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***Observational Study***

**Detection of metastatic cancer cells in mesentery of colorectal cancer patients**

Luo XL *et al*. Metastasis V in CRC

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**Abstract**

***AIM***

To detect the existence of isolated cancer cells in the mesentery of colorectal (named as Metastasis V), and investigate its clinical significance in colorectal cancer patients.

***METHODS***

Sixty-three colorectal cancer patients who received radical excision between January 2012 and September 2015 were included. All the patients received laparoscopy-assisted radical colorectomy or proctectomy (with CME or TME) with R0 dissections at the Department of Gastrointestinal Surgery, Tongji Hospital, Tongji Medical College in Huazhong University of Science and Technology. The location and size of the primary lesions were recorded immediately after the tumor was removed, with the surrounding mesenterium completely separated along the intestinal wall. Each dissected mesentery sample was analyzed for hematoxylin-eosin staining and immunohistochemistry by using cytokeratin 19 antibody. Image Pro Plus Software 6.0 (Media Cybernetics, CA, United States) was used to semi-quantitatively measure the concentration of the cytokeratin 19 immunohistochemistry. The correlation of metastasis found in mesentery with clinicopathological characteristics was examined. The prognosis of patients was also evaluated by preoperative serum CEA level.

***RESULTS***

Metastasis V was detected in 14 of 63 (22.2%) colorectal cancer patients who received laparoscopy-assisted radical colorectomy or proctectomy (CME or TME) with R0 dissections in our hospital between January 2012 and September 2015. There was no significant difference of patients with Metastasis V in age, gender, tumor size, and tumor location (*P* > 0.05). Metastasis V was more likely to occur in poorly differentiated tumor (5/11; 45.5%) rather than moderately (8/46; 17.4%) and well differentiated one (1/6; 16.7%). The incidence of Metastasis V in N2 stage (9/14; 64.3%) was more frequent than in the N0 stage (3/35; 8.6%) or N1 stages (2/14; 14.3%). In addition, Metastasis V was positively related to the tumor invasive depth (T1:0/1, 0%; T2:1/12, 8.3%; T3:7/39, 17.9%; T4:6/11, 54.5%). Furthermore, preoperative serum CEA level in Metastasis V-positive patients was significantly higher than Metastasis V-negative patients (4.27 ng/ml *vs* 3.00 ng/ml).

***CONCLUSION***

Metastasis V might be associated with the poor prognosis of colorectal cancer patients.

**Key words:** Colorectal cancer; Radical surgery; Metastasis V; Colorectal mesentery; serum CEA level

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**Core tip:** Local–regional recurrence of colorectal cancer is common in patients who received R0 resection. Our previous study proposed a novel type of metastasis designated as “Metastasis V” in gastric cancer. Metastasis V is defined as the appearance of cancer cells in the mesentery in broad sense, and may be a risky factor for the poor prognosis after radical surgery. In this study, we demonstrated that Metastasis V was also detected in the mesentery of colorectum, and it might be associated with the poor prognosis of colorectal cancer.

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**INTRODUCTION**

Three decades ago, R.J. Heald introduced total mesorectal excision (TME) in the treatment of rectal cancer[1]. Subsequently, complete mesocolic excision (CME) was popularized by W Hoehenbuerg to reduce tumor relapse and improve prognosis in colon cancer[2]. These new approaches greatly contributed to the complete mesentery excisions and improved harvested lymph nodes. Furthermore, with the development of surgical technology, neo-adjuvant chemotherapy and efficient perioperative management, the prognosis of patients with colorectal cancer has been improved. However, local-regional recurrence of colorectal cancer is still often noted in those patients who received radical R0-resection with no lymphatic metastasis[3].

Direct invasion, lymphatic drainage, hematogenous spread and peritoneal dissemination are the four classical routes through which local-regional recurrence or distant implants of cancer cells can be determined. In addition to these pathways, people have also reported the existence of metastatic cancer cells in the mesentery of colorectum, and this type of metastasis was named as ‘extra-capsular spreads’ or ‘extra-nodal metastasis’[4-7]. In our previous study, we proposed a novel type of tumor metastasis designated as Metastasis V in gastric cancer. Metastasis V is defined as the appearance of cancer cells in the mesogastrium with perigastric adipose tissue, and it may be a risk factor for patient survival after radical surgery[8]. Here, in this study, we further determined whether metastasis V can be detected in the mesocolorectum, and examined its clinic significance.

**MATERIALS AND METHODS**

***Patients, samples, and sectional tissue analysis***

Sixty-three patients who underwent surgery for colorectal cancer at the Department of Gastrointestinal Surgery, Tongji Hospital, Tongji Medical College in Huazhong University of Science and Technology between January 2012 and September 2015 were included in this study. The patients consisted of twenty-five female patients and thirty-eight male patients. All of these patients received laparoscopy-assisted radical colorectomy or proctectomy (with CME or TME) with R0 dissections. All participants signed informed written consents in this research. This study was approved by the Tongji Hospital Ethics Committee.

Clinical samples of colorectal cancer were obtained from the resected colorectal tissue. The location and size of the primary lesions were recorded immediately after the tumor was removed, and then the mesenterium was completely separated from the intestinal wall along with the anterior edge. All specimens were fixed in 10% formaldehyde before processing. The lymph nodes were obtained from the mesenterium. Next, the mesentery was cut into strip-shape with a width of 2 cm in an outward-to-inward manner, vertical to the intestinal wall. The strip-shaped samples were cut into cube samples with a mean length of 2 cm. All of the small samples were numbered by numbers from inside (proximal end of the cancer) to outside (Figure 1). These samples were embedding in 10% formaldehyde. Demographic characteristics of the 63 patients were described in Table 1. The clinic-pathological stage was determined by the 7th edition of the AJCC Cancer Staging Manual.

***Immunohistochemistry analysis of Metastasis V***

Immunohistochemistry was performed using the cytokeratin 19 antibody. Cytokeratin 19 is specifically expressed in intestine epithelia cells and colorectal cancer (CRC) cells[9]. Hence, it has been proven to be a highly sensitive marker for colorectal cancers.

Immunostaining was performed according to the standard streptavidin-biotin method. First, the slices were de-paraffinized and rehydrated. To retrieve antigenicity, the slices were boiled in 0.01 mol/l, pH 6.0 sodium citrate buffer for approximately 15 min. Endogenous peroxidase was restrained with 0.3% hydrogen peroxide for roughly 25 min. After blocking with 3% BSA for 30 min, the anti-cytokeratin 19 monoclonal antibody was now arranged in blocking buffers and the slices were incubated at 4 ℃ overnight. After being washed in pH 7.4 PBS 3 times (5 min each time), the corresponding secondary antibody, retained at room temperature for approximately 50 min and marked by HRP was added. After once more being washed with pH 7.4 PBS for 15 min, the chromogenic reagent DAB was added to the colorate. The slices were counterstained with Harris hematoxylin, dehydrated in a graded alcohol series (75% alcohol for 6 min, 85% alcohol for 6 minutes, absolute ethyl alcohol I for 6 min, and absolute ethyl alcohol II for 6 min), cleared in xylene for 5 min, and mounted. Individual tumor cells in mesenteries, separated from primary lesion and lymph nodes, were identified to be Metastasis V.

***Immunohistochemistry image analysis***

Image Pro Plus Software 6.0 (Media Cybernetics, CA, United States) was used to semi-quantitatively measure the concentration of the cytokeratin 19 immunohistochemistry. This procedure, was split into six steps: (1) discovering and surveying the section of interest; (2) adjusting of the optical densities; (3) obtaining, transforming and preserving images; (4) amending the background and background staining; (5) configuration of the section of interest to survey the optical density; and (6) surveying optical densities.

***Statistical analysis***

The Fisher’s exact test, *χ²* test, and Mann-Whitney *U* test were used to inspect the significance of the differences between the covariates. We considered a *P*-value of less than 0.05 statistically significant. Standard statistical analyses were executed by SPSS version 20.0.

**RESULTS**

***Detection of Metastasis V in mesenterium of colorectal cancer patients***

Metastasis V was detected in 14 out of the total 63 (22.2%) patients by immunohistochemistry (IHC) staining. These isolated cancer cells separating from the primary lesion and lymph nodes were observed in the same slice (Figure 2). In terms of “T” stage, only one case showed limited muscular layers invasion, seven cases had sub-serosa invaded, and the rest had penetrated the serosa. In these patients, there were three lymph node negative patients with different invasive depths (Table 2).

***Correlation between Metastasis V and clinical factors***

The demographic characteristics of patients and the pathologic features of tumors with Metastasis V were shown in Table 3. There was no significant difference in age, gender, tumor size, and tumor location (*P* > 0.05). Metastasis V was more likely to occur in poorly differentiated tumor (5/11; 45.5%) rather than moderately (8/46; 17.4%) and well differentiated one (1/6; 16.7%). The incidence of Metastasis V in N2 stage (9/14; 64.3%) was more frequent than in the N0 stage (3/35; 8.6%) or N1 stages (2/14; 14.3%). In addition, Metastasis V was positively related to the tumor invasive depth (T1:0/1, 0%; T2:1/12, 8.3%; T3:7/39, 17.9%; T4:6/11, 54.5%).

***Correlation between Metastasis V and patients preoperative CEA levels***

Previous studies have demonstrated the correlation between increased preoperative CEA and the poor prognosis of rectal cancer patients[10,11]. Here, we further determined the correlation between Metastasis V and preoperative CEA levels of patients. Totally forty-two CRC patients who underwent operations in our department were retrospectively studied. Metastasis V was detected in nine patients according to the pathological reports. Six patients were excluded because their preoperative CEA data was missed. The CEA levels were divided into two grades. The CEA levels more than 3 times of normal range (15.00 ng/ml) were classified as high grade, while those less than that were designated as low grade. Our data indicated that preoperative serum CEA levels in Metastasis V-positive patients were significantly higher than Metastasis V-negative patients (4.27 ng/ml *vs* 3.00 ng/ml) (Table 4).

**DISCUSSION**

In this study, we have showed metastatic cancer cells can reside in the mesentery of the colorectum of CRC patient. These tumor cells show no directly attachment to the primary lesions, with no involvement of vessel-lymphatic drainage system, and are confined to the fatty tissues enveloped by the fascia proper (Figure 1). In our previous study, we proposed a novel type of tumor metastasis designated as Metastasis V in gastric cancer patients[8]. Thus, we believe that Metastasis V could be detected in the mesenteries of both gastric and colorectal cancer patients. Similar to our previous studies, the incidence of Metastasis V was closely related to the tumor invasion of colorectal cancer patients, which was mainly detected in the T3 and T4 tumors (Table 2). Metastasis V was also correlated with lymphatic metastasis. Lymphatic nodes staging N1-N3 had larger percentages of Metastasis V than N0 (Table 2). Therefore, immunohistochemical analysis of Metastasis V in these patients is strongly recommended in clinical practice.

More interesting, the preoperative serum CEA levels in Metastasis V-positive patients were significantly higher than Metastasis V-negative patients (4.27 ng/ml *vs* 3.00 ng/ml) (Table 4). These data indicated that the prognosis of the patients with Metastasis V-positive tumors was worse than those with Metastasis V-negative tumors. Due to the limited number of patients in the current study, a larger sample of cohort patients should be enrolled for further analysis.

Little is known about the exact route or mechanism of Metastasis V. It was hypothesized that primary tumor lesions penetrates the intestinal wall, and then the cancer cells could probably scatter into the fatty tissues enveloped by proper fascia. In this way, Metastasis V occurs not only in CRC and gastric cancers, but also in oral head-neck, pancreas and papilla of vater tumors[12-20].

It has been well known in practice that radical surgery for cancers should include *en bloc* resections of the primary tumor and neighboring tissues. However, it is difficult to understand the exact boundaries of *en bloc* resection within gastrointestinal tumors. “TME” or “CME” can reduce the tumor recurrence and increase patient survival, however, the possible reason was still unknown. In our opinion, the mesenteries of colorectum enveloped by the proper fascia not only propose a precise boundary for resection, but also function as pathologic barriers of Metastasis V spreading. Thus, our findings are clinically significant because TME or CME should be the useful choice for surgical excision of Metastasis V. Further studies on the efficiency of Metastasis V excision through TME or CME will be investigated.

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**COMMENTS**

***Background***

local-regional recurrence of colorectal cancer is still often noted in those patients who received radical R0-resection with no lymphatic metastasis.

***Research frontiers***

In addition to the classical four metastatic patterns, people have also reported the existence of metastatic cancer cells in the mesentery of colorectal, and this type of metastasis was named as ‘extra-capsular spreads’ or ‘extra-nodal metastasis’. Examination of the clinical and oncological significance of isolated tumor cells found in mesentery of colorectum in colorectal cancer patients is one of the main frontiers in surgical oncology.

***Innovations and breakthrough***

Metastasis V is defined as the appearance of cancer cells in the mesogastrium, and may be a risky factor for the poor prognosis after radical surgery. In this study, we demonstrated that Metastasis V was also detected in the mesentery of colorectum, and it might be associated with the poor prognosis of colorectal cancer.

***Applications***

It is clinically useful to be assisted in evaluation of prognosis of colorectal cancer patients.

***Peer-review***

In this paper, the authors well described an interesting study about isolated cancer cells in the mesentery of colorectum (named as Metastasis V). This is an interesting point, and the issue of metastasis in patients with colorectal cancer is of main importance.

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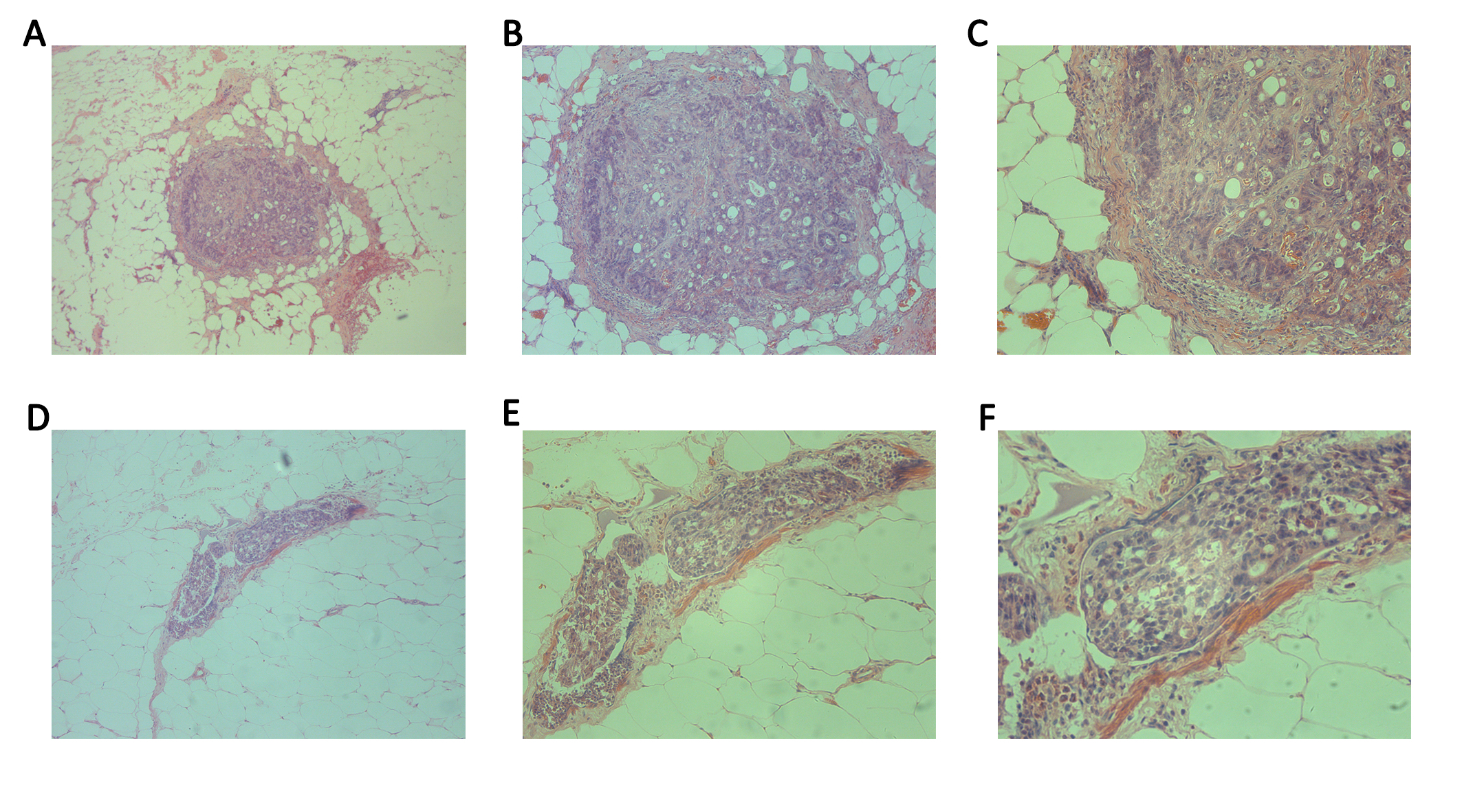
Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0



**Figure 1 Obtaining samples of mesenterium from colorectal cancer patients.** Large cross sectional tissue samples analysis of mesentery of colorectum from surgically resected specimens.



**Figure 2 Detection of Metastasis V in colorectal cancer patients.** Isolated cancer cells were detected in the mesentery of colorectal by HE staining. A (10 ×), B (20×) and C (40 ×) represent the same slide; D (10 ×), E (20 ×) and F (40 ×) represent the same slide.

**Table 1 Data about 63 colorectal cancer patients**

|  |  |
| --- | --- |
| **Parameters** | **Results** |
| Gender |  |
| Female | 25 |
| Male | 38 |
| Age (yr, mean, range) | 55.5 ( 28-86 ) |
| Location of cancer |  |
| Colon | 11 |
| Rectum | 52 |
| Differentiated grade |  |
| Well | 6 |
| Moderately | 46 |
| Poorly | 11 |
| Affected lymph nodes |  |
| N0 | 35 |
| N1 | 14 |
| N2 | 14 |
| Invasive depth |  |
| T1 | 1 |
| T2 | 12 |
| T3 | 39 |
| T4 | 11 |

**Table 2 Data about Metastasis V positive patients**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patients** | **Sex** | **Age/yr** | **Cancer size/cm** | **Cancer location** | **Differentiated grade** | **Invasive depth** | **Affected lymph nodes** | **TNM** |
| 1 | F | 37 | 2.5 | rectum | poorly | serosal | 8/15 | pT4N2M0 |
| 2 | F | 42 | 3 | rectum | Aoderately | subserosal | 5/17 | pT3N2M0 |
| 3 | M | 33 | 2 | Rectum | moderately | Serosal | 5/15 | pT4N2M0 |
| 4 | M | 48 | 6 | Sigmoid | Well | Serosal | 12/21 | pT4N2M0 |
| 5 | F | 63 | 3 | rectum | Poorly | Muscularis | 0/15 | pT2N2M0 |
| 6 | F | 45 | 3 | Rectum | Moderately | Serosal | 5/28 | pT4N2M0 |
| 7 | M | 58 | 3 | Rectum | Poorly | Subserosal | 8/12 | pT3N2M0 |
| 8 | M | 64 | 4 | Rectum | Poorly | Subserosal | 7/15 | pT3N2M1 |
| 9 | M | 54 | 6 | Rectum | Moderately | Subserosal | 2/13 | pT3N1M0 |
| 10 | F | 60 | 2 | descending colon | moderately | Subserosal | 0/17 | pT3NxM0 |
| 11 | M | 54 | 3 | Rectum | Moderately | Serosal | 5/12 | pT4N2M0 |
| 12 | M | 58 | 3 | Rectum | Moderately | Subserosal | 0/16 | pT3N0M0 |
| 13 | F | 57 | 3 | Rectum | Poorly | Serosal | 2/21 | pT4N1M1 |
| 14 | M | 47 | 4 | rectum | moderately | subserosal | 24/32 | pT3N2M0 |

**Table 3 Comparison between Metastasis V positive patients and Metastasis V negative patients**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Metastasis V** | | ***P* value** |
| **Negative** | **Positive** |
| Age (yr) |  |  | NS |
| < 45 | 8 | 3 |  |
| ≥ 45 and ≤ 59 | 19 | 8 |  |
| ≥ 60 | 22 | 3 |  |
| Cancer size (cm) |  |  | NS |
| ≤ 3 | 24 | 10 |  |
| > 3 and < 5 | 14 | 2 |  |
| ≥ 5 | 11 | 2 |  |
| Gender |  |  | NS |
| Female | 19 | 6 |  |
| Male | 30 | 8 |  |
| Cancer location |  |  | NS |
| Colon | 9 | 2 |  |
| Rectum | 40 | 12 |  |
| Differentiated grade |  |  | NS |
| Well | 5 | 1 |  |
| Moderately | 38 | 8 |  |
| Poorly | 6 | 5 |  |
| Affected lymph nodes |  |  | < 0.05 |
| N0 | 32 | 3 |  |
| N1 | 12 | 2 |  |
| N2 | 5 | 9 |  |
| Invasive depth |  |  | < 0.05 |
| T1 | 1 | 0 |  |
| T2 | 11 | 1 |  |
| T3 | 32 | 7 |  |
| T4 | 5 | 6 |  |

**Table 4 Comparison preoperative CEA levels between Metastasis V positive and negative patients**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Metastasis V** | | ***P* value** |
| **Negative** | **Positive** |
| Median (ng/ml) | 3.00 | 4.27 | < 0.05 |
| Grade |  |  | < 0.05 |
| High (> 15.00 ng/ml) | 5 | 5 |  |
| Low (≤ 15.00 ng/ml) | 73 | 16 |  |