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# The relationship between serum tumor-associated trypsin inhibitor levels and clinicopathological parameters in patients with gastric cancer

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**Abstract. – BACKGROUND: Tumor-associated trypsin inhibitor (TATI) is expressed with trypsinogen in tumors. We studied the clinical-pathologic association and significance of preoperative serum levels of TATI in gastric cancer patients.**

**PATIENTS AND METHODS: Pre-treatment serum levels of TATI in patients with gastric cancer and healthy controls were analyzed by a specific enzyme-linked immunosorbent assay (ELISA).**

**RESULTS: Statistically significant differences were found in serum TATI levels between patients with gastric cancer and healthy controls ($p < 0.0001$). There was a significant relationship between the serum levels of TATI and clinicopathological parameters. However, serum levels of TATI were significantly higher in patients with an advanced T stage (T3) ($p < 0.001$), lymph node metastasis ($p < 0.001$) and an advanced TNM stage (stage III or IV; $p < 0.001$).**

**CONCLUSIONS: Our study suggests that TATI may be used to identify potentially high-risk groups of upper gastric carcinoma. Elevated level of TATI was associated with progressive disease or advanced stage.**

*Key Words:*
Tumor-associated trypsin inhibitor, Clinical-pathological features, Gastric cancer.

## Introduction

One of the most important problems in the diagnosis of gastric cancer is the management of metastatic spread to loco-regional lymph nodes. Gastric cancer is the second leading cause of cancer death in the world and in Turkey[1,2]. The prognosis of gastric cancer varies conspicuously by the stage of gastric cancer with the 5-year comparative survival rate reaching 90% in stage I but less than 5% in stage IV[3]. As the stage of disease has a definite influence on survival, early diagnosis is highly critical. Therefore, early detection of gastric cancer is an important determinant to reduce the mortality and improves the prognosis of gastric cancer. Lymphatic spread of gastric cancer cells to the regional lymph nodes is one of the earliest events associated with distant metastasis and poor prognosis[4]. Depth of invasion, lymph node metastasis and the presence of distant metastasis have all been found to be obligatory prognostic factors[5].

Tumor-associated trypsin inhibitor (TATI) is a low-molecular weight (6 kDa) trypsin inhibitor that has been used as a marker for various cancer types[6, 7].

TATI is same to the previously described pancreatic secretory trypsin inhibitor (PSTI) (7), which is also called as the Kazal inhibitor[6].

TATI is encoded by a gene comprising four exons located on chromosome 5. The promoter region of the gene contains an interleukin-6 (IL-6)-responsive element[8]. TATI consists of 56 amino acids, has a molecular weight of 6242, and contains three disulphide bridges[7]. TATI is expressed at significant concentrations in other healthy tissues, particularly in the gastrointestinal and urogenital tissues[9,10].

TATI may behave as an acute-phase reactant, but increased serum concentrations are inspected only in connection with strong inflammatory reactions. *Because* TATI is expressed by the liver and the gene contains an IL-6-responsive element[11].

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The purpose of the present study was to examine the importance of the serum levels of TATI collected from ninety patients with gastric cancer. Furthermore, the serum levels of TATI were assessed, along with the association between the serum levels of TATI and clinical-pathological features.

## Patients and Methods

### Patients

Preoperative serum levels of TATI were determined in 90 patients with gastric carcinoma between 2010-2012. All patients who had surgery were eligible and were studied. No patients were excluded or refused consent. Each patient underwent the following procedures; chest radiograph, stomach imaging (ultrasound or CT scan), gastroscopy, computed tomography (CT) scan of the other organs, clinical diagnosis, and hematological and biochemical profiles. The clinical-pathological parameters were studied including age, tumor differentiation, TNM stage, distant metastasis, peritoneal carcinoma, lymph invasion, T stage.

The patient group was comprised of ninety (45 females and 45 males) patients with gastric cancer and the control group was comprised of fourty healthy (18 females and 22 males) subjects. The age range was 40-65 years (mean 55.7 ± 9.6 years) for the gastric cancer group and 39-63 years (mean 52.5 ± 7.5 years) for the control group. Healthy was defined as being free from acute illness and without any previous hospitalization for any illness within the past 2 years.

None of the cases involved in our study had undergone chemotherapy and radiotherapy prior to sampling. The pathological evaluation was based on the criteria outlined by the American Joint Committee and Cancer staging criteria. Informed consent was obtained from all participants for the use of their blood samples in the study. This project was approved by the Ethics Committee of the Medical School of Yuzuncu Yil University, Van.

### Biochemical Analysis

Serum samples were drawn prior to surgical treatment and stored at -70ºC until analysis. The samples were analysed using a time-resolved immunofluorometric assay (12). MAb 6E8 was used as a capture antibody for TATI and a europium (Eu) labelled antibody 11B3 was used as a tracer. Fluorescence was measured with a 1234 Delfia 2 time-resolved fluorometer (Turku, Finland). The lower limit of detection for TATI is 0.1 µg/L and the measuring range was 0.5-150 µg/L.

### Statistical Analysis

Shapiro Wilk normality and the Kolmogorov-Smirnov one-sample tests, assessement and histogram charts were drawn. Defining the average values, standard deviation, median, min. and max. given in the form**.** Analysis, independent samples *t*-test (normally distributed variables), Mann-Whitney U test, Kruskal-Wallis one-way analysis of variance and Benferroni correction after the Mann-Whiyney U test were used for binary comparisons. Limit of significance was set at $p < 0.05$. Analyses were performed using SPSS 17.0 program (SPSS Inc., Chicago, IL, USA).

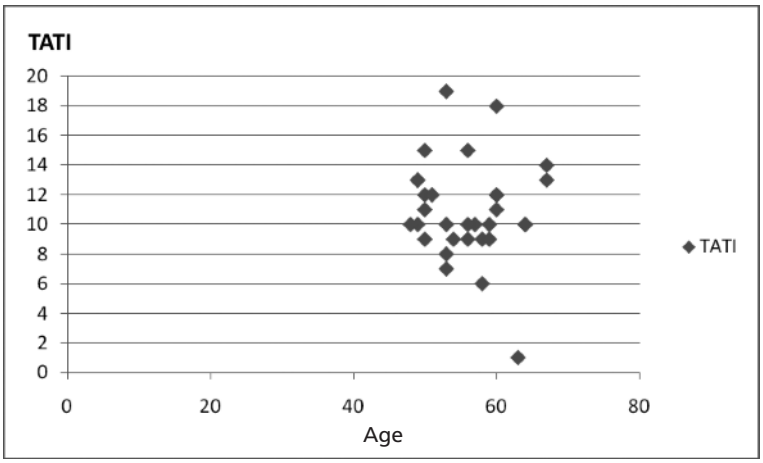


**Figure 1.** The graphic of controls.

**Table I.** The expression of TATI in the normal controls and in the patients with gastric cancer.

| | Controls n = 40 | Patients n = 90 | *p* |
|---|---|---|---|
| Age (y) | 55.7 ± 9.6 | 52.5 ± 7.5 | > 0.05 |
| TATI | 12 ± 4 | 82 ± 25 | < 0.0001 |

## Results

Serum TATI levels were detected in all patients and healthy controls. Serum TATI level varied in healthy donors, and the median level was 11 [range: 7-18, 95% confidence interval (CI): 10-16]. In gastric cancer, the median serum TATI level was 77(range: 19-123, 95% CI: 42-105). A highly significant difference was found in the median TATI levels between the gastric cancer patients ($p < 0.0001$) and healthy donors (Table I).

We used receiver operating characteristics (ROC) curves to determine the cut-off values and sensitivity and specificity of serum TATI test in the patients. The cut-off value was chosen according to the ROC curve coordinate points and the cut-off point for serum TATI was equal to its mean value. The sensitivities and specificities determined from the ROC curves at a cut-off level of 70 were 95% and 97% for serum TATI respectively.

There was a significant relationship between the serum levels of TATI and clinicopathological parameters. However, serum levels of TATI were significantly higher in patients with an advanced T stage (T3) ($p < 0.001$), lymph node metastasis ($p < 0.001$) and an advanced TNM stage (stage III or IV; $p < 0.001$) (Table II).

**Table II.** The association of TATI with the clinicopathologic parameters of the gastric cancer patients.

| Clinicopathologic Variables | TATI |
|---|---|
| Tumor differentiation | |
| Well | 25 ± 10 |
| Moderate | 54 ± 29 |
| Poor | 98 ± 17 |
| TNM stage | |
| TI | 19 ± 2 |
| TII | 67 ± 19 |
| TIII | 85 ± 10 |
| TIV | 94 ± 9 |
| Distant metastasis | |
| Absent | 18 ± 2 |
| Present | 87 ± 9 |
| Carcinoma peritonei | |
| Absent | 16 ± 3 |
| Present | 85 ± 11 |
| Lymph invasion | |
| Negative | 19 ± 2 |
| Positive | 80 ±17 |
| T stage | |
| T 1 | 18 ± 2 |
| T 2 | 60 ± 13 |
| T3 | 83 ± 9 |
| T4 | 95 ± 8 |

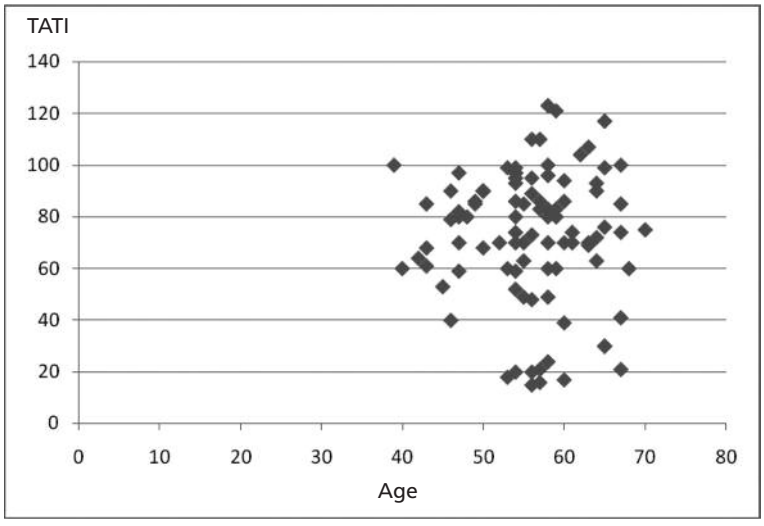


**Figure 2.** The graphic of patients.

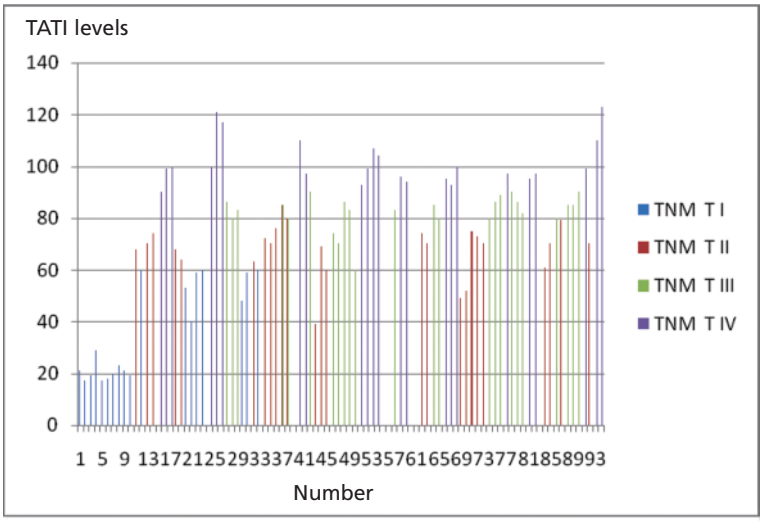


**Figure 3.** The graphic of TNM stage.

## Discussion

TATI is a 6000 Daltons peptide, which is synthesized by several tumors and cell lines[11,13]. This peptide is also produced by the mucosa of the gastrointestinal tract[14, 15], where it is thought that it protects the mucosal cells from proteolytic breakdown. Elevated serum and urine levels of TATI occur in connection with many types of cancer, especially mucinous ovarian cancer[16].

Increased levels may also occur in nonmalignant diseases, e.g. in pancreatitis[14], severe infections and tissue destruction[17]. Thus, TATI may behave as an acute phase reactant. Tumors producing TATI often express tumor-associated trypsinogen.

Therefore, increased levels of TATI in cancer and pancreatic disease are associated with the expression of trypsin[14], but such a connection has not been demonstrated in inflammatory diseases.

TATI can inhibit trypsin-mediated degradation of extracellular matrix by tumor cells. Therefore, its role may be to control the activation of tumor-associated trypsinogen. TATI has also been shown to possess growth factor activity *in vitro*, but it is not known whether this is a physiological function[18].

Solakidi et al[19] suggested that leakage from the tumor cells into the circulation might be the mechanism behind the elevated TATI levels, but the lack of a correlation between the serum TATI and tissue TATI levels in this study implies that leakage from the tumor into the circulation is not a major cause of the elevated serum TATI levels.

TATI was promptly shown to be identical to the SPINK1 (serine protease inhibitor kazal-type 1) based on the identical $NH_2$-terminal amino acid sequence[20]. As tumors often expressed both TATI and trypsinogen, TATI initially thought to have a similar function as in the pancreas, that is, the protection of the tumor against the destructive activity of trypsin within tumor cells. However, sequence similarities were detected between human epidermal growth factor (EGF) and human PSTI in 1983[21], leading to the idea that SPINK1 was shown to stimulate growth of several cell lines including cancer cells[22,23]. In 1987, the human SPINK1 gene was identified as being approximately 7.5kb consisting of 4 exons and was located on chromosome 5[24]. The gene product consists of 79 amino acids including a 23 amino acid signal peptide with three intramolecular disulfide bridges (Cys9-Cys33, Cys16-Cys35, and Cys24-Cys56)[24]. SPINK1, with the molecular weight of 6.2 kD, is secreted by the acinar cells of the exocrine pancreas into the pancreatic juice. Later on SPINK1 was shown to compete with EGF in binding to Swiss 3T3 fibroblast cells to almost the same extent, suggesting that SPINK1 binds to the EGF receptor (EGFR)[22]. The growth factor activity of SPINK1, roles of SPINK1 in cancer were suggested by many investigations, such as a relationship with survival, invasion, recurrence. However, the studies have not been performed and explained. *In vitro* studies using several cancer lines revealed that the SPINK1 stimulates growth of cancer cells lines through the EGF receptor and its downstream signaling pathway, MAPK/ERK

(mitogen-activated protein kinases extracellular signal-regulated kinases). The possibility that SPINK1 was a growth factor was raised when SPINK1 was shown to have structural similarities with EGF. They share approximately 50% amino acid sequence homology[21,25] and have similar numbers of amino acid residues (56 and 53, respectively; molecular weights of about 6 kDa) and three intra-chain disulfide bridges[26].

Actually, SPINK1 was shown to stimulate the growth of NIH 3T3 fibroblasts[27], human endothelial cells[23], and human epithelial cells[28].

Trypsinogen is thought to participate in tumor associated protease cascades mediating tumor invasion. Thus, the increased expression of SPINK1 was initially thought to inhibit the activated tumor-associated trypsinogen[18,29]. Tomlins et al[30] found that SPINK1 expression is an independent predictor of biochemical recurrence after resection and that SPINK1 knockdown in a prostate cancer cell line attenuated invasion. Tonouchi et al[31] reported that the SPINK1 expression was associated with early recurrence of intrahepatic cholangiocarcinoma after resection. Gouyer et al[32] found that SPINK1 triggered collagen type I invasion by several colon and breast cancer cells through phosphoinositide-3-kinase, protein kinase C, and Rho-GTPases/Rho kinase-dependent pathways.

## Conclusions

In our study, higher levels of circulating TATI were associated with clinical-pathological variables. In particular, higher TATI levels were related to advanced stage.

An altered endothelial cell metabolism is indicated in several detrimental pathways. Based on these observations, the most important of these pathways from a mortality perspective may be primarily gastric cancer-related ones. Circulating TATI levels may be promising markers of those endothelial cell metabolism alterations. We encourage the investigation of these associations in other general population samples, and the investigation of the relationships between the other markers of cell metabolism and hard endpoints in the general population. This is a novel group of cancer progression risk reflecting pathways for which development of drugs is ongoing. Before gastric cancer treatment can be useful in clinical practice, tools, for identification of target groups and for monitoring treatment need to be developed.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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