The relationship between tissue inhibitor metalloproteinase-1 with colorectal cancer and tissue inhibitor metalloproteinase-1 as a predictor of colorectal cancer

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Abstract---Colorectal cancer is reported as the third most common case worldwide in men and accounts for 10% of all cancers. Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent endopeptidases that affect the degradation and remodeling of extracellular components and contribute to the three Hallmarks of cancer. TIMP-1 is an endogenous MMP inhibitor with multiple functions related to the tumor microenvironment and colorectal cancer development. This study used a prospective cohort study design to prove that colorectal mucosa with high TIMP-1 expression is a prognostic factor in the incidence of colorectal cancer compared to normal/low TIMP values. This study involved 53 samples consisting of 27 polyp patients and 26 colorectal cancer patients. The bivariate analysis results showed a significant difference between TIMP-1 levels in cancer patients and colorectal polyps (p<0.001; 95%CI: 33.65-67.31). The cut-off value of 66.50 obtained a sensitivity value of 80.8% and a specificity value of 77.8% (p<0.001). Spearman rho correlation
test found a significant positive correlation between TIMP-1 levels and cases of colorectal cancer (p<0.001) of 0.680. This study shows a significant relationship between TIMP-1 levels and colorectal cancer, where TIMP-1 is a good predictor of colorectal cancer incidence.

**Keywords**---colorectal cancer, TIMP-1, MMP1, MMPs, Colorectal polyps

**Introduction**

Colorectal cancer (CRC) is reported as the third most common case worldwide in males, with an incidence of 1,361,000 cases, accounting for 10% of all cancers. It was also mentioned that CRC was the second most common type of cancer in women, with 614,000 cases representing 9.2% of all cancers (Granados-Romero et al., 2017). Based on the Surveillance, Epidemiology, and Final Results program, in the United States, an estimated 132,7000 new cases of colorectal cancer in 2015. With a mortality rate of 8.1/100,000 population and an estimated 49,700 deaths per year, it is also stated that the incidence of CRC is more common in states with developing countries (25.1/100,000), compared to less developed countries (3.9/100,000) (Bray et al., 2018).

In Indonesia, colorectal cancer is the third most common type of cancer. In 2008, Indonesia was ranked fourth among ASEAN countries, with an incidence rate of 17.2 per 100,000 population, and this figure is predicted to continue to increase from year to year (Jan et al., 2012).

TIMP-1 is a family of TIMPs, which are multifunctional proteins that act directly on cell apoptosis and metastasis and are characterized as endogenous MMP inhibitors. In addition to its inhibitory activity against MMPs, TIMP-1 promotes cell proliferation in various cell types. As a cytokine and a significant regulator of ECM degradation, TIMP-1 has multiple functions related to the tumor microenvironment and cancer development. The higher the TIMP-1, the higher the activity of inhibited MMPs so that it can be used as a marker in showing the proliferation of a tumor (Min et al., 2012; Vočka et al., 2019).

As with other types of cancer, optimal therapy depends on accurate diagnosis and early detection, which are critical factors in reducing mortality in CRC. Screening to discover the early stages of CRC requires invasive and/or noninvasive examination. Examinations that use invasive techniques are flexible sigmoidoscopy, colonoscopy, double contrast barium enema, and noninvasive tests such as fecal occult blood testing (FOBT), examination of tumor markers (CEA, FIT, TIMP-1, etc.). For people over 50 years of age with moderate risk, CRC screening is done by noninvasive methods. As for people at high risk, the screening method is invasive. Detection using invasive methods such as colonoscopy is still an option because of the high accuracy of up to 95%. However, multiple complications, high cost, and lack of patient compliance reduce the applicability and sensitivity of the test (Heitman et al., 2009).
For this reason, an alternative examination is needed to establish the diagnosis of CRC at an early stage with minimal complications and low prices without reducing applicability and sensitivity to reduce morbidity and mortality from colorectal cancer patients. In this study, researchers will assess the amount of TIMP-1 in Colorectal Polyps and CRC. Where this test has the potential for screening and diagnostic of CRC, it is easy to perform, where TIMP-1 serum is taken in peripheral venous blood.

**Method**

This study used a two-stage study design, the first stage was a prospective cohort study to determine whether high TIMP-1 expression was a prognostic factor in the incidence of colorectal cancer, and the second stage was a cross-sectional study to determine whether high TIMP-1 expression was associated with colorectal cancer incidence.

**Inclusion Criteria for Case Groups in a Prospective Cohort Study Design**

1. Paraffin blocks preparations from surgery or colonoscopy of colorectal tumors with histopathology of colorectal cancer.
2. Complete clinical data include age, gender, and tumor location listed on SIMARS or medical records.
3. The paraffin blocks were in good condition and contained sufficient tissue for re-cutting.

**Control Group Inclusion Criteria in the Prospective Cohort Study Design**

1. Preparation paraffin block of surgical materials or colonoscopy of colorectal tumors showing well-differentiated dysplasia without malignancy.
2. Complete clinical data include age, gender, and tumor location listed on SIMARS or medical records.
3. The paraffin blocks were in good condition and contained sufficient tissue for re-cutting.

**Exclusion Criteria in the Prospective Cohort Study Design**

1. Paraffin block with no appearance or in a damaged condition
2. Incomplete medical records
3. Inadequate immunohistochemical results
4. Inadequate TIMP-1 serum levels
5. Subjects with an active autoimmune disorder
6. Subjects with generalized acute inflammatory condition (Sepsis or SIRS)

7. Subjects who are taking immunosuppressant drugs

8. Subjects with diseases that suppress the immune system (HIV/AIDS)

Cross-sectional study design inclusion criteria are:

1. Patients with colorectal cancer will undergo a biopsy through a colonoscopy or a biopsy operation.

2. Complete clinical data include age, gender, and tumor location listed on SIMARS or medical records.

3. The paraffin block is in good condition and still contains sufficient tissue for re-cutting

Data analysis was carried out with the help of SPSS 23 with bivariate, multivariate and correlation tests. Significant value <0.05.

**Results and Discussion**

This study involved 53 samples consisting of 27 polyp patients and 26 colorectal cancer patients. The characteristics of the data are presented in Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case Colorectal Polyps (n=27)</th>
<th>Case Colorectal Cancer (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD)</td>
<td>53.96±12.35</td>
<td>57.15±10.61</td>
</tr>
<tr>
<td>Gender n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>17 (63.0%)</td>
<td>15 (57.7%)</td>
</tr>
<tr>
<td>Woman</td>
<td>10 (37.0%)</td>
<td>11 (42.3%)</td>
</tr>
<tr>
<td>Location n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>23 (85.2%)</td>
<td>5 (19.2%)</td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>4 (14.8%)</td>
<td>21 (80.8%)</td>
</tr>
<tr>
<td>Histology Grade n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>13 (48.1%)</td>
<td>7 (26.9%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>5 (18.6%)</td>
<td>19 (73.1%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>9 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Histology Type n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adeno</td>
<td>25 (92.6%)</td>
<td>23 (88.5%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1 (3.7%)</td>
<td>2 (7.7%)</td>
</tr>
<tr>
<td>Fibroepithelial</td>
<td>1 (3.7%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Squamous</td>
<td>0 (0.0%)</td>
<td>1 (3.8%)</td>
</tr>
<tr>
<td>HistoryDM n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>There is</td>
<td>7 (25.9%)</td>
<td>12 (46.2%)</td>
</tr>
<tr>
<td>There is not any</td>
<td>20 (74.1%)</td>
<td>14 (53.8%)</td>
</tr>
</tbody>
</table>
History of Hypertension
n(%)  
There is 8 (29.6%)  8 (30.8%)
There is not any 19 (70.4%)  18 (69.2%)

Genetic History n(%)  
There is 0 (0.0%)  4 (15.4%)
There is not any 27 (100.0%)  22 (84.6%)

SD: Standart deviation

Numerical variables in this study, namely age and TIMP-1 levels, were tested for data normality. The results of the data normality test using the Kolmogorov-Smirnov test found that the age variable was normally distributed, and the TIMP-1 level variable was not normally distributed. Therefore, the significance value for the age variable used the Independent T-Test, while the TIMP-1 level variable used the Mann-Whitney test. The results of the bivariate analysis showed a significant difference between TIMP-1 levels in cancer patients and colorectal polyps (p<0.001; 95%CI: 33.65-67.31) (Table2)

Table 2 Bivariate Test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case</th>
<th>Mean Diff</th>
<th>P-Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancer</td>
<td>Polyps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>57.15±10.61</td>
<td>53.96±12.35</td>
<td>3.19</td>
<td>0.319</td>
</tr>
<tr>
<td>TIMP-1 levels</td>
<td>97.04±36.96</td>
<td>46.56±22.62</td>
<td>50.48</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Furthermore, determine the most optimal cut-off value for colorectal cancer cases. From the Receiver Operating Curve (ROC) analysis, the area under the curve (AUC) was 0.892, indicating that TIMP-1 levels can be used as a predictive value for the incidence of colorectal cancer cases. A cut-off value of 66.50, a sensitivity value of 80.8%, and a specificity value of 77.8% (p<0.001) were obtained (Table 3 and Figure 1).

Table 3 ROC test

<table>
<thead>
<tr>
<th>Case</th>
<th>Parameter</th>
<th>AUC</th>
<th>95% CI</th>
<th>Cut-off Value</th>
<th>Sn</th>
<th>Sp</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal Cancer</td>
<td>TIMP-1 levels</td>
<td>0.892</td>
<td>0.810 0.975</td>
<td>66.50</td>
<td>80.8%</td>
<td>77.8%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
To find out more about the relationship between the incidence of colorectal cancer and TIMP-1 levels, a multivariate analysis was conducted to see the effect of TIMP-1 levels >66.50 independently without being affected by other variables. From the results of multivariate analysis using logistic regression, it was found that there was a significant relationship between the incidence of colorectal cancer and TIMP-1 levels >66.50 (p<0.001). The adjusted RR value was found to be 14.70 with 95% CI: 3.880-55.696, which means that TIMP-1 levels > 66.50 have a 14.7 times chance of developing colorectal cancer compared to patients with TIMP-1 levels <66.50 (Table 4).

Table 4 Multivariate Test

<table>
<thead>
<tr>
<th>TIMP-1 level &gt;66.50</th>
<th>B</th>
<th>SE</th>
<th>p-value</th>
<th>Adjusted RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-1,435</td>
<td>0.498</td>
<td>0.004</td>
<td>0.238</td>
<td>3.880-55.696</td>
</tr>
<tr>
<td>TIMP-1 level &gt;66.50</td>
<td>2,688</td>
<td>0.680</td>
<td><strong>&lt;0.001</strong></td>
<td>14.70</td>
<td></td>
</tr>
</tbody>
</table>

To further review the relationship between TIMP-1 levels and cases of colorectal cancer, a correlation test was performed using Spearman Rho because TIMP-1 levels were not normally distributed. From the Spearman Rho correlation test, it was found that there was a significant positive correlation between TIMP-1 levels and cases of colorectal cancer (p<0.001) of 0.680 (Table 5).

Table 5. Spearman Rho. Correlation Test

<table>
<thead>
<tr>
<th>R-value</th>
<th>p-value</th>
</tr>
</thead>
</table>

Figure 1. ROC curve
TIMP-1 levels |     0.680     | <0.001
-----|--------------|------
Colorectal Cancer Cases | | |

The balance of activity between the enzyme tissue inhibitors of metalloproteinase (TIMP) and the enzyme matrix metalloproteinase (MMP) in the extracellular matrix is known to have an important role in the process of tumor cell invasion. The tissue inhibitor of metalloproteinase-1 (TIMP-1) is an enzyme that can inhibit the proteolytic activity of MMP by forming a stoichiometric complex and regulating the remodeling balance of the extracellular matrix during the matrix degradation process (Batra et al., 2012). The findings obtained in various types of tumors namely increased levels of TIMP-1 are associated with the rate of invasion of cancer cells, metastasis, and survival of cancer patients. The study conducted by Song et al. showed that TIMP-1 expression level correlated with patient clinical outcome (Song et al., 2016)

Colon cancer patients with high TIMP-1 expression levels have a worse survival when compared to patients with low TIMP-1 expression levels. TIMP-1 overexpression is also associated with the activation of FAK, a regulator of the PI3K-AKT signaling pathway (Kostourou et al., 2013; Oudart et al., 2016; Song et al., 2016). AKT phosphorylation will upregulate cyclin D1 and downregulate p21 and p27 proteins, which are cyclin D kinase (CDK) inhibitors, thereby promoting the cell cycle’s progression. In addition, AKT phosphorylation will further phosphorylate and activate BAD, activating the anti-apoptotic Bcl-2 protein. Song et al. also found that TIMP-1 can downregulate E-cadherin and increase fibronectin which can facilitate the process of invasion and metastasis of colon cancer cells. Based on this mechanism, TIMP-1 has an important role in cancer proliferation, metastatic ability, and anti-apoptosis. Other studies have also shown that the detection of colorectal cancer metastases can be done by checking serum TIMP-1 levels (Vočka et al., 2019). Meanwhile, in this study, examination of TIMP-1 levels using serum samples from colorectal cancer patients found similar results in a relationship between TIMP-1 levels with cancer and colorectal polyps (p<0.001). However, TIMP-1 levels in colorectal cancer were higher than in colorectal polyps.

Tissue inhibitors of matrix metalloproteinases (TIMPs) are naturally occurring matrix network metalloproteinases (MMPs) inhibitors partially regulate the proteolytic activity of MMPs, stimulate tumor growth and inhibit tumor cell apoptosis, and also act as functional regulators of malignant transformation. In addition, some literature reports that an imbalance between MMPs and TIMPs is a risk factor for tumorigenesis. Currently, there are four types of TIMPs known, namely TIMP-1, TIMP-2, TIMP-3, and TIMP-4. The main functional TIMP is TIMP-1, encoded by a gene located on chromosome Xp11.23-11.4 and mainly found in the intercellular matrix and plasma. The over-regulated expression of TIMP-1 is observed in various tumor tissues and is a significant indicator of cancer invasion, metastasis, and survival of patients with cancer (Møller Sørensen et al., 2008). Many studies convincingly report that TIMP-1 can regulate apoptosis and proliferation independently of MMPs and play a role in colorectal carcinogenesis (Møller Sørensen et al., 2008). In particular, many studies have shown that TIMP-1 can be used as a biomarker for prognosis in CRC patients and as a diagnostic
marker for detecting CRC. (Niewiarowska et al., 2014). The above conditions are in line with the findings of this study, TIMP-1 value above 66.50 has a sensitivity of 80.8% and a specificity of 77.8%. The TIMP-1 value above 66.50 in this study also indicates a 14.70 times greater chance of becoming a CRC.

According to previous studies, plasma TIMP-1 was equally efficient in detecting left and right-sided colonic adenocarcinoma. More importantly, TIMP-1 can detect early-stage colon cancer with sensitivity and specificity comparable to those obtained when detecting advanced cancers (Møller Sørensen et al., 2008). Another study analyzing total TIMP-1 levels in 210 individuals scheduled for endoscopy showed an association between CRC and plasma TIMP-1. At endoscopy, it was found that 30 patients had colon cancer, and an ELISA of plasma obtained before endoscopy was performed. The results showed that TIMP-1 plasma could detect these 30 colon cancer patients with a sensitivity of 60% with a specificity of 98% (Waas et al., 2005). Another study demonstrated a relatively specific role of TIMP-1 in predicting CRC, with a sensitivity of 0.65 and a specificity of 0.87. Estimation diagnostic odds ratio (DOR) showed superior diagnostic accuracy for diagnosing CRC, with a score of 12.73. Next, the results summary receiver operator characteristics (SROC) showed that TIMP-1 produced an AUC of 0.77, indicating that the efficiency of TIMP-1 for the diagnosis of CRC is quite large. TIMP-1 also performed well in clinical utility when the likelihood ratio was used to simulate clinical scenarios (Meng et al., 2018).

TIMP-1 serum level can also be a prognostic factor in colorectal cancer patients. A study by Vodka (2019) showed that an increase in serum TIMP-1 in colorectal cancer patients significantly worsened the prognosis of colorectal cancer. This study showed that an increase in TIMP-1 was associated with a decrease in median overall survival (MOS) compared with patients with low serum TIMP-1 levels (mOS 4.4 months; P < 0.001). (Vočka et al., 2019). It was also revealed that decreased survivability was associated with higher metastasis rates in patients with increased TIMP-1 expression.

Early diagnosis is also very important in patients with distant metastases previously treated for non-metastatic cancer to determine the possibility of radical resection. The study by Holten-Andersen (2006) showed a correlation between pre-and postoperative plasma TIMP-1 levels with poor prognosis in patients with resectable CRC. (Holten-Andersen et al., 2006). The study by Sørensen et al. demonstrated a poorer prognosis with higher plasma TIMP-1 levels at the start of treatment in patients with metastatic CRC (and HR 3.80, P < 0.001)(Sørensen et al., 2007). In contrast, Ishida et al., 2015 reported no correlation between TIMP-1 levels and prognosis. The entry-level of TIMP-1 appears to be a highly correlated prognostic factor (Ishida et al., 2003). This can be explained by the strong correlation between TIMP-1 levels and liver involvement in patients without pulmonary metastases. The patients with some liver metastases greater than 100 mm had significantly higher TIMP-1 levels. Another explanation is that patients with unresected primary tumors, due to poor performance status, have higher TIMP-1 levels. This explanation could be the unsatisfactory treatment result (Vočka et al., 2019).
High levels of TIMP1 also correlated significantly with the increased risk of CRC patients with metachronous liver metastases and intrahepatic recurrence after resection of liver metastases, with a 5-year disease-free survival rate of 15.9%. Univariate and multivariate analysis showed that high TIMP1 levels were predictors of poorer prognosis (Min et al., 2012). Overexpression of TIMP1 in CRC tissues was significantly associated with regional lymph node metastasis \((p = 0.033)\), distant metastases \((p = 0.039)\), and vascular invasion \((p = 0.024)\). It is stated that TIMP-1 has multiple functions, one of which is based on MMP-dependent anti-proteolytic activity with other MMP-independent cell growth activity. TIMP-1 depletion can suppress CRC cell proliferation, migration, and invasion in vitro and suppress tumorigenicity and metastasis of cancer cells in vivo. In addition, TIMP-1 can also enhance anti-apoptosis in CRC through the BAD-mediated phosphorylation pathway. These findings suggest that TIMP-1 not only has an important role as a predictor of CRC in proliferation, invasion, and metastasis but can also be an important therapeutic target for CRC patients (Song et al., 2016)

This study has limitations sample one health center. The following research can be done to use TIMP-1 serum to predict chemotherapy response in colorectal cancer patients and treatment response in colorectal polyp patients. In addition, research can be done that combines TMIP with other biomarkers that can be used to predict chemotherapy and treatment resistance in cancer patients and colorectal tumors. Tissue Inhibitor Mettaloprotease (TIMP-1) is a biomarker of CRC events based on its relationship with cancer cell proliferation, migration, and invasion in vitro. It can suppress tumorigenicity and cancer cell metastasis in vivo. However, the use of TIMP-1 is still limited.

The examination often used is the Fecal Occult Blood Test, which has a sensitivity of 30-52% and a specificity of 95%. Other tests that are used and invasive, such as flexible sigmoidoscopy, give a sensitivity value of 58%-75% for small lesions and a specificity of 94%. (Granados, 2017). In this study, TIMP-1 obtained from peripheral venous blood samples had a sensitivity of 80.8% and specificity of 77.8%, the relatively specific role of TIMP-1 in predicting CRC.

Based on this study, the chance of developing Colorectal Cancer if the TIMP-1 level with a cut-off point > 66.5 has a 14.70 times greater chance of becoming a CRC with a sensitivity of 80.8% and specificity of 77.8. So TIMP-1 is a biomarker that can predict CRC events in Indonesia. TIMP-1 levels are also obtained through venous blood sampling so that it can be an alternative if the patient chooses a noninvasive procedure related to colorectal cancer predictors. Based on this, this research has a novelty value that must be investigated further to advance the health sector, especially the medical field in Indonesia.

**Conclusion**

From the results of this study, it can be concluded that there is a significant relationship between TIMP-1 levels and colorectal cancer, where TIMP-1 is a good predictor of colorectal cancer incidence and has high sensitivity and specificity values.
Acknowledgments

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