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Original Article

CIRCULATING P53 ANTIBODIES IN PATIENTS WITH COLORECTAL CANCER: RELATION TO CLINICOPATHOLOGICAL FEATURES AND TUMOR MARKERS

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ABSTRACT

Background: P53 antibodies (p53 Abs) have been detected in the serum of a proportion of colorectal cancer (CRC) patients. The development of these antibodies is associated with the presence of mutant oncoprotein p53. However, it is not yet known at which stage during CRC progression p53Abs appear in serum.
Aim of the Work: To identify the prevalence of p53 Abs in CRC and their association with clinicopathological features of the tumor.
Patients and Methods: The study included 30 CRC patients (16 males and 14 females) their age ranged between 20-73 years. All patients were subjected to full clinical examination and laboratory studies including tumor markers (CEA, CA 19.9). Colonoscopy was done for pathological grading and staging of the tumor according to TNM and Dukes' staging system. P53 antibodies in patients' sera were detected using ELISA technique.
Results: We found that 16.6 % of our CRC patients had positive anti-p53 Abs. No association was observed between the presence of these Abs and clinicopathological features including age, tumor location, tumor size, T-stage, Dukes' staging or even distant metastasis. A statistically significant association was found between the presence of p53 Abs and lymph node status ($p < 0.001$) and with histological grading ($p=0.031$).
Conclusion: The presence of p53 Abs in sera of patients with CRC indicates a more advanced tumor histopathological stage and could be utilized as a complementary prognostic tool to colonoscopy in high risk patients.

Key Words: Colonoscopy- CRC- p53 Abs.

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INTRODUCTION

The p53 tumor suppressor gene is located on the short arm of chromosome 17 and encodes a 53-kd nuclear phosphoprotein that regulates the cell cycle. Mutational inactivation of the p53 is one of the most frequent genetic changes in the development of human malignancies and can be detected in 40-70% of all colorectal adenocarcinomas[1-4].

As most mutations modify the configuration and the stability of the p53 protein with its accumulation in the nucleus of tumor cells, there has been intensive investigation screening for p53 alterations by immunohistochemical analysis[5].

Mutant p53 protein and other tumour-specific antigens, may be a target of the host's immune response[6,7]. Studies have shown that 9-26% of patients with different carcinomas have mounted a humoral immune response (antibodies) to abnormal p53 protein[8-10]. Thus, anti-p53 antibodies (Abs) may be a surrogate marker of p53 mutation and poor prognosis[11].

Many studies have shown increased serum antibody levels against mutant p53 protein in patients with breast[7,12] and lung[13,14] cancers. Moreover, p53 Abs were found in 13-32% of colorectal cancer (CRC) patients[15,16]. Previous studies in breast cancer patients have indicated that the occurrence of p53 Abs may be a useful determinant regarding poor prognosis[6,7]. This was also reported in CRC patients[15].

The purpose of our study is to:

1. Detect the prevalence of anti- p53 Abs among patients with CRC.
2. Correlate the presence of anti-p53 with the clinico-pathological findings and with the widely used tumor markers; the carcinoembryonic antigen (CEA) and the carbohydrate antigen (CA) 19.9.
3. Assess the predictive value of anti-p53 regarding tumor staging in view of selecting patients for neoadjuvant treatment.

PATIENTS AND METHODS

Thirty patients, 16 males and 14 females, presenting to Clinical Oncology department, Kasr El Aini, Cairo University, were enrolled in the study. Their age ranged from 20-73 years. Informed consent was taken from patients before participation in the study.

All patients were subjected to full clinical examination and laboratory studies including ESR, complete blood count (CBC), liver function tests (LFT) and kidney function tests (KFT). Imaging studies as x-ray chest, CT abdomen and pelvis were also done for patient assessment.

Colonoscopy was done for detection of the site of the lesion, evaluating tumor size and gross pathology. Biopsies were obtained from each suspected lesion for histopathological classification. The tumors were graded according to the WHO classification[17] and staged according to the Dukes' classification system[18]. Operable cases were subjected to abdominoperineal resection, anterior resection or resection anastomosis for either palliative or curative intent.

CEA and CA 19.9 were tested with commercially available Abbott Axsym system based on microparticle enzyme immunoassay (MEIA) technology. Cut off levels of 5 ng/ml and 37 U/ml, respectively, were recommended by the manufacturers. Serum samples of the 30 patients were collected preoperatively and before starting radiotherapy or chemotherapy and stored at -80°C. The measurements of anti-p53 were performed using enzyme-linked immunosorbent assay (ELISA) supplied by IBL product, Germany. All samples were assayed in duplicate and considered positive at an optical density above the low positive control sample. The cut-off level was estimated according to the manufacturer as 0.074. Results less than this cut-off were considered negative for p53 Abs and those greater than the cut-off were positive.

Statistical Methods:

Statistical Package for Social Sciences (SPSS) version 12 was used. Quantitative variables were summarized using mean and SD, median and ranges. Qualitative data were summarized using frequencies and percentages. Comparison between different groups was done using Mann-Whitney test. Differences were considered significant when p value was ≤ 0.05 and highly significant when p value ≤ 0.01.

RESULTS

The study included 30 patients diagnosed as colorectal adenocarcinoma. Their ages ranged between 20 to 73 years (median: 52 yrs). The demographic and clinical characteristics of patients are shown in Table (1).

Table 1: Patients characteristics.

	Cases (n)	Percentage (%)
Age (all patients)	30	100%
< 30	3	10%
> 30	27	90%
Gender		
Female	14	46.6%
Male	16	53.4%
Presentation		
Bleeding per rectum	20	66.6%
Constipation	11	36.7%
Intestinal obstruction	3	10%
Pain	3	10%
Abdominal distension	1	3.33%
Mass	1	3.33%
Lesion		
Ulcerative	13	43.3%
Fungating	11	36.7%
Stricture	3	10%
Cauliflower	3	10%
Site		
Rectum	19	63.3%
Colon	11	36.7%
Grade		
I + II	20	66.6%
III + IV	10	33.4%
T		
I + II	2	6.6%
III + IV	28	93.4%
N		
0	15	50%
1	11	36.7%
2	4	13.3%
M		
+ve	10	33.4%
-ve	20	66.6%
Dukes' staging		
A + B	15	50%
C + D	15	50%

Anti-p53 Abs were increased in 16.6% (5/30) of the CRC patients. Association between anti-p53 Abs expression with clinicopathological and laboratory parameters is shown in Table (2).

There was a significant association between the presence of p53 Abs and lymph node status ($p<0.001$) and with tumor differentiation ($p=0.031$) as shown in (Figures 1,2). No significant association was observed regarding other clinical parameters (Table 2).

Association between laboratory parameters and anti-p53: The median value of CA 19.9 was 42 U/ml (range: 7-817) and 10 U/ml (range: 1-800) in anti-p53 positive and negative patients, respectively. Moreover, the median value of CEA was 1.6 ng/ml (range: 0.1-4) and 3 ng/ml (range: 0.1-500) in anti-p53 positive and negative patients, respectively. The association of CA19.9 and CEA with anti-p53 Abs was not significant ($p= 0.190$, $p=0.220$, respectively).

Table 2: Association between P53-Abs and clinicopathological features of CRC patients.

Parameter	No	Positive		Negative		P-value
		No	%	No	%	
Age						
< 30 years	27	5	100	22	88	0.432
> 30 years	3	0	0	3	12	
Sex						
Male	16	3	60	13	52	0.754
Female	14	2	40	12	48	
Grosspathology						
Ulcerative	13	3	60	10	40	0.678
Fungating	11	1	20	10	40	
Stricture	3	1	20	2	8	
Cauliflower	3	0	0	3	12	
Tumor location						
Rectum	19	5	100	14	56	0.129
Color	11	0	0	11	44	
Histopatholgical grading:						
I / II	20	1	20	19	76	0.031*
III / IV	10	4	80	6	24	
Tumor size						
II	2	0	0	2	8	1.00
III + IV	28	5	100	23	92	
Nodal stages						
N 0	15	0	0	15	60	
N 1	11	1	20	10	40	<0.001**
N 2	4	4	80	0	0	
Distant metastases						
Positive	10	2	40	8	32	1.00
Negative	20	3	60	17	68	
Dukes' staging						
A + B	15	1	20	14	56	0.330
C + D	15	4	80	11	44	

* = significant (p<0.05)
**=Highly significant (p<0.01)

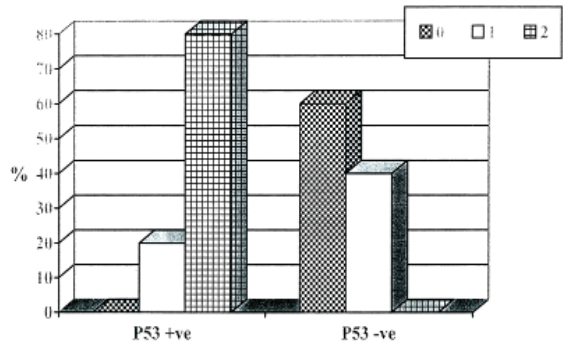

Figure 1: Relation between LN status and anti-p53 Abs (p<0.001).

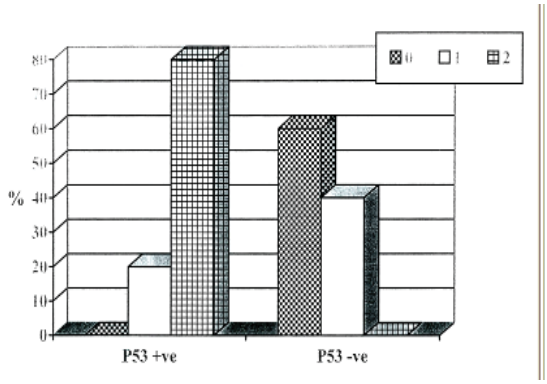

Figure 2: Relation between tumor grade and serum anti-p53 Abs (p=0.031)

DISCUSSION

Alterations in p53 tumor suppressor gene have prognostic value in colon, breast and gastric cancers making their recognition of importance in clinical practice[19-22]. Mutational inactivation of the p53 is one of the most frequent genetic events in the development of human cancer including CRC. The inactive mutant p53 usually acumulates in the nucleus of tumor cells leading to generation of p53 Abs.

In the current study, 16.6% of our patients were expressing positive p53 Abs, which is consistent with previous reports of 18–32%[11, 22-26].

The reported frequencies in individual studies vary due to different detection methods and cut-off values. Although our patients' number is limited, yet our results are in agreement with a large single study using an approved immunoassay technique which reported 13% sero positivity in a Taiwanese population[26].

The authors attributed their results which is lower than other ELISA-based studies to differences in population genetics as most other studies were performed on European populations. The mean seropositivity reported from many other studies was 17.8% which is nearly similar to our results[9-13, 27-32].

In the present study, the proportion of p53 Abs positivity was independent of age, sex, clinical presentation, gross pathology of the lesion, tumor size or distant metastasis, which coincides with other studies reporting no association between anti-p53 positivity and clinico-pathological features of CRC[11,22-26].

Although all positive patients for p53 Abs were above 30 years of age, with rectal involvement, T3, T4 stages, Dukes' stages C and D, yet the increased level of anti-p53 Abs was not statistically significant.

Since distinct mechanisms may be involved in the carcinogenesis of proximal and distal colon cancer [33] and a greater proportion of distal colon tumors express p53 mutation or accumulation[34,35], it was not surprising that patients with distal colon cancer have a higher frequency of p53 Abs in the serum than those with proximal tumors[26].

Regarding the Dukes' staging, the level of serum p53 Abs was increased with Dukes' stages C and D, however, the difference was not statistically significant. This is in agreement with Suppiah et al.[11], who reported no association between anti-p53 and Dukes' staging (p = 1.00) although other studies reported correlations between anti-p53 Abs and CRC with advanced Dukes' C/D, Stage IV disease[15,26,32].

Among patients with N1 lymph node metastases (36.7%), only one case (3.3 %) was positive for serum anti-p53 Abs. On the other side, all patients with N2 metastases (13.3%) were positive for anti-p53 Abs indicating a highly significant association between p53 Abs level and lymph node status ($p < 0.001$).

In this context, Tang et al. in 2001 reported higher autoantibody frequency in patients with advanced N3 nodal stage compared to early nodal disease (N1/2, pericolic/perirectal nodes). Whereas no difference was encountered between N0 and N1/2 disease[26]. This suggests that advanced nodal status may induce autoantibody production[11]

In the present study, 66.6% of patients were presenting with low grade tumors (GI, II), only one case (3.3%) of those patients was positive for the anti p53, whereas among the rest of patients (33.4%) with high grade tumors (GIII, IV), 13.4% were positive for anti-p53 Abs. The correlation between tumor grading and anti-p53 Abs was statistically significant (P=0.031).A significant correlation was also reported by Houbiers et al.,[15] who found association between anti-p53 Abs and histological grading ($p = 0.02$).

This association was not found by Kressner et al.[36] who reported that the proportion of p53 positivity in serum was independent of tumor differentiation[36].

In the present study and in accordance with Tang et al.[26], anti-p53 Abs level was not correlated with laboratory parameters including ESR, hemoglobin level, CEA or CA19.9

At present, CEA and CA 19.9 are the most extensively used tumor markers for the management of patients with colorectal cancer. However, it has been shown that false positive increases in CEA may occur in smokers and false positive increases in CA 19-9 have been found in patients with cirrhosis, pancreatitis, or acute cholestasis[37].

Hammel et al. suggested that CEA measurement and CA 19.9 and p53 Abs testing may be complementary methods for the management of patients with colorectal cancer[22]. Recently, it was concluded that multiple assessments of both serum p53 Abs and CEA during postoperative monitoring increase the probability of early detection of recurrence or metastasis in patients with colorectal cancer and normal CEA level before surgery[38].

In our study, 3 patients with non-metastatic colorectal cancer showed p53 Abs. This is in agreement with the results of Hammel et al.[22] who reported similar results[22]. It has been shown that p53 Abs can even precede tumor detection[12].

A possible future role for anti-p53 autoantibodies is in screening and/or monitoring disease progression. The low sensitivity makes it unsuitable for screening the general population but it may have a role in high-risk groups who require more frequent evaluation. Anti p53 Abs serum testing is faster, cheaper, non-invasive and hence more repeatable than colonoscopy[11]. It is independent of CEA and highly specific for malignancy[10, 22,32].

CONCLUSION

The presence of p53 Abs in sera of patients with CRC indicates more advanced tumor histopathological staging. Their monitoring after surgery and adjuvant chemotherapy has predictive potential for early diagnosis of tumor relapse in p53 Abs positive cases especially if other markers were normal preoperatively.

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