

INTRODUCTION

Endometrial cancer (EC) is the most common malignancy of the female genital tract in industrialized countries, and occurs predominantly after menopause (*Uharcek, 2008; Odicini et al., 2008; Benevolo et al., 2007; Brys et al., 2007; Santin et al., 2008; Villella et al., 2006; Grushko et al., 2008; Santin et al., 2005; Dahmoun et al., 2003; Burton and Wells, 1988; Kapucuoglu et al., 2007; Macwhinnie and Monaghan, 2004; Mariani et al., 2006*). Histological grade is strongly associated with prognosis, stage, lymph-node metastasis and myometrial invasion. Grade is one of the prognostic factors applied in clinical decisions regarding treatment. The most frequently used grading criteria are the ones of International Federation of Gynecology and Obstetrics (FIGO) and the World Health Organization (WHO), which include both architectural and nuclear features (*Uharcek, 2008*). The presence of metastases in the lymphatic or vascular spaces of the uterus is an important prognostic factor for relapse of disease and poor survival, and it is independent of histological grade or depth of myometrial invasion (*Uharcek, 2008*). In recent years, the molecular analysis of EC has identified abnormalities in the expression, structure, or activity of oncogene products which can contribute to the development

and maintenance of the malignant phenotype (*Benevolo et al., 2007*).

HER-2, also known as c-erbB-2, is an 185kd transmembrane receptor protein encoded by HER-2/neugene, which is localized on chromosome 17. HER-2 is a member of the epidermal growth factor receptor family with tyrosine kinase activity together with HER-1, HER-3, and HER-4. Epidermal growth factor (EGF) receptor related with growth factors has a regulatory role, particularly by influencing the mitogenic activity (*Odicini et al., 2008; Benevolo et al., 2007; Brys et al., 2007; Santin et al., 2008; Villella et al., 2006; Macwhinnie and Monaghan, 2004; Mariani et al., 2006*).

When HER-2 is normally expressed, it leads to the combination of a few copies of HER-2 heterodimers and the HER-2-mediated signaling is weak, resulting in a normal cell growth (*Odicini et al., 2008*). Over-expression of HER-2 has been associated with a more aggressive biological behavior of human tumors including breast and ovarian cancer, prostate, bladder, cervical cancer, and endometrial cancer (*Odicini et al., 2008; Brys et al., 2007; Santin et al., 2008*).

Over-expression of the HER-2 oncogene occurs in about 10% to 40% of EC and has been associated with other adverse prognostic factors, including advanced stage, higher grade and worsened overall survival (*Uharcek, 2008; Villella et al., 2006*).

AIM OF THE WORK

The aim of the present study was to study tissue expression of HER 2 receptors in patients with endometrial cancer and its potential role in prognosis of these cases.

EPIDEMIOLOGY OF ENDOMETRIAL CANCER

Endometrial cancer is the most common gynaecological cancer and the fourth most common cancer in women (*Strom et al., 2006*). The incidence of endometrial cancer rises steadily until the age of 65–70 years and then declines (*Persson and Adami, 2002*). This incidence varies widely across continents, with the highest annual incidence rates in developed countries (*Parkin et al., 1997*). In North America and Europe, endometrial cancer accounts for about 8–10% of all cancer cases in women, whereas in Africa and Asia, it represents only 2–4% of cancers (*Purdie and Green, 2001*). Endometrial carcinomas (EC) are grouped into two broad categories based on clinical, light microscopic appearance and prognostic factors (*Bokhman, 1983*). The first group is referred to as type I and is mainly composed of endometrioid carcinomas. These cancers are oestrogen-related, are often preceded by endometrial hyperplasia and usually show a low grade malignancy (*Ryan et al., 2005; Hecht and Mutter, 2006*). These tumours make up about 80% of ECs in both pre- and early postmenopausal women and have a good prognosis with a 5 year survival rate of 74% (*Hecht and Mutter, 2006; Emons et al., 2000; Salvesen et al., 2000*).

The second group is referred to as type II and is composed of serous or clear cell ECs (non-endometrioid

carcinomas). These cancers are not related to oestrogen and are not preceded by endometrial hyperplasia. They occasionally arise in endometrial polyps or from precancerous lesions, endometrial intraepithelial carcinoma, in the vicinity of atrophic endometrium (*Ambros et al., 1995; Wheeler et al., 2000*). They are usually high grade and have a poor prognosis with a 5 year survival rate of 27–42% (*Salvesen and Akslen, 2002*). Type II EC occur mainly later in postmenopausal women and the only risk factors that can be assumed to date are age and pelvic irradiation (*Ryan et al., 2005; Hecht and Mutter, 2006; Emons et al., 2000*).

There is some overlap between the two categories since 30% of endometrioid carcinomas are associated with atrophic endometrium. In addition, mixed endometrioid/serous carcinomas exist and, in 46% of cases, are associated with endometrial hyperplasia (*Emons et al., 2000*).

RISK FACTORS FOR ENDOMETRIAL CARCINOMA

Obesity:

According to the 2008 national health survey in the United Kingdom, approximately 25% of women are obese, and 32% are overweight (*Department of Health, Health survey for England 2008*). This will continue to increase over the next decade; 1.2 million more women are classified as obese in 2010 than in 2003. The association between obesity and endometrial cancer is well documented. Five percent of all cancers in postmenopausal women are attributable to being overweight or obese. For women with endometrial cancer, this increases to 51% of cases (*Reeves et al., 2007*). The risk is increased in both premenopausal and postmenopausal women with a high body mass index, unlike breast cancer where the risk is increased only in postmenopausal women (*Ellis and Ghaem-Maghani, 2010*).

Obesity increases the level of endogenous estrogen synthesis as a result of the increase in the aromatization of C19 steroids in the adipose tissue and skin. Adipose tissue produces the enzyme aromatase and 17 α hydroxysteroid dehydrogenase. This leads to the increased production of androstenedione, which is converted into estrone in the peripheral tissues. Increased circulating unopposed estrogen levels have a

stimulatory effect on the endometrium, which can induce endometrial carcinogenesis (*Genazzani et al., 2001*).

Endometrial cancer patients who are obese tend to have a poorer outcome. They tend to have shorter survival rates and significantly more co-morbidities than their non-obese counterparts (*von Gruenigen et al., 2003*). Strategies to reduce obesity have been well documented. Lifestyle adaptation that includes weight loss, regular exercise, and a diet low in fat and high in fiber has been shown to reduce obesity and reduce the risk of endometrial cancers. Bariatric surgery is another such strategy to reduce weight loss. The National Institute for Health and Clinical Excellence (NICE) recommends bariatric surgery as first line option in women with a body mass index of more than 50 kg/m² (*NICE clinical guideline 43, 2006*). The cancer risks in obese patients, who have undergone surgery, have been shown to be decreased by 60%. There was also a significant reduction in diabetes (*Adams et al., 2007; Sjostrom et al., 2007*).

Polycystic Ovarian Syndrome:

Polycystic ovarian syndrome (PCOS) affects 4% to 7% of women in the reproductive-age group (*Ehrmann, 2005*). It is a heterogeneous disorder characterized by infertility, menstrual dysfunction, polycystic ovaries, anovulation, and hyperandrogenism. Obesity and hyperinsulinemia are also associated with PCOS. An association between PCOS and endometrial cancer

has been well described in the literature over the years (*Jackson and Dockerty, 1957; Pierpoint et al., 1998; Niwa et al., 2000; Giudice, 2006*).

Both PCOS and endometrial cancer have common risk factors, which includes obesity hyperinsulinemia and hyperandrogenism. Whether women are at an increased risk of endometrial cancer because of PCOS per se, independent of the common risk factors, is debatable. Recent reports suggest that the incidence and mortality of endometrial cancer may not be increased in PCOS (*Navaratnarajah et al., 2008*). It has been suggested that endometrial cancer in women with PCOS has a better prognosis; however, this still remains controversial (*Pilay et al., 2006*).

The link between PCOS and insulin resistance is well established. Insulin resistance increases insulin levels, which can increase the risk of endometrial cancer. According to *Lukanova et al. (2003)* this can be achieved by a number of mechanisms. Increased insulin levels downregulate IGF-1 binding protein, which increases circulating IGF-1 levels. This results in an excess of estrogen, which stimulates the endometrium and increases the mitotic activity of the endometrial cells. Insulin can also act as a growth factor, stimulating cell proliferation and inhibiting apoptosis through its receptors. Elevated insulin concentrations contribute to excess ovarian androgen production, which can cause chronic anovulation and progesterone deficiency. Finally, insulin can

induce the inhibition of the hepatic synthesis of the sex hormone binding globulin protein, which can result in increased estrogen levels (*Ellis and Ghaem-Maghani, 2010*).

Family History:

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant inherited disorder caused by germ-line mutations of the mismatch repair genes, most commonly MLH1, MSH2, and MSH6 (*Peltomaki and Vasen, 2004*). Endometrial carcinoma is the second most common cancer to develop in patients with HNPCC, after colorectal cancer, with 30% to 60% of women developing endometrial cancer. There is also a predisposition to other gynecologic malignancies such as ovarian cancer, with a cumulative risk of up to 12% (*Rijcken et al., 2003; Watson et al., 2001*) and breast cancer (*Ellis and Ghaem-Maghani, 2010*).

Women with HNPCC generally develop endometrial cancer 10 years earlier than the general population, with a peak incidence between the ages of 40 and 60 years. They generally tend to have a good prognosis. Up to 3% of women with endometrial cancer will have mutations in the MMR genes (*Ellis and Ghaem-Maghani, 2010*).

Hereditary nonpolyposis colorectal cancer related endometrial cancers are rare making up 0.5% of the total cases of endometrial cancers. Those women who are at risk of

HNPCC should be genetically tested to identify those with the disorder. Educating these women with regard to menstrual irregularities or postmenopausal bleeding and the need to see their doctor if abnormal bleeding does occur should be reinforced. The use of pelvic ultrasound or hysteroscopy and endometrial biopsy as screening tools for endometrial cancer, in those women at risk of HNPCC, have been shown not to be beneficial (*Lecuru et al., 2008*). Consideration of a hysterectomy should be given to those women who have completed their families, as this would reduce their risk completely. Those women who have not completed their families could be offered the oral contraceptive pill or progestogens. Both have been shown to reduce the risk of endometrial cancers (*Schleesselman, 1997*).

CLASSIFICATION OF ENDOMETRIAL CANCER

It is true that endometrial carcinomas occurring during the reproductive years are all, or nearly all, well-differentiated endometrioid adenocarcinomas (so-called type I carcinomas). However, the reverse contention may not be necessarily correct as, indeed, only a quarter of the tumours developing after the menopause are non-endometrioid carcinomas. In addition, it is not always realised that as many as 55% of endometrial carcinomas at menopause are also of the grade 1 (G1) endometrioid type and a further 20% are grade 2/ grade 3 (G2/G3) endometrioid tumours (*Sivridis et al., 1998; Sivridis, 1986; Buckley and Fox, 2002; Sivridis and Giatromanolaki, 2001; Sivridis et al., 2011*). The prototypes of non-endometrioid carcinomas (type II carcinomas), the serous papillary and the clear cell tumours, share no more than 15% of the total, (*Cirisano et al., 1999; Trope et al., 2001; Fadare and Zheng, 2009; Creasman et al., 2004; Clement and Young, 2004; Hamilton et al., 2006; Faratian et al., 2006; Mendivil et al., 2009; Mazur and Kurman, 1995*) and there is, of course, a group of around 10% of rather unusual types of nonendometrioid carcinomas (*Sivridis et al., 2011*) (Table 1).

Table (1): Classification of endometrial carcinomas at menopause (*Sivridis and Giatromanolaki, 2011*)

Low-grade endometrial adenocarcinomas

< *Endometrioid*

- Usual type
- With squamous differentiation
- Papillary
- Secretory
- Ciliated cell
- Sertoliiform
- With trophoblastic differentiation
- Oxyphil cell

< *Non-endometrioid*

- Mucinous

High-grade endometrial carcinomas

< *Endometrioid*

- Solid type
 - < Non-endometrioid
 - Serous papillary
 - Clear cell
 - Squamous cell
 - Transitional cell
 - Mixed types
 - Undifferentiated
 - Verrucous
 - Glassy cell'
-

Obviously, these data have an impact on patient survival. The many well-differentiated endometrioid adenocarcinomas originating from an atrophic postmenopausal endometrium are similar in all respects to those endometrioid adenocarcinomas

of equal grade and stage that are associated with atypical hyperplasia in young premenopausal women (*Sivridis et al., 1998; Buckley and Fox, 2002*); they all have an almost excellent prognosis, with reported 5-year survival rates varying from 87% to 97% (*Abeler and Kjorstad, 1991; Kuwabara et al., 2005*). The serous papillary and clear cell carcinomas, on the other hand, are aggressive tumours that have an increased metastatic potential, high tendency for relapses, and a 5-year survival rate that may be as low as 15% (*Christopherson et al., 1982; Matthews et al., 1997*), although the usual survival rate quoted has varied from 30% to 68% (*Christopherson et al., 1982; Hamilton et al., 2006; Hendrickson et al., 1982; Kato et al., 1995; Malpica et al., 1995; Slomovitz et al., 2003; Sagr et al., 2007; Benito et al., 2009; Murphy et al., 2003; Abeler et al., 1996*). With the exception of mucinous carcinomas, the moderately and poorly differentiated endometrioid carcinomas and the various non-endometrioid types have a similar ominous clinical course (**table 1**) (*Abeler et al., 1991; Altrabulsi et al., 2005; Soslow et al., 2007*). Recently, *Soslow et al. (2007)* who investigated a series of high-grade endometrial neoplasms of different histological type, namely G3 endometrioid, serous papillary and clear cell carcinomas, found that they were all associated with an almost similar clinical outcome; the corresponding 5-year survival rates of 45%, 36% and 50% were not statistically significant.

There can be no doubt that a diverse collection of lethal tumours, such as the serous papillary, the clear cell, other nonendometrioid tumours, together with the G2/G3 endometrioid carcinomas, would make the survival of postmenopausal women worse. Yet, it has to be admitted that these tumours attracted a degree of attention disproportionate to their frequency, while the many well-differentiated endometrioid adenocarcinomas of the postmenopausal age, with an unquestionably favourable prognosis, have been almost totally ignored. Thus, the longstanding assertion that endometrial carcinomas developing during menopause are of poor prognosis is too wide a generalisation (*Sivridis and Giatromanolaki, 2011*).

It has been thus far illustrated that endometrial carcinomas occurring at menopause form a heterogeneous group of tumours, the prognosis of which depends on the histological type (whether endometrioid or non endometrioid) (**table 1**) and on the degree of differentiation (whether G1, G2 or G3) (**table 2**) (*Sivridis and Giatromanolaki, 2004; Clarke and Gilks, 2010; Zaino, 2009*).

However, grading of endometrioid adenocarcinomas can be highly subjective, particularly in distinguishing between International Federation of Gynecology and Obstetrics (FIGO) grade G1 (<5% solid growth) and FIGO G2 (>5% solid growth) tumours (*Buckley and Fox, 2002; Clarke and Gilks, 2010*) and, indeed, many G2 endometrioid adenocarcinomas may

appear less aggressive than G1 endometrioid adenocarcinomas. Nevertheless, it has been suggested that a binary grading system that divides endometrial tumours into low-grade and high-grade is more effective in assessing prognosis and has greater reproducibility than the presently used three-tier FIGO grading system. This has been claimed for all two-tier grading systems proposed (*Gemer et al., 2009; Alkushi et al., 2005; Scholten et al., 2004; Lax et al., 2000; Taylor et al., 1999*), whether based solely on the proportion of solid growth (*Scholten et al., 2004; Taylor et al., 1999*), or on solid growth and additional histological criteria, such as mode of invasion and tumour cell necrosis (*Lax et al., 2000*) or nuclear atypia and mitotic activity (*Alkushi et al., 2005*) (table 2).