RESEARCH ARTICLE

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Mismatch Repair Protein Deficiency Does Not Affect Disease Free Survival in Type I Endometrial Carcinoma

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Abstract

Background: This study aimed to analyze the correlation between the 3-year disease-free survival (DFS) and mismatch repair (MMR) protein levels in patients with type 1 endometrial carcinoma. Many studies have reported different results regarding the role of MMR in the prognosis of endometrial carcinoma; therefore, we aimed to identify this association in our hospital. **Methods:** This observational study employed a historical cohort design and included patients with type 1 endometrial carcinoma who underwent surgery at Dr. Soetomo Hospital between January 2017 and December 2019. Medical records and paraffin blocks meeting these criteria were obtained. MMR proteins (MLH1 and MSH2) were assessed using immunohistochemistry. **Results:** A total of 46 patients with type 1 endometrial carcinoma were analyzed. We observed MMR deficiency (dMMR) in 12 patients (26.1%) and MMR proficiency (pMMR) in 34 patients (73.9%). Of the 12 patients with dMMR, nine cases (75%) were diagnosed as stage I and 7 (58.33%) as low grade. The 3-year DFS in patients with dMMR and pMMR was 83.3% and 67.6%, respectively (Hazard Ratio 2.31, 95% CI 0.5135-10.475, p=0.27). Higher stages had a 5.42 times increased risk of recurrence (95% CI 1.3378-21.9358, p=0.018). Higher histopathological grades were also associated with 8.65 times increased risk of recurrence (95% CI 2.5020-29.8738, p=0.001). **Conclusion:** Patients with dMMR had a better DFS compared to those with pMMR; however, the difference was not statistically significant. The tumor stage and histopathological grade were independent risk factors for recurrence.

Keywords: Endometrial carcinoma- mismatch repair deficiency- disease free survival

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Introduction

In this study we aim to analyze the correlation between DFS and dMMR in patients with type 1 endometrial carcinoma. No study on MMR status in patients with endometrial carcinoma has been conducted in Indonesia. This is the first study on MMR status in patients with endometrial carcinoma in Surabaya, Indonesia.

Endometrial carcinoma is the sixth most common cancer in women. Its incidence is higher in developed countries and is annually increasing. Its incidence in North America is 21.1/100,000 and in Southeast Asia is 6.6/100,000, with a mortality rate of 2/100,000 (Sung et al., 2021).

There are several risk factors for endometrial carcinoma, including obesity, nulliparity, age, race, unopposed estrogen levels, and genetics. Only 5-10% of endometrial carcinomas are caused by genetic mutations (Lynch syndrome). Lynch syndrome is an autosomal-dominant disease caused by germline mismatch repair mutations. Most (30–40%) mutations in MMR proteins are sporadic mutations: 54% in mutL Homolog-1 (MLH1), 21% in mutS Homolog-2 (MSH2), 16% in mutS Homolog-6 (MSH6), and post-meiotic segregation-2 (PMS2). MMR proteins are heterodimers of MLH1, MSH2, MSH6, and PMS2. MMR deficiency is defined as the loss of one of the proteins, determined using immunohistochemistry (IHC) (Concin et al., 2021).

The proportion of sporadic MMR mutations is affected by race. Asian women are more likely to develop dMMR than Caucasian women (25.1% vs. 23.6%). Furthermore, black women tend to have p53 mutations (49%) (Jumaah et al., 2021).

In 2013, The Cancer Genome Atlas (TCGA) classified endometrial carcinoma into four categories: POLE mutation, low copy number, microsatellite instability (MSI), and p53 abnormality (Siegenthaler et al., 2022). The TCGA molecular classification was used to identify the prognosis in addition to the clinicopathological prognosis. MSI is an MMR protein phenotype with an intermediate prognosis. POLE mutations are associated with the best prognosis, whereas p53 abnormalities are

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associated with a worse prognosis. MSI is usually found in type I endometrial carcinoma (95–98%) and p53 abnormalities in type II endometrial carcinoma (Arciuolo et al., 2022).

ESGO/ESTRO/ESP recommends MMR screening using IHC for all endometrial carcinoma cases to determine the prognosis of the disease. The MMR status can be used to administer immunotherapy based on the 2019 FDA guidelines for recurrent or metastatic endometrial carcinoma. Almost 99% of endometrial carcinomas with dMMR have cytotoxic T-cell infiltration and high tumor neoantigen levels; therefore, pembrolizumab/PD-1 blocking agents play a role in dMMR endometrial carcinomas (Njoku et al., 2022).

Previous studies have reported different results regarding the role of MMR in the prognosis of endometrial carcinoma. Study from Donostia Hospital Spain, there was no difference in the overall survival (OS) in endometrial carcinoma with dMMR or pMMR (56.4 months versus 56.6 months) (Ruiz et al., 2014). Another study from Canada found that endometrial carcinoma with dMMR had a worse prognosis than that with pMMR, with a progression-free survival (PFS) of 24 months in dMMR and 27 months in pMMR (p=0.04) (Kim et al., 2020). A study from Japan reported that patients with dMMR had a better prognosis than those with pMMR, with a PFS of 92% versus 78% (p<0.01) (Kato et al., 2015).

We conducted this study due to the different results regarding the role of MMR in the prognosis of endometrial carcinoma and the effect of race on dMMR; therefore, we aimed to determine this prognosis in our hospital. Endometrial carcinoma with dMMR has a high neoantigen production and cytotoxic T cell lymphocyte infiltration therefore PD-1 blocking agent has a benefit. The National Comprehensive Cancer Network (NCCN) recommended immunohistochemistry (IHC) study for detecting dMMR in endometrial carcinoma and PD-1 blocking agent/pembrolizumab for standard therapy in advanced stage or reccurence endometrial carcinoma with dMMR. In addition, the FDA has also approved pembrolizumab/PD-1 blocking agents for recurrent and metastatic endometrial carcinoma with dMMR (Yen et al., 2020).

Materials and Methods

This analytical observational study employed a historical cohort design and was conducted at Dr. Soetomo Hospital in Surabaya, Indonesia. We obtained the specimen which had preserved a good tissue morphology, consisted 80% tumor cells, and no necrotic areas. The specimen was placed into formalin-fixed paraffin-embedded blocks (FFPE). We took tissue from the original FFPE to make an Array block. Then we placed a thin layer of tissue on a slide and did the IHC for MLH1 and MSH2 protein. We used G168-728 antibody for MLH1 IHC and G219-1129 antibody for MSH2 IHC. Positive staining (>10%) for two proteins was interpreted as MMR proficiency (pMMR) and one negative staining (<10%) was interpreted dMMR. The 3-year DFS was obtained from medical records. This study was approved by the hospital's ethics committee (certificate number: 0616/KEPK/III/2023).

The inclusion criteria were patients with type 1 endometrial carcinoma who underwent primary surgery at Dr. Soetomo Hospital between January 2017 and December 2019. The patients were required to have complete medical records and paraffin blocks obtained during their surgeries. Primary surgery included total hysterectomy, bilateral salpingo-oophorectomy, and lymph node dissection if indicated. Patients with type 2 endometrial carcinoma, residual disease, or neoadjuvant therapy prior to the primary surgery were excluded.

Patient demographic data included age at diagnosis, body mass index (BMI), parity, menopausal status, stage according to the 2009 International Federation of Gynecology and Obstetrics (FIGO), tumor grade, lymphovascular invasion, and depth of myometrial invasion.

Specimens that preserved good tissue containing 80% cancer cells and no necrotic areas were placed in formalin-fixed paraffin-embedded blocks. IHC was performed for two MMR proteins (MLH 1 and MSH2). The slides were reviewed by a pathologist. The intensity of nuclear staining was assessed: specimens with <10% of tumor cells were labelled as negative, and those with >10% of tumor cells were labelled as positive (Doghri et al., 2019). Positive results for both proteins were interpreted as MMR proficiency (pMMR), and negative results for one of the two proteins were interpreted as MMR deficiency (dMMR) (Pina et el., 2018).

The sample size was calculated using the hypothesis of two proportional populations (Relative Risk) and was found to be 46 patients. We used 80% statistical power and a two-sided alpha error of 0.05 for the calculated sample size. Patient demographic data and characteristics were assessed using chi-squared tests. Survival analysis was performed using the log-rank test to compare the DFS between the dMMR and pMMR groups. Cox regression analysis was used to evaluate the association between dMMR, patient characteristics, and DFS. The DFS for each group is presented using Kaplan–Meier graphs. The event was death and censor was defined as no death until the time of study. We confirmed the event and censor by tracing the medical record in 3 year study from 2017 until 2019. Statistical significance was set at p=0.05 and 95% confidence intervals. Statistical analyses were performed using Stata Version 12.1.

Results

A total of 83 patients were included in the study. Thirty-five patients were excluded because of the loss of paraffin blocks, and two patients were excluded because we could not obtain their medical records. A total of 46 patients were included in the data analysis. Twenty-two patients (47.83%) were diagnosed with stage 1 disease, 16 (34.78%) with stage 2 disease, and 8 (17.39%) with stage 3 disease. Regarding tumor grade, 21 patients (45.65%) were diagnosed with grade 1, 15 (32.61%) with grade 2, and 10 (21.74%) with grade 3 tumors. Thirteen patients (28.26%) experienced a recurrence from January 2017 to December 2019. The three-year DFS of type 1 endometrial carcinoma at Dr. Soetomo Hospital was

Table 1. Demographic Data and Clinicopathology Characteristics

Characteristics	MMR Status		p-value
	dMMR (%)	pMMR (%)	
Age			
<50 years	1 (8.33%)	5 (14.71%)	0. 573
>50 years	11 (91.67%)	29 (85.29%)	
Body mass index			
<30	2 (16.67%)	6 (17.65%)	0. 407
>30 - <35	6 (50%)	22 (64.71%)	
>35 -<40	2 (16.67%)	5 (14.71%)	
> 40	2 (16.67%)	1 (2.94%)	
Parity			
Nulipara	2 (16.67%)	14 (41.18%)	0. 125
Multipara	10 (83.33%)	20 (58.82%)	
Menopausal Status			
Premenopouse	1 (8.33%)	3 (8.82%)	0. 959
Menopouse	11 (91.67%)	31 (91.18%)	
Stage			
I	9 (75%)	13 (38.24%)	0. 056
II	3 (25%)	13 (38.24%)	
III	0 (0%)	8 (23.53%)	
IV	0 (0%)	0 (0%)	
Tumor grade			
Gr 1	7 (58.33%)	14 (41.18%)	0. 384
Gr 2	4 (33.33%)	11 (32.35%)	
Gr 3	1 (8.33%)	9 (26.47%)	
Lymphovascular invasion			
Positive	2 (16.67%)	20 (58.82%)	0. 012*
Negative	10 (83.33%)	14 (41.18%)	
Myometrial invasion			
<50%	4 (33.33%)	10 (29.41%)	0. 800
>50%	8 (66.67%)	24 (70.59%)	

* p-value is significant at the 95% confidence interval

Table 2. Disease Free Survival According MMR Status

MMR Status	Recurrency (%)	DFS (%)	HR	CI 95%	p
dMMR	11 (32.3%)	23 (67.6%)	2.31	0.5135-10.475	0. 27
pMMR	2 (16.6%)	10 (83.3%)			

71.74%, regardless of the MMR status.

Of the 46 patients, 12 (26.1%) were categorized as having dMMR and 34 (73.9%) as having pMMR. Regarding MMR protein loss, we found a loss of MLH1 in only nine patients (75%), a loss of MSH2 in only two patients (16.67%), and a loss of both proteins in one patient (8.33%).

Patient demographic data and clinicopathological characteristics according to MMR status are shown in Table 1. Patients with dMMR and pMMR were diagnosed at >50 years of age (91.67% and 85.29%, respectively). Obesity was present in 38 patients: 50% of patients with dMMR and 64.71% of patients with pMMR were categorized as obese grade 2. Most patients diagnosed with type 1 endometrial carcinoma were multiparous: 83.33% with dMMR and 58.8% with pMMR. In this study, 91.67% of patients with dMMR were postmenopausal, and only one patient was premenopausal. Patients with dMMR were diagnosed with stage 1 (75%) or 2 (25%) disease, and none were diagnosed with stage 3 or 4 disease. Seven out of 12 patients (58.8%) with dMMR were diagnosed

Table 3. Multivariate Analysis for Disease Free Survival

	AHRR (Adjusted Hazard Rate Ratio)	95% CI	p
MMR	1.66	0,2124 - 13,0303	0, 628
Body Mass Index	1.63	0,6354 - 4,1754	0, 310
Parity	3.07	0,7197 - 13,0872	0, 130
Stage	5.42	1,3378 - 21,9358	0, 018*
Tumor Grade	8.65	2,5020 - 29,8738	0, 001*

* p-value is significant at the 95% confidence interval

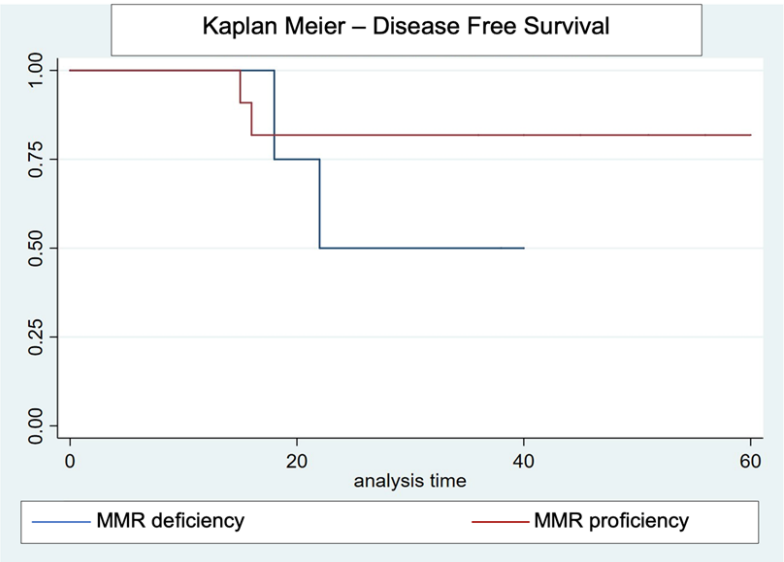

Figure 1. Kaplan–Meier Disease-Free Survival of dMMR and pMMR. The three-year DFS was 83.3% in dMMR group and 67.6% in pMMR group (p=0.27)

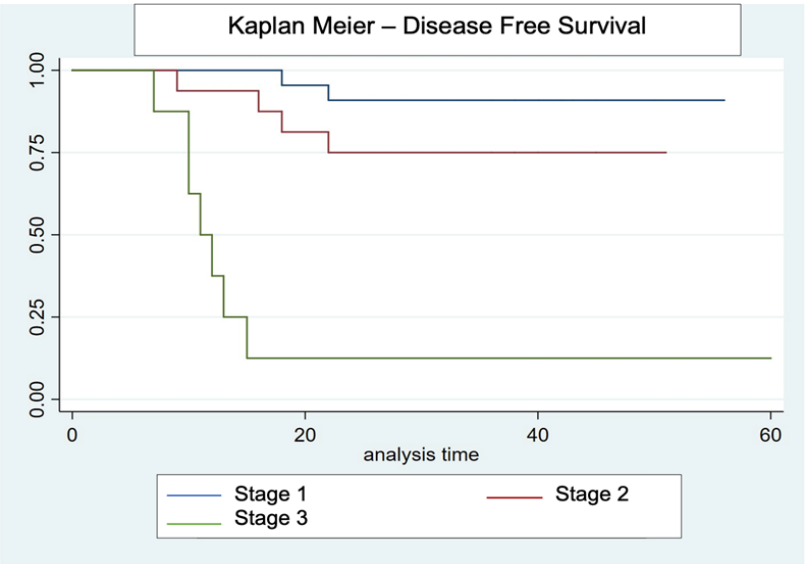

Figure 2. Kaplan-Meier Disease-Free Survival in All Endometrial Carcinoma by Stage. The three-year DFS in stage 1 was 90.1%, stage 2 was 75%, and stage 3 was 12.5%

with grade 1 tumors and only one (8.33%) with a grade 3 tumor. Nine patients (26.47 %) with pMMR were diagnosed with grade 3 tumors. Among the 12 patients with dMMR, eight (66.67%) had myometrial invasion greater than 50%. Twenty-four patients (70.59%) with pMMR had myometrial invasion greater than 50%. There were no statistically significant differences in age, BMI, parity, menopausal status, stage, tumor grade, or myometrial invasion between the two groups. In this study, we found a statistically significant difference in lymphovascular invasion between the dMMR and pMMR groups (p=0.012). Lymphovascular invasion was not observed in 10 patients (83.33%) with dMMR, whereas it was observed in 20 patients (58.82%) with pMMR.

We used the log-rank test to compare the DFS between the dMMR and pMMR groups. We found that 13 patients had recurrences after 36 months or 3 years: 2 with dMMR and 11 with pMMR. The three-year DFS of endometrial carcinoma was 83.3% with dMMR and 67.6% with pMMR (HR 2.31, 95% CI: 0.5135–10.475, p=0.27). The DFS was not significantly different between the dMMR and pMMR groups; nevertheless, the pMMR group had a 2.31 times higher risk of recurrence, as shown in Table 2 and Figure 1.

Stage and tumor grade were identified as independent risk factors for the recurrence of type 1 endometrial carcinoma in the multivariate analysis. The higher the stage, the higher the risk of recurrence (5.42 times higher) (HR 5.42; 95% CI: 1.3378–21.9358, p=0.018). The higher the grade, the higher the risk of recurrence (8.65 times higher) (HR 8.65, 95% CI: 2.5020–29.8738, p=0.001). MMR status was not a statistically significant risk factor for recurrence; however, pMMR increased the risk of recurrence (HR 1.66, 95% CI: 0.2124–13.0303, p=0.628),

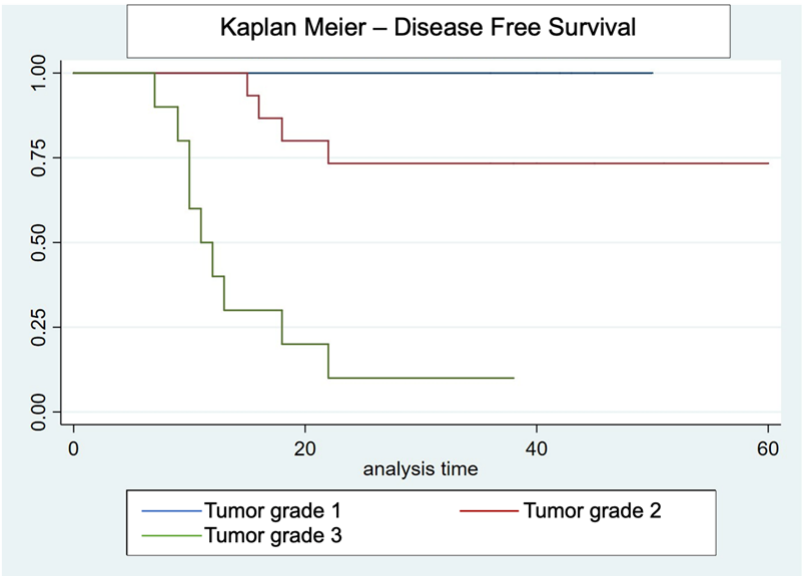

Figure 3. Kaplan–Meier Disease-Free Survival in All Endometrial Carcinoma by Tumor Grade. The three-year DFS in grade 1 was 100%, grade 2 was 73.3%, and grade 3 was 10%

as shown in Table 3.

In our study, we found that the 3-year DFS of endometrial carcinoma was 90.1% with stage 1, 75% with stage 2, and 12.5% with stage 3 diseases, as shown in Figure 2. The 3-year DFS rates for tumor grades 1, 2, and 3 were 100 %, 73.3%, and 10%, respectively (Figure 3).

Discussion

In this study, we found that the DFS of type 1 endometrial carcinoma was 71.74%, regardless of MMR status. A study conducted in Spain in 2013 reported that the DFS of endometrial carcinoma was 82.3%., which was similar to that in our hospital. Tumor stage and grade are risk factors for recurrence in endometrial carcinoma (Tejerizo et al., 2013). We also found that tumor stage and grade were independent risk factors for recurrence.

Regarding the demographic data of patients with endometrial carcinoma, we found that the age at diagnosis for patients with dMMR and pMMR was >50 years. These results are similar to those reported by Suede (2014). Age is also a risk factor for the development of carcinoma (Ruiz et al., 2014). Patients were mostly obese (grade 2) in the dMMR and pMMR groups. Previous studies have reported that every 5-point increase in BMI increases the risk of developing endometrial carcinoma (Lu et al., 2020). In our data, we found no statistically significant differences in age, BMI, parity, or menopausal status between the two groups, which is similar to those in previous studies (Fountzilas et al., 2019; Kato et al., 2015).

Patients with endometrial carcinoma with dMMR are mostly diagnosed at stage 1 or grade 1, with no lymphovascular invasion. Our study showed similar results to those of a previous study, in which 73.3% of patients with dMMR were diagnosed at stage 1, and only 9.4% had lymphovascular invasion (Ruiz et al., 2014). A study from Japan also reported that 63% of endometrial carcinomas with dMMR were diagnosed at stage 1, and 35% had myometrial invasion >50% (Shikama et al., 2016). In contrast, we found that 66.67% of patients with endometrial carcinoma with dMMR had myometrial invasion >50%.

Our study reported that the 3-year DFS in patients with endometrial carcinoma was 83.3% with dMMR and 67.6% with pMMR. pMMR was associated with a 2.31 times higher risk of recurrence than dMMR, although this result was not statistically significant. This study is similar to that conducted by Ruiz (2014). Ruiz et al. found that the PFS was 53.9 months with dMMR and 54.1 months with pMMR (p=0.43). The OS was 56.4 months with dMMR and 56.6 months with pMMR (p=0.65) (Ruiz et al., 2014). In contrast, a study conducted in Canada reported that patients with dMMR had a poorer prognosis. The DFS was 66% with dMMR and 89% with pMMR (p=0.001). Another study reported that patients with dMMR were older and had higher tumor grades, myometrial invasion greater than 50%, and larger uterine sizes; therefore, their prognoses were poorer (Kim et al, 2020). In contrast, Kato et al. showed that patients with dMMR had a better prognosis, with a PFS of 93% with dMMR and 78% with pMMR (p=0.013). MMR status was reported to be an independent prognostic factor for OS in endometrial carcinoma (HR 0.24, 95%CI 0.08-0.70, p<0.01) (Kato et al., 2015).

We conducted this study because a novel study reported that patients with dMMR may benefit from immunotherapy with pembrolizumab. Patients with dMMR and advanced-stage or recurrent disease may respond to immunotherapy (Sloan et al., 2017). In addition, many studies have reported different results regarding the prognosis of endometrial carcinoma in patients with dMMR.

This study had some limitations. We used only two proteins to detect MMR status; therefore, some diagnoses may have been missed. In addition, this study may have been conducted on a large scale with a larger sample size.

In conclusion, this study found that patients with MMR deficiency had a better DFS compared to those with MMR proficiency; however, the difference was not statistically significant. Furthermore, the authors found that the tumour stage and histopathological grade were independent risk factors for recurrence. These results are expected to be relevant to oncological clinical practice today and are expected to guide future clinical decisions for patients with endometrial carcinoma.

Author Contribution Statement

We would like to acknowledge the following author contributions: Yoan Alexandria Angelina for conceptualization, methodology, investigation, writing, data analysis, and editing; Brahmana Askandar Tjokroprawiro for cconceptualization, methodology, validation, and reviewing; and Willy Sandhika for pathologist review and editing.

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Ethical Declaration

This study was approved by the hospital's ethics committee (certificate no. 0616/KEPK/III/2023).

Conflict of Interest

There is no conflict of interest declared by all of authors.

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