Prognostic meaning of tissue inhibitors of matrix metalloproteinases TIMP-1 and TIMP-2 in patients with colorectal cancer

Elena Kostova¹*, Slavica Shubeska Stratrova²

¹Department of Preclinical and Clinical Pharmacology and Toxicology, Medical Faculty, Ss. Cyril and Methodius University, 50 Divizija 6, 1000 Skopje, Republic of North Macedonia
²University Clinic of Endocrinology, Diabetes and Metabolic disorders, Medical Faculty, Ss. Cyril and Methodius University, Mother Theresa 17, 1000 Skopje, Republic of North Macedonia

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Abstract

The aim of this study was to analyze TIMP-1 and TIMP-2 serum levels in patients with colorectal cancer (CRC) and to correlate the results with the pathological stage of the disease and outcome in order to evaluate the role of TIMP-1 and TIMP-2 serum levels as prognostic markers.

The investigation has been made on 82 patients with operable CRC without distant metastases, who had undergone blood tests in order to determine the TIMP-1 and TIMP-2 serum levels in the following points of time: preoperatively, as well as 3, 6, 9 and 12 months postoperatively.

Significant differences were found between serum levels of TIMP-1 and TIMP-2 obtained preoperatively and postoperatively, as well as significant association of serum TIMP-1 levels obtained preoperatively in CRC patients in stage I and III, in the 3rd and in the 6th month (p<0.001) postoperatively as defined points of time with the outcome of CRC patients. Serum TIMP-2 levels obtained preoperatively was significantly associated with the outcome of the CRC patients. Analysis of the obtained TIMP-1 and TIMP-2 serum levels in CRC patients showed statistically significant differences with: disease progression, occurrence of liver metastasis, prior to and post chemotherapy treatment.

The results derived a conclusion that the serum levels of TIMP-1 and TIMP-2 could be indicators for occurrence and progression of CRC, as well as valuable and useful markers for following the effects of chemotherapy treatment.

Keywords: colorectal cancer, TIMP-1, TIMP-2, prognosis

Introduction

Colorectal cancer (CRC) prognosis predominantly depends on the disease stage, but new prognostic factors are being investigated in order to determine disease progression and outcome in patients as well as postsurgical pharmacology treatment (Ferlay et al., 2010). The correct staging of each CRC patient is crucial in order to plan an optimal treatment regimen. It is widely recognised that prognostic information based on clinical and histopathological investigation may be insufficient, although tumour stage and lymph node involvement are the main prognostic tools in evaluating cancer specific survival. It is questionable to expose a large number of patients to adjuvant treatment with considerable side
Makitalo, 2010; DeClerck et al., 1992; Nagase et al., 2006). Elevated TIMP-1 and TIMP-2 serum levels in patients with CRC and we correlated the results with the pathological stage of the disease and outcome in order to evaluate the role of TIMP-1 and TIMP-2 serum levels as a prognostic markers and markers that may indicate the changes in cancer disease progression.

**Materials and methods**

The study included a total of 82 previously untreated CRC patients, 30 females and 52 males (age range from 43 to 75 years, averaged 67.85) with operable CRC, without detectable distant metastases, who respected the medical instructions and were available for follow-up. All the patients underwent surgical resection of the primary neoplasm at the University Clinic for Abdominal Surgery in Skopje in the period of 2 years.

Blood samples from all the patients were drawn before surgical treatment, as well as 3, 6, 9, and 12 months postoperatively, in order to examine the TIMP-1 and TIMP-2 serum levels. None of the CRC patients had received chemotherapy before blood sample collection. To standardize clotting conditions, all serum samples were separated within 1 h after blood collection, aliquoted and stored at −80 °C until assayed.

Serum levels of TIMP-1 and TIMP-2 were determined using a quantitative solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, USA) according to the manufacturer's instructions. High concentrations of TIMP-1 and TIMP-2 were diluted with calibrator, to produce samples with values within the dynamic range of the assay.

The histopathological analysis of surgically removed operative material was made at the Institute of Pathology at the Faculty of Medicine, Skopje, where the pathological stage was defined for every patient according to the International Union Against Cancer (UICC-pTNM) and American Joint Committee on Cancer (AJCC), 2010.

Forty-three patients with Stage II B and III (A, B, C) received adjuvant chemotherapy, postoperatively at the Institute for Radiotherapy and Oncology in Skopje.

Correlations were made between the MMPs serum levels and the pathological parameters.

**Statistical analysis**

Descriptive statistics data are given according to normality of the distribution. Normality of the distribution was determined by Kolmogorov-Smirnov’s test. Analysis of variance with Kruskall-Wallis test was used in the analysis of different sample types. In the case of significant results, the analyses were continued by pairing the variables and analyzing them with Mann-Whitney’s U-test. Fisher’s exact probability test and Pearson’s Chi-Square test (r) were used for testing the association (linearity of the correlation of serum concentrations) between TIMPs and major prognostic variables in CRC, such as grade and stage. P-values less than 0.05 (p<0.05) were considered as statistically significant.
Results

There have been 17 (20.73%) patients in stage I, 40 (48.78%) patients in stage II and 25 (30.48%) patients in stage III of the CRC. Lymph node metastases were substantiated in 25 (30.48%) patients and were not find in 57 (69.51%) patients with different pT category.

The majority of patients were with pT3N0M0 (26.82%), i.e. patients in stage II A of the disease, and the smallest number of patients were with pT4aN1M0 (4.87%), i.e. patients in stage III B of the CRC.

The explanation of this condition would be identical as in previously displayed results of the other investigated parameters, i.e. due to the change of condition of the disease in patients most of the stage III, in which is shown the largest percentage of lethal outcomes.

The mean TIMP-1 levels in patients in all stages of the disease decreased after tumor resection, whereas the mean serum values in stage III demonstrated most intensive changes, unlike the mean serum values in CRC patients in stage I and II which showed mild changes. All three groups of followed patients showed decreasing values postoperatively, where the decline was most evident in 3\textsuperscript{th} month postoperatively in all CRC patients at all determined stages.

Mean serum levels of TIMP-1 in CRC patients in stage II remained approximately at the same level in further defined points of time, with a slightly increase in 9\textsuperscript{th} and 12\textsuperscript{th} month postoperatively.

Mean TIMP-1 serum levels of CRC patients in stage I showed approximately the same movement, while the curve mean TIMP-1 serum levels of CRC patients in stage III showed a plateau values from 3\textsuperscript{th} to 6\textsuperscript{th} month postoperatively, then abruptly increases at 9\textsuperscript{th} month and again achieves plateau up to 12 months postoperatively.

Most evident decline in mean serum levels of CRC patients was observed at 6\textsuperscript{th} month postoperatively in all determined stages, and the most pronounced increase in mean serum levels was observed in CRC patients in stage III, especially in the defined points from 6\textsuperscript{th} to 9\textsuperscript{th} months postoperatively.

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
\textbf{Stage} & \textbf{pTNM} & \textbf{N} & \textbf{Percent} \\
\hline
I & pT1N0 M0 & 8 & 9.75 \\
I & pT2 N0 M0 & 9 & 10.97 \\
II A & pT3 N0 M0 & 22 & 26.82 \\
II B & pT4a N0 M0 & 18 & 21.95 \\
III B & pT3 N1b M0 & 7 & 8.53 \\
III B & pT3 N2a M0 & 9 & 10.97 \\
III B & pT4a N1b M0 & 4 & 4.87 \\
III C & pT4a N2b M0 & 5 & 6.09 \\
\hline
\end{tabular}
\caption{Staging of CRC patients according to AJCC}
\end{table}

The mean serum TIMPs (ng/mL) values in terms of stages and defined points of time

\begin{table}[h]
\centering
\begin{tabular}{lcccccccc}
\hline
\textbf{Defined points} & \textbf{TIMP-1 Stage I} & \textbf{TIMP-1 Stage II} & \textbf{TIMP-1 Stage III} & \textbf{TIMP-2 Stage I} & \textbf{TIMP-2 Stage II} & \textbf{TIMP-2 Stage III} \\
\hline
\textbf{in time} & & & & & & & & \\
Preoperat. & 176.2 & 228.66 & 348.62 & 150.01 & 222.56 & 333.29 \\
SD & 46.87 & 66.11 & 102.97 & 31.35 & 65.11 & 97.24 \\
Max & 277.5 & 362.5 & 633 & 220.5 & 329.4 & 586 \\
Min & 115.4 & 89.5 & 175 & 102 & 65.5 & 162 \\
3 months postop. & 157.74 & 215.05 & 257.98 & 240.91 & 201.49 & 245.99 \\
SD & 54.82 & 215.05 & 50.98 & 48.3 & 65.53 & 50.49 \\
Max & 355 & 64.86 & 372.4 & 324.3 & 313.5 & 334.3 \\
Min & 137.5 & 50.6 & 176 & 136.4 & 58.5 & 126.7 \\
6 months postop. & 156.16 & 215.51 & 257.74 & 121.78 & 194.69 & 240.91 \\
SD & 34.3 & 64.19 & 54.82 & 28.83 & 64.86 & 48.3 \\
Max & 209 & 345.5 & 355 & 170.3 & 307.4 & 324.3 \\
Min & 95 & 55.5 & 137.5 & 79.8 & 29.2 & 136.4 \\
9 months postop. & 161.11 & 216.43 & 334.18 & 132.05 & 201.27 & 310.5 \\
SD & 40.45 & 59.54 & 109.13 & 30.61 & 62.44 & 104.8 \\
Max & 225 & 360 & 603 & 199 & 305.6 & 561 \\
Min & 82 & 100.5 & 150.2 & 80.8 & 70.4 & 135.6 \\
12 months postop. & 169.23 & 219.82 & 336.69 & 138.17 & 203.36 & 321.45 \\
SD & 44.88 & 60.05 & 102.09 & 28.82 & 58.57 & 96.02 \\
Max & 262 & 155.2 & 610 & 205 & 300.4 & 550.5 \\
Min & 110 & 110.5 & 165 & 92.5 & 90.7 & 147 \\
\hline
\end{tabular}
\caption{Mean serum TIMPs (ng/mL) values in terms of stages and defined points of time}
\end{table}
In Table 3 are presented significant associations in TIMP-1 and TIMP-2 and outcome of the CRC patients, where it is shown that serum levels of TIMP-1 and TIMP-2 preoperatively, as well as the TIMP-2 serum levels in 3rd and 6th month postoperatively, are in a significant correlation with the outcome of the CRC patients.

Table 3. Significant correlations of serum levels of TIMPs (ng/mL) and CRC patients’ outcome

<table>
<thead>
<tr>
<th>Parameter</th>
<th>p</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>&lt;0.001</td>
<td>0.635</td>
</tr>
<tr>
<td>pT</td>
<td>0.005</td>
<td>0.331</td>
</tr>
<tr>
<td>pN</td>
<td>&lt;0.005</td>
<td>0.618</td>
</tr>
<tr>
<td>TIMP-1 preoperatively</td>
<td>&lt;0.001</td>
<td>4.0279</td>
</tr>
<tr>
<td>TIMP-1; 3 months postop.</td>
<td>&lt;0.001</td>
<td>4.0595</td>
</tr>
<tr>
<td>TIMP-1; 6 months postop.</td>
<td>&lt;0.001</td>
<td>4.6553</td>
</tr>
<tr>
<td>TIMP-1; 9 months postop.</td>
<td>NS</td>
<td>/</td>
</tr>
<tr>
<td>TIMP-1; 12 months postop.</td>
<td>NS</td>
<td>/</td>
</tr>
<tr>
<td>TIMP-2 preoperatively</td>
<td>&lt;0.001</td>
<td>4.5372</td>
</tr>
<tr>
<td>TIMP-2; 3 months postop.</td>
<td>NS</td>
<td>/</td>
</tr>
<tr>
<td>TIMP-2; 6 months postop.</td>
<td>NS</td>
<td>5.1124</td>
</tr>
<tr>
<td>TIMP-2; 9 months postop.</td>
<td>NS</td>
<td>/</td>
</tr>
<tr>
<td>TIMP-2; 12 months postop.</td>
<td>NS</td>
<td>/</td>
</tr>
</tbody>
</table>

NS – not significant

There were significant differences between the mean serum levels of TIMP-1 before tumor resection and 3rd and 6th month (p<0.001) postoperatively in CRC patients in stage I and III, between 3rd and 6th month and 9th and 12th month postoperatively in CRC patients in stage III (p<0.001 among all) and between preoperative and postoperative levels during defined control points of time in CRC patients in stage II.

Regarding mean serum levels of TIMP-2 obtained prior to surgical intervention and 6th month in all CRC patients stages postoperatively we found statistically significant differences between mentioned features (p<0.001), as well as between 3rd and 6th month and 9th and 12th month postoperatively in CRC patients in stage III (p<0.001 among all).

The CRC patients with different stages who received and who didn’t receive chemotherapy treatment, and their outcome are shown in Table 4.

In twenty patients that were particularly monitored to the appearance of liver metastasis and subsequently were administered first-line chemotherapy treatment (in patients with stage I and II A) and additional second-line chemotherapy treatment (in patients with stage II B and III).

The liver metastasis was substantiated by imaging techniques and liver biopsy with histological confirmation with the following distribution: in 2 patients of stage I, 3 patients of stage II A, 4 patients of stage II B, 8 patients of stage III B and in 3 patients of Stage III C.

In these patients’ blood samples were drawn additionally for quantifying of TIMPs serum levels prior to and post chemotherapy treatment in order to determine...
variability of serum levels for their role as markers, which could indicate a change in the condition of the disease in CRC patients, that indicate possible progression and occurrence of metastases.

The mean serum levels of TIMP-1 and TIMP-2 in CRC patients obtained prior to chemotherapy treatment reached approximately the levels of the mean values of the group of CRC patients in stage IIIC in 12th month separately, while the mean serum levels abruptly decreased (p<0.001) post chemotherapy treatment, i.e. similar values were obtained after resection of the primary neoplasm.

![Graph](image_url)

Fig. 2. Mean serum TIMP-1 (ng/mL) and TIMP-2 (ng/mL) levels prior to and post-chemotherapy treatment.

### Discussion

TIMPs are also linked with a wide variety of other functions of cell growth and survival, and at least some of these functions seem to be independent of MMP inhibition. TIMP-1 and TIMP-2 were first identified as proteins potentiating the epi-effect on proliferation and differentiation of erythroid progenitor cell (Gasson et al., 1985).

Additionally, the cell-growth promoting effects of TIMP-1 and -2 have been shown in various normal and malignant cell lines (Hayakawa et al., 1998; Yamashita et al., 1996). There is some evidence supporting the hypothesis that TIMPs’ role as growth stimulators and MMP inhibitors is functionally as well as possibly structurally separated (Chesler et al., 1995).

The role of TIMPs in cancer is very complex, and acknowledging the fact that angiogenesis and ECM degradation are very crucial in tumor cell spreading and metastasis formation, it is obvious that TIMPs have a role of some importance in cancer. Previously the TIMPs’ role as MMP inhibitors seemed most important, and there is clear evidence that downregulation of TIMP-1 and TIMP-2 expression is associated with increased invasiveness of tumors, while overexpression leads to reduced tumor growth and metastasis formation in tumors of various origins (Chesler et al., 1995).

In meta-analysis for determining plasma and serum levels TIMP-1 as predictors of the outcome of CRC patients, Lee et al. (2011) reported that the elevated TIMP-1 values obtained in blood samples, as well as expression of TIMP-1, are associated with shorter survival time.

Contemporary researches have been made in order to determine the significance of the serum TIMP-1 levels in patients of pre-invasive to invasive CRC, in order to diagnose the malignancies and to determine TIMP-1 influence on the disease’s outcome (Pellegrini et al., 2000).

TIMP-2 (tissue inhibitor of metalloproteinases-2, CHIAMP-chondrocyte-derived inhibitor of angiogenesis and metalloproteinase activity, CSC-21K) could either promote the activity of MMP-2 through interaction with Mo1-MMP or to inhibit through direct action (Stetler-Stevenson, 2008). TIMP-2 can reduce proliferation of endothelial cells, fibroblasts and some tumor cell lines. In a mouse model, TIMP-2 could protect from CRC and reduce the existing metastasis (Baker et al., 1998; Brand et al., 2000; Stetler-Stevenson and Seo, 2005).
Some researchers investigated TIMP-1 and -2 serum levels in 97 CRC patients and discovered a significant correlation between elevated TIMP-1 serum levels and shorter survival time, which led them to the conclusion that the elevated TIMP-1 serum levels are an independent prognostic factor for survival in patients with CRC (Giaginis et al., 2009).

In the examination conducted by Oberg et al. (2000) on 158 CRC patients was confirmed that the elevated serum TIMP-1 levels are significantly higher in patients with an advanced stage of the disease, and that the increased values of TIMP-2 were in correlation with worse prognosis.

### Conclusion

In the present study significant differences were found between serum levels of TIMP-1 and TIMP-2 obtained preoperatively and postoperatively, as well as significant association of serum TIMP-1 levels obtained preoperatively, in the 3rd and in the 6th month as defined points of time with the outcome of CRC patients. Serum TIMP-2 levels obtained preoperatively was significantly associated with the outcome of the CRC patients.

Analysis of the obtained TIMP-1 and TIMP-2 serum levels in CRC patients showed statistically significant differences with: disease progression, occurrence of liver metastasis, prior to and post chemotherapy treatment.

The results derived a conclusion that the serum levels of TIMP-1 and TIMP-2 could be indicators for occurrence and progression of CRC, as well as valuable and useful markers for following the effects of chemotherapy treatment.

### References


Резиме

Прогностично значење на ткивните инхибитори на матрикс металопroteинази ТИМП-1 и ТИМП-2 кај пациенти со колоректален карцином

Елена Костова¹, Славица Шубеска Стратрова²

¹Институт за претклучничка и клиничка фармакоолошија со токсикологија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, 50 Дивизија 6, 1000 Скопје, Република Северна Македонија
²Универзитетска клиника за ендокринологија, дијабетес и метаболички болести, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Мајка Тереза 17, 1000 Скопје, Република Северна Македонија

Клучни зборови: колоректален карцином, ТИМП-1, ТИМП-2, прогноза

Целта на оваа студија беше да се анализираат серумските нивоа на ТИМП-1 и ТИМП-2 кај пациенти со колоректален карцином (КРК), да се корелираат резултатите со патолошкиот стадиум на болеста и со исходот, и притоа да се евалуира улогата на серумските нивоа на ТИМП-1 и ТИМП-2 како прогностички маркери.

Истражувањето беше направено на 82 пациенти со опериран КРК без далечни метастази, од кои без земен крви примероци за одредување на серумските нивоа на ТИМП-1 и ТИМП-2 во следните временски точки: предоперативно, како и 3, 6, 9 и 12 месеци постоперативно.

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Беа најдени сигнификантни разлики помеѓу серумските нивоа на ТИМП-1 и ТИМП-2 предоперативно и постоперативно, исто така беше утврдена сигнификантна поврзаност на серумските нивоа на ТИМП-1 предоперативно кај пациентите со КРК во стадиум I и III, во 3-от и 6-от месец (p<0,001) постоперативно. Серумските нивоа на ТИМП-2 добиени предоперативно беа сигнификантно поврзани со исходот на пациентите со КРК. Анализата на добиените ТИМП-1 и ТИМП-2 серумски нивоа кај пациентите со КРК покажа статистички значајни разлики со прогресијата на болеста, со вредностите пред и после хемотерапиското лекување.

Од резултатите се сугерира заклучок дека серумските нивоа на ТИМП-1 и ТИМП-2 би можеле да служат како индикатори за појавата и прогресијата на КРК, а воедно може да бидат вредни и корисни маркери за следење на ефектите од хемотерапиското лекување.