Peripheral and mesenteric serum levels of CEA and cytokeratins, staging and histopathological variables in colorectal adenocarcinoma

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INTRODUCTION

The estimates of cancer incidence in Brazil for the year 2006, published by INCA, indicate that colorectal cancer is the fifth most common malignant tumor type among men (11,390 new cases) and the fourth among women (13,970 new cases). The greatest incidence of cases occurs in the age group between 50 and 70 years old, but the possibility of developing this disease is already increasing after the age of 40 years is reached [1-3].

In 2004, in a study carried out in the 25 member countries of the European Union, 2,886,800 new cases of cancer and 1,711,000 deaths were recorded. The most common type was lung cancer (13.3%) followed
by colorectal cancer (13.2%) and breast cancer (13%). Lung cancer was also the greatest cause of death (341,800 cases) followed by colorectal cancer (203,700 cases)\[4\].

When colorectal cancer is detected in its initial stage, it may even be curable. However, the overall survival of patients with colorectal cancer does not exceed 40%. The mean five-year survival for patients with early diagnosis (stage I) is approximately 70%, while it is 6% for advanced cases of the disease (stage IV)\[1,2\].

Tumor markers are substances produced by the neoplasia than can be identified in the neoplastic tissue itself and in patients’ biological fluids. Many studies have been conducted to evaluate serum tumor markers at different stages of diagnosis and follow-up of colorectal carcinoma cases. Carcinoembryonic antigen (CEA) is distinguished as the most important marker\[1,2,5-9\].

CEA was first identified in 1965, and is a high-molecular-weight glycoprotein that is found in the cytoplasmic membrane of digestive system cells in the fetal phase and in neoplastic cells\[10\]. Cytokeratins form part of the microtubules of the cellular cytoskeleton, and they are released into the bloodstream during processes in which there is intense cell proliferation or apoptosis\[11\].

There is controversy regarding whether or not there are differences in the serum levels of the markers according to the location of the blood sample collection: from peripheral veins or from blood flowing directly out of the lesions. If there were a difference between the mesenteric and peripheral serum levels of such markers, the former might more accurately reflect the real levels produced by the tumors than would the latter. Some authors have found a significant difference between the mesenteric and peripheral levels of CEA, while others have not reproduced these results. Some studies have also demonstrated relationships between high marker levels in mesenteric serum and the histopathological variables of colorectal tumors\[12-16\].

The objective of the present investigation was to analyze the mesenteric and peripheral levels of CEA and cytokeratins in patients with colorectal adenocarcinoma and observe their correlation with the staging and certain histopathological variables.

**MATERIALS AND METHODS**

The patients were volunteers and were treated in accordance with a protocol approved by the Research Ethics Committee of the institution. In this study, 138 patients with colorectal adenocarcinoma were retrospectively analyzed. These patients were attended to and surgically treated by the Coloproctology Group, Discipline of Surgical Gastroenterology, Department of Surgery, Federal University of São Paulo-Escola Paulista de Medicina (UNIFESP-EPM). The operations were performed at Hospital São Paulo between December 1993 and March 2000. Surgical resection was performed on 124 patients, while the tumors were considered irresectable in 14 patients.

Patients who had had some other benign or malignant neoplasia at some previous time, and those for whom it was not possible to collect the data needed for the proposed analysis, were not included in the study.

With regard to the ethnic group to which the patients belonged, 68.1% were white, 22.5% brown, 6.5% yellow and 2.8% black. With regard to gender, 57.2% were female. The patients’ ages ranged from 19 to 87 years, with a mean of 61.7 years.

The variables analyzed in the present investigation were: staging of the colorectal neoplasia by means of the TNM classification, degree of cell differentiation, diameter of the neoplasia, presence or absence of venous invasion and presence or absence of lymphatic invasion.

According to the TNM classification, 34 patients were in stage I, 21 in II, 34 in III and 49 in IV. With regard to the degree of cell differentiation, there were 55 patients with well-differentiated tumors, 66 with moderately-differentiated tumors and three with poorly-differentiated tumors. Regarding the diameter of the neoplasia, 16 patients had tumors of ≤3.9 cm; 76 of 4.0-7.9 cm and 32 ≥8.0 cm. The presence of venous invasion was identified in the lesions of 23 patients, while lymphatic invasion was identified in 41 patients.

The collection of peripheral venous blood was done by means of direct puncture in the arm that was free of endovenous hydration, while anesthesia was being induced. Samples of 10 mL of blood were collected in dry tubes. These were centrifuged to obtain the serum from the sample, and this was stored at -20°C.

The mesenteric blood for assaying the marker levels was collected by dissection, sectioning and catheterization of the inferior mesenteric vein, when the tumor was located in the left colon or the rectum. For tumors in the right colon, collection was via the wide tributary vein of the superior mesenteric vein, and for tumors in the transverse colon, collection was via the middle colic vein. The corresponding vein was dissected and repaired with 00 cotton thread; the vein was sectioned obliquely and the catheter was introduced towards the tumor, with the collection of 10 mL of blood. This procedure was carried out before any manipulation of the tumor. The blood was centrifuged, with separation of the serum and storage in the same way as done for the peripheral blood samples.

The method utilized for assaying the CEA levels was DELFIA®. The CEA levels were considered to be normal when they were less than the limit of 5.0 mg/L.

For assaying the cytokeratin (TPA-M) levels, the LIA-mat® TPA-M Prolifigen® method was utilized, (AB Sangtec Medical®), which utilizes a reference value of 72 U/L as the cutoff point between normal and abnormal values, and this point was taken for the present investigation. The apparatus utilized for carrying out the serum assays was the Lumat LB 9501® luminometer, (EG&G Berthold).

**Statistical analysis**

For the statistical analysis of the data obtained in this study, t test and the marginal homogeneity test were
utilized. To study the correlations using the TNM variable, the Bonferroni multiple comparisons method was utilized.

In the tests utilized, the level of statistical significance for rejection of the nullity hypothesis was set at 0.05% or 5\% (\( \alpha \leq 0.05 \)), thereby indicating the results that were considered significant.

**RESULTS**

**Analysis of the mesenteric and peripheral levels of the markers**

Two statistical analysis methods were performed (one numerical and the other categorical), and each of the markers was analyzed in relation to its peripheral and mesenteric concentrations. With regard to the numerical descriptive measurements of the CEA levels, the mean for CEA (M) was 39.10 mg/L ± 121.19 mg/L, and the mean for CEA (P) was 38.5 mg/L ± 122.55 mg/L, with a statistically significant difference (\( P < 0.05 \)). Comparison between the proportions of positive rates of mesenteric and peripheral CEA was done by means of the marginal homogeneity test. No statistical difference was found from this.

**DISCUSSION**

Many studies have been conducted on tumor markers, seeking greater understanding of all the possible ways of using them in diagnoses, staging, prognoses and Table 1 Descriptive measurements of the tumor markers and the histopathological variables and staging of the colorectal adenocarcinoma (mean ± SD)

| Variables       | CEA (M) ug/L | CEA (P) ug/L | TPA-M (M) U/L | TPA-M (P) U/L |
|-----------------|-------------|-------------|---------------|---------------|
| TNM             |             |             |               |               |
| I               | 14.96 ± 43.02 | 15.82 ± 52.37 | 178.91 ± 116.08 | 168.44 ± 137.84 |
| II              | 3.71 ± 3.23  | 3.10 ± 2.63  | 148.56 ± 79.25  | 126.13 ± 82.13  |
| III             | 11.33 ± 21.21 | 8.00 ± 16.71  | 214.94 ± 164.20 | 151.55 ± 131.75 |
| IV              | 90.28 ± 190.14 | 90.69 ± 190.88 | 578.52 ± 812.53 | 511.02 ± 692.70 |
| P               | 0.001        | 0.001        | 0.001          | 0.001          |
| Cell differentiation |         |             |               |               |
| PD              | 22.06 ± 72.07 | 22.40 ± 72.24 | 227.35 ± 214.74 | 197.28 ± 234.32 |
| MD              | 40.95 ± 116.87 | 40.52 ± 127.30 | 392.67 ± 695.62 | 338.50 ± 589.94 |
| BD              | 11.40 ± 17.84 | 9.97 ± 14.84  | 111.67 ± 46.50  | 72.53 ± 43.22  |
| P               | 0.816        | 0.632        | 0.212          | 0.164          |
| Diameter (cm)   |             |             |               |               |
| Up to 3.9       | 44.80 ± 155.57 | 53.88 ± 190.73 | 194.84 ± 139.81 | 193.03 ± 230.85 |
| 4.0 to 7.9      | 27.96 ± 86.94 | 27.18 ± 85.14 | 331.94 ± 645.83 | 294.35 ± 561.49 |
| ≥ 8.0           | 34.64 ± 89.26 | 31.51 ± 89.48 | 325.34 ± 314.19 | 248.44 ± 234.79 |
| P               | 0.106        | 0.186        | 0.104          | 0.197          |
| Macroscopic ulcerated |         |             |               |               |
| No              | 38.21 ± 105.39 | 36.82 ± 108.08 | 530.45 ± 997.46 | 457.95 ± 811.36 |
| Yes             | 30.01 ± 96.84 | 30.26 ± 104.16 | 248.99 ± 257.75 | 214.44 ± 276.23 |
| P               | 0.433        | 0.736        | 0.014          | 0.009          |
| Vegetating      |             |             |               |               |
| No              | 29.19 ± 105.53 | 30.94 ± 114.16 | 233.59 ± 229.94 | 217.40 ± 284.20 |
| Yes             | 34.44 ± 93.07 | 32.51 ± 95.45 | 388.99 ± 706.77 | 319.81 ± 583.88 |
| P               | 0.035        | 0.197        | 0.057          | 0.18           |
| Infiltrative    |             |             |               |               |
| No              | 15.71 ± 41.64 | 15.22 ± 45.76 | 281.19 ± 394.86 | 244.83 ± 376.19 |
| Yes             | 47.00 ± 129.00 | 47.23 ± 137.53 | 341.94 ± 637.18 | 292.49 ± 532.86 |
| P               | 0.132        | 0.07         | 0.415          | 0.321          |
| Venous invasion |             |             |               |               |
| Present         | 48.41 ± 129.86 | 53.23 ± 158.57 | 347.10 ± 282.17 | 255.56 ± 477.84 |
| Absent          | 28.09 ± 89.57 | 26.85 ± 88.36 | 304.68 ± 575.348 | 330.35 ± 391.40 |
| P               | 0.034        | 0.029        | 0.163          | 0.094          |
| Lymp. invasion  |             |             |               |               |
| Present         | 49.76 ± 129.89 | 46.75 ± 125.35 | 447.89 ± 845.14 | 227.83 ± 286.49 |
| Absent          | 23.02 ± 77.03 | 24.33 ± 92.70 | 245.69 ± 251.79 | 353.65 ± 691.80 |
| P               | 0.095        | 0.15         | 0.137          | 0.527          |

With regard to the numerical descriptive measurements of the TPA-M levels, the mean for TPA-M (M) was 325.06 U/L ± 527.29 U/L and the mean for TPA-M (P) was 279.48 U/L ± 455.81 U/L (\( P < 0.01 \)). To compare the evaluations of mesenteric and peripheral TPA-M, the marginal homogeneity test was utilized, from which it was found that rate of positive results was greater for mesenteric TPA-M (\( P < 0.05 \)).

**Associations**

For both markers and for both mesenteric and peripheral blood, the levels were related to advanced stage of the neoplasia, and especially to stage IV of TNM. In addition to this association, CEA (M) and CEA (P) presented correlations with venous invasion, and CEA (M) alone correlated with vegetating lesions. Both the mesenteric and peripheral levels of TPA-M were high in non-ulcerated lesions (Table 1).
The impact on postoperative survival among patients with colorectal cancer. A study published in Japan in 1990 demonstrated that patients with a mesenteric-peripheral CEA gradient greater than 10 ng/mL would have a worse prognosis.

Another study of interest showed that the mesenteric levels and the mesenteric-peripheral gradient were more effective than the utilization of the peripheral levels alone for predicting liver metastases. A study published in Japan in 1998 compared patients with advanced colorectal cancer divided into two groups: with and without liver metastases. The mean mesenteric CEA level and mesenteric-peripheral gradient were greater than the peripheral level in the group with postoperative liver metastases. This suggests that mesenteric assaying of this marker would be more effective for predicting this event.

Subsequent studies conducted by other authors have not shown significant differences between the peripheral and mesenteric CEA levels. This may be related to the small size of the samples analyzed in these studies.

In the present investigation, the sample was composed of 138 patients who were analyzed retrospectively. All of them underwent peripheral and mesenteric assaying of the CEA and cytokeratin (TPA-M) levels, which were evaluated in relation to seven histopathological variables. Statistically significant differences were found between the peripheral and mesenteric CEA and cytokeratin levels when numerical analysis was performed. When only the positive frequency of the markers was investigated, there was only a difference for cytokeratins. This may signify that the main drainage route for the markers is the portal system.

Both of these markers had high levels in TNM stage IV, both for mesenteric and for peripheral blood. Thus, the markers had significantly higher levels when the neoplastic disease was no longer limited to the colon. This corroborates the findings of Fernandes et al. (2005), which showed higher marker levels in cases of patients with extra-colonic disease, perhaps signifying the presence of liver or occult lymph node micrometastases.

The mesenteric and peripheral CEA levels were higher in the presence of venous invasion, and this reproduces the results from previous studies. This may corroborate the hypothesis that drainage via the portal vein system is the fundamental principle for the distribution of this marker.

In the present study, the cytokeratin levels were also higher in the presence of non-ulcerated lesions. No studies presenting an association between peripheral and mesenteric CEA and cytokeratin levels and the macroscopic characteristics of the lesion were found in a search of the medical literature. In the present investigation, there were associations between mesenteric CEA and vegetating lesions and between mesenteric and peripheral cytokeratins and non-ulcerated lesions. It is believed that subsequent studies will be necessary, in order to analyze and compare ulcerated, vegetating and infiltrative lesions in relation to...
to survival and the peripheral and mesenteric levels of biological tumor marker, so as to obtain greater depth for the conclusions.

In summary, the present results allow it to be concluded that, for the patients analyzed, there was a significant difference between the CEA and cytokeratin tumor marker levels, with higher levels in the samples collected from the portal vein system than in those obtained from the peripheral blood. The levels increased in accordance with the progression of neoplastic dissemination. High mesenteric and peripheral CEA levels were associated with venous invasion. There were higher assayed cytokeratin levels in patients with non-ulcerated colorectal adenocarcinoma lesions.

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