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Case-case study of factors associated to hMLH1, hMSH2, and hMSH6 protein expression among endometrial cancer patients of the University District Hospital of San Juan, Puerto Rico

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Abstract

Lynch syndrome (LS) is an autosomal dominant disorder caused by DNA mismatch repair (MMR) system deficiencies. Women affected by LS present a 40 to 60% lifetime risk of endometrial cancer (EC).

Objective—This case-case study aims to determine the frequency of the hMLH1, hMSH2, and hMSH6 MMR proteins and the factors (age, family history of cancer [FHC] related to LS, and body mass index [BMI]) associated to their absence in EC patients attending the University District Hospital of San Juan, Puerto Rico.

Method/Materials—Twenty cases were preliminary evaluated for the MMR protein expression by immunohistochemistry testing and classified as positive-cases (presence of protein) or negative-cases (absence of protein). The statistical analysis was based on the logistic regression model using the Maximum Likelihood estimation (MLE). The Bayesian approach was used to determine the posterior probability {posterior $\Pr[OR > 1]$ }.

Results—Results showed absence for at least one MMR protein in 25% of the cases; 15% for hMLH1 and 10% for hMSH2. None of the cases showed an absence for hMSH6. The MLE demonstrated that women diagnosed with EC before the age of 50 (OR: 12.4; 95%CI = 0.5–322.7), having FHC related to LS (OR: 17.7; 95%CI = 0.6–534.6), and having lower BMI (OR: 2.38; 95%CI = 0.39–14.28) present higher odds than their counterparts of lacking an MMR protein, once adjusting for potential predictors ($p > .05$). The posterior probability that an excess risk of lacking an MMR protein occurs was 95% for each predictor.

Conclusion—Our study in this Hispanic population supports previous studies in that younger age, FHC, and lower BMI are associated with increased odds of having an absence of MMR protein expression. Further studies with larger sample sizes should be performed.

Keywords

endometrial cancer; lynch syndrome; HNPCC; MMR proteins

Introduction

Lynch syndrome (LS), also known as hereditary nonpolyposis colorectal cancer, is an autosomal dominant disorder with variable penetrance, caused by germline mutations in any one of the mismatch repair (MMR) genes resulting in abnormal function or absence of the MMR proteins.¹ Persons with LS have a genetic predisposition to various cancers, including colorectal, endometrial and ovarian cancer.¹ The MMR genes frequently associated to LS are *hMLH1*, *hMSH2*, and *hMSH6*. They are implicated in post replication repair, DNA damage signaling, and apoptosis when it is overwhelmed by DNA damage.²

Endometrial cancer (EC) is the most common gynecological type of cancer in Puerto Rico (PR)³ and the United States,⁴ and is the most common cancer among women with LS.⁵ Previous studies have reported a lifetime risk of developing EC of 40 to 60% for women with LS,^{5,6} compared to < 3% in the general population.⁷ This study aimed to determine the absence of the MMR proteins (hMSH2, hMSH6, and hMLH1) expression in a clinic-based sample of EC cases in PR and its association with age, body mass index (BMI), and family history of cancer (FHC).

Materials and Methods

A case-case study design was used. The study's population was composed of 20 women 21 years old with EC treated at the University District Hospital of PR during the years 2004 to 2008 and who participated in a previous case-control study.⁸ The cases had an histological confirmation of EC, no previous cancer diagnosis and a valid telephone number.⁸ Eligible cases were invited to participate and recruited consecutively from the gynecology clinics of the Medical Sciences Campus (MSC), University of PR. Data related to socio-demographic, lifestyle and medical history was obtained through phone interviews using a standardized questionnaire. Medical records and surgical pathological reports of patients from the Clinics of the Medical Services Administration of PR (ASEM) were used as sources for clinical and pathological data. For this sub-study, participants' tumors were obtained from ASEM's tumor bank in order to perform an immunohistochemistry test (IHC) and identify those tumors with presence (positive protein expression-cases) or absence (negative protein expression-cases) of the MMR proteins (hMLH1, hMSH2, and hMSH6). Negative protein expression-cases were tumors presenting < 10% of nuclear staining for at least one of the MMR proteins. External (tissue of normal colon) and internal controls (normal tissue adjacent to the EC tumor and lymphocytes) were used during the IHC testing in order to ensure staining quality. Slide evaluation and scoring was performed by two pathologists who were blinded from the tissue IDs. Approval of the MSC-IRB was obtained for this study.

An epidemiologic profile of the participants was performed using descriptive statistics. Logistic regression modeling was performed to determine the magnitude of association between age at diagnosis (< 50 years old/ 50 years old), FHC related to LS (yes/no), and BMI (kg/m²) with the absence of MMR proteins using Maximum Likelihood estimation (MLE).⁹ Due to the small sample available, the Bayesian approach was used to compute the following posterior probability: $\Pr[OR > 1]$ with WinBugs program. For the Bayesian approach, a total of 500 simulations were run after discarding the first 501 "burn-in" iterations to reach convergence of the simulation results and ensure validity using a non-informative prior of uniform distribution over a fixed range (-3, 3), (-1, 1) or (-0.5, 0.5).¹⁰

Results

On average, the age at diagnosis of EC was 55.2 ± 14.1 and the BMI of the participants was 33.4 ± 8.2 (table 1). Type 1 adenocarcinomas were the most common histology (95 %) while 70% presented stage I tumors. The proportion of cases lacking at least one of the MMR proteins was 25%; the most commonly absent was hMLH1 (15%), followed by hMSH2 (10%) while none of the cases showed an absence for hMSH6 (Figure 1). According to the MLE results, EC patient diagnosed 50 years and those with a FHC related to LS had higher odds of lacking MMR protein expression than their counterparts, after adjusting for potential predictors (Table 2). Moreover, EC patients with lower BMIs presented more than twofold the odds of having an absence of an MMR protein compared to those EC patients with higher BMIs (OR: 2.4; 95% CI = 0.4–14.3). The Bayesian approach showed that our sample had over 94% posterior probabilities of an excess risk of lacking the

expression of an MMR protein among the EC patients diagnosed ≥ 50 years, with FHC related to LS, or presenting lower BMIs (Table 2).

Discussion

To our knowledge, this is the first study that evaluates EC in PR through molecular epidemiological approaches and the expression of MMR proteins in a sample that is 100% Hispanic. The proportion of patients that presented tumors lacking at least one MMR protein in our study (25%) is similar to previous studies in other populations^{6,11} ranging between 23 to 26%. Other studies have shown different proportions,^{5,12–13} which might be due to the number of MMR proteins and/or the age group evaluated. Similarly to other studies^{6,11,13} the MMR protein most commonly absent was hMLH1. The absence of the hMLH1 protein may be secondary to somatic methylation of this gene, but our study did not examine hMLH1 methylation; nonetheless our cases had a phenotype consistent with LS. Meanwhile, our results may be affected by selection bias as only 20 consecutive cases of the original sample were evaluated. However when we compared our cases with the rest of the original sample (cases without IHC performed) with respect to demographic and clinical covariates, no differences were observed ($P>0.10$; data not shown).

Age at EC diagnosis, FHC related to LS, and lower BMI seem to play an important role in the absence of these MMR proteins. Our observed positive association between absence of MMR proteins in EC patients and younger age at diagnosis (age < 50 years), FHC related to LS, and lower BMI is consistent with previous studies^{6,12} and suggests a genetic defect in one of the MMR genes as the underlying etiology for the observed lack of protein expression. Patients with age at diagnosis ≥ 50 years and FHC related to LS presented more than a 90% chance of having an excess risk of lacking an MMR protein. This high probability is not rare since both characteristics have been acknowledged as criteria to identify LS cases.

Meanwhile, those EC patients with lower BMI presented the higher probability (99.6%) for an excess risk of not expressing these proteins. Our observation of increased likelihood of absence of MMR protein among those EC patients with lower BMI compared to their counterparts is consistent with previous studies^{6,14} showing that non-obese EC patients have higher risks of having defects of the MMR system. Although increased BMI is associated with increased risk of EC, it has been suggested a different pathway for the association between decreased BMI and microsatellite instability positive tumors (somatic change in length on allele, as a result of mutations in the MMR genes).¹⁵

In conclusion, although our results might not be generalizable to all EC cases in PR, our study in this Hispanic population supports previous studies in that younger age, FHC, and lower BMI are associated with increased odds of having an absence of MMR protein expression. EC patients with these characteristics should be considered for genetic testing in order to determine the presence of LS. Identifying these patients may have an important impact on the development of personalized medicine. Further studies with larger sample sizes are warranted.

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Figure 1.

IHC[†] results for hMLH1, hMSH2, and hMSH6 proteins. (n = 20)

[†] IHC =Immunohistochemistry; [‡] MMR =Mismatch Repair; * Two tumors showed a negative internal control for one particular protein (hMSH6). These tumors had a negative expression for at least one MMR protein.

Table 1

Study group description. (n=20)

<i>Continuous data</i>	Mean $\pm$ SD
Body Mass Index	33.4 $\pm$ 8.2
Age at diagnosis	55.2 $\pm$ 14.1
<i>Categorical data</i>	n (%)
Socio-demographic data	
Education	
Less than High School	8 (40)
High School graduate	6 (30)
College Graduate or Beyond	6 (30)
Medical Insurance	
Private	6 (30)
Health Reform	8 (40)
Medicare	6 (30)
Histopathological data	
Pathology	
Type 1 Adenocarcinoma	19 (95)
Type 2 Adenocarcinoma	1 (5)
Stages	
I	14 (70)
II	3 (15)
III or IV	3 (15)
Grades^a	
G1	16 (84)
G2	1 (5)
G3	2 (11)
LS associated cancer in at least one first degree relative^b	
	3 (15)

^aPercentages were based on 19 cases due to one unknown grade tumor.^bLynch syndrome associated cancer included: endometrial, colon, kidney, stomach, bowel, ovaries, and brain

Table 2

Magnitude of the association between MMR[†] protein expression status and BMI, age at diagnosis, and FHC related to LS, adjusting for potential predictors. (n = 20)

Characteristic	MLE* OR _{adjusted} (95%CI)	Bayesian approach ^b Pr (OR _{adjusted} >1 data)
BMI^a		
Difference between two BMI = SD	2.38 (0.39–14.28)	0.996 ^c
Age at endometrial cancer diagnosis		
< 50 years old	12.4 (0.5–322.7)	0.96
50 years old	1.00	
Family history of first-degree relative with cancer associated to Lynch Syndrome		
Yes	17.7 (0.6–534.6)	0.95
No	1.00	

[†]MMR = Mismatch repair;

^aReference group was the highest BMI (1/OR);

^bLogistic Regression using non-informative priors based on 500 simulations after discarding the first 501 iterations. The distribution used was the following: dunif(−3,3) for age at diagnosis and family history of cancer and dunif(−1,1) for BMI. The step function was used to determine the probability;

^cPr[(1/OR_{adjusted})>1|data];

* All the variables were used in the model (MLE = maximum likelihood estimation).