Usefullness of Serum Insulin-Like Growth Factor (IGF-1) and Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) for Colorectal Cancer Diagnosis

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ABSTRACT

Insulin-like growth factor (IGF-1) is a potent mitogen and an anti-apoptotic agent which is regulated by insulin-like growth factor binding protein-3 (IGFBP-3). IGFBP-3 carries IGF-1 through the bloodstream to the target tissues and protects it from proteolytic degradation. IGFBP-3 reduces tumorigenic potential of colon cancer cells in vitro. IGFBP-3 may inhibit target cells directly. In the present study, we assessed whether serum levels of IGF-I and IGFBP-3 could be used as tumor markers in patients with colorectal cancers and evaluated its relationship with known tumor markers, carcinoembryogenic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9).

Our study enrolled 40 patients diagnosed with colorectal cancer histopathologically and 40 completely healthy, age-matched subjects (control group). Serum IGF-I levels were found significantly higher in patients with colorectal cancers compared to control group ($p< 0.01$). Serum IGFBP-3 levels were significantly lower in patients with colorectal cancers compared to control group ($p< 0.05$). The observed increase in CEA and CA19-9 levels in the same subjects were found statistically significantly higher compared to control group ($p< 0.05$). However, no significant association could be found between change in IGF-1 and IGFBP-3 levels and CEA or CA 19-9. Increased serum levels of IGF-I might be considered as a significant predictor of tumor presence in patients with suspected colorectal cancer. Agents that could increase IGFBP-3 levels might be promising for the treatment of colorectal cancers.

Key Words: Colorectal cancer, IGF-I, IGFBP-3, CEA, CA 19-9

ÖZET

Kolorektal Kanser Tanısında İnsülin Benzeri Büyüme Faktörü-1 (IGF-1) ve İnsülin Benzeri Büyüme Faktör Bağlayıcı Protein-3 (IGFBP-3)'ün Kullanılabilirliği

İnsülin benzeri büyüme faktörü-1 (IGF-1), etkisi insülin benzeri büyüme faktör bağlayıcı protein-3 (IGFBP-3) tarafından düzenlenen, potent bir mitojen ve antiapoptotik ajandır. IGFBP-3, IGF-1'i dolaşımda taşır, hedef dokulara yönlendirir, onu proteolitik degradasyondan korur. IGFBP-3 in vitro şartlarda kolon kanser hücrelerinin tümörojenik potansiyellerini azaltır. IGFBP-3 hedef hücreleri doğrudan inhibe edebilir.

Çalışmamızda kolorektal kanserli hastalarda IGF-I, IGFBP-3'ün tümör markırı olarak kullanılıp, kullanılamayacağı ve bilinen tümör markırlarından karsinoembriyonik antijen (CEA), karbonhidrat antijeni 19-9 (CA19-9) ile olan ilişkisi incelendi. Çalışmamıza histopatolojik olarak kolorektal kanser teşhisi konulmuş 40 hasta ile, kontrol grubu olarak benzer yaş grubundan ve tamamen sağlıklı 40 kişi dahil edildi.

Kontrol grubu ile kıyaslandığında kolorektal kanserli hastalarda IGF-I düzeyi anlamlı derecede yüksek bulundu ($p< 0.01$). IGFBP-3 düzeyleri kolorektal kanserde kontrol grubu ile kıyaslandığında anlamlı derecede düşük bulundu ($p< 0.05$). Yine aynı vakalarda çalışılan CEA ve CA19-9 düzeylerinde gözlenen artış kontrol grubu ile kıyaslandığında istatistiki açıdan anlamlı olarak yüksek bulundu ($p< 0.05$). Fakat IGF-1 ve IGFBP-3 düzeylerindeki değişimi ile CEA ve CA 19-9 arasında anlamlı ilişki tespit edilemedi. Sonuç olarak, IGF-I düzeyindeki artış, kolorektal kanser şüphesi olan hastalarda tümör varlığı yönünden anlamlı bir gösterge olarak kabul edilebilir. IGFBP-3 düzeyini artışına neden olabilecek ajanlar kolorektal kanser tedavisinde yeni ufuklar açabilir.

Anahtar Kelimeler: Kolorektal kanser, IGF-I, IGFBP-3, CEA, CA 19-9

INTRODUCTION

Despite recent breakthrough advances in cancer therapy, about half of malignant diseases are diagnosed when clinical symptoms arise or when they reach a stage when curative therapy is no longer possible because of metastatic condition. Tumor markers are used for diagnostic purposes, for evaluation of response to treatment and for early detection of recurrences in clinical oncology.[1,2]

Tumor determinants might be quantitatively measured in patient's tissue, blood or other body fluids by using biochemical or immunohistochemical methods. These include hormones, enzymes, metabolites, immunoglobulins, several proteins, tumor associated antigens, oncogens and substances that contains oncogen products.[3] IGF system including Insulin-like growth factors (IGF-I ve IGF-II), insulin-like growth factor receptors (IGF-IR ve IGF-IIR) and insulin-like growth factor binding proteins (IGFBPs) plays an important role in epithelial growth, anti-apoptosis and mitogenesis.[4] IGF-1 is a potent mitogen and anti-apoptotic agent which is regulated by IGFBP-3. IGFBP-3 carries IGF-1 through the bloodstream to the target tissues and protects it from proteolytic degradation and regulates its interaction with IGF-IR. IGF-1 prevents cell death by modulating the expression of apoptotic Bcl and Bax proteins. In addition, IGFBP-3 may exert an apoptotic effect by interacting with specific cell surface receptors independently of IGF.[5,6] An increase in p-53 dependent apoptosis within 24 hours of DNA damage has been observed in colonic epithelium induced by gamma radiation in the presence of IGFBP-3. [7]

IGF-IR mediates the growth promoting effect of IGFs on cancer cells via its tyrosine kinase feature.[4] IGFBP-3 activates phosphotyrosine phosphatase which dephosphorylates IGF-IR by impairing cellular signalling and shows an inhibitory effect.[8]

IGF receptors mediate all known proliferative effects of IGF-I and IGF-II. While in vitro studies and animal models support the functional importance of IGF-IR for cancer cell growth, clinical and prognostic value of IGF-IR expresion levels for human malignancies is yet unknown.[8,9]

CEA is a glycoprotein produced by epithelial cells secreting mucine in the fetus. Normally it is an oncofetal antigen synthesized during embryonic and fetal life.[10] CEA was first shown in colon adenocarcinoma and reported to be absent in normal adult tissues.[11-13] CA 19-9 was first detected in patients with colorectal cancer and has been a marker very widely used by many clinics for detecting metastasis and recurrences in gastrointestinal system cancers and for monitoring treatment response.[14,15] In our study, we assessed whether IGF-I and IGFBP-3 could be used as tumor markers in patients with colorectal cancers and evaluated its relationship with other tumor markers CA19-9 and CEA.

MATERIAL AND METHOD

Our study enrolled 40 patients who admitted to the Internal Medicine outpatient clinics with complaints of weight loss, change in defecation routine and bloody stools and histopathologically diagnosed with colorectal cancer as a result of assessments. Control group comprised 40 age-matched healthy subjects with no infectious or systemic diseases by clinic and laboratory analyses, without a history of use of any drugs that could affect IGFI and IGFBP-3 levels.

All patients were evaluated by imaging methods (ultrasonography, computed tomography). All of

our patients have been histopathologically diagnosed with recent adenocarcinoma, with no prior surgery, chemotherapy or radiotherapy.

Serum IGF-I and IGFBP-3 Analyses

Blood samples drawn into nonadditive vacutainer tubes were centrifuged at 3500 g at 4°C for 10 min, and sera were stored at -80°C until analysis. Serum IGF-I and IGFBP-3 concentrations were measured using a two-site coated tube immunoradiometric assay (IRMA; Diagnostics Systems Laboratories, Webster, TX, USA). Assays were performed according to the manufacturer's instructions. These assays are based on noncompetitive IRMA in which the analyze to be measured is sandwiched between two antibodies. The IGF-I IRMA has a minimum detection limit of 2.06 ng/ml and intra- and interassay coefficients of variation of 4.5 and 5.2%, and the IGFBP-3 IRMA has a minimum detection limit of 0.5 ng/ml and intra- and interassay coefficients of variation of 3.2 and 3.8%, respectively.

CEA levels were analysed by using chemiluminimetric method (IMMULITE-2000 DPC –Diagnostic-USA). The same method was applied for CA-19-9 levels (HITACHI E170 Tokyo-Japan).

Statistical Analysis

For statistical analysis of data, SPSS 11.0 for Windows statistical software. Non-parametric Mann Withney-U test was used because of the heterogenous nature of variants. Difference between group means was assessed by student's t test for significance. Data were expressed as means (±) standard deviation (X ± SD). p= 0.05 and lower values were considered significant.

RESULTS

Mean age of our cancer patients (32 males, 18 females) was 51.80±14.51 (28-75) and 53.96±12.71 (22-90) for control group (25 males, 15 females). Serum IGF-I levels were found significantly higher in patients with colorectal cancers compared to control group ($p< 0.01$). Serum IGFBP-3 levels were significantly lower in patients with colorectal cancers compared to control group ($p< 0.05$). Also, The observed increase in CEA and CA19-9 levels in the same patients with colorectal cancers were found statistically significantly higher compared to control group ($p< 0.05$). However, no significant association could be found between change in IGF-I and IGFBP-3 levels and CEA or CA 19-9 ($p> 0.05$) (Table 1).

DISCUSSION

Growth factors have drawn substantial attention because of their role in cancer development. Several oncogen products show similarity to growth factor receptors with respect to transmembrane tyrosine kinase activity. Also, it has been demonstrated that growth factors promote transcription of protooncogens that regulate transcription of other genes necessary for stimulation of cell replication.[16] Epidemiologic studies performed in various regions reported that increased serum levels of IGFs and decreased serum levels of IGFBP-3 could be associated with increased risk for several cancer types including premenopausal breast cancer, prostate cancer, lung cancer, colorectal cancer, endometrial cancer and gallbladder cancer.[8,17]

Surgery may provide cure for colorectal cancers if the disease could be detected at an early stage. However, metastasis has already developed in more than 40% of patients with colorectal cancer at the time of diagnosis. Currently, the association between IGFs and colorectal cancers is based on evidence from in vitro studies. IGFBP-3 reduces tumorigenic potential of colon cancer cells in vitro. In one study it has been shown that IGFBP-3 could induce early differentiation of Caco-2 cells and inhibit proliferation.[4]

IGF-I levels were found to be quite low in normal colonic mucosa. Studies have demonstrated that increased IGF expression is a marker that indicates growth potential of intestinal cells.[18] In their experimental studies on rats, Wu et al.[19] showed that IGF-I induced an increase in crpyt cell population, in addition to an increase in linear and cross-sectional mucosal and muscular layers of intestine.

In their study Giovannucci et al.[20] reported that IGF-I increased the risk of cellular transformation by increasing cell turnover via IGF-IR and they implied overexpression of IGF-I receptors in the neoplastic cells as an evidence for this indication. Mishra et al.[21] showed in their study that IGFBP-2 mRNA levels were increased in colon cancer by 4-8 times compared to control group. Thus, they indicated that IGFBP-2 may be an important marker for metastasis and prognosis of colon cancer.

Table 1. Parameters measured in patient and control groups: Mean values (X) of IGF-I and IGFBP-3 levels and their standard deviations (SD); Mean values (X) of CEA and CA19-9 and their standard errors (SE)

	Control	Colorectal cancer	p
Cases (n)	40	40	–
Age	53.80 ± 20.66	51.80 ± 14.51	–
IGF-I	129 ± 29.8	174.2 ± 50	p< 0.01
IGFBP-3	2144.9 ± 747	1722 ± 317	p< 0.05
CEA	1.8 ± 0.1	70.1 ± 31.2	p< 0.05
CA19-9	10.8 ± 0.9	108 ± 13.1	p< 0.05

Ma et al.[22] found that 193 patients among 14 916 patients with basal serum samples obtained developed colorectal cancer at the end of 14 years of follow-up. They have observed an increased risk for development of colorectal cancer in patients with increased levels of IGF-I compared to age-matched control group (p< 0.02). These researchers found a lower risk for development of colon cancer in patients with elevated IGFBP levels (p< 0.005).

Giovannucci et al.[23] found that 79 patients out of 32 826 patients with basal serum samples taken developed colorectal cancer at the end of 6 years of follow-up. They indicated that elevated serum IGF-I levels and low serum IGFBP-3 levels could be independent risk factors for developing colon cancer in comparison to control group. Manousos et al.[24] found in their case-control study that while IGF-I levels were directly proportional to colorectal cancer risk, IGFBP-3 levels were inversely proportional. They reported that increased IGF-I levels in circulation might be associated with risk of colorectal cancer, although there was not any statistically significant association between them. Similary, in our patients a statistically significant increase in IGF-I levels (p< 0.01) and a statistically significant decrease in IGFBP-3 levels were found compared to control group (p< 0.05). Our results were consistent with the results of Mishra[21], Ma[22], Giovannucci[23] and Manousos[24].

Forones et al.[25] found increased CEA levels in 42% of 74 patients with colorectal cancer and increased levels of CA-19-9 in 35% at the time of diagnosis. Relapse was observed in 33 patients and 73% of them were reported to have increased levels of CEA and/or CA19-9. They found increased levels of CEA and/or CA19-9 in 93% of patients with recurrence at first year and indicated that both elevated preoperative serum CEA and CA19-9 levels predicted increased cancer-related mortality. Nakayama et al.[26] found increased levels of CA19-9 (15%) and CEA (19.5%) among 264 patients with colorectal cancer. They reported that despite lower sensitivity of CA19-9 for early stages of colorectal cancer compared to CEA, combinations of CA19-9 and CEA were better in the detection of recurrences compared to CA19-9 or CEA alone.

Increase in CEA and CA19-9 levels in our patients was statistically significantly higher compared to control group (p< 0.05). Our results were consistent with studies in literature. However, neither the association of increased IGF-1 levels, nor decrease in IGFBP-3 levels with increased CEA and CA 19-9 levels was statistically significant (p> 0.05).

In conclusion, elevated IGF-I levels might be used as a tumor marker for early diagnosis of colorectal cancer. Interventions directed to antagonization of IGF-IR stimulation or promotion of IGFBP-3 function could result in new modalities for treatment of colorectal cancer. However we believe that more comprehensive studies are needed to elaborate this suggestion.

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