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Histoprognostic markers role in colorectal cancer

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Abstract

Malignant tumors with digestive location are, according to the *World Health Organization* (WHO), a major cause of morbidity and mortality worldwide. In Romania, the constant increase of prevalence and incidence of colorectal cancer awarded it the status of priority public health problem. The study aimed to identify specific aspects of colorectal cancer histoprognosis that may be associated with a higher frequency of the disease. Data were collected from records and registers within Clinic of Medical Oncology, Emergency County Hospital, Craiova, Romania. Were analyzed and associated demographics and epidemiological data, clinical features, anatomotopographical, histopathological and immunohistochemical. The cases studied were adenocarcinomas with a balanced gender distribution and a worrying incidence for Craiova. The age group with the highest incidence was that of 55–64 years. Topographic, rectum and rectosigmoidian junction are the first two locations. More than half of the cases (55.55%) are adenocarcinomas with moderate differentiation and belong to the pT3 category, as extension of colorectal tumor degree. 32.5% of patients were identified with mutations in the K-Ras oncogenes and were found Ki67 positive immunoreacted and heterogeneity of antigen expression in tumor areas studied. Colorectal cancer recorded a worldwide steady increase in the incidence; growth trend in our country is above the European average. Dolj County faces with an increased incidence and mortality rates by this disease. To limit the disease at the population level and pre-malignant diagnosis is necessary to establish histo-prognostic value and predictive of tumor markers.

Keywords: colorectal cancer, indicators, immunohistochemistry, tumor markers, public health.

Introduction

Colorectal cancer currently represents a major public health problem in medicine today. Worldwide, the last decade has been recorded a steady increase in all indicators specific to this local digestive malignant tumors (morbidity, mortality, incidence and prevalence), cancerous disease itself is considered in recent decades as one of the most complex, difficult and priority human pathologies [1]. According to data published by the *International Agency for Research on Cancer* (IARC), GLOBOCAN 2012 – Colorectal cancer: estimated incidence, mortality and prevalence worldwide in 2012, *World Health Organization* (WHO), this type of cancer is the third most common in men (746 000 cases 10.1% of total) and second in women (614 000 cases, 9.2% of the total), worldwide. According to the same sources, in the European Union member states (EU 28), lung cancer, responsible for the deaths of 266 000 people is followed by colorectal cancer (152 000 or 11.9%). In the EU 28, in 2012, there were 345 000 cases of colorectal cancer and 152 000 deaths from this type of malignancy. More than half of all cases are registered in economically developed regions, ranging incidence rates 10 times in both sexes worldwide. The highest incidence rates are estimated for Australia (44.8 and 32.2 to 100 000 men and women, respectively) and lowest for West Africa (from 4.5 and 3.8 per 100 000) [2].

In Romania, the main cause of death is attributed to cardiovascular diseases and neoplasms then, in recent years malignancy specific mortality is registering a growth trend higher than the European average. WHO's statistics reveals that colorectal cancer mortality records is a tendency in our country, permanently increasing in the last two decades [3]. In the hierarchy of death causes from cancer disease, colorectal location for our country stands after the lung and before the stomach. It was considered necessary this statistics list of elements considered to highlight several aspects: the major impact and severity of these diseases on human health, impaired quality of life and colorectal location to digestive malignancies in this context [4, 5].

In the multifactorial context of increasing life expectancy, modern treatments and innovative techniques in the field of genetic research, this increase of cancers' incidence, including colorectal location, became manifest today. The literature highlighted a link between the high rate of mortality from colon cancer and diagnosis in advanced stages of the disease because of its insidious onset. It also contributes to this lack of access to health policy, allowing cancer patients to national strategies for prevention, screening and surveillance and weaknesses in risk identification for families with a genetic predisposition for this location [6].

Molecular changes underlying the initiation and progression of colorectal cancer are under intense scrutiny in the past decade, trying to determine the nature of interactions between environmental factors, hereditary factors and those related to the human body. In 2009,

Sanford Markovitz issued the hypothesis of multifactoriality in colorectal carcinogenesis. Results of studies have been made on this type of neoplasia to maintain its status as a model, to illustrate the molecular underpinnings of carcinogenesis. Determinants of tumor progression and heterogeneity evidences are generating selection variants with the best survival rate, growth and invasion. Acquired specific mutations of interest and genes encoding proteins are progressively involved in cellular differentiation and proliferation, which are those that will then dictate tumor phenotype and the presence of metastases [7].

By correlating elements of molecular biology and pathology to clinical aspects of colorectal cancer, we can identify new predictive and prognostic factors. Modern molecular techniques currently used can facilitate clinical management of disease and the treatment can be properly directed to the patients most likely to respond. By examining the cellular mechanisms involved in cell cycle control, immunohistochemical markers can identify the correlations between the tumoral expression degree and aggressiveness and survival of patients with colorectal cancer [8]. Because of involvement in the cell cycle and transmitting signals initiating cell proliferation, Ras proto-oncogenes (responsible for encoding G proteins of low molecular weight) may be associated with the earliest stages of colorectal cancer genesis [9]. More than half of the colorectal cancers' literature confirm mutations at codons 12 and 13 of K-Ras gene (Kirsten Ras gene) [10]. This finding was associated with a poor prognosis but without demonstrable link with disease stage. The protein located on the internal face of the cell membrane exhibit GTP, acting as intracellular signal transduction and playing an important role in cell division, differentiation and apoptosis. Detection of K-Ras gene mutations was studied for a potential predictive role in determining response to chemotherapy.

The correlations between mutational activation, by binding to EGFR (epidermal growth factor receptor) as a transmembrane receptor, triggering cascades of intracellular signal transduction to the nucleus, support the role of these mutations in the predictability of response to specific therapy [11]. Known research data on this receptor expression are variable and cannot demonstrate that EGFR is an independent indicator of prognosis in colorectal cancers [12].

Patients positive for K-Ras gene mutations, by poorly responding to therapy that interfere with the functional domains of the EGFR, show the impact of this status in the signaling pathway mediated by this receptor and the association with advanced stages of disease, increased metastatic potential and lower prognosis. The mutations described are responsible for altering the mechanism of EGFR inhibition, through which therapy with anti-EGFR monoclonal antibodies can no longer exert inhibitory effect on cell proliferation [13, 14].

The study of potential biomarkers is very important to identify susceptible individuals at the population level, timing of onset and disease detection in the early stages of colorectal cancer progression.

Patients, Materials and Methods

Our study is part of a wider research whose theme is the correlation of risk factors for digestive cancers and which was achieved through an observational study of specific records, namely: statistical sheets for cancer patients, records of inpatient cancer (ONC1 and ONC2) and charts with general clinical observation of patients hospitalized and diagnosed with digestive cancers within the Clinic of Medical Oncology, Emergency County Hospital, Craiova, Romania. From the 2790 patients diagnosed with colorectal cancer in the time frame 2002–2014, group on which there was a retrospective study – longitudinal study, we extracted a subset of 81 patients of both genders, aged less than 75 years, with the average residence Craiova or one of three Sub-regions of Dolj County (North, South-East and South-West). For our study, we took into account many variables considered important, namely: demographics data (belonging to one age group, gender, residence – urban/rural), incidence, anatomical location of tumoral formation, degree of differentiation, tumoral stage, extension of the tumor, and results of the histopathological examination, immunohistochemical and molecular biology analyses. The focus was on observing cases that it is possible to detect K-Ras gene mutations, to determine the gene type ("wild" – WT or mutant – MT), using molecular biology tests, and to analyse, by immunohistochemical techniques, the EGFR and Ki67 molecular biomarkers, whose overexpression may be a histoprognostic indicator for our cases, according to current scientific data. All patients who benefited from surgery, complex cancer treatment (radio-chemotherapy) with complete or partial remission, relapse or died, have been identified. By analyzing the correlations between all the above-mentioned variables, predictive or prognostic importance and value of molecular biomarkers and clinical factors, and the effectiveness of specific therapies can be demonstrated through immunohistochemical tests targeted to early diagnosis.

Results

Analysis of incidence according to gender does not show major differences in the study group: 44 (54.32%) men and 37 (45.68%) women (Figure 1).

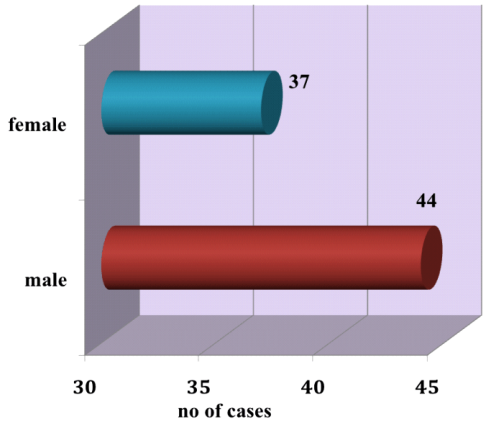

Figure 1 – ***Distribution by gender of patients with colorectal cancer.***

Regarding the distribution of cases studied by the six age groups considered, it can be seen that the highest incidence (41.97% and 32.11%) were registered for the groups 55–64 and 65–74 years, less than 45% of the values having insignificant incidence (Figure 2).

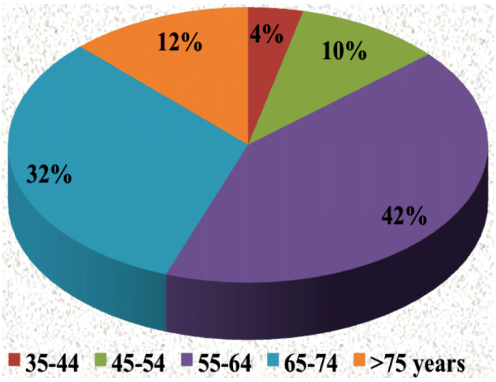

Figure 2 – ***Distribution of patients with colorectal cancer by age group.***

Regarding the residential area, we made a distribution of cases for Craiova and of the three major Sub-regions of Dolj County, respectively North, South-East and South-West. The analysis showed that more than half of the cases are of patients residing in Craiova (53%) and in the Sub-regions a slightly higher incidence was recorded for the South-West Sub-region of Dolj County (18.51%) (Figure 3).

The main anatomical locations of tumors are, as follows, in descending order: rectum – 38 (46.91%) cases, rectosigmoid junction – 13 (16.04%) cases, cecum and ascending colon – 10 (12.34%) cases, sigmoid colon – 7 (8.64%) cases. Other localizations (hepatic and splenic flexure, transverse and descending colon) and unspecified locations had a balanced percentage distribution.

For the 81 studied cases, analysis of the results of differentiation degree has highlighted the aspects shown in Figure 4. More than half of the cases (55.55%) are represented by moderately differentiated malignancies (G2), followed by the well and poorly differentiated or G1 (24.69%) and G3 (19.75%). We note that for 79 of the observed cases (81), histopathological examination revealed adenocarcinoma. Also, most of the cases were discovered in stage III to stage IV, according to pTNM staging. The degree of anatomical tumor extension into the adjacent structures, in terms of histological category reflected by the degree of tumoral invasiveness (T), is shown in Table 1. It is noted that more than half of the cases (55.55%) belong to the advanced lesions category or pT3 (Figure 4).

Table 1 – ***Distribution of cases according to pT stage (extent of primary tumor)***

Stage (pT)	No. of cases	Percent
Tx	0	0%
Tis	0	0%
T1	5	6.18%
T2	18	22.22%
T3	45	55.55%
T4	13	16.04%

Of the 81 colorectal adenocarcinoma cases studied and confirmed by histopathological examination, at the half (40) K-Ras gene specific tests have been carried out to establish the molecular biology status. Thus, for 27 (67.5%) of the 40 patients to which DNA was investigated after extraction from tissue fragments, molecular biology tests did not detect activating mutations in K-Ras gene level (to "wild" type – WT). For the other 13 (32.5%) patients, genetic tests have identified genetic mutations (Figure 5). By using the polymerase chain reaction (PCR) amplification technique (direct sequencing method of DNA), mutations have been identified in codons 12 and 13 by sequencing exon 2 (Figure 5).

For this subset of 40 colorectal cancer cases examined through molecular biology, the results of various analyzes were observed also by immunohistochemical techniques for EGFR and Ki67 biomarkers reaching statistical significance. Ki67 antigen is a protein useful in the identification of tumoral proliferating cells, the results being expressed as a percentage of positive tumor cells. Ki67 positive immunoreaction and expression heterogeneity have been found in tumoral areas. This shows an uneven proliferative activity in adenocarcinoma compared to conventional integral colic mucosa (Figures 6 and 7). Positivity for Ki67 nuclear antigen varied between 30% and 80% for 29 of the 40 studied cases, <30% for 10 patients and one case by 80%. Positivity >50% as well as moderately differentiated adenocarcinoma have been confirmed for all cases. No statistically significant associations were observed for Ki67 positivity and variables such as gender, age, residence, pT stage, K-Ras WT or MT. Percentage of EGFR-positive cases was <50%, with poorly or moderately positive immunomarking (more than 30% of tumor cells) (Figures 8 and 9).

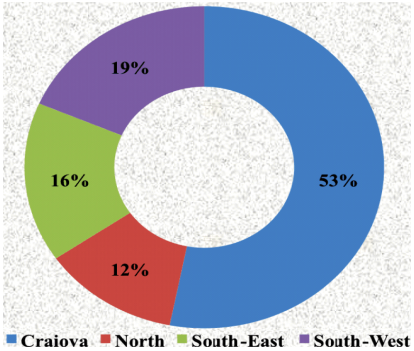

Figure 3 – ***Distribution of patients according to their residence.***

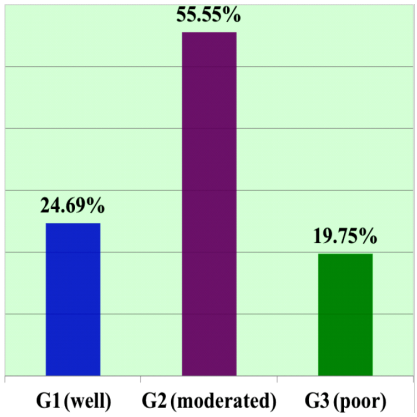

Figure 4 – ***Distribution of cases according to the differentiation degree (histopathological grading).***

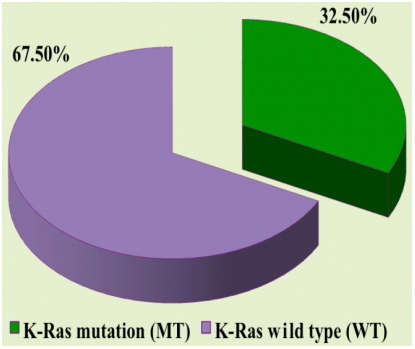

Figure 5 – ***Distribution of cases by K-Ras gene MT/WT types.***

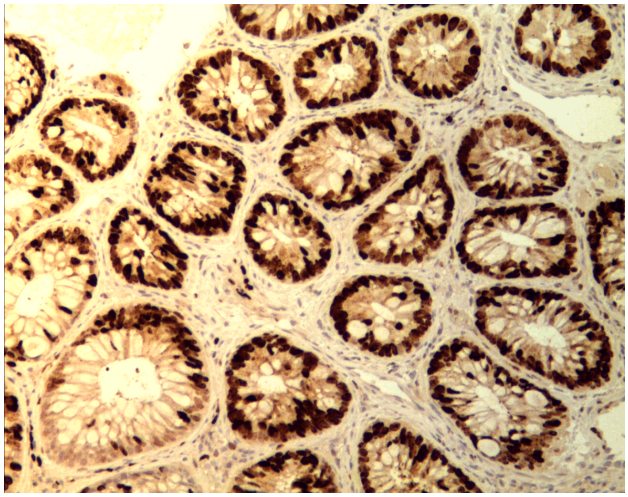

Figure 6 – ***Colic mucosa: Ki67 nuclear basal immuno-marking, ×100.***

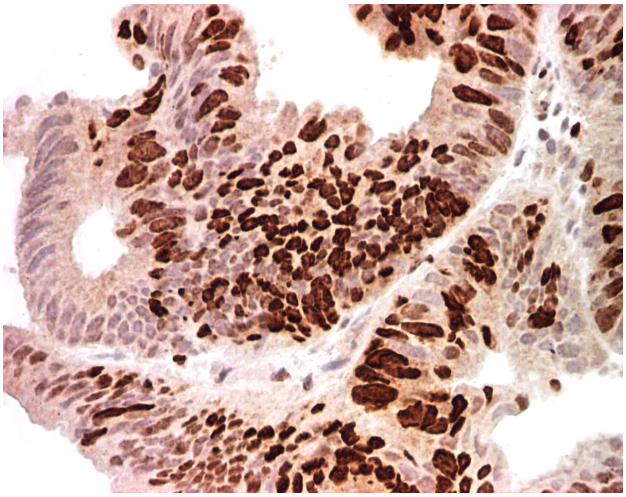

Figure 7 – ***Well-differentiated adenocarcinoma: Ki67 nuclear immunomarking, ×200.***

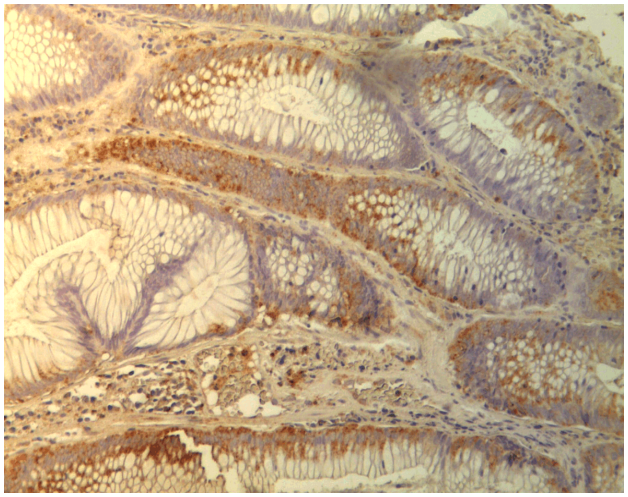

Figure 8 – ***Colic mucosa, immunomarking for EGFR: focal basal membrane with low intensity, ×100.***

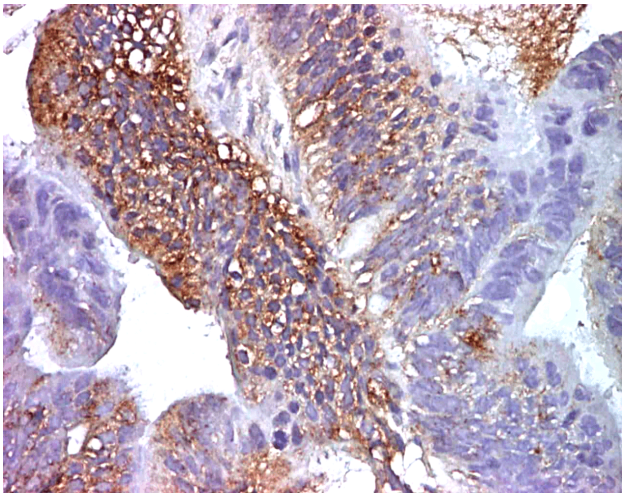

Figure 9 – ***Well-differentiated adenocarcinoma: EGFR heterogeneous membrane immunomarking, ×200.***

Discussion

The contemporary period is characterized by a continued increase in the incidence of cancer disease. This become manifest today as a major public health problem and disability-free life expectancy is an universally recognized indicator of European structural importance [15]. Reevaluation of the concept of primary prevention, whose major goal is to reduce the specific causal factors, not only through research, identification and quantification, is itself an objective of the proposed research. In this context, within the first three digestive locations (gastric, colorectal and esophageal) represents almost a quarter of all new cases of cancer registered in Dolj County, in the 2002–2014 period. Colorectal cancer is the second in the hierarchy of digestive cancers and accounts for more than half of all digestive cancers. This data confirms the results of current clinical and epidemiological scientific studies and proves that malignant colorectal represents a major medical problem with a great social impact but also its epidemic character through its shared structural profile of morbidity and general mortality [16]. Although investigative techniques from various fields (genetics, molecular biology, biochemistry, imaging) showed their role in the early diagnosis of colorectal cancer, its incidence is in constant growth [17, 18].

Epidemiological and clinical studies conducted on a case similar to our study showed that gender did not register major differences [19]. The epidemiological profile of colorectal cancer studied cases indicate an increased incidence for Dolj County, compared to other counties in the Macro-region III – South-West Oltenia –, with a maximum incidence for Craiova. Regarding the residence, incidence of colorectal cancer has a relatively balanced distribution in the three subregions of Dolj but with a worrying incidence for Craiova (more than half of new cases registered during the survey period), which conforms to the results of previous studies on a populational level. The age groups in the study that patients were diagnosed reveal a peak record incidence in the age group of 55–64 years. Regarding malignant tumors, rectum and sigmoid are the most common locations. The results of the study for the degree of differentiation suit the current study data, more than half being moderately differentiated adenocarcinoma grade (G2), and the degree of dominance of the pT3 tumor extension [20, 21].

The current state of research on K-Ras oncogene mutations highlight their predictive value in patients'

responses to chemotherapy due to the association of mutations in colorectal cancers in advanced stages of development. Over 75% of the mutations identified in extensive scientific research aimed exon 2 or codon 12. Although K-Ras status is analyzed in general to support targeted therapy, literature associated mutations in codon 12 with a poor prognosis, contact stage extension of the tumor is not yet well defined [22, 23].

Clinical, histopathological and imaging examination, which determine the degree of extension, the proper staging and the degree of differentiation, are key factors without which the studies of clinicomolecular phenotypes cannot be performed. K-Ras mutation frequency is consistent with the outcome of similar studies in patients with colorectal cancer in advanced stages of disease, resistant to cancer treatment and without an association with tumor differentiation grade and prognosis. Mutations confirmed by biomolecular tests correlated with high levels of biomarkers and the weak tumor differentiation grade seem to have a poor prognosis, which conforms to the results of other studies. On the other hand, low levels of biomarkers are associated with well-differentiated tumors, small extension stages and a favorable prognosis regardless of the K-Ras type gene. Extensive studies have demonstrated high expression of EGFR association with T3 tumor [24].

Analysis of the Ki67 positivity rate is closely correlated in most studies with epidemiological, clinical and biological variables, to support its role in assessing the prognosis of patients with colorectal cancer. Although variable, immunoreactions are the most common, being moderately or strongly positive, which shows an increased capacity for proliferation of colorectal adenocarcinomas. There has been shown a correlation with the survival rate of patients, so relationship positivity rate–prognosis remains unclear. Some specialized studies argue that tumors with Ki67 positive nuclear immunomarking higher than 35% have a greater risk of relapse, therefore a poor prognosis. The proliferative activity of the tumor determines the malignant potential and correlates with the metastatic recurrence rate [25, 26].

Conclusions

This study conducted by observing and analyzing the existing data from the reporting sheets and records within the Clinic of Medical Oncology, Emergency County Hospital of Craiova, allows us to reach to the following conclusions. By increased crude and standardized mortality rate (according to the National Institute of Statistics), by geographic profile and characteristics of its population, Dolj County was consistently included in epidemiologic studies for comparative interpretation of correct results. Research, identification and quantification of specific causal factors of colorectal cancers may elucidate the limiting disease mechanisms, at the population level. Thus, we highlighted the importance of epidemiological studies correlating with all other aspects of colorectal neoplastic disease. Exact control of colorectal cancers phenomenon requires proper evaluation of the disease incidence through optimum operation of the Regional Cancer Registry. A correct combination of their survival and disease-free interval, achieving statistically significant results, requires studies involving a larger number of observed cases. Demonstrations of the prognostic and predictive values preferably with an independent character of the markers are elements that legitimize continued oversight of the population for improving the screening and early diagnosis of the colorectal cancer.

Conflict of interests

The authors declare that they have no conflict of interests.

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