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Relationship between *Mismatch* Repair (MMR) Status and Chemotherapy Response in Colorectal Carcinoma

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ABSTRACT

Background
Colorectal carcinoma has several pathways that play a role in the development of normal colonic mucosa into carcinoma, one of which is the *microsatellite instability* (MSI) pathway. This pathway results from a deficiency in one of the MMR proteins that normally repair *genome* damage, leading to *microsatellite instability high* (MSI-H) and excessive mutations. *Microsatellite instability high* is closely associated with *Lynch* syndrome and often experiences resistance to chemotherapy treatment, one of which is 5-fluorouracil (5-FU), so it is necessary to conduct initial screening in colorectal carcinoma, especially patients with *Lynch* syndrome to assess MMR status. This study aims to see the relationship between MMR status and chemotherapy response in colorectal carcinoma.

Methods
This study is a cross sectional study using 30 archival block and slaid samples of all colorectal carcinoma cases from hemicolectomy and biopsy results that have been histopathologically diagnosed in the Anatomic Pathology Section of the Faculty of Medicine Unsri / RSMH Palembang from January 2017 to December 2021. Each sample was immunohistochemically stained using four antibodies namely anti-MLH1, anti-MSH2, anti-MSH6 and anti-PMS2 (ventana). Interpretation was qualitative by assessing cell positivity. Categorized into intact and missing with a cut-off point value of >10% positive declared intact. MMR deficiency was considered if there was loss of cell positivity of at least one MMR CPI marker and MMR proficiency if all MMR CPI were intact. Analysis of the relationship between MMR status and chemotherapy response was performed using Fisher's exact test analysis.

Results
Fisher's exact test showed an association between MMR status and chemotherapy response, and the results were significantly significant with a p value of 0.045 where MMR deficiency status had a 6 times chance of not responding to chemotherapy compared to MMR proficiency. There was no association between MMR status and age, gender, histopathological subtype and tumor location with p values of 0.139, 1.000, 0.657 and 0.174 respectively.

Conclusion
There was a significant association between MMR status and chemotherapy response.

Keywords: MMR status, colorectal carcinoma, chemotherapy response, histopathologic subtype

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INTRODUCTION

Colorectal carcinoma is a heterogeneous disease with diverse clinical and biological features that lead to differences in progessivity and response to therapy in each case. Tumor stage, tumor location and several molecular markers including *mismatch repair* (MMR) status, *RAS* and *BRAF* mutations have been widely used for specific therapy in colorectal carcinoma patients.[1,2]

The pathogenesis of colorectal carcinoma has three main pathways that play a role in the development of normal colon mucosa into carcinoma, namely *chromosomal instability* (CIN) as much as 75%, *microsatellite instability* (MSI) as much as 15% and CpG *island methylator phenotype* (CIMP) as much as 20%. [7,8] Colorectal carcinoma originating from the MSI pathway occurs due to the failure of *mismatch* repair (MMR) proteins to repair *genome* damage, especially at *microsatellite*. MMR proteins consist of MLH1, MSH6, MSH2 and PMS2 which play a role in repairing DNA damage. A deficiency in any of these MMR proteins will lead to *microsatellite instability high* (MSI-H) and excessive mutations in DNA. *Microsatellite instability high* is closely associated with *Lynch* syndrome caused by *germline* mutations in the MMR gene. Therefore, relatives of patients with *Lynch* syndrome will carry the pathogenic MMR gene for about 50% and may suffer from other carcinomas besides colorectal carcinoma.[9]

Microsatellite testing with *genotyping* has been conducted since 1996. In the past 1 year, it has been found that MMR immunohisto-chemical examination can replace *micro-satellite genotyping* examination. MMR protein examination has been used as a screening and therapeutic basis in patients with syndromic or sporadic colorectal adenocarcinoma. There is a clinical *guideline* that recommends all colorectal carcinoma patients must be screened for MMR/MSI either through immunohistochemical examination or *genotyping*.[10] However, in Indonesia itself screening MMR/MSI status has not become a standardized standard in colorectal carcinoma patients, especially in Palembang.

METHODS

This study is an analytical descriptive study with a *cross sectional* design, to determine the relationship between *mismatch repair* (MMR) status and chemotherapy response in colorectal carcinoma at the Anatomic Pathology Department of Sriwijaya University / RSUD Dr. Mohammad Hoesin Palembang from January 1, 2017 to December 31, 2021.

This study used 30 archival samples of hematoxylin eosin preparations and *paraffin blocks/formalin fixed paraffin embedded* (FFPE) of all colorectal carcinoma cases from hemicolectomy and biopsy results that had been diagnosed histopathologically. The study sample was taken retrospectively from the target population using *total sampling* technique that met the inclusion and exclusion criteria.

Immunohistochemical staining using four antibodies: anti-MLH1 (M1) mouse *monoclonal primary antibody ready to use*, ventana anti-MSH6 (SP93) *rabbit monoclonal primary antibody ready to use,* ventana anti-MSH2 (G219-1129) *mouse monoclonal primary antibody ready to use*, ventana anti-PMS2 (A 16-4) *mouse monoclonal primary antibody ready to use* brand Ventana *Medical System*, Inc, *Tucson*, AZ.

MMR status was categorized into MMR deficiency characterized by loss of cell positivity of at least one MMR IHK marker and MMR proficiency characterized by all intact MMR IHK results. Interpretation was carried out by three people, namely the researcher (dr. Soraya Sagita Desmaradd) and two supervisors (dr. Suly Auline Rusminan, Sp.PA and dr. Ika Kartika, Sp.PA) who were determined using a qualitative method, which is based on the positivity of cells that are stained brown in the cell nucleus with a 10% cut point declared intact. [79] (Figures 1 and 2). The first examination used weak magnification (100 times) to assess whether the tumor cells were intact or missing. Next, the area was examined under strong magnification (400 times) to confirm and compare with the positive internal control. After that, photographs were taken using a DP 21 camera on an Olympus type BX51 binocular light microscope as documentation. Positive controls in this study were only taken through internal controls, namely lymphocytes, colonic krypta cells and stromal cells. Negative controls were taken from cases by not being given primary antibodies. The chromogen used in this study was *Diaminobenzidine tetrahydrochloride* (DAB).

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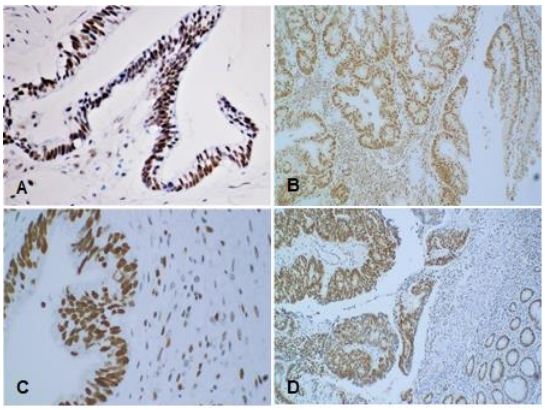

Figure 1. IHK expression of intact MMR. A. MLH1 expression (400 times magnification, case number 18). B. MSH2 expression (magnification 400 times, case number 4). C. MSH6 expression (magnification 400 times, case number 13). D) PMS2 expression (magnification 400 times, case number 7).

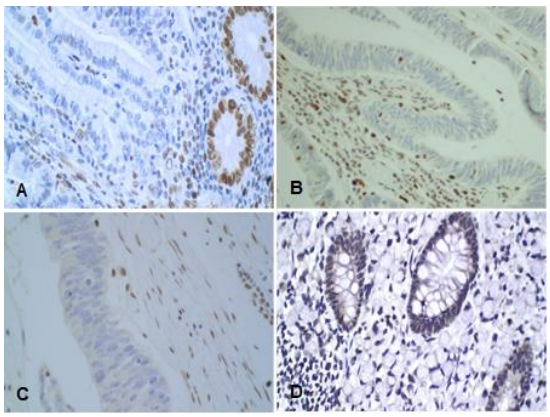

Figure 2. CPI expression of missing MMR. A) MLH1 expression (400 times magnification, case number 15). B) MSH2 expression (magnification 400 times, case number 19). C) MSH6 expression (magnification 400 times, case number 19). D) PMS2 expression (magnification 400 times, case number 1).

Univariate analysis (descriptive) determined the proportion of study subjects based on clinicopathologic variables including age, gender, tumor location, diagnosis as well as based on independent variables, namely MMR status and dependent variables, namely chemotherapy response. Bivariate analysis to see if there is an association between MMR status and chemotherapy response in colorectal carcinoma. This analysis was also used to see if there was an association between MMR status and clinicopathological characteristics including gender, tumor location and histopathological subtype using *Fisher's exact* test. The *p* value was considered significant if $p<0.05$ with a 95% confidence interval. Data analysis used *Statistical Program for Social Sciences* (SPSS) version 26.0. This study has received ethical approval at the Doctor Mohammad Hoesin Palembang Hospital, according to seven WHO standards 2011 with ethical number 161/kepkrsmh/2022.

RESULTS

Clinicopathologic Characteristics of Colorectal Carcinoma

The distribution of clinicopathological characteristics of age, gender, tumor location, histopathological subtype, MMR status and chemotherapy response of colorectal carcinoma can be seen in table 1. Based on table 1, it was found that the largest age group was <50 years old as many as 18 samples (60.0%) while the age group of ≥50 years old as many as 12 samples (40.0%). Male gender was 17 samples (56.7%) and female was 13 samples (43.3%). Tumor location in the right colon was found in 11 samples (36.7%), left colon in 6 samples (20.0%) and rectum in 13 samples (43.3%). Histopathological subtypes based on WHO digestive in 2019 were *poorly differentiated adenocarcinoma* as many as 14 samples (46.7%), *mucinous adenocarcinoma* 11 samples (36.7%) and *signet ring cell carcinoma* 5 samples (16.7%). MMR status consisted of MMR deficiency obtained in 20 samples (66.7%) and MMR proficiency in 10 samples (33.3%). Giving chemotherapy to the samples of this study showed a response of 10 samples (33.3%) and no response as many as 20 samples (66.7%).

Table 1. Sample characteristics based on clinicopathologic, MMR status and chemotherapy response.

Variables	n	%
Age		
<50 years old	18	60.0
≥50 years old	12	40.0
Gender		
Male	17	56.7
Female	13	43.3
Tumor location		
Right colon	11	36.7
Left colon	6	20.0
Rectum	13	43.3
Histopathologic subtype		
Poorly differentiated adenocarcinoma	14	46.7
Mucinous adenocarcinoma	11	36.7
Signet-ring cell carcinoma	5	16.7
MMR Status		
Deficiency	20	66.7
Proficiency	10	33.3
Chemotherapy response		
Response	10	33.3
No response	20	66.7

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Relationship between MMR status and chemotherapy response

Analysis using fisher's exact test in table 2 shows that there is an association between MMR status and chemotherapy response, and the results are significant with a *p*-value of 0.045. The odd ratio (OR) value is 6. This means that MMR deficiency status has a 6-fold possibility of not responding to chemotherapy compared to MMR proficiency.

Table 2. Relationship between MMR status and chemotherapy response.

MMR Status	Chemotherapy response				Total		*p
	No response		Response				
	n	%	n	%	n	%	
Deficiency	16	80.0	4	40.0	20	66.7	0.045
Proficiency	4	20.0	6	60.0	10	33.3	

**Fisher's exact* test, significant if *p<0*.05.

Relationship between MMR status and clinicopathologic characteristics

Relationship between MMR status and age

The association between MMR status and age was analyzed using Fisher's exact test (Table 3). Table 3 shows that there was no association between MMR status and age (*p*=0.139). The 97 age group in MMR deficiency was more common at the age of 50 years old, with 6 samples (30.0%). In contrast, the results obtained at MMR proficiency was more common in the age group >50 years old as many as 6 samples (60.0%) and age Table 3 Relationship between MMR status and age.

Table 3. Relationship between MMR status and age.

Age	MMR Status				Total		*p
	Deficiency		Proficiency				
	n	%	n	%	n	%	
<50 years old	14	70.0	4	40.0	18	60.0	0.139
≥50 years old	6	30.0	6	60.0	12	40.0	

**Fisher's exact* test, significant if *p*<0.05.

Relationship between MMR status and gender

The association between MMR status and gender was analyzed using *Fisher's exact* test (Table 4). The results showed that samples with MMR deficiency and MMR proficiency were mostly found in the male gender, namely 11 samples (55.0%) and 6 samples (60.0%). While in the female gender, there were 9 samples (45.0%) and 4 samples (40.0%). Analysis using *fisher's exact* test showed no significant relationship between MMR status and gender. (*p*=1.000).

Table 4. Relationship between MMR status and gender.

Gender	MMR Status				Total		*p
	Deficiency		Proficiency				
	n	%	n	%	n	%	
Male	11	55.0	6	60.0	17	56.7	1.000
Female	9	45.0	4	40.0	13	43.3	

**Chi square* test, significant if *p* < 0.05.

Relationship between MMR status and histopathologic subtype

Analysis between MMR status and histopathologic subtype was performed using the *sommers correlation* test (Table 5). The majority of samples with MMR deficiency were found in the histopathologic subtype of *poorly differentiated adenocarcinoma*, namely 9 samples (45.0%), while for *mucinous adenocarcinoma* there were 7 samples (35.0%) and *signet ring cell carcinoma* as many as 4 samples (20.0%). Samples with MMR proficiency were found in *poorly differentiated adenocarcinoma* with 5 samples (50.0%), *mucinous adenocarcinoma* with 4 samples (40.0%) and *signet ring cell carcinoma* with 1 sample (10.0%). However, there was no significant association between MMR status and diagnosis (p=0.657).

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Table 5. Relationship between MMR status and histopathologic subtypes.

Histopathologic subtype	MMR Status				Total		*p
	Deficiency		Proficiency				
	n	%	n	%	n	%	
Poorly differentiated adenocarcinoma	9	45.0	5	50.0	14	46.7	
Mucinous adenocarcinoma	7	35.0	4	40.0	11	36.7	0.657
Signet ring cell carcinoma	4	20.0	1	10.0	5	16.7	

**Spearman* test, significant if *p<0*.05.

Relationship between MMR status and tumor location

Analysis between MMR status and tumor location was performed using the *sommers correlation* test (Table 6). Table 6 shows that there was no significant relationship between MMR status and tumor location (p=0.174). MMR deficiency status was most commonly found in the right colon with 10 samples (50.0%), followed by rectum with 8 samples (40.0%) and left colon with 2 samples (10.0%). While MMR proficiency status was most often found in the rectum as many as 5 samples (50.0%), followed by the left colon as many as 4 samples (40.0%) and the right colon as many as 1 sample (10.0%).

Table 6. Relationship between MMR status and tumor location.

Tumor location	MMR Status				Total		*p
	MSI-H		MSS				
	n	%	n	%	n	%	
Right colon	10	50.0	1	10.0	11	36.7	
Left colon	2	10.0	4	40.0	6	20.0	0.174
Rectum	8	40.0	5	50.0	13	43.3	

**Chi square* test, significant if p<0.05.

DISCUSSION

Research based on Taieb *et al* shows that the average age of colorectal carcinoma patients in the current era is increasing at the age below 50 years old. This is due to changes in the pattern of increasing risk factors (reduced smoking) and increasing cancer screening, especially colorectal carcinoma. This is in accordance with the results obtained in this study where colorectal carcinoma patients on average occur at the age below 50 years old. Early screening is also increasingly being done especially in young patients with a family history of colorectal carcinoma or polyps and with genetic predisposition.[1] You et al stated that genetic predisposition and hereditary syndromes contribute to this trend. Early detection and prevention are important in the current era. The most common genetic mutation associated with hereditary cancer syndrome at a young age is Lynch syndrome, which causes a deficiency in the DNA mismatch repair mechanism. Therefore, in this study, the age of 50 years old was used as a cut off, considering that one of the objectives of this study was to analyze the relationship between MMR status and age where most colorectal carcinomas increase with age.[2]

Research conducted by Abualkhair *et al* also stated the same thing that increased screening carried out at a young age would reduce the incidence of colorectal carcinoma at the age of over 50 years old because before that age the symptoms of colorectal carcinoma usually do not appear and are only detected through screening. However, if screening is not done early, the symptoms of colorectal carcinoma will only appear at the age of >50 years old due to disease progression and diagnosis becomes more frequent in this age group.[3]

Research conducted by Siegel et al among various countries in the world states that the incidence of colorectal carcinoma increases under the age of 50 years old. This is attributed to fast food restaurants being popular among young people leading to increased body mass index and obesity. About 20% of obese patients are found to be associated with colorectal carcinoma. Another factor contributing to the increased risk of colon adenoma formation is the high prevalence of antibiotic use since childhood. Some studies have reported about 30% of patients with colon adenomas in countries with the highest rates of pediatric antibiotic consumption.[4] Long-term antibiotic use alters the gut microbiota. Studies suggest that there is a depletion of phylabacteroidetes, clostridia and proteobacteria, and an increase in fusobacteria in patients with colorectal carcinoma. The interaction of microbiota dysbiosis with the immune system in the mucosa and epithelial cells will initiate colorectal carcinogenesis.[5] The prevalence of colorectal carcinoma at a young age is also associated with smoking, alcohol consumption,

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lack of fiber and fruit consumption, and consumption of processed foods and meats that create an environment conducive to the proliferation of colonic mucosa that eventually leads to colorectal carcinoma.[4]

This study shows that colorectal carcinoma is more common in men (56.7%) and is in line with several studies that have been done before which state that men are a risk factor for colorectal carcinoma. This is because men consume alcohol and smoke more often. In addition, decreased physical activity and obesity are also risk factors for colorectal carcinoma in men. Men who work in factories are also at high risk of colorectal carcinoma due to exposure to carcinogens such as asbestos and other factory chemicals.[6-8]

The most common tumor location in this study was the rectum (43.3%) and in line with research conducted by Anthonysamy *et al* which states that the location of the rectum has a tendency for colorectal carcinoma. This is related to the function of the rectum in defecation, where it is known that the trigger factor for cancer is food. Low-fiber foods increase fecal concentration and increase food transit, which leads to longer contact between carcinogenic ingredients on the rectal mucosa. Moreover, the rectal mucosa will be directly exposed to the carcinogenic material as it is not coated by the alkaline mucus normally found in the colon.[9-10]

Relationship between MMR status and chemotherapy response

Mismatch repair (MMR) plays a role in DNA replication and is involved in the repair of DNA damage caused by chemical agents. It is known that DNA repair and cell cycle control are closely related in response to any DNA damage. Pors *et al* stated that the loss of one of the MMR protein functions can lead to resistance to some chemotherapeutic agents by inhibiting the cell's ability to detect drug-induced damage. In addition, cells with MMR defects are unable to stimulate cell death after exposure to drugs, resulting in the mutated cells continuing to divide and multiply.[11-15] However, in addition to MMR status, other factors that may affect chemotherapy response such as signet-ring histology features, high clinical stage, lymph node positivity, lymphovascular invasion, high TILS density, CEA levels and some of these factors were not included in the study.[16-18]

Alkylating agents react with DNA directly, causing base pair abnormalities by releasing electrophilic methyl diazonium ions through spontaneous chemical degradation and damaging DNA. The methyl diazonium ion is known to react at the O^6 position of guanine to produce DNA damage to the unpaired base chain through the action of DNA polymerase. The class of drugs developed resistance in MMR-deficient cells and 100-fold less toxicity in MMR-deficient cells compared to proficient cells. Guanine methylation at $O^{6Me}G/T$ and $O^{6Me}G/C$ stimulates repair processes mediated by MSH2/MSH6. There is some evidence that mutations in one of the heterodimers, hMutSα (comprising MSH2 and MSH6) or hMutLα (comprising MLH1 and PMS2) lead to resistance from exposure to cytotoxic alkylating agents. These agents can also activate apoptosis in MMR-proficient cells by cell cycle arrest at G2/M phase and p53 accumulation, but this is not the case in MMR-deficient cells.[19]

Antimetabolites are small molecules that work by altering the base chain in DNA, affecting the function of enzymes required for cellular metabolism and protein synthesis. One such drug is 6-thioguanine (TG) which works by inhibiting the synthesis of purine nucleotides by joining DNA and RNA. This methylation will cause G and A changes that stimulate MMR to recognize the *mispair* formed between $6^{Me}TG$ and thymine or cytosine during DNA replication. This results in G2/M phase cell cycle arrest in MMR-proficient cells and activates anti-apoptotic kinases such as Akt/PKB, but this cannot occur in MMR-deficient cells. Another antimetabolite drug, *5-fluorouracil* (5-FU), is usually metabolized by normal cells and tumor cells. These metabolites cause cell damage by inhibiting *thymidylate synthetase*, thus inhibiting cell division or disrupting RNA and protein synthesis. Intact MMR proteins can recognize them and incorporate them into DNA, but defective MMR can be one of the mechanisms for tumor resistance to 5-FU and tumor cells with MMR deficiency are more resistant to this drug than those with MMR proficiency. At the DNA and RNA levels, the 5-FU:G pair will be recognized by the MMR MutSα complex consisting of MSH2 and MSH6. Then the MutLα heterodimer consisting of MLH1 and PMS2 initiates a repair response or triggers the activation of apoptotic signaling through ATR/CHK1 activation. This complex mechanism of MutSα and MutLα does not occur in MMR deficiency, so the metabolite

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product of 5-FU cannot activate the apoptotic pathway of tumor cells.[19,20]

Platinum group drugs such as *cisplatin*, *carboplatin* and oxaloplatin are used in the treatment of a broad spectrum of human cancers. The hydrolyzed materials of these drugs react with DNA by forming *crosslinks* at N^7G-N^7G and N^7G-$N(^7)$A. These agents are biologically inactive and will incorporate into DNA via 1,2-ApG and 1,2-GpG which are recognized by MSH2 and MSH6 heterodimers. In MMR deficiency states, this drug will continue to be in an inactive state and will not incorporate into DNA.[1,9]

Immunotherapy has been developing rapidly for decades and has excellent antitumor effects, which is a hope for patients with advanced cancer and colon cancer with MMR deficiency. This therapy kills cancer cells by activating human antitumor immunity. The type of immunotherapy used in colon cancer patients with MMR deficiency is PD-1/PD-L1 *inhibitors*. *Programmed death* 1 (PD-1) is an immune *checkpoint* receptor expressed by activating T cells and stimulating immuno-suppression, where *programmed death ligand* 1 (PD-L1) binds to PD-1 causing T cell anergy and apoptosis. Therefore, PD-1/PD-L1 *inhibitors* prevent such T cells from undergoing dysfunction and apoptosis, thereby enhancing T cell activation, providing a novel option for the treatment of MMR or MSI-H deficient cancers. Examples of such drugs are nivolumab, pembrolizumab, atezolizumab, durvalumab and avelumab.[20]

Relationship between MMR status and age

A study by Chang *et al* showed that there was a high frequency of MMR deficiency in patients <50 years old and this is consistent with our study. The most likely cause is that there is undetected *Lynch* syndrome in patients with young age, due to late initial screening, where the syndrome is associated with young age.[15] Microsatellite instability (MSI-H) is a *hallmark of* Lynch syndrome, which is inherited in an autosomal dominant manner. It is caused by a *germline* mutation in one of the four MMR genes, resulting in loss of function of the protein coding for DNA repair. If on immuno-histochemical examination there is a deficiency in all four markers, it is most likely a Lynch syndrome. Defects in DNA repair will increase the frequency of mutations in cancer cells and increase the likelihood of mutations in important oncogenes (APC and BAX) and tumor suppressor genes. Errors in DNA replication that are not repaired, will cause the damaged DNA to continue to replicate and accumulate in the genome permanently. This phenomenon underlies MMR deficiency.[21] In addition, a defect in one of the four MMR genes at a young age causes a *germline* deletion in exon 3 of the epithelial cell adhesion molecule (EPCAM, also known as the TACSTD1 gene). Deletion in EPCAM causes MSH2 to be inactivated which is called MSH2 epimutation and EPCAM protein expression can be seen using immunohistochemistry, being one of the methods to identify *Lynch* syndrome with *germline* deletion of EPCAM.[22]

A multicenter study performing MMR panel screening in patients with colorectal carcinoma before the age of 50 years old showed the presence of *germline* mutational deletions in approximately 16% of individuals, most of whom had *Lynch* syndrome. Given this consistent data, patients diagnosed with colorectal carcinoma under the age of 50 years old should be screened with an MMR panel. Assessment of *Lynch* syndrome should begin with a family history of cancer on both the maternal and paternal side for at least three generations including the age at cancer diagnosis. Genetic testing will continue in the family considering screening in the form of Amsterdam and Bethesda criteria.[23-25]

Young age can also be affected by MLH1 hypermethylation which is one of the sporadic factors, where the MMR DNA system is inactivated by hypermethylation of the MLH1 promoter leading to loss of MLH1 expression. Loss of MLH1 expression leads to MSI, a form of genetic instability characterized by alterations in DNA microsatellite sequencing. In addition, MLH1 hypermethylation also often leads to activation of BRAF mutations that stimulate cell proliferation by signaling through the MAPK pathway (BRAF/MEK/ERK). The study conducted by Bhattarai et al, showed that 108 patients were young.[26]

Relationship between MMR status and gender

This study showed that MMR deficiency was more common in males compared to females although it was not statistically significant. This is because men consume more cigarettes and alcohol. Cigarettes have several genotoxic contents that vary in the form of polycyclic aromatic hydrocarbons, nitro-samines, heterocycles and aromatic amines. These carcinogens can reach the colon directly through the intestines and blood vessels,

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causing damage to the colorectal mucosa. Patients with *Lynch* syndrome are very susceptible to these carcinogens due to MMR deficiency so that the damaged colorectal mucosa cannot be repaired and increases the risk of tumors with MSI-H.[21] In a study conducted by Dashti *et al* stated that the mechanism of carcinogenesis of MMR gene mutations associated with alcohol is not yet known for certain. Alcohol contains acetaldehyde, a metabolite of ethanol found in high concentrations in the colon in patients who frequently consume alcohol and plays a role in carcinogenesis. Acetaldehyde affects DNA synthesis and repair, damages the structure and function of glutathione (an antioxidant peptide) and increases colonic mucosal proliferation.[(2)(7]

Research conducted by Tsai *et al* stated that colorectal carcinoma with MMR deficiency is more common in older women and younger men.[22] This is due to the role of estrogen as a protective factor from colorectal carcinoma with MMR deficiency. Estrogen acts as a pro-apoptotic effect usually mediated by MLH1 on cancer cells which inhibits colorectal cancer carcinogenesis. Estradiol increases MMR protein expression through Erβ. Some studies also show *hormone replacement therapy* (HRT) significantly increases MMR protein expression and works as an Erβ agonist. Estrogen receptor β is the predominant estrogen receptor on human colonic epithelium and its expression is reduced during carcinogenesis. Lack of Erβ expression is usually associated with decreased differentiation and reduced apoptosis, providing strong evidence for its role in antitumorigenic effects on colon cancer cells.[2(8]

Relationship between MMR status and histopathologic subtype

Some theories state that there is no difference in MMR status between colorectal carcinoma histopathologic subtypes of *signet ring cell*, *mucinous* and *poorly differentiated adenocarcinoma*, because both have high MMR deficiency and have the same mutation mechanism, so there is no significant difference as done in this study. The genetic background in these subtypes both have high BRAF mutations and these mutations are often found in MSI-H cases. *CpG island methylator phenotype* (CIMP) is a phenotype in which gene expression is suppressed by methylation of *promoter* genes. Colorectal carcinomas with positive CIMP are often reported to have high KRAS, BRAF mutations and often followed by methylation of the MMR gene MLH1 which causes MMR deficiency in colorectal carcinomas.[2(9]

Relationship between MMR status and tumor location

Luisetto *et al* stated that carcinoma in the left colon predominantly follows the CIN molecular pathway, while carcinoma in the right colon predominantly follows the MSI pathway. Some studies also state that colorectal carcinoma with a high frequency of MMR deficiency is often found in the right colon. This is consistent with our study but the results are not statistically related. The colon is one and the same organ, but it develops through two different primitive embryonic areas. The *midgut* develops into the small intestine up to the proximal two-thirds of the transverse colon and *the hindgut* develops into the distal third of the transverse colon up to the upper anal canal. Despite the fact that the midgut also develops into different organs namely most of the small intestine and appendix, carcinomas arising from these organs have different mutations and are clinically treated differently. As many as 1000 genes are differentially expressed in the right and left intestine, which are acquired during embryonic formation. These two intestines have different responses to environmental factors (e.g. exposure to bile acids and bacteria) and have different procarcinogenesis factors. There is an inherent trait in the right colon where it is more susceptible to the initiation or progression of cellular transformation through a pathway that begins with cells losing their ability to recognize and repair nucleotide damage. These different characteristics result in an increased number of MMR-deficient tumors in the right colon, including several other genetic and clinicopathological features.[30-32] The precursor lesion in the right colon is usually a *serrated polyp*. This *serrated* pathway transforms into malignancy, high BRAF mutation, associated with damage to the *mismatch repair* system in DNA, hypermethylation of CpG *island* which causes loss of tumor *suppressor gene* function (CpG *island methylator phenotype/CIMP*). This colonic location is also associated with lymphocyte infiltration and immune system activation resulting in a high density of TILs, hypermethylation and activation of the Wnt pathway. Mutations or hypermethylation in the *promoter region of* the MMR gene cause *microsatellite instability* (MSI).[7,33,34]

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Colorectal cancer is a heterogeneous disease, having various genetic alterations depending on its location, namely the right colon and left colon. There are differences in embryologic, histologic, genetic and immunologic origin between the two sites. The left colon predominantly follows the CIN molecular pathway, while the right colon follows the MSI pathway. The colon is a single organ, but develops from two distinct embryologic areas: the midgut, which develops into the small intestine up to two-thirds of the colon transversum. Hindgut which develops into the distal third of the transverse colon up to the upper anal canal. Functionally, the proximal colon absorbs most of the water from the food content and the rest of the colon plays a peristaltic role for motility and lubricates the food residue as it becomes harder as it moves to the rectum. The right colon, upon detecting bile acids and other metabolites migrating from the small intestine, activates detoxification mechanisms before the bacterial colonies act on the metabolites in the left colon by fermenting the unabsorbed nutrients. Several hydrolytic enzymes of bacteria (β-glucoronidase, β-glucosidase, arylsulphatase, azoreductase, nitroreductase) are involved in the production of mutagenic or genotoxic metabolites that are usually abundant in the left colon and rectum, which play a role in carcinogenesis at these locations. The cecum is the first part of the colon to be involved in digestion, and the appendix shares the same embryological origin but is not involved in digestion. Both have immune responses and a high concentration of lymphocytes. Histologically, inflammation is more common in the right colon compared to the left colon due to these immune reactions. This will lead to activation of the MSI pathway due to the presence of tumor infiltrating lymphocytes (TILs) as one of its characteristics.[35-37]

Research conducted by Watanabe et al stated that colon carcinoma with MSI-H mostly occurs in the right colon. However, sporadic MSI-H colorectal carcinoma can also occur in the left colon and the underlying mechanism is not yet known. This may be due to the ABCB1 and PLAGL1 genes which are tumor suppressor genes that are highly expressed in MSI-H in the left colon. The expression of these two genes is inhibited by methylation of the MLH1 promoter which is overexpressed in the left colon 113. Therefore, MSI-H can not only occur in the right colon but can also occur in the left colon. Therefore, the results of this study are not significantly related.[38]

CONCLUSIONS

There was a significant association between MMR status and chemotherapy response in colorectal carcinoma. However, there was no association between MMR status and age, gender, diagnosis and tumor location.

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