THE ROLE OF SERUM CK-18, MMP-9 AND TIMP-1 LEVELS IN PREDICTING R0 RESECTION IN PATIENTS WITH GASTRIC CANCER

From the 1Haseki Training and Research Hospital, Department of General Surgery, and 2Istanbul Training and Research Hospital, Department of General Surgery, Istanbul, Turkey.

ABSTRACT - Background: Gastric cancer is the 3rd most common cause of death in men and the 5th common in women worldwide. Today, surgery is the only curative therapy. Currently available advanced imaging modalities can predict R0 resection in most patients, but it can only be detected with certainty in the perioperative period. Aim: To determine the role of serum CK18, MMP9, TIMP1 levels in predicting R0 resection in patients with gastric cancer. Methods: Fifty consecutive patients scheduled for curative surgery with gastric adenocarcinoma diagnosed between 2013-2015 were included. One ml of blood was taken from the patients to analyze CK18, MMP9 and TIMP1. Results: CK18, MMP9 and TIMP1 levels were positively correlated with pathological N and the stage (p<0.05). CK18, MMP-9 and TIMP-1 averages in positive clinical lymph nodes and in clinical stage 3 were found to be higher than the averages of those with negative clinical lymph nodes and in clinical stage 2 (p<0.05). Conclusion: Although serum CK-18, MMP-9 and TIMP-1 preoperatively measured in patients scheduled for curative surgery did not help to evaluate gastric tumor resectability, they were useful in predicting N3-stage.

INTRODUCTION

Cytokeratins are keratin-containing proteins of the family of intermediate filaments, found in the intracytoplasmic cytoskeleton of the epithelial tissue. CK18 is involved in important signaling pathways such as apoptosis, cell cycle, and cancer progression that regulate cell behavior. Especially with caspase-mediated cell death, the serum level is elevated. Matrix metalloproteinase 9 (MMP-9) disrupts the extracellular matrix and affects almost all aspects of cell biology. MMP-9 is one of the most complex matrix metalloproteinases. The tissue inhibitor of metalloproteinase-1 (TIMP 1) plays a negative role in the invasion and metastasis of tumor cells by forming a 1:1 complex with MMP-9 and inhibiting its enzymatic activity.5,10,16-18

Gastric cancer is the 3rd most common cause of death in men, and the 5th in women worldwide.15 Today, surgery is the only curative therapy for stomach cancer. One of the main determinants of survival is the type of surgical dissection done. Currently available advanced imaging modalities can predict R0 resection in most patients, but it can only be detected in the perioperative period.

The aim of this study was to determine the role of serum CK18, MMP9 and TIMP1 levels in predicting R0 resection in patients with gastric cancer.
This study was conducted with the approval of the Ethical Committee for Non-Drug Clinical Researches dated 01.01.2016 and numbered 302.

In the Department of General Surgery, Haseki Training and Research Hospital, Istanbul, Turkey, 50 consecutive patients scheduled for curative surgery for gastric cancer diagnosed between 2013 and 2015 were included in the study. Esophagogastroduodenoscopy was performed preoperatively and endoscopic biopsy results were determined as adenocarcinoma. Clinical staging of the patients was performed with IV contrast-enhanced thorax and whole abdominal CT. Patients inoperable and/or unresectable in clinical staging, with neoadjuvant treatment, and with a pathologic diagnosis outside of adenocarcinoma, were excluded. One day prior to the operation, 1 ml of blood was taken from them to analyze CK18, MMP9 and TIMP1, which were taken for routine investigations, and placed in separator gel-containing biochemical tubes. The blood was centrifuged at 10,000 rpm for 5 min and stored at -50°C.

The cost of kit purchase for CK18, MMP9 and TIMP1 was met by the Advisory and Monitoring Committee of the Thesis and Academic Studies. All of the patients were informed about the study and their written consents were obtained.

Statistical analysis
SPSS 15.0 for Windows, Mann-Whitney U test, Kruskal-Wallis test, Bonferroni correction were used for statistical analysis. Statistical significance level of alpha was accepted as p<0.05.

RESULTS

Of the 50 patients studied, 31 (68%) were male and 19 (32%) female and the mean age was 64 (min-max: 42-88). Twenty (40%) were ASA 1 and 30 (60%) ASA 3. The mean gastric tumor size was 34.1±6.7 mm (min-max: 21-47 mm). Six (12%) underwent palliative surgery and 44 (88%) R0 resection. Subtotal gastrectomy + D2 lymph node dissection was performed in 24 (48%) patients and total gastrectomy + D2 lymph node resection in 20 (40%). D2 lymph node dissection range varied between 14-24 nodes. Ten (20%) patients underwent additional organ resection, two of whom (4%) had colon resection, three (6%) cholecystectomy and five (10%) splenectomy. Seven (14%) were in stage 2B, six (12%) stage 4 and 37 (74%) stage 3. Average follow-up time 21.28 (Min-max: 1-40) months.

CK18, MMP9 and TIMP1 levels were positively correlated with pathological N and the stage (p<0.05, Tables 1 and 2).

TABLE 1 - CK18, MMP9 and TIMP-1 averages of the patient group

| Biomarker | Mean ± SD (Min-Max) |
|-----------|---------------------|
| CK18 (ng/ml) | 6.0±6.4 (2-29) |
| MMP9 (ng/l) | 1542.1±2347.7 (129-8237) |
| TIMP1 (pg/ml) | 511.2±452.4 (183-1923) |

TABLE 2 - Relationship between biomarker levels and tumor size, number of pathologic lymph nodes and pathologic stage

| Biomarker | Rho | p  | Rho | p  | Rho | p  |
|-----------|-----|----|-----|----|-----|----|
| CK18      | 0.093 | 0.521 | -0.052 | 0.718 | -0.019 | 0.897 |
| MMP9      | 0.491 | 0.001 | 0.396 | 0.008 | 0.497 | 0.001 |
| TIMP1     | 0.278 | 0.050 | 0.346 | 0.014 | 0.359 | 0.011 |

The CK-18, MMP-9 and TIMP-1 averages of those with positive clinical lymph nodes (pathologic lymph nodes in preop tomography) and those in clinical stage 3 were found to be higher than the averages of those with negative clinical lymph nodes and those in clinical stage 2 (p<0.05, Table 3).

The serum averages in the palliative operation group were found to be higher than the curative for all three markers, but as a result of the statistical analysis of these markers, no cut-off point could be predicted, which would suggest that the case was inoperable. As a result of the curve fitting analysis, the maximum effect of markers was determined for over 7.7, the number of metastatic lymph nodes resected in the operation (Table 4). When CK-18 >5.6 ng/ml, the probability of the number of metastatic lymph nodes being eight and over increased by 18 times in the 95% confidence interval. When MMP-9 >1528 ng/l, the probability of the number of metastatic lymph nodes being eight and over increased by seven times in the 95% confidence interval; similarly, when MMP-9 =495.7 pg/ml, was found the probability of the number of metastatic lymph nodes being eight and over increased by seven times (Table 5). Although these analyzes were not related to T-phase, and did not predict inoperability, they showed that N-stage was over 3.

TABLE 3 - Relationships of biomarker averages with clinical T, clinical N, and accordingly with clinical stage

| Biomarker | Avg. ± SD | Avg. ± SD | Avg. ± SD |
|-----------|-----------|-----------|-----------|
| CK-18     | 5.8±6.0  | 1619.5±2427.7 | 503.5±4533 |
| MMP-9     | 6.5±8.0  | 1234.2±2082.5 | 452.1±4717.7 |
| TIMP-1    | 0.942  | 0.416  | 0.482  |

TABLE 4 - Curve fitting analysis

| Biomarker | Mean | SD | Median |
|-----------|------|----|--------|
| CK-18     | 7.7  | 3.5 | 6.3    |
| MMP-9     | 7.6  | 2.3 | 6.4    |

TABLE 5 - Logistic regression analysis of the probability of the number of metastatic lymph nodes being eight and over

| Biomarker | p  | OR  | 95.0% C.I.for EXP(B)  |
|-----------|----|-----|----------------------|
| CK18  >5.6 | 0.010 | 18.200 | 1.979 | 167.337  |
| MMP9 >1528 | 0.012 | 7.111 | 1.336 | 32.912  |
| TIMP1 >495.7 | 0.016 | 7.111 | 1.336 | 32.912  |

DISCUSSION

It has been reported that CK18 may exhibit an abnormal expression in various cancers and may provide information about the prognosis. Makino et al. reported that an increase in CK18 expression in esophageal squamous cell carcinoma is associated with poor prognosis. Messai et al. reported about the prognosis. Messai et al. reported that an increase in CK18 expression in colorectal cancer was associated with poor prognosis. Buhler et al. emphasized that transfection of...
the CK18 gene in human breast cancer cells led to induction of adhesion proteins and dramatic retreat of in vitro and in vivo tumor invasion. Zhang et al.16 reported that high CK18 expression was determined in 101 (78.3%) of 129 patients. CK18 expression, correlated significantly with clinical stage, lymph node metastasis, number of pathologically positive lymph nodes, recurrence and metastasis. Scott et al.13 reported that the pre-operative CK-18 levels in patients with gastric adenocarcinoma and that a significant fall in these levels after treatment allowed the assessment of the tumor response to the treatment. Oyama K et al.17 mentioned the importance of the levels of caspase-dependent (M30) and total (M65) fractions in CK-18 serum in predicting the prognostic factor in gastric adenocarcinoma. Bilici et al.5, reported that CK-18 fragments (M30 and M65) could be used in evaluating chemotherapeutic tumor response in these patients.

Matrix metalloproteinase-9 (MMP-9) can disrupt the major components, extracellular matrix (ECM), type IV and V collagen and gelatin, so their activity is closely related to the invasion and metastatic ability of tumor cells. Increased expression of matrix metalloproteinases (MMPs) constitutes the ability to digest tumor cells, in particular basal membranes covering blood vessels, bas. In tissue cancer Zhang et al.18 relating that motility. The tissue inhibitor of metalloproteinase-1 (TIMP 1) plays a negative role in the invasion and metastasis of tumor cells by forming a 1:1 complex with MMP-9 and inhibiting its enzymatic activity.15, 10, 16-18

Zhang et al.19 reported that serum MMP-9 levels correlated with tumor invasion in patients with gastric adenocarcinoma. Shan et al.19 examined the association of HER-2 gene signal with MMP-9 activity in cells from gastric mucosa and reported that MMP-9 increased via HER2 in gastric cancer pathogenesis. Zhang et al.19 in their study in patients with gastric adenocarcinoma, reported that levels of many proteins of the metalloprotease family, including MMP-9, and TIMP-1 were significantly higher than healthy humans, regardless of histological type.

The main objective of this study was to determine significant differences between the groups in whom curative surgery could be performed and could not be performed (pathological stage > 3) by measuring preoperative serum CK-18, MMP-9 and TIMP-1 levels in gastric adenocarcinoma considered to be operable (clinical stage <4). In the cases of gastric cancers, preoperative diagnostic methods considerably determine the operability in patients; however, the laparotomies done with the hope of resection cannot reach its goal in a considerable number of patients. In some studies, this rate is over 30%; palliative operation in this study was 12%.

CONCLUSION

Although serum CK-18, MMP-9 and TIMP-1 preop measurements in patients scheduled for curative surgery due to gastric adenocarcinoma did not help to gain any idea of tumor resectability, they were valid to predict N3-stage.

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