ORIGINAL ARTICLE

Predictive value of tissue inhibitor of metalloproteinases-1 and carcinoembryonic antigen plasma levels in response to chemotherapy in patients with metastatic colorectal cancer

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Background: Colorectal cancer is one of the most important causes of morbidity and mortality in the world. It has been suggested that tissue inhibitor of metalloproteinase-1 (TIMP-1) plays a particular role in cancer promotion. The objective of the present study was to compare the effect of repeated measurement of carcinoembryonic antigen (CEA) and TIMP-1 plasma levels in a follow-up response to chemotherapy treatment in patients with unresectable metastatic colorectal cancer by surgery. **Materials and Methods:** All consecutive patients between 18 and 75 years old in both genders with rectal adenocarcinoma who referred to Sayed Al-Shohada Medical Center, during 6 months of study period enrolled in the study. Level of CEA and TIMP-1 were assessed before and after two cycles of chemotherapy. Furthermore, patients underwent computed tomography scan to assess response to chemotherapy. The sensitivity, specificity, and areas under the receiver operating characteristic curves (AUC) calculated for baseline level of CEA and TIMP. **Results:** The mean age of the studied patients was 52.7 ± 7.5 years, 12 patients (48%) were male. Response to chemotherapy after two cycles was 80%. In patients who responded to chemotherapy level of TIMP after treatment was significantly decreased compared to before treatment ($P < 0.0001$). Before and after treatment in patients who responded to chemotherapy the level of TIMP was significantly lower than who did not response to chemotherapy ($P < 0.05$). The critical values for the prediction response to chemotherapy for CEA was >63 (AUC: 0.54) and for TIMP was ≤8823 (AUC: 0.68). **Conclusion:** The present study has identified a strong significant association between high plasma TIMP-1 levels and short survival in patients with colorectal cancer. In addition, results demonstrated that the TIMP-1 provides stronger prognostic information than CEA.

Key words: Carcinoembryonic antigen, chemotherapy, metastatic colorectal cancer, predictive value, tissue inhibitor of metalloproteinase-1

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INTRODUCTION

Colorectal cancer is one of the most important causes of morbidity and mortality in the world.[1,2] More than 9% of all cancer incidences are belonged to it.[3] Colorectal cancer is the fourth most frequent cause of death and third most common cancer worldwide that is more widespread in developed countries.[4,5] Nowadays, following the response to therapy in patients with cancer, who receives chemotherapy, is an important issue. Toward this end, various biological markers have been introduced as potential biomarkers in colorectal cancer cases. Carcinoembryonic antigen (CEA) is a glycoprotein that would be generated in gastrointestinal tissue at some stage in fetal maturity and plays some roles in cell adhesion. Inasmuch as CEA exists only at scarce levels in the blood of healthy adults, and arise only in some kinds of cancer, usually would be applied as a tumor marker in clinical experiments.[6-9] The specificity of CEA is usually associated with the site of metastases. In metastatic colorectal cancer of lungs, the CEA elevation is not sufficiently enough and also could be significantly affected by chemotherapy; totally, cures ascribable to CEA monitoring are commonly scarce.[10,11] Despite the aforesaid limitations, CEA is currently used as a serological diagnostic factor. In some literature, it has been suggested that tissue inhibitor of metalloproteinase-1 (TIMP-1) plays a particular role in cancer promotion.[12] They have expressed that there is an association between high plasma TIMP-1 levels

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and low prognosis of colorectal cancer patients.[13-15] In a study, 45% increase in plasma TIMP-1 levels significantly decreased the overall survival, while in individuals who had TIMP-1 plasma reduction from the baseline level, have had superior overall survival.[16] Overall, in the setting of improving the diagnostic power for following the response to chemotherapy in patients with metastatic colorectal cancer, since CEA assay is not a self-determining test and also has considerable rates of false positivity and false negativity monitoring, its accompany with another biomarker such as TIMP-1 sounds logical. In this regard, the present investigation tries to compare the effect of repeated measurement of CEA and TIMP-1 plasma levels in a follow-up response to chemotherapy treatment in patients with unresectable metastatic colorectal cancer by surgery.

MATERIALS AND METHODS

All consecutive patients with rectal adenocarcinoma who referred to Sayed Al-Shohada Medical Center in Isfahan, Iran, Between September 2013 and March 2014, were scheduled to enroll in the present study (30 consecutive patients with primary rectal adenocarcinoma). Patients between 18 and 75 years old in both genders that had evidence of distant metastasis with no previous or concurrent other malignancy and did not receive previous or concurrent chemotherapy were eligible. Also patients with current infection, severe uncontrolled diabetes, central nervous system metastases, unresolved bowel obstruction, uncontrolled inflammatory bowel disease, current history of chronic diarrhea, or any other serious medical condition or that hindered treatment with chemotherapy were excluded from the study. The internal board and ethics committee of the Isfahan University of Medical Sciences investigated and approved this study. All of the participants were counseled, and a written informed consent was obtained from all of them.

Based on the physician's judgment and good clinical practice at the time of treatment, patients in the study received two cycles of standard chemotherapy (3 weeks in every cycle). Before and after the first and the second cycles of chemotherapy blood samples were collected from an antecubital vein using a light tourniquet. Samples were drawn into tubes with ethylenediaminetetraacetic acid as an anticoagulant for plasma and without additives for serum. Then the supernatants were separated from the blood cells at room temperature and stored at −80°C until samples had been collected from the patients and weekly were analyzed.

The collected information included age, gender, serum level of CEA and TIMP-1, and also a response to chemotherapy. Plasma level of TIMP-1 in the cytosols was measured using an established, validated in-house enzyme-linked immunosorbent assay (ELISA). Plasma level of CEA analyses was measured using the principle of solid-phase ELISA. Level of CEA and TIMP-1 were assessed before and after two cycles of chemotherapy. Furthermore, patients underwent computed tomography scan to assess response to chemotherapy, which were evaluated by the same radiologist and were graded according to the response evaluation criteria in solid tumors. In this study, disappearance of all target and nontarget lesions and reduction in the longest diameters of target lesions lower than 10 mm was defined as a response to chemotherapy and all other condition defined as no response to chemotherapy.

SPSS version 20 (SPSS Inc.; Chicago, IL, USA) was used to managed and analyzed the data. Descriptive data are reported as mean ± standard deviation or number (percent) as appropriate. Due to a small number of samples Mann-Whitney test and Wilcoxon, where appropriate, were used to compare continues data between patients with successful or unsuccessful chemotherapy. For serum level of CEA and TIMP, a receiver operating characteristic (ROC) curve analysis was used to establish the cut-off values that optimized the prediction of response to chemotherapy. Sensitivity, specificity, and areas under the ROC curves (AUC) were then calculated for baseline level of CEA and TIMP. All probability tests used were two-tailed, and alpha was set at 5%.

RESULTS

During two cycles of chemotherapy five patients were excluded from the study and 25 patients were followed to assess chemotherapy response. The mean age of the studied patients was 52.7 ± 7.5 years, 12 patients (48%) were male and 13 patients (52%) were female.

Response to chemotherapy after two cycles was 80% (20 out of 25 patients), and 20% (5 of 25) did not response to chemotherapy. Table 1 shows the level of CEA and TIMP in studies patients by response to chemotherapy. As shown in first cycle of chemotherapy, the level of CEA and TIMP before and after treatment was not significantly different between patients who response to chemotherapy compared to whom did not response to chemotherapy ($P > 0.05$). In patients who responded to chemotherapy, the level of CEA and TIMP after treatment was decreased compared to before treatment but these were no statistically significant ($P > 0.05$). In patients who did not respond level of CEA and TIMP after treatment was increased compared to before treatment which these were no statistically significant ($P > 0.05$). In addition, after second cycle of chemotherapy, the level of CEA before and after treatment between and within patients who responded to chemotherapy and who did not response to chemotherapy was not significantly different ($P > 0.05$).

In patients who responded to chemotherapy level of TIMP after treatment was significantly decreased compared to before treatment ($P < 0.0001$). Before and after treatment in patients who responded to chemotherapy the level of TIMP was significantly lower than who did not response to chemotherapy ($P < 0.05$).

Figures 1 and 2 show the results on a comparison of the trend of level of CEA and TIMP between patients who responded to treatment and who did not response to treatment. The level of CEA in patients who responded to treatment was decreased during two cycles of chemotherapy but in whom did not response to treatment was increased during chemotherapy, the difference in the trend of CEA was not statistically significant between patients ($P = 0.37$) [Figure 1]. The trend of the level of TIMP in patients who responded to treatment decreased and in whom did not response to treatment was increased during two cycles of chemotherapy which was statistically significant ($P = 0.025$) [Figure 2].

To determine the critical values that could predict response to chemotherapy, ROC curves were generated for baseline level of CEA and TIMP. The curve constructed for CEA was >63 (sensitivity, 40, 95% confidential interval [CI], 6.5-84.6; and specificity, 90, 95% CI, 68.3-98.5; and AUC, 0.540, $P = 0.79$). The curve constructed for TIMP was TIMP ≤8823 (sensitivity, 80, 95% CI, 28.8-96.7; and specificity, 80, 95% CI, 56.3-94.1; and AUC, 0.680, $P = 0.15$). There was no significant relationship between the baseline level of CEA and TIMP with response to chemotherapy in obtained cut-off points [Figure 3].

Table 1: CEA and TIMP in studies patients by response to chemotherapy

First cycle	Before chemotherapy	After chemotherapy	P†
CEA			
Response to treatment	25.4±30.9	24.9±29.1	0.76
No response	27.9±32.9	35.1±42.9	0.19
P*	0.78	0.95	
TIMP			
Response to treatment	11582.7±3473.9	10377.1±3560.7	0.11
No response	9934.8±4298.3	13135.2±3007.6	0.05
P*	0.22	0.11	
Second cycle			
CEA			
Response to treatment	20.6±30.3	16.7±27.4	0.06
No response	40.6±48.8	41.5±53.4	0.73
P*	0.17	0.39	
TIMP			
Response to treatment	7559.4±4085.7	4090.4±3488.1	0.0001
No response	12548.4±3405.8	11977.7±4314.2	0.47
P*	0.02	0.002	

Data are mean ± SD. *P* values calculated by *Mann-Whitney test and †Wilcoxon test. CEA = Carcinoembryonic antigen; TIMP = Tissue inhibitor of metalloproteinase; SD = Standard deviation

DISCUSSION

Efficient predictive biomarker should maintain patients from unsuccessful treatments and thereby from the

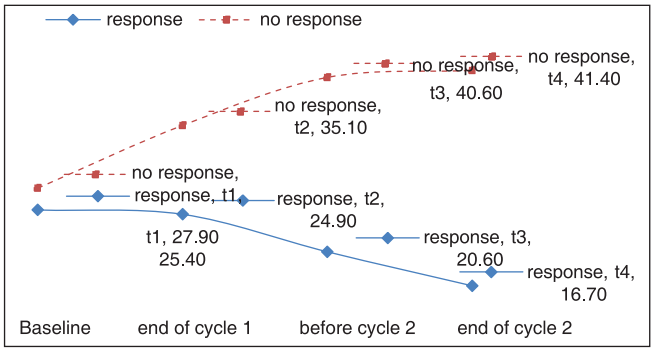

Figure 1: Comparison of carcinoembryonic antigen (CEA) between patients who response to treatment and who did not response to treatment. The difference of the trend of CEA was not statistically significant between patients ($P = 0.37$)

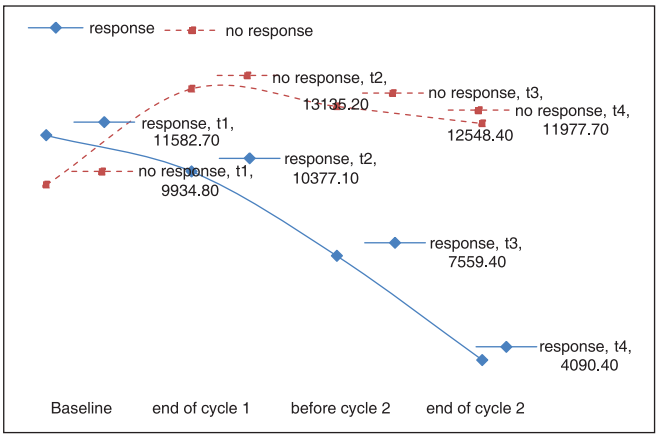

Figure 2: Comparison of tissue inhibitor of metalloproteinase-1 (TIMP) between patients who response to treatment and who did not response to treatment. The difference of the trend of TIMP was statistically significant between patients ($P = 0.025$)

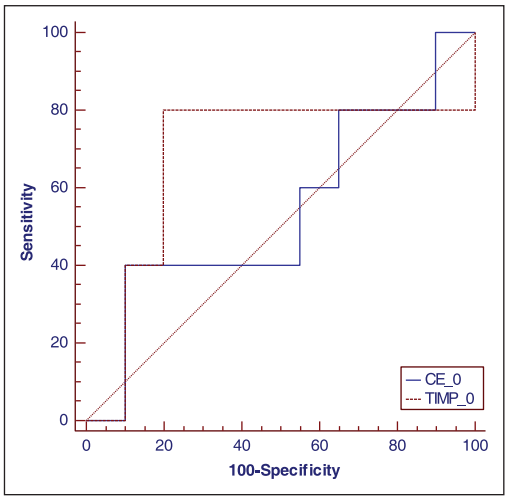

Figure 3: Receiver operating characteristic curves for carcinoembryonic antigen (area under the curve, 0.540; standard error [SE], 0.15; $P = 0.79$) and tissue inhibitor of metalloproteinase-1 (area under the curve, 0.680; SE, 0.12; $P = 0.15$) for predicting response to treatment in patients with rectal adenocarcinoma underwent chemotherapy

unpleasant impacts caused by chemotherapy. Therefore, due to ameliorating the diagnostic power for following the response to chemotherapy in patients with metastatic colorectal cancer, present investigation compares the effect of repeated measurement of CEA and TIMP-1 plasma levels in a follow-up response to chemotherapy treatment, in patients with unresectable metastatic colorectal cancer by surgery.

Our investigation indicated that in patients who responded to chemotherapy, the level of TIMP-1, after treatment was significantly decreased compared to before treatment ($P < 0.0001$). Meanwhile, the level of CEA before and after treatment between and within patients who responded to chemotherapy and who did not response to chemotherapy was not significantly different.

Our findings are keeping with an independent prospective validation study conducted by Birgisson *et al*. They expressed that there are a significant association among preoperative plasma TIMP-1 levels and survival of colorectal cancer cases. Our observation also indicated that the trend of TIMP-1 level in patients who responded to chemotherapy treatment decreased and in those cases that did not respond to treatment has increased during two cycles of chemotherapy. Another consistency between our results and theirs are relied on this fact that TIMP-1 provided stronger prognostic information than CEA.[17] Although the level of CEA in patients who responded to treatment has decreased during two cycles of chemotherapy and in those who did not respond to treatment increased during chemotherapy, the difference in the trend of CEA was not statistically significant between patients. Reports have demonstrated that in colorectal cancer, breast cancer and lung cancer the TIMP-1 mRNA levels in tumor tissues are significantly associated with patient.[18-20] The survival of colorectal cancer patients had a strong association with preoperative plasma TIMP-1 levels, so that higher plasma TIMP-1 levels was associated with poor outcome; in Holten-Andersen *et al*. study. They expressed plasma TIMP-1 as a strongly predictive biomarker in prognosis of colorectal cancer.[15]

Studies conducted based on ELISA measurements of total TIMP-1 in tumor tissues that confirms our results are also suggests that increased plasma ratios of the TIMP-1 inhibitor are associated with shorter survival.[21-23]

Several studies found that CEA serum level is an appropriate biomarker in chemotherapy monitoring.[24-26]

Also in patients with unresectable metastasis of colorectal cancer, the CEA kinetic has been suggested as an accurate, easy, and noninvasive technique to recognize the disease progression.[8] Taking these data together with the results of our screens, we could conclude that although the level of CEA was not statistically significant between patients; we should not neglect the fact that CEA in patients who responded to treatment decreased and in those who did not respond to treatment has increased during the chemotherapy.

In fact, one of the limitations that our study suffers from is few sample size that could effects on accurate estimation of biomarker positions that should be considered in future studies.

CONCLUSION

The present investigation has identified a strong significant association between high plasma TIMP-1 levels and short survival in patients with colorectal cancer. In addition, results demonstrated that the TIMP-1 provides stronger prognostic information than CEA. Nonetheless, supplementary researches with more sample size are considered necessary to clarify the significance of TIMP-1 in cancer progression as well as the potential clinical use and its superiority over CEA.

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AUTHOR'S CONTRIBUTION

MR, HE, PA, and AR designed the study and contributed to the conception and design, collection and assembly of data, manuscript editing, final approval of the manuscript. MA performed statistical analysis of data, contributed to design, interpretation, manuscript writing, and final approval of the manuscript.

REFERENCES

1. Haggar FA, Boushey RP. Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg 2009;22:191-7.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
3. Boyle P, Langman JS. ABC of colorectal cancer: Epidemiology. BMJ 2000;321:805-8.
4. Jitawatanarat P, Wee W. Update on antiangiogenic therapy in colorectal cancer: Aflibercept and regorafenib. J Gastrointest Oncol 2013;4:231-8.
5. International Agency for Research on Cancer. Colorectal Cancer Incidence: Mortality and Prevalence Worldwide in 2008 Summary. Available from: http://www.globocan.iarc.fr/Pages/fact_sheets_cancer.aspx. [Last cited on 2014 Mar 07].

6. Hammarström S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol 1999;9:67-81.
7. Thomas SN, Tong Z, Stebe KJ, Konstantopoulos K. Identification, characterization and utilization of tumor cell selectin ligands in the design of colon cancer diagnostics. Biorheology 2009;46:207-25.
8. Iwanicki-Caron I, Di Fiore F, Roque I, Astruc E, Stetiu M, Duclos A, *et al.* Usefulness of the serum carcinoembryonic antigen kinetic for chemotherapy monitoring in patients with unresectable metastasis of colorectal cancer. J Clin Oncol 2008;26:3681-6.
9. Wu XZ, Ma F, Wang XL. Serological diagnostic factors for liver metastasis in patients with colorectal cancer. World J Gastroenterol 2010;16:4084-8.
10. Fakih MG. Carcinoembryonic antigen monitoring in metastatic colorectal cancer: Words of caution. J Clin Oncol 2008;26:E7.
11. Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Tangen C. An evaluation of the carcinoembryonic antigen (CEA) test for monitoring patients with resected colon cancer. JAMA 1993;270:943-7.
12. Waas ET, Hendriks T, Lomme RM, Wobbes T. Plasma levels of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 correlate with disease stage and survival in colorectal cancer patients. Dis Colon Rectum 2005;48:700-10.
13. Holten-Andersen MN, Christensen IJ, Nielsen HJ, Stephens RW, Jensen V, Nielsen OH, *et al.* Total levels of tissue inhibitor of metalloproteinases 1 in plasma yield high diagnostic sensitivity and specificity in patients with colon cancer. Clin Cancer Res 2002;8:156-64.
14. Curran S, Dundas SR, Buxton J, Leeman MF, Ramsay R, Murray GI. Matrix metalloproteinase/tissue inhibitors of matrix metalloproteinase phenotype identifies poor prognosis colorectal cancers. Clin Cancer Res 2004;10:8229-34.
15. Holten-Andersen MN, Stephens RW, Nielsen HJ, Murphy G, Christensen IJ, Stetler-Stevenson W, *et al.* High preoperative plasma tissue inhibitor of metalloproteinase-1 levels are associated with short survival of patients with colorectal cancer. Clin Cancer Res 2000;6:4292-9.
16. Frederiksen C, Qvortrup C, Christensen IJ, Glimelius B, Berglund A, Jensen BV, *et al.* Plasma TIMP-1 levels and treatment outcome in patients treated with XELOX for metastatic colorectal cancer. Ann Oncol 2011;22:369-75.
17. Birgisson H, Nielsen HJ, Christensen IJ, Glimelius B, Brünner N. Preoperative plasma TIMP-1 is an independent prognostic indicator in patients with primary colorectal cancer: A prospective validation study. Eur J Cancer 2010;46:3323-31.
18. Ree AH, Florenes VA, Berg JP, Maelandsmo GM, Nesland JM, Fodstad O. High levels of messenger RNAs for tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) in primary breast carcinomas are associated with development of distant metastases. Clin Cancer Res 1997;3:1623-8.
19. Murashige M, Miyahara M, Shiraishi N, Saito T, Kohno K, Kobayashi M. Enhanced expression of tissue inhibitors of metalloproteinases in human colorectal tumors. Jpn J Clin Oncol 1996;26:303-9.
20. Fong KM, Kida Y, Zimmerman PV, Smith PJ. TIMP1 and adverse prognosis in non-small cell lung cancer. Clin Cancer Res 1996;2:1369-72.
21. Sørensen NM, Byström P, Christensen IJ, Berglund A, Nielsen HJ, Brünner N, *et al.* TIMP-1 is significantly associated with objective response and survival in metastatic colorectal cancer patients receiving combination of irinotecan, 5-fluorouracil, and folinic acid. Clin Cancer Res 2007;13:4117-22.
22. McCarthy K, Maguire T, McGreal G, McDermott E, O'Higgins N, Duffy MJ. High levels of tissue inhibitor of metalloproteinase-1 predict poor outcome in patients with breast cancer. Int J Cancer 1999;84:44-8.
23. Zucker S, Lysik RM, DiMassimo BI, Zarrabi HM, Moll UM, Grimson R, *et al.* Plasma assay of gelatinase B: Tissue inhibitor of metalloproteinase complexes in cancer. Cancer 1995;76:700-8.
24. Hanke B, Riedel C, Lampert S, Happich K, Martus P, Parsch H, *et al.* CEA and CA 19-9 measurement as a monitoring parameter in metastatic colorectal cancer (CRC) under palliative first-line chemotherapy with weekly 24-hour infusion of high-dose 5-fluorouracil (5-FU) and folinic acid (FA). Ann Oncol 2001;12:221-6.
25. Ward U, Primrose JN, Finan PJ, Perren TJ, Selby P, Purves DA, *et al.* The use of tumour markers CEA, CA-195 and CA-242 in evaluating the response to chemotherapy in patients with advanced colorectal cancer. Br J Cancer 1993;67:1132-5.
26. Goslin R, O'Brien MJ, Steele G, Mayer R, Wilson R, Corson JM, *etal.* Correlation of Plasma CEA and CEA tissue staining in poorly differentiated colorectal cancer. Am J Med 1981;71:246-53.

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