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Research Article

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Association Between Microsatellite Instability (MSI) based on MLH1 and PMS2 Marker with Clinicopathological Features of Colorectal Cancer Patients

Rizka Vidya Lestari[1*], Neni Arshita[1], Dewi Kartikawati Paramita[2] and Susana H. Hutajulu[3]

[1]Postgraduate of Biomedical and Medical Science Study Program, Main Concentration of Histology and Cell Biology Medical Faculty of Gadjah Mada University

[2]Department of Histology and Cell Biology Medical Faculty of Gadjah Mada University

[3]Division of Hematology and Medical Oncology, Department of Internal Medicine, Medical Faculty of Gadjah Mada University

[*]Corresponding Author
Rizka Vidya Lestari, Department of Histology and Cell Biology, Gadjah Mada University, Indonesia.

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Abstract

Background: *Microsatellite instability (MSI) is one of the important pathways involved in development of colorectal cancer (CRC). MSI occurs due to mutations or hypermethylation of negative MMR proteins MLH1 and PMS2. The accumulation of mutations at microsatellite locus accelerated the development of CRC. Characteristics of CRC patients with MSI are not sensitive to 5FU chemotherapy and have a good clinical outcomes that can be used as a prognostic factor and a predictor of therapy. Therefore MSI detection is needed.*

Method: *A retrospective cross-sectional study of 80 CRC slides obtained from DR. Sardjito Hospital Yogyakarta and clinical laboratory in 2010-2016. Immunohistochemical staining with anti-MLH1 and anti PMS2 antibodies to see MSI status. Negative MLH1 expression is called MSI MLH1 positive and PMS2 negative expression is called positive MSI PMS2. The association between MSI MLH1 and PMS2 with clinicopathology parameters was analyzed using Chi square.*

Results: *Colorectal cancer patients MSI MLH1 as much as 44 (61.1%), MSI PMS2 as much as 21 (29.2%) and, MSI MLH1 and PMS2 simultaneously as much as 18 (25.0%). MSI MLH1 and PMS2 positive are found in ≥50 years old. The number of male patients is more than women. Most of the patients had a T3-T4 tumor size with advanced stage and well differentiated. MSI MLH1 associated with tumor differentiation (p = 0.011).*

Conclusion: *MSI MLH1 is associated with tumor differentiation (p = 0.011), but, no association with the other clinicopathology parameters. MSI PMS2 is not associated to the overall clinicopathology parameters. MSI MLH1 and PMS2 are not simultaneously associated with all clinicopathology parameters (p> 0.05).*

Keywords: Microsatellite Instability, MLH1, PMS2, Colorectal Cancer.

1. Introduction

Colorectal cancer (CRC) is a malignancy caused by abnormal growth of cells in the colon and rectum. Pinheiro et al (2010) states that 25% - 35% of CRC are in rectum and the other in colon. In Indonesia, the incidence of CRC by 100,000 population every years is 19.1 in males and 15.6 in females [11]. One of the important pathway involved in the development of CRC is Microsatellite instability (MSI). CRC cases of MSI only for 15% in overall CRC cases. The MSI pathway involves germline and hypermethylation mutations in the protein coding genes that play a role in maintaining and repair of DNA damage (mismatch repair protein/ MMRp) such as MLH1 and PMS2 [7]. Both of these proteins will form heterodimers to correct base pairs of errors during replication. In CRC with MSI, MLH1 and PMS2 heterodimers are not formed so that DNA repair process does not occur [27]. Characteristics of CRC patients with MSI are not sensitive to 5FU chemotherapy

and have good clinical outcomes that can be used as a prognostic factor and a predictor of therapy. Therefore, the detection of MSI is necessary for patient's management [24].

Research of MSI in Indonesia is still very limited. The research has been done is only to observe the association between the absence of expression of MMR protein separately with clinicopathological parameters such as age, sex, tumor size, tumor location and tumor differentiation. For clinicopatholog parameters such as tumor stage have never been evaluated. Therefore, this study aims to observe MSI status based on the expression of MMR proteins that have a corresponding role either separately or together and are associated with the clinicopathology parameters. The molecular examination is remains a standard inspection for the detection of MSI, but the method is relatively expensive so that the immunohistochemical method (IHC) is often to use as an option when molecular examination cannot be performed.

2. Method

This research is descriptive observational with cross sectional design. This research has received permission from Medical Research and Medical Ethics Committee Medical Faculty of Gadjah Mada University, with reference number KE / FK / 90 / EC / 2016. The sample of research are 80 slide of adenocarcinomas type of CRC that collected from DR. Sardjito Hospital in Yogyakarta and the laboratory clinic in 2010-2016 based on inclusion and exclusion criteria. Eight samples did not contain tumor tissue and not colon, so total samples that have been analyzed were 72 samples.

Immunohistochemical staining with anti-MLH1 antibodies (Biocare Medical, CM 220 AK, BK, CK) and anti PMS2 (Biocare Medical, CM 344 AK, BK) to observe MSI status. Negative MLH1 expression is called MSI MLH1 positive and negative PMS2 expression is called positive PMS2 MSI (Figure 1). The immunohistochemical kit used is Star Trek Universal HRP Detection System (STUHRP700 H, L10). Positive control for MLH1 (Sigma-Aldrich Cell Marque, with catalog number 285S), positive control for PMS2 (Sigma-Aldrich Cell Marque, with catalog number 285S) and normal colon tissue from laboratory collection of Anatomy Pathology Faculty of Medicine GMU which gave the results of staining same as positive control. Negative control used is human tonsil tissue from laboratory collection of Anatomical Pathology Faculty of Medicine GMU. Descriptive data on the frequency of MSI status based on protein expression were presented in number (%) .The association between MSI MLH1 and PMS2 with clinicopathology parameters analyzed by Chi square. A p value of 0.05 was statistically significant.

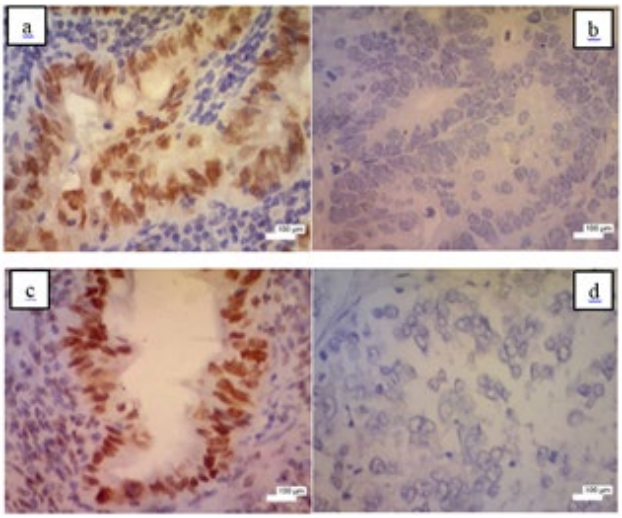

Figure 1: Immunohistochemical staining using anti-MLH1 and anti PMS2 antibodies with 400x magnification. a) Positive MLH1 expression or MSI MLH1 negative, b) Negative MLH1 expression or MSI MLH1 positive, c) Positive PMS2 expression or MSI PMS Negative. d) PMS2 negative expression or MSI PMS2 Positive.

3. Results

3.1 Frequency of MSI MLH1 and PMS2 in CRC Patients

Descriptive analysis of MSI MLH1 and MSI PMS2 frequency showed that there were 44 (61.1%) MSI MLH1, 21 (29.2%) MSI PMS2 and 18 (25.0%) MSI MLH1 and PMS2 (Table 1).

MSI Status	MLH1	PMS2	MLH1 and PMS2*
MSI positive	44 (61,1%)	21 (29,2%)	18 (25,0%)
MSI negative	28 (38,9%)	51 (70,8%)	54 (75,0%)
Total	72 (100%)	72 (100%)	72 (100%)

*Both of MMRp negative

Table 1: Frequency of MSI MLH1 and PMS2 in CRC Patients

3.2 Association between MSI MLH1 with Clinicopathology Parameters of CRC Patients

In this study seen the association between MSI MLH1 with some clinicopathological parameters such as age, sex, size, location, stage and tumor differentiation. CRC patients with MSI MLH1 who were <50 years old were 11 (15.3%) and those aged ≥50 years were 33 (45.8%). CRC patients with MSI MLH1 in male as many as 29 (40.3%) and women as many as 15 (20.8%). CRC patients with MSI MLH1 had a 4 (6.6%) T2 tumor size and T3-T4 tumor size of 31 (50.8%). CRC patients with MSI MLH1 who had tumors in colon were 30 (41.7%) and in rectal were 14 (19.4%). CRC patients with MSI MLH1 early stage were 15 (21,1%) and advanced stage were 28 (39,4%). CRC patients with MSI MLH1 have well differentiation as many as 42 (58,3%) and poor differentiation as many as 2 (2,8%). The result of statistical analysis using chi square showed that there was a association between MSI MLH1 with tumor differentiation in CRC patients (p = 0,011). However there was no association between MSI MLH1 with other clinicopathology parameters (p> 0.05) (Table 2).

Parameters	MSI-MLH1	P value	OR
Age	n= 72	0, 113	2,250
<50 y.o	11 (15,3%)		
≥50 y.o	33 (45,8%)		
Gender	n= 72	0,102	0,448
Male	29 (40,3%)		
Female	15 (20,8%)		
Tumor size	n= 61	0,286	0,310
pT1-pT2	4 (6,6%)		
pT3-pT4	31 (50,8%)		
Tumor location	n= 72	0,510	0,721
Colon	30 (41,7%)		
Rectum	14 (19,4%)		
Tumor stage	n= 71	0,579	0,747
Early	15 (21,1%)		
Late	28 (39,4%)		
Tumor differentiation	n= 72	0,011	0,143
Well	42 (58,3%)		
Poor	2 (2,8%)		

Table 2: Association between MSI MLH1 with Clinicopathology Parameters of CRC Patients

3.3 Association between MSI PMS2 with Clinicopathology Parameters of CRC Patients

The status of MSI PMS2 was found as much as 6 (8.3%) at <50 years old and 15 (20.8%) at ≥50 years old. Male patients with MSI PMS2 were found as many as 15 (20.8%) and women as many as 6 (8.3%). All MSI PMS2 patients had a T3-T4 tumor size that is 17 (27.9%). CRC patients with MSI PMS2 had 14 (19.4%) tumor in colon and 7 (9.7%) in rectum. CRC patients with MSI PMS2 early stage were 5 (7.0%) and advanced stage were 16 (22,5%). Patients CRC with MSI PMS2 who have well differentiation as many as 20 (27,8%) and poorl differentiation as many as 1 (1,4%). The result of statistical analysis using chi square shows that there is no association between MSI PMS2 with all clinicopathology parameters of CRC patients (p> 0,05) (Table 3).

Parameters	MSI-MLH1	P value	OR
Age	n= 72	0,694	1,250
<50 y.o	6 (8,3%)		
≥50 y.o	15 (20,8%)		
Gender	n= 72	0,148	0,450
Male	15 (20,8%)		
Female	6 (8,3%)		
Tumor size	n= 61	0,147	-
pT1-pT2	0 (0%)		
pT3-pT4	17 (27,9%)		
Tumor location	n= 72	0,874	0,917
Colon	14 (19,4%)		
Rectum	7 (9,7%)		
Tumor stage	n= 71	0,316	1,800
Early	5 (7,0%)		
Late	16 (22,5%)		
Tumor differentiation	n= 72	0,203	0,269
Well	20 (27,8%)		
Poor	1 (1,4%)		

Table 3: Association between MSI PMS2 with Clinicopathology Parameters of CRC Patients

3.4 Association between MSI MLH1 and PMS2 with Clinicopathology Parameters of CRC Patients

In this study, MSI was also assessed based on the absence of MLH1 and PMS2 expression. MSI positive if both of the MMR (MLH1 and PMS2) proteins are not expressed together. MSI status was positive in patients under the age of <50 years old as much as 4 (5.6%) and the age of ≥50 years old was 14 (19.4%). MSI was positive in men as many as 13 (18.1%) and in women as many as 5 (6.9%). All MSI-positive patients had a T3-T4 tumor size of 15 (24.6%). Positive MSI patients who had tumors in colon were 13 (18.1%) and in rectum 5 (6.9%). Patients of MSI were positive for 5 (7.0%) and advanced stage were 13 (18.3%). Patients with positive MSI had well differentiation of 17 (23.6%) and poorl differentiation of 1 (1.4%). The result of statistical analysis using chi square showed that there was no association between MSI MLH1 and PMS2 together with all clinicopathology parameters of CRC patients (p> 0,05) (Table 4).

Parameters	MSI (+)	MSI (-)	P value	OR
Age	n= 72		0,307	1,900
<50 y.o	4 (5,6%)	19 (26,4%)		
≥50 y.o	14 (19,4%)	35 (48,6%)		
Gender	n= 72		0,168	0,446
Male	13 (18,1%)	29 (40,3%)		
Female	5 (6,9%)	25 (34,7%)		
Tumor size	n= 61		0,183	-
pT1-pT2	0 (0,0%)	5 (8,2%)		
pT3-pT4	15 (24,6%)	41 (67,2%)		
Tumor location	n= 72		0,475	0,654
Colon	13 (18,1%)	34 (47,2%)		
ReCtum	5 (6,9%)	20 (27,8%)		
Tumor stage	n= 71		0,628	1,337

Early	5 (7,0%)	18 (25,4%)		
Late	13 (18,3%)	35 (49,3%)		
Tumor differentiation	n= 72		0,304	0,338
Well	17 (23,6%)	46 (63,9%)		
Poor	1 (1,4%)	8 (11,1%)		

Table 4: Association between MSI with Clinicopathology Parameters of CRC Patients

4. Discussion

4.1 Clinical Parameters of CRC Patients

Based on the clinicopathology data of the CRC patients obtained, it can be seen that the CRC sufferers are more commonly found at the age of ≥50 years old. Over 90% of CRC cases developed over the age of 50 years old with a 50-fold incidence of CRC at the age of 60-79 years old [11]. The incidence of CRC in men is more common than women. Men and women have a different patterns of nutritional and metabolic intake. Nutrition affects about 30% of CRC events. The involvement of hormonal factors such as estrogen in women is also known to play a role in the development of CRC [16]. Clinicalopathology parameters such as size, location, stage and degree of tumor differentiation determine the prognosis of CRC patients [18]. Based on the clinicopathology data obtained, it is known that most of the CRC patients have T3-T4 tumors. T3 and T4 tumor sizes generally have wide invasive diameters and distances and are associated with poor prognosis [1,2]. The study of Bohorquez et al., (2016) showed that the majority of CRC patients had a T3-T4 tumor size (78.9%) with a high mortality rate. Differences in tumor sites represent different risk factors and molecular profiles [22]. Family history and heredity are closely related to the risk of colon tumor than in rectum [3]. In contrast, the habit of consuming alcohol is closely related to the risk of rectal cancer than in colon [6]. In this study, CRC patients who had tumors in colon more than in rectum. Xiao et al., (2013) states that patients with tumor sites in colon have a poor prognosis and low life expectancy [29].

Staging is also the most important prognostic factor for colorectal cancer. Patients with CRC diagnosed at an early stage have a better prognosis than advanced stage [18]. In this study, patients of advanced stage of CRC (III-IV) more than the early stage (I-II). This is in line with the study of Nahas et al. (2015) which shows that as many as 88% of CRC patients are found in advanced stage III and IV. Another clinicopatholog parameter related to prognosis is the degree of tumor differentiation. Tumor differentiation is significantly associated with tumor stage and metastatic risk to surrounding tissues. The degree of differentiation is closely related to the survival of the CRC patient [8]. In general, adenocarcinoma type CRC patients have a good to moderate degree of differentiation [19]. In this study all patients were adenocarcinoma type. CRC patients with well differentiation were found to be more numerous than poor differentiation.

4.2 Frequency of MSI MLH1 and PMS2 in CRC Patients

In this study found that the frequency of MSI MLH1 in CRC patients more than MSI PMS2. Alteration of PMS2 expression affects only a small proportion of cases with MSI of 1.5% [18]. Negative MLH1 expression affects PMS2 stability, so that the heterodimer is not formed from either of these proteins [10]. MLH1 has a stronger stability than PMS2. MLH1 remains expressed even without PMS2 because it can still bind to the other DNA repair proteins such as MLH3 or PMS1. When MLH1 is not expressed due to mutation or hypermethylation, the heterodimer complex is not formed so that PMS2 tends to be unstable and easily degraded [27].

4.3 Association Between MSI MLH1 and PMS2 with Age

The incidence of MSI MLH1 and PMS2 either separately or together is found in older age compared to young age. MSI MLH1 is the most common compared to MSI PMS2. This is in line with research conducted by Sudoyo et al., (2010) which states that MLH1 negative is more prevalent in elderly patients (50.4%). Furthermore, the results of the study of Yiu et al. (2005) also showed that patients aged> 50 years old experienced an MLH1 mutation of 83% and hypermethylation of MLH1 promoters by 62%.

4.4 Association Between MSI MLH1 and PMS2 with Gender

Colorectal cancer is also one of the causes of high mortality worldwide in both men and women [26]. In this study, CRC with MSI MLH1 and PMS2 were separately or together found mostly in males than females. This is in line with the study [31] which states that MMR protein deficiency is found in males than females. Young men tend to have MSI. However, as we get older, the condition of MSI is more common in women. Even women over the age of 50 tend to experience MSI-H with low survival rates (4, 14).

4.5 Association Between MSI MLH1 and PMS2 with Tumor Size

The size of the tumor plays an important role in staging, this is known by looking at the diameter of the tumor and the distance of invasion to the surrounding tissue [23]. The majority of MSI MLH1 and PMS2 CRC patients were known to have tumor size of T3-T4 group, whereas patients with T1-T2 tumor size were not found. Tumor group T3 and T4 tumors generally have large enough invasive diameters and distances [28]. The invasion mechanism and tumor metastasis capability are caused by MAPK pathway activation resulting in signal transduction for adhesion and cell migration. This condition is triggered by an interruption in the MMR protein leading to MSI [29].

4.6 Association Between MSI MLH1 and PMS2 with Tumor Location

Studies of tumor location such as proximal, distal and rectal are often combined with each other. The evidence suggests that the location of different tumors is associated with risk factors and molecular profiles that cause cancer [22]. The results of Mojarad et al. (2016) showed that MSI is mostly found in colon than rectum [20]. Proximal colon especially has a worse prognosis than other colonic regions. This is because the proximal region is very susceptible to mutation of the MMR protein or hypermethylation of the MLH1 promoter [22]. In line with previous studies, MSI MLH1 and PMS2 both separately and together in this study occurred in colon rather than rectum. Xiao et al., (2013) states that tumors in colon especially in the proximal region are more aggressive than other colonic regions. A flat tumor type causes the tumor to be difficult to distinguish from colonoscopy, so early detection is difficult [29]. Lifestyles such as smoking and alcohol consumption are also risk factors for MSI in colon [9].

4.7 Association Between MSI MLH1 and PMS2 with Tumor Stage

Staging of the tumor is one of the important prognostic predictors of clinical outcomes of CRC patients [20]. In this study, MSI MLH1 and PMS2 both separately and together were found in patients with advanced stage III and IV stages. This is consistent with the characteristics of CRC patients with MSI which tend to be present in advanced stages with a poor prognosis [18]. CRC patients with MSI are often associated with reduced tumor recurrence rates, and have better survival compared with non-MSI CRC patients, especially if the tumor is still at an early stage. However, it continues to decline as the tumor stage progresses further so that the patient will show a poor prognosis [13].

4.8 Association Between MSI MLH1 and PMS2 with Tumor Differentiation

Another clinicopathology characteristic of CRC patients with MSI is poor differentiation [29]. MSI patients with poor differentiation are commonly found at younger ages (<50 years old), tumors often present in large-scale colon, and lymphovascular invasion. This condition illustrates a poor prognosis. However, when compared with non-MSI, MSI CRC patients with poor differentiation had a better clinical outcomes [17]. Kazama et al., (2007) states that MSI is a non-aggressive subtype compared to a non-MSI of poor differentiation. This is characterized by metastatic ability to the lymph nodes and progression to a lower advanced stage [15]. In contrast to previous studies, in this study, MSI MLH1 and PMS2 both separately and together largely had a well differentiation, MSI MLH1 statistically had a association with tumor differentiation significantly ($p = 0.011$). Well differentiation is usually indicated by tumors located in the distal colon, at the early stage with low aggressiveness [13].

5. Conclusion

Based on the result of the research, it can be concluded that the patients of CRC MSI MLH1 are 44 (61,1%), MSI PMS2 are 21 (29,2%) and MSI MLH1 and PMS2 together are 18 (25,0%). MSI MLH1 was associated with tumor differentiation ($p = 0.011$), but was not related to the other clinicopathology parameters. MSI PMS2 is not related to all clinicopathology parameters. MSI MLH1 and PMS2 also are unrelated to all clinicopathology parameters ($p > 0.05$).

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