The significance of combined measurement of p53 antibody and other tumor markers for colorectal cancer after curative resection.

Daisuke Kawahara [1], Fumihiko Fujita [1], Hideki Hayashi [2], Toshifumi Matsuyama [2], and Susumu Eguchi [1]

[1] Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, 852-8501, Japan

[2] Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences

Address correspondence: Susumu Eguchi, Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

Tel: +81-95-819-7316, Fax: +81-95-819-7319,

E-mail: sueguchi@nagasaki-u.ac.jp

Abstract

Background/Aims

Though serum p53 antibody has been widely used, it is mentioned that it is not related to clinical parameters of colorectal cancer (CRC) and has no prognostic significance in long-term follow-up. The aim of this study was to explore the possibility to increase the value of p53 antibody in combination with carcinoembryonic antigen (CEA) and CA19-9 for post operation follow up. We also investigated the relevance of KRAS mutation and p53 antibody.

Methodology

One hundred twenty-eight patients with primary CRC who underwent surgery were enrolled in this study. Serum p53 antibodies ,CEA and CA19-9 were measured before and after the surgery and their impact on CRC staging patient survival. DNA was extracted from colorectal cancer tissue and KRAS mutation analysis was determined by direct sequencing.

Results

Thirty seven point five patients were positive for serum p53 antibodies

before the operation. Total 67.2% patients were positive with any tumor markers, but it was only 3.9% that were positive with all markers. Compared with other tumor markers, positive rate of p53 antibody could detect patients with earlier stage cancer. Positivity of p53 antibody levels tended to become negative slowly after surgery. In overall survival, there was no significant difference between patients with or without positive p53 antibody. There was no association between KRAS mutation and p53 antibody level.

Conclusions

The combined use of tumor markers can be an effective screening method for detection of colorectal cancer. If serum p53 antibody level continues to be positive for long after resection, the case should require subsequent intense follow-up.

INTRODUCTION

The use of various tumor markers for monitoring of CRC has been described. Although CEA monitoring has been widely used for CRC (1), CEA is elevated especially in advanced stages of cancer, therefore it is not useful for early cancer. Recently, serum p53 antibodies are widely reported to overcome this limitation (2). The p53 antibodies were first described by Crawford et al. in the patients with breast cancer (3). By subsequent researches, circulating anti-p53 antibodies can be found in sera of patients with various human malignancies; esophageal cancer, colorectal cancer, lung cancer, and uterine cancer (4, 5, 6).

Some studies have reported that serial measurement of p53 antibodies provides useful clinical information during follow-up among cancer patients (7, 8), and mutations in p53 are associated with poor prognosis in CRC in common with many other tumors (9, 10). The Japanese Ministry of Health and Welfare approved the use of p53 antibodies as a new tumor marker for various human cancers on Nov 2007. However, According to some studies

report, p53 antibodies are not related to clinical parameters of CRC and have no prognostic significance in long-term follow-up, indicating its controversial significance (11).

The aim of this study was to clarify the effect of combined measurement of p53 antibodies, CEA and CA19-9 in CRC patients especially with long-term follow up. We also examine the correlation between p53 antibodies and KRAS mutations for deeper insight.

METHODOLOGY

Patients

One hundred twenty-eight patients with primary colorectal cancer who underwent surgery at Department of Surgery of Nagasaki University between January 2008 and December 2011 were enrolled in this study. Serial serum samples of all the patients were measured from before the operation and from after the surgery. In each serum sample, p53 antibodies, CEA, and CA19-9 were measured. Patients in positive p53 antibodies group before surgery were also analyzed KRAS mutation.

Patients who had multiple primary cancers or received preoperative radiotherapy or chemotherapy were excluded. All the patients were followed-up at physical examination, blood tests, interval abdominal computer tomography and colonoscopy. Patients were offered adjuvant chemotherapy based on pathological Stage III/IV cancer in the study of following levels of serum p53 antibodies. There were 58 male and 70 female patients, with a mean age of 65.6 years (range 35-90). The International

Tumor-Node-metastasis (TMN) classification according to the World Health Organization (WHO) was established on the pathologic examinations of resected specimens. Four tumors were in stage 0. 19 were in stage I, 48 were in stage II, 38 were in stage III, and 19 were in stage IV. The mean follow-up was 42.3 months with a rage of 10-69 months.

Biochemical analysis

Serum level of p53 antibodies, CEA and CA19-9 were measured with Chemiluminescent Immunoassay at SRL, Inc. (Tokyo, Japan). In this study, the cutoff values for serum p53 antibodies, CEA and CA19-9 were 1.3 U/ml, 5.0 ng/ml and 37.0 U/ml, respectivery.

DNA extraction and KRAS mutation determination

Formalin-fixed paraffin-embedded colorectal cancer tissue blocks were used. Tissue was sliced 2 to 4 section of 5μm in thickness. Paraffin was removed predominantly through immersion in 100% xylene following Kraffit et al (12).

Samples were immersed in xylene for 5 minutes, centrifuged to pellet the tissue and to enable removal of the xylene, washed twice in ethanol (1×85% and 1×100%), then allowed to air dry at 75℃ for 5 minutes (13). The extraction buffer was composed of 20mM Tris [hydroxymethyl] aminomethane hydrochloride (Tris-HCl), pH 7.6, 20mM ethylenediaminetetraacetic acid, disodium salt (EDTA), 1% sodium dodecyl sulfate (SDS), 0.5mg/ml proteinase K (Takara Bio Inc, Japan). The extraction buffer was add, 500μl/pellet, and the sample was incubated in a 55℃ water bath for 48 hr. After incubation, two phenol/chloroform extractions were performed and mixed 50μl 3M sodium acetate, pH5.5 20μg glycogen. The aqueous phase from the final extraction was placed in a fresh tube, and washed twice with 100% ethanol (12). KRAS mutation analysis was determined by direct sequencing at BML Inc. (Tokyo, Japan).

Statistical analysis

The differences in clinicopathological parameters between seronegative and

seropositive groups were tested using Student's t test for continuous variables and Fisher's exact or chi-square test for categorical variables. Survival probabilities were calculated by the product limit method of Kaplan and Meier from the time of surgery. Differences between groups were tested with the log-lank test. A *p* value of 0.05 was considered significant.

RESULTS

In Figure 1, the sensitivity of combination with three markers is shown. The 48 (37.5%) of the 128 patients were positive for serum p53 antibodies before operation. It was only 13.2% that both p53 antibodies and CEA were positive. It was only 3.9% (5/128) that all markers were positive. On the other hand, it was 32.8% (42/128) that all markers were negative. Twenty two point six percent (29/128) patients were only positive for p53 antibodies. As show in Table 1, there was no significant association in gender, age, and tumor location between seronegative and seropositive patients about serum p53 antibodies. The positive rate in serum p53 antibody was 25.0% (1/4) in stage

0, 31.6% (6/19) in stage I, 31.3% (15/48) in stage II, 44.7% (17/38) in stage III, 47.4% (9/19) in stage IV (Fig 2). Sensitivity of the serum p53 antibodies was not associated with each stage. Compared with other tumor markers positive rate of p53 antibodies can be detected more by early stages. Fig3 shows positive rate of p53 antibodies after the surgery. At one month after the surgery, the rate was still 37%, and 32% at 6 months. Positive p53 antibodies levels tend to change to negative slowly after the surgery. On the other hand, positive rate of CEA and CA19-9 transited to negative after surgery, immediately. Relationship between changes in serum p53 antibodies positivity after surgery and recurrence rate was shown in Table 2. Total 117 patients who underwent curative A/B resection were enrolled. Six patients (33.3%) who showed positive serum p53 antibodies even after surgery had clinical recurrence. Only one (3.8%) of 26 patients with positivity for serum p53 antibodies before surgery who showed negative conversion after surgery had recurrent disease. Patients that changed serum p53 antibodies to positive after surgery had all recurrent disease. Figure 4 shows

Kaplan-Meier analysis of overall survival by p53 antibodies and CEA presence. The median overall survival time in all patients was 62 months. Preoperative p53 antibodies presence was not associated with overall survival (63 months vs 62 months, p=0.74). One month after surgery, p53 antibodies presence also showed no association with overall survival. Preoperative CEA presence was associated with overall survival (65 months vs 58 moths, p=0.03). Figure 5 shows change in level of serum p53 antibodies with adjuvant chemotherapy. In no-recurrence group, almost all the p53 antibody levels tended to decrease slowly. In recurrence group, the p53 antibody levels tended to increase after surgery. Forty eight patients were in positive p53 antibodies group before surgery. Of 48, 46 patients could be analyzed KRAS codons 12 and 13 mutation. KRAS mutations were found in 14/46 (30.4%) cases, 85.5% of these mutations were in codon 12 and 21.4% were in codon 13. One patient can be analyzed KRAS mutations both codon 12 and 13. As shown in Table 3, there was no significant association between KRAS wild-type and KRAS mutation with any clinicolpathological

characteristics.

DISCUSSION

The role that serum p53 antibodies could play in the follow-up CRC patients after curative resection has not been adequately elucidated. Some studies reported that monitoring of serum p53 antibodies might help physicians to follow up CRC patient (14), others reported that serum p53 antibodies are not related to clinical parameters of CRC and have no prognostic significance in long-term follow-up (11). Our results can not indicate that p53 antibodies levels before or after surgery were a reliable tumor maker for overall survival. However, CEA levels may be a reliable tumor maker for overall survival and recurrence during follow-up for CEA seropositive patients with curatively resected CRC. One possible reason of the results is that p53 antibodies levels tend to change to negative slowly after curative resection. Alteration of the p53 gene is the most frequent genetic alteration in human cancer and leads to the accumulation of mutant p53 in the nucleus of tumor

cells (15). Normal p53 protein is diminished easily, but mutant p53 protein is remained longer in the tumor cells. It is because the half life of mutant p53 protein is longer than that of normal, and its antibody is released and staying long in blood serum. In the cases, where serum p53 antibody was positive even after surgery, there was a high risk of recurrence. On the other hand, another results indicated that serum p53 antibodies levels can exhibit a high sensitivity for detecting early stages of CRC. CEA has previously been demonstrated to exhibit a high sensitivity as a maker for detecting advanced CRC (16), and the similar results are indicated in this study. So, the combined use of serum p53 antibody and CEA can be an effective screening method for detection of colorectal cancer. Indeed, before surgery, total 67.2% patients were positive with any tumor markers; serum p53 antibodies, CEA or CA19-9.

We also investigated the relevance of KRAS mutation and serum p53 antibodies in CRC patients. Since the KRAS mutation is seen as important events in the tumor progression and based their relatively high incidence, it

has been proposed as prognostic biomarkers for CRC (17). However, there is no significant association between KRAS mutation and p53 antibody level. They have independent influences on CRC development and there could be alternative genetic pathways to CRC.

CONCLUSIONS

The combined use of serum p53, CEA, and CA19-9 can be an effective screening method for detection of colorectal cancer even in the early stage. If serum p53 antibody level continues positive for a long time after resection, the case will require subsequent intense follow-up. KRAS mutation status does not seem to represent as a variable marker for CRC.

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FIGUER LEDENDS

FIGURE 1.

Positive rate of tumor markers before surgery

FIGURE 2.

Sensitivity of preoperative makers in stages

FIGURE 3.

Positive rate of tumor markers after surgery

FIGURE 4.

Overall survival curves according to changes of serum p53 antibodies.

a. Survival according to preoperative status of serum p53 antibodies. **b**. Survival according to preoperative status of serum CEA. **c**. Survival according to status of one month after surgery of serum p53 antibodies. **d**. Survival according to status of one month after surgery of serum CEA.

FIGURE 5.

Change in level of serum p53 antibodies with adjuvant chemotherapy.

a. no-recurrence group (n=20). **b**. recurrence group (n=8).

Fig.1

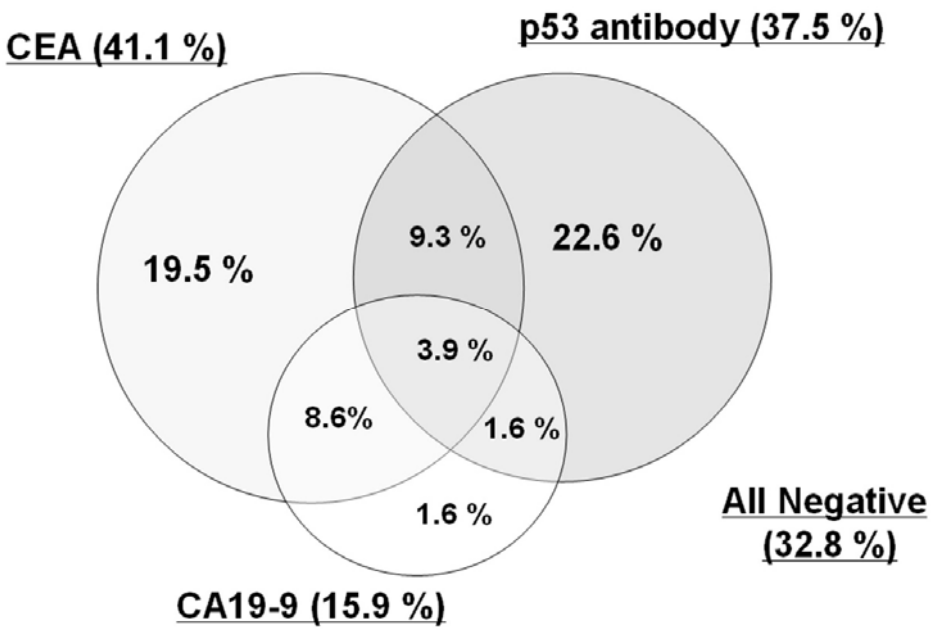
CEA (41.1 %)
p53 antibody (37.5 %)
19.5 %
9.3 %
22.6 %
3.9 %
8.6%
1.6 %
1.6 %
All Negative
(32.8 %)
CA19-9 (15.9 %)

Fig.2

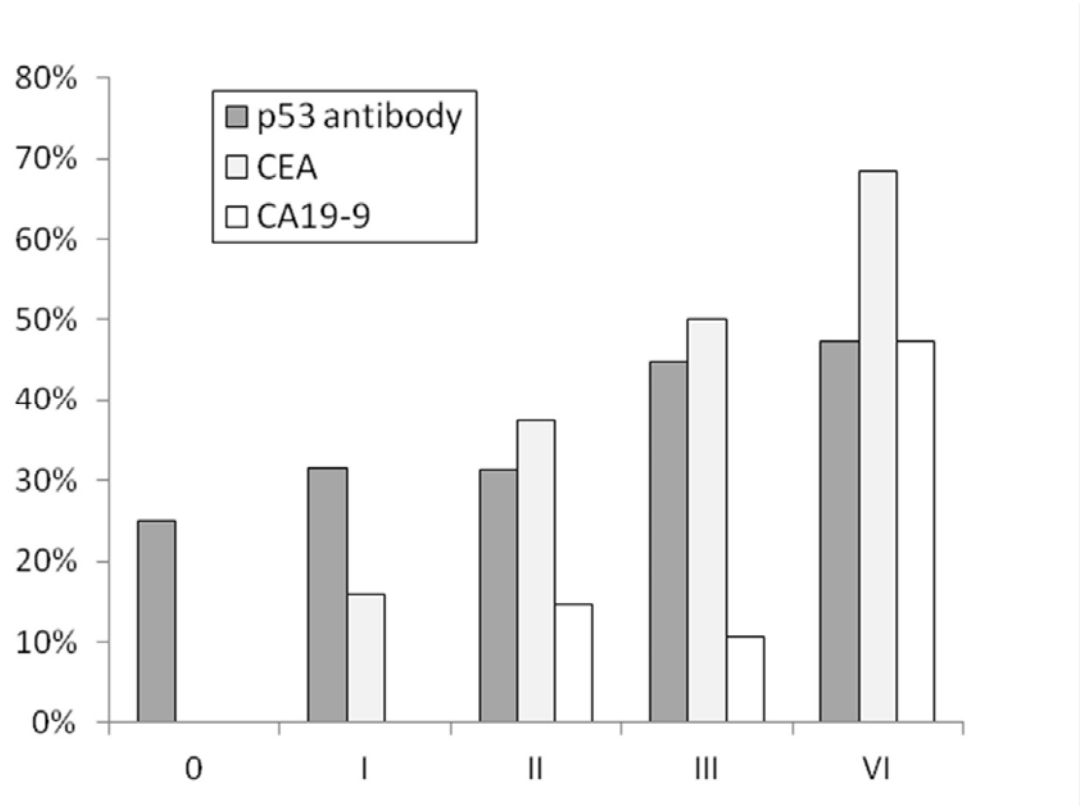

Fig.3

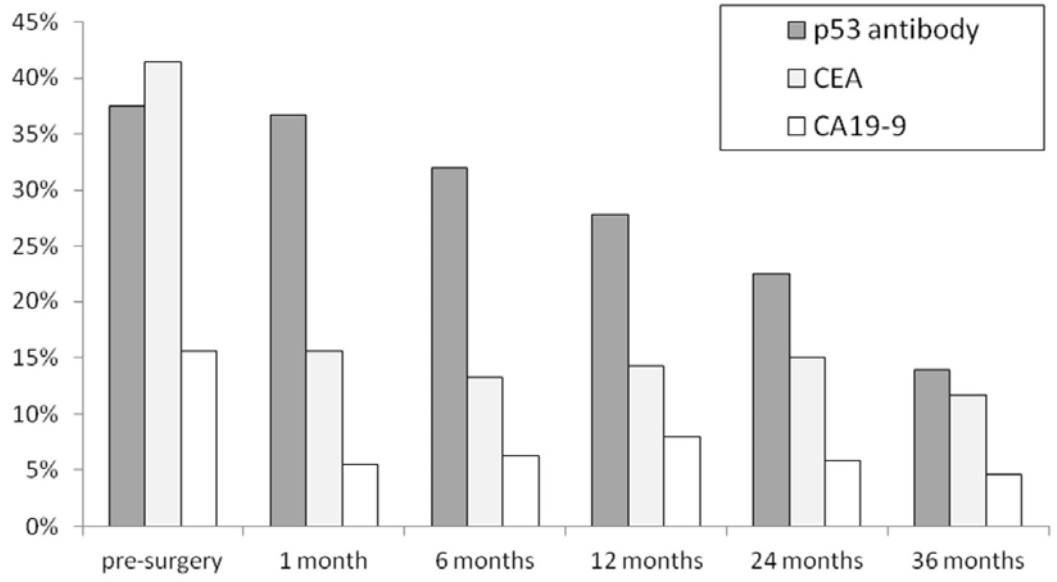
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
p53 antibody
CEA
CA19-9
pre-surgery
1 month
6 months
12 months
24 months
36 months

Fig.4

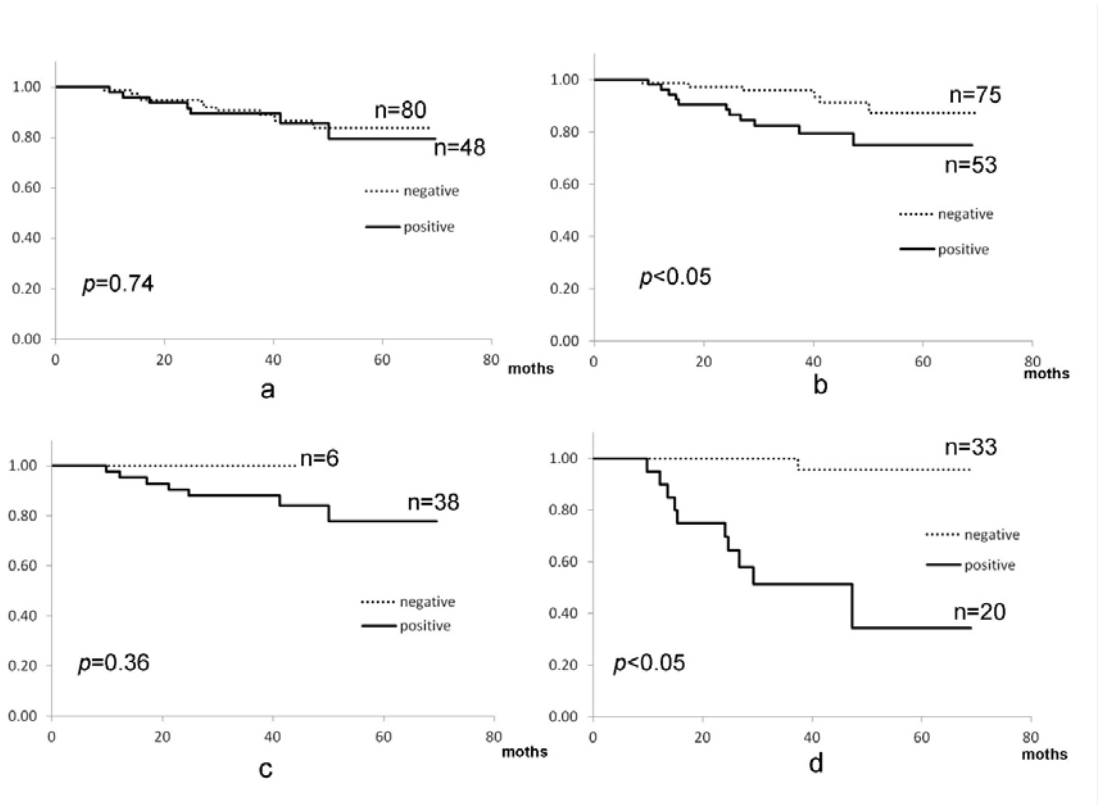
1.00
0.80
0.60
0.40
0.20
0.00
0
20
40
60
80
moths
n=80
n=48
negative
positive
p=0.74
a
n=75
n=53
negative
positive
p<0.05
b
n=6
n=38
negative
positive
p=0.36
c
n=33
n=20
negative
positive
p<0.05
d

Fig.5

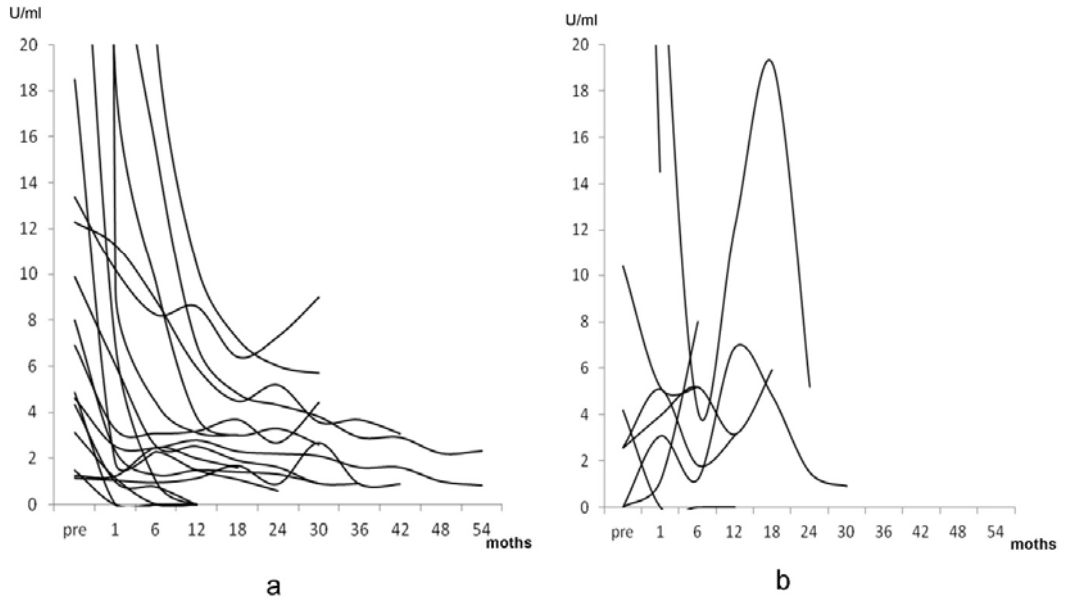
U/ml
20
18
16
14
12
10
8
6
4
2
0
pre 1 6 12 18 24 30 36 42 48 54
moths
a
U/ml
20
18
16
14
12
10
8
6
4
2
0
pre 1 6 12 18 24 30 36 42 48 54
moths
b

TABLE 1 Characteristics of patients with preoperative serum p53 antibodies

	Positve (n=48)	Negative (n=80)	P value
Age (year)	65.2 (35-90)	65.9 (42-86)	0.23
Gender			
Male	23	35	
Female	25	45	0.64
Tumor location			
Proximal	10	13	
Transverse	2	8	
Descending	4	6	
Sigmoid	7	18	
Rectum	25	35	0.54

TABLE 2 Recurrence rate with changing in serum p53 antibodies after surgery

Serum p53 antibody		recurrence		
Pre-surgery	Post-surgery	(-)	(+)	%
+	+	12	6	33.3
+	-	25	1	3.8
-	+	0	2	100
-	-	59	12	16.9
Total		96	21	17.9

TABLE 3 Association of KRAS codons 12 and 13 mutation status with clinicolpathological characteristics

	No. (%)	KRAS wild-type (%)	KRAS mutation (%)	P value
	46	32 (69.6)	14 (30.4)	
Age				
<50	4 (8.7)	3 (9.4)	1 (7.1)	
50-69	27 (58.7)	20 (62.5)	7 (50.0)	
≤70	15 (32.6)	9 (28.1)	6 (42.9)	0.65
Gender				
Male	22 (47.8)	16 (72.7)	6 (27.3)	
Female	24 (52.2)	16 (66.7)	8 (33.3)	0.61
Tumor location				
Proximal	10 (21.7)	6 (18.8)	4 (28.6)	
Transverse	2 (4.4)	1 (3.1)	1 (7.1)	
Descending	4 (8.7)	3 (9.3)	1 (7.1)	
Sigmoid	6 (13.0)	6 (18.8)	0 (0)	
Rectum	24 (52.2)	16 (50.0)	8 (57.2)	0.47
Stage				
I	5 (10.9)	3 (9.4)	2 (14.3)	
II	15 (32.6)	9 (23.1)	6 (42.9)	
III	17 (37.0)	12 (37.5)	5 (35.7)	
IV	9 (19.5)	8 (25.0)	1 (7.1)	0.48
p53 antibodies (U/ml)				
Pre-surgery	111.8 [1.3-2960]	141.3 [1.3-2960]	44.5 [1.5-122]	0.35
Post-surgery	33.8 [0-942]	40.3 [0-942]	18.9 [0-64]	0.21