Gynecologic Oncology 135 (2014) 81–84

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Lynch Syndrome in patients with clear cell and endometrioid cancers of the ovary

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HIGHLIGHTS

- Women with clear cell/endometrioid ovarian cancer are at risk for Lynch Syndrome.
- Synchronous and metachronous malignancies are more common in these patients.
- Screening for MMR expression is recommended in patients under 53 with these tumors.

ARTICLE INFO

Article history:
Received 17 May 2014
Accepted 28 July 2014
Available online 2 August 2014

Keywords:
Lynch Syndrome
Ovarian cancer
Microsatellite instability
Mismatch repair
Endometrioid ovarian carcinoma
Clear cell ovarian carcinoma

ABSTRACT

Objective. Patients with Lynch Syndrome are at an increased risk for a variety of malignancies, including ovarian cancer. Ovarian cancers associated with Lynch Syndrome are predominantly clear cell or endometrioid in histology. Lynch Syndrome is characterized by germline mutations in mismatch repair (MMR) genes. The current study aims to assess the prevalence of loss of MMR expression in patients with endometrioid and clear cell ovarian carcinoma.

Methods. A retrospective review identified 90 patients with endometrioid and/or clear cell carcinomas. Slides made from tumor tissue microarray blocks were evaluated using immunohistochemical stains with antibodies against MLH1, PMS2, MSH2, and MSH6. Statistical analysis was performed.

Results. Seven of the 90 cases (7.8%) had loss of MMR expression. The mean age of patients with loss of MMR expression (47 years) was significantly younger than those with retained MMR expression ($p = 0.014$). Loss of MMR expression was present in 20% of patients under the age of 53 with clear cell or endometrioid cancers. Genetic studies found that 3 of the 5 patients with loss of MMR expression carried mutations consistent with Lynch Syndrome; acquired hypermethylation of MLH1 was noted in one patient. Six of 7 patients (86%) whose tumors lacked MMR expression had synchronous or metachronous primary malignancies, a significantly greater prevalence than those with retained MMR expression ($p < 0.001$).

Conclusion. Patients under the age of 53 with clear cell or endometrioid ovarian carcinomas are at a clinically significant risk for loss of MMR expression and Lynch Syndrome; routine screening with immunohistochemical staining should be considered.

Introduction

The role of genetic evaluation in patients with ovarian cancer has historically focused on BRCA testing in patients with serous cancers of the ovary [1,2]. Women with Lynch syndrome (hereditary non-polyposis colorectal carcinoma), however, are also at increased risk of ovarian cancer [3]. Commonly associated with an increased risk of colon and endometrial cancer, Lynch syndrome may also be associated with ovarian, urothelial, and pancreatic malignancies [4,5]. The risk of ovarian cancer in women with Lynch Syndrome is estimated at 7–12% [6,7]. These malignancies are primarily non-serous in histology, and the majority are clear cell or endometrioid [8–15]. Lynch syndrome is characterized by loss of expression of mismatch repair (MMR) genes. The four clinically significant MMR genes are MSH6, MSH2, MLH1, and PMS2 [16–18]. Immunohistochemical staining can be performed on fixed tumor tissue to assess for loss of MMR expression [19–22]. Although women with serous ovarian cancers are often referred for

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http://dx.doi.org/10.1016/j.ygyno.2014.07.100
0090-8258/

Table 1
Clinocopathologic characteristics of ovarian endometrioid and clear cell carcinomas with and without MMR protein loss of expression (LOE).

	MMR LOE (n = 7)	Normal MMR (n = 83)	P value
Age at diagnosis	47 y (39–53 y)	58 y (38–84 y)	0.014
Incidence under age 53			
≤53 y	100% (7)	34% (28)	<0.001
>54 y	0% (0)	66% (55)	
Synchronous or metachronous malignancy			
Other primary malignancy	86% (6)	13% (11)	<0.001
No other primary malignancy	14% (1)	87% (72)	
Histology			
Clear cell	14% (1)	38% (32)	n.s.
Endometrioid	86% (6)	57% (47)	
Mixed	0% (0)	5% (4)	
Tumor grade			
1	29% (2)	20% (17)	n.s.
2/3	71% (5)	80% (66)	
FIGO stage			
I	71% (5)	72% (60)	n.s.
II-IV	29% (2)	28% (23)	
Five year overall survival rate	50%	57%	n.s.

BRCA testing, women with clear cell or endometrioid ovarian cancers are not routinely screened for Lynch Syndrome [23]. The current study was undertaken to assess the prevalence of Lynch Syndrome in patients with clear cell and endometrioid cancers of the ovary. A high prevalence of MMR deficiency in this population would support routine screening for Lynch Syndrome.

Methods

This is a retrospective review of cases at the Queens Medical Center in Honolulu diagnosed between January 1, 1995 to April 12, 2013 with clear cell cancer and endometrioid cancer of the ovary. The study was approved by the Institutional Review Board. Patients were identified through the hospital tumor registry and the Pathology Department database. Ninety cases were identified for inclusion. All paraffin-embedded tissue blocks were retrieved for immunohistochemical analysis and patient charts were reviewed for clinicopathologic variables.

A gynecologic pathologist (DS) reviewed hematoxylin and eosin-stained whole-slide sections of the selected cases. Upon confirmation of tumor histologic type per World Health Organization (WHO) guidelines [24], formalin-fixed, paraffin-embedded tissue microarray blocks were constructed from 2.0 mm cores of representative tumor samples. In cases demonstrating mixed endometrioid and clear cell histology, representative areas from each histologic type were selected. Synchronous primary ovarian and endometrial carcinomas were verified as independent primaries using criteria outlined by the WHO [24].

Antigen retrieval was performed with EnVision FLEX Target Retrieval Solution, High pH (Dako, Carpinteria, CA) at 97 degrees C for 20 minutes. Evaluation of MMR protein expression was performed on 4-μm sections of the tissue microarray blocks using antibodies to MLH1 (clone G168-15, 1:75 dilution, Biocare Medical, Concord, CA), PMS2 (clone A16-4, 1:300 dilution, Biocare Medical), MSH6 (clone BC/44, 1:100 dilution, Biocare Medical) and MSH2 (clone FE11, 1:100 dilution, Biocare Medical). Detection was obtained using the MACH 3 Mouse HRP-Polymer Detection Kit (Biocare Medical). Chromogenic detection was achieved with diamniobenzidine (Dako) and sections were counterstained with hematoxylin (Dako).

Results were evaluated by a gynecologic pathologist experienced in MMR protein immunohistochemical interpretation. Staining of any tumor nuclei was interpreted as positive, with expression by lymphocytes and/or stromal cells considered a positive internal control. Ten tissue microarray results without nuclear staining were then validated with whole-slide sections of the tumor by the aforementioned staining and interpretative procedure. Seven of these cases had validated and confirmed MMR loss of expression.

Clinicopathologic data were obtained via review of electronic medical records, paper charts, and the institutional cancer registry. For all cases, variables collected included age at diagnosis, tumor grade, International Federation of Gynecology and Obstetrics (FIGO) stage, synchronous or metachronous primary malignancies, vital status, disease status, and date of last contact. For cases demonstrating loss of MMR protein expression, results of germline testing DNA sequence analysis, if available, were also obtained (Ambry Genetics, Aliso Viejo, CA; Myriad Genetic Laboratories, Salt Lake City, UT). One case exhibiting loss of MLH1 expression was sent to a reference laboratory for MLH1 promoter hypermethylation PCR analysis (Mayo Medical Laboratories, Rochester, MN).

Statistical analyses were performed using the two sample t-test and Fisher's exact test as appropriate for continuous and categorical variables. Overall survival was assessed and a Kaplan-Meier curve constructed. A p value of <0.05 was considered statistically significant.

Results

At Queen's Medical Center, Honolulu, 438 cases of epithelial ovarian cancer were identified between January 1, 1995 and April 12, 2013. Ninety (20%) had clear cell or endometrioid histology; 53 endometrioid tumors, 33 clear cell tumors, and 4 with mixed clear cell and endometrioid histology. Seven (7.8%) of the tumors demonstrated confirmed loss of MMR expression. Clinical and pathologic characteristics are summarized in Table 1. The mean age of patients with MMR protein loss of expression was 47 years, significantly younger than those with normal expression ($p = 0.014$). Within the subgroup of patients under the age of 53, 7 of 35 (20%) demonstrated loss of MMR expression. A majority of patients with loss of MMR expression (86%) were diagnosed with a synchronous or metachronous primary malignancy, significantly more than those with normal MMR expression ($p < 0.001$). Differences in histology, grade, stage, and overall survival were not statistically significant.

Clinicopathologic characteristics of the 7 patients with MMR protein loss of expression are displayed in Table 2. Five of the 7 patients with MMR deficient tumors underwent subsequent genetic analysis. Three of these patients were found to carry a deleterious MMR gene mutation consistent with Lynch Syndrome; one patient had hypermethylation of the MLH1 promoter region, and another patient was found to have an MSH6 variant of uncertain significance. Two patients with loss of

Table 2
Clinicopathologic features of patients with MMR protein loss of expression (LOE).

Case	Age	MMR LOE	Histologic subtype	Grade	FIGO Stage	Synchronous or metachronous primary malignancy	Additional genetic testing
1	39	MLH1	Endometrioid	1	I	Endometrial	MLH1 promoter hypermethylation
2	42	MSH2	Endometrioid	1	I	Endometrial	Deleterious mutation R621X (Lynch syndrome)
3	47	MLH1	Endometrioid	2	II	Colorectal	Deleterious mutation Q426X (Lynch syndrome)
4	48	MSH2	Endometrioid	3	III	None	Not performed
5	51	MSH6	Endometrioid	2	I	Endometrial	Not performed
6	52	MSH6	Endometrioid	3	I	Endometrial	Deleterious mutation E1163X (Lynch syndrome)
7	53	MSH6	Clear cell	3	I	Pancreatic	MSH6 variant of uncertain significance (G1216E)