]	2001	China	30			Molecular	19			73.3%
Mohiuddin et al[36]	2013	India	56	58.3 ± 13	85	Molecular	11	43		
Zhang et al[37]	2017	China	192	64		Molecular	67			

Abbreviations: HPV, human papilloma virus; IHC, immunohistochemistry; PCR, polymerase chain reaction.

Author Contributions

Bita Geramizadeh: Concept and idea of the research, looking at the slides, writing the paper. Alireza Mohammadian: Analysis and extracting the data, literature search, Alireza Shojazadeh: Literature search and helping to write the paper, Sahand Mohammadzadeh: Helping to look at the slides.

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