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Mismatch repair protein expression in 1049 endometrial carcinomas, associations with body mass index, and other clinicopathologic variables

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HIGHLIGHTS

- We analyzed data from 1049 hysterectomy specimens with endometrial carcinoma.
- BMI and MMR correlation is explained by young women with MSH2/MSH6 loss and low BMI.
- Higher BMI correlated with lower stage and grade of endometrial carcinoma; lower BMI correlated with increased MMR protein loss.

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ABSTRACT

Objective. Links between obesity, with its attendant estrogen abnormalities, and the endometrial carcinoma (EC) DNA Mismatch Repair Protein (MMR) system have recently been proposed. We investigated relationships between body mass index (BMI) and clinicopathological correlates including MMR expression in a large single institution EC cohort.

Methods. Clinical and pathological databases from 2007 to 2012 were used to identify consecutive hysterectomy specimens with EC. Univariate and multivariate analyses were used to explore relationships between BMI, age, stage, tumor type and immunohistochemical results for MLH1, PMS2, MSH2 and MSH6.

Results. 1049 EC were identified. Overall, BMI was higher amongst women with normal MMR ($p = 0.002$). However, when stratified by age and specific MMR, statistically significant differences localized exclusively to women <50 years old with loss of MSH2 and/or MSH6 ($p = 0.003$ and $p = 0.005$ respectively). Higher BMI correlated with endometrioid FIGO 1 and 2 tumors ($p < 0.001$) and with stage 1a ($p < 0.001$). Conversely, MMR abnormalities did not show significant associations with stage ($p = 0.302$) or histologic grade ($p = 0.097$).

Conclusions. BMI showed statistically significant associations with MMR expression, tumor grade and stage amongst 1049 consecutive EC. Obesity correlates with lower grade and stage EC. A link between BMI and maintenance of the MMR system is not supported by our data because the only statistically significant association occurred in women <50 years old with MSH2 and/or MSH6 abnormalities where Lynch syndrome related cases are expected to cluster.

Introduction

Excess body weight is a well-established risk factor for endometrial carcinoma (EC) [1–3]. Obesity, defined as body mass index (BMI) >30 kg/m^2, afflicts over 35% of women in the United States, resulting in a 41% population attributable risk for EC [4]. The relationship between obesity and EC is complex and involves multiple mechanisms including increased free estrogens [1] and a low grade inflammatory milieu [5]. Recently, other EC investigators have also suggested a link between BMI with its attendant estrogen levels and the highly conserved DNA mismatch repair (MMR) system [6]. Moreover, Miyamoto et al. have contributed in-vitro evidence that estradiol increases cell proliferation, MLH1/MSH2 expression, and MMR activity in cultured glandular endometrial cells as well as an endometrial cancer cell line [7]. Because the MMR system corrects DNA mismatches generated during replication [8] its impairment leads to accumulation of mutations and microsatellite instability (MSI), a phenotype observed in 17–31% of EC [9,10]. MSH2 and MSH6 (*Escherichia coli* MutS homologs); and MLH1 and PMS2 (*E. coli* MutL homologs) work as heterodimers that recognize

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and initiate mismatch repair [11]. The pathologic loss of expression of one or more of these MMR proteins can be detected with immunohistochemistry (IHC) [12]. Since they are "obligatory partners" of their respective heterodimer, loss of expression of MLH1 or MSH2 results in concurrent loss of expression of PMS2 and MSH6 respectively [12]. Conversely, mutations of the *PMS2* or *MSH6* genes result in isolated protein losses. While most MSI in sporadic EC can be attributed to hypermethylation of the *MLH1* gene promoter [13,14] instead of MMR gene mutations [15], EC is the second most common cancer in women with Lynch syndrome (LS), a condition caused by a hereditable autosomal dominant germ line mutation of one of the MMR genes [16,17].

The main objective of the present study is to correlate BMI with MMR IHC, including MLH1, PMS2, MSH2 and MSH6, in 1049 consecutive EC. Associations with other clinicopathological variables including tumor type, grade and stage are also presented.

Methods

The institutional pathology database at the Ohio State University Wexner Medical Center (OSUWMC) was searched for the terms endometrial carcinoma and endometrial carcinosarcoma in hysterectomy specimens received between July 1, 2007 and April 15, 2012. Tumor stage, histological type and MMR IHC results were extracted from original pathology reports. Our institution routinely performs MLH1 (NovoCastra, clone: ES05), PMS2 (BD, clone: A16-4), MSH2 (Calbiochem, clone: FE11), and MSH6 (Epitomics, clone: EP 49) on all endometrial carcinoma specimens using clinically-validated Bond (MLH1, MSH6) and Dako (MSH2, PMS2) immunostainers, each at a dilution of 1/200 and with colon cancer as control tissue. IHC for any of the four MMR proteins is considered positive if definite nuclear staining is detected in neoplastic cells. The patients were grouped by presence or absence of the MMR proteins (both individually and as a group), and further clinical data including height, weight, and age was extracted from the institution's electronic medical record; BMI data was calculated. Endometrial carcinoma was classified as type 1 or low grade if the tumor was diagnosed as endometrioid carcinoma FIGO grade 1 or 2. FIGO grade 3 endometrioid tumors were classified as type 2 or high grade, along with serous carcinomas, carcinosarcomas, clear cell carcinomas, mixed carcinomas, and undifferentiated carcinomas.

Relationships between MMR defects, BMI, age, tumor type, and tumor stage were investigated. Comparison groups for MLH1, PMS2, MSH2, and MSH6 deficiencies were defined as the subjects with each absent individual protein. The normal or control group for the comparisons of each of the individual protein deficiencies consisted of the 814 patients in the cohort who had all four MMR proteins present by IHC. Age and BMI as continuous variables were compared between tumor types and protein defects using Wilcoxon rank-sum tests. Categorical variables were compared using Chi-square tests; however, Fisher's exact test was utilized when the number of patients in any comparison group was small. Since numerous tests were performed, and to control the type I error rate, a p-value of 0.01 or less was considered significant for these tests. An analysis of variance model with Tukey–Kramer adjustment for multiple comparisons was used to compare the continuous BMI between tumor stages. All statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC).

The study was performed under appropriate IRB approvals (OSU 2007C0081 and 2001C0203).

Results

Patient characteristics

A total of 1054 eligible patients were identified within the study dates. Five patients were removed from the study; four of these had insufficient tumor for MMR IHC and one had complete coagulative necrosis from previous endometrial ablation precluding accurate assessment. Overall patient age ranged from 25 to 93 years (median 61). 814 patients had type 1 tumors (age range 25–93, median 59.5) and 235 patients had type 2 tumors (age range 36–91, median 65) including 65 FIGO 3 endometrioid adenocarcinomas, 52 serous carcinomas, 11 clear cell carcinomas, 36 carcinosarcomas, 2 poorly or undifferentiated carcinomas, and 69 mixed pattern carcinomas. The majority of patients presented with stage 1a disease (695, 66.3%) while 176 (16.8%), 75 (7.2%), and 103 (9.8%) patients presented with stage 1b, stage 2, and stage 3 or 4 disease, respectively. BMI ranged from 14.7 kg/m^2 to 85.0 kg/m^2 (median 35.3 kg/m^2). Overall, BMI was significantly higher for women younger than 50 years ($n = 148$, median BMI = 39.0 kg/m^2, range 17.8–85.0 kg/m^2) versus women ≥50 years ($n = 901$, median BMI = 34.9 kg/m^2, range 14.7–82.4 kg/m^2), $p < 0.001$. (Table 1)

MMR associations (Fig. 1, 2)

One or more MMR proteins were absent in 235 (22.4%) of the 1049 tumors investigated. PMS2 loss was the most common abnormality, followed by absence of MLH1 (Table 1). Combined loss of MLH1 and PMS2 occurred in 165 cases (15.7%), while isolated PMS2 loss occurred in 21 cases (2.0%). Combined loss of MSH2 and MSH6 occurred in 20 (1.9%) cases, while isolated loss of MSH6 occurred in 31 (3%) cases.

MMR abnormalities were associated with patient BMI. Women whose tumors had abnormal MMR IHC were thinner than those women with normal MMR expression (median BMI 34.2 kg/m^2, range 17.8–74.6 kg/m^2 vs. 35.8 kg/m^2, range 14.7–85.0 kg/m^2, $p = 0.002$, Wilcoxon Rank Sum test) (Fig. 1). However, women whose tumors lacked both MLH1 and PMS2 (the most common defect, $n = 165$ cases) had a similar BMI compared with those who had normal MMR expression ($n = 814$) (median BMI 34.6 kg/m^2, range 19.2–85 kg/m^2 vs. 35.8 kg/m^2, range 14.7–85.0 kg/m^2; respectively, $p = 0.040$, Wilcoxon Rank Sum test). On the other hand, the BMIs of those women whose tumors lacked MSH2 and/or MSH6 ($n = 51$) were markedly lower than women with normal MMR expression (MSH2 deficient women with median BMI 27.1 kg/m^2, range 17.8–54.2; MSH6 deficient women with median BMI 31.2 kg/m2, range 17.8–64.0) (see Fig. 1 and Table 1 for breakdown by individual protein loss).

Loss of MMR was also associated with age at diagnosis. Women with absent MLH1 (median age 63, range 35–92) and/or PMS2 (median age 63, range 35–92) were older than women who had all proteins present (median age 60, range 25–93), $p < 0.001$ for both comparisons. In contrast, women with absent MSH2 and/or MSH6 on IHC (median age 56, range 35–80 for each) were significantly younger than those with all proteins detected (median age 60, range 25–93), $p = 0.006$ and $p = 0.003$, respectively.

More interestingly, when stratified by age, significant BMI differences were seen only in women <50 years old with loss of MSH2 and/or MSH6. BMI was significantly higher in women when all proteins were present ($n = 124$, median BMI = 39.3 kg/m^2) than in the young women with MSH2 ($n = 7$) and/or MSH6 ($n = 11$) loss (median BMI = 26.4 kg/m^2 for each), $p = 0.003$ and $p = 0.005$, respectively (Fig. 2).

There was no BMI difference in women ≥50 years old who retained all proteins or lost expression of MSH2 and/or MSH6 (35.0 kg/m^2 compared to 31.0 kg/m^2 and 33.8 kg/m^2, respectively), $p = 0.174$ and $p = 0.460$. Likewise, no significant differences in BMI were seen between those who retained all proteins or lost MLH1 and/or PMS2, regardless of age.

There was no difference in stage distribution between those who had MMR protein loss by IHC and those who did not, $p = 0.302$. Additionally, there were no differences in MMR protein loss between type 1 and type 2 tumors (21.3% type 1 v. 26.4% type 2), $p = 0.097$.