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Estradiol metabolites as biomarkers of endometrial cancer prognosis after surgery

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ABSTRACT

Endometrial cancer (EC) is the most common gynecologic malignancy prevailing after menopause. Defining steroid profiles may help predict the risk of recurrence after hysterectomy, which remains limited due to the lack of reliable markers. Adrenal precursors, androgens, parent estrogens and catechol estrogen metabolites were measured by mass spectrometry (MS) in preoperative serums and those collected one month after hysterectomy from 246 newly diagnosed postmenopausal EC cases. We also examined the associations between steroid hormones and EC status by including 110 healthy postmenopausal women. Steroid concentrations were analyzed in relation to clinicopathological features, recurrence and overall survival (OS). The mean follow-up time was 65.5 months and 26 patients experienced relapse after surgery for a recurrence incidence of 10.6% (6.4% Type I and 29.5% Type II). Recurrence and OS were related to a more aggressive disease but not linked to body mass index. Preoperative levels of estriol (E_3) and estrone-sulfate (E_1-S) were inversely associated with recurrence in a multivariate logistic regression analysis (Hazard ratios (HRs) of 0.31, $P = 0.039$ and 3.01, $P = 0.024$; respectively). All circulating steroids declined considerably after surgery almost reaching those of healthy women, except 4-methoxy-E_2 (4MeO-E_2) for which postoperative levels increased by 35% and were associated to a 68% decreased risk of recurrence (HR = 0.32, $P = 0.015$). Women diagnosed with both histological types of EC present significantly higher levels of steroids, in support of their mitogenic effects. The estrogen precursor E_1-S, the anticancer metabolite 4MeO-E_2, and E_3 that exert mixed antagonist and agonist estrogenic activities and immunological effects, are potential independent prognostic factors.

1. Introduction

Endometrial cancer (EC) is the most common gynecologic cancer and the fourth most frequent neoplasm in women in North America, predominantly occurring in postmenopausal women. Furthermore, EC is the only gynecologic cancer with a rising incidence and mortality [1]. Curative surgery, alone or combined with adjuvant radiation therapy, is performed when cancer is limited to the uterus. However, a subset of EC patients experience recurrence, shorter survival and display inadequate response rates to cytotoxic chemotherapy [2]. The prognosis of EC is determined primarily by disease stage, grade and histologic subtype, reinforcing the need to explore novel prognostic markers.

EC is a heterogeneous disease comprising two types based on histology. The most common type, which accounts for nearly 80% of cases, is the endometrioid or Type I adenocarcinoma, associated with unopposed estrogen stimulation and generally has good prognosis. Type II

Abbreviations: DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate; 5-Diol, androstenediol; 4-Dione, androstenedione; DHT, dihydrotestosterone; ADT, androsterone; ADT-G, ADT-glucuronide; 3α-diol-3G, androstane-3α, 17β-diol 3-glucuronide; 3α-diol-17G, androstane-3α, 17β-diol 17-glucuronide; E_1, estrone; E_1-S, estrone-sulfate; E_2, estradiol; E3, estriol; 2OH-E_1, 2-hydroxyestrone; 2OH-E_2, 2-hydroxyestradiol; 4OH-E_1, 4-hydroxyestrone; 4OH-E_2, 4-hydroxyestradiol; 2MeO-E_1, 2-methoxyestrone; 2MeO-E_2, 2-methoxyestradiol; 4MeO-E_1, 4-methoxyestrone; 4MeO-E_2, 4-methoxyestradiol; 16OH-E1, 16α-hydroxyestrone; BMI, body mass index; LVSI, lymph-vascular space invasion; ANCOVA, analysis of covariance; 95% CI, 95% confidence interval; CYP, Cytochrome P450; SRD5A, Steroid 5α-reductase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; SULT, Sulfotransferase; CYP19, aromatase; OC, oral contraceptives; HRT, hormone replacement therapy; LC, liquid chromatography; GC, gas chromatography; MS, mass spectrometry; OR, odds ratio

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is nonendometrioid that includes serous, clear cell, mixed carcinoma, with higher-grade histology and carries an adverse prognosis. Studies originally described Type I EC as estrogen-dependent whereas Type II was not. However, recent studies indicate that steroid hormones may play a significant etiological role in both types [3]. EC prevails after menopause when ovaries have ceased to secrete potent estrogens. Obesity is a known risk factor of EC [4] and this may be partly related to the fact that adipose tissue represents a major source of estrogen synthesis in postmenopausal women, actively converting adrenal and androgen precursors to estrogens resulting in increased serum bioavailable estradiol (E_2) [5,6]. Previous work by our group and others has revealed that the potent estrogen E_2 primarily derive from conversion of estrone-sulfate (E_1-S) in EC tumors rather than aromatization of androgens by the aromatase (CYP19), which has barely detectable expression levels in EC cells [7–10]. Besides, E_2 and E_1 may be converted into numerous biologically active derivatives with varying mitogenic and genotoxic properties by the action of various cytochrome P450 and catechol-*O*-methyl transferase enzymes [11]. This metabolism involves the irreversible hydroxylation (OH) at the C-2, C-4, or C-16 positions of the steroid ring and the methylation of C-2 or C-4 hydroxyl group. The latter prevents formation of mutagenic catechol quinones derived from hydroxyl estrogens that form stable and depurinating DNA adducts. In vitro and in vivo studies further support that 2-methoxyestradiol (2-$MeOE_2$) has strong anticancer activity [12,13]. In addition, these metabolites can be converted to their inactive sulfate and glucuronide conjugates. Recent studies have assessed the risk of EC in relation to steroid hormones, yet none have explored their association with prognosis [4,14].

In a cohort of 246 postmenopausal women undergoing hysterectomy for a newly diagnosed endometrial cancer, we analyzed the levels of 27 steroids in serums collected the morning of surgery and one month after surgery. Steroid measures included the assessment of endogenous concentrations of adrenal precursors, androgens, potent estrogens and catechol estrogens using sensitive and specific mass spectrometry (MS) validated assays. Our primary goal was to evaluate the association between circulating steroid levels, clinicopathological features and the risk of recurrence after surgery. A group of 110 healthy postmenopausal women was also included to examine the association between steroid hormones and EC status.

2. Materials and methods

2.1. Study populations

All participants provided a written informed consent for their participation to the study and the use of their specimens. The current study was reviewed and approved by our institutional review boards. Recruitment of healthy postmenopausal women, as well as specimen collection and treatments, have been described elsewhere [15]. Briefly, women were recruited in a mammography clinic in Quebec City (QC, Canada) between July 2003 and March 2004. To be eligible, women had to: 1) be of postmenopausal status, 2) have no history of health problems related to steroid hormone metabolism, 3) have no history of hepatic, thyroid, or adrenal diseases, and 4) have not taken hormone replacement therapy (HRT) during the three months preceding enrolment. Recruitment methods and specimen collection of EC cases have been described [16]. Participants were all recruited at the Hôtel-Dieu de Québec Hospital (Québec City), between 2002 and 2013. All women were of postmenopausal status, undergoing surgery for EC (hysterectomy and bilateral salpingo-oophorectomy) and had not taken HRT in the three weeks prior to surgery. Blood samples were collected the morning of surgery and one month after surgery as part of a follow-up appointment. Samples were immediately processed, separated in aliquots and stored at −80 °C until analysis. EC recurrence was ascertained by computerized tomography scan. For both cohorts, demographic and anthropometric data were collected through nurse-administered questionnaires, whereas information regarding drug use (including oral contraceptive and HRT) and obstetric history were collected at the same time. A pathologist assessed the histopathological characteristics of the hysterectomy specimen. Systematic assembling and review of medical records was performed by one of the treating gynecologic oncologist (J.G.).

Table 1
Clinicopathological features of endometrial cancer cases treated by radical hysterectomy.

Features	Endometrial cancer cases (n = 246) Mean ± SD	
Age (yr)	65.1 ± 8.9	
Weight (kg)	75.0 ± 19.0	
Height (cm)	158.4 ± 6.4	
Follow-up (months)	65.5 ± 36.7	
5-year survival (%)	90.2	
5-year recurrence rate (%)	9.8	
	n	(%)
Body mass index (BMI)[1]		
Normal Weight	70	(28)
Overweight	66	(27)
Obese	108	(44)
Missing	2	(1)
Histological Type[2]		
Type I	202	(82)
Type II	44	(18)
Grade		
1	90	(37)
2	94	(38)
3	61	(25)
Missing	1	(0)
Stage		
1	197	(80)
2	12	(5)
3	28	(11)
4	9	(4)
Myometrial invasion		
< 50%	187	(76)
≥50%	59	(24)
Lymph-vascular space invasion		
Absence	183	(74)
Presence	58	(24)
Undetermined	5	(2)
Presence of metastatic nodes		
No	220	(89)
Yes	26	(11)
Relapse after surgery[3]		
No	220	(89)
Yes	26	(11)

[1] Categories of BMI according to WHO Guidelines: normal weight: BMI < 25 kg/m^2, overweight: BMI between 25 and 30 kg/m^2, and obese: BMI ≥ 30 kg/m^2.
[2] Type I only comprises endometrioid carcinomas. Included in Type II are papillary serous carcinoma, mixed carcinoma, clear cells carcinoma, undifferentiated carcinoma, and malignant mixed Müllerian tumor.
[3] Clinicopathological features of endometrial cancer cases in relation to recurrence post-surgery are presented in Table 2.

2.2. Reagents and material

Parent estrogens standards were purchased from USP reference standard (Rockville, MD, USA), while other steroids were purchased from Steraloids (Newport, RI, USA). Deuterated standards were from C/D/N Isotopes (Montréal, QC, Canada), except d3-DHEA, which was synthesized by the Organic Synthesis Service of the CHU de Québec Research Center (Québec, QC, Canada). All chemicals and solvents used in this study were HPLC or reagent grade. Methanol, chlorobutane, dichloromethane, ethyl acetate and acetone were purchased from VWR (Montréal, QC, Canada). Ascorbic acid, sodium bicarbonate, β-glucuronidase/sulfatase (*Helix Pomentia* Type HP-2) and dansyl chloride were purchased from Sigma (Oakville, ON, Canada).