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Potential Prognostic Value of Circulating Levels of Vascular Endothelial Growth Factor-A in Patients with Gastric Cancer

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Abstract. *Objective: The aim of this study was to evaluate the potential prognostic value of the circulating levels of vascular endothelial growth factor-A (VEGF-A) in patients with gastric cancer. Materials and Methods: An ELISA was used to quantify the serum and plasma levels of VEGF-A in 135 patients with gastric cancer and 48 controls with benign gastric diseases diagnosed by gastroendoscopy and histology of the biopsied specimens. Serum VEGF-A levels were assayed in controls (n=10) and patients with gastric cancer (n=10) prior to surgery. Further samples from 16 patients with gastric cancer, 3 weeks after tumour excision, were collected. Results: VEGF-A levels were significantly higher in both serum and plasma from patients with gastric cancer than those from the controls (serum: 342.1 pg/ml vs. 80.0 pg/ml, p<0.01; plasma: 74.1 pg/ml vs. 23.7 pg/ml, p<0.01). In both cancer patients prior to surgery and controls, serum VEGF-A levels appeared to be unchanged over a 7-day period. However, examination of paired samples from 16 cancer patients collected prior to surgery and 3 weeks post-surgery showed that tumor excision resulted in a significant decrease in VEGF-A levels (Paired t-test, t=5.4, p<0.0001). Conclusion: Serum and plasma VEGF-A levels were significantly higher in patients with gastric cancer than in the controls. Serum VEGF-A levels correlated with tumour burden, as VEGF-A levels were significantly lowered following tumour excision in patients with gastric cancer.*

Gastric cancer is still the most common malignant tumour and the leading cause of cancer death in China and it remains a major public health problem in many other countries (1). The poor prognosis of gastric cancer is related to its aggressive nature, which is determined by the mutations of various genes and abnormalities in several growth factors and their receptors (including VEGF-A) (2). Since the discovery of VEGF-A, its high expression has been observed in a wide variety of malignant tumor tissues, VEGF-A being important for tumour angiogenesis and metastasis (3-5). Elevated levels of VEGF-A were also detected in serum and other fluids from patients with gastrointestinal tumours (6, 7). However, little is known about VEGF-A levels either in serum and plasma from the same cohort of cancer patients or the effect of surgery on VEGF-A levels (8, 9). Here, these two issues were addressed and their clinical significance was evaluated.

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Materials and Methods

Patients. Samples from 135 patients with gastric cancer and 48 controls with benign gastric diseases, diagnosed by gastroendoscopy and histology, were collected at the time of diagnosis prior to any treatment. Five ml blood samples were drawn by vein puncture preoperatively and divided into vials without any anticoagulant or with the anticoagulant EDTA. Within an hour of collection, the samples were centrifuged at 500 xg for 10 minutes and aliquots were frozen at –80˚C. In addition, serum samples were collected from 10 controls with benign gastric diseases and 10 patients with gastric cancer prior to surgery. To compare VEGF-A levels in pre- and post-operative samples, blood samples from 16 patients with gastric cancer were collected prior to surgery and 3 weeks post-surgery.

ELISA for VEGF-A. VEGF-A was quantified using commercial sandwich enzyme-linked immunosorbent assay kits (Human VEGF Quantikine™, R&D Systems, Minneapolis, MN, USA). The OD_{450} of samples was measured by BIO-RAD model 550 spectrophotometry and VEGF-A levels were determined utilizing a standard curve.

Statistical analysis. Statistical analysis was performed using the STATA $V_{5.0}$ Statistical software package. VEGF-A levels were compared between different groups using the *t*-test. *P* values ≤ 0.05 were considered significant.

Table I. *Circulating VEGF-A levels in patients with gastric cancer and in controls with gastric benign diseases.*

Patients with	No.	Plasma VEGF-A (pg/ml±S.D)	*p* value cancer *vs.* controls	Serum VEGF-A (pg/ml±S.D)	*p* value cancer *vs.* controls
CSG*	18	20.0±8.6		68.0±23.9	
CAG	15	23.3±12.9		84.4±33.9	
GU	15	24.8±16.2		95.4±51.5	
TOTAL	48	23.7±16.9		80.0±36.4	
Gastric cancer	135	74.1±96	<0.001	342.1±277.8	<0.0001

*CSG: chronic superficial gastritis; CAG: chronic atrophic gastritis; GU: gastric ulcer.

Table II. *The relationship between circulating VEGF-A levels and clinical features in 135 patients with gastric cancer.*

Parameter	No.	Plasma VEGF-A (pg/ml±S.D)	*p* value	Serum VEGF-A (pg/ml±S.D)	*p* value
Gender					
Male	96	81.1±114.9	>0.05	367.3±271	>0.05
Female	39	55.8±72		302.2±284.4	
Age(year)					
<60	36	79.2±75.3	>0.05	339.7±185.1	>0.05
≥60	99	71.5±51.5		344.6±199.2	
Location					
Cadia and fodus	22	58.9±35.7	>0.05	308.7±191.6	>0.05
Body	17	76.6±34.9		384.2±196.9	
Antrum	67	68.5±39.4		323.2±158.5	
Diffuse	29	95.5±103.6		390.8±257.7	
Type of cancer					
Early cancer	7	57.9±30.7	>0.05	290.6±154.9	>0.05
Borr. I	10	60.0±39.6		306.4±170.6	
Borr. II	55	73.5±38.7		348.4±208.8	
Borr. III	39	86.8±34.9		412.1±231.3	
Borr. IV	24	108.3±108.1		441.6±137.9	
Differentiation					
High	20	96.3±103.9	>0.05	388.6±233.8	>0.05
Medium	22	62.5±36.4		318.2±215.8	
Low	93	71.5±49.3		339.2±180.3	

Borr.: Borrmann's classification of gastric cancer

Results

Circulating VEGF-A levels in patients with gastric cancer and controls with benign gastric diseases. VEGF-A levels were significantly higher in both serum and plasma from 135 patients with gastric cancer compared with 48 controls (serum: 342.1 pg/ml *vs.* 80.0 pg/ml, $p<0.0001$; plasma: 74.1 pg/ml *vs.* 23.7 pg/ml, $p<0.001$) (Table I).

Relationship between circulating VEGF-A levels and clinical features of patients with gastric cancer. Circulating VEGF-A levels were not significantly affected by sex, age, location of gastric cancer, type or grade of differentiation (Table II).

Serum VEGF-A levels in 10 controls and 10 cancer patients prior to surgery. VEGF-A levels were compared in samples taken on day 7 and day 1 prior to surgery. The results showed that serum VEGF-A levels were 264.3±148.2 on day 7 and 265.2±142.9 pg/ml on day 1 in cancer patients ($p>0.05$). In controls, the VEGF-A levels were 71.4±16.2 and 78.0±14.3 pg/ml at the same time-intervals ($p>0.05$). These results indicate that VEGF-A levels were stable over a period of one week in both preoperative cancer patients and the controls.

Serum VEGF-A levels in pre- and post-operative patients. To investigate whether VEGF-A levels were associated with

tumour burden, blood samples were collected before and three weeks after tumour removal. Data pooled from 16 patients showed that serum VEGF-A levels were 328.4 pg/ml in preoperative samples, whilst the VEGF-A levels dropped to 103.5 pg/ml after operation (paired *t*-test, t=5.4, $p<0.0001$).

Discussion

Solid tumour remains avascular and dormant, reaching a maximum diameter of 1-2 mm (10), until it is supplied with an adequate vascularization (angiogenesis). Tumuor angiogenesis requires the coordinated action of various growth factors and their receptors. Among these growth factors, VEGF-A is the most potent, playing a critical role in both physiological and pathological angiogenesis. The degree of angiogenesis is indicative of tumour progression, therefore the levels of VEGF-A may be of prognostic value in various malignant diseases. In the current study, we measured serum and plasma VEGF-A levels by ELISA in the same cohort of patients with gastric cancer (n=135) and controls (n=48). Both serum and plasma VEGF-A levels were significantly higher in patients with gastric cancer than in the controls ($p<0.01$). Serum VEGF-A levels were higher than those in plasma, but were not affected by age, gender, location, tumour type or degree of differentiation. The reason that higher VEGF-A levels exist in serum is that VEGF-A is stored and released from platelets into the serum. Serum VEGF-A levels in cancer patients and controls examined one week and one day prior to surgery were similar, suggesting that our VEGF-A assay is reliable and the levels of VEGF-A are stable. An important finding to emerge is that surgical excision of tumours in 16 cancer patients resulted in a significant reduction in VEGF-A levels, demonstrating that VEGF-A was secreted by the tumour mass (9, 11). These patients are being followed-up to evaluate whether circulating levels of VEGF-A predict reccurrence and prognosis of the tumour.

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