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

The objective of the research was to investigate the p53 expression in colorectal cancer and compare the expression levels of p53 with the macroscopic appearance and manner of growth of the tumours, followed by detection and identification of the expression levels of p53 as an important prognostic factor of the further course, outcome and data for the selection of appropriate therapy.

Research was implemented as a prospective, clinical and experimental study. We used a postoperative material obtained by resection of colorectal cancer from 63 patients of both sexes.

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 have shown that three tumours of infiltrative forms were p53 negative and 17 of them were p53 positive. In tumours with ulcerating form, eight were p53 negative, and 16 of them were no p53 positive tumours. In vegetative tumour forms, four were p53 negative, and 15 of the were p53 positive.

Positive p53 status was present in 52.4% of tumours. Expression of p53 was absent in 47.6% of the tumours and does not correlate with the macroscopic appearance and manner of tumour growth. Expression levels of p53 do not have a diagnostic, predictive and prognostic potential, while its importance is undeniable in the understanding of oncogenesis. Acta Medica Medianae 2011; 50(3):22-26.

Key words: colorectal cancer, p53, correlation, macroscopic appearance, growth pattern
errors in the duplication of genetic material) as well as controlling the cell cycle, proliferation and differentiation. Also, when it comes to the accumulation of genetic errors, p53 allows apoptosis, which is why it is known as „guardian of the cell genome”. The data obtained as a result of various clinical and basic studies suggest that damage or mutation of p53 leads to gene amplification of p53 and loss of control of cell cycle and apoptosis. The level of these changes has reflected the degree of unfavourable prognosis in patients, the very reason that tumours with over-expression of p53 have shown resistance to radiation and most chemotherapeutics (7).

The objective of this study was to determine the possible correlation between the morphological parameters and the expression levels of p53 and identification of possible prognostic significance of this correlation. In accordance with the basic objective, the following specific tasks have been set:

1. Examination of p53 expression at the operational material-resectioned segments from patients with colorectal carcinoma.
2. Correlation between p53 expression with the macroscopic appearance and manner of tumour growth.
3. Detection and identification of p53 tumour suppressor gene expression levels as an important prognostic factor for further progress and outcome of disease and the data for the choice of suitable, optimal, target, individual anti-tumour therapy.

Materials and methods

The study was implemented as a prospective, clinical and experimental study. We used a postoperative material obtained by resection of the colorectal tumours of 63 patients of both sexes, from the Surgery Clinic of Clinical Centre in Kragujevac. In order to obtain as much relevant histopathological data, the routine haematoxylin - eosin (HE) and immunohistochemical methods were performed at the Centre for Pathological-Anatomical Diagnosis of Clinical Centre “Kragujevac” in Kragujevac. Routine HE method was used for pathohistological 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 (8-10).

Immunohistochemical methods were used to identify the expression of antigen in colorectal cancer resection sample. The procedure for immunohistochemical staining included the unmasking of antigens, blocking of endogenous peroxidase, incubation with primary antiserum preparation and the procedure of immunohistochemical methods - LSAB+ - HRP (11).

For detection of p53 murine monoclonal antibody was applied (DAKO p53 DO-7 monoclonal mouse clone, 1:200) and highly sensitive specific streptavidin - biotin immunohistochemical method (LSAB+/HRP, DAKO, Denmark), in which the streptavidin is labelled with peroxidase and as a chromogen 1,3 – diaminobenzidine was used . As a general solvent of antiserum and rinsing solution between the different steps in the immunohistochemical staining procedure 0.1 M phosphate buffer pH 7.4 was used. Cell nuclei were stained with Mayer’s haematoxylin.

Cut off is the selected threshold value to distinguish positive and negative expression of p53. Cut off in this study to assess positivity was > 30% (score of overexpression if more than 30% tumour cells showed immunoreactivity for p53). Scoring system is based on determination of the percentage of immunoreactive nuclei of tumour cells as well as the intensity of immunoreactive staining. Adding points for the percentage of immunoreactivity and intensity gives the total, maximum possible points for the evaluation of expression (Table 1).

<table>
<thead>
<tr>
<th>% IMMUNOREACTIVE NUCLEI</th>
<th>INTENSITY OF IMMUNOREACTIVE STAINING</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 = &lt;5% stained nuclei</td>
<td>0 = no nuclei stained</td>
</tr>
<tr>
<td>1 = 5 do 30% stained nuclei</td>
<td>1 = poor intensity of nuclei staining</td>
</tr>
<tr>
<td>2 = 30 do 50% stained nuclei</td>
<td>2 = moderate intensity of nuclei staining</td>
</tr>
<tr>
<td>3 = 50 do 70% stained nuclei</td>
<td>3 = very intensive staining</td>
</tr>
<tr>
<td>4 = 70 do 90% stained nuclei</td>
<td></td>
</tr>
<tr>
<td>5 &gt; 90% stained nuclei</td>
<td></td>
</tr>
</tbody>
</table>

Immunohistochemical staining was carried out with the control of quality and specifics 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 SPSS software package and methods of descriptive statistics were used (frequencies, percentages, median, and percentiles). Testing according to 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 regression multivariant analysis.

**Results**

Nuclear expression of p53 was present in 33/63, i.e. in 52.4% (grade of positivity for more than 30% tumour cells showing immunoreactivity for p53). In 30/63 i.e. 47.6% had no expression of p53 (Table 2).

**Table 2. Expression of p53**

<table>
<thead>
<tr>
<th>P53 expression</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>33</td>
<td>52.4</td>
</tr>
<tr>
<td>Positive</td>
<td>30</td>
<td>47.6</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100.0</td>
</tr>
</tbody>
</table>

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 3).

**Table 3. Macroscopic manifestation of tumour**

<table>
<thead>
<tr>
<th>Macroscopic manifestation of tumour</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exophytic</td>
<td>18</td>
<td>28.6</td>
</tr>
<tr>
<td>Ulcerating</td>
<td>9</td>
<td>14.3</td>
</tr>
<tr>
<td>Infiltration</td>
<td>36</td>
<td>57.1</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The results show that three tumours of infiltrative forms were p53 negative and 17 of them were p53 positive. In tumours with ulcerating form, eight were p53 negative, and 16 of them were no p53 positive tumours. In vegetative tumour forms, four tumours were p53 negative, and 15 of the were p53 positive (Table 4).

**Table 4. Correlation of macroscopic manifestation and expression of p53**

<table>
<thead>
<tr>
<th>Macroscopic manifestation of tumour</th>
<th>P53-</th>
<th>P53+</th>
<th>Percentage (%) -</th>
<th>Percentage (%) +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative form</td>
<td>4</td>
<td>15</td>
<td>21.05</td>
<td>78.95</td>
</tr>
<tr>
<td>Ulcerating form</td>
<td>8</td>
<td>16</td>
<td>33.33</td>
<td>66.67</td>
</tr>
<tr>
<td>Infiltrative form</td>
<td>3</td>
<td>17</td>
<td>15</td>
<td>85</td>
</tr>
</tbody>
</table>

Macroscopic manifestation of the tumour and p53 are independent (p=0.104).

In order to obtain reliable parameters to examine the various factors that could affect the expression of p53, binary logistic regression was performed.

The presented results showed that p53 is not dependent on clinical and histological parameters (Figure 1-4).
Discussion

Colorectal cancer (CRC) is one of the most common causes of morbidity and mortality in both western world and our region. Classifications and established prognostic parameters, which are today 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 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 tumour (12-14).

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 would complement them, mostly in terms of recognizing the response to the therapy (genetic changes), or in terms of application in improving the screening of high-risk categories to allow for timely and successful treatment (15).

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 that nuclear expression of p53 was present in 33 cases or 52.4% of the total, with a rating of positivity evaluated as p53 expression in more than 30% of tumour cells. In 30 samples, or 47.6% of the total number of analyzed CRC there was no p53 expression. The data are consistent with data from the literature on the expression of p53 markers in colorectal cancer, according to which the normal p53 expression in all carcinomas is 45-70%, while the expression of this marker in colorectal carcinomas is 42-67% (16, 17).

The results of various studies suggest that, in regards to prognosis, it is not sufficient to follow only the expression of p53, but it is necessary to monitor the expression of other tumour markers, whose behaviour can also lead to better therapeutic approaches for the treatment of malignant tumours (18-21).

P53 expression does not depend on the macroscopic manifestations of tumours and tumour growth pattern, i.e., there is no correlation between p53 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.

Although the influence of macroscopic manifestation of the tumour on the p53 expression was not statistically significant (p=0.124), it was found that in ulcerating forms the possibility that p53 is positive is many times lower (odds ratio 0.032, p=0.017).

Conclusion

Expression of p53 was present in 52, 5% of the tumours and does not correlate with the macroscopic manifestation or the growth pattern of colorectal carcinoma.

Based on the aforesaid, it could be concluded that in previous studies the role of p53 as an important prognostic indicator has not been proven. Evaluation of the genes and molecular profiling can help identify groups of patients with overexpression of p53, which might imply a particular therapeutic intervention and an important therapeutic target for screening purposes.

Expression level of p53 does not have diagnostic, predictive and prognostic potentials, while its importance is undeniable in the understanding of oncogenesis.

References

KORELACIJA EKSPRESIJE P53 TUMOR SUPRESOR GENA SA MAKROSKOPSKOM MANIFESTACIJOM I NAČINOM RASTA KOLOREKTALNIH KARCINOMA

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 bio je ispitivanje ekspresije p53 kod kolorektalnog karcinoma kao i komparacija nivoa ekspresije p53 sa makroskopskim izgledom i načinom rasta tumora, zatim detekcija i identifikacija nivoa ekspresije p53 kod kolorektalnog karcinoma u pacijentima, objašnjenje za izbor adekvatne terapije.

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

Dobijeni rezultati su pokazali da se najčešći broj tumora, 36/63, tj. 57.1%, manifestovao u vidu infiltrativne forme, 9/63, tj. 14.3% kao ulcerišća i posivi, a 18/63, tj. 28.6% tumora imalo je polipoidni - egzofiti izgled.


Ključne reči: kolorektalni karcinom, p53, korrelacija, makroskopski izgled, način rasta