RESEARCH ARTICLE

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Comparison of Tissue Mismatch Repair Protein Deficiency between Early- and Advanced-Stage Endometrial Cancer

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Abstract

Objective: ESGO/ESTRO/ESP guidelines recommend that DNA mismatch repair (MMR) proteins or microsatellite instability tests should be performed in all cases of endometrial cancer. This study aims to clarify the relationship of MMR protein deficiency (dMMR) between early and advanced stages of endometrial cancer. Secondary objective is to identify dMMR affecting factors in endometrial cancer. **Methods:** This cross-sectional study was conducted on endometrial cancer patients who underwent surgery at HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University, between May 2013 and April 2021. Patients with endometrial cancer whose tumor tissue was available for analysis were identified. The expression of MMR proteins was assessed by immunohistochemistry, including MLH1, MSH2, MSH6, and PMS2. Then, the pathological specimens were reviewed. **Results:** A total of 207 patients with endometrial cancer were assessed for data analysis. MMR deficiency was observed in 92 cases (44.4%). We found patients with dMMR in both the early and advanced stages of endometrial cancer-68/155 cases (43.9%) and 24/52 cases (46.2%), respectively (P = 0.774). Statistically significant differences were found only in myometrial invasion (adjusted prevalence odds ratio 2.35, 95% CI 1.21 to 4.57, P = 0.012). **Conclusion:** Our study showed no difference in tissue dMMR between early- and advanced-stage endometrial cancer. The dMMR was not associated with improved outcomes in patients with endometrial cancer. Even though ESGO/ESTRO/ESP guidelines recommend the performance of MMR IHC or MSI tests in all endometrial cancer cases, we can select the appropriate patients those categorized as "advanced stage" or "recurrent"-who may gain the most benefits from the immunotherapy modality of treatment.

Keywords: Endometrial cancer- immunohistochemistry-mismatch repair proteins- microsatellite instability

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Introduction

Endometrial cancer is one of the most common malignancies of the female genital tract. In Thailand, it is the third most common female genital cancer (Ferlay et al., 2021). Some cases of endometrial cancer are known to be associated with Lynch syndrome, an autosomal dominant disease caused by germline mutations of the mismatch repair (MMR) gene (Long et al., 2014). Lynch syndrome patients possess a 40–60% lifetime risk of being diagnosed with endometrial and colon cancers (Long et al., 2014; Meyer et al., 2009; Valu M et al., 2017). The estimated cumulative risk of developing endometrial cancer by age 70 is 54% for mutations of MLH1, 21% for mutations of MSH2, and 16% for mutations of MSH6 (Burke et al., 2014). Molecular and immunohistochemistry (IHC) studies of tumor tissue for microsatellite instability (MSI) or MMR gene defects are performed before proceeding to a genetic test.

ESGO/ESTRO/ESP guidelines recommend that MMR IHC or MSI tests should be performed in all cases of endometrial cancer, irrespective of the histologic subtype of the tumor (Concin et al., 2021), while NCCN guidelines recommend that screening for genetic mutations should be considered in all patients with endometrial cancer, especially in those younger than 50 years of age (Network, 2022). Although genetic testing remains the gold standard for the detection of MSI, IHC is a method adopted to detect the expression of MMR proteins, which consist of mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2). They can indirectly reflect the status of MSI (Li et al., 2020). The IHC method is more convenient and inexpensive, and the two tests are comparable in function (Tangjitgamol et al., 2017). A meta-analysis was performed to assess the accuracy of IHC for MMR proteins as a surrogate for MSI molecular testing in endometrial cancer. Studies of IHC for all four MMR proteins showed a sensitivity of 0.96, a specificity of 0.95, and high diagnostic accuracy (Raffone et al., 2020). The accuracy of the test was about

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89–95% (Li et al., 2020).

Acknowledging MMR status may guide proper adjuvant treatment. Recently, the FDA approved immunotherapy—namely Pembrolizumab, a programmed cell death ligand 1 (PD-L1) blocking agent—as a treatment modality for unresectable or metastatic microsatellite instability high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed despite prior treatment (Li et al., 2020). Since a patient's MMR status may affect treatment plans, IHC would be a good option for those who would gain the most benefits from immunotherapy.

Data on the prevalence of dMMR vary, with some sources reporting 20–40% of cases (McMeekin et al., 2016) and others reporting up to 55% of cases (Tangjitgamol et al., 2017). The outcome concerning the relationship between dMMR and stage, along with the prognosis of the disease, is still inconclusive (Puangsricharoen et al., 2020; Tangjitgamol et al., 2017). In this study, we aimed to determine the relationship of dMMR between early- and advanced-stage endometrial cancer.

Materials and Methods

A cross-sectional study was conducted at HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University, with the approval of the institutional review board, certificate No. SWUEC/E-007/2564. The inclusion criteria were endometrial cancer patients who underwent primary surgery between May 2013 and April 2021. Patients were required to have available pathology reports and paraffin tissue blocks of specimens obtained from their hysterectomy. Surgery generally included a total hysterectomy, bilateral salpingo-oophorectomy, and lymph node dissection, if indicated. Exclusion criteria were patients who had radiation or chemotherapy as a primary treatment prior to surgery, those who did not undergo a hysterectomy, and those who had no available or inadequate tissue from specimens obtained from the hysterectomy.

Demographic data such as age at diagnosis, parity, menopausal status, body mass index (BMI), a personal or family history of cancer, 2009 International Federation of Gynecology and Obstetrics (FIGO) staging, pathological data (histopathology, tumor grade, depth of myometrium invasion, lymphovascular invasion, lymph node metastasis, peritoneal washing), residual tumor, duration of follow-up, and living status were collected. FIGO stages 1 and 2 were grouped in the early stages, while stages 3 and 4 were grouped in the advanced stages.

The specimen, which had preserved good tissue morphology and no necrotic areas or artifacts, was placed into formalin-fixed paraffin-embedded blocks. An ArrayBlock is created by taking tissue from a regular block and placing it into a recipient block (the ArrayBlock) in an array format. An ArraySlide is created by cutting an ArrayBlock and placing a thin layer of tissue on a slide. The IHC for the four MMR proteins (MLH1, MSH2, MSH6, PMS2) and hematoxylin and eosin (H&E) stain were dyed. The slide was reviewed by the pathologist, who reported the official MMR status. Positive IHC staining for all four proteins was interpreted as MMR proficiency (pMMR) (Berek, 2020). Cases with negative staining reach the criteria were assigned as dMMR. The IHC results and their likely defective genes are shown in Appendix 1.

Sample size calculation was conducted using two independent proportion formulas; a sample size of 207 patients was needed. It was necessary to recruit 155 patients from the early-stage endometrial cancer group and 52 patients from the advanced-stage endometrial cancer group. We used 80% statistical power and a two-sided alpha error of 0.05 for the calculated sample size. Statistical analysis was performed using Stata Version 13 (Statacorp, College Station, TX, USA). The Shapiro-Wilk test was used to determine the normality of the distribution of data on the ratio scale. Further demographic data were assessed using the Chi-squared test, Mann-Whitney U test, or T-test. Multivariable logistic regression analysis was used to evaluate the association between the outcome of IHC and the factors that might have affected it.

Survival analysis was tested by a log-rank test to compare progression-free survival (PFS) and overall survival (OS) between pMMR and dMMR patients, and Cox regression was used to evaluate the hazard ratio between the two groups. The PFS and OS were presented with a Kaplan-Meier graph. Other statistical methods were also used, including percentages, means, interquartile range, 95% confidence interval, prevalence, prevalence ratio, and prevalence odds ratio. Statistical significance was defined as a P value of 0.05 in this study.

Results

A total of 249 patients were included in this study. Twenty-four patients were excluded due to inadequate specimens. An additional 18 patients were excluded due to an inability to interpret their MMR status. A total of 207 patients were included in the data analysis. There were 155 patients from the early stage and 52 patients from the advanced stage of endometrial cancer. Our study found that 115 cases (55.6%) were categorized as having pMMR, and another 92 cases (44.4%) were categorized as having dMMR. In terms of the profile of MMR-related protein loss in endometrial cancer patients, in this study, we found a loss of MLH1 in 46 patients (22.2%), a loss of MSH2 in 16 patients (7.7%), a loss of MSH6 in 19 patients (9.2%), and a loss of PMS2 in 11 patients (5.3%).

Patient characteristics according to MMR status are shown in Table 1. The mean age of the patients was 56.9 ± 11.16 years. Ninety-five (45.9%) patients were nulliparous, and the other 112 (54.1%) patients were multiparous. In this study, there were 165 (79.7%) postmenopausal women. Obesity with a BMI of 30 or higher was present in 64 cases (30.9%). We compared the demographic data and pathologic outcomes between the dMMR and pMMR groups. There were no statistically significant differences in age, parity, menopausal status, or BMI.

Of the 15 patients who had a family history of colorectal cancer, 10 (66.7%) had dMMR, and the others had pMMR. In our study, three patients had a family history of endometrial cancer, of which two (66.7%) had dMMR. Only 1 of 4 (25%) patients with a personal

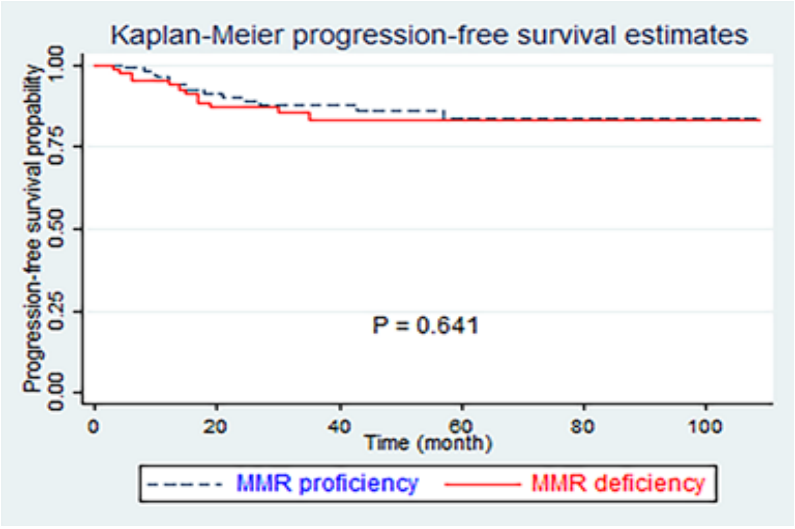

Figure 1. Kaplan-Meier Survival Curves of pMMR and dMMR in All Endometrial Cancers Progression-Free Survival between the dMMR and pMMR Groups. The five-year PFS was 83.9% in the pMMR group and 83.5% in the dMMR group.

history of colorectal cancer had dMMR. There were no statistically significant differences in patients with either a family history of endometrial/colorectal cancer or a personal history of colorectal cancer.

The majority of the histology was endometrioid carcinoma, consisting of 173 cases (83.6%). There were grade 2–3 tumors in 141 cases (68.1%). Of the 116 patients with myometrial invasion less than 50%, 41 (35.3%) had dMMR. Among 91 patients whose myometrial invasion was equal to or greater than 50%, 51 patients (56.0%) had dMMR. We found 40 cases (19.3%) of lymphovascular invasion. In 34 cases (16.4%), lymph node metastasis was found. After finishing the operation, 197 patients had complete cytoreduction without residual tumor. Of those, 89 patients (45.2%) had dMMR. While in optimal surgery, the patients with residual tumor of less than 1 cm, 3 out of 4 patients (75%) had dMMR. We found that six patients who had residual tumor greater than 1 cm in each area after surgery, suboptimal groups. All patients were categorized as advanced stage and had pMMR status.

Concerning the pathological outcome, there were no statistically significant differences in histology, tumor grade, lymphovascular invasion, lymph node status, peritoneal washing, or residual tumor. Statistically significant differences were shown only concerning myometrial invasion between both groups using multivariate analysis. Patients whose myometrium invasion was equal to or greater than 50% had a significantly higher chance of detecting the deficiency of MMR proteins (prevalence rate ratio 1.58, 95% CI 1.17–2.15, P = 0.003). After multivariable analysis was completed, the adjusted prevalence odds ratio was 2.35, with a 95% CI 1.21–4.57, and P = 0.012. The results are shown in Table 3.

In the early-stage group, which included 155 patients, 68 patients (43.9%) were dMMR. On the other hand, out of the 52 patients in the advanced-stage group, 24 patients (46.2%) were dMMR, as shown in Table 2. We found no

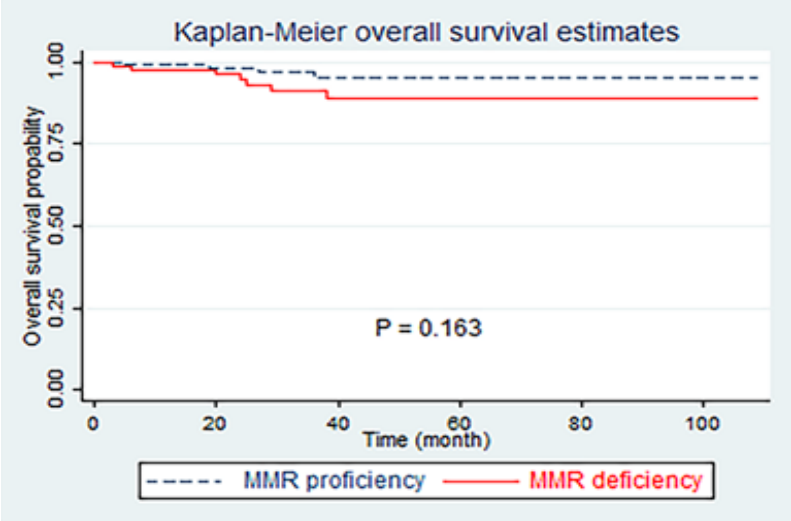

Figure 2. Kaplan-Meier Survival Curves of pMMR and dMMR in All Endometrial Cancers. Overall survival between the dMMR and pMMR groups. The five-year OS was 95.1% in the pMMR group and 88.6% in the dMMR group

Table 1. Demographic Data

	All cases (N = 207)	MMR proficiency (N = 115)	MMR deficiency (N = 92)	P value
Age (mean ± standard deviation)	56.9 ± 11.16	56.6 ± 11.76	57.2 ± 10.42	0.887
Parity				
Nulliparous (n, %)	95 (100%)	46 (48.4%)	49 (51.6%)	0.057
Multiparous (n, %)	112 (100%)	69 (61.6%)	43 (38.4%)	
Menopausal status				
Premenopause (n, %)	42 (100%)	25 (59.5%)	17 (40.5%)	0.562
Postmenopause (n, %)	165 (100%)	90 (54.6%)	75 (45.4%)	
BMI				
< 30 (n, %)	143 (100%)	83 (58.0%)	60 (42.0%)	0.282
≥ 30 (n, %)	64 (100%)	32 (50.0%)	32 (50.0%)	
Family history of cancer				
No (n, %)	184 (100%)	105 (57.1%)	79 (42.9%)	0.216
Yes (n, %)	23 (100%)	10 (43.5%)	13 (56.5%)	
Family history of colorectal cancer				
No (n, %)	192 (100%)	110 (57.3%)	82 (42.7%)	0.072
Yes (n, %)	15 (100%)	5 (33.3%)	10 (66.7%)	
Family history of endometrial cancer				
No (n, %)	204 (100%)	114 (55.9%)	90 (44.1%)	0.586 □
Yes (n, %)	3 (100%)	1 (33.3%)	2 (66.7%)	
Personal history of colorectal cancer				
No (n, %)	203 (100%)	112 (55.2%)	91 (44.8%)	0.631 □
Yes (n, %)	4 (100%)	3 (75%)	1 (25%)	
Histology				
Endometrioid (n, %)	173 (100%)	92 (53.2%)	81 (46.8%)	0.121
Non-endometrioid (n, %)	34 (100%)	23 (67.6%)	11 (32.4%)	
Tumor grade				
Grade I (n, %)	66 (100%)	41 (62.1%)	25 (37.9%)	0.193
Grades II–III (n, %)	141 (100%)	74 (52.5%)	67 (47.5%)	
Myometrium invasion				
Less than 50% (n, %)	116 (100%)	75 (64.7%)	41 (35.3%)	0.003*
Equal or more than 50% (n, %)	91 (100%)	40 (44.0%)	51 (56.0%)	
Lymphovascular invasion				
No (n, %)	167 (100%)	96 (57.5%)	71 (42.5%)	0.254
Yes (n, %)	40 (100%)	19 (47.5%)	21 (52.5%)	
Lymph node metastasis				
No (n, %)	173 (100%)	96 (55.5%)	77 (44.5%)	0.967
Yes (n, %)	34 (100%)	19 (55.9%)	15 (44.1%)	
Peritoneal washing				
Negative (n, %)	173 (100%)	94 (54.3%)	79 (45.7%)	1
Positive (n, %)	23 (100%)	14 (60.9%)	9 (39.1%)	0.555
Not done (n, %)	11 (100%)	7 (63.6%)	4 (36.4%)	-
Residual tumor				
None (n, %)	197 (100%)	108 (54.8%)	89 (45.2%)	1
Optimal < 1 cm (n, %)	4 (100%)	1 (25%)	3 (75%)	0.334†
Suboptimal > 1 cm (n, %)	6 (100%)	6 (100%)	0 (0%)	0.036†

†, Using Fisher's exact test; *P value is significant at a 95% confidence interval

Table 2. The Relationship of Mismatch Repair Protein Deficiency between Early- and Advanced-Stage Endometrial Cancer

Stage of endometrial cancer	All cases (N = 207)	MMR proficiency (N = 115)	MMR deficiency (N = 92)	P value
Early stage EMC (I-II) (n, %)	155 (100%)	87 (56.1%)	68 (43.9%)	0.774
Advanced stage EMC (III–IV) (n, %)	52 (100%)	28 (53.8%)	24 (46.2%)	

statistically significant differences in MMR status between early- and advanced-stage endometrial cancer (P = 0.774). After multivariable analysis with stage, parity, family history of colorectal cancer, family history of endometrial cancer, personal history of colorectal cancer, histology, tumor grade, myometrial invasion, and residual tumor, neither early- nor advanced-stage endometrial cancer had statistically significant differences in MMR status (adjusted prevalence odds ratio of 0.91, 95% CI 0.41–2.02, P = 0.816), as shown in Table 3.

During the surveillance of 207 endometrial cancer patients, 181 patients (87.4%) had no recurrences of the disease. Another 26 patients showed either disease progression or recurrence. In early-stage endometrial cancer, 8 of 155 patients (5.2%) had recurrence, including 4 patients from the pMMR groups and 4 patients from the dMMR groups (P = 0.567). Furthermore, in advanced-stage endometrial cancer, 18 of 52 patients (34.6%) had recurrence, including 10 patients from the pMMR groups and 8 patients from the dMMR groups (P = 0.924). Considering the timing of recurrent endometrial cancer, 20 patients had a recurrence within 12 months. There were no recurrences of the disease after five years (60 months) of remission. Based on the results of our study, the PFS between the pMMR and dMMR groups did not show statistically significant differences after controlling for the factors of stage, parity, family history of colorectal cancer, family history of endometrial cancer, personal history of colorectal cancer, histology, tumor grade, myometrial invasion, and residual tumor (crude HR 1.20, 95% CI 0.56–2.60, P = 0.641). The adjusted HR was 1.28, with a 95% CI of 0.53–3.09 and a P = 0.577.

The death rate in our study was 11 patients two patients in the early stage and nine patients in the advanced stage of endometrial cancer. Both patients (100%) in the early stage and five of the nine (55.6%) in the advanced stage of endometrial cancer had dMMR. We found that after the first four years (48 months), no one else died of the disease. OS also did not show statistically significant differences after adjusting for the same factors as PFS (crude HR 2.40, 95% CI 0.70–8.20, P = 0.163; adjusted HR 2.63, 95% CI 0.66–10.38, P = 0.167). As shown in Figure 1 and 2, there were no statistically significant differences in PFS or OS between the pMMR and dMMR groups.

Discussion

There are several risk factors for the development of endometrial cancer, most of which are related to prolonged, unopposed estrogen stimulation of the endometrium, and Lynch syndrome (Berek, 2020). Women with Lynch syndrome, a cancer susceptibility syndrome with germline mutations in the MMR genes MLH1, MSH2, MSH6, and PMS2, have a 40–60% lifetime risk of endometrial and colon cancer (Berek, 2020; Long et al., 2014; Meyer et al., 2009; Valu M et al., 2017) . Thus, we aimed to study IHC for MMR proteins that can reflect MMR genes. The prevalence of dMMR in our study was 44.4%, depicting a similar trend as previous studies in Thailand, Tangjitgamol et al., 2017 reporting 55.1% and Puangsricharoen et al., 2020 , reporting 35.9%.

In our study, MMR status between early and advanced stages did not show statistically significant differences, which is compatible with previous studies in Southeast Asia (Puangsricharoen P et al., 2020; Woo et al., 2014). However, some papers have illustrated that dMMR is more common in early-stage endometrial cancer (Tangjitgamol et al., 2017).

Table 3. The Association of MMR Deficiency on Univariable and Multivariable Analysis

Factors	Prevalence rate ratio	Univariable analysis Crude prevalence odds ratio	Multivariable analysis Adjusted prevalence odds ratio
Stage			
Early	1	1	1
Advanced	1.05	1.09	0.91
	95% CI 0.75–1.48,	95% CI 0.59–2.05,	95% CI 0.41–2.02,
	P-value 0.774	P-value 0.774	P-value 0.816
Myometrial invasion			
Less than 50%	1	1	1
Equal to or more than 50%			
	1.58	2.33	2.35
	95% CI 1.17–2.15,	95% CI 1.33–4.08,	95% CI 1.21–4.57,
	P-value 0.003	P-value 0.003	P-value 0.012

*Including factors in the multivariable analysis were stage, parity, family history of colorectal cancer, family history of endometrial cancer, personal history of colorectal cancer, histology, tumor grade, myometrial invasion, and residual tumor.

Focusing on the characteristics of patients with endometrial cancer, our study found that there were no statistically significant differences in age, parity, menopausal status, and BMI between the pMMR and dMMR groups, which mirrors the results as previous studies (Fountzilas et al., 2019; Hashmi et al., 2019; Kato et al., 2015; Puangsricharoen P et al., 2020; Woo et al., 2014). However, some previous studies reported an association between MMR status and age (McMeekin et al., 2016; Tangjitgamol et al., 2017) and between MMR status and BMI (McMeekin et al., 2016). Some studies found that dMMR showed statistically significant differences in patients younger than 60 (Tangjitgamol et al., 2017).

Concerning family history of colorectal and endometrial cancer, have been reports association with Lynch syndrome. A germline mutation in MMR genes is the cause of dMMR in patients with Lynch syndrome. These tumors showed MSI-H. It was discovered that having a family history of endometrial/colon cancer caused a statistically significant difference in the expression of MSI (Hashmi et al., 2019). In contrast, our study found no statistically significant difference between a family history of colorectal and endometrial cancer and a deficiency in MMR status. Because this is not our primary objective, the sample size may not be large enough to show significant differences.

Pathological factors that might influence the prognosis or stage of endometrial cancer were addressed, such as histologic cell type, tumor grade, depth of myometrial invasion, lymphovascular invasion, lymph node metastasis, and peritoneal cytology results. In our study, when considering various factors, all patients had no statistically significant differences in MMR status, except for myometrial invasion, between the groups. A study of Southeast Asian endometrial cancer patients (Woo et al., 2014) reported no significant differences in histopathologic characteristics and clinical outcomes between dMMR cases and controls. Like a prior paper in Thailand, which reported that dMMR is not related to stage, histology, tumor grade, myometrial invasion, lymphovascular invasion, or lymph node involvement (Puangsricharoen P et al., 2020). On the other hand, some previous studies reported an association between MMR status and histopathology (Kato et al., 2015), tumor grade (Fountzilas et al., 2019; Kato et al., 2015; McMeekin et al., 2016), lymphovascular invasion (McMeekin et al., 2016), and lymph node metastasis (Hashmi et al., 2019; Tangjitgamol et al., 2017). A larger systematic review or more comprehensive meta-analysis may be needed for confirmation.

The presence of a residual tumor has an impact on the rate of recurrence and prognosis. Our study shows that patients in the suboptimal group have a higher chance of detecting MMR proficiency when compared with those in the no-residual subgroup. In a study by Kato et al., the presence of residual tumor did not show a statistically significant difference in MMR status (Fountzilas et al., 2019) . This paper divided subgroups into the absence or presence of residual tumors. However, in our study, we divided the subgroups into no residual disease, residual disease less than 1 cm (optimal surgery), and residual disease greater than 1 cm (suboptimal surgery). Thus, the outcomes may be different. Furthermore, because there were very few cases in the suboptimal group in our study (only six) and all of them were pMMR, with no dMMR in suboptimal groups, we can't evaluate and conclude the difference between the two groups.

Various factors might affect the PFS or OS of endometrial cancer, especially at an early or advanced stage. In our study, there were no statistically significant differences in PFS or OS between pMMR and dMMR for any endometrial cancer. If the group were subdivided into early stage or advanced stage, there would still be no difference in PFS or OS. Research conducted by Woo et al., showed that dMMR protein expression in a Southeast Asian endometrial cancer cohort was not correlated with disease outcome (Woo et al., 2014). The dMMR status was not related to the recurrence of disease (Hashmi et al., 2019). However, Cohn et al. claimed a significant improvement in disease-free survival in patients with normal MLH1 and MSH2 expression compared with those with abnormal expression (estimated five-year survival of 92% versus 81%, P = 0.035) (Cohn et al., 2006). Another paper reported that MMR deficiency was associated with an increased risk of cancer-related death after controlling for confounders (hazard ratio of 2.0) (Loukovaara et al., 2021). On the other hand, some papers have reported that the MMR deficiency group was associated with improved overall survival when compared with MMR proficiency (Fountzilas et al., 2019). A paper by Kato et al. showed that five-year PFS was 92% in dMMR patients and 78% in pMMR patients (P = 0.013), and five-year OS was 94% in dMMR patients and 78% in pMMR patients (P = 0.009) (Kato et al., 2015). The treatment outcomes may be diverse because standard treatment in the past comprised surgery, radiation, and chemotherapy, which did not include immunotherapy in either the pMMR or dMMR groups.

Our study was conducted because knowing MMR deficiency status may benefit the patient and guide proper adjuvant treatment. Patients with advanced-stage or recurrent disease with MMR deficiency may respond to immunotherapy. A study in 2019 concluded that the clinical benefits of anti-programmed death-1 therapy with pembrolizumab among patients with previously treated unresectable or metastatic MSI-H/dMMR noncolorectal cancer (Marabelle et al., 2020). The FDA has officially approved the use of pembrolizumab for adult and pediatric patients with unresectable or metastatic MSI-H dMMR solid tumors. This was the FDA's first approval of a tissue-site agonist (Arend et al., 2018). Tumor testing with IHC is less expensive and more available in most pathology laboratories. If there is an absence of MMR protein expression in any gene, the patient should be offered genetic counseling and further appropriate genetic testing.

There are some limitations to this study. First, using a tissue microarray did not include the whole specimen and, therefore, may have missed some lesions. Second, inadequate tissue fixation can result in weak or equivocal staining patterns that make the results less reliable. However, using this technique is less expensive.

Consequently, this can guide specific MMR genes that are likely to have germline mutations. Further studies to increase the diagnostic accuracy of germline and somatic mutations may involve alternate methods, such as microsatellite status assessed by next-generation sequencing or fluorescent multiplex polymerase chain reaction and capillary electrophoresis, which may be used to determine the status of MSI.

In conclusion, our study has not shown a difference in tissue mismatch repair protein deficiency between early-stage and advanced-stage endometrial cancer. Even ESGO/ESTRO/ESP guidelines recommend the performance of MMR IHC or MSI tests in all endometrial cancer cases. However, in our study, which was conducted in a Thai population, we were able to identify the appropriate patients categorized as "advanced stage" or "recurrent" who may gain the most benefits from the immunotherapy modality of treatment.

Author Contribution Statement

Kamonporn Chaowiwatkun: Conceptualization, Methodology, Validation, Writing - Review & Editing. Therkiat Trongwongsa: Validation, Investigation. Nopporn Rodpenpear: Formal analysis, Review & Editing. Pattiya Nutthachote: Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing, Funding acquisition.

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Ethical Declaration

The approval of the institutional review board, Strategic Wisdom and Research Institute, Srinakharinwirot University, certificate No. SWUEC/E-007/2564.

Conflict of Interest

All of authors declare no conflict of interest.

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