RELATIONSHIPS BETWEEN INSULIN-LIKE GROWTH FACTOR I AND SELECTED CLINICO-MORPHOLOGICAL PARAMETERS IN COLORECTAL CANCER PATIENTS

ADAM KUKLIŃSKI¹, ZBIIGNIEW KAMOCKI¹, DARIUSZ CEPOWICZ¹, MARIUSZ GRYKO¹, JOLANTA CZYŻEWSKA³, KRYSZYNA PAWLAK², BOGUSŁAW KĘDRA¹

¹2nd Department of General and Gastroenteral Surgery
   Kierownik: prof. dr hab. B. Kędra
   Department of Nephrology and Transplantology with Dialysis Unit²
   Kierownik: prof. dr hab. M. Myśliwiec
   Department of Clinical Laboratory Diagnostics³
   Kierownik: prof. dr hab. H. Kemona
   (Medical University in Białystok)

Insulin Like Growth Factor (IGF I) as the one of the strongest growth factors which can affect cancers development including colorectal cancer. IGF I induces processes of the cells growth and division. It regulates cells cycle and inhibits apoptosis. There is limited data about correlation between IGF I and staging of the tumor.

The aim of the study was estimation of the clinical usefulness of IGF I concentration in the serum of the patients with colorectal cancer.

Material and methods. We have examined 125 individuals with colorectal cancer. The age range was 36 to 92 years. They have been operated in the 2nd Department of The Gastrointestinal Surgery of the Medical University in Białystok. Serum concentration of the IGF I have been estimated using immunoassay ELISA before and after operation. Correlation between serum level of IGF I and clinico-pathologic features: age, gender, localisation of the primary tumor, TNM stage of tumor, histological type and histological grade (G) of the cancer have been estimated.

Results. Our study revealed statistically significant increased serum concentration of IGF I in patients with locally advanced colorectal cancer (pT3 and pT4) comparing to less advanced (pT2) The investigations showed higher serum concentration of IGF I in patients with poorly differentiated cancers (G3) than in moderately differentiated. Similarly higher serum concentration of IGF I were found in male, in patients older than 60 years and in mucigenous colorectal cancers.

Conclusions. Our results indicated that IGF I can be one of the factors of the prognosis in colorectal cancer development.

Key words: IGF I, colorectal cancer, clinico-morphological parameters, surgery.

Colorectal cancer is the third most common malignancy diagnosed in the United States (1). Surgical resection of this malignancy leads to a 94% survival rate in patients in whom proliferation is limited to the intestinal wall (stage A according to Dukes) and a 2.4% survival rate in patients with advanced disease (Dukes D) (2).

Cancer markers that are currently in use are not effective in the screening of asymptomatic individuals for colorectal cancer (3). Some hope may lay with a group of insulin-like growth factors (IGFs) that induce cell proliferation and growth, regulate the cell cycle and inhibit apoptosis (4). According to some studies, IFG II may serve as a diagnostic marker for colorectal adenomas (1, 5). The IGF system is comprised of two types of receptors (IGF IR and IGF IIR), two polypeptides (IGF I and IGF II) and six IGF-binding proteins (IGFBP) (6, 7).

The system is responsible for the growth and proliferation of cells. IGF I mostly medi-
ates the effects of growth hormone. Moreover, it stimulates amino acid uptake, protein synthesis and glucose metabolism. IGF I is a mitogene, cell cycle regulator, and an inhibitor of apoptosis (4). This factor also induces proliferation and growth of either normal or neoplastic cells. Increased IGF I concentrations were revealed in lung, breast, colon, prostate, liver and adrenal cortex cancers, Wilms's tumors, gliomas, sarcomas, and pheochromocytomas (8-15). Isolated from serum or tumor stroma, IGF I was able to stimulate the growth of cancer cell lines (16).

Manousos et al. revealed that increased plasma levels of IGF I and IGF II are associated with an elevated risk of colon cancer (17). In other studies, an increase in IGF I along with a decrease in IGF-binding protein BP3 were observed in colon cancer patients (7, 18, 19). On the other hand, other clinical studies did not reveal significant associations between serum concentrations of IGF I or IGF BP and the risk of colon cancer (20).

The purpose of this study was to analyze IGF I concentrations in colorectal cancer patients before and after curative surgery along with correlations between these parameters and some clinicopathological findings, i.e.: 1) TNM stage of tumor, 2) histological type of cancer, 3) tumor histological grade, and 4) location of primary tumor.

MATERIAL AND METHODS

Material

This study included 125 patients with primary colorectal cancers who were treated surgically at the 2nd Department of General and Gastroenterologic Surgery, Medical University in Białystok (Poland) between 1998 and 2003.

The study group comprised of 74 (59.2%) males and 51 (40.8%) females aged from 36 to 92 years of age (mean 66.1 years). Based on their ages, the patients were divided into two categories: subjects up to the 60th year of life (n=32, 24.6%) and those older than 60 years of age (n=93, 74.4%). Depending on the location of the primary tumor, the study group comprised of rectal cancer patients (n=59, 47.2%), and colon cancer subjects (n=66, 52.8%). Histopathology revealed that the study group included 100 (80%) adenocarcinomas, 11 (8.8%) mucinous adenocarcinomas, 10 (8%) partially mucinous adenocarcinomas, 2 (1.6%) undifferentiated adenocarcinomas, along with 1 (0.8%) ulcerative adenocarcinoma, and 1 (0.8%) signet ring cell carcinoma.

The stage of disease was classified based on histological criteria by WHO and TNM clinicopathological classification. No T1 subjects were found in the group studied. Stage T2, T3 and T4 tumors were revealed in 9 (7.2%), 93 (74.4%), and 23 (18.4%) patients respectively. Lymph node involvement (group N0 according to TNM) was absent in 62 (49.6%) patients. N1, N2 and N3 subjects comprised 31 (24.8%), 28 (22.4%), and 4 (3.2%) cases respectively. Distant metastases (M1 according to TNM) were found in 11 (8.8%) subjects. No distant metastases (M0) were observed in the remaining 114 (91.2%) patients. No cases of highly differentiated tumors (grade G1) were found in the studied subjects. Moderately differentiated tumors (G2) were found in 82 (65.6%) patients, and poorly differentiated ones (G3) in 43 (34.4%) subjects.

All patients were treated surgically. Abdominoperineal resection of the rectum according to Miles was performed in 26 (20.8%) subjects, along with 33 (26.4%) anterior resections of the rectum with end-to-end anastomoses, 36 (28.8%) sigmoid resections, 1 (0.8%) Hartman surgery, 20 (16%) right hemicolectomies, 6 (4.8%) left hemicolectomies, and 3 (2.4%) partial resections of the transverse colon.

The control group comprised of 24 healthy volunteers, with a mean age of 64 years, with no diagnosed cancer, metabolic disorders and/or malnutrition.

Methods

Serum IGF I concentrations were determined using ELISA. Blood was collected from the basilic vein directly before the surgery and 11 days thereafter. After collection, the blood was left for 30 minutes at room temperature in order to obtain clotting and subsequently centrifuged for 15 minutes in a temperature of 4°C at 1000 x g. The obtained supernatant was stored at -80°C.

The commercial ELISA kit from R&D System (USA) was used for serum IGF I determination following the manufacturer's instructions. The absorbance was measured using a
spectrophotometer at the 450 nm wave length. Results were determined based on a calibration curve and presented in ng/ml.

The histological type and grade (G) of the tumor, along with the clinicopathological stage were analyzed using routine histopathological examination.

The results were subjected to statistical analysis. The patient’s gender and age, along with histological type of tumor, its location and histopathological stage were used as grouping variables.

Two age groups were distinguished amongst the patients: individuals up to and over 60 years of age. Referring to the histological type of tumor, analysis included only adenocarcinoma and mucinous adenocarcinoma patients since the frequency of other types of tumors was too low. Based on tumor location, patients were divided into rectal cancer and colon cancer subjects. Based on the pT parameter of the TNM classification system, patients were divided into pT2, pT3 and pT4 groups. No pT1 cases were found in the sample subjects. Based on pN and pM parameter distributions, the patients were divided into those with and those without metastases, designated as pN+ and pN- respectively. Depending on the histological tumor grade, only G2 and G3 groups were distinguished due to the lack of highly differentiated tumors (G1) in the studied subjects.

Mean preoperative and postoperative IGF I concentrations were compared between subgroups distinguished based on age, gender, tumor location, histological type, clinicopathological stage, pT, pN and grade using the Mann-Whitney U test. Wilcoxon test was used for comparisons between mean preoperative and postoperative IGF I values. Spearman’s coefficients of correlation were calculated in the entire study group as well as within the subgroups distinguished, based on patients’ genders and ages, tumor location, histological type, grade and clinicopathological stage.

Calculations were performed using the SPSS 8.0 PL package, with statistical significance defined as p≤0.05.

### RESULTS

Before surgery mean serum IGF I concentration in the entire group of colorectal cancer patients amounted to 79.98±37.39 ng/ml and did not differ significantly when compared to the control group.

No significant decrease in serum IGF I (mean 76.04 ng/ml) was observed 10 to 12 days post surgery (tab. 1).

A significant positive correlation between IGF I concentrations before and after surgery was observed in the entire group of colorectal cancer patients (r=0.52; p<0.001; tab. 1)

#### IGF I and patient’s age

Significantly higher serum IGF I concentrations were noted in subjects below 60 years of age (105.3±47.5 ng/ml) when compared to patients over 60 years of age (72.3±29.8 ng/ml, p<0.001).

Post surgery, IGF I decreased in both age groups, being significantly higher amongst patients up to 60 years (88.12±28.74 ng/ml) when compared to subjects over 60 years of age (71.3±26.3 ng/ml, p<0.01; fig. 1).

A significant negative correlation between serum IGF I and patient’s age was observed before and after surgery (r=-0.36, p<0.001, and r=-0.32, p<0.001, respectively).

#### IGF I and patient’s gender

Significant differences in IGF I concentrations were also noted depending on the pa-
IGF I and clinical parameters in colorectal cancers

Patient’s gender. Both before and after surgery, serum IGF I was significantly higher in males. Before surgical intervention, mean IGF I concentrations in males amounted to 86.1±31.5 ng/ml, compared to 71.0±43.4 ng/ml in females (p<0.001). The parameter decreased postoperatively to 81.9±27.7 ng/ml and 67.6±26.0 ng/ml in men and women respectively, all the while being significantly higher in males (p<0.01; fig. 2).

IGF I and tumor location

Tumor location did not significantly influence IGF I concentrations. Preoperative values of this parameter were slightly higher in rectal cancer patients (78.3±30.5 ng/ml) compared to subjects with colon tumors (81.5±42.8 ng/ml) but this difference proved insignificant.

IGF I concentrations decreased slightly post surgery in both the groups and amounted to 74.5±27.9 ng/ml and 77.4±27.9 ng/ml for rectal and colon tumors respectively. This difference was statistically insignificant (fig. 3).

IGF I and histological type of tumor

Preoperatively, IGF I concentrations were higher, although insignificantly, in patients with mucinous adenocarcinomas (96.9±60.7 ng/ml) when compared to adenocarcinoma subjects (76.4±29.4 ng/ml). After surgery, IGF I concentrations decreased slightly and were similar for both of the groups (fig. 4).

IGF I and the clinicopathological stage of tumor

Preoperative IGF I levels were found to decrease with the clinical stage of the tumor, amounting to 67.48±11.37 ng/ml, 79.85±40.06 ng/ml, and 85.18±32.51 ng/ml for pT2, pT3 and pT4 cases respectively. All differences were statistically insignificant. Postoperatively, a decrease in mean IGF I concentration was observed in pT2 and pT4 patients, whereas the value for pT3 patients was similar to the one
determined before surgery. Postoperative IGF I concentrations differed significantly when group pT2 values were compared to pT3 and pT4 groups (fig. 5).

Preoperative concentrations of IGF I were higher in patients without metastases involvement (83.3±39.57 ng/ml) compared to metastases positive subjects (76.51±34.96 ng/ml), but this difference proved insignificant. After surgery, IGF I concentrations in both the groups decreased to similar levels (fig. 6).

Both preoperatively or postoperatively, IGF I concentrations in patients with poorly differentiated tumors (G3) were higher than in subjects with moderately-differentiated cancers (G2). However, these differences remained insignificant. Postoperatively, serum IGF I decreased in both of the groups and the difference was close to the significance threshold (fig. 7).

**DISCUSSION**

This study showed no significant differences in serum IGF I concentrations in patients before surgery compared to controls. Glass and Atiq also did not find associations between increased IGF I concentrations and colorectal cancer. These studies, however, included small groups, comprised of at most of 30 subjects. Blood samples were obtained upon diagnosis of cancer (21, 22).

The study by Palmqvist et al. proved that cancer risk increases along with IGF I concentrations only for colon cancer. Similar relationships were not observed for rectal cancers, where an inverse correlation was found between cancer risk and serum IGF I concentrations (23).

Probst-Hensch et al. did not observe significant associations between the risk of cancer and elevated serum IGF I concentrations in patients diagnosed with colorectal cancer (24). In a cohort study by Nomura et al. on 9 345 males, including 177 colon cancer patients and 105 rectal cancer subjects, a slight increase in IGF I was observed amongst colon cancer patients when compared to the control group. No differences however, were noted in serum IGF I concentrations in rectal cancer patients when compared to controls. According to their hypothesis, the authors believed that the lack of significant differences in the studied parameters resulted from other than clinical factors which may have had an influence on serum IGF I concentrations in colorectal cancer pa-
IGF and clinical parameters in colorectal cancers

In the aforementioned study, serum IGF was determined 20 years after blood collection. However, no studies on the stability of frozen serum IGF I have been performed thus far (19).

In our study, IGF I concentrations differed between patients with rectal cancer compared to subjects with other parts of the colon involved. Similar to Nomura, higher concentrations were found in subjects with colon cancer, but this difference was insignificant.

Several prospective studies were published recently, dealing with the prognostic role of increased IGF I concentrations in the detection of colorectal cancer. They revealed that significantly higher IGF I concentrations were noted in subjects with colorectal adenomas (25, 26, 27).

According to Hassan and Macaulay, individuals with an elevated risk of colorectal cancer may be identified from amongst the healthy population based on higher serum IGF I concentrations (28).

The generally accepted theory on an association between colorectal cancer development and increased serum IGF I concentrations was confirmed by in vitro and animal studies as well as by prospective studies on patients with colorectal adenomas (25, 26, 27).

Published clinical research on IGF I in colon cancer patients is scant. Wei et al. performed a clinical study on 32 862 female students in medical school. A total of 182 colorectal cancer cases were identified in this group and compared to 350 healthy controls in terms of concentrations of IGF system components. This study revealed that increased serum concentrations of IGF I, along with decreased level of IGF I binding protein (IGF BP3), were associated with a higher risk of colorectal cancer (29).

A study of 200 women from New York revealed slight positive association between high concentration of IGF I and colorectal cancer risk (20).

Similar but statistically insignificant associations were noted in a small (n=41) cohort of colorectal cancer patients from Greece (17).

Our results did not confirm the widely adopted hypothesis that IGF I concentrations in colorectal cancer patients are higher compared to healthy individuals. In this study, IGF I concentration determination was performed on blood collected upon diagnosis of colorectal cancer. Moreover, we cannot exclude the influence of other factors which may have increased IGF I concentrations in the control group. According to Ma et al., these factors include diabetes, hyperlipidemia, cigarette smoking, obesity and alcohol consumption (30).

In this study, IGF I concentrations were determined either before or after curative surgery. No significant differences in IGF I were found between these two time points although the postoperative concentrations were slightly lower compared to the preoperative ones. No similar comparisons of IGF I concentrations were published in literature thus far.

The plasma half-life of IGF I amounts to around 15 hours (4). In our study, IGF I was determined 11 days post surgery, guaranteeing a decrease in the pool of IGF I potentially synthesized by a tumor in an autocrine manner. Based on our results, only a slight fraction of IGF I seems to originate from autocrine secretions in advanced colorectal malignancies (over 80% of a sample studied comprised of Dukes B and C tumors). Consequently, most of the IGF I measured in this study belonged to the endocrine pool of this factor. This finding raises possibility of using pharmacologic modulation of the Growth Hormone – IGFs axis in cancer prevention and treatment (7).

It is of interest whether autocrine IGF I secretions decrease along with tumor growth. Some of the previously mentioned studies revealed increased IGF I concentrations in adenoma patients. Hence, it is likely that carcinogenesis is induced solely by an endocrine pool of IGF I since elevated blood levels of this factor were observed before cancer detection.

Our study revealed that IGF I concentrations were significantly higher in patients below 60 years of age when compared to subjects over 60 years of age. Age-related changes in IGF I concentrations were described in detail by Hall et al. Levels of IGF I start to increase from the 6th month of life while growth is continued up to puberty with a slight decrease thereafter. Then the concentration remains relatively constant to decrease again along with age (4).
Clinical studies on Japanese males revealed inverse correlations between IGF I and IGF BP3 concentrations and patient age (27).

In our study, IGF I concentrations were significantly higher in males than in females. According to Clemmons, both estrogens and androgens modulate somatomedin concentrations with similar impact (31). A small number of clinical research papers published on gender-related differences in IGF I levels do not allow for any further comparisons with our study findings. Most of the published research deals with patient groups homogenous in terms of gender. Moreover, gender-related differences in IGF I concentrations found preoperatively and postoperatively in our study may have resulted from different body masses of the male and female patients.

In this study, preoperative or postoperative IGF I concentrations were higher in subjects with advanced colorectal cancers (pT3 and pT4) and with poorly differentiated tumors (G3). Significant differences were found in preoperative IGF I levels in pT3 and pT4 subjects compared to pT2 patients. No increase in IGF I concentration was noted however in patients with metastases. The amount of clinical research published on IGF I concentrations and the clinical features of colorectal cancers is limited, further hindering any possible comparisons with our results.

Michell et al. observed that IGF II expression is associated with the Dukes stage of colorectal cancer. These authors hypothesized on the possibility of a similar relationship existing in the case of IGF I (32).

Bauer et al. confirmed in vitro that an elevated IGF concentration is reflected with increased migration and invasion of neoplastic cells (33). Consequently, higher concentrations of IGF I could be expected in patients with higher-stage, i.e. more invasive, colorectal cancers.

Wu et al., in their study on cecal cancer in mice, observed an association between increased synthesis of VEGF or angiogenesis itself and elevated blood concentrations of IGF I. Based on these findings, the aforementioned authors suggested that increased serum concentrations of IGF I may stimulate tumor growth and metastasis formation with the resulting correlation between serum IGF and tumor stage (15).

According to the previously mentioned scores IGF I determination could serve as an effective tool in the early identification and management of patients with more aggressive colorectal cancers. However, similar to the findings of other authors, we have not found marked differences in IGF I concentrations in subjects already diagnosed with cancer when compared to the control group.

CONCLUSIONS

1. No significant differences in IGF I concentrations were observed in colorectal cancer patients preoperatively and postoperatively when compared to healthy controls. Consequently, serum IGF I does not seem to be a sufficiently sensitive marker of already existing neoplastic process.

2. Since higher concentrations of IGF I were found in patients with more aggressive tumors (low-grade tumors and mucinous adenocarcinomas) and higher clinical stages of neoplastic processes, this marker could be used for identification of subjects with worse prognosis and their qualification to more aggressive therapy.

REFERENCES


Received: 6.04.2011 r.
Adress correspondence: 15-276 Białystok, ul. M. Skłodowskiej-Curie 24a