

Lipid Peroxidation and Transforming Growth Factor- β 1 Levels in Gastric Cancer at Pathologic Stages

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ABSTRACT

Objective: High levels of TGF- β 1 and enhanced TGF- β 1 receptor signaling are related to the pathology of gastric cancer. This effect is caused by oxidative stress and lipid peroxidation products. The aim of this study was to investigate the levels of TGF- β 1 and lipid peroxidation products in gastric cancer patients and their correlation with pathologic stage.

Material and Methods: Lipid peroxidation products and TGF- β 1 levels were studied in the serum samples of 50 gastric cancer patients and 18 control subjects.

Results: HNE-protein adducts and TGF- β 1 levels were significantly higher in T2, T3 and T4 gastric cancers than in either the T1 stage or controls ($p<0.001$). Pathologic stage was correlated with TGF- β 1 levels ($r=0.702$, $p<0.05$).

Conclusion: These markers production may contribute to tumor angiogenesis and aid in the prognosis of the gastric cancer.

Key Words: Gastric cancer, lipid peroxidation, TGF- β 1

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Introduction

In terms of incidence and mortality, gastric cancer is the second most widespread of all cancers in Turkey (1). Gastric cancer accounts for 3-10% of all cancer-related deaths (2). The five-year survival rate in patients with resectable gastric cancer ranges from 10-30% (3). Molecular biological analysis is helpful in the determination of cancer progression and prognosis.

Transforming growth factor- β 1 (TGF- β 1) is a prototypical member of the TGF protein superfamily, and is a multi-potent cytokine recognized as having an important role in cell growth, differentiation, motility and angiogenesis (4, 5). Two other isoforms (β 2, β 3) with important homology and functional similarity have also been identified in mammalian tissues (6-8). Signaling through the TGF- β receptor system has a negative impact in cancers of the stomach and colon, prostate, breast and lung (9-12). TGF- β is an important transcriptional regulator of the extracellular matrix, and is involved in two important events, promoting carcinogenesis and angiogenesis, that are associated with a poor prognosis.

The overall lipid peroxidation level is identified by the concentrations of MDA and 4-HNE, two of several byproducts of the lipid peroxidation process. MDA is a naturally occurring endogenous product of lipid peroxidation and prostaglandin biosynthesis, but it is also mutagenic and tumorigenic. The

end product of membrane lipid peroxidation, 4-hydroxyneonenal (HNE), may make an important contribution toward up-regulating TGF- β 1 expression (13). However, low levels of lipid peroxidation have been illustrated in the cell membranes of cancer cell lines and in carcinogenesis models (14).

The aim of this study was to investigate the levels of lipid peroxidation end products such as malondialdehyde (MDA) and 4-hydroxyneonenal (HNE) as well as TGF- β 1 in patients with gastric cancer, and to determine their correlation with staging.

Material and Methods

Fifty patients with gastric cancer (20 females, 30 males; mean age 48.72 ± 7.2 years) and 18 subjects as a control group (nine females, nine males; mean age 50.16 ± 5.7 years) were enrolled in our study. History and clinical parameters were recorded, and samples were collected after the Haseki Education and Research Hospital Ethics Committee approved this study. Informed consent was obtained from all patients. All of the pathology reports were reviewed, and data on tumor histology were recorded. Stage was defined according to the 1997 American Joint Committee on Cancer Staging System (15).

Preoperative serum samples of the 50 patients diagnosed with gastric cancer were collected. Patients with gastric cancer were excluded if they had a history of alcohol abuse,

smoking, previous treatment with chemotherapy or radiotherapy, brain metastases, second malignancy, acute or chronic infection, dysphagia, active infection, hypertension, diabetes mellitus, hyperlipidemia, chronic respiratory insufficiency, rheumatoid arthritis, cirrhosis, renal disease, coronary heart disease or cerebrovascular disease.

Control subjects with negative preoperative serum samples were free of gastrointestinal symptoms and had a normal endoscopic exam. Control subjects were asymptomatic, with an unremarkable medical history and a normal physical examination.

Biochemical markers of lipid peroxidation and TGF- β

The TBARS assay was performed as described by Jentzsch et al. (9). In the TBARS assay, one molecule of malondialdehyde (MDA) reacts with two molecules of thiobarbituric acid (TBA), and thereby produces a pink pigment with an absorption peak at 535 nm. Amplification of peroxidation during the assay is prevented by the addition of the chain-breaking antioxidant butylhydroxytoluene (BHT).

Plasma (400 μ L) prepared by hydrolysis with 1, 1, 3, 3-tetramethoxypropane (Sigma Chemical Co.) was mixed with 400 μ L of orthophosphoric acid (0.2 mol/l) (Sigma Chemical Co.) and 50 μ L of BHT (2 mmol/l) (Sigma Chemical Co.) in 12x72 mm tubes. A total of 50 μ L of the TBA reagent (0.11 mol/l in 0.1 mol/l NaOH) (Fluka Chem.) was then added and the contents were mixed. Subsequently, the contents were incubated at 90°C for 45 min in a water bath. The tubes were then kept on ice to prevent further reaction. TBARS were extracted once with 1000 μ L of n-butanol (Sigma Chemical Co.). The upper butanol phase was read at 535 nm and 572 nm, to correct for baseline absorption, on a Shimadzu UV-1601 UV-spectrophotometer (Shimadzu). MDA equivalents (TBARS) were calculated by the difference in absorption at the two wavelengths, and quantification was done with a calibration curve (10, 16). 4-HNE was assayed by a fluorimetric method (17, 18). Serum TGF- β levels were measured by enzyme-linked immunosorbent assay (ELISA).

Table 1. Serum levels of MDA, HNE and TGF- β 1 in the patient and control groups

	MDA content (nmol/mL)	MDA-protein adducts (AFU/mL)	HNE-protein adducts (AFU/mL)	TGF- β 1 (pg/mL)
Patients	0.65 \pm 0.34	21 \pm 9	27 \pm 9	38.5 \pm 11.1
Controls	0.21 \pm 0.09*	5 \pm 1*	8 \pm 2*	11.7 \pm 5.5*

Values are mean \pm SD, *p<0.0001 between the control group and all patients in all parameters

Table 2. MDA and HNE levels according to tumor stage

Tumor stage	MDA content (nmol/mL)	MDA-protein adducts (AFU/mL)	HNE-protein adducts (AFU/mL)
T1 (8)	0.38 \pm 0.12	19 \pm 8	20 \pm 11
T2 (10)	0.67 \pm 0.14*	20 \pm 7*	21 \pm 9*
T3 (22)	0.73 \pm 0.11*	21 \pm 8*	29 \pm 8*
T4 (10)	0.75 \pm 0.12*	24 \pm 7*	30 \pm 9*

Values are mean \pm SD, *p<0.0001 between the control group and all patients in all parameters

Statistical analyses

Statistical analyses were performed with the Shapiro-Wilk test of normality as well as histogram charts. Data are shown as mean \pm standard deviation; median (normal distribution, display number) was given in the form. Normally distributed variables with a normal distribution, display number and independent groups t test were analyzed using the Mann-Whitney U test. The Kruskal-Wallis test was performed between the two groups. Pearson correlations are shown between variables in the patient group or Spearman (not normally distributed) correlation tests are given. The limit of statistical significance was set at p<0.05. Analyses were performed using the SPSS 17.0 statistical program.

Results

There were no statistically significant differences between gastric cancer patients and the control group in respect to gender and mean age (all p>0.05).

All serum markers were statistically higher in patients than in the control group. Also, all markers were found to be higher in patients with gastric cancer in stages T2, T3 and T4 compared with stage T1 gastric cancer (p<0.001) (Table 1, 2).

Pathologic stages were correlated with TGF- β 1 levels ($r=0.702$, p<0.05) (Table 3).

Discussion

In our study, we observed that MDA, HNE-protein adducts and TGF- β 1 levels were significantly higher in T2, T3 and T4 gastric cancers than in controls or patients with stage T1 cancer. In our findings, the elevated TGF- β 1 protein continuously found in T2-T4 gastric cancers is of peculiar interest, as this elevation may be a further mechanism for managing the cell growth that occurs *in vivo*.

According to a number of studies, TGF- β 1 exerts its effects through a serine/threonine kinase complex involving both

Table 3. TGF- β 1 levels according to tumor stage

Tumor stage	TGF- β 1 (pg/mL)
T1 (8)	20.4±9.2
T2 (10)	31.6±11.5*
T3 (22)	40.9±9.8*
T4 (10)	49.7±10.4*

Values are mean±SD, The levels of TGF- β 1 were higher in stages T2-T4 compared to stage T1, *(p<0.001)

TGF- β 1 receptors I and II (19-21). However, the participation of these receptors, in particular type I, has not yet been completely characterized, and a differential role has been shown for receptor I and receptor II proteins in the pleiotropic effects of the cytokine (22, 23). Trans-differentiation of epithelial cells to mesenchymal cells is one probable event, originally shown in the mammary gland, which is inducible by TGF- β 1 (22). Under this hypothesis, mesenchymal-like cells would secrete more TGF- β 1, which in turn might influence the cell differentiation in an autocrine loop. Where the mechanism(s) of changed expression of TGF- β 1 in the disease cases under observation are concerned, the fairly continuous direct correlation of cytokine levels with those of tissue lipid peroxidation is challenging.

There is a significant body of literature on the association between oxidative stress, that is, the prevalence within the cell potential, and enhanced fibrogenic cytokines, in particular TGF- β 1. This association has primarily been found in several disease processes characterized by excessive fibrogenesis, such as atherosclerosis, liver cirrhosis and lung fibrosis (24, 25). Moreover, an important role of oxidative stress in the upregulation of TGF- β 1 in fibrotic diseases has been shown in both in vitro and in vivo systems, as supplementation of cells or animals with sufficient amounts of antioxidants not only prevented lipid peroxidation, but also inhibited the synthesis of the fibrogenic cytokine TGF- β 1 (26-28).

Currently, therapeutic markers in cancer patients are being examined by many researchers (29). We investigated the most important ones among these markers: the levels of TGF- β 1 and lipid peroxidation products. We believe that TGF- β 1 is a predictive marker that is associated with cancer stage, and thus can be of clinical benefit. In the present study, this result can be explained by the development of metastasis. High TGF- β 1 levels are associated with tumor invasion in peripheral tissues.

In recent years, *Helicobacter pylori* and some cytokines have been associated with the initiation of gastric cancer (30, 31). In addition, tissue levels of TGF- β 1 are associated with survival time in patients with gastric cancer (32). Also, high TGF- β 1 levels stimulate the growth regulation induced by gastric cancer cells (33).

Conclusion

Increased TGF- β 1 levels were found in advanced phases of human gastric cancer and were associated with the progression of cancer. In gastric cancer, the pro-apoptotic effect

of TGF- β 1 and the increased level of this cytokine within the tumor may be a general mechanism of cell growth regulation. Damage to the cell membrane, leading to lipid oxidation, appears to be related to tumor progression, and favors this process through increases in aldehyde end-products, known to stimulate the expression and synthesis of TGF- β 1.

Conflict of Interest

No conflict of interest was declared by the authors.

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