Original Article

The Effect of P53 Protein Over-Expression and Its Clinical Features on the Response to Preoperative Chemoradiotherapy of Esophageal Squamous Cell Carcinoma

Mehdi Seilanian Toosi[1], Mohammad Reza Ghavam Nasiri[1], Kamran Ghafarzadegan[2], Azar Fani Pakdel[1], Roham Salek[1], Kazem Anvari[1]

1. Dept. of Radiotherapy Oncology and Cancer Research Center, Mashhad Univiversity of Medical Sciences, Mashhad, Iran.

2. Dept. of Pathology and Cancer Research Center, Mashhad Univiversity of Medical Sciences, Mashhad, Iran.

ABSTRACT

Background and Objective: **P53 is a suppressive gene that plays a key role in DNA repair and apoptosis. The purpose of this study was to investigate the effect of P53 protein over-expression and some clinicopathological factors on the esophageal squamous cell carcinoma (SCC) response to neoadjuvant chemoradiotherapy.**

Patients and Methods: **In this retrospective cohort study, 44 patients with localized esophageal SCC undergoing neoadjuvant chemoradiotherapy (cisplatin + 5FU and 40 Gy in 20 fractions of irradiation) and surgery were evaluated. Pretreatment specimens were immunohistochemically assessed for p53 over-expression and scored according to the frequency of stained cells. The pathologic response in resected specimens was categorized as follows: complete response (CR), no evidence of malignant cell; partial response (PR), small foci of malignant cells and negative lymph nodes and minor response, macroscopic residual tumor or positive lymph nodes.**

Results: **It was found out that p53 protein over-expression exists in 29 cases (65.9%). Following chemoradiotherapy, CR and PR were found in 9 (20.5%) and 19 cases (43.2%) respectively. There were also no significant association between tumor response and clinicopathological features such as sex ($p = 1$), age ($p = 0.82$), dysphagia grade ($p = 0.82$) and longitudinal length of the tumor ($p = 0.59$). No significant correlation was found between p53 expression and pathological response to preoperative chemoradiotherapy ($p = 0.94$).**

Conclusion: **These findings suggest that p53 protein expression is not reliable for predicting the response to neoadjuvant chemoradiotherapy. There were also no correlations between pathological response to chemoradiotherapy and clinical features such as age, sex, dysphagia grade and longitudinal diameter of the tumor.**

Key words: Esophagus, Squamous cell carcinoma, Combined Modality Therapy, Chemotherapy, Radiotherapy, Tumor Suppressor Protein p53

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Address communications to: Dr. Mehdi Seilanian Toosi, Department of Radiotherapy Oncology, Omid Hospital, Mashhad, Iran.
Email: mehdi.seilaniantoosi@gmail.com

Introduction

Esophageal squamous cell carcinoma (SCC) is a highly lethal and aggressive disease (1). Most cases are diagnosed in advanced stages (2) which is due to vague symptoms at early stages, lack of serosal envelop and the rich submucosal lymphatic network.

The long-term results of surgery (2,3) or radiotherapy alone (4) for the treatment of localized esophageal carcinoma have been poor. Combined modality approaches have been introduced for improving both local control and survival. Several randomized and non-randomized trials with different protocols have been conducted to illustrate the role of preoperative chemoradiotherapy in resectable esophageal carcinoma (5). In a meta-analysis by Gebski et al on ten randomized trial comparing neoadjuvant chemoradiotherapy (CRT) versus surgery alone in local operable esophageal carcinoma, a significant survival benefit was shown for neoadjuvant CRT. The hazard ratio for all-cause mortality for CRT plus surgery versus surgery alone was 0.81 (95% CI 0.70-0.93; p = 0.002) (6). Similarly, a meta-analysis of randomized trials by Urschel et al showed a 3-year survival benefit and reduced local recurrence for neoadjuvant CRT as compared to surgery alone (7). However, some earlier randomized trials had not shown any survival advantage for neoadjuvant CRT as compared to surgery alone (8,9).

The results of multiple trials proved that tumor response to induction chemotherapy or CRT is an important predictor of survival (10-12). Therefore, it would be advantageous if we being able to predict the response to CRT and recognize a group of patients who gain the most benefit from this treatment. As a result, we could avoid toxic regimens for patients with unresponsive tumors. In addition, defining responsive tumors would be useful for selecting patients who are more suitable for definitive CRT programs.

Tumor genetic factors contribute to resistance to chemotherapy and/or radiotherapy. The roles of several biological factors including p53, Ki67, bax, VEGF, cyclin D1, bcl-2, metallo-thionein and CDC25B in the response to CRT have been assessed(13-18). The p53 gene is a tumor suppressor located on the short arm of chromosome 17q13. This gene encodes a 53-kDa phosphor-protein which is involved in DNA repair and induces apoptosis when DNA damage can not be repaired (19,20). Many chemotherapeutic agents including cisplatin and 5-FU as well as radiotherapy induce apoptosis by DNA damage. Therefore, it can be postulated that alteration in apoptotic pathway could result in unresponsiveness of tumors to treatment.

In this study, we analyzed the association between immunohistochemical (IHC) over-expression of p53 protein and response to neoadjuvant chemoradiotherapy in patients with esophageal SCC. The prognostic value of p53 expression was also evaluated.

Patients and Methods

This retrospective cohort study was conducted at Cancer Research Center (Mashhad University of Medical Sciences, Mashhad). Eligible cases were patients with localized esophageal SCC who underwent chemoradiotherapy followed by esophagectomy between April 2000 and April 2005 and their histological specimens were available for the evaluation. The pretreatment evaluation consisted of barium swallow X-ray, chest radiography, abdominal ultrasonography and in some cases chest CT scan. The longitudinal diameter of the lesion was measured based on barium X-ray findings. The severity of dysphagia was graded as follows: grade 1: normal swallowing, grade II: difficulty in swallowing some hard solids but can swallow semisolids, grade III: can not swallow any solids but can swallow liquids, grade IV: difficulty in swallowing liquids, grade V: can not swallow saliva. A total radiation dose of 40 Gy in 20 fractions was prescribed by cobalt 60 unit. Whole mediastinum and celiac lymph nodes were treated with anterior and posterior fields. Chemotherapy consisted of cisplatin 70-80 mg/m2 intravenous infusion and 5-FU 750-1000 mg/m2 as a continuous 24 h infusion for 4 consecutive days.

In post surgical specimens, the response to chemoradiotherapy was evaluated as follows: complete response (CR), no evidence of malignant cells; partial response (PR), small cluster of malignant cells and negative resected lymph nodes; minor response, macroscopic residual tumor and/or positive resected lymph nodes. Complete and partial responses were regarded as major responses.

Immunohistochemical study

Pretreatment paraffin-embedded blocks were used for p53 immunostaining. The prepared 4-μm sections were deparaffinised and treated with 3% H2O2 in methanol to block endogenous peroxidase activity. Antigen-retrieval procedure was performed by Trilogy solution (Cell Marque). Then, monoclonal antibody against P53 protein (clone DO-7; Dako), was applied to the sections and incubated for 30 minutes

at room temperature. The antigen-antibody complex was visualized using biotin-streptavidin-peroxidase staining technique. The color was developed with diaminobenzidine (DAB) solution and the sections were lightly counterstained with hematoxylin. Slides were then dehydrated and mounted. Immunoreactivity for p53 was scored as follows: negative (<10% positively stained cells); weak (+, 10-40% positively stained cells); moderate (++, 40-75% positively stained cells) and intense (+++, >75% positively stained cells).

Statistical analysis

The data were analyzed using SPSS (v. 11.5) in March 2007. The differences between groups were evaluated using chi-square test. In this respect, a p value less than 0.05 was considered as significant.

Results

Forty-four eligible patients were evaluated in this study. The median age of patients was 59 years (range: 32-69). The clinical characteristics of patients are illustrated in Table 1. p53 protein over-expression was negative in 15 (34.1%), 1+ in 5 (11.4%), 2+ in 10 (22.7%), and 3+ in 14 cases (31.8%). Therefore, 29 cases (65.9%) were considered as positive for p53 protein over-expression. Pretreated paraffin-embedded blocks were used for p53 immunostaining. The prepared 4-µm sections were deparaffinised and treated with 3% H2O2 in methanol to block endogenous peroxidase activity. Antigen-retrieval procedure was performed by Trilogy solution (Cell Marque). Then, monoclonal antibody against P53 protein (clone DO-7, Dako) was applied to the sections and incubated for 30 minutes at room temperature. The antigen-antibody complex was visualized using biotin-streptavidin-peroxidase staining technique. The color was developed with diaminobenzidine (DAB) solution and the sections were lightly counterstained with hematoxylin. Slides were then dehydrated and mounted. Immunoreactivity for p53 was scored as follows: negative (<10% positively stained cells); weak (+, 10-40% positively stained cells); moderate (++, 40-75% positively stained cells) and intense (+++, >75% positively stained cells). Figure.1 illustrates a moderately positive p53 nuclear staining in a pretreatment esophageal SCC accompanied with normal esophageal epithelium. The response to the neoadjuvant chemoradiotherapy was as follows: 9 (20.5%) complete response, 19 (43.2%) partial response, and 16(36.4%) minor response. Accordingly, 28 cases (63.6%) had major response to the preoperative treatment.

Table 1. Clinical characteristics of patients

Characteristics	n (%)
Sex:	
Male	21 (47.7)
Female	23 (52.2)
Dysphagia grade:	
Grade 1	3 (6.8)
Grade 2	5 (11.3)
Grade 3	18 (40.9)
Grade 4	10 (22.7)
Grade 5	2 (4.5)
Unspecified	6 (13.6)
Longitudinal diameter:	
<5 cm	16 (36.3)
5-10 cm	23 (52.3)
>10 cm	5 (11.3)
Total	44

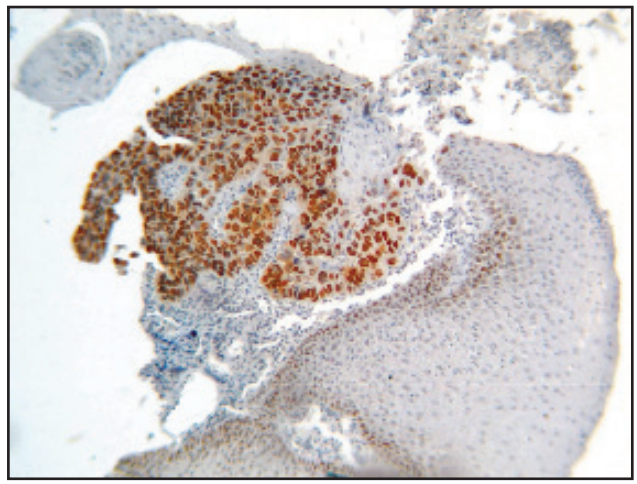

Figure 1. Moderately positive p53 nuclear staining in a pretreatment esophageal squamous cell carcinoma accompanied with normal esophageal epithelium (x40)

As it is shown in Table 2, there were no significant association between major tumor responses (complete plus partial responses) and clinicopathological features such as sex (male versus female; p = 1), age (>60 vs. < 60; p = 0.82), dysphagia grade (grades 1, 2, 3 vs. 4, 5; p = 0.82) longitudinal length of the disease (≥5 cm vs. < 5 cm; p = 0.59). The major pathologic response rates with respect to p53 expression scores

were as follows: negative, 10/15 (66.7%); weakly positive, 3/5 (60%), moderately positive, 6/10 (60%); and intensely positive, 9/14 (64.3%). Accordingly, there were no significant correlation between tumor response to chemoradiotherapy and p53 protein expression (p = 0.94).

Table 2. The relationship between clinical features, immunoexpression of p53 protein and the response of esophageal squamous carcinoma to preoperative chemoradiotherapy

Variables	Whole study number	Number of significant responses (%)
Age (years):		
<60	22	14 (63.6)
≥60	22	14 (63.6)
		p = 1
Sex:		
Male	21	13 (61.9)
Female	23	15 (65.2)
		p = 0.82
Dysphagia grade:		
Grade, 1,2,3	26	12 (46.2)
Grade, 4,5	12	6 (50)
		p = 0.82
Longitudinal diameter:		
<5 cm	16	11 (68.8)
≥5 cm	28	17 (60.7)
		p = 0.59
P53 protein over-expression:		
Negative	15	10 (66.7)
1+	5	3 (60)
2+	10	6 (60)
3+	14	9 (64.3)
		p = 0.94

Discussion

The results of our study did not show a correlation between p53 protein over-expression and response to neoadjuvant chemoradiotherapy. Clinical features such as age, sex, longitudinal diameter of lesions and pretreatment dysphagia grade had also no significant effect on the response rate.

Neoadjuvant chemoradiotherapy is a modality which is used frequently for the treatment of esophageal SCC. However, only a proportion of patients respond to the treatment. In our study, the major response rate and complete response rate were in compatible with those of previous studied, reported to be 50-70% and 20-30% respectively (21).

It has been shown that response to the chemoradiotherapy is the most important predictor of survival and only those patients with major responses benefit from neoadjuvant treatment (10-12). Therefore, many trials conducted to define factors which could predict the response to chemoradiotherapy. In a study by Szumilo et al, there were no significant associations between the response to preoperative chemotherapy and clinical indices (including age, sex, stage and tumor longitudinal diameter and lymph node metastasis) or histopathological features (grade of differentiation, degree of keratinization, nuclear polymorphism and mitotic index) (14). Several biologic factors, some of which are apoptosis mediators have been proposed as the potential predictors of response to chemotherapy and/or radiotherapy.

In our study, 65.9% of patients with esophageal carcinoma were p53 protein positive. In most references, this percentage ranges from 60% to 70%. Several clinical studies analyzed the correlation between p53 protein expression in esophageal cancer and response to treatment with inconsistent results. Although some trials showed poor response to neoadjuvant radiotherapy (22), chemotherapy (23) or chemoradiotherapy (16,24-26) in p53-positive tumors, some others did not support this association (14,18,27)

Even in trials showing significant association between p53 alteration and response, there have been p53 positive tumors with good response to the treatment and vice versa. Apparently, analysis of p53 gene status by IHC techniques is not a reliable method for predicting response in esophageal carcinoma. One explanation is that negative p53 over-expression does not always indicate normal p53 gene status. The reason described for the presence of mutation in absence of p53 over-expression is the deletion mutations or nonsense mutations which results in stop codon or production of truncated p53 protein which is undetectable (28,29).

The existence of alternative pathways in apoptosis is the reason for p53 protein expression inaccuracy in predicting the treatment response. The p21Waf1/Cip1 gene which encodes a potent cycline-dependant kinase inhibitor is necessary for p53 mediated G1 arrest (30). In a study by Miyazaka et al on 61 patients with esophageal SCC undergoing chemoradiotherapy (31

patients) or radiotherapy (30 patients), significantly better responses were shown for p53-negative as compared to p53-positive and p21-positive as compared to p21-negative tumors (26). In a trial on patients with stage III/IV esophageal SCC undergoing chemoradiotherapy, Nakamura observed that in cases with p53-positive lesions, survival of those patients with p21-positive tumors was significantly higher as compared to those with p21-negative tumors. However, in this study p53 status had no effect on tumor response or survival per se (18).

One of the p53-independent pathways is G2-M cell cycle checkpoint. Cdc25 phosphatases activate a set of cycline-dependant kinases (cdk/cyclines) involving in cell cycle regulation. Cdc25B activate CDC2 which in turn promote G2-M transition (31). A high expression of cdc25B has been shown in many human cancers. The over-expression of cdc25b may interfere with G2-M arrest after DNA damage which would lead to mitotic death (32). In studies of Shiozaki et al and Kishi et al, a correlation between cdc25b over-expression and good response of esophageal carcinoma to radiation (22) or chemoradiation (16) was found out. However, this result was not revealed in a study by Nakamura (18).

Conclusion

We did not find a correlation between p53 protein expression and response of esophageal carcinoma to chemoradiation. According to the literature, response to chemoradiation is a complicated matter which involves multiple pathways and molecules. Further studies are warranted for recognition of the role of these molecules in chemoradiation-induced apoptosis.

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