Prospective clinical study of the association between plasma level of free IGF-1 and myometrial invasion in patients with endometrial adenocarcinoma

Badanie prospektywne zależności pomiędzy poziomem wolnego insulinopodobnego czynnika wzrostu (IGF-1) w surowicy krwi a zaawansowaniem nowotworu u pacjentek z rakiem *endometrium*

Baloglu Ali, Bezircioglu Incim, Cetinkaya Burcu, Hicyılmaz Levent

1st Department of Obstetrics and Gynecology, Izmir Ataturk Training and Research Hospital, Izmir, Turkey

Abstract

Introduction: *Endometrial carcinoma is a common malignancy of the female genital tract. There is a strong correlation between myometrial invasion and clinical prognosis. Increasing myometrial penetration is associated with an increasing risk of pelvic and para-aortic lymph node metastasis, adnexal metastasis, positive peritoneal cytology, local vault recurrence, and hematogenous spread. The causal role for Insulin-like growth factor-1 and insulin in endometrial carcinogenesis is well supported and insulin and IGF system have mitogenic and antiapoptotic activity. Endometrial cancer cell lines express high-affinity insulin receptors, consistent with there being a direct biological effect of insulin and IGF system on the growth and myometrial invasion of endometrial cancer cells.*

Material and methods: *Patients with endometrial carcinoma have been divided into three groups: tumor confined to the endometrium (stage IA, n:24), endometrial carcinoma with a minimal invasion (less than 50% of the myometrium; stage IB, n:32), and the control group (n:40). Demographic factors, estradiol and free IGF-1 plasma levels have been compared in all groups.*

Results: *Lower Free IGF-1 plasma levels were found in patients with myometrial invasion when compared to the patients without myometrial invasion.*

Conclusions: *In the following work we have presented the current understanding of endometrial carcinoma, association between free IGF-1 plasma levels and myometrial invasion in patients with endometrial adenocarcinoma in terms of management and survival.*

Key words: **insulin-like growth factor-1** / **endometrial carcinoma** / / **myometrial invasion** /

Corresponding author:
Ali Baloglu
1st Department of Obstetrics and Gynecology, Izmir Ataturk Training and Research Hospital,
Izmir, Turkey
Talatpasa Street Number: 61/4 Alsancak, Izmir, Turkey 35220
Phone: +90 232 4224493, Fax: +90 232 4644969
e-mail: alibaloglu@yahoo.com

Otrzymano: **10.01.2010**
Zaakceptowano do druku: **15.06.2010**

Ginekol Pol. 2010, 81, 501-505

Streszczenie

Wstęp: *Rak trzonu macicy jest częstym nowotworem narządu rodnego. Istnieje zależność pomiędzy stopniem naciekania macicy a rokowaniem. Wraz z zaawansowaniem nacieku na ścianę mięśniową macicy wzrasta ryzyko wystąpienia przerzutów do węzłów chłonnych miednicy i okołoaortalych, nacieku na przydatki, wznów w obrębie sklepienia pochwy, obecności komórek nowotworowych w badaniach cytologicznych płynu z jamy otrzewnowej oraz rozsiewu komórek nowotworowych za pośrednictwem krwi.*

Insulina i insulinopodobny czynnik wzrostu (IGF-1) pobudzają komórki do rozrostu, mają właściwości antyapoptotyczne oraz są znanymi czynnikami ryzyka raka trzonu macicy. Jednocześnie komórki nowotworowe wywodzące się z endometrium wykazują wysoką ekspresję receptorów o silnym powinowactwie wobec insuliny.

Materiał i metodyka: *Pacjentki z rakiem endometrium podzielono na 3 grupy: chore z rakiem ograniczonym do endometrium (n:24), następnie pacjentki, u których naciek nie przekraczał ½ grubości ściany mięśniowej (n:32) i grupę kontrolną (n:40). U pacjentek analizowano czynniki demograficzne, badano poziom estradiolu i IGF-1 w surowicy krwi.*

Wyniki: *Poziomy IGF-1 w surowicy krwi były niższe u pacjentek z obecnym naciekiem ściany mięśniowej w porównaniu z chorymi z naciekiem ograniczonym do endometrium.*

Wnioski: *Przedstawiliśmy obecną wiedzę na temat raka trzonu macicy, zależności pomiędzy poziomem wolnego IGF-1 w surowicy krwi a stopniem naciekania na ścianę endometrium u pacjentek z rakiem endometrium, pod kątem postępowania i przeżycia chorych.*

Słowa kluczowe: **insulinopodobny czynnik wzrostu** / **rak *endometrium*** /
/ **naciekiem na ścianę mięśniową** /

Introduction

With a lifetime risk among women of 2-3%, endometrial cancer is the most common pelvic gynecologic malignancy in industrialized countries [1]. Approximately 75% of cases are diagnosed at an early stage, with tumor confined to the uterine corpus. Although most patients are cured by surgery alone, about 15-20% with no signs of locally advanced or metastatic disease at primary treatment recurs, with limited responsiveness to systemic therapy [2].

Prognostic factors in early stage endometrial cancer are histological type, tumor grade, and depth of myometrial invasion. Myometrial invasion is a significant prognostic factor in Stage I endometrial carcinoma as it defines the frequency of lymph node metastases, recurrence, and survival from disease. Superficially invading tumor carries a risk of 3-5% for pelvic and paraaortic lymph-node metastasis, while deeply invasive tumor increases it substantially to 17-25% respectively [3].

Obesity is a major risk factor for endometrial cancer. The relationship between obesity and endometrial cancer is due to elevated serum estradiol and insulin levels. IGF-1, which shares extensive amino acid sequence homology and downstream signaling pathways with insulin, has similarly garnered research interest as a potential risk factor for endometrial cancer, especially because insulin-like growth factor-1 (IGF-1) has much stronger mitotic and antiapoptotic activity than insulin [4]. Within endometrial tissue, IGF-1 may mediate the proliferative effects of estrogen. IGF-I are produced not only in the liver, by far the major source of the peptide in the circulation, but also by most other tissues, including the endometrium. IGF-I levels in the blood circulation mainly circulates bound to IGF-binding proteins (IGFBP). Only 1% of IGF-1 circulates free in the serum. However, as with estrogen, the free fraction may be the most biologically active form of IGF-1 [5].

IGF-1 and IGF-2 are expressed in the endometrial stromal cells, and their expression is associated with endometrial differentiation.

Higher levels of IGF-1 have been associated with an increased risk of a number of epithelial cancers [6]. Correlations between higher estrogen levels and IGF-1 expression and between higher progesterone levels and IGFBP-1 levels provide a potential link between the IGF system, the estrogen-progesterone balance, and endometrial cancer risk [7].

Epidemiological studies have shown evidence for a relationship between high circulating levels of insulin like growth factors and the increased risk of several cancers, including breast, colon, prostate, lung, cervix, endometrium cancer [8].

Objectives

We present the current understanding of endometrial adenocarcinoma, association between free IGF-1 plasma levels and myometrial invasion in terms of patient management and survival.

Materials and methods

The case control study was conducted on patients who were hospitalized and underwent surgery for endometrial adenocarcinoma at Gynecologic Oncology Department of Izmir Ataturk Training and Research Hospital, between 2006-2009. The control group consisted of patients from the same age group, with no history of cancer, who came to Outpatient Clinic due to benign gynecological diseases. Patients who were enrolled into the study signed an informed consent.

Fasting serum samples were collected preoperatively from patients with endometrial cancer and also for in outpatient department from controls. In all cases serum concentration of the free IGF-1 was measured using ELISA by DSL, Webster, TX® kits.

Table I. Free IGF-1 and other variables in three different groups of patients (control group and, endometrial adenocarcinoma group with or without myometrial invasion)

	mean±SD	*interval*	*mean±SD*	*interval*	***P value***
	Control Group. (Patients without endometrial adenocarcinoma)		***Patients with endometrial adenocarcinoma***		
Age	61.50±5.12	54-72	62.29±6.29	53-81	0.693
BMI	30.66±3.43	26.00-38.63	31.53±3.47	22.65-39,26	0.230
Period after the menopause	11.75±5.39	4-21	13.57±6.71	1-34	0.159
Parity	2.42±1.29	1-5	2.75±1.51	1-7	0.365
Estradiol (pg/ml)	22.85±8.07	12-36	23.34±8.52	7-38	0.778
Free IGF-1 level (µg/l)	122.68±82.15	26-314	71.21±8.52	7-218	**0.001**
	Endometrial adenocarcinoma confined to endometrium		***Endometrial adenocarcinoma with myometrial invasion***		
Age	62.25±5.383	54-71	62.31±6.981	53-81	0.971
BMI	31.36±3.274	22-36	31.65±3.657	24-40	0.761
Period after the menopause	13,46±6.413	1-25	13.66±7.033	4-34	0.914
Parity	2.67±1.633	1-7	2.81±1.447	1-7	0.484
Estradiol (pg/ml)	22.21±8.84	7-38	24.19±8.32	10-38	0.395
Free IGF-1 level (µg/l)	104.92±63.921	12-258	45.94±32.796	7-102	**0.000**

The results of the patients with endometrial adenocarcinoma were evaluated and divided into two groups, according to the histopathological results. First group consisted of patients whose endometrial carcinoma confined to the endometrium alone (International Federation of Gynecology and Obstetrics; stage IA, Grade I), while the second group comprised patients with minimal invasion (less than 50% of the myometrium; stage IB, Grade I). Patients with advanced stage endometrium carcinoma were excluded due to its low incidence. Demographic factors such as age, height, weight, body mass index, parity and postmenopausal period were recorded individually for each case. Exclusion criteria were alike for all groups of patients: current and previous hormone replacement therapy, diabetes mellitus together with other metabolic systemic diseases, and endometrial carcinoma at an advanced stage and grade.

Statistical analyses were made using SPSS 16.0 software program and parametric data was tested for its normal distribution. Mean and standard deviation of continued variables were calculated. Time after the menopause, BMI, and plasma estradiol level were tested with student-t and one way ANOVA tests. Age, parity, height, weight, and plasma free IGF-1 levels of the patients were compared by means of Mann-Whitney-U and Kruskal Wallis ANOVA tests. Spearman's test was used to investigate the correlation between myometrial invasion and variables. Logistic Regression Analysis determined the significance of the variables. A value of $p<0.005$ was considered statistically significant.

Results

56 patients who had been diagnosed with stage IA and IB endometrial carcinoma were included into our study. 24 patients with tumors confined to the endometrium (stage IA) comprised the first group. 32 patients with tumors invading less than 50% of myometrial invasions constituted the second group (stage IB). The control group consisted of 40 patients suffering from various benign gynecological diseases. All patients were postmenopausal.

The mean and standard deviations of the patients regarding age, BMI, time after the menopause, parity, estradiol and free IGF-1 levels are presented in Table I. Binary comparison of the variables (control group versus patients with endometrial adenocarcinoma, and patients with stage IA endometrial adenocarcinoma versus patients with stage IB endometrial adenocarcinoma) are summarized in Table I.

Results for binary and trinity analysis of variables showed no statistically significant difference between these three groups except for free plasma IGF-1 levels. As far as the IGF-1 levels are concerned, free IGF-1 plasma levels of the patients with endometrial carcinoma were found to be lower than those of patients without endometrial carcinoma. Similarly, the results of patients with stage IB endometrial carcinoma were also lower than in case of stage IA. The difference between the free IGF-1 plasma levels of the groups was statistically significant (p:0.001, $p<0.001$ respectively).

The correlation between myometrial invasion and demographic data and IGF levels was investigated. There was weak correlation between BMI and free IGF-1 plasma levels (rho:0.297, p<0.028), and moderate correlation between free IGF-1 plasma levels and myometrial invasion (rho:-0.490, p<0.001). According to these findings, there is logarithmic correlation between increasing levels of free plasma IGF-1 and BMI. Also, there is a negative correlation between decreasing free IGF-1 plasma levels and myometrial invasion. LRA (Logistic Regression Analysis) allows us to estimate that free IGF-1 plasma level is one of the most important markers for predicting myometrial invasion of endometrial carcinoma.

Discussion

In this prospective case control study the plasma levels of free IGF-1 were evaluated in patients with or without endometrial carcinoma. Postmenopausal patients with an early stage endometrial carcinoma which have similar age, BMI, and estradiol levels were found to have lower free IGF-1 levels. In endometrial carcinoma groups, the free IGF-1 levels of the patients with myometrial invasion were found to be lower than of those with endometrial carcinoma confined to the endometrium.

Numerous epidemiological studies have shown evidence for a relationship between high circulating levels of insulin-like growth factors (IGFs) and an increased risk of several cancers. Most of IGF-I in the circulation originates from the liver. The main physiological stimulus for hepatic IGF-I synthesis is growth hormone. The only unbound and free fraction is 1% of total and acts in an autocrine or paracrine fashion. In our study, we have focused exclusively on free IGF-1 levels in our patients. In endometrial tissue, however, estrogens provide the main stimulus for IGF-1 synthesis. Given these differences in physiology, it is quite possible that, contrary to several other forms of cancer [9], endometrial cancer risk is relatively independent of circulating IGF-I levels.

Circulating IGF-1 levels change substantially by age, increasing slowly from birth to puberty, surging in puberty, and declining with age thereafter [10]. On the other hand, individual levels of free IGF-1 are known to be stable over postmenopausal years, and failure to have optimally detected variations in serum values would most likely have caused bias toward the null [11]. In our study, all of the patients were postmenopausal and there was no difference in the mean age of the enrolled cancer cases and the controls.

IGF-1 expression was significantly decreased in the myometrium by age, which we have previously shown to become proliferatively quiescent [11]. In our study, all patients were postmenopausal and had limited ability to stimulate endometrial cell proliferation, so plasma IGF-1 could act as such a survival factor by inhibiting apoptosis. A recent study that directly measured IGF-1 mRNA levels in human endometrial cancer specimens (rather than in cell lines) found IGF-1 mRNA levels to be low [12].

Obese individuals are presumed to be at higher risk for the development of endometrial cancer due to the increased bioavailability of estrogen. Free and total plasma levels of IGF-2 were found to be increased in both non-diabetic obese cases and type 2 diabetics, whereas IGF-1 remained unchanged [13]. However, although the control group was not BMI-matched with the rest of the subjects, it was found similar to the BMI of the endometrial cancer groups. We found week positive correlation between BMI and IGF-1 levels.

Gunter MJ et al. found that estradiol level itself was positively associated with endometrial cancer risk, even after adjustment for free IGF-1, BMI, and age. IGF-1 was not significantly associated with the risk for endometrial cancer [4]. In our study we found no difference in the levels of estradiol between stage IA, IB endometrial carcinoma and the controls.

IGF-1 pathway plays an important role in the normal endometrial glandular and stromal cell proliferation [8]. At carcinogenesis, despite a very clear evidence to support the direct role of insulin in endometrial tumorigenesis, it is also possible that insulin like growth factor is simply a presenting clinical and pathological marker of endometrial cancer development and invasion.

There is a strong correlation between disease mortality and depth of myometrial invasion in endometrial cancer. Depth of invasion is a well-established prognostic factor in stage I cases, as it defines the risk and existence of lymph-node metastases, recurrence, and survival from the disease [1]. A superficially invading tumor (less than 50% of the myometrium) carries a risk of 5% and 3% for pelvic and paraaortic node metastasis, in contrast, among deeply invasive tumor (more than 50% of the myometrium) the risk increases substantially to 25% and 17%, respectively [1]. Recognition of the prognostic significance of myometrial invasion, in addition to other factors as well, led to the adoption of surgical staging in *Endometrial Cancer by FIGO* (*International Federation of Gynecologists and Obstetricians*) in 1988.

The clinic-based studies directly evaluated circulating IGFs and IGFBPs in women with and without endometrial cancer. The results of these studies conflicted. The patients with and without endometrial cancer were compared in the study of Rutanen EM et al, and they found lower IGF-1, IGF-2 and IGFBP3 levels in patients with endometrial cancer [14].

Petridou et al compared the cases of histologically confirmed endometrial cancer with a control group of women who had undergone minor gynecological operations [15]. They found that endometrial cancer was positively associated with IGF-2 and inversely with IGF-1.

In the study by Lukanova, which included primary invasive endometrial cancer and matched controls, it was shown that endometrial cancer risk increased with increasing level of c-peptide and IGFBP-1, but the risk was unrelated to levels of IGF-1 [9]. Weiderpass E. et al also presented in their study that serum IGF-1, IGFBP-1, IGFBP-3 and insulin levels seem unrelated to endometrial cancer risk [16]. Lacey JV et al also suggested that serum IGF-1 was inversely associated with endometrial cancer [17]. Oh CJ et al demonstrated similar findings as in our study that the mean values of IGF-1 were significantly lower among the studied cases than controls [18].

On the other hand, Ayabe et al compared IGF-1 and IGFBP-1 levels in women with and without endometrial cancer and they concluded that an increased circulating concentration of IGF-1 and decreased circulating concentration of IGFBP-1 are associated with endometrial cancer, especially in postmenopausal women [19]. The BMI was significantly higher in the endometrial cancer group than in the control group.

In our study, the counter correlation between free plasma IGF-1 and myometrial invasion was surprising, particularly given our a priori hypothesis that predicted a positive relationship, in keeping with the mitogenic effects of plasma IGF-1 in myometrial tissue. In the present study, IGF levels are decreased when myometrial invasion exists. The seemingly paradoxical inverse association of free IGF-1 and myometrial invasion has several potential explanations. A disconnection between local uterine IGF-1 levels and IGF-I levels in circulation is believed to exist. Autocrine IGF-1 signaling may be an important regulator of tissue growth in the normal myometrium and that dysregulated expression of growth factor is observed in uterine cancers.

Conclusions

As a summary, our data suggests that IGF levels increased when BMI increased. In the existence of myometrial invasion, free IGF-1 plasma levels decreased.

Free IGF-1 plasma levels would probably be an important factor determining myometrial invasion in endometrial cancer. To the best of our knowledge, our study is the first prospective study in the literature which evaluates the association between myometrial invasion and IGF-1 levels in endometrial cancer patients.

A much larger further prospective cohort studies of endometrial cancer should be conducted, accompanied by continuous efforts to better understand and clarify the role of free IGF-1 plasma levels in endomyometrial invasion of tumors.

References

1. Parkin D, Bray F, Ferlay J, [et al.]. Global cancer statistics, 2002. *CA Cancer J Clin.* 2005, 55, 74-108.
2. Engelsen I, Akslen L, Salvesen H. Biologic markers in endometrial cancer treatment. *APMIS.* 2009, 117, 693-707.
3. Sorosky J. Endometrial cancer. Obstet Gynecol. 2008, 111, 436-447.
4. Gunter M, Hoover D, Yu H, [et al.]. A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. *Cancer Epidemiol Biomarkers Prev.* 2008, 17, 921-929.
5. Juul A, Holm K, Kastrup K, [et al.]. Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. *J Clin Endocrinol Metab.* 1997, 82, 2497-502.
6. Rosen C, Pollak M. Circulating IGF-I: new perspectives for a new century. *Trends Endocrinol Metab.* 1999, 10, 136-141.
7. Liu H, He Z, Mele C, [et al.]. Hormonal regulation of expression of messenger RNA encoding insulin-like growth factor binding proteins in human endometrial stromal cells cultured in vitro. *Mol Hum Reprod.* 1997, 3, 21-26.
8. Pavelic J, Matijevic T, Knezevic J. Biological and physiological aspects of action of insulin-like growth factor peptide family. *Indian J Med Res.* 2007, 25, 511-522.
9. Lukanova A, Zeleniuch-Jacquotte A, Lundin E, [et al.]. Prediagnostic levels of C-peptide, IGF-I, IGFBP -1, -2 and -3 and risk of endometrial cancer. *Int J Cancer.* 2004, 108, 262-268.
10. Kaklamani V, Linos A, Kaklamani E, [et al.]. Age, sex, and smoking are predictors of circulating insulin-like growth factor 1 and insulin-like growth factor-binding protein 3. *J Clin Oncol.* 1999, 17, 813-817.
11. Missmer S, Spiegelman D, Bertone-Johnson E, [et al.]. Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. *Cancer Epidemiol Biomarkers Prev.* 2006, 15, 972-978.
12. Bermont L, Lamielle F, Fauconnet S, [et al.]. Regulation of vascular endothelial growth factor expression by insulin-like growth factor-I in endometrial adenocarcinoma cells. *Int J Cancer.* 2000, 85, 117-123.
13. Frystyk J, Skjaerbaek C, Vestbo E, [et al.]. Circulating levels of free insulin-like growth factors in obese subjects: the impact of type 2 diabetes. *Diabetes Metab Res Rev.* 1999, 15, 314-322.
14. Rutanen E, Stenman S Blum W, [et al.]. Relationship between carbohydrate metabolism and serum insulin-like growth factor system in postmenopausal women: comparison of endometrial cancer patients with healthy controls. *J Clin Endocrinol Metab.* 1993, 77, 199-204.
15. Petridou E, Koukoulomatis P, Alexe D, [et al.]. Endometrial cancer and the IGF system: a case-control study in Greece. *Oncology.* 2003, 64, 341-345.
16. Weiderpass E, Brismar K, Bellocco R, [et al]. Serum levels of insulin-like growth factor-I, IGF-binding protein 1 and 3, and insulin and endometrial cancer risk. *Br J Cancer.* 2003, 89, 1697-1704.
17. Lacey J Jr, Potischman N, Madigan M, [et al.]. Insulin-like growth factors, insulin-like growth factor-binding proteins, and endometrial cancer in postmenopausal women: results from a U.S. case-control study. *Cancer Epidemiol Biomarkers Prev.* 2004, 13, 607-612.
18. Oh J, Wu W, Tortolero-Luna G, [et al.]. Increased plasma levels of insulin-like growth factor 2 and insulin-like growth factor binding protein 3 are associated with endometrial cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2004, 13, 748-752.
19. Ayabe T, Tsutsumi O, Sakai H, [et al.]. Increased circulating levels of insulin-like growth factor-I and decreased circulating levels of insulin-like growth factor binding protein-1 in postmenopausal women with endometrial cancer. *Endocr J.* 1997, 44, 419-424.