P-ISSN 0215-7284
e-ISSN 25279106
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Correlation between Metalloproteinase-9 Matrix Expression and Clinicopathology in Colorectal Adenocarcinoma Not Otherwise Specified

Rica Friska Sembiring, T. Kemala Intan, T. Ibnu Alferraly
Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra
Medan

Correspondence writer: dr. Rica Friska Sembiring, dr. T. Kemala Intan.
Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra
Jl. University No. 1, Medan 20155.
E-mail: sembiringricafriska@gmail.com; t.kemala@.ac.id

Received : 19-10-2022
Accepted : 22-11-2022
Published: 31-05-2024

ABSTRACT

Background
Matrix metalloproteinase-9 (MMP-9) is a member of the metalloproteinase enzyme that is often found in colorectal adenocarcinoma. MMP-9 is able to destroy basement membrane proteins and extracellular matrix which play a role in tumor cell invasion.

Method
Cross-sectional analytic study with 30 paraffin block samples diagnosed as colorectal adenocarcinoma NOS at H. Adam Malik General Hospital Medan. MMP-9 expression was assessed using immunohistochemical staining in the cytoplasm of tumor cells and cells in the stroma. Expression was assessed using the Quick Score method and was declared weak if the total value was 0-5 and strong if the total value was ≥6. Statistical analysis between MMP-9 expression and depth of invasion was carried out using Fisher's Exact and Mann-Whitney U tests.

Results
MMP-9 expression was significantly related to stage (p=0.01), depth of invasion(p=0.03), and stromal Tils (p=0.03) and not significantly related to gender (p=0.28) , age (p=0.82), tumor location (p=0.69), grading (p=0.26), lymph node involvement (p=0.53), metastasis (p=0.53), perineural invasion (p=0.25), lymphatic invasion (p=1.00), IMVI (p=0.531.00), EMVI (p=1.00), tumor budding (0.25).

Conclusion
MMP-9 expression can be used as an indication of prognosis and also helps clinicians, especially in the management of patients with colorectal adenocarcinoma NOS.

Keywords: colorectal adenocarcinoma NOS, MMP-9, clinicopathology.

Maj Patol Indones 2024; 33(3): 795-802
DOI: 10.55816/mpi.v33i3.629

P-ISSN 0215-7284
e-ISSN 25279106
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INTRODUCTION

Colorectal carcinoma is a malignancy that is still feared by most of the world's people and is one of the main causes of cancer-related death. Data from the Global Burden of Cancer (GLOBOCAN) in 2020 shows that colorectal carcinoma is the third most common cancer in the world with an estimated 1,931,590 new cases (10%) and 935,173 deaths (9.4%).[1]

World Health Organization defines colorectal cancer is a malignant tumor of the colon, originating from the epithelial component and the presence of invasion of tumor cells between the stroma of the muscularis mucosal layer to the submucosal layer. Histopathologically, 90% of colorectal malignancies are adenocarcinomas.[2-4] The most common type of adenocarcinoma is N.otherwise specified (NOS), which is a type of colorectal adenocarcinoma that does not have specific features and certain molecular characteristics of colorectal adenocarcinoma.

Reporting of colorectal carcinoma according to the Standardized Pathology Report for Colorectal Cancer, 2nd edition 2020 covers, specimen origin, histopathological type, tumor location, macroscopic features, tumor size, margin of resection, vascular and lymphatic invasion, depth of invasion, regional lymphnode involvement, tumor budding, preoperative chemoradiotherapy, pre-existing adenoma, DNA mismatch repair, Microsatellite instability (MSI), KRAS/BRAF/NRAS mutation analysis.[8]

One of the components of the extracellular matrix that is interesting to talk about is Matrix Metalloproteinase-9 (MMP-9). MMP-9 is an endopeptidase that has a proteolytic effect and plays a role in degrading the extracellular matrix, and is secreted by various cells such as tumor cells, inflammatory cells, and fibroblasts. Hersenyi et al in their research stated that MMP-9 acts as a potential biomarker in patients with colorectal carcinoma because it has a consistently higher diagnostic sensitivity when compared to conventional biomarkers such as Carcino Embryonic Antigen (CEA) or Carbohydrate Antigen (CA 19-9).[9] Several studies stillexpress controversial opinions regarding MMP-9 expression in colorectal carcinoma, namely the study of Buhmeida et al in 2009 and Fahry's research in 2014.[10]

METHOD

This research is an analytical study with a cross sectional approach. The study was conducted at the Department of PA FK USU from April 2022 to September 2022. The population in this study were paraffin slides or blocks of Colorectal Adenocarcinoma NOS patients undergoing colectomy surgery. The sample size in this study was calculated based on the Slovin sample size formula and a total of 30 samples were obtained.[12] Metalloproteinase-9 expression matrix is an immunohistochemical assessment of MMP-9 protein using BZ- 0898450-AP Rabbit Anti MMP-9 Polyclonal Antibody with a dilution of 1:300 (40^0C, overnight) from the manufacturer Bioenzy. MMP-9 expression was assessed semiquantitatively using an Olimpus CX21 binocular light microscope with an 18mm eyepiece and the assessment was made based on the 'Quick Score', namely Q=PxI, where P is the percentage of cells stained positively and I is the staining intensity. MMP-9 immunohistochemical expression was assessed in the cytoplasm of tumor cells and cells in the stroma. the ten great visual fields. To see the intensity of staining on positively stained cells using a strong magnification of 400 times. The assessment is calculated based on the area proportion score and the intensity score: 17 16 Assessment of the percentage of cells that were positively stained was carried out by looking in 10 large fields of view (100 times magnification) and taking the average number of the ten large fields of view. To see the intensity of stainingon positively stained cells using a strong magnification of 400 times. The assessment is calculated based on the area proportion score and the intensity score: 17 16 Assessment of the percentage of cells that were positively stained was carried out by looking in 10 large fields of view (100 times magnification) and taking the average number of the ten large fields of view. To see the intensity of staining on positively stained cells using a strong magnification of 400 times. The assessment is calculated based on area proportion score and the intensity score:17

1. The score for the proportion of the area of the stained cells is divided into: 16,18
   0=negative
   1=<25%
   2=≥25%-≤50%
   3=>50%
2. The staining intensity score is divided into: 18
   0=uncolored 1+=colored weak
   2+=medium colored,
   3+=strongly colored

Maj Patol Indones 2024; 33(3): 795-802
DOI: 10.55816/mpi.v33i3.629

P-ISSN 0215-7284
e-ISSN 25279106
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The final result is by multiplying the area proportion score and the intensity score, andinterpreted to be:18
Weak expression: if the total value is 0-5
Strong expression: if total score ≥6

Data analysis was carried out using the Fisher's Exact and Mann-Whitney U tests. The value ofp <0.05 was stated to be statistically significant.

RESULTS

Table 1. Frequency distribution of clinical characteristics of samples of patients with colorectal adenocarcinoma not otherwise specified.

Variable	n	%
Gender		
Man	19	63.3
Woman	11	36.7
Age		
<24 years old	1	3.3
24-39 years old	5	16.7
40-55 years old	16	53.3
56-71 years old	5	16.7
>71 years old	3	10.0
Location		
Right Colon	5	16.7
Left Colon	19	63.3
Rectum	6	20.0
Stadium		
Stage 1	7	23.3
Stage 2	14	46.7
Stage 3	5	16.7
Stage 4	4	13.3

The youngest is 22 years old and the oldest is 83 years with an average age of 50.1 years. The largest age group is aged 40-55 years with 16 samples (53.3%). The male sex was more numerous, namely 19 samples (63.3%). Most colorectal adenocarcinoma locations were in the left colon of 19 samples (63.3%). The most stage is stage II of 14 (46.7%).

The histopathological frequency distribution of colorectal adenocarcinoma NOS samples was lower grade, namely 18 samples (60%). The highest depth of tumor invasion (T) was T3, namely 20 samples (66.7%). Without the involvement of the KGB, there were 21 samples (70%). Without more metastases, namely 26 samples (86.7%). Without perineural invasion, there were 20 samples (66.7%). Without invasion of lymphatic vessels more, namely 16 samples (53.7%). Without IMVI more 20 samples (66.7%). With EMVI found more, namely 18 samples (60%). The most budding tumors were low budding with a total of 21 samples (70%). Stromal Tils are more Tils rich by 20 samples (60%).

Table 2. Frequency distribution of histopathological characteristics of samples of colorectal adenocarcinoma not otherwise specified.

Variable	n	%
Grading		
Low Grade	18	60.0
High Grade	12	40.0
The depth of the invasion		
T1	1	3.3
T2	6	20.0
T3	20	66.7
T4	3	10.0
Lymph node involvement		
N0	21	70.0
Metastases	9	30.0
M0	26	86.7
M1	4	13.3
Perineural Invasion		
Negative	20	66.7
Positive	10	33.3
Lymphatic Invasion		
Negative	16	53.3
Positive	14	46.7
Intramural Vascular Invasion		
Negative	20	66.7
Positive	10	33.3
Extramural Vascular Invasion		
Negative	12	40.0
Positive	18	60.0
Budding tumor		
Low budding	21	70.0
Intermediate budding	5	16.7
High buddingStromal TILs	4	13.3
Tils rich	20	66.7
Tils poor	10	33.3

Tumor infiltrating lymphocytes (Tils)

Table 3. Frequency distribution of metalloproteinase-9 matrix expression in samples of patients with colorectal adenocarcinoma not otherwise specified.

MMP-9 expression	Amount (n)	Percentage (%)
Weak	3	10
Strong	27	90

In this study, 3 samples (10%) had weak MMP-9 immunohistochemical expression and 27 samples (90%) had strong expression.

Maj Patol Indones 2024; 33(3): 795-802
DOI: 10.55816/mpi.v33i3.629

P-ISSN 0215-7284
e-ISSN 25279106
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Table 4. Frequency distribution and relationship of metalloproteinase-9 matrix expression with clinical characteristics of samples with colorectal adenocarcinoma not otherwise specified.

Variable	MMP-9 expression			p-value
	Weak expression n (%)	Strong expression n (%)	Total n (%)	
Gender				
Man	3 (100)	16 (59.3)	19 (63.3)	0.28*
Woman	0 (0)	11 (40.7)	11 (36.7)	
Age				
<24 years old	0 (0)	1 (3.7)	1 (3.3)	
24-39 years old	0 (0)	5 (18.5)	5 (16.7)	
40-55 years old	3 (100)	13 (48.2)	16 (53.3)	0.82*
56-71 years old	0 (0)	5 (18.5)	5 (16.7)	
>71 years old	0 (0)	3 (11.1)	3 (10)	
Location				
Right colon	0 (0)	5 (18.5)	5 (16.7)	
Left colon	2 (66.7)	17 (63.0)	19 (63.3)	0.69**
Rectum	1 (13.3)	5 (18.5)	6 (20.0)	
Stadium				
Stadium 1	3 (100)	4 (14.8)	7 (23.3)	
Stadium 2	0(0)	14 (51.8)	14 (46.7)	0.01**
Stadium 3	0 (0)	5 (18.6)	5 (16.7)	
Stadium 4	0 (0)	4 (14.8)	4 (13.3)	

Matrix Metalloproteinase-9 (MMP-9), Fisher's Exact test*, Mann-Whitney U** test.

Statistical test results showed that there was no significant relationship between gender and MMP-9 expression with a value of p=0.28 (p>0.05), nor was there a significant relationship between age and MMP-9 expression with a value of p=0.82 and there was no significant relationship between tumor location and MMP-9 expression with p=0.69. However, there was a significant relationship between stage and MMP-9 expression with a value of p=0.01.

Table 5. Frequency distribution and correlation between mmp-9 immunohistochemical expression and histopathological characteristics of samples with colorectal adenocarcinoma not otherwise specified.

Variable	MMP-9 expression			p-value
	Weak expression n (%)	Strong expression n (%)	Total n (%)	
Grading				
Low Grade	3 (100)	15 (55.6)	18 (60.0)	0.26*
High Grade	0 (0)	12 (44.4)	12 (40.0)	
The depth of the invasion				
T1	1 (33.4)	0 (0)	1 (3.3)	0.03**
T2	2 (66.7)	4 (14.8)	6 (20.0)	
T3	0 (0)	20 (74.1)	20 (66.7)	
T4	0 (0)	3 (11.1)	3 (10.0)	
KGB involvement				
N0	3 (100)	18 (66.7)	21 70.0)	0.53*
N1	0 (0)	9 (33.3)	9 (30.0)	
Metastases				
M0	3 (100)	23 (85.2)	26 (86.7)	
M1	0 (0)	4 (14.8)	4 (13.3)	1.00*
Perineural Invasion				
Negative	1 (33.3)	19 (70.4)	20 (66.7)	
Positive	2 (66.7)	8 (29.6)	10 (33.3)	0.25*
Lymphatic invasion				
Negative	2 (66.7)	14 (51.8)	16 (53.3)	
Positive	1 (33.3)	13 (48.2)	14 (46.7)	1.00*
IMVI				
Negative	3 (100)	17 (63.0)	20 (66.7)	
Positive	0 (0)	10 (37.0)	10 (33.3)	0.53*
EMVI				
Negative	1 (33.3)	11 (40.7)	12 (40.0)	
Positive	2 (66.7)	16 (59.3)	18 (60.0)	1.00*
Budding tumor				
Low budding	3 (100)	18 (66.7)	21 (70)	
Intermediate budding	0 (0)	5 (18.5)	5 (16.7)	0.25**
High budding	0 (0)	4 (14.8)	4 (13.3)	
Stromal TILs				
TIls rich	3 (100)	7 (25.9)	10 (33.3)	
Tilspoor	0 (0)	20 (74.1)	20 (66.7)	0.03*

Maj Patol Indones 2024; 33(3): 795-802
DOI: 10.55816/mpi.v33i3.629

P-ISSN 0215-7284
e-ISSN 25279106
Accredited by KEMENRISTEKDIKTI/Sinta-3

Lymph nodes (KGB), Intra Mural Vascular Invation(IMVI), Extra Mural Vascular Invasion (EMVI),Fisher's Exact Test * Mann-Whitney U Test**

Statistical test results showed that there was no significant relationship between grading and MMP-9 expression with a value of p=0.26 (p>0.05). There is a significant relationship between the depth of tumor invasion and MMP-9 expression with p=0.03. There was no significant relationship between KGB involvement and MMP-9 expression with p=0.53. There was no significant relationship between metastases and MMP-9 expression with a p=1.00. There was no significant relationship between Perineural Invasion and MMP-9 expression with p=0.25, likewise there was no significant relationship between LymphaticInvasion and MMP-9 expression with p=1.00 and there was no significant relationshipbetween IMVI with MMP-9 expression with p=0, 53 and there was no significant relationshipbetween EMVI and MMP-9 expression with p=1.00. There was no significant relationship between tumor budding level and MMP-9 expression with a p-value=0.25 but there was asignificant relationship between stromal TILs and MMP-9 expression with a p=0.03.

DISCUSSION

Colorectal adenocarcinoma NOS in this study was found more in males than females. This is in accordance with the literature which explains that this malignancy affects more men due to differences in hormones between men and women. 4 of 11 colorectal adenocarcinoma NOS samples involving female patients in this study, most were aged over 50 years, namely a total of 8 samples. This indicates that most female patients have reached menopause, resulting in a decrease in endogenous estrogenlevels which triggers a decrease in the antineoplastic activity of estrogen against colorectal carcinoma. Besides that, there is a significant relationship between gender and MMP-9 expression with a value of p=0.28. This is in accordance with previous research conducted by Ashoor et al.[20]

In this study, the average age of patients with colorectal carcinoma in this study was 50.1 years old, with the most common age group being 40-55 years old. This is in accordance with the theory in WHO Classification of Digestive Tumors System Fifth edition in 2019.[1,21] Currently, several recent studies, such as the study of Lugito et al shows an increased incidence in patients who are young adults (under 40 years). 17,22 Patients with colorectal adenocarcinoma who are under 40 years of age generally have genetic abnormalities. There is a significant relationship between age and MMP-9 expression with p=0.82. This study is in accordance with previous research conducted by Ashoor et al.[20]

The most colorectal adenocarcinoma NOS location in this study was in the left colon. This is in accordance with the research of Wang et al who also reported the right colon as the rarest location. In this study also there is not a significant relationship between location and MMP-9 expression with a value of p=0.69. This study is in accordance with previous researchconducted by Ashoor ZF et al.[11.20]

The stage of colorectal adenocarcinoma in this study was assessed based on the criteriaof the TNM system of the AJCC. The most clinical stage that the researchers got was stage IIas many as 14 samples (46.7%). This is in accordance with research conducted by Gunasekaran et al in Bali in 2019.23 In this study also There was a significant relationship between stage and MMP-9 expression with p=0.01. This study is in accordance with previous research conducted by Ashoor ZF et al.[20]

The histopathology of colorectal adenocarcinoma grading was more common in low grade, namely 18 samples (60%). These results are in accordance with research conducted by Schwartz et al and Zlobec et al in 2020.[24] In this study there was no significant relationship between grading and MMP-9 expression with a p-value=0.26. This is not in accordance with the research of Fahri et al, Morini et al, and Ashoor et al who reported MMP-9 expression had a significant relationship with the histopathological degree.[11,20,25]

In this study, tumor invasion depth is most common at T3. This research is in line withthe research by Bedge et al in 2012.[26] Depth of invasion exceeding T1 allows infiltration of the vascular, lymphatic and distant metastases. In this study found significant relationshipbetween the depth of tumor invasion and MMP-9 expression with a value of p=0.03. This is inaccordance with research by Yang et al (2014).[13]

In this study, lymph node involvement (N) was found in 21 cases (70%) and 9 cases

Maj Patol Indones 2024; 33(3): 795-802
DOI: 10.55816/mpi.v33i3.629

P-ISSN 0215-7284
e-ISSN 25279106
Accredited by KEMENRISTEKDIKTI/Sinta-3

(30%) without lymph node involvement. Lymph node involvement is a poor prognostic indicator in colorectal adenocarcinoma patients which is related to survival rates where patientswith lymph node involvement have a shorter survival rate than colorectal carcinoma patients without lymph node involvement. this is not found significant relationship between KGB involvement and MMP-9 expression with a value of p=0.53.

In this research, metastases were found in 26 cases (86.7%) and without metastases in 4 cases (13.3%). The main role of MMP-9 in degrading collagen IV is a key role in the process of invasion, metastasis, migration and angiogenesis so that it can be used as an indicator of poor prognosis. In this study not found significant association between metastases and MMP-9 expression with p=1.00. Hal this is not in accordance with research Farina et al.[27]

On this study, found more EMVI invasion than IMVI. This is in accordance with the research conducted by Betge et al 2012.[26] Vascular invasion has been associated with the occurrence of lymph node metastases and distant metastases. In this study, there was not a significant relationship between MMP-9 and EMVI with p=1.00, also not significantly relatedto IMVI with p=0.53 and not significantly related to lymphatic invasion with p=1.00 and notsignificantly related to PNI with a value of p=0.25.

From budding tumor inspection, found the most is low budding of 21 cases (70%). This is consistent with a study conducted by van Wyk et al.[28] In colorectal cancer, budding tumors are associated with an increased risk of metastases to the KGB and decreased survival and recurrence. In this study, no results were found meaningful relationship between budding tumor with MMP-9 expression with a value of p=0.25.

In this study, rich stromal TILs were found in 20 samples (66.7%) and poor stromal TILs in 10 samples (33.3%). This explains that MMP-9 is produced by inflammatory cells ofstromal lymphocytes around the tumor. The interaction between tumor cells and surrounding stromal cells has an important role in assisting the process of tumor cell invasion and metastasis. In this study, there was a significant relationship between stromal TILs and MMP-9 expression with a p-value=0.03.

The difference in results between several studies is thought to be due to the heterogeneity of the criteria for assessing MMP-9 expression with a certain cut-off point and also due to factors that were not controlled for in this study, for example whether the patient had a comorbid disease such as chronic colitis where in several previous studies by Walter et al, stated that in certain conditions such as chronic colitis, MMP-9 tends to play a protective role by activating itTgM9 (Transgenic Mice-9) which can reduce tumor invasion, increase apoptosis and activate the action of Notch1, p53, p21, so that it can reduce tumor cell proliferation, stop the cell cycle and reduce DNA damage to cells. So, under these conditions it seems that MMP-9 works as a tumor suppressor.[3]

CONCLUSION

Clinical characteristics of colorectal adenocarcinoma NOS samples in this study were found more frequently in males, age group 40-55 years, left colon location, and at stage T2. The histopathological characteristics of colorectal adenocarcinoma NOS samples were more common in low grading, T3 invasion depth, without KGB involvement, without metastases, without PNI, without lymphatic invasion, without IMVI, with EMVI, low budding, and rich TILs. MMP-9 expression is more common in strong expression. Frequency distribution and relationship between MMP-9 immunohistochemical expression and clinical characteristics of patients with colorectal adenocarcinoma NOS showed that MMP-9 expression was stronger in males, age group 40-55 years, left colon location and stage T2 and found a non-significant relationship based on type gender, age, tumor location, However, a significant association was found in clinical stage. Frequency distribution and relationship between MMP-9 immunohistochemical expression and histopathological characteristics of patients withcolorectal adenocarcinoma NOS showed that MMP-9 expression was stronger at low grading, depth of T3 invasion, without lymph node involvement, without metastases, without PNI, without LI, without IMVI, with EMVI, low budding, stromal rich and found no significant relationship based on grading, KGB involvement, metastasis, PNI, LI, IMVI, EMVI, tumor budding and found a significant relationship on the depth of invasion and stromal TILs.

Maj Patol Indones 2024; 33(3): 795-802
DOI: 10.55816/mpi.v33i3.629

P-ISSN 0215-7284
e-ISSN 25279106
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