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Hormone receptor expression patterns in the endometrium of asymptomatic morbidly obese women before and after bariatric surgery

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HIGHLIGHTS

- Occult endometrial hyperplasia is present in a notable minority of asymptomatic morbidly obese women.
- Obese women with hyperplasia express higher levels of estrogen receptor, but these levels appear to decrease with weight loss.

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ABSTRACT

Objective. Obesity increases risk for endometrial neoplasia, but neither the pathophysiology nor the effects of weight loss on the risk are well established. We attempted to characterize the molecular profile of the endometrium of asymptomatic women with morbid obesity before and following bariatric surgery-induced weight loss.

Methods. 59 asymptomatic, morbidly obese women underwent endometrial sampling before bariatric surgery; 46 (78%) of these returned one year later for re-biopsy (median weight loss of 41 kg). Duplicate samples from these specimens were scored for expression of estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), and Ki-67 by two independent, blinded pathologists using an H-score [staining intensity (0–3) × (percent of tissue involved)].

Results. The prevalence of hyperplasia pre-operatively was 7% overall and 10% among patients not on an anti-estrogen. ER H-scores were similar before and after surgery overall (median 190 and 196 respectively, $p = 0.82$), but patients with hyperplasia had higher pre-operative H-scores (median 256, $p < 0.001$) and experienced greater H-score drops, than those without hyperplasia (-112 vs $+50$, $p = 0.028$). In two patients with persistent hyperplasia at one year, ER H-scores fell to levels that were similar to those without pathology. One patient who developed hyperplasia during the study period had a rising ER H-score. Patients with hyperplasia had higher median PR H-scores pre-operatively (284 vs 188, $p = 0.01$), which normalized through greater drops (75 vs 0, $p = 0.053$). AR H-scores dropped significantly after surgery (13 vs 2, $p = 0.015$), but were similar between patients with and without hyperplasia ($p = 0.33$). Weight loss did not affect Ki-67 proliferation index.

Conclusion. Asymptomatic morbidly obese patients have a high prevalence of occult hyperplasia, characterized by relatively high hormone receptor expression. These profiles appear to normalize with weight loss and in advance of pathologically identifiable changes. These data suggest a potential role for screening this population as well as the possibility that weight loss may be a valid treatment strategy for risk reduction.

Introduction

Increasing obesity in women is associated with a progressively worsening estrogen/progesterone imbalance [1]. The causal effect of unopposed estrogen on the induction of endometrial cancer is singularly well established through historical study of early hormone replacement efforts [2]; but there is increasing epidemiologic evidence indicating that obesity increases the risk of adenocarcinoma in other endocrine sensitive organs such as the breast, ovary, and prostate [3–5]. Simple estrogen imbalance, however, does not account for all of the increased risk attributable to obesity. Obese patients also have an increased risk for adenocarcinomas of the pancreas and colon which are not traditionally considered endocrine-sensitive, suggesting that alternate or multiple molecular pathways may be involved [6].

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Despite a rising incidence and relative mortality of endometrial cancer, coinciding with a national epidemic of obesity, no prevention or screening strategy has proven cost-effective to date [7]. This is largely owing to a relatively low prevalence of endometrial pathology in the asymptomatic general population and a tendency for detection at early stages because of symptomatic bleeding. Recently however, our group has completed a natural history study of the effect of bariatric surgery-induced weight loss, in which we observed that the rate of occult hyperplasia in untreated, asymptomatic, morbidly obese women is up to 10% and that weight loss reduced but did not eliminate this risk [8]. This level of premalignant disease suggests that in morbidly obese women screening or proactive intervention may be cost effective.

Ultimately, effective screening efforts will require the treating clinicians to be able to stratify risk based on objective and reproducible measures, while prevention efforts will require an understanding of the natural history of disease progression and the range of potential treatment options. Multiple studies have demonstrated that the risk of coincident or subsequent endometrial cancer rises along a continuum of endometrial architectural complexity and cytologic atypia [9]. Despite these findings, traditional pathologic assessments have high inter-observer variability, and are increasingly unreliable at the lower end of the risk spectrum. Efforts to simplify the grading system using endometrial intraepithelial neoplasia (EIN) nomenclature have encouraging initial results, but have been slow to be adopted, and still rely heavily on microscopic, as opposed to molecular features [10].

The expression patterns of both the estrogen receptor (ER) and the progesterone receptor (PR) in normal endometria and to a lesser extent in malignant and premalignant endometria are well characterized [11]. However, the significance of the expression patterns in obese women, the implications of androgen receptor (AR) expression, and the effect of weight loss on hormone expression patterns remain unknown. The present study was undertaken to determine if morbidly obese patients with premalignant changes had detectably different expression profiles of markers known or suspected to be involved in the development of endometrial cancer; and to determine if weight loss affected these profiles.

Methods

Tissue acquisition

After obtaining institutional review board approval, 59 patients were enrolled in a prospective non-interventional trial to assess the effect of Roux-en-Y gastric bypass surgery on the prevalence of endometrial pathology. The details of this study are reported elsewhere; but briefly, all morbidly obese women who were asymptomatic by self report (no abnormal vaginal bleeding in the previous 6 months), had a negative pregnancy test or prior sterilization, and gave consent were eligible. We initially excluded patients using hormonal therapies in order to homogenize the patient population, however as our natural history design precluded actively preventing the use of hormonal agents (especially contraceptive therapy) in the intervening year between biopsies and because the prevalence of neoplasia in the morbidly obese on progestins is unknown we eliminated this exclusion criterion. Patients on oral contraceptives, progesterone (oral or IUD), or aromatase inhibitor were deemed "on anti-estrogen therapy." All patients underwent biopsy at the time of bariatric surgery and 46 patients (78%) returned for follow-up biopsy 12 months after bariatric surgery. Of the 13 patients without follow-up biopsy eight declined follow-up despite initial consent, 4 patients moved geographically and were unavailable for biopsy, and 1 patient was pregnant at the time of planned follow-up and was excluded; all had normal pre-operative biopsies.

Processing

For the purposes of this analysis, a tissue microarray (TMA) was created using tissue from formalin fixed, paraffin imbedded blocks. After expert review of the original hematoxylin and eosin stained sections, paired cores of 0.6 mm diameter were extracted from each specimen block with sufficient tissue, and arranged randomly across 3 blocks by the BioNet service University of Minnesota. 8 μm sections were cut from the TMA and processed as follows. Antigen retrieval was performed in an automated fashion using the Benchmark Immunostainer (Ventana Medical Systems, Tucson, AZ) using CC1—a tris-based buffer with a slightly basic pH. Primary antibody staining was with undiluted monoclonal rabbit anti-human antibodies Ki-67 (30-9), ER (SP1), and PR (1E2) (Ventana). AR antibody (Dako) was diluted 1:1000 in primary staining. Slides were counterstained with biotin streptavadin (iVIEW DAB Detection, Ventana), after blocking for endogenous biotin.

Specimens were assigned a semi-quantitative "H-score" by two independent pathologists with expertise in gynecologic pathology who were blinded to the layout of the TMA and to each other's report. The H-score was the product of the percentage staining and an ordinal value designating staining intensity (0 for absent, 1 for weak, 2 for moderate, and 3 for intense). Histocores had a potential range of 0 (100% unstained) to 300 (100% intensely staining) and the final H-score was the average of the available measurements (2 cores × 2 readers when all cores were adequate). The Ki-67 proliferation index was calculated by estimating and averaging the percentage of positive staining nuclei, and averaging as per the H-score analyses.

Statistical considerations

H-scores were correlated with pathologic diagnosis and compared using t-test or Wilcoxon rank sum where appropriate for continuous variables and chi-squared test for proportions. All continuous data analyses were two-tailed, and a p-value of <0.05 was considered statistically significant throughout. Sample size was dictated by the availability of specimens harvested in the previous study, and power calculations were therefore not performed.

Results

Clinical results

59 morbidly obese women underwent initial sampling. Patient demographics are listed in Table 1, most patients were Caucasian, less than 50 years old, had good access to health care and relatively few medical co-morbidities. All patients underwent Roux-en-Y bypass and endometrial biopsy without overt complication, though in five pre-operative (8.5%) and one post-operative (2.2%) specimens there was insufficient tissue for diagnosis. Twelve months post-operatively the

Table 1
Patient characteristics (N = 59).

Age (years)	39 (20–60)
Caucasian	57 (97%)
Other	2 (3%)
Pre-operative weight (kg)	125 (87–195)
Pre-operative BMI (kg/m^2)	46 (36–64)
Diabetes	16 (27%)
Diet controlled	5 (8%)
Oral agent	10 (17%)
Both oral agents and insulin	1 (2%)
Menopausal status	
Pre-menopausal	49 (83%)
Postmenopausal	10 (17%)
Use of hormonal therapy	
No	43 (73%)
Yes	16 (27%)
Oral contraceptives	12 (20%)
Progestin (IUD or oral)	3 (5%)
Anastrozole	1 (2%)

Continuous variables described by medians (ranges).