Pre- and Post-operative Plasma Big Endothelin-1 Levels in Patients with Gastric Carcinoma Undergoing Radical Gastrectomy

XIAO-JUN TENG[1], ZHI-XIANG SHEN[1], JIN-JIAN XIANG[2],
LEI SHEN[1], LIN YUAN[2], JIE GUO[1] and XIAO-LING WANG[1]

Departments of [1]Gastroenterology and [2]General Surgery,
Renming Hospital, Wuhan University, Wuhan 430060, China

Abstract. *Background: Big Endothelin-1 levels increase significantly in patients with various tumors, and raised plasma concentrations are associated with worse outcome. The aim of this study was to investigate plasma Big Endothelin-1 levels in patients with gastric carcinoma before and after radical gastrectomy and to explore its clinical significance. Materials and Methods: One hundred and six patients with gastric carcinoma and 20 controls were studied. Big Endothelin-1 plasma levels in patients with advanced gastric cancer were examined by enzyme-linked immunosorbent assay before and on days 1, 3 and 10 after curative surgery and were then tested every 3 months. Results: All patients, except those with stage I gastric cancer, had significantly higher mean plasma Big Endothelin-1 levels compared with the normal controls (p=0.000). The plasma Big Endothelin-1 levels were markedly increased on the first post-operative day (1st POD) in all patients but decreased on the 3rd POD with no significant difference compared to the pre-operative levels. On the 10th POD, patients with stages I and II gastric cancer showed a marked reduction in the plasma Big Endothelin-1 levels (p=0.010 and p=0.000, respectively), whereas no significant difference was observed in stage III and IV patients. During the follow-up, plasma Big Endothelin-1 levels immediately before recurrences occurred in stage II patients were significantly higher compared with the levels on the 10th POD (p=0.011). Conclusion: Plasma Big Endothelin-1 levels might be a reliable marker to determine the severity of gastric carcinoma. Monitoring plasma Big Endothelin-1 levels after curative resection in stage II gastric cancer patients was valuable in predicting recurrences.*

Endothelin (ET)-1, a recognized vasoconstrictor peptide, is implicated in numerous physiological and pathological conditions, including hypertension, cardiac failure and disseminated intravascular coagulation. In addition to its potent vasoconstrictor properties, ET-1 is believed to play a role in a diversity of tumor biological processes such as mitogenesis, apoptosis, angiogenesis, tumor invasion and metastases (1-5). Many tumor cell lines, including gastric carcinoma, can release ET-1 (6-9). This is also reflected *in vivo*, where elevated ET-1 levels can be detected in the plasma of patients with solid malignant tumors, implicating it as a reliable tumor marker (10-12). Due to the low circulating levels and a short plasma half-life, the measurement of plasma ET-1 levels is difficult. Big ET-1, the biological precursor of ET-1, has a circulation half-life of 23 min compared with only 3.5 min for ET-1 (13, 14), and was demonstrated to be a more sensitive indicator of Endothelin system activation (15, 16). Reported data have shown elevated plasma Big ET-1 levels in patients with various tumors, such as colorectal, non-small cell lung and hepatocellular carcinoma, compared with those in healthy controls, which were associated with worse outcome (16-18). To date, however, data concerning Big ET-1 in gastric carcinoma are lacking.

The purpose of this study was to investigate the changes in plasma Big ET-1 levels in patients with gastric carcinoma before and after radical gastrectomy and to explore their clinical significance. Furthermore, a follow-up study was conducted in patients with advanced gastric cancer regarding whether alterations of the plasma Big ET-1 levels might be an indicator for disease recurrence.

Materials and Methods

Patients and methods. From October 2001 to March 2003, 106 patients were diagnosed with gastric cancer at the General Surgery Department, Wuhan University Renmin Hospital, China. The age range of the 70 males and 36 females was from 23 to 79 years (mean age, 57). All the patients were classified using the international

Table I. *Plasma Big ET-1 levels and TNM stage of gastric carcinoma.*

	n	Big ET-1 (pg/ml)	*p* value
Normal controls	20	2.07±0.33	0.000*
TNM stage			
I	13	3.36±0.63	
II	43	5.42±0.42	0.022**
III	36	5.85±0.54	0.007**
IV	14	6.18±0.93	0.010**

Values are mean±SEM.
*Big ET-1 values in stages II, III and IV patients *vs.* those in normal controls.
**Big ET-1 values in stages II, III and IV patients *vs.* those in stage I patients.

unifying new TNM staging of gastric cancer: I a, 9; Ib, 4; II, 43; IIIa, 20; IIIb, 16; IV,14. All the patients underwent radical gastrectomy for the treatment of these diseases. The control group was comprised of 20 healthy subjects with no known history of cancer who were age- and sex-matched with patients. This study was approved by the local ethics committee.

Peripheral venous blood samples from patients and healthy controls were taken at four time-points: pre-operatively and on post-operative days 1, 3 and 10. Furthermore, blood samples were collected in post-surgical patients every 3 months until recurrence occurred. All specimens were collected in ethylenediamine tetra-acetic acid-containing specimen tubes, placed immediately over crushed ice and centrifuged for 15 min. The plasma supernatant was drawn off, snap-frozen in liquid nitrogen and stored at –80˚C. The concentrations of Big ET-1 were measured within 3 months using a one-step sandwich enzyme-linked immunoassay (ELISA) kit (Biomedica, Divischagossc, Austria), in accordance with the manufacturer's protocol. All standards and patient samples were analyzed in duplicate and the mean value was taken. The detection range for this assay is 0.125-39 pg/ml and the cross-reactivity with human Big ET-1 (22-38), ET-1, ET-2 and ET-3 is <1%. The intra- and inter-assay coefficients of variation for this assay kit were 5% and 8%, respectively. The plasma Big ET-1 concentrations were calculated by extrapolation from a standard curve. A separate standard curve was constructed for each ELISA batch.

Follow-up and recurrence. Ninety-three stage II-IV patients were followed-up using a standard protocol after discharge from the hospital. The mean follow-up period was 19 months (2-32 months). There were five missing cases in the duration (II, 2; III, 2; IV, 1) and two patients who died within 3 months post-surgically were excluded from the recurrence cohort. Big ET-1 plasma levels were examined every 3 months with diagnostic imaging and endoscopy after curative surgery.

Statistical analysis. The results are expressed as mean±SEM and comparisons among groups were performed with the Student's *t*-test and one-way ANOVA test. All tests were two-sided. The statistical analysis was performed using the SPSS 10.0 statistical software. A value of *p*<0.05 was considered statistically significant.

Table II. *Plasma Big ET-1 levels and clinicopathological parameters of gastric carcinoma.*

	n	Big ET-1 (pg/ml)	*p* value
Gender			
Male	70	5.18±0.33	0.279
Female	36	5.86±0.59	
Age			
<70	89	5.40±0.32	0.928
≥70	17	5.47±0.80	
Tumor size(mm)			
<50	54	5.14±0.35	0.360
≥50	52	5.69±0.48	
Histological type			
Well- or moderately-differentiated	60	5.65±0.41	0.354
Poorly-differentiated	46	5.10±0.42	
Depth of invasion			
Without infiltration of serosa	38	4.74±0.38	0.035
Infiltration of serosa	68	6.00±0.45	
Lymph node metastasis			
Negative	35	4.43±0.49	0.020
Positive	71	5.89±0.36	

Results

All patients, except those with stage I gastric cancer, had significantly higher mean plasma Big ET-1 levels compared with the healthy controls (*p*=0.000). The plasma levels of Big ET-1 in patients with advanced gastric cancer (stages II, III and IV) were higher than those in patients with early gastric cancer (*p*=0.022, *p*=0.007 and *p*=0.010, respectively), but the levels in stage II, III and IV gastric cancer patients were comparable (Table I). Patients with lymph node metastases and infiltration of the serosa had higher plasma levels of Big ET-1 than their counterparts (*p*=0.020 and *p*=0.035, respectively). There were no significant differences for Big ET-1 plasma levels related to patient age (> or < 70 years), gender, tumor size (> or <50 mm) and histological type (Table II).

The mean plasma Big ET-1 levels were markedly increased on the first post-operative day (1st POD) in stage I and II patients (*p*=0.007 and *p*=0.000, respectively), but decreased on the 3rd POD with no significant difference compared to the pre-operative levels. However, on the 10th POD, these levels declined significantly compared to the pre-operative concentrations (*p*=0.010 and *p*=0.000, respectively) (Figure 1). In stage III and IV patients, the plasma levels of Big ET-1 were also markedly increased on day 1 after operation (*p*=0.001 and *p*=0.011, respectively), whereas no significant changes were observed on days 3 and 10 after operation (Figure 2).

During the follow-up, recurrences occurred in 48 out of 86 patients (stage II 14, stage III 23, stage IV 11). Recurrent
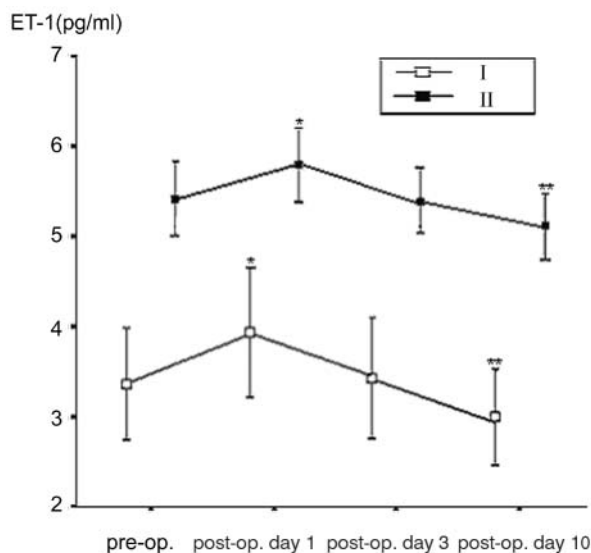
Figure 1. *Mean plasma Big ET-1 levels in stage I and II patients *elevated on post-operative day 1 (p=0.007, p=0.000, respectively) and **reduced on post-operative day 10 (p=0.010, p=0.000, respectively) compared with the pre-operative levels.*
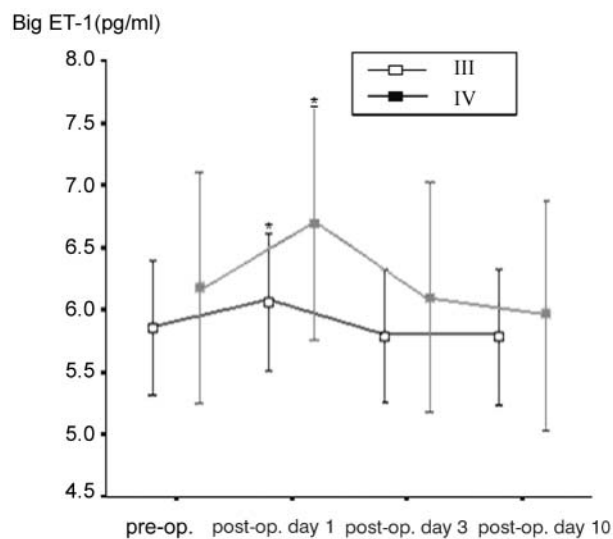

Figure 2. *Mean plasma Big ET-1 levels increased in stage III (p=0.001) and stage IV patients (p=0.011) on post-operative day 1 compared to the pre-operative levels.*

disease patterns included distant only 18, peritoneal only 13, all sites combined seven, locoregional only three, distant/locoregional four and peritoneal/locoregional three. The plasma Big ET-1 levels immediately before relapse in stage II patients were significantly increased compared with the levels of the 10th POD (6.16±0.80 *vs.* 6.56±0.88, *p*=0.011). However, no similar alterations were observed in the stage III and IV recurrent patients (6.06±0.79 *vs.* 6.15±0.79, 6.45±1.10 *vs.* 6.56±1.11, *p*=0.087 and *p*=0.099, respectively). In patients without recurrences, no significant changes in plasma levels of Big ET-1 were found during the follow-up compared with the levels on the 10th POD. There were no significant differences in age, gender or surgical procedure between recurrent patients and those with no recurrence.

Discussion

Curative resection is the mainstay for treating gastric cancer. The clinicopathological staging and histopathological classification after gastrectomy, to some degree, may serve as an evaluation of tumor severity and prognosis. However, the results are unsatisfying due to the limitations of subjective judgments by surgeons and pathologists. Thus, simpler and more useful methods, such as the detection of tumor markers in the blood, are needed. Interest in the role of ET-1 in cancer has grown over the last decade, following reports by many researchers who demonstrated an increase in ET-1 production in a number of cancers (16-18). In this study, measurement of the more stable Big ET-1 suggested

that it may be a more accurate indicator of the degree of the activation of the Endothelin system in patients with gastric cancer.

To date, raised plasma levels of Big ET-1 have been detected in patients with various solid tumors, including hepatocellular, colorectal and lung caner and further increased levels in advanced patients have been observed (18-20). In this study, the levels of Big ET-1 in pre-operative plasma samples were found to be significantly higher in patients with advanced gastric cancer(stages II, III, IV) than those in healthy controls and in patients with early gastric cancer (stage I), but the levels in stage II, III and IV patients were comparable. Similarly, patients with lymph node metastases and infiltration of serosa had raised plasma levels of Big ET-1 compared with their counterparts, suggesting a particular association with tumor progression. Recent studies have demonstrated that the ET-1 system participates in the promotion of angiogenesis and invasion/metastases in cancers (4, 21-22), indicating that tumor cells may acquire the capability to produce and secrete Big ET-l after malignant transformation. This may result in the significantly raised plasma Big ET-1 levels in the late stage of this disease, which suggest that the peripheral blood Big ET-1 levels increase with the progression of cancer and that the detection of this peptide may be a reliable indicator for tumor severity in patients with gastric cancer.

Tissue damage, ischemia and surgical stress during operation could raise circulating ET-1 levels (23), and the elevated ET-1 levels related to surgery frequently peaked

between 1 and 12 hours in the immediate post-operative period and declined gradually thereafter (24-26). Shito *et al.* (27) and Itoh *et al.* (28) have shown that plasma ET-1 levels in patients with gastric cancer were not changed significantly during peri-operation. This may be due to the small samples included in those studies and/or lower sensitivity of ET-1 plasma levels. Our study indicated that Big ET-1 plasma levels in all patients increased significantly on the 1st POD compared to the levels before operation, in agreement with the Nelson *et al's* (15) report that Big ET-1 is more sensitive than ET-1. On the 3rd POD, the Big ET-1 plasma levels declined, but were comparable to those before operation, suggesting that the increased levels of Big ET-1 as response to surgical stress were lessened. However, on the 10th POD, patients with stages I and II gastric cancer showed a marked reduction in plasma Big ET-1 levels compared to the pre-operative levels, whereas no significant difference was observed in stage III and IV patients. The lower levels of plasma Big ET-1 on the 10th POD in stage I and stage II patients may be due to the tumor's localization and less metastasis compared with stage III and stage IV patients, as well as to its more thorough removal by operation. These findings suggested that a reduction of plasma levels of Big ET-1 on the 10th POD may be associated with the early stage of gastric cancer.

Simpson *et al.* (20) demonstrated elevated plasma levels of Big ET-1 in patients with primary colorectal cancer compared with healthy controls, and that patients with liver metastases had significantly higher levels than those with localized disease. Arun *et al.* (17, 18) showed that the detection of plasma Big ET-1 levels in patients with non-small cell lung cancer and colorectal cancer might predict their prognoses. During the follow-up in our study, plasma Big ET-1 levels immediately before disease recurrences in stage II gastric cancer patients were significantly increased compared with the levels on the 10th POD. However, no similar alterations were observed in stage III and IV recurrent patients. To our knowledge, the present study is the first to specifically evaluate plasma Big ET-1 levels in patients with advanced gastric cancer. The results indicated that stage II patients had markedly raised Big ET-1 plasma levels immediately before recurrences occurred, suggesting that monitoring of plasma Big ET-1 levels after operation was useful in predicting the relapses in these patients.

In conclusion, the plasma Big ET-1 levels in patients with gastric cancer increased with tumor progression. The elevated plasma levels of Big ET-1 before operation, together with its significant changes peri-operatively, suggest that Big ET-1 might serve as a tumor marker in the clinical evaluation of patients with gastric cancer. Monitoring plasma Big ET-1 levels after operation in stage II could benefit the early diagnosis of recurrent disease.

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