ANTICANCER RESEARCH *24*: 3029-3034 (2004)

The Role of Insulin-like Growth Factor 1 and Insulin-like Growth Factor Binding Protein 3 in Human Esophageal Cancer

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Abstract. *Background: Insulin-like growth factors (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) play important roles in cell growth and differentiation. The aim of this work was to investigate the roles of IGF-1 and IGFBP-3 in esophageal cancer. Materials and Methods: We examined the circulating IGF-1 and IGFBP-3 concentration in 18 healthy controls and 66 esophageal cancer patients by ELISA and a ligand capture immunoassay. Immunohistochemistry for IGF-1 was performed on surgical specimens obtained from 93 patients with esophageal cancer. Results: The serum IGF-1 and IGFBP-3 levels were significantly elevated in patients compared with healthy subjects and there was a positive correlation between IGF-1 and IGFBP-3. There was a significant correlation between IGF-1 level and depth of invasion and pathological stage. Poor prognosis was significantly correlated with increasing IGF-1 levels. The survival rates of high IGF-1 expression immunohistochemical study patients were poorer than those of low expression patients. Conclusion: Elevated serum IGF-1 levels may be an important predictor of risk for esophageal cancer. IGF-1 related to the progression of esophageal cancer may depend on an autocrine function of IGF-1.*

The insulin-like growth factor (IGF) family of peptides and binding proteins (IGFBPs) are important for normal human growth and development. IGFs are expressed not only in the liver, which is the sole producer, but also in different cancer cells and they may play an important role in the propagation of these malignancies (1, 2). The possible autocrine, paracrine and endocrine role of IGFs in human tumors has been suggested (3). The IGFBPs are a family of six proteins that bind to IGF peptide with very high affinity. Of the six binding proteins, IGFBP-3 which is the most abundant IGFBP, binds more than 95 percent of the IGF in serum (4).

Recently, several epidemiological investigations have shown that there is a correlation between IGF-1 and IGFBP-3 in various cancers (5-11).

The expression of IGF-1 protein was not detected, except in thyroid cancer and its metastases, by immunohistochemistry (12). Few data are available on the expression and biological functions of the IGF system in esophageal cancer. Two previous reports demonstrated that IGF-1 acted as a mitogen in some human esophageal cancer cell lines (13, 14). Chen *et al.* suggested that IGF-1 plays a role as an autocrine growth factor for CE48T/VGH esophageal cancer cells (15).

The aim of this study was to determine the role of IGF-1 and IGFBP-3 in esophageal cancer.

Materials and Methods

Patients. For ELISA preoperatively, 66 serum samples from patients of the Department of Surgery I, Gunma University Faculty of Medicine, Japan, who had esophageal cancer between 1998 and 2002, were assayed for circulating IGF-1 and IGFBP-3. Eighteen serum samples from 18 healthy volunteers (average age 65.4 years) were assayed as normal controls. The mean follow-up period for the 66 patients was 19.7 months (range, 1.3-46 months).

For immunohistochemistry, surgical specimens were obtained from patients with esophageal cancer who underwent potentially curative surgery without preoperative therapy at the same department, between 1983 and 2001. The age of the patients ranged from 40 years to 78 years, with a mean age of 61.6 years. Tumor stage was classified according to the fifth edition of the

Abbreviations: IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3.

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Key Words: IGF-1, IGFBP-3, esophageal carcinoma, ELISA.

TNM classification of the International Union Against Cancer (UICC). The mean postoperative follow-up period for the 93 patients was 27.5 months (0.2-192.2).

Measurement of serum IGF-1 and IGFBP-3 concentration. Venous blood samples were drawn into sterile vacuum tubes when esophageal carcinoma was detected. They were centrifuged at 3000 rpm for 10 minutes and stored at -80˚C until assayed. For quantitative measurements of serum IGF-1 levels, we used a quantitative sandwich enzyme immunoassay (Quantities Human IGF-1 ELISA kit; R&D Systems, Minneapolis, MN, USA). IGFBP-3 was measured by a ligand capture immunoassay (IGFBP-3 ELISA kit; Diagnostic System Laboratories, Webster, TX, USA).

Immunohistochemistry for IGF-1. Immunohistochemical staining was performed according to the method described previously (16). The sections were heated in phosphate-citrate buffer at 120˚C for 3 minutes and then cooled to 30˚C. Slides were incubated with normal rabbit serum for 30 minutes and then blotted. The sections were then incubated with anti-IGF-1 monoclonal antibody (clone Sm1.2; Upstate Biotechnology, Corporation, Lake Placid, NY, USA) at a dilution of 1:2000 in phosphate-buffered saline(PBS) containing 1% bovine serum albumin at 4˚C overnight, then washed in PBS, and incubated with secondary antibody for 30 minutes at room temperature. Immunohistochemistry was performed using the Histofine SAB-PO(M) kit (Nichirei Co, Tokyo, Japan). The chromogen was 3,3'-diaminobenzidine tetrahydrochloride applied as a 0.02% solution containing 0.0055% H_2O_2 in 50 mM ammonium acetate-citric acid buffer, pH 6.0. The sections were counterstained lightly with hematoxylin.

Evaluation of serum IGF-1 and IGF-1 expression. We evaluated the prognostic value of IGF-1 and IGFBP-3 with cut-off values set at a mean (82.21, 3.735, respectively). IGF-1 levels above the cut-off value were classified as IGF-1 high group (n=27) and IGF-1 levels below the cut-off value were classified as IGF-1 low group (n=39).

The expression of IGF-1 in tumor cells was compared with IGF-1 expression in normal epithelium. When the staining in tumor cells was stronger than the staining in normal epithelium, the sample was classified as IGF-1-positive; when the staining in tumor was weaker than or as weak as the staining in normal epithelium or when there was no staining at all, the sample was classified as IGF-1-negative.

Statistical analysis. Statistical analysis was performed using the Chi-squared test, the Fisher's exact test and the One factor ANOVA test. Survival curves of the patients were calculated by using the Kaplan-Meier method and analysis was performed using the log-rank test. Statistical significance in this study was set as $p<0.05$.

Result

Measurement of serum IGF-1 and IGFBP-3 concentration and their correlation with clinicopathological features. Figure 1A shows the serum IGF-1 level of esophageal cancer patients and healthy volunteers. Among the 18 healthy volunteers, the median concentration of circulating IGF-1 was 50.83ng/ml (range, 20.63-81.03ng/mL; mean±standard error of the mean[SEM], 54.68±3.97). Among the 66 esophageal carcinoma patients, the median concentration of circulating IGF-1 was 142.27ng/mL(30.74-253.8 ng/mL; mean±SEM, 82.21±5.29), which was considerably higher than that of the healthy controls ($p=0.013$). Figure 1B shows the serum IGFBP-3 levels of esophageal cancer patients and healthy volunteers. Among the 18 healthy volunteers, the median concentration of circulating IGFBP-3 was 2.480ng/mL(range, 0.475-4.492 ng/mL; mean±SEM, 2.593±0.287). Among the 66 esophageal carcinoma patients, the median concentration of circulating IGFBP-3 was 3.527 ng/mL (0.696-6.358 ng/mL; mean±SEM, 3.735±0.131), which was considerably higher than that of the healthy controls ($p=0.016$). The correlations among the clinicopathological characteristics of patients with esophageal cancer and serum IGF-1 and IGFBP-3 levels in patients are summarized in Table I. There was a significant correlation between serum IGF-1 concentration, depth of invasion ($p=0.001$) and pathological stage ($p=0.042$). However, there was no significant association with other clinicopathological features.

Although the serum IGFBP-3 level in esophageal cancer was substantially higher than that of controls, there was no correlation between IGFBP-3 level and the clinicopathological features. Also, there was a positive correlation between IGF-1 and IGFBP-3 (Figure 1C: $p<0.0001$).

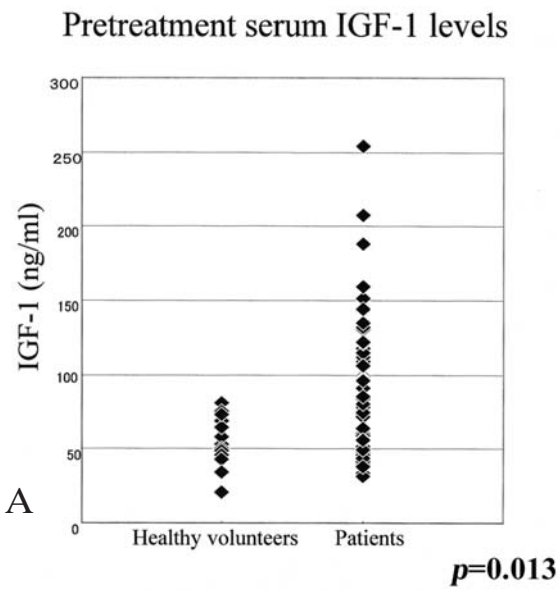
Immunohistochemistry of IGF-1. In normal esophageal epithelium, immunostaining of IGF-1 was detected in the cytoplasm of the basal and parabasal regions (Figure 2A). There was positive immunostaining for IGF-1 in the cytoplasm in primary esophageal carcinoma (Figure 2B) and in tumor cell nests (Figure 2C). The correlations among the clinicopathological characteristics of patients with esophageal cancer and IGF-1 expression in tumors are summarized in Table II. There is no correlation between the IGF-1 expression and clinicopathological features.

Prognostic significance of IGF-1 and IGFBP-3 expression. The survival rate of patients with high levels of serum IGF-1 was significantly lower than for those with low levels of serum IGF-1 (Figure 1D; $p=0.0398$). No significant association was observed between the IGFBP-3 level and overall survival (Figure 1E; $p=0.4397$).

The survival rate of patients with IGF-1-positive tumors was significantly lower than for those with IGF-1-negative tumors ($p=0.047$, Figure 2D).

Discussion

Previously no association has been shown between serum IGF-1 or IGFBP-3 and increased risk of developing esophageal cancer. In addition, the IGF-1 protein secreted from esophageal cancer had not been identified immunohistochemically.



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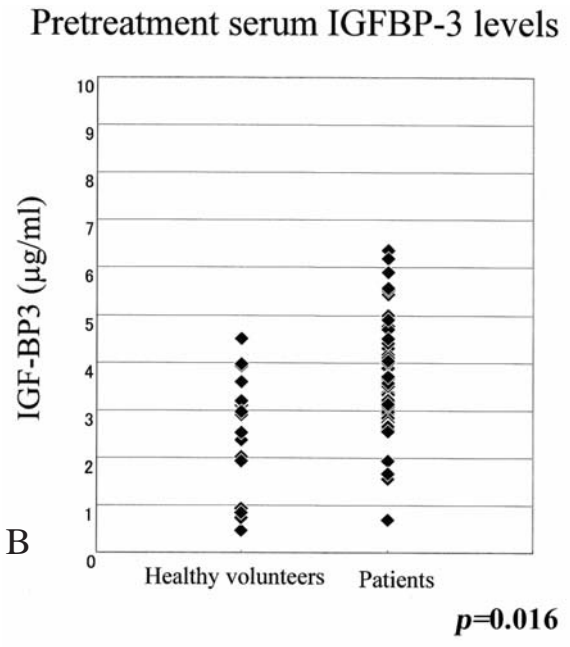

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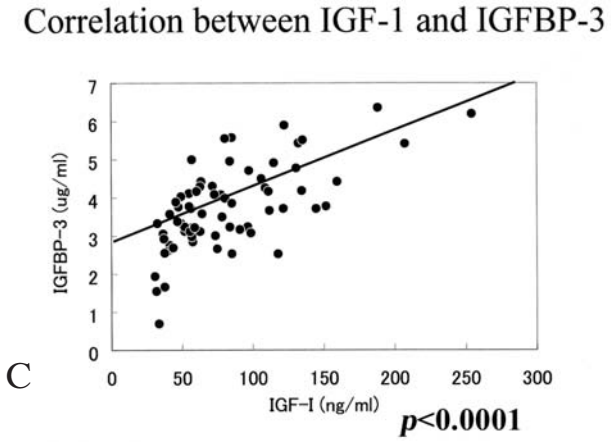

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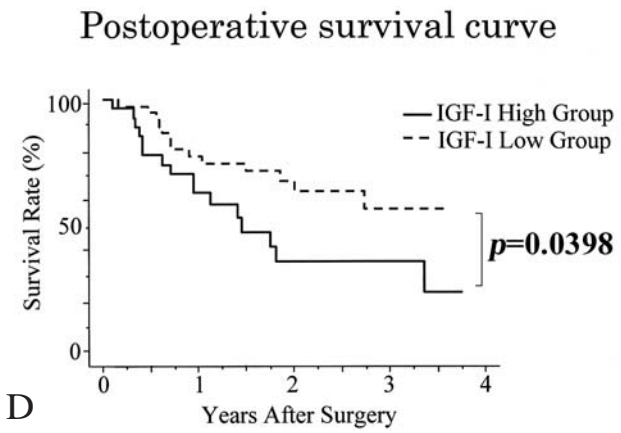

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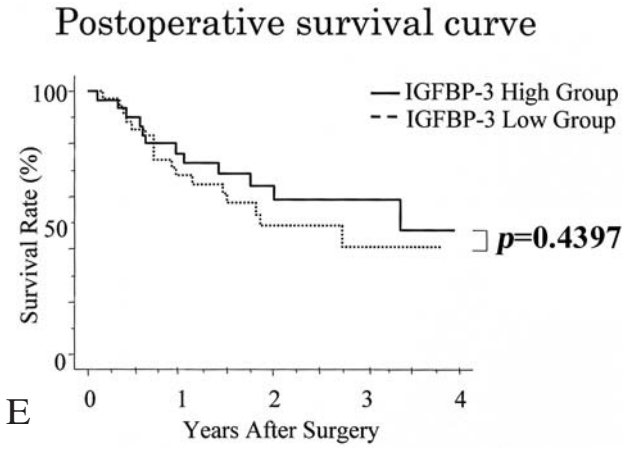

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Figure 1. *(A) Pretreatment serum IGF-1 levels. Comparison between 18 normal volunteers and 66 patients with esophageal carcinoma. The mean preoperative serum IGF-1 level was 82.21±5.29ng/mL in patients with esophageal carcinoma and 54.68±3.97ng/mL in the normal volunteers. The IGF-1 level in patients was significantly higher than in the 18 healthy controls (p=0.013). (B) Pretreatment serum IGFBP-3 levels. Comparison between 18 normal volunteers and 66 patients with esophageal carcinoma. The mean preoperative serum IGFBP-3 level was 3.735±0.131 μg/mL in patients with esophageal carcinoma and 2.593±0.287μg/mL in the normal volunteers. The IGFBP-3 level in patients was significantly higher than in the 18 healthy controls (p=0.016). (C) Correlation between IGF-1 and IGFBP-3. In esophageal carcinoma, note the positive correlation between IGF-1 and IGFBP-3. (D) Postoperative survival curve of patients with esophageal carcinoma. Serum IGF-I level (the 3-year survival rate: high group 35.5%, low group 58.1%). (E) Postoperative survival curve of patients with esophageal carcinoma. Serum IGFBP-3 level (the 3-year survival rate: high group 59.9%, low group 41.1%)*

Table I. *The correlation between clinicopathological characteristics and IGF-1 or IGFBP-3 serum level.*

Parameters	IGF-1 (mean±SEM) ng/ml	*p* value	IGFBP-3(mean±SEM) μg/ml	*p* value
Gender				
Male	81.9±5.69	0.848	3.70±0.14	0.438
Female	85.3±14.5		4.004±0.35	
Location				
Upper	66.9±9.92	0.642	3.42±0.40	0.427
Midthoracic	85.9±8.77		3.88±0.19	
Lower	85.6±7.02		3.69±0.16	
Differentiation				
Well	96.2±18.0	0.228	4.22±0.31	0.572
Moderate	67.5±5.41		3.52±0.19	
Poorly	94.7±12.5		3.75±0.30	
Adenocarcinoma	93.6±18.7		4.00±0.34	
Small cell carcinoma	69.0±27.6		3.00±0.24	
TNM clinical classification				
pT				
T1	63.6±5.77	0.001	3.56±0.22	0.116
T2	74.2±8.75		4.15±0.38	
T3	85.3±6.32		3.61±0.16	
T4	129±24.9		4.50±0.49	
pN				
N0	77.8±6.50	0.439	3.67±0.17	0.645
N1	86.1±8.14		3.79±0.20	
pM				
M0	80.7±5.45	0.542	3.67±0.14	0.247
M1	89.3±16.4		4.07±0.36	
p Stage				
1	60.6±5.69	0.042	3.55±0.22	0.359
2	94.7±9.70		3.80±0.28	
3	81.8±7.59		3.56±0.22	
4	100±18.3		4.18±0.35	

SEM: standard error of mean

In our study, serum IGF-1 and IGFBP-3 levels were significantly elevated in patients with esophageal cancer compared to healthy volunteers. Our data also showed a significant correlation between IGF-1 level and depth of invasion, pathological stage and prognosis. Concerning serum IGFBP-3 levels in esophageal cancer, there was no significant correlation with clinicopathological features. These data concerning IGF-1 are in agreement with previous data and may indicate that IGF-1 is a powerful predictor in esophageal cancer. However, IGFBP-3 had no predictive value in the prognosis of esophageal cancer.

The expression of IGF-1 protein was immunohistochemically detected only in thyroid cancer and its metastases. Our results demonstrated that there was an autocrine function of IGF-1 in esophageal cancer. There are no previous reports demonstrating that IGF-1 protein is secreted from esophageal cancer cells. We also found that IGFBP-3 is not expressed in esophageal cancer cells (data not shown).

In conclusion, elevated serum IGF-1 levels are associated with low survival rates in esophageal cancer. IGF-1 related to the progression of esophageal cancer depends on autocrine function. Identification of serum IGF-1 as a predictor of esophageal cancer risk may have implications for risk reduction and treatment.

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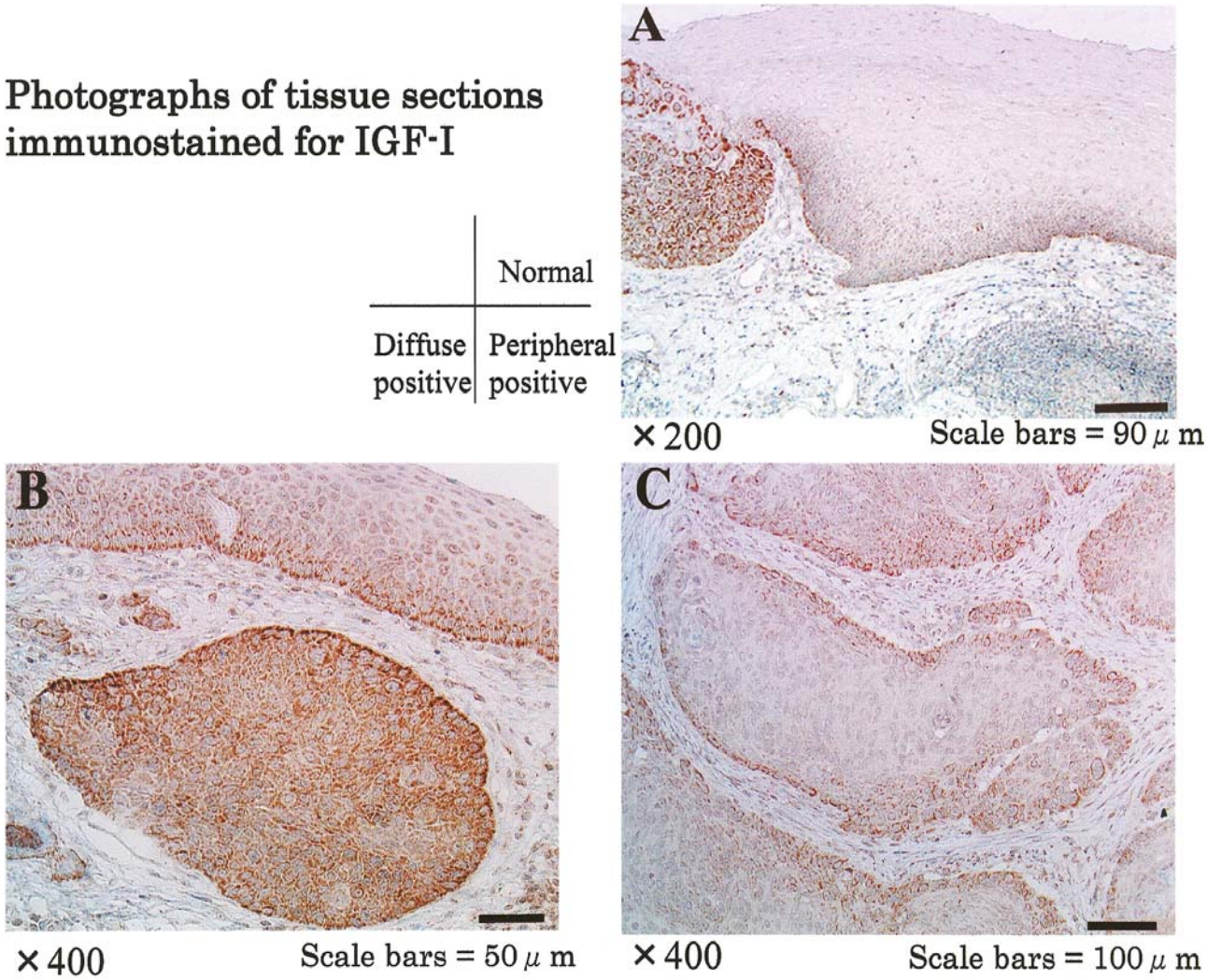

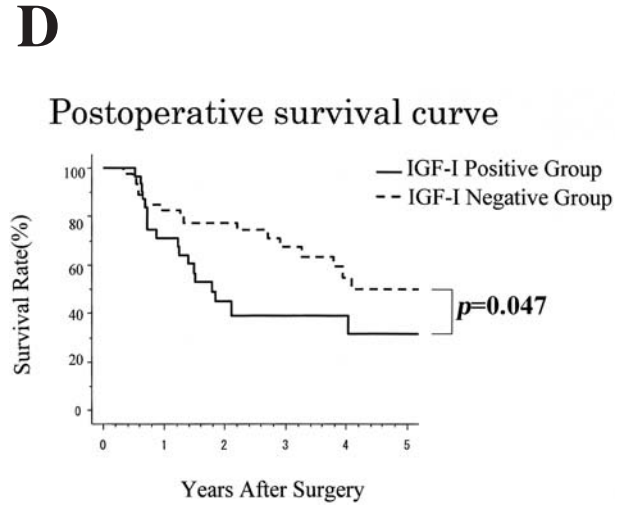

Figure 2. *Photographs of tissue sections immunostained for IGF-I. (A) IGF-1 was detected in the cytoplasm of basal and parabasal regions in normal esophageal epithelium(X100:Right side). Primary superficial esophageal squamous carcinoma also showed positive staining for IGF-1 in the cytoplasm (X100: Left side). Scale bars = 90 μm. (B) Overexpression of IGF-1 was detected in primary squamous cell carcinoma (X400). Scale bars = 50 μm. (C) IGF-1 was also detected in tumor cell nests at the peripheral site (X200). Scale bars = 100 μm. (D) Postoperative survival curve of patients with esophageal carcinoma in correlation with immunohistochemical IGF-1 expression (the 5-year survival rate: positive expression 32.1%, negative expression 51.2%)*

Table II. *The correlation between clinicopathological characteristics and IGF-1 expression in immunohistochemistry.*

Parameters	IGF-1		Total	*p* value
	positive	negative		
Age (mean±SD; yrs)	60.0±7.5	62.0±8.6	0.273	
Gender				
Male	28	50	78	0.8492
Female	5	10	15	
Location				
Upper	4	8	12	0.3677
Midthoracic	24	33	57	
Lower	5	19	24	
Differentiation				
Well	5	19	24	0.1076
Moderate	16	29	45	
Poorly	12	12	24	
TNM clinical classification				
pT				
T1	11	25	36	0.7801
T2	4	9	13	
T3	15	22	37	
T4	3	4	7	
pN				
N0	10	27	37	0.1659
N1	23	33	56	
pM				
M0	26	49	75	0.5152
M1	7	11	18	
p Stage				
1	7	17	24	0.6304
2	8	20	28	
3	11	12	23	
4	7	11	18	
Total	33	60	93	

SD: standard deviation

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Received March 1, 2004
Revised June 17, 2004
Accepted July 7, 2004