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

Bita Geramizadeh1,2, Alireza Mohammadian1, Alireza Shojazadeh2 and Sahand Mohammadzadeh1

1Department of Pathology, Medical School of Shiraz University, Shiraz University of Medical Sciences, Shiraz, Iran. 2Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

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 or 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

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...
according to the documented criteria. 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 quantitatively 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. 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. 

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. 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. 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 POSITIVE | P16 NEGATIVE | P53 POSITIVE | P53 NEGATIVE | P VALUE |
|----------------------------|--------------|--------------|--------------|--------------|---------|
| Age                       |              |              |              |              |         |
| <60                        | 4            | 7            | 9            | 2            | .718    |
| ≥60                        | 9            | 11           | 19           | 1            | .281    |
| Gender                     |              |              |              |              |         |
| Male                       | 8            | 12           | 18           | 2            |         |
| Female                     | 5            | 6            | 10           | 1            |         |
| Ulcerative mass            |              |              |              |              | .036*   |
| Yes                        | 3            | 11           | 12           | 2            | .567    |
| No                         | 10           | 7            | 16           | 1            |         |
| Perforation                |              |              |              |              | .058    |
| Yes                        | 0            | 5            | 4            | 1            | .422    |
| No                         | 13           | 13           | 24           | 2            |         |
| Grade                      |              |              |              |              | .134    |
| Poor diff                  | 4            | 1            | 5            | 0            |         |
| Others                     | 9            | 17           | 23           | 3            |         |
| Lymphnode involvement      |              |              |              |              | .349    |
| Yes                        | 2            | 2            | 3            | 1            |         |
| No or unclear              | 11           | 16           | 25           | 2            |         |
| Stage                      |              |              |              |              | .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 |
|----------------------------|------|----------|--------------|-----------------|----------------|-------------------|--------------|--------------|
| Abbaszadegan et al16       | 2003 | Khorasan | 45           | –               | –              | Molecular and IHC | 8            | –            | 74%          |
| Abdirad et al17            | 2012 | Tehran   | 93           | 58.84 ± 11      | –              | Molecular         | 8            | –            |
| Emadian et al18            | 2011 | Mazandaran | 40        | 37.59 ± 1.33    | 40             | Molecular         | 15           | 5            |
| Far et al19                | 2007 | Tehran   | 140          |                 | 140            | Molecular         | 33           | 12           |
| Farhadi et al20            | 2005 | Tehran   | 38           | 54.2 ± 13       | 38             | Molecular         | 14           | 5            |
| Haeri et al21              | 2013 | Tehran   | 30           | 59.6            | 30             | Molecular         | 0            | 0            |
| Mehran22                   | 2010 | Guilan   | 45           | 64              | –              | Molecular         | 17           | –            |
| Moradi and Mokhtari-Azad23  | 2006 | Golestan | 85           |                 | 31             | Molecular         | 42           | 18           |
| Noori et al24              | 2012 | Fars     | 92           | 20              | Molecular       | 0              | –            |

(Continued)
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 |
|-------------------------|------|-----------------------|--------------|----------------|----------------|-----------------------------|---------------|---------------|
| Soheili et al\(^4\)     | 2016 | Kermanshah           | 58           | 62.63          | –              | Molecular                   | 7             | –             |
| Yahyapour et al\(^5\)   | 2013 | Mazandaran            | 177          | –              | 49             | Molecular                   | –             | 11.6%         | 67.5%         |
| Yahyapour et al\(^6\)   | 2016 | Mazandaran            | 51           | 69.1           | 45             | Molecular                   | 16            | 20            |
| This Study              | 2019 | Fars                  | 31           | 64.92 ± 12.15  | –              | IHC                         | 28            | –             | 42.9%         | 66.12%        |
| Cao et al\(^7\)         | 2014 | North China (Shandong)| 105          | 60             | 29             | In situ Hybridization       | 7             | –             |
| Pastrez et al\(^2\)     | 2017 | Brazil                | 87           | 65.2 ± 9.2     | 12             | Molecular                   | 11.6%         | 67.5%         |
| Antonnson et al\(^8\)   | 2010 | Australia             | 222          | 62.1           | 8              | Molecular                   | 1.8%          |
| Castillo et al\(^9\)    | 2006 | South America         | 73           | 63.6 ± 12.9    | 5              | Molecular                   | 16            |
|                         |      | Columbia              | 47           | 63.6 ± 12.9    |                |                             |
|                         |      | Chile                 | 26           | 72.3 ± 8.9     |                |                             | 5             |
| Castillo et al\(^10\)   | 2011 | Pakistan              | 42           | 64             | 11             | Molecular                   | 9.63%         |
|                         |      | Columbia              | 49           | 64             |                |                             |
|                         |      | Japan                 | 75           | 64             |                |                             | 11            |
| Ding et al\(^11\)       | 2010 | North China (Henan)   | 17           | 62.63          | 8              | Molecular                   | 11.70%        |
| Doxtader and Katzenstein\(^12\) | 2012 | USA                   | 20           | 62.1           | 1              | Insitu Hybridization        | 5.00%         |
| Herbster et al\(^13\)   | 2012 | Brazil                | 264          | 62.1           | 34             | Molecular                   | 2.65%         |
| Koshtiel et al\(^14\)   | 2010 | North China (Linxian)| 272          | 60             | 3              | Molecular                   | 0.00%         |
| Löfdahl et al\(^15\)    | 2012 | Sweden                | 204          | 64.3 ± 10.5    | 20             | Molecular                   | 1.96%         |
| Malik et al\(^16\)      | 2011 | USA                   | 25           | 64.3 ± 10.5    | 0              | Insitu Hybridization        | 0.00%         |
| Shuyama et al\(^17\)    | 2007 | China                 | 59           | 61 ± 10        | 19             | Molecular                   | 0.00%         |
| Teng et al\(^18\)       | 2014 | East China (Shanghai)| 177          | 64.92 ± 12.15  | 1              | Molecular                   | 2.82%         |
| Vaiphei et al\(^19\)    | 2013 | India                 | 23           | 64.92 ± 12.15  | 20             | Molecular                   | 0.75%         |
| Sitas et al\(^20\)      | 2012 | Inter SCOPE Study     | 133          | 63.3           | 10             | Molecular                   | 6             |
| Astori et al\(^21\)     | 2001 | Italy                 | 14           | 63.3           | 6              | Molecular                   | 0             |
| Bellizzi et al\(^22\)   | 2009 | USA                   | 31           | 64.3 ± 10.5    | 8              | Molecular                   | 0             |
| da Costa et al\(^23\)   | 2017 | Brazil                | 87           | 60.9 ± 10.3    | 12             | Molecular                   | 12.2%         | 66.2%         |
| Katiyar et al\(^24\)    | 2005 | India                 | 101          | 64.92 ± 12.15  | 19             | Molecular                   | 16.8%         |
| Kawaguchi et al\(^25\)  | 2000 | East Asia             | 75           | 64.92 ± 12.15  | 17             | Molecular                   | (Continued)    |

Abbreviations: HPV, human papilloma virus; IHC, immunohistochemistry; PCR, polymerase chain reaction.
Table 5. (Continued)

| AUTHOR | YEAR | COUNTRY | NO. OF CASES | AGED, MEAN ± SD | NO. OF CONTROLS | METHOD | POSITIVE PCR | POSITIVE IHC |
|--------|------|---------|-------------|----------------|----------------|--------|--------------|--------------|
| Lu et al \(^{36}\) | 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 Shojaezadeh: Literature search and helping to write the paper, Sahand Mohammadzadeh: Helping to look at the slides.

ORCID iD
Bita Geramizadeh: https://orcid.org/0000-0003-1009-0049

REFERENCES
1. Cao F, Han H, Zhang F, et al. HPV infection in esophageal squamous cell carcinoma and its relationship to the prognosis of patients in northern China. Sci World J. 2014;2014:804738.
2. Pastrez PRA, Mariano VS, da Costa AM, et al. The relation of HPV infection and expression of p53 and p16 proteins in esophageal squamous cells carcinoma. J Cancer. 2017;8:1062-1070.
3. Kumar R, Ghosh SK, Verma AK, et al. p16 expression as a surrogate marker for HPV infection in esophageal squamous cell carcinoma can predict response to neo-adjuvant chemotherapy. Asian Pac J Cancer Prev. 2015;16:7161-7165.
4. Ludmiz EB, Stephens SJ, Palma M, Willett CG, Cizito BG. Human papillomavirus tumor infection in esophageal squamous cell carcinoma. J Gastrointest Oncol. 2015;6:287-295.
5. Abbassadegan M, Omidia A, Niyazi A, et al. Prevalence of human papillomavirus type 16 and 18 and p53 mutant protein expression in esophageal squamous cell carcinomas. Iran J Basic Med Sci. 2003;6:3.
6. Abdirad A, Eram N, Behzadi AH, et al. Human papillomavirus detected in esophageal carcinoma of the esophagus from Turkmen-Sahra, Iran. J Clin Diagn Res. 2016;22:667-672.
7. Emadian O, Eram N, Behzadi AH, et al. Human papillomavirus detected in esophageal carcinoma in Guilan province. J Infect Dis. 2007;39:58-62.
8. Farhadi M, Tahmasebi Z, Merat S, et al. Human papillomavirus in squamous cell carcinoma of the esophagus in a high-risk population. World J Gastroenterol. 2005;11:1200-1203.
9. Haeri H, Mardanoy A, Asadi-Amoli F, Shahshah R. Human papilloma virus and esophageal squamous cell carcinoma. Acta Med Iran. 2013;51:242-245.
10. Mehran MS. Detection of human papilloma virus in esophageal squamous cell carcinoma in Gisian province. J Clin Diag Res. 2010;4:2373-2377.
11. Moradi A, Mohktari-Azad T. Detection of HPV in cancerous and non-cancerous esophageal tissues from Turkmen-Sahra, Iran. Int J Cancer Res. 2006;2:113-118.
12. Noori S, Monabati A, Ghaderi A. The prevalence of human papilloma virus in esophageal squamous cell carcinoma. Iran J Med Sci. 2012;37:126-133.
13. Sobedi F, Heidary N, Rahbar M, et al. Human papillomavirus and its clinical relevance in esophageal squamous cell carcinoma in a Kurdish population in the west of Iran. Infect Dis (London). 2016;48:270-273.
14. Yahiyoupo Y, Shamisi-Shahradabi M, Mahmoadi M, et al. High-risk and low-risk human papillomavirus in esophageal squamous cell carcinoma at Mazandaran, Northern Iran. Pathol Oncol Res. 2013;19:385-391.
15. Yahiyoupo Y, Sadeghi F, Alizadeh A, Rahbini R, Siddati S. Detection of Merkel cell polyomavirus and human papillomavirus in esophageal squamous cell carcinomas and non-cancerous esophageal samples in Northern Iran. Pathol Oncol Res. 2016;22:667-672.

16. da Costa AM, Fregnan JHTG, Pastrez PRA, et al. HPV infection and p53 and p16 expression in esophageal cancer: are they prognostic factors? Insect J Clin Diagn Res. 2017;12:54.
17. Antonsson A, Nancarrow DJ, Brown IS, et al. High-risk human papillomavirus in esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2010;19:2080-2087.
18. Castillo A, Aguayo F, Koriyama C, et al. Human papillomavirus in esophageal squamous cell carcinoma in Colombia and Chile. World J Gastroenterol. 2006;12:6188-6192.
19. Castillo A, Koriyama C, Higoshi M, et al. Human papillomavirus in upper digestive tract tumors from three countries. World J Gastroenterol. 2011;17:5295-5304.
20. Ding GC, Ren JL, Chang FB, et al. Human papillomavirus DNA and P16(INK4A) expression in concurrent esophageal and gastric cardia cancers. World J Gastroenterol. 2010;16:5901-5906.
21. Doostader EE, Katebzian AL. The relationship between p16 expression and high-risk human papillomavirus infection in squamous cell carcinomas from sites other than uterine cervix: a study of 137 cases. Hum Pathol. 2012;43:327-332.
22. Herbst S, Ferraro CT, Koff NK, et al. HPV infection in Brazilian patients with esophageal squamous cell carcinoma: interpopulation differences, lack of correlation with surrogate markers and clinicopathological parameters. Cancer Lett. 2012;326:52-58.
23. Koshto J, Wei WQ, Kreimer AR, et al. No role for human papillomavirus in esophageal squamous cell carcinoma in China. Int J Cancer. 2010;127:93-100.
24. Lodahl HE, Du J, Naisan A, et al. Prevalence of human papillomavirus (HPV) in oesophageal squamous cell carcinoma in relation to anatomical site of the tumour: PLoS ONE. 2012;7:e46538.
25. Malek SM, Nevin DT, Cohen S, Hunt JL, Palazos JP. Assessment of immunohistochemistry for p16INK4A and high-risk HPV DNA by in situ hybridization in esophageal squamous cell carcinoma. Int J Surg Pathol. 2011;19:31-34.
26. Shuyama K, Pastrez A, Aguayo F, et al. Human papillomavirus in high- and low-risk areas of esophageal squamous cell carcinoma in China. Br J Cancer. 2007;96:1554-1559.
27. Teng H, Li X, Liu X, Wu J, Zhang J. The absence of human papillomavirus in esophageal squamous cell carcinoma in East China. Int J Clin Exp Pathol. 2014;7:281-287.
28. Vaipehi K, Kochhar R, Bhadravat S, Dutta U, Singh K. High prevalence of human papillomavirus in esophageal squamous cell carcinoma: a study in paired samples. Dis Esophagus. 2013;26:282-287.
29. Sitias F, Egger S, Urban MI, et al. InteSCOPE study: associations between esophageal squamous cell carcinoma and human papillomavirus serological markers. J Natl Cancer Inst. 2012;104:147-158.
30. Astori G, Merluzzi S, Arrese A, et al. Detection of human papillomavirus DNA and p53 gene mutations in esophageal cancer samples and adjacent normal mucosa. Digestion. 2001;69:14.
31. Bellizi AM, Woodford RL, Moskaluk CA, Jones DR, Kozower BD, Stelow EB. Basaloid squamous cell carcinoma of the esophagus: assessment for high-risk human papillomavirus and related molecular markers. Am J Surg Pathol. 2009;33:1608-1614.
32. Kotyur S, Heidau S, Jain N, et al. p53 gene mutation and human papillomavirus (HPV) infection in esophageal squamous cell carcinoma from three different endemic geographic regions of India. Cancer Lett. 2005;218:69-79.
33. Kawaguchi H, Ohno S, Arai K, et al. p53 polymorphism in human papillomavirus-associated esophageal cancer. Cancer Res. 2000;60:2753-2755.
34. Lu Z, Chen K, Guo M. [Detection of HPV in human esophageal cancer in high-incidence area and its correlation with p53 expression]. Zhonghua Zhong Liu Za Zhi. 2001;23:220-223.
35. Mohiuddin MK, Chava S, Upenrud P, et al. Role of human papilloma virus infection and altered methylation of specific genes in esophageal cancer. Asian Pac J Cancer Prev. 2013;14:4187-4193.
36. Zhang D, Zhang W, Liu W, et al. Human papillomavirus infection increases the chemoradiation response of esophageal squamous cell carcinoma based on P53 mutation. Radiatther Oncol. 2017;12:155-160.