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CORRELATION OF HER 2 PROTO-ONCOGENE EXPRESSION WITH THE MACROSCOPIC MANIFESTATION AND GROWTH PATTERN OF COLORECTAL CANCER

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Colon carcinoma is one of the most common malignant tumours and the second cause of cancer deaths in developed countries.

The objective of this study is to investigate the expression of HER2 in colorectal cancer and compare the expression levels of HER2 with the macroscopic appearance and manner of growth of the tumours, followed by detection and identification of the expression levels of proto-oncogene HER2 as an important prognostic factor of the further course, outcome and the data for the selection of appropriate therapy.

This was a prospective, clinical and experimental study. Postoperative material obtained by resection of colorectal cancer from 63 patients of both sexes was used in the study.

HER2 status was positive in 9.5% of the tumours. The results showed that the majority of tumours 36/63, i.e. 57.1% were manifested as infiltrative forms, 9/63, i.e. 14.3% as ulcerating form, and 18/63, i.e. 28.6% of tumours had a polypoid – exophytic form. The results show that 31 tumours of infiltrative forms were HER2 negative and 5 of them were HER2 positive. In tumours with ulcerating form 9 were HER2 negative, and there were no HER2 positive tumours. In vegetative tumour forms 17 were HER2 negative, and only 1 was HER2 positive.

HER2 expression was absent in 90.5% of the tumours and does not correlate with the macroscopic appearance and manner of tumour growth. Expression level of HER2 does not have a diagnostic, predictive and prognostic potential, while its importance is undeniable in the understanding of oncogenesis. *Acta Medica Medianae 2011; 50(4):5-10.*

Key words: *colorectal cancer, HER 2, correlation, macroscopic appearance, growth pattern*

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Introduction

Colon carcinoma is one of the most common malignant tumours and the second cause of cancer deaths in developed countries (1).

Processes of division, differentiation and cell death are strictly controlled, and a disorder in the regulation of any of them gives rise to clones of cells that independently and inappropriately breed and produce tumour mass. Onset of tumour is a complex process involving many genetic and molecular mechanisms. Oncogenesis is the result of accumulation of disorder in the structure and function of genes regulating cell proliferation mechanisms, reparation of DNA molecule or programmed cell death. These genes are: oncogenes, tumour suppressor genes, genes that are the matrix for the synthesis of the reparatory system enzymes, as well as genes that control apoptosis (2).

The human genome has a normal presence of proto-oncogenes, whose protein products participate in the transmission of signals that control cell proliferation. Mutated protooncogenes are oncogenes and their products are oncoproteins (3), and via receptors and soluble factors that bind to them, a cell communicates with the environment, and disturbances in the transmission of signals from cell membrane to the nucleus can lead to changes of mitotic activity or the ability of differentiation (4). HER 2 (human epidermal growth factor receptor) from the EGFR family is a transmembrane, glycoprotein receptor gene which is encoded by a gene on the chromosome 17. In terms of cell transformation - modified physiological structures and functions in regards of control of cell cycle and death, increased expression classifies HER 2 as an oncoprotein (5).

Ligand for HER2 has not yet been identified, but it is considered that this molecule

belongs to HRG (heregulin) family. HRG and HER2 genes are genes that are most spliced i.e. with the most exons and introns, which means they have lots of protein forms (isoforms). Members of the EGFR and HER family are bivalent and thus HER family receptors homo-and hetero-dimerize, binding themselves to different isoforms of the ligand, thus creating a specific network of signal transduction through the cell, because each combination of receptor-ligand has their own way of signal transmission through the cell. This further modifies the expression of genes that change cell phenotype and function in terms of changed presentation of molecules on the surface of cells and synthesis of specific proteins (6-8). The result of hyperactivity of these signalling pathways through the cell, leads to expression and activation of proteins that lead to the development of the cell cycle and prevention of cell death. Wrong expression of any enzyme or protein from the signal transmission cascade through the cell is sufficient to affect the balance of development control of cell cycle and apoptosis.

There are several morphological types of colorectal cancer: a ring (annular), vegetative (polypoid), infiltrative and ulcerating tumours (9).

On the left side the majority of colorectal cancers are ringed lesions that significantly narrow the lumen, and consequently often cause expansion of proximal intestine. The edges of the ring are raised and firm, while the central part is usually ulcerated. These tumours over time (in years) penetrate the bowel wall and can occur as sub-serous or serous, a solid whitish thickening. Their metastases spread to regional lymph nodes and liver, and later distant organs (9,10).

Right colon cancers are generally polypoid, with mushroom-like appearance and protrude into the lumen like "cauliflower" masses. These lesions are rarely ulcerative type or in the form of plaque. Regardless of the macroscopic appearance, these tumours penetrate the bowel wall and spread to mesentery and regional lymph nodes. It is possible that they further disseminate in the liver and other organs (9-11).

The aim of this study was to examine the expression of HER2 at the operational material-resectioned segment of patients with colorectal cancer, comparing the expression levels of HER 2 with the macroscopic appearance and manner of tumour growth, identification of possible prognostic significance of this correlation, and the detection and identification of HER2 expression levels as an important prognostic factor in the further course of illness and the data for the choice of suitable and individual antitumor therapy.

Material and methods

This was a prospective, clinical and experimental study. Postoperative material obtained by resection of the colorectal tumours of 63 patients of both sexes was used, from the Surgery Clinic of the Clinical Centre "Kragujevac", in Kragujevac. In order to obtain as much relevant histopathological data, the routine hematoxylin - eosin (HE) and immunohistochemical methods were performed at the Centre for the pathological-anatomical diagnosis of CC "Kragujevac", Kragujevac.

Routine HE method was used for patho-histological verification of tumours, and histopathological analysis. In the routine processing of products, tissue samples were fixed in 4% neutral buffer formalin solution, in 24 hours, at room temperature. Upon completion of fixation, they were dehydrated through a series of alcohols of increasing concentration (70%, 96% and 100%), stained in xylol and embedded in paraffin. Tissue sections, 4 μm thick, were cut with microtomes Leica SM 2000R and Leica Reinhart Austria.

After deparaffinization in xylol and hydration in decreasing order of alcohol, sections were stained with haematoxylin according to Mayer, stained in 2% eosin, then dehydrated, stained and mounted on a plate with Canada balsam (12-14).

Immunohistochemical methods were used to identify the expression of antigen in colorectal cancer resection sample. The procedure for immuno-histochemical staining included the unmasking of antigens, blocking of endogenous peroxidase, incubation with primary antiserum preparation and the procedure of immuno-histochemical methods - LSAB+ - HRP (15).

Evaluation of HER2 expression results was performed according to the criteria recommended by the manufacturer („DAKO - Hercep Test"). The estimate is based on the determination of three parameters: the percentage of tumour cells whose membranes show immunoreactivity (limit is 10%), of continuity, i.e. discontinuity and intensity of immunoreactive staining of the membrane. Immunological criteria for the assessment of HER2 expression:

0 – no staining or membrane staining is at least 10% of tumour cells

1+ - barely stained membrane in more than 10% of tumour cells

2+ - weak or intermediate staining of the entire membrane in more than 10% of tumour cells

3+ - strong staining of the entire membrane in more than 10% of tumour cells

0 and 1 + are the negative result of a 2 + and 3 + are the positive results

Cytoplasmic expression was not included in the assessment of positivity.

Immunohistochemical staining was carried out with the control of quality and specificity of staining, using positive and negative controls according to the propositions of UK NEQAS (UK National External Quality Assessment for immuno-cytochemistry). For statistical data processing the SPSS software package and methods of descriptive statistics were used (frequencies, percentages, medians, percentiles). Analysis of two descriptive variables was carried out using Chi-square test and Fisher test. Investigation of the effect of several variables on a binary variable was performed using binary logistic multivariant regression.

Results

The largest number of tumours 36/63, i.e. 57.1% was manifested in the form of infiltrative forms, 9 / 63, i.e. 14.3% as ulcerating form, and 18/63, i.e. 28.6% of tumours had a polypoid - exophytic appearance (Table 1).

Expression of HER2 was present in 57/63, i.e. 90.5% of tumours. HER2 status was positive in 6 / 63, i.e. 9.5% of tumours, i.e. demonstrated a continuous membrane immunoreactivity of moderate and high intensity (more than 10% tumour cells) (Table 2, Figure 1).

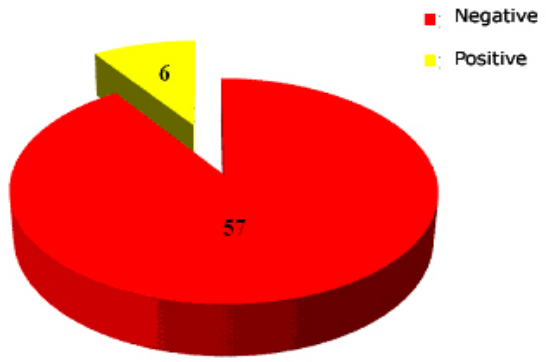

Figure 1: HER2 expression

Table 1. Macroscopic manifestation of tumour

Macroscopic manifestation of tumour	Frequency	Percentage (%)
Vegetative	18	28,6
Ulcerating	9	14,3
Infiltrative	36	57,1
Total	63	100,0

Table 2. HER2 expression

HER-2 expression	Frequency	Percentage (%)
Negative	57	90,5
Positive	6	9,5
Total	63	100,0

Table 3. Correlation between macroscopic manifestation of tumour and HER2

Macroscopic manifestation of tumour	HER2-	HER2+	Percentage (%) -	Percentage (%) +
Vegetative form	17	1	94.44	5.56
Ulcerating form	9	0	100	0
Infiltrative form	31	5	86.11	13.89

The results showed that 31 infiltrative form tumours were HER2 negative and 5 of them were HER2 positive. In tumours of ulcerating form 9 were HER2 negative, and there were no positive HER2 tumours. Vegetative forms tumours had 17 HER2 negative, and only 1 was HER2 positive (Table 3).

Macroscopic manifestation of the tumour and HER2 are independent (p=1.355).

In order to obtain reliable parameters to examine the various factors that could affect the expression of HER2, binary logistic regression was performed.

The presented results showed that HER2 is not dependent on clinical and histological parameters (Figures 2-5).

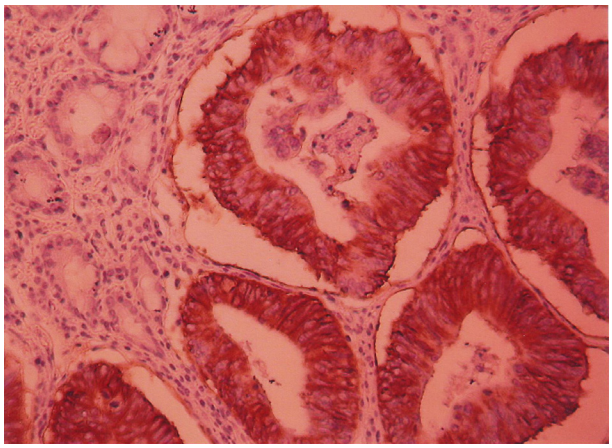

Figure 2: HER2 – negative internal control (IHH,x 200)

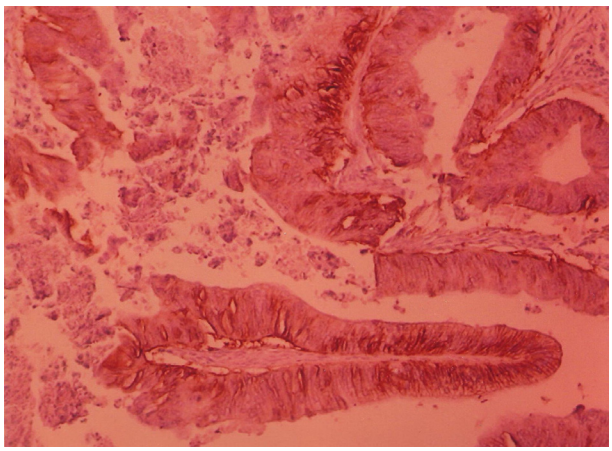

Figure 3: HER 2=1, weak staining of the entire membrane of more than 10% of tumour cells(IHH x 200)

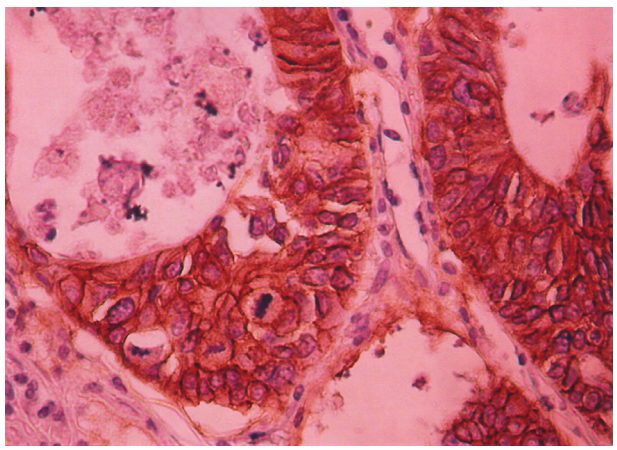

Figure 4: HER2=2+, moderate staining of the entire membrane of more than 10% of tumour cells (IHH x 400)

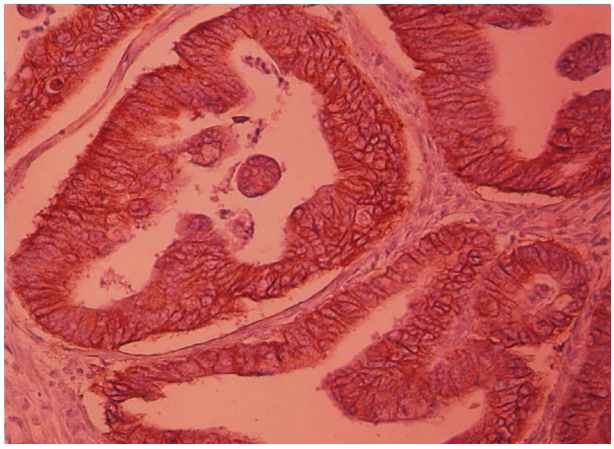

Figure 5: HER2=3+, more than 10% tumour cells showing high intensity continuous immunoreactivity (IHH x 200)

Discussion

Colorectal cancer (CRC) is one of the most common causes of morbidity and mortality in the western world and in our region. Classifications and established prognostic parameters, which are used in the treatment of CRC still only partially provide information about the course and outcome of this disease; hence, there is a need to improve the existing and identify new diagnostic and prognostic markers. In this regard, it is especially important to identify molecular markers that would provide insight into the potential behaviour or aggressiveness of the tumours (16-18).

Since the current clinical and morphologic parameters (histological type of tumour, degree of differentiation, tumour stage, nodal status, invasion of vascular structures and surgical margins) still retain their dominant role in diagnostic procedures, molecular profiling will contribute to their completion, usually in terms of recognition of response to applied therapy (genetic changes), or in terms of improving the screening of high-risk categories to allow for timely and successful treatment (19).

The largest number of tumours 36/63, i.e. 57.1% was manifested in the form of infiltrations, 9 / 63, i.e. 14.3% as ulcerating form, and 18/63, i.e. 28.6% of tumours had a polypoid – exophytic look.

The results of this study showed expression in 9.5% of the tumours, where the range of HER2 expression is from 0 to 85% in the available literature, which correlates with the results presented (20). However, the same literature shows contradictory results that were obtained when another method was applied for determining HER2 status (Hercept Test - kit (DAKO, Glostrup, Denmark)), which suggests that a methodology could affect the obtained expression result.

Research shows that only ¼ of patients with HER2 overexpression has a favourable response to treatment with Herceptin. The goal is to determine the potentials role of this drug, as there are valid methods for detection of patients with CRC. This result is a consequence of lack of standardized methods, types of antibodies, the tissue used, inadequate storage and cytoplasmic presentation of HER2, which is not targeted by Herceptin. FISH uses more objective scoring, sensitivity of 96% and specificity 100%. IHH is cheaper, more available and requires routine microscopy. FISH is an expensive method, which requires fluorescent microscopy and has difficulty distinguishing tumour cells from the stroma (21).

HER2 expression does not depend on the macroscopic manifestations of tumours and tumour growth pattern, i.e., there is no correlation between HER2 expression and the macroscopic manifestation and manner of growth of CRC, which would serve as a prognostic factor in the further course and outcome of the illness.

Conclusion

Expression of HER 2 was absent in 90.5% of the tumours and does not correlate with the macroscopic manifestation or the growth pattern of colorectal carcinoma.

From all the foregoing, it can be concluded that previous studies have not demonstrated the role of HER 2 as an important prognostic indicator. Evaluation of the genes and molecular profiling can help identify groups of patients with HER 2 overexpression, which could imply a particular therapeutic intervention and an important screening for target therapeutic purposes.

An expression level of HER 2 has diagnostic, predictive and prognostic potential, while its importance is undeniable in the understanding of oncogenesis.

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KORELACIJA EKSPRESIJE HER 2 PROTOONKOGENA SA MAKROSKOPSKOM MANIFESTACIJOM I NAČINOM RASTA KOLOREKTALNIH KARCINOMA

Jelena Lukić-Flora, Dušica Petrović, Vesna Stanković, Miloš Milosavljević i Vladimir Bulatović

Karcinom debelog creva jedan je od najčešćih malignih tumora i drugi po redu uzročnik smrti od karcinoma u razvijenim zemljama.

Cilj istraživanja bilo je ispitivanje ekspresije HER2 kod kolorektalnog karcinoma kao i komparacija nivoa ekspresije HER2 sa makroskopskim izgledom i načinom rasta tumora, zatim detekcija i identifikacija nivoa ekspresije protoonkogena HER2 kao značajnog prognostičkog faktora daljeg toka, ishoda bolesti i podatka za izbor adekvatne terapije.

Istraživanje je urađeno kao prospektivna, kliničko-eksperimentalna studija. Korišćen je postoperativni materijal dobijen resekcijom kolorektalnog karcinoma od 63 bolesnika oba pola.

Pozitivan HER2 status bio je u 9.5% tumora. Dobijeni rezultati pokazali su da se najveći broj tumora 36/63, tj. 57.1% manifestovao u vidu infiltrativne forme, 9/63, tj. 14.3% kao ulcerišuća forma, a 18/63, tj. 28.6% tumora imalo je polipoidni – egzofitičan izgled. Rezultati su pokazali da je 31 tumor infiltrativne forme bio HER2 negativan a 5 ih je bilo HER2 pozitivno. Kod tumora ulcerišuće forme 9 je bilo HER2 negativno, a nije bilo pozitivnih HER2 tumora. Kod tumora vegetativne forme 17 je bilo HER2 negativno, a samo 1 je bio HER2 pozitivan.

Ekspresija HER2 je izostala u 90,5% tumora i ne korelira sa makroskopskim izgledom i načinom rasta tumora. Nivo ekspresije HER2 nema dijagnostički, prediktivni i prognostički potencijal, dok je neosporna njegova važnost u razumevanju onkogeneze.
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***Ključne reči**: kolorektalni karcinom, HER 2, korelacija, makroskopski izgled, način rasta*