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Original Research

Retrospective Analysis of Molecular Markers in Endometrial Cancer: Single Center Experience

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Abstract

Background: To emphasize the effect of molecular markers on prognosis in endometrial cancer, in addition to the International Federation of Gynecology and Obstetrics (FIGO) 2009 classification. **Methods**: The records of 160 patients with endometrial cancer between 2008 and 2022 were retrospectively reviewed. Staging was done according to FIGO 2009 criteria. Patients were divided into 4 groups according to molecular classification. If one had polymerase epsilon (*POLE*) mutation, the patient was included in *POLE* ultramutated (POLEmut) group. In case of intakt *POLE*, but abnormal staining of mismatch repair (MMR), the group was diagnosed as mismatch repair defciency (MMRd). If there was only *p53* abnormal results detected, that group was p53-abnormal (p53mut). If no mutation at all, that group was categorized as non-specific molecular profile (NSMP). The Kaplan-Meier method was used to evaluate overall survival and progression-free survival. Survival rates were compared for molecular markers. **Results**: According to the molecular analysis, 4 patients (2.5%) were classifed as POLEmut group, 53 patients (33.1%) in the MMRd group, 18 patients (11.3%) had p53mut, and 85 patients (53.1%) into the NSMP group. 5-year overall survival was 79.4%, 5-year progression-free survival was 90%. 5-year overall survival was 75% in POLEmut group, 84.9% in MMRd group, 38.9% in p53mut group and 84.7% in NSMP group ($p = 0.001$). 5-year progression-free survival was 100% in POLEmut group, 96.2% in MMRd group, 77.8% in p53mut group and 88.2% in NSMP group ($p = 0.082$). **Conclusion**: Our study shows the prognostic value of the molecular endometrial cancer classification. Patients with p53mut have a poor progression-free survival, POLEmut endometrial cancer have a good prognosis. In this study, we wanted to demonstrate the importance of molecular markers in endometrium cancer and their contribution to prognosis.

Keywords: endometrial carcinoma; molecular markers; survival

1. Introduction

Endometrial carcinoma (EC) is the sixth most ordinarily analyzed disease in ladies around the world [1]. The American Cancer Society reports that there will be an estimated 61,880 new cases and 12,550 women will die due to EC in the United States in 2022 [2]. EC has been divided into two subtypes based on histopathological features, expression of hormone receptors, and grade. However, recently molecular classification based on immunohistochemistry has emerged and become accepted [3]. In 2013, The Cancer Genome Atlas Research Network (TCGA) performed characterization of EC in 373 cases [4]. Polymerase epsilon (POLE)-ultramutated, MSI (Microsatellite instability) hypermutated, copy-number low (endometrioid) and copy-number high (serous-like) are the subgroups of this molecular classifcation. In 2015, Talhouk *et al*. [5] developed the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), which demonstrates an easy classification system for ECs. POLE-ultramutated (POLEmut), p53-abnormal (p53abn), mismatch repair deficient (MMRd) and No Specific Molecular Profile Subgroup (NSMP) are the subgroups of this molecular classifcation [5]. With the increasing number of studies emphasizing the importance of molecular markers in EC, International Federation of Gynecology and Obstetrics (FIGO) published a new staging system in 2023 and molecular markers are now included in the staging system [6]. The molecular EC characterization has shown great prescient worth utilizing this strategy [7]. This study aimed to retrospectively screen and present molecularly classified endometrial cancer cases in our clinic.

2. Methods

The study was approved by the Afyonkarahisar Health Sciences University Ethics Committee decision numbered 2011-KAEK-2 (dated 02/06/2023) and the research was

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continued in accordance with the Declaration of Helsinki. The inclusion criteria of the study; the patients were diagnosed with endometrial cancer by endometrial biopsy and they were operated in our clinic. Exclusion criteria are sarcoma in the postoperative pathology report results, synchronous tumor and patients who do not come for follow-up. As a result, 160 patients who were operated on with the diagnosis of endometrial cancer between 2008 and 2022 were included in the study. Patients' age, height and weight were recorded. Carbohydrate antigen 125 (CA125) levels were checked from the laboratory values of the patients. Participants were categorized into two groups, namely, non-obese ($<$30.0) and obese ($\geq$30.0), according to the World Health Organization classification [8]. Endometrial cancer cases were staged according to the FIGO 2009 system. Serous and clear cell cancer types were accepted as grade 3. The cases were examined in 4 groups according to molecular classification. Mismatch repair defcient (MMRd) including PMS homolog 2 (*PMS-2*), MutS homolog 6 (*MSH-6*), MutL homolog 1 (*MLH-1*), MutS homolog 2 (*MSH-2*) and *p53* were evaluated immunohistochemically (IHC) from selected formalin-fixed paraffin-embedded blocks. Sequence analyzes of DNA polymerase epsilon, catalytic subunit (*POLE*) gene 9th and 13th exons were performed from genomic DNA by Sanger Sequencing method. If one had *POLE* mutation, the patient was included in POLEmut group. In case of intakt POLE, but abnormal staining of MMR, the group was diagnosed as MMRd. If there was only *p53* abnormal results detected, that group was p53mut. If no mutation at all, that group was categorized as NSMP.

The collected data were recorded in the IBM SPSS Statistics for Windows (Version 26.0. Armonk, NY, USA) and statistical analyzes were performed. For continuous variables, standard deviation, mean and median were calculated. Chi-square test was used for categorical variables. As a result of statistical analysis, values of $p < 0.05$ were considered significant. Kaplan-Meier curves were drawn to show progression free survival (PFS) and overall survival (OS).

3. Results

In our study, 160 patients were included, the mean age was 59.94 $\pm$ 9.2 and the mean body mass index (BMI) was 33.54 $\pm$ 6.2 kg/m^2. BMI $<$30 in 54 (33.8%) patients, BMI $\geq$30 in 106 (66.2%) patients. The median CA125 value was 16 IU/mL (3 IU/mL–4344 IU/mL). The mean tumor size was 3.97 $\pm$ 2.1 cm. The most frequently detected histopathological type was endometrioid type endometrial cancer and was detected in 136 patients (85%). We detected recurrence in 16 patients. 33 of the patients died. 127 patients are alive and continue to be followed up. In the study, the mean follow-up time was 35 months (range: 2–124 months). Of the endometrial cancer cases consisting of 160 patients, 67.5% had stage I, 7.5% had stage II, 16.9% had stage III and 8.1% had stage IV cancer. It was seen that the majority of the patients were in the stage 1 group. Table 1 shows the postoperative pathology results. While 84 (52.5%) patients were treated laparoscopically, 76 (47.5%) patients underwent laparotomic surgery. According to postoperative pathology results, 54 patients were followed up without treatment, 43 patients were given radiotherapy treatment, 29 patients were given chemotherapy, and 34 patients were given chemoradiotherapy treatment.

Table 1. Pathology findings.

	Number of patients
Surgical stage	
IA	64 (85%)
IB	44 (27.5%)
II	12 (7.5%)
IIIA	14 (8.8%)
IIIB	0 (0%)
IIIC1	12 (7.5%)
IIIC2	1 (0.6%)
IVA	0 (0%)
IVB	13 (8.1%)
Histology	
Endometrioid adenocarcinoma	136 (85%)
Non-endometrioid adenocarcioma	24 (15%)
Clear cell carcinoma	2 (1.3%)
Serous carcinoma	20 (12.5%)
Malignant Mix Mullerian Tumor	1 (0.6%)
Mucinous carcinoma	1 (0.6%)
Grade	
1	56 (35%)
2	62 (38.8%)
3	42 (26.2%)
Lymphovascular space invasion	
Negative	111 (69.4%)
Positive	49 (30.6%)
Myometrial invasion	
$<$1/2	80 (50%)
$\geq$1/2	80 (50%)
Cervical involvement	
Positive	31 (19.4%)
Negative	129 (80.6%)
Abdominal cytology	
Positive	27 (16.9%)
Negative	133 (83.1%)

According to the molecular analysis, 4 patients (2.5%) were classifed as POLEmut group, 53 patients (33.1%) in the MMRd group, 18 patients (11.3%) had p53mut, and 85 patients (53.1%) into the NSMP group. In the MMRd group, 9 patients had *MSH-2* mutation, 7 patients had *MSH-6* mutation, 45 patients had *PMS-2* mutations and 33 patients had *MLH-1* mutation. Double mutation was seen in 46 patients, triple mutation in 2 patient, and quadruple mutation in 1 patient. In our study, stage (stage IA and stage IB–IVB), histological subtype, cervical involvement,

Table 2. Comparison of clinical and pathological findings with molecular markers.

	Number of Patients					*p* value
	POLEmut	MMRd	P53mut	NSMP	Total group	
Surgical stage						
IA	1	20	6	37	64	<0.001*
IB–IVB	3	33	12	48	96	
Histology						
Endometrioid adenocarcinoma	3	52	7	74	136	<0.001*
Non-endometrioid adenocarcinom	1	1	11	11	24	
Grade						
1	1	17	4	34	56	<0.001*
2	2	27	1	32	62	
3	1	9	13	19	42	
LVSI: lymphovascular space invasion						
Negative	2	37	9	63	111	<0.001*
Positive	2	16	9	22	49	
Cervical involvement						
Positive	3	40	13	73	129	<0.001*
Negative	1	13	5	12	31	
Abdominal cytology						
Positive	4	47	11	71	133	<0.001*
Negative	0	6	7	14	27	

*, $p < 0.05$. POLEmut, polymerase epsilon ultramutated; MMRd, mismatch repair deficiency; p53mut, p53-abnormal; NSMP, non-specific molecular profile.

lymphovascular space invasion (LVSI), cytology, and grade had significant relationship with the molecular subtype. Table 2 shows comparison of clinical and pathological findings with molecular markers.

5-year OS and PFS rates were compared in terms of POLEmut, MMRd, P53mut and NSMP. In our study, 5-year OS of endometrial cancers was 79.4%, 75% in POLEmut group (one POLEmut patient died due to cardiovascular reasons during follow-up), 84.9% in MMRd group, 38.9% in p53mut group and 84.7% in NSMP group (p = 0.001) (Fig. 1).

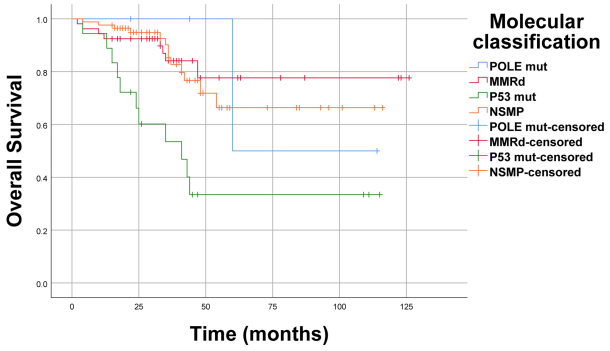

Fig. 1. Overall survival curves. POLEmut, POLE ultramutated; MMRd, mismatch repair deficiency; P53mut, p53-abnormal; NSMP, non-specific molecular profile.

Vaginal examination, CA125 levels and imaging methods (magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography and computed tomography (PET-CT)) were used to detect recurrence. During the postoperative follow-up of the patients, recurrence was detected in 16 of 160 patients. In our study, 5-year PFS of endometrial cancers was 90%. No recurrence was detected in any patient with *POLE* mutation. 5-year PFS was 100% in POLEmut group, 96.2% in MMRd group, 77.8% in p53mut group and 88.2% in NSMP group (p = 0.082) (Fig. 2).

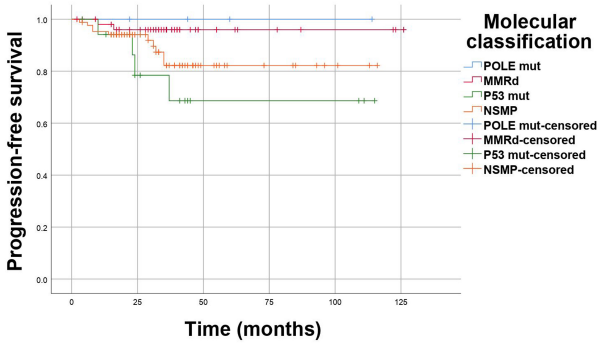

Fig. 2. Progression-free survival curves.

4. Discussion

Endometrial cancer is usually seen in the postmenopausal period and the average age of incidence is 63 years [9]. Endometrial cancer cases are rare in women un-

der the age of 45 and in the premenopausal period. Survival was found to be better in patients aged 40 years and younger than patients over 40 years of age [10]. In a large-scale study conducted in our country, patients were divided into 4 groups in terms of age (<40 years, 40–49 years, 50–59 years, >60 years) and the *p* value was found to be significant in terms of 5-year survival [11]. In our study, the mean age of incidence of endometrial cancer was 59.94 ± 9.2 years, similar to the literature.

5-year survival is 74–91% for stage 1–2, 57–66% for stage 3, 20–26% for stage 4 endometrial cancer [12]. In a recent study conducted in our country, the overall survival of endometrial cancer was 85% [11]. Hamilton *et al*. [13] reported that the 5-year PFS was 80% for stage I/II, 29% for stage III and 10% for stage IV. Eltabbakh *et al*. [14] reported that the 5-year PFS and OS of the study group was 95.2% and 96.4%. In our clinic, similar to the literature, 5-year OS was 79.4%, and 5-year PFS was 90%.

Molecular markers can be used to predict recurrence risk and therefore survival [15]. It has been found that adding molecular classification to pathological diagnosis may be very important in improving the clinical approach of cases with EC. Piulats *et al*. [16] propose the incorporation of TCGA molecular classification as an option for assessing prognosis in EC patients. They found that disease-specific survival was 100% in the POLEmut group, 82% in the microsatellite instability group, 42.9% in the serous like group and 77.8% in the copy-number low group [16]. Leon-Castillo *et al*. [17] reported that 5-year recurrence rates was 36.7% for cases with *p53* abnormal group, 0% for POLEmut group, 13.4% for MMRd group and 42.9% for NSMP group. A recent study by Bilir *et al*. [18] reported that the POLEmut group had the highest OS. The molecular distribution of endometrial cancer in a study by Jamieson *et al*. [19], including 172 patients, was as follows: 21 POLEmut (12.2%), 47 MMRd (27.3%), 74 NSMP (43.1%) and 30 p53mut (17.4%). They found that molecular classification in EC was associated with lymph node metastasis and that molecular classification could be obtained in preoperative biopsies [19]. Van Gool *et al*. [20] supported the reduction of adjuvant therapy in early-stage endometrial cancer cases with *POLE* mutation. *POLE* mutations in cases of metastatic endometrial cancer may help identify candidates for targeted therapies. Recent studies are promising for POLEmut endometrial cancer cases [21,22]. Similar to the literature, we found that the PFS was 100% in POLEmut group and the lowest PFS (77.8%) was in p53mut group. No relapse was detected in the follow-up of 4 patients with *POLE* mutation (2 patients stage 1b grade 2, 1 patient stage 3a grade 2, 1 patient stage 1 grade 1).

As it is known, FIGO changed the staging of endometrial cancer in 2023 [6]. The committee found that risk stratification, including the recently developed molecular classification, helps better define the clinical management of endometrial cancer. In addition to molecular markers, LVSI is also included in the FIGO new staging system [23]. Many studies have shown that risk assessment in early stage endometrial carcinoma is improved by adding molecular factors [24–26]. These changes to the endometrial staging system by FIGO have shed light on the clinical management of endometrial cancer since the publication of the 2009 system [27].

Our study has some limitations. The majority of our patient group has early stage endometrial cancer and our study is a retrospective single center study. The exclusion criteria of sarcoma or synchronous tumor in the postoperative pathology report results may have caused the higher surveillance of uterine cancers in our study. Another limitation of our study is that it is single-center and the number of patients is therefore small.

5. Conclusion

Molecular classification of endometrial cancer has become important with the FIGO 2023 staging system. We wanted to retrospectively screen and present molecularly classified endometrial cancer cases in our clinic. Our study shows the clinical impact of molecular EC classification in addition to grade and histopathological type in endometrial cancer. Patients with p53mut have a poor PFS, POLEmut EC have a good prognosis. In this study, we wanted to demonstrate the importance of molecular markers in endometrium cancer and their contribution to prognosis.

Availability of Data and Materials

All data are available from the corresponding author upon reasonable request.

Author Contributions

CYO: Concept, Performed the research, Writing the Article. DTA: Concept, Control, Supervision, Critical Revision. NC: Performed the research, Validation, Drafting the manuscript. MC: Analyzed the data, Literature Review, Critical Revision. FB: Analyzed the data, Writing the Article. CO: Pathological evaluation of molecular markers, Analyzed the data, Critical Revision. HD: Literature Review, Analyzed the data, Writing the Article. ESAS: *POLE* gene analysis, Analyzed the data, Drafting the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was approved by the Afyonkarahisar Health Sciences University Ethics Committee decision numbered 2011-KAEK-2 (dated 02/06/2023) and the research was continued in accordance with the Declaration of Helsinki. Consent was obtained from all patients during their hospitalization.

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Conflict of Interest

The authors declare no conflict of interest.

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