Determining the Risk of Premenopausal Endometrial Carcinoma by Analysing Expression of c-eErb-B2 in Patients with Polycystic Ovary Syndrome

Feyza ARMAN[1], Tayfun GÜNGÖR[1], A. Levent ŞİRVAN[2], S. Özlem ALTINKAYA[1], Banu MAT[1] Yeksin HELVACIOĞLU[1], Ümit BİLGE[1], Leyla MOLLAMAHMUTOĞLU[1]✍

Ankara, Turkey

OBJECTIVE: The present study aims to determine the risk of premenopausal endometrial carcinoma by analyzing c-erb-B2 overexpression in patients with polycystic ovary syndrome (PCOS).

STUDY DESIGN: A total of 20 women with PCOS, 20 premenopausal women under the age of 50 with endometrial carcinoma and 20 healthy women were eligible for the study.

RESULTS: 18 (90%) of the endometrial cancers were endometrioid adenocarcinoma. C-erb-B2 overexpression, was found in 4 (20%) of endometrial cancer cases. Women with endometrial cancer were found to have significantly higher c-erb-B2 overexpression compared to women with PCOS and with normal endometrial biopsy (p=0.037). Polycystic ovaries (PCO), as a marker of PCOS, were investigated histologically from 10 of the 20 (50%) women with endometrial cancer. Comparing women with endometrial cancer on their own in terms of c-erb-B2 expression, no significant difference was found in PCO existence (p>0.05), and in terms of PCO existence, there was no significant difference in c-erb-B2 expression and age however, PCO (+) women were slightly younger than PCO (-) ones. All of the PCO (+) women had obesity, as compared to PCO (-) ones (p=0.005).

CONCLUSION: C-erb-B2 overexpression appears to play a role in endometrial carcinomas. In women with endometrial cancer, although no significant difference is found, lower mean age of PCO (+) patients preoccupies the possibility of developing cancer at early ages in PCOS. c-erb-B2 overexpression is not found in the endometrial tissues from women with PCOS and is therefore not a determining factor for endometrial cancer among these women.

Key Words: c-erb-B2, Endometrial carcinoma, PCOS

Gynecol Obstet Reprod Med;16:1 (45-50)

Introduction

Endometrial carcinoma is the most common malignant tumor of the female genital tract and the fourth most common cancer in women after carcinomas of breast, colorectum and lung. Two different clinicopathological subtypes of endometrial carcinoma recognized: the estrogen-related (type 1, endometrioid) and the non-estrogen related (type 2, non-endometrioid). The morphologic differences in these cancers are mirrored in their molecular genetic profile with type 1 showing defects in DNA-mismatch repair and mutations in PTEN, K-ras, and beta-catenin, PIK3CA, and type 2 showing c-erb-B2 (Her-2/neu) expression, aneuploidy and p-53 mutations.[1-2] These genetic changes may occur singly or in various combinations which differ between individual cases.

Epidermal growth factor type II transmembrane tyrosine kinase receptor encoded by the Her-2 cellular oncogene is amplified in several types of human carcinomas and provides an attractive target.Her-2/neu, the transmembrane receptor encoded by the c-erb-B2 gene, is overexpressed by immunohistochemistry in <25% of ovarian cancers and 20-30% of breast cancers, and 10% of endometrial cancers.[3]

Endometrial carcinoma typically arises in the sixth or seventh decade of life, with a mean age at diagnosis of 61 years. Risk factors for endometrial cancer include obesity, diabetes, late menopause, unopposed estrogen therapy and nulliparity. Although, the majority of women with endometrial cancer are

Dr. Zekai Tahir Burak Women's Health Care Education and Research Hospital [1]Department of Gynecologic Oncology and [2]Department of Pathology, Ankara,

Address of Correspondence: *S. Özlem Altınkaya*
Oğuzlar Mah. 39 Sok. Çağdaş Apt. 3/6,
06520, Balgat, Ankara,
altinkayaozlem@yahoo.com

Submitted for Publication: *18.08.2009*
Accepted for Publication: *08.12.2009*

✍*: These data were presented in part (poster presentation) at the 15th International Meeting of the European Society of the Gynecological Oncology (ESGO), which was held in Berlin, Germany, 2007.*

postmenopausal, 5-30% are under the age of 50 years at the time of the diagnosis.[4] A large cohort of young premenopausal women who developed endometrial cancer under the age of 50 years, reported that a high proportion of the women to be overweight or obese.[5] Women with polycystic ovary syndrome (PCOS) are also at significant risk of developing endometrial hyperplasia and/or endometrial cancer. Chronic anovulation, obesity, and hyperinsulinemia are all associated with PCOS as well as with endometrial carcinoma. Chronic lack of progesterone, accompanying anovulation, hyperinsulinemia and hyperandrogenemia can translate into a net stimulatory effect on endometrial proliferation, poor endometrial development, and endometrial hyperplasia and cancer.[6]

The present study aims to determine the risk of premenopausal endometrial carcinoma by analyzing the expression of c-erb-B2 in patients with PCOS.

Material and Method

The present study was approved by the Ethical Committee and Institutional Review Board of Dr. Zekai Tahir Burak Women's Health Care Education and Research Hospital where the study was conducted. Written informed consents were obtained from all participants.

Case Selection

A total of 20 women with PCOS (group 1), 20 premenopausal women under the age of 50 with endometrial carcinoma (group 2) and 20 healthy women (group 3) with normal endometrial biopsy were eligible for the study. Body mass index (BMI) was calculated for all participants. Subjects with a BMI value of ≥30 kg/m^2 was considered to be obese.

Group 1(women with PCOS)

Group 1 included 20 women, with an ultrasound diagnosis of PCOS who were initially investigated to recruit those with a "certain" diagnosis of PCOS. They were finally diagnosed as PCOS using clinical and/or biochemical signs of hyperandrogenism and clinical criteria for ovulatory dysfunction (chronic anovulation) according to the most widely accepted criteria, from the 1990 National Institute of Child Health and Human Development Conference on PCOS.[7] Ultrasound diagnosis was based on evaluation by transvaginal ultrasonography, using a 7.5 MHz transvaginal probe with an SSD-1000 ultrasound scanner (Aloka, USA). Certified sonographers checked for endometrial thickness, ovarian volume, ovarian stromal thickness and number of small follicles in the periphery of the ovary. The criteria used to establish an ultrasound diagnosis of PCOS were increased ovarian volume (>10 cm^3), increased ovarian stroma thickness and increased number (>10) of small (2-8 mm) follicles in the periphery of the ovary.[8] Therefore, all PCOS women included in the study had additional to the NIH criteria and the ultrasound diagnosis of PCOS. Endometrial sampling with pipelle curettage was performed on all patients.

Group 2 (women with endometrial carcinoma)

Group 2 included 20 premenopausal women, under the age of 50, with a histopathological diagnosis of endometrial carcinoma from the endometrial biopsy specimens. Personal and family history of cancer was obtained. All of the patients underwent staging surgery including peritoneal washing, total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, appendectomy and pelvic, para-aortic lymphadenectomy. Staging assignment was made according to the International Federation of Gynecology and Obstetrics (FIGO) for endometrial cancer.[9] Architectural grading was based on the degree of glandular differentiation in accordance with the FIGO guidelines.

Group 3 (healthy women)

Group 3 included 20 healthy women. Endometrial sampling with pipelle curettage was performed on all patients.

Histopathological evaluation and immunohistochemical staining

Endometrial samples from group 1 and 3, and hysterectomy materials from group 2 were evaluated histopathologically and immunohistochemically by a certified pathologist. Formalin-fixed, paraffin-embedded tissue samples from diagnostic curettage and hysterectomy were available for analysis. Tissue sections were subjected to conventional hematoxylin and eosin staining (H and E). 5µm sections of representative blocks from each case were deparaffinized, rehydrated, and immunostained by the peroxidase method (Envision System, DAKO, Carpinteria, CA, USA). Slides were then incubated for 30 min with the c-erb- B2 rabbit polyclonal antihuman antibody (DAKO) at a 1/250 dilution. The chromogen, diaminobenzidine (DAB), was used for developing. Cell count was performed in 10 high power fields (x40) for each section. A positive control of breast cancer section was always run in the assay. C-erb-B2 immunoreactivity was restricted to the intensity and percentage of membranous staining. 0, no staining was observed or membrane staining in less than 10% of cells; 1+, weak membrane staining was seen in more than 10% of the cells; 2+, moderate membrane staining was observed in more than 10% of the cells; 3+, strong complete membrane staining was observed in more than 10% of the cells. 2+ and 3+ membrane staining was considered as positive.

Statistical analyses

Statistical analysis was done by Statistical Package for Social Sciences (SPSS) 11.5 software (SPSS Inc., Chicago, IL, United States). Continuous variables were shown as mean ± standard deviation and categorical variables were shown as frequency and percentages where appropriate. The differences among groups were evaluated by Mann-Whitney U test for continuous variables and Chi-Square test for categorical vari-

ables. P values less than 0.05 were considered to be statistically significant.

Results

Distribution of demographic and clinical characteristics is shown in Table 1. None of the women with endometrial carcinoma had family history of cancer.

Table 1: Distributions of demographic and clinical characteristics

	Women with PCOS (Group 1) (n=20)	Women with EC (Group 2) (n=20)	Healthy Women (Group 3) (n=20)	*P value*
Age (years) (mean±SD)	29.1±5.5	44.9±5.9	33.3±6.3	>0.05* <0.001**
Nulliparity	5 (25)	5(25)	2(10)	>0.05* >0.05**
Infertility	11(55)	7(35)	2(10)	<0.001* >0.05**
BMI (kg/ m^2) (mean±SD)	28.8±3.4	29.5±4.2	23.5±2.2	<0.001* >0.05**

EC: Endometrial carcinoma
Values are expressed as mean ±SD. Figures in parantheses are percentages.
**Comparison for group 1 and 3,*
***Comparison for group 1 and 2*

Among the 20 women with PCOS, 8 (40%) had proliferative endometrium, 7 (35%) had secretory endometrium, 2 (10%) had irregular proliferation and 3 (15%) had chronic endometritis, histologically. None of them had c-erb-B2 expression.

Table 2 shows the distributions of clinicopathologic characteristics in 20 patients with endometrial carcinoma. Among the 20 women with endometrial carcinoma, 3 had diabetes mellitus (DM), 3 had hypertension (HT), 1 had both DM and HT. 18 (90%) of the endometrial tumors were endometrioid adenocarcinoma and 2 (10%) of the endometrial tumors were mixed type of papillary serous and endometrioid cancers. 12 (60%) of the endometrial tumors were associated with complex atypical hyperplasia. 4 (20%) subjects had c-erb-B2 overexpression (2+ or 3+), whereas 6 (30%) subjects had weak (1+) and 10 subjects had no c-erb-B2 expression. Polycystic ovaries (PCO), as a marker of PCOS, were investigated histologically from 10 of the 20 (50%) women with endometrial cancer. Comparing women with endometrial cancer on their own in terms of PCO existence, there was no significant difference in c-erb-B2 overexpression and age, however PCO (+) women were slightly younger than PCOS (-) ones (mean age 43.5±7 and 46±2.9 respectively). All of the PCO (+) women with endometrial carcinoma had obesity (BMI ≥30 kg/m2), as compared to PCO (-) women with endometrial carcinoma (p=0.005). Comparing women with endometrial cancer on their own in terms of c-erb-B2 overexpression, no significant difference was found in PCO existence, age, parity, infertility, obesity, stage, grade, myometrial invasion and histologic type (p>0.05).

Table 2: Distribution of clinicopathologic characteristics in 20 patients with endometrial carcinoma

Characteristics	**Frequency (percent)**	*c-erb-B2 overexpression*
Total	20 (100)	4 (100)
Histology		
Endometrioid	18 (90)	3 (75)
Non-endometrioid	2 (10)	1 (25)
Stage		
1	15 (75)	2 (50)
2	2 (10)	1 (25)
3	2 (10)	1 (25)
4	1 (5)	0 (0)
Grade		
1	14 (70)	2 (50)
2	6 (30)	2 (50)
PCO		
Positive	10 (50)	2 (50)
Negative	10 (50)	2 (50)
Obese (BMI>30 kg/ m^2)	14 (70)	2 (50)
Non-obese (BMI≤30 kg/ $m^{2)}$	6 (30)	2 (50)

Figures in parantheses are percentages.

Among the 20 healthy women, 11 (55%) had proliferative and 9 (45%) had secretory endometrium, respectively. 2 (10%) women had weak (1+) c-erb-B2 expression.

Women with endometrial cancer were found to have significantly higher c-erb-B2 overexpression compared to women with PCOS and with normal endometrial biopsy (p=0.037, Table 3)

Table 3: Distribution of c-erb-B2 immunoreactivity

	Women with PCOS (Group 1) (n=20)	Women with EC (Group 2) (n=20)	Healthy Women (Group 3) (n=20)	P value
c-erb-B2 positive				
2+	0	4	0	0.037***
3+	0	0	0	
c-erb-B2 negative				
1+	0	6	2	
0	20	10	18	

****comparison for group 2 with group 1 and 3*

Discussion

Endometrial carcinoma is the most common malignant tumor of the female genital tract. PCOS is the most common endocrine disturbance affecting women, but disagreements in diagnostic criteria make it difficult to compare epidemiological studies on long term health risks such as cancer. However, women with PCOS are at significant risk of developing endometrial hyperplasia and/or endometrial cancer. The risk of developing endometrial cancer has been shown to be adversely influenced by a number of factors including obesity, unopposed estrogens, nulliparity, and infertility. Gao et al.[10] conducted a study on 52 cases of endometrial carcinoma aged 45 years and younger and stated that there were high incidences of infertility, irregular menstruation, endometrial hyperplasia, obesity and polycystic ovaries in patients under 45 years and younger, indicating the relationship between endometrial carcinoma and estrogen. Niwa et al.[11] also mentioned that untreated ovarian dysfunction such as PCOS with unopposed estrogenic action in the endometrium may be associated with development and growth of endometrial carcinoma in younger women. The present study also demonstrated that, in women with endometrial cancer, although no significant difference is found, lower mean age of PCO (+) patients preoccupies the possibility of developing cancer at early ages in PCOS. Recent evidence in cellular and molecular oncology revealed that estrogens act by genetic and epigenetic mechanisms on cancer cells, and a close relationship between estrogens, growth factors, and oncogenes is important for human cancer. In the present study we investigated the immunohistochemical expression of c-erb-B2 in patients with PCOS, premenopausal endometrial carcinoma and healthy women in order to determine the risk of premenopausal endometrial carcinoma in patients with PCOS.

While most serous (type 2) cancers contain mutations of p53, endometrioid adenocarcinomas demonstrate larger numbers of genetic changes in which the temporal sequence of mutation, and the final combination of defects differ substantially between individual examples. Common genetic changes in endometrioid endometrial cancers include, but are not limited to, microsatellite instability, or specific mutation of PTEN, K-ras, and β-catenin genes.[1]

The c-erb-B2 oncogene is one of the most frequently altered genes in human cancer. It encodes a 1255-aminoacid, 185-kDa receptor tyrosine kinase (p185neu). The protein is a transmembrane receptor, whose expression is associated with malignant transformation. Her-2/neu activation results in an increased mitogen activated protein kinase and PI3K cell signaling. High levels of Her-2/neu overexpression in various human tumors including breast, ovarian, and endometrial carcinomas, are associated with resistance to chemotherapy and poor survival, suggesting that tumor cells overexpressing Her-2/neu behave more aggressively and may have a selective growth advantage over Her-2/neu negative tumor cells. There are prognostic and therapeutic implications associated with the overexpression of this transmembrane protein. Herceptin, a humanized murine monoclonal antibody directed against the extracellular domain of the Her-2/neu protein, is being used to treat breast cancer that overexpress Her-2/neu.

A connection between c-erb-B2 overexpression and endometrial carcinoma has been investigated to determine whether it might be useful in the prognostic and therapeutic implications. There are several studies reporting Her-2/neu, the transmembrane receptor encoded by the c-erb-B2 gene, is overexpressed in endometrial cancers.[3-12-13-14-15] The reported percentage of endometrial cancers that overexpress her-2-neu protein varies considerably. Her-2-neu overexpression is predictive of unfavorable outcomes and aggressive histology in some studies,[3,12,14,16] but not in others.[17,18] A possible explanation for the lack of concordance in the literature with respect to Her-2-neu protein expression includes differences in populations, techniques, antibodies used and interpretation of results. Tissue preparation, fixation, and storage techniques may vary and yield inconsistent results. Inherent intraobserver variability in immunohistochemistry may also account for the large differences in reported positivity among publications.[3]

Mariani et al.[12] mentioned an insignificant overexpression of Her-2/neu in 12% of endometrioid tumors and 22% of non-endometrioid tumors within hysterectomy specimens from 125 patients, however they concluded that Her-2/neu overexpression may be an important step in hormone independent growth and proliferation in a subgroup of endometrial cancers according to the estrogen and progesterone receptor levels. Lambropoulou et al.[19] conducted a study on 110 patients with endometrial carcinoma and stated that cytoplasmic expression of c-erb-B2 to be observed more frequently than membranous (69.1% vs. 5.5%) expression. They also reported that synchronous cytoplasmic and membranous signaling was noticed in 7.9% of cases. Although 90% of the subjects in the present study were endometrioid cancers, membranous c-erb-B2 overexpression was found in 20% of the tumors. In addition, a statistically significant difference was found in c-erb-B2 overexpression in patients with endometrial carcinoma as compared to patients with PCOS and healthy women. Evidently, the present study claims that c-erb-B2 overexpression appears to play a role in endometrial carcinomas, reinforcing the majority of previous similar studies.

Manavi et al.[20] examined specimens from 25 normal, 31 hyperplastic and 72 malignant samples of the human endometrium using differential polymerase chain reaction. Their data indicated that c-erb-B2 plays a role in the early development of endometrioid carcinomas and non-amplification of

the c-erb-B2 gene was associated with the absence of immunoreactivity. In the present study, as no significant difference was found in patients with PCOS compared to healthy women, we could not determine the risk of premenopausal early developing endometrial carcinoma in patients with PCOS by analyzing the c-erb-B2 expression immunohistochemically. These results may suggest that c-erb-B2 is an important oncogene, as it was pointed out in various publications, in high grade and late stage endometrial cancer, but plays a minor role in the much more common low grade and stage tumors that encompass the majority of clinical practice. To our knowledge this is the first study to evaluate the expression of c-erb-B2 in patients with PCOS in order to determine the premenopausal endometrial cancer risk. In addition, comparing women with endometrial cancer on their own in terms of c-erb-B2 overexpression, the present study found no significant difference in PCO existence, age, parity, infertility, obesity, stage, grade, myometrial invasion and histologic type. A high ratio of low grade tumors and early stage of our cancer population may explain these results. Morrison et al.[15] conducted a study in a large cohort of 483 patients and found that both expression and amplification of Her-2 was associated with high grade and high stage endometrial cancer. Engelsen et al.[21] also stated that pathological Her-2/neu staining identifies endometrial carcinomas with an aggressive phenotype, high proliferation and patients with poor survival in a population based setting. The reported rates of overexpression and amplification range from 17 to 38% in several studies.[22-25] In the present study, histologic stage, histologic grade, depth of invasion did not correlate with Her-2 oncoprotein expression consistent with Kohlberger's[24] study which stated that Her-2 oncoprotein expression did not seem to be a late event in the natural history of endometrial cancer. Some of the studies [22-23-25] demonstrated that Her-2 gene amplification was of prognostic value, associated with poor prognosis cell types (clear cell and serous), high risk of disease recurrence, and decreased survival. The rate of Her-2 gene amplification in endometrial carcinosarcomas in Livasy's[26] study appears similar to that reported for endometrial carcinomas.

Similar to some other previous studies, our study is limited by the small sample size; however we demonstrate that endometrial cancer is associated with overexpression of c-erb-B2 which exists in 20% of samples when evaluated by immunohistochemistry in spite of the low grade and early stage tumors. Moreover, c-erb-B2 overexpression is not found in the endometrial tissues from women with PCOS and is therefore not a determining factor for endometrial cancer among these women. Additional translational larger studies are needed to identify molecular and genetic alterations with potential for therapeutic interventions.

Polikistik Over Sendromlu Hastalarda c-erb-B2'nin İmmünohistokimyasal Analizi İle Premenopozal Endometrium Kanseri Riskinin Belirlenmesi

AMAÇ: Bu çalışmada polikistik over sendromu (PCOS) hastalarında premenopozal endometriyum kanseri riskinin c-erb-B2'nin immünohistokimyasal analizi ile belirlenmesi amaçlandı.

GEREÇ VE YÖNTEM: Çalışma için 20 tane polikistik over sendromu olan hasta, 20 tane 50 yaşından küçük premenopozal endometriyum kanseri olan hasta ve 20 tane de sağlıklı kadın seçildi. Sağlıklı kadınlardan ve PCOS olan hastalardan pipel biyopsi ile endometrial biyopsi alındı. Endometrial biyopsi materyallerinde ve endometriyum kanseri olan hastaların histerektomi materyallerinde immünohistokimyasal olarak c-erb-B2 analizi yapıldı.

BULGULAR: Endometriyum kanseri vakalarının %90'ı (18/20) endometrioid adenokarsinom idi. Bu hastalardan 4 tanesinde (%20) c-erb-B2 overekspresyonu izlendi. Endometriyum kanseri hastalarında c-erb-B2 overekspresyonu, PCOS'lu hastalara ve sağlıklı kadınlara göre anlamlı olarak yüksek bulundu (p=0.037). Endometriyum kanseri olan 20 hastanın 10'unda (%50) histolojik olarak, PCOS belirteci olan, polikistik overler (PCO) izlendi. Endometriyum kanseri hastalarını kendi içinde, c-erb-B2 overekspresyonu gösterenler ve göstermeyenler olarak grupladığımızda, histolojik PCO varlığı açısından anlamlı fark bulunmadı (p>0.05). Yine endometriyum kanseri hastalarını kendi içinde, histolojik PCO olanlar ve olmayanlar olarak grupladığımızda, c-erb-B2 ekspresyonu ve yaş bakımından anlamlı fark bulunmamakla birlikte, PCO(+) hastaların PCO(-) hastalara göre yaş ortalaması biraz daha düşüktü (sırasıyla 43.5±7 ve 46±2.9). PCO(+) hastalarda obesite oranı, PCO(-) olanlara göre anlamlı olarak yüksekti (p=0.005).

SONUÇ: c-erb-B2 overekspresyonu endometriyum patogenezinde önemli rol oynamaktadır. Her ne kadar istatistiksel olarak anlamlı fark bulunmasa da; endometriyum kanserli hastalarda PCO (+) olan hastaların yaş ortalamasının, PCO(-) olanlara göre daha düşük olması, PCOS'lu hastalarda daha erken yaşlarda endometriyum kanseri gelişebileceğini düşündürmektedir. c-erb-B2 overekspresyonu PCOS'lu hastaların endometriyal dokularında tespit edilememiştir, dolayısıyla bu hastalarda premenopozal endometriyum kanseri için belirleyici bir faktör olmadığı sonucuna ulaşılmıştır.

Anahtar Kelimeler: c-erb-B2, Endometriyum kanseri, PCOS

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