IMMUNOHISTOCHEMICAL STUDY IN COLON CANCER PATIENTS

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ABSTRACT

Colorectal cancer is an important public health problem; it stands at the top of oncologic pathology, in Romania and in the world; colon cancer is the third most frequent cancer diagnosed in men and women.
Immunohistochemistry plays an important role in differentiating tumor types, assessment of aggressiveness and recognizing of metastasis origin. Although the molecular analyses are increasingly used, many therapeutical protocols are still based on histological types and immunohistochemical phenotypes.
We present the results of an immunohistochemical study on 120 patients with colorectal cancer.

Key words: colorectal cancer; immunohistochemistry; endoscopy

INTRODUCTION

The study of colon cancer is a main preoccupation for the researchers worldwide due to the high incidence of the disease, current important opportunities in entire colon imaging and biopsy sampling used for histopathological and genetic studies.

Insidious onset and deficiency of national population screening programs are the main underlying causes for colon cancer is diagnosed in advanced stages when treatment possibilities are limited and the chances for survival are lower. The result is the increased morbidity and mortality from colon cancer seen in many countries, both European and worldwide.

Reviewing the literature of our country we found the small number of immunohistochemical research studies on colon cancer. This has formed the basis for choosing the immunohistochemical study in colon cancer as a research theme, the aim being to identify correlations between immunohistochemical aspects and the prognosis of patients with colon cancer.

MATERIAL AND METHODS

We included 120 patients with colon cancer admitted in the Department of Internal Medicine of the Emergency County Craiova Hospital between 2004 and 2008. The study was prospective; the patients were assessed at the time of hospitalization with a follow-up after surgery at 1 year period.

The diagnosis was based on available imaging techniques (irigography, ultrasound, and colonoscopy) and was confirmed by histopathological examination of colonic biopsy fragments and pieces of surgical resection. Resection pieces were initially processed and examined in the Pathology Laboratory of Emergency Clinical Hospital Craiova.

After morphopathological examination, tissue sections fixed on slides previously coated with polylysin were analyzed immunohistochemically in the Center for Studies of Microscopic Morphology and Immunology at the University of Medicine and Pharmacy of Craiova. We investigated the following cell markers: protein P53, BCL2 protein, E-cadherin, CD44, α1-fetoprotein (AFP), VEGF receptor (VEGF-R), TGFβ-RI and TGFβ-RII, PCNA.

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RESULTS

120 patients were included (74 men – 61.66% and 46 female – 38.33%). The average age was of 65.3 ± 13.2 years for males and 67.3 ± 12.8 years for women.

Studying the distribution by age, separately for the two sexes, there were the following results: maximum incidence in men was in the age group 60-70 years (27 patients, 36.48% of the group of men) and in women maximum incidence was between 60-70 years (18 patients, 39.13% of women). Depending on the area of origin it was observed that both in women and men, dominated the urban area (52.7% men, 56.52% of women).

In the analysis of risk factors, 70.27% of men were smokers, smoking was statistically significant ($p = 0.014$) more often in young men with colon cancer. Family history of neoplastic disease was identified in 14 men (18.91%) and 6 women (13.04%). The statistical analysis showed a significant correlation between male gender and presence of the family history of neoplastic disorders ($p = 0.04$). The existence of neoplastic diseases in patients' family history was statistically significantly correlated with younger patient age ($p = 0.03$).

The presence of a history of polyps was observed in 12 men (16.21%) and 3 women (6.52%).

Clinical symptoms were dominantly bowel disorders at 62.5%, abdominal pain in 56.66%, lower gastrointestinal bleeding in 51.66%, weight loss in 59.16%, and palpable tumor mass in 17.5%. Regarding the correlations between symptoms and other factors, we found that bowel disorders were correlated with palpable tumor mass ($p = 0.04$), abdominal pain occurred more frequently in patients without signs of gastrointestinal bleeding ($p = 0.02$), and weight loss was significantly more common in those with palpable tumor mass ($p = 0.005$).

Regarding tumor location detected by colonoscopy, most of those were represented at the sigmoid and rectum localization (72.5%), followed by cecum and ascending colon (23.33%) and transverse colon (4.16%); localization differences were statistically significant (all $p < 0.05$ for comparison between groups).

Also in colonoscopic examination, it was observed that most tumors were infiltrating, ulcerated (46.66%), and followed by infiltrating-stenosed (29.16%) and protrusive (24.18%). Differences between groups were statistically significant (all $p < 0.05$).

Depending on the staging, 37.5% of tumors were classified in stage III, stage IV – 31.6%, 26.6% – stage II and 4.16% in stage I.

The vast majority were adenocarcinomas (97.5%); there was only one case of epidermoid carcinoma, sarcoma and carcinoid tumor (each 0.83% of tumors) – all in women.

In terms of the degree of tumor differentiation (histopathological grading), the most common forms were the moderately differentiated (75%), followed by poorly differentiated (15%). Undifferentiated tumors and well differentiated were rare (6.66% and 3.33%) (Figure 1).

FIGURE 1. *Corelation of tumor grading with VEGF, P53 and CEA markers.*

Immunohistochemical analysis showed a moderately increase in PCNA density and moderate density of E-cadherin in neoplastic cells and other tumor markers showed a moderately low (as in P53 – Figure 2) or low density (as in the case of BCL2, CD44, AFP, VEGF, TGFβRI, TGFβRII).

VEGF was more expressed in younger patients ($p = 0.02$) and BCL2 was found to be expressed more frequently in patients from rural areas (0.04). Other markers were not found to be influenced by gender, age or area of origin of patients. P53 and VEGF markers were common in smokers ($p = 0.02$, respectively $p = 0.03$). P53 and CD44 markers were expressed more frequently in patients with normal body mass index ($p = 0.04$, respectively $p = 0.02$).

Location in the rectum and left colon was commonly associated with expression of VEGF ($p = 0.002$) or PCNA ($p = 0.01$), while the location in the right colon was associated with expression of TGFβRII ($p = 0.02$) and p53 ($p = 0.03$) (Table 1).

TABLE 1. *Corelation of markers with the location of the tumor*

	VEGF	P53	PCNA	TGF-RII
Right colon	33%	78%	18%	63%
Left colon	62%	46%	56%	32%
Rectum	82%	12%	76%	14%

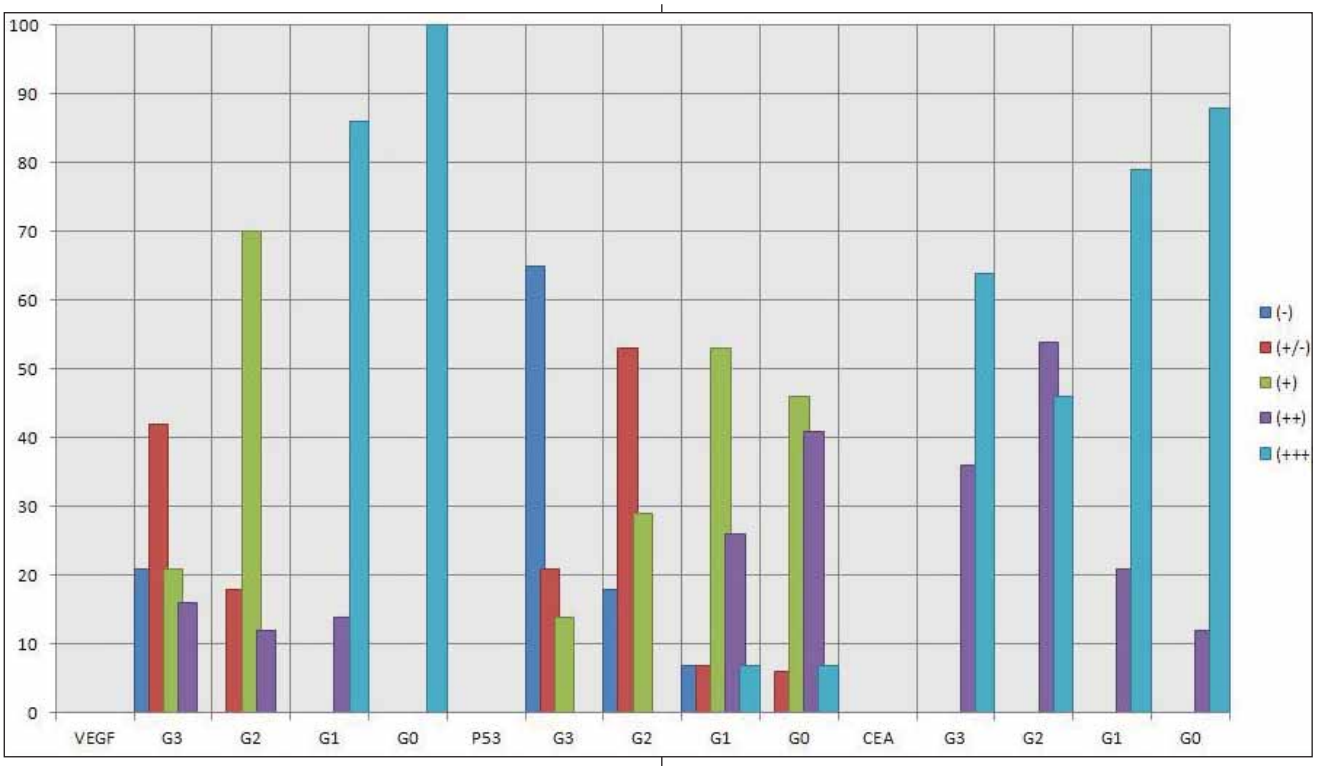

FIGURE 2

E-cadherin marker was present predominantly for infiltrating-stenosed tumor (p = 0.0002) and VEGF showed a higher density in infiltrating tumors (p = 0.04).

Advanced TNM stages were associated with increased expression of PCNA, CD44 and VEGF (p = 0.01, p = 0.0004, p = 0.001 respectively). Markers PCNA, BCL2 and VEGF had a higher density, and E-cadherin was less expressed in poorly differentiated tumors (p = 0.002, p = 0.02, p = 0.03, respectively p = 0.01). Markers PCNA and VEGF have been better cast in advanced stages of primary tumor extension (p = 0.001 respectively p = 0.01).

CD44 and VEGF expression was increased in tumors with lymphatic extension (p=0.01, respectively p=0.004) or in the presence of distant metastases (p = 0.0004, p = 0.002 respectively). BCL2 was a better marker for vascular invasion and expressed the presence of synchronous neoplasia (p=0.001, respectively p = 0.01). Simultaneous expression of several tumor markers was found in the association of P53 and CD44 (p = 0.001), between AFP and PCNA (p = 0.01) between BCL2, CD44 and AFP (p = 0.01 respectively p = 0.02), between CD44 and VEGF (p = 0.0004).

The degree of expression of the marker PCNA was not found to have a statistically significant influence on survival of patients (p = 0.2), although its high-level expression was correlated with survival probability (OR = 0.68). Neither the expression of E-cadherin or TGFβRII markers proved to have a statistically significant influence on survival of patients (p = 0.4, respectively p = 0.3) even though, in the case of TGFβRII, a higher density was associated with a low probability of survival (OR = 0.51).

Cox regression analysis showed that the risk of death was higher for the following markers: PCNA (OR = 1.2), BCL2 (OR = 1.3), CD44 (OR = 1.42), AFP (OR = 1.48), TGFβRI (OR = 1.5), TGFβRII (OR = 1.34).

DISCUSSIONS

Colorectal cancer is one of the most common life-threatening gastrointestinal diseases encountered in clinical practice but with a good healing potential depending on the stage it is discovered. Thus 5-year survival rate in colorectal cancer is different depending on the stage the disease is detected, being 80-90% when the neoplasia is localized strictly to the colon wall, 40-60% when there is regional extension, and only about 5% in case of distant metastases (1,2). This explains the permanent concern of the medical world to devise viable strategies for early detection of this cancer, in curable stages.

Colorectal carcinogenesis process is complex, and incompletely understood. In pursuing this issue we must take into account the involvement of predisposing conditions, and risk factors (3).

The vast majority of colorectal tumors are adenocarcinomas, most resulting from malignant transformation of preexisting adenomatous lesions, polypoid lesions (4-6). Most colonic adenocarcinomas are moderately or well differentiated. About 20% of them are poorly differentiated or undifferentiated, the latter associated with poor outcome. Most adenocarcinomas secrete a small or moderate amount of mucin, but 10-20% of tumors are described as mucinous or colloidal, due to major mucin production; they are associated with a worse survival at 5 years compared with those without mucin secretion (7,8).

Several classifications have been used for staging. Duke's classification presented in 1930 is still used. Because of its limitations especially related to the number of lymph nodes involved, the presence and quantification of distant metastases and the definition of carcinoma in situ, it was proposed and is currently used the TNM classification (9,10).

CONCLUSIONS

Research of immunohistochemical markers may be useful in early detection of aggressive forms of colon cancer, even if only part of the markers studied, and only in a limited number of studies, have been shown to influence the likelihood of patient survival. There are required much larger population studies and research on a variety of tumor markers in order to obtain results with high statistical significance.

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