Expression of p53 Protein in Colorectal Cancer and Association With Prognostic Factors in Northeast Iran

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Abstract- Colorectal cancer (CRC) is one of the most prevalent cancers in the world. The p53 protein is the most commonly mutated protein in human cancer, and it is a frequent abnormality in colorectal cancers. In this study, we evaluated the expression of p53 protein and its association with clinicopathological findings as prognostic factors and mortality rate in 95 colorectal cancer patients' in Northeast Iran, over a 5 years period (2010-2015). In this retrospective study, the method of immunohistochemistry (IHC) was applied to determine the expression of the p53 protein and pathological features of tumors and mortality rate were examined between 2010 and 2015 at Gorgan City (IRAN). Multiple logistic regression models were used to estimate odds ratios and confidence intervals between clinicopathological and mortality rate as the outcome variable, adjusted for potential confounders. Immunoreactivity was found in 58.9% of specimens from 95 patients with colorectal cancer, and the mortality rate was estimated 14.7 percent. The mortality rate of patients with colorectal cancer is significant, and findings indicate that it relates to pathologic factors such as vascular involvement [OR=0.02, CI95%: 0.006-0.11], lymph node involvement [OR=0.17, CI95%: 0.05-0.56], round neural invasion, depth of invasion, tumor size and grade. The findings confirmed that the P53 gene mutation could be considered as a prognostic factor affecting mortality in cancer and specifically colorectal cancer. Although the P53 protein expression shows no relationship with histopathological features, the mortality rate of the patients demonstrated a strong association.

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Introduction

Colorectal cancer is a major cause of morbidity and mortality throughout the world (1). Colorectal cancer in Iran is the fourth common cancer in men and the second one among women (2). Abnormalities of the p53 gene have been identified in many malignancies, with reports of aberration in over half of colorectal, lung, breast, and hepatocellular carcinoma cases (3). The normal gene acts as a recessive oncogene, while mutations change the apparent function to that of a dominant oncogene(4).

The p53, also known as TP53 or tumor protein is a gene that codes for a protein that regulates the cell cycle and hence functions as tumor suppression. It is very important for cells in multicellular organisms to suppress cancer (5). Somatic TP53 mutations occur in almost every type of cancer at rates from 38% to 50% in ovarian, esophageal, colorectal, head and neck, larynx, and lung cancers and about 5% in primary leukemia, sarcoma, testicular, malignant melanoma, and cervical cancers (6). The p53 tumor suppressor gene is a frequent abnormality in colorectal cancers (7).

Previous studies have suggested that p53 was found to be most frequently mutated in colorectal cancer, which reports frequencies between 50 and 70 percent (8,9). Recently, p53 expression has also been proposed as a prognostic factor in some human cancers in different geographical areas (10).

The existence of important variations in mutation patterns between different groups of patients with same cancer suggests that further mutation fingerprints related to environmental exposures are still to be discovered. In the present study, our aim was to examine the prevalence of the expression of p53 protein, and differences in clinicopathological presentation and outcome in colorectal cancer, over a period of 5 years (2010-2015) in Northeast Iran.

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Materials and Methods

In this retrospective study, the method of immunohistochemistry (IHC) was applied to determine the expression of the p53 protein in 95 patients with colorectal cancer and pathological features of tumors and mortality rate were examined over a 5-year period from 2010 through 2015, at Golestan Path. Laboratory and pathology department of 5Azar hospital medical center, affiliated to Golestan University of Medical Sciences in Gorgan city. Gorgan is in the center of Golestan province in northern Iran, southeast of the Caspian Sea (11). This study obtained its ethics approval from the Ethical Committee of Golestan University of Medical Science (Ethical Code: 27962893111921).

The method of immunohistochemistry (IHC) was performed to determine the P53 protein expression in colorectal cancer paraffin-embedded tissues (12). After sectioning, the tumor specimens were fixed by formaldehyde (10%) and finally embedded in paraffin, and sliced into 4-pm thick sections. The sections were stained with a monoclonal antibody against p53 (Clone DO-7; Dako Cytomation, Glostrup, Denmark). The sections were deparaffinized with xylene and progressively dehydrated in decreasing concentrations of alcohol. Endogenous peroxidase activity was blocked by incubation in hydrogen peroxidase 1% in methanol for 30 minutes. The sections were covered with normal goat serum for 15 minutes to reduce nonspecific staining and incubated with a 1:20 dilution of primary antibody at room temperature for 2 hours. The sections were washed with Tris-buffered saline, incubated with a 1:30 dilution of biotinylated goat anti-mouse immunoglobulin G(Tago, Burlingame, CA) at room temperature for 30 minutes, and then covered with a 1:lOO dilution of streptavidin-biotin-peroxidase complex (Dakopatts, Copenhagen, Denmark) at room temperature for 30 minutes. The antibody was localized with 3, 3'-diaminobenzidine tetrahydrochloride. The slides were stained with methyl green 0.3% for 30 minutes. Negative control studies were made without the primary antiserum top53. A tumor was classified as p53 positive when nuclear staining was observed in 5% or more of the cells counted in 10 high power fields (13).

The pathologic features include tumor grade (grade), lymph node involvement, the space around nerve involvement, vascular involvement, tumor size, depth of invasion, metastasis of patient, information on patients' medical history which is often obtained from where they were registered (date of diagnosis and date of death of the patients).

The quantitative and qualitative data were described as mean (SD) and frequency (percentage), respectively. Differences in categorical variables between expressions of p53 protein groups were analyzed using two-tailed Fisher's exact tests, and Continuous variables were compared with Student *t*-test. Multiple logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) between clinicopathological (neural invasion, lymph node metastasis, vascular invasion, depth of invasion and etc.) and mortality rate as the outcome variable was adjusted for potential confounders (age and sex). Overall significant levels were set at $P<0.050$. Stata software, version 11 (Stata Corp, College Station, TX, USA) was used for all statistical analyses.

Results

Colorectal carcinoma specimens from 95 patients were investigated for p53 immunoreactivity from 2010 to 2015 in Golestan province. The mean age of the patients was 59.5±10.3 (range 38-90) years, and of the 95 subjects, 55 (57.9%) were male, and all of the patients were in clinical stage 3.

Lymph node metastasis was absent in 72 patients (75.8%) but present in 23 (24.2%). The majority of the histological grade was grade I and II in 84 (90%). The characteristics and clinicopathological features as prognostic factors of the colorectal cancer patients separately by expression of p53 protein are presented in table 1.

The prevalence of P53 protein expression in patients with colorectal cancer is 58.9 percent. Immunohistochemical analysis of p53 protein was performed in colorectal carcinomas specimens from 95 patients. Positive expression of p53 protein was defined as nuclear staining of >5% tumor cells. The p53 protein was detected in the nuclei in 56 (58.9%) of 95 colorectal carcinomas. The relationship between p53 expression and clinicopathological factors in colorectal cancer patients are summarized in table 1. P53 expression was significantly correlated with tumor size (P=0.03) and histological grade (P=0.04).

Over a period of 5 years, mortality occurred in 14 cases (14.7%). The mortality rate of patients with colorectal cancer is significant and it is related with pathologic factors such as vascular invasion [OR=0.02, CI95%: 0.006-0.11], lymph node metastasis [OR=0.17, CI95%: 0.05-0.56], neural invasion and depth of invasion as shown in table 2. In other words, the odds of death in colorectal cancer patients without vascular and

neural invasion are 2% less than in with vascular and neural invasion. The odds of death in colorectal cancer patients without lymph node metastasis are 17% less than in with lymph node metastasis.

Table 1. Description of the colorectal cancer patients separately by expression of p53 protein in Northeast Iran (2010-2015)

Characteristic		Total (N=95)	Presence of p53 protein mutation (N=56)	Absence of p53 protein mutation (N=39)	*P* *
			Values are mean ±SD		
Age (years)		59.54± 10.3	59.5±10.7	59.4±10.06	0.09
Tumor size (cm)		2.31± 0.6	2.39± 0.6	2.19± 0.5	0.03
			Values are n (%)		
Sex	**Male**	55 (57.9)	27 (69.2)	28 (50)	0.091
	Female	40 (42.1)	12 (30.8)	28 (50)	
Neural invasion	**Negative**	84 (88.4)	49 (87.5)	35 (89.7)	0.93
	Positive	11 (11.6)	7 (12.5)	4 (10.3)	
Depth of invasion	**2**	32 (33.7)	16 (50)	16 (50)	0.51
	3	46 (48.4)	31 (67.4)	15 (32.6)	
	4	17 (17.9)	9 (52.9)	8 (47.1)	
Lymph node metastasis	**Negative**	72 (75.8)	43 (76.8)	29 (74.4)	0.81
	Positive	23 (24.2)	13 (23.2)	10 (25.6)	
Vascular invasion	**Negative**	80 (84.2)	45 (80.4)	35 (89.7)	0.26
	Positive	15 (15.8)	11 (19.6)	4 (10.3)	
Histological grade	**GI**	62 (65.3)	38 (61.3)	24 (38.7)	0.04
	GII	22 (23.1)	10 (45.5)	12 (54.5)	
	GIII	9 (9.5)	6 (66.7)	3 (33.3)	
	GIV	2 (2.1)	2 (100)	--	
Mortality	**No**	81 (85.3)	8 (14.3)	6 (15.4)	0.98
	Yes	14 (14.7)	48 (85.7)	33 (84.6)	

*absence vs. presence

Table 2. Multiple logistic regression models using mortality rate as the outcome in colorectal cancer patients in Northeast Iran (2010-2015)

Risk factors	B	OR	95%CI	*P*
Neural invasion (Negative=0, Positive=1)	-3.91	0.02	0.006-0.13	0.0001
Lymph node metastasis (Negative=0, Positive=1)	2.83	0.17	0.05-0.56	0.002
Vascular invasion(Negative=0, Positive=1)	-3.91	0.02	0.006-0.11	0.0001
Depth of invasion	-3.50	0.03	1.36-6.09	0.01

Non-significant: Sex, Age, Tumor size, Histological Grade

Discussion

In this study, we observed the prevalence p53 protein expression and differences in clinicopathological presentation and outcome in colorectal cancer in Northeast Iran, over a period of 5 years. This finding confirms that immunoreactivity was found in 58.9 percent of specimens from 95 patients with colorectal cancer which is in line with the 32%–69% range of p53 positivity in previous reports (13). Various studies showed that the p53 gene mutation factor as a factor influencing the prognosis is not yet a definitive expression and it is still under controversial debates. Bazan and Russo's studies reported that P53 gene mutation is proposed as a prognostic factor (14,15), while other studies reported this factor to be ineffective in prognosis (9,16). Sporadic studies are available on mutation frequency of p53 among colorectal cancers across Iran. Previous studies showed overexpression of mutant p53 in 59-63 percent of patients with colorectal cancer using the immunohistochemical method, while sequencing of amplified DNA samples revealed the rate as 23-44 percent (17,18). Several studies using the 5-10% cutoff scoring method describe a high degree of concordance between pathologists evaluating positive and negative tumors (19, 20).

According to results of Malekzadeh *et al*., p53 gene mutation in Iranian CRCs occurs as frequent as in other series, but the proximal and distal side of colon show different p53 mutation patterns, which may suggest different tumorigenesis pathways of the proximal and distal colon (21).

The results from the one systematic review on 18766 patients indicated that abnormal p53 had more of an

impact on survival in patients whose underlying prognosis was better. This suggests that abnormalities in p53 may have an independent adverse impact upon prognosis. In patients with better underlying prognosis, that is, survival rates of 465 percent after surgery; abnormal p53 has an adverse effect on the outcome. Rectal tumors containing proven mutations in p53 are less likely to respond to radiation, or chemoradiation, than rectal cancers without evidence of mutant p53 (22).

In the present study, p53 expression was significantly correlated with tumor size and histological grade. We could not see a significant relationship between P53 expression and other clinicopathological factors. Likewise, Zaanan's study stated that there is a relationship between P53 gene mutation and tumor location, but there is no relationship between P53 protein mutations and their stage nor the grade of tumors (23). Increased tumor cell apoptosis can have a positive or a negative impact on survival and local recurrence depending on the location of the tumor in the large bowel (24). Zlobec *et al*., reported Immunoreactivity was found in approximately 72% of specimens from 87 pretreatment formalin-fixed paraffin embedded diagnostic rectal biopsy tissues (20). Zhang *et al*., showed that there is no significant correlation between the expression of p53 and the histologic grade, tumor size, serosal invasion, lymphatic invasion, venous invasion, lymph node metastasis, or liver metastasis (25).

Our study demonstrates that the mortality rate of colorectal cancer patients was 14.7 percent over a period of 5 years. Worldwide attributable mortality to colorectal cancer is approximately half of that incidence in this study. Nearly 530,000 deaths were recorded in 2002, that is ~8% of all cancer deaths (26). In the US, colorectal cancer incidence and mortality rates are about 30% to 40% higher in men than in women (27).

The majority of colorectal cancer cases occur in individuals without a family history of colorectal cancer or a predisposing illness. Nevertheless, up to 20% of people who develop colorectal cancer have other family members who have been affected by this disease (28).

The mortality rate of patients with colorectal cancer is significantly related with pathologic factors such as vascular invasion, lymph node metastasis, neural invasion and depth of invasion. Gonzalez *et al*., have reported the results of a systematic review indicating that four parameters clearly emerged as significant predictors of poor outcome after colorectal cancer; synchronous LM, the involvement of thoracic lymph nodes, multiple LM and elevated prethoracotomy CEA level (29).

While the current study has supplied much useful information about the prevalence p53 protein expression and differences in clinicopathological presentation in colorectal cancer in Northeast of Iran, it has several limitations that must be acknowledged. This study provided information about 95 colorectal cancer patients, and we did not have a control group to compare demographic characteristics and clinical symptoms. The study was limited exclusively to patients of pathology department of 5Azar hospital, affiliated to Golestan University of Medical Sciences in Gorgan city and not the whole community of Iran. More studies are needed to investigate the p53 protein expression in other studies population.

The findings confirmed that the P53 gene mutation might be considered as a prognostic factor affecting mortality in colorectal cancer in Northeast Iran. In addition, we cannot see the relationship between P53 protein expression and pathological features, but the mortality rate of the patients demonstrated a strong association.

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References

1. Schreuders EH, Ruco A, Rabeneck L, Schoen RE, Sung JJ, Young GP, et al. Colorectal cancer screening: a global overview of existing programmes. Gut 2015;64:1637-49.
2. Dolatkhah R, Somi MH, Bonyadi MJ, Asvadi Kermani I, Farassati F, Dastgiri S. Colorectal cancer in Iran: molecular epidemiology and screening strategies. J Cancer Epidemiol 2015;2015:643020.
3. Vousden KH, Lane DP. P53 in health and disease. Nat Rev Mol Cell Biol 2007;8:275-83.
4. Lima CRdO, Rabelo RE, Vulcani VAS, Cardoso LD, Sousa NLdM, Moura VMBDd. P53 gene: major mutations in neoplasias and anticancer gene therapy. Ciência Rural 2012;42:845-53.
5. Al-Salihi KA, Azlina A. P53 gene mutation and protein expression in ameloblastomas. Braz J Oral Sci 2015;5:1034-40.
6. Olivier M, Hollstein M, Hainaut P. TP53 mutations in

human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol 2010;2:a001008.
7. Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 tumor suppressor gene important milestones at the various steps of tumorigenesis. Genes Cancer 2011;2:466-74.
8. Muller PA, Vousden KH. p53 mutations in cancer. Nat Cell Biol 2013;15:2-8.
9. Duffy M, Van Dalen A, Haglund C, Hansson L, Holinski-Feder E, Klapdor R, et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Eur J Cancer 2007;43:1348-60.
10. Pages F, Galon J, Dieu-Nosjean M, Tartour E, Sautes-Fridman C, Fridman W. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene 2010;29:1093-102.
11. Mehravar F, Rafiee S, Bazrafshan B, Khodadost M. Prevalence of asthma symptoms in Golestan schoolchildren aged 6–7 and 13–14 years in Northeast Iran. Front Med 2016;10:345-50.
12. Akkiprik M, Ataizi-Celikel C, Dusunceli F, Sonmez O, Gulluodlu BM, Sav A, et al. Clinical significance of p53, K-ras and DCC gene alterations in the stage I-II colorectal cancers. J Gastrointestin Liver Dis 2007;16:11.
13. Zhao D-p, Ding X-w, Peng J-p, Zheng Y-x, Zhang S-z. Prognostic significance of bcl-2 and p53 expression in colorectal carcinoma. J Zhejiang Univ Sci B 2005;6:1163-9.
14. Bazan V, Agnese V, Corsale S, Calo V, Valerio M, Latteri M, et al. Specific TP53 and/or Ki-ras mutations as independent predictors of clinical outcome in sporadic colorectal adenocarcinomas: results of a 5-year Gruppo Oncologico dell'Italia Meridionale (GOIM) prospective study. Ann Oncol 2005;16:iv50-iv5.
15. Russo A, Bazan V, Iacopetta B, Kerr D, Soussi T, Gebbia N. The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment. J Clin Oncol 2005;23:7518-28.
16. Ren J, Li G, Ge J, Li X, Zhao Y. Is K-ras gene mutation a prognostic factor for colorectal cancer: a systematic review and meta-analysis. Dis Colon Rectum 2012;55:913-23.
17. Mahdavinia M, Bishehsari F, Verginelli F, Cumashi A, Lattanzio R, Sotoudeh M, et al. P53 mutations in colorectal cancer from northern Iran: Relationships with site of tumor origin, microsatellite instability and K ras mutations. J Cell Physiol 2008;216:543-50.
18. Golmohammadi R, Namazi MJ, Nikbakht M, Salehi M, Derakhshan MH. Characterization and prognostic value of mutations in exons 5 and 6 of the p53 gene in patients with colorectal cancers in central Iran. Gut Liver 2013;7:295-302.
19. Zu Y, Steinberg SM, Campo E, Hans CP, Weisenburger DD, Braziel RM, et al. Validation of tissue microarray immunohistochemistry staining and interpretation in diffuse large B-cell lymphoma. Leuk Lymphoma 2005;46:693-701.
20. Zlobec I, Steele R, Michel RP, Compton CC, Lugli A, Jass JR. Scoring of p53, VEGF, Bcl-2 and APAF-1 immunohistochemistry and interobserver reliability in colorectal cancer. Mod Pathol 2006;19:1236-42.
21. Malekzadeh R, Bishehsari F, Mahdavinia M, Ansari R. Epidemiology and molecular genetics of colorectal cancer in Iran: a review. Arch Iran Med 2009;12:161-9.
22. Munro A, Lain S, Lane D. P53 abnormalities and outcomes in colorectal cancer: a systematic review. Br J Cancer 2005;92:434-44.
23. Zaanan A, Cuilliere-Dartigues P, Guilloux A, Parc Y, Louvet C, De Gramont A, et al. Impact of p53 expression and microsatellite instability on stage III colon cancer disease-free survival in patients treated by 5-fluorouracil and leucovorin with or without oxaliplatin. Ann Oncol 2010;21:772-80.
24. Heer Pd. Molecular and biological interactions in colorectal cancer [Dissertation]. Netherlands: Leiden Univ., 2007.
25. Zhang M-F, Zhang Z-Y, Fu J, Yang Y-F, Yun J-P. Correlation between expression of p53, p21/WAF1, and MDM2 proteins and their prognostic significance in primary hepatocellular carcinoma. J Trans Med 2009;7:110.
26. Marmot M, Atinmo T, Byers T, Chen J, Hirohata T, Jackson A, et al. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. (Accessed January 2018, 2, at http://www.aicr.org/assets/docs/pdf/reports/Second_Expert_Report.pdf).
27. Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin 2008;58:130-60.
28. Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg 2009;22:191-7.
29. Gonzalez M, Poncet A, Combescure C, Robert J, Ris HB, Gervaz P. Risk factors for survival after lung metastasectomy in colorectal cancer patients: a systematic review and meta-analysis. Ann Surg Oncol 2013;20:572-9.