Comparison of Carcinoembryonic Antigen Level and E-Cadherin Expression between Metastatic and Non-Metastatic Colorectal Carcinoma in RSUP, Dr. Sardjito Yogyakarta-Indonesia

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Abstract

Background: WHO has reported 34,189 (8.6%) colorectal carcinoma cases out of 396,914 total cancer cases in Indonesia. Accumulated gene mutation and the environment can affect cell regulation, growth, and differentiation, impacting the methylation of tumor suppressor genes. Carcinoembryonic antigen (CEA) is a biomarker used to detect the presence of colorectal carcinoma. Moreover, the E-cadherin gene has an essential role in tissue homeostasis, the adhesion between cells at embryogenesis, tissue morphogenesis, differentiation, and carcinogenesis stages. During instability and dysfunction in its regulation, the E-cadherin gene induces tumor progression. This study aimed to compare the level of CEA and E-cadherin expression in metastatic and non-metastatic sample groups.

Method: The present study is descriptive with a quantitative approach using ANOVA one-way, unpaired t-test, and Pearson correlation analysis for the measurement and comparison of the CEA level and relative gene expression value from the reverse transcription-quantitative polymerase chain reaction analysis.

Results: The obtained results suggested increasing CEA level and decreasing E-cadherin expression on the metastatic sample. Statistically, E-cadherin proven to show a negative r value or correlation value of CEA, even though it has a significant P-value. In other parameters, alanine transaminase and aspartate aminotransferase indicated a positive r value and a significant P-value.

Conclusion: These findings indicated the potential clinical benefit of E-cadherin in detecting tumor progressivity, supported by other significant parameters, such as alanine transaminase and aspartate aminotransferase. Furthermore, E-cadherin was found beneficial in diagnosing the colorectal carcinoma with liver metastatis. Nonetheless, further research is needed to determine the role of E-cadherin regulation in colorectal cancer metastatis.

Keywords: Colorectal neoplasms, Neoplasm metastatic, Non-metastatic, Carcinoembryonic antigen, E-Cadherin
Introduction

Colorectal carcinoma (CRC) is an epithelial malignant tumor in the colon and rectum area. In 2020, World Health Organization (WHO) reported that CRC is the most prevalent cancer diagnosed in males (ranked third with 10%) and females (ranked fourth with 9.4%) out of the total global cancer cases. Meanwhile, in the same year, The International Agency for Research on Cancer also reported that CRC is ranked as the third cause of death. The incidents and death rate of CRC vary greatly depending on the geographical location. Around 55% of colorectal cases are recorded in industrial countries, while the highest death rate is found in developing countries. Siegel et al. (2020) explain that around 147,950 cases were recorded in the United States of America, with 53,200 death cases, estimated as 9% of the total death rate of cancer mortality. In 2020, Global Cancer Observatory also reported 34,189 (8.6%) cases of CRC in Indonesia, out of a total of 396,914 and 12,425 cancer cases in males (ranked second) and females (ranked fourth), respectively.

The causes and pathogenesis of CRC are correlated with genetic and environmental factors. The mutation accumulation has a role in the cell growth regulation and differentiation, which affects the methylation of the tumor suppressor gene, inactivating and prompting neoplasia. Additionally, the environment contributes to the increase in CRC. Consumption of alcohol, nicotine, processed meat, lack of physical activities, heredity, and obesity enhances the risks of CRC, as well as the increase in age and gender.

One of the markers capable of detecting CRC is carcinoembryonic antigen (CEA) within a serum. CEA is a glycoprotein extracted from CRC tissue. It is an oncofetal antigen produced by epithelial tumor cells and endodermal derived in the gastrointestinal tract. Detection with CEA is considered safe, non-invasive, inexpensive, and easy to perform. CEA is also used in laboratory tests, as the biomarker for the screening, diagnosis, and treatment. Moreover, Nadeem, M. (2018) reported that around 74% of patients present increasing CEA levels, following the advancement of CRC stages.

Generally, the primary process of distant metastatic is the transmission of epithelial-mesenchyme, with the tumor cell impairing the normal epithelial structure and substituting it with a fibroblast-like phenotype. This epithelial-

![Figure 1](image_url)

**Figure 1.** This figure illustrates the E-cadherin gene expression levels in different tissues of the CRC patients.

* Significant with $P \leq 0.0$ with ANOVA one way test; CRC: Colorectal cancer
mesenchyme results in decreasing the adhesion between cells, stronger infiltration power, and increasing cell apoptotic resistance. It is correlated with the zinc-finger factors, functioning as transcription factors, such as Snail1 and ZEB1/2. These two genes also affect the E-cadherin expression in CRC. Kaszak et al. (2020) explained that the E-cadherin gene possesses an essential role in tissue homeostasis as it is liable for cell adhesion during embryogenesis, tissue morphogenesis, differentiation, and carcinogenesis. The instability and disfunction during its regulation results in E-cadherin triggering the tumor development.

Based on this background, the CRC that has been developed into distant metastatic activates the epithelial-mesenchymal transition process. Therefore, we conducted the present work as it is essential to investigates and compare the level of CEA and E-cadherin gene expression on CRC before and after the liver metastatic; thus, new diagnostic and treatment approaches for CRC would be identified.

**Materials and Method**

*Research samples*

This descriptive study used a quantitative approach. Meanwhile, a quantitative approach was utilized in measuring the CEA level and E-cadherin gene expression on 45 samples, consisting of 20 CRC non-metastatic colon tissues, 12 CRC hepatic metastatic colon tissues, and 13 normal colon tissues from different patients diagnosed with CRC. The surgeon team of Dr. Sardjito Central General Hospital, Yogyakarta, collected the CRC tissue samples. We conducted this study from December 2021 to February 2022. Subsequently, data measurement was carried out in the Central Laboratory of Advanced Minerals and Materials, Universitas Negeri Malang, and Central Laboratory of Biological Sciences, Universitas Brawijaya.

*Human rights and informed consent*

This study attained ethical clearance from the Health Research Ethics Committee of Dr. Sardjito Central General Hospital, Yogyakarta, under the number KE/FK/0938/EC/2021. The samples were obtained from the patients with confirmed CRC. This study applied a purposive sampling method, with the inclusion criteria being only the colorectal patients who had undergone surgery, either with or without liver metastases. Written informed consent was received from all the patients before study. The exclusion criteria, on the other hand, were having agreed to participate in other studies, having peritoneal metastases, not having received

![Figure 2](image_url)

*Figure 2. This figure illustrates E-cadherin and CEA profile in the non-metastatic and metastatic CRC patients. Significant with $P \leq 0.05$ with ANOVA one way test; CRC: Colorectal cancer; CEA: Carcinoembryonic antigen*
preoperative chemotherapy or radiotherapy, not meeting the inclusion criteria.

**Measurement of baseline characteristic parameters**

Anthropometric data were measured for all the study subjects. Briefly, the study subjects were rested for 15 minutes, after which the patient's blood pressure was measured on the right arm in a sitting position (normal). All the patient parameters/baseline characteristics were measured with standard clinical methods according to the basic anthropometric examination protocol. The baseline characteristic data included age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), white blood cells (WBC), red blood cells (RBC), blood urea nitrogen (BUN), platelet count (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), CEA. In addition, we checked the pathological grading (under the categories of well, moderate, and poor) and tumor location.

**Gene expression measurement**

We carried out a quantitative gene expression test by isolating the total RNA from our patients’ tumor samples, which was tested via real-time quantitative polymerase chain reaction (RT-qPCR). The sample was a fresh tumor containing tumor tissue (diagnosed as colonic adenocarcinoma) at Dr. Sardjito Hospital, Yogyakarta. To this end, 100 mg of fresh tumor samples were weighed, following which the sample was ready for the total RNA isolation process using kit TRISURE TM / Qiazol (BIOLINE, UK). The RNA samples were then stored for RT-PCR at -20°C in the refrigerator. Afterwards, via reverse transcription PCR, we carried out a conversion from RNA to cDNA using a reverse transcription kit with ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan). Then, we utilized RT-PCR with Analytik Jena qTower Machine, using mixed solution following the formula from SensiFAST SYBR Green No-ROX kit (BIOLINE, UK). The primers for E-cadherin and β-actin used in our study included: E-cadherin as a target gene in this study, forward 5’ AAAGGCCCATTTCC-TAAAAACCT 3’ and reverse 5’ TGCGTTCTCTATCCAGGCT 3’.19 The housekeeping gene using β-actin, forward 5’-CATGTACGGTGCATCCAGG-3’ and reverse 5’-CTCCCTT AATGTCACGCACGAT-3’.20 The RT-qPCR temperature set by following the Kit SYBR Green 2-step cycling as follows: 1 cycle at 95°C for 2 minutes for polymerase activation, followed by 40 cycles at 95°C for 10 seconds for the denaturation step and with the Tm primary temperature set at 30 seconds for the annealing/extension step.

**CEA level measurement**

Body surface area (BSA) blocked bead–antibody complexes, and labeled primary antibodies and fluorescence-labeled secondary antibodies were added to the centrifuge microfluidic device and incubated at room temperature for 2 hours. The clinical serum samples, collected and provided by the affiliated Hospital of Dr. Sardjito Yogyakarta, Indonesia, from both cancer metastatic and non-metastatic individuals, were then added to the centrifuge chip and spun at 2500 rpm for 2.5 min. After obtaining the fluorescence images using the fluorescence microscope and processing with the ImageJ software, we used the standard concentration curve for obtaining the experimental CEA concentrations.

**Data analysis technique**

Measuring the mRNA level, we investigated the expression of the E-cadherin gene, while the RT-PCR resulted in the cycle threshold (Ct). The mRNA level (relative gene expression) was analyzed using Relative Quantification and the 2^ΔΔCt formula. The RT-PCR results were then analyzed using JMP.6 software (Chicago, IL; North Carolina, USA). The data normality was also investigated with the Kolmogorov-Smirnov test. Subsequently to compare the data from each group (between non-metastatic and metastatic), the data was analyzed with Anova-One Way. We identified the E-cadherin levels between the sample groups. Then, we compared the level of relative gene expression of E-cadherin and CEA via t-test and univariate correlation using Pearson correlation. The significance level of 5% or \[ P \leq 0.05 \] indicated a significant statistical difference.
This study was carried out on 45 samples, consisting of 20 CRC non-metastatic tissues, 12 metastatic CRC/liver metastatic tissues, and 13 normal colon tissues from different patients diagnosed with CRC. The results of the examination revealed that 20 non-metastatic and 12 metastatic CRC patients experiencing a significant increase in CEA level were also observed on the metastatic CRC samples. The data in table 1 show the serology test ANOVA results (baseline characteristic parameters) of the CRC metastatic and non-metastatic groups. These data suggested significant differences in terms of BMI, fasting blood sugar, as well as the level of ALT, AST, and CEA between the metastatic and non-metastatic groups. Based on the results of the Pearson correlation test represented in table 2, it is known that the ALT, AST, and CEA parameters had significant \( P \)-values. Nonetheless, the CEA levels had a negative r value (correlation). Similarly, the sex parameters, BMI, RBC, albumin, creatinine, and PLT had negative r values as well.

The results of Ct values, calculated using \( 2^{\Delta\Delta C_t} \), represented the relative expression of E-cadherin and CEA and were visualized using GraphPad Prism 5 (Figures 1 and 2). According to the results, the lowest E-cadherin expression belonged to the group of tumors that had liver metastatic compared with the tumor group without metastatic and non-tumor group (Figure 1). We observed the increasing level of CEA (Figure 2, right side) and lower E-cadherin expression (Figure 2, left side) in the liver metastatic group in comparison with the non-metastatic group, meaning they were inversely proportional.

**Discussion**

In this study, we used the level of CEA and E-cadherin expression as the marker for diagnosing the incidence of CRC. This study revealed that CEA levels increased drastically in the group of metastatic patients (Figure 2, right side).
side). This is in line with the results of Saito et al. (2016) who reported increasing CEA concentration levels in CRC patients. CEA adheres to the cell membrane through a glycosylphosphatidylinositol bond and can be released as solute formed by phospholipase C or phospholipase D. CEA presumably serves as an adhesion molecule contributing to cancer invasion and metastatic. Su et al. (2012) also explained that CEA modulates the adhesion between collagen and epithelium cells in the colon. Increased concentrations of CEA levels can also be caused by environmental factors, namely obesity, lack of physical activity, alcohol consumption, and nicotine. The increased risk of CRC incidence can also be caused by age and gender.

The results showed different E-cadherin expressions in the non-metastatic and metastatic CRC sample groups, with significantly decreasing E-cadherin expression in the metastatic CRC sample group. E-cadherin is associated with the tumor suppressor gene, while decreased expression is associated with poor prognosis and metastatic. Downregulation of E-cadherin is associated with poor differentiation, vascular invasion, metastatic, and advanced stage of cancer. Moreover, decreased expression of E-cadherin gene facilitates the epithelial-mesenchymal transition (EMT), the indicator of epithelial cells transition into mesenchymal cells, under physiological and pathological situations. Therefore, EMT is presumed to be one of the primary mechanisms that determine the invasive spread and metastatic of cancer cells. CRC that undergoes EMT can invade and metastasize to other organs, such as the liver, through direct diffusion as well as blood and lymphatic circulation. Decreasing E-cadherin expression on the cell membrane weakens the interaction between cells, inhibits the activation of transcription factors (Snail, Slug, Twist dan ZEB-1), and results in EMT.

Sterneck et al. (2020) also mentioned that the Snail family gene is the transcription factor suppressing the E-cadherin gene. E-cadherin depends on Ca (calcium) for efficient adhesion between cells; when calcium deficiency occurs, it causes instability of cell structure, increases EMT, and facilitates gaining mobility and invasion for cells.

The findings herein also suggested the increase in ALT and AST, the markers of liver fibrosis. ALT is an enzyme that changes protein into the energy used by the liver cells, while AST is an

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R</th>
<th>E-cadherin</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Sex</td>
<td>-0.156</td>
<td>0.394</td>
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<td>Age (yrs)</td>
<td>0.285</td>
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<td>BMI (kg/m²)</td>
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<tr>
<td>DBP (mmHg)</td>
<td>0.062</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>0.091</td>
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<tr>
<td>Haematocrit (%)</td>
<td>0.015</td>
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<tr>
<td>WBC (10³/µl)</td>
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<td>RBC (10⁶/µl)</td>
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<td>Creatinine (mg/dL)</td>
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<tr>
<td>PLT (10³/µl)</td>
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<td>ALT (U/L)</td>
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<tr>
<td>AST (U/L)</td>
<td>0.742</td>
<td>0.000*</td>
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<tr>
<td>CEA (µg/L)</td>
<td>-0.359</td>
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</table>

* Significant with $P \leq 0.05$ with Pearson product-moment correlation test; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; WBC: White blood cells; RBC: Red blood cells; BUN: Blood urea nitrogen; PLT: Platelet count; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CEA: Carcinoembryonic antigen
enzyme with a role in the metabolism of amino acids.\textsuperscript{30} ALT can be found in the liver, kidneys, heart, muscles, and pancreas, whereas AST is found in RBCs, liver, heart, muscle tissue, pancreas, and kidneys. ALT and AST levels are used to determine the level of hepatocellular damage. When hepatocellular damage occurs, liver cells release these enzymes into the bloodstream, which raises levels of ALT-AST.\textsuperscript{31} The constant increase in these two enzymes escalates the risk of CRC.\textsuperscript{31,32}

The results of BMI data showed a significant difference with the level of relative gene expression of E-cadherin. People with excess BMI are classified as obese and have high lipoprotein levels.\textsuperscript{33} Obesity increases the risk of diabetes mellitus 2, cardiovascular diseases, and some cancers.\textsuperscript{34} High triglyceride levels cause fat accumulation in liver tissue, which is known as fatty liver disease and can cause cancer. Accumulation of free fatty acids triggers the production of oxidants, thereby inhibiting lipogenesis and causing inhibition of triacylglycerol clearance. Triacylglycerol causes an increase in blood triglyceride levels (hypertriglyceridemia), causing cardiovascular events.\textsuperscript{35} The fasting glucose levels showed a significant difference. The insulin system or IGFs (Insulin-like growth factors) are involved in the pathogenesis of colon cancer. IGF1 and IGF-1R overexpression levels were detected in CRC and activated Src, thereby leading to increased proliferation and migration (metastatic) of colon cancer.\textsuperscript{36,37} Insulin binds to the IGF-1 receptor, thus increasing cell proliferation and the occurrence of colon cancer.\textsuperscript{38–41} The proliferation of these cells occurs through the activation of the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway. This activation plays a role in the transformation and growth of colon cancer cells.

Based on the obtained results, an increase in CEA level along with a significant decrease in E-cadherin were observed in the metastatic sample group. Furthermore, an increase in ALT and AST was also found in the same group. Therefore, the results of this study can be used as the baseline data for further research development. These findings are expected to help the identification of preventive methods and treatments for CRC patients. Nevertheless, this study has certain limitations due to the limited sample size and areas of sample; therefore, further research is needed to cover other areas.

**Conclusion**

The increase in CEA level and a significant decrease in E-cadherin were observed in the metastatic sample group. Additionally, a significant increase in ALT and AST was found in the same metastatic group. This indicates that the E-cadherin and CEA are capable of becoming candidate biomarkers, for CRC in particular, in the future. E-cadherin expressed in the samples can also be coupled with the other parameters to determine the expression pathway. This can help the improvement of diagnostic and prognostic information for patients and further research.

**Acknowledgments**

We express our gratitude toward the medical surgery team of Dr. Sardjito Central General Hospital, Yogyakarta, for supplying the samples, and the biotechnology team of Universitas Negeri Malang for their assistance in RT-qPCR molecular analysis.

**Conflict of Interest**

None declared.

**References**

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