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Abstract

Background—Gastric cancer remains a major cause of cancer mortality globally but no good prognostic tumour marker is available. Soluble fragment of E-cadherin protein has been reported to increase in the sera of patients with cancer and recently was found to be elevated in 67% of patients with gastric cancer.

Aims—To investigate if serum soluble E-cadherin is a valid prognostic marker in gastric cancer.

Methods—Concentrations of soluble E-cadherin from 116 patients with histologically confirmed gastric adenocarcinoma and 40 healthy subjects were measured using an immunoenzymometric method with a commercially available sandwich ELISA kit based on monoclonal antibodies.

Results—The logarithm of the means of soluble E-cadherin concentration was significantly higher in patients with gastric cancers (mean 3.85 (SD 0.28)) than in healthy subjects (3.71 (0.18)) ($p=0.001$), and in palliative/conservatively treated cancers (3.91 (0.35)) than in operable cancers (3.78 (0.19)) ($p=0.015$). The logarithm of the concentrations correlated with tumour size ($p=0.032$) and carcinoembryonic antigen concentrations ($p=0.001$). The cut off value calculated from discriminant analysis on operability and inoperability/palliative treatment was 7025 ng/ml. Soluble E-cadherin concentrations higher than this cut off value predicted tumour (T4) depth invasion ($p=0.020$, confidence interval (CI) 1.008–1.668) and palliative/conservative treatment ($p=0.023$, CI 1.038–2.514). In contrast, the relative risks for lymph node (N2) metastasis, distant metastasis, and stage III/IV disease were 1.41, 1.33, and 1.55 respectively, despite not reaching statistical significance.

Conclusion—Serum soluble E-cadherin is a potential valid prognostic marker for gastric cancer. A high concentration predicts palliative/conservative treatment and T4 invasion.

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Keywords: E-cadherin; gastric cancer; tumour marker

Gastric cancer remains the second major cause of cancer related deaths in the world.¹ However, there is currently no satisfactory tumour marker for diagnosis or monitoring disease

progress. The most frequently used tumour markers in gastric cancer are carcinoembryonic antigen (CEA) and CA19-9, but only a modest proportion of patients have elevated levels of these markers.

The cadherins are a major class of adhesion molecules which play an important role in the homotypic cell-cell adhesion and hence cancer cell metastasis and invasion. E-cadherin is a member of the cadherin family which is expressed in all epithelial cells. The role of E-cadherin in metastasis and invasion is evidenced by the fact that the invasiveness of epithelial tumour cell lines was inhibited in vitro by transfection and expression of E-cadherin cDNA, and induced again by exposure to anti-E-cadherin monoclonal antibodies.²⁻⁴ Under expression of E-cadherin molecule has been found in various malignancies and has potential value as a prognostic marker.⁵

Serum soluble E-cadherin is the degradation product of the cellular E-cadherin molecule. It is found in the circulation of normal individuals but is particularly elevated in patients with malignancies. Serum soluble E-cadherin has been shown to be a potentially valuable prognostic marker for carcinoma of the bladder.⁶ However, its prognostic value has not been proved in colorectal cancer,⁷ and its value in gastric cancer is controversial. Velikova and colleagues⁸ were unable to show a significant difference in serum soluble E-cadherin between patients with gastric cancer and normal subjects, while Gofuku and colleagues⁹ showed that concentrations were significantly elevated in 67% of patients. The aim of the present study was to investigate the value of serum soluble E-cadherin as a prognostic marker in patients with gastric cancer.

Methods

PATIENT SELECTION

All patients admitted from 1 January 1997 to 30 September 1998 to the Departments of Medicine and Surgery, Queen Mary Hospital, with histologically proved gastric carcinoma, including both operable and inoperable tumours, were recruited. The sera of 125 patients were collected after gastric cancer was confirmed histologically and before operation or initiation of chemotherapy. Nine patients were excluded from the present analysis because two were non-Chinese, one had serum collection after tumour debulking, one had another

Abbreviations used in this paper: CEA, carcinoembryonic antigen.

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synchronous tumour, and five had coincidental liver cirrhosis. Therefore, the total number of patients included in the analysis was 116. A group of 40 healthy subjects was recruited as controls.

PATIENT ASSESSMENT AND DEFINITIONS OF TREATMENTS

After gastric cancer was confirmed histologically by endoscopic biopsy, the extent of disease was assessed by chest x ray, endoscopic ultrasound, and computer tomography or ultrasound of the abdomen. Curative resection was defined as UICC R0 resection. Palliative treatment included UICC R1 or R2 resection, gastrojejunostomy, or palliative chemotherapy. Conservative treatment referred to those patients receiving symptomatic support only.

STAGING AND CLASSIFICATION OF GASTRIC CANCER

Tumour was staged according to the criteria of the Japanese Research Society for Gastric Cancer¹⁰ and classified histologically according to the WHO and Lauren's classification.¹¹

ASSAY OF SOLUBLE E-CADHERIN

Venous blood samples were collected into plain tubes, allowed to clot, and within one hour of collection were centrifuged at 800 *g* for 10 minutes at 4°C to obtain serum. Serum was removed, aliquoted, and stored at -70°C until assay. Concentrations of soluble E-cadherin were measured with a commercially available sandwich ELISA kit based on monoclonal antibodies (Zymed Laboratories Inc., South San Francisco, California, USA). All blood samples were measured by an investigator who was blinded to the clinical details and coded data sheet. Each sample was measured twice.

The assay method has been described previously.¹² In brief, the first monoclonal antibody, HECD-1, was coated onto microtitre plate wells to create the solid phase. Non-specific binding was blocked by a blocking buffer. Serum samples from patients and standard solutions supplied were incubated in the microtitre plate wells. The second monoclonal antibody, SHE 13-1, labelled with peroxidase was added. During incubation, human E-cadherin molecule was trapped by the two monoclonal antibodies as a sandwich. The reaction between the peroxidase and substrate solution (H₂O₂ and tetramethylbenzidine) resulted in colour development with intensities proportional to the concentration of human E-cadherin present in the samples and standards. The colour developed was measured with the microtitre plate reader for measurement of absorbance at 450 nm. Accurate sample concentrations of human E-cadherin were determined by comparing specific absorbances with those obtained from the standards plotted on a standard curve.

STATISTICAL METHODS

Data were collected and analysed using the Statistical Package for the Social Sciences. Logarithmic transformation was performed on soluble E-cadherin data for conversion to a

normal distribution. Clinical and biochemical parameters of patients are expressed as mean (SD). Comparisons were performed using the independent sample Student's *t* test and the χ^2 test. Differences were considered significant when $p < 0.05$, and approaching statistical significance when $p < 0.1$ and ≥ 0.05 . Cut off values of soluble E-cadherin concentration were calculated by discriminant analysis.

Results

There were 75 men and 41 women in the patient group with a mean age of 66 (14) years. There were 19 men and 21 women in the control group with a mean age of 31 (10) years. The sizes of the tumours measured from the pathological specimens obtained after resection ranged from 0.5 cm to 18 cm (mean 4.8 (3.2) cm). Forty eight per cent of tumours were located in the gastric antrum. Of those with gastric resection specimen available for pathological examination, 61% were of the intestinal-type, 30% were the diffuse-type, and 9% were a mixed-type according to Lauren's classification. The percentage of patients with stage I, II, III, and IV disease were 12.6%, 18.4%, 28.2%, and 40.8%, respectively. Therefore, most patients presented at advanced stages.

The means of the logarithm of soluble E-cadherin concentration in patients with gastric cancer were significantly higher than those of normal healthy subjects (3.85 (0.28) *v* 3.71 (0.18); $p = 0.001$). In contrast, the means of the logarithm of soluble E-cadherin concentration in patients with T4 invasion, liver metastasis, distant metastasis, and stage III/IV disease were higher than the means of other tumour depth invasion, absence of liver metastasis, absence of distant metastasis, and stage I/II disease, respectively, with *p* values approaching statistical significance ($p = 0.057, 0.067, 0.082, 0.086$, respectively). The logarithm of soluble E-cadherin concentration correlated with the size of the tumour ($p = 0.032$) and with the logarithm of CEA concentration ($p = 0.001$).

Fifty four patients underwent curative gastric resection while 43 patients received palliative treatment. Another 10 patients received conservative treatment only. Nine patients were excluded from further analysis of their treatment results because eight were operable but medically unfit and one committed suicide before receiving any treatment. The means of the logarithm of soluble E-cadherin concentrations in patients receiving palliative/conservative treatment and those receiving curative resection were 3.91 (0.35) and 3.78 (0.19), respectively ($p = 0.015$).

The cut off value for serum soluble E-cadherin of normal subjects and patients with gastric cancer was calculated as 5994 ng/ml; 27.5% of normal subjects and 51.7% of patients were above this cut off value. The cut off value for curative treatment and palliative/conservative treatment was calculated as 7025 ng/ml. Concentrations higher than 7025 ng/ml were used to predict the relative risks of various poor prognostic factors (table 1). Patients with soluble E-cadherin concentrations above the

Table 1 Prediction of relative risks of various prognostic factors using concentrations of soluble E-cadherin higher than the cut off value (> 7025 ng/ml)

Factor	Category	Relative risk	p Value	Confidence interval
T	T4 v T1 or T2 or T3	1.30	0.020	1.008–1.668
N	N2 v N0 or N1	1.41	0.064*	0.944–2.118
M	M1 v M0	1.33	0.073*	0.954–1.859
Staging	Stage III or IV v stage I or II	1.55	0.164	0.82–2.913
Operability	Palliative/conservative v operable	1.62	0.023	1.038–2.514

T, tumour; N, lymph node; M, metastasis.

p<0.05 is considered statistically significant and is indicated by bold type; p<0.1 but >0.05 is considered approaching statistical significance and is indicated by an asterisk.

cut off value were more likely to have T4 invasion (p=0.020, confidence interval (CI) 1.008–1.668) and palliative/conservative treatment (p=0.023, CI 1.038–2.514). The relative risks of N2 metastasis, distant metastasis, and stage III/IV disease were 1.41, 1.33, and 1.55, respectively (NS).

Discussion

During carcinogenesis, tumour cells have to dissociate from one another before they can invade or metastasise. Therefore, adhesion molecules are expected to play an important role in carcinogenesis and especially metastasis. Decreased membranous expression of E-cadherin molecules has been found in gastric cancer^{13 14} and other malignancies such as colon,^{15 16} pancreas,¹⁷ oesophagus,¹⁸ liver,¹⁹ prostate,²⁰ bladder,^{21 22} breast,^{23 24} and head and neck tumours.²⁵ Disruption of membranous expression of E-cadherin could be caused by disturbed polarisation of the cell, or mutations or partial deletions of the E-cadherin gene,²⁶ resulting in a protein which is not transported to the cell membrane. Direct correlation between E-cadherin and grade of tumour differentiation has been observed in some tumours.^{20–22} In a multivariate retrospective study of 413 patients with gastric cancer, E-cadherin positive tumours had significantly better three and five year survival rates than E-cadherin negative tumours.²⁷

Soluble E-cadherin, a 80 kDa peptide, is considered to be the degradation product of the 120 kDa intact E-cadherin generated by a Ca²⁺ ion dependent proteolytic action.^{28 29} The peptide was found in the circulation of healthy subjects and was not dependent on age or sex. It was elevated in patients with gastric carcinoma and other malignancies.¹² Increased serum soluble E-cadherin concentrations have also been found in pemphigoid or pemphigus skin conditions,^{30–32} and in multiorgan failure.³³ As E-cadherin is expressed in all epithelial cells, any condition with rapid epithelial cell turnover may lead to an increase in its concentration. Therefore, patients with these conditions and those with chronic inflammatory diseases were excluded from our study. In addition, patients with cirrhosis were also excluded as we believe that in cirrhosis cell turnover is rapid and may result in higher levels of soluble E-cadherin. This has been confirmed in our unpublished data. However, it is also important to identify other conditions that may significantly affect soluble E-cadherin concentrations.

The present study confirmed the observation that concentrations of soluble E-cadherin in patients with gastric cancer were higher than those in healthy subjects. However, higher soluble E-cadherin concentrations were observed both in our healthy subjects and patients compared with those reported in the literature, with a mean value of 5616 ng/ml versus 2515 ng/ml in healthy subjects, and 9344 ng/ml versus 4735 ng/ml in patients with gastric carcinoma.⁹ Only Chinese subjects were recruited to the study because we do not know if racial differences have any effect on soluble E-cadherin concentrations. Differences in biological behaviour in gastric cancer between Japan and the Western world have been suggested and may account for the observed differences in prognosis.³⁴ Therefore, higher E-cadherin concentrations in our patients and normal controls could be due to racial differences. In addition, the fact that most of our patients had advanced disease may also explain in part the high concentrations of soluble E-cadherin. Consequently, each laboratory should have its own reference range.

Our results showed that soluble E-cadherin concentrations were elevated in patients receiving palliative/conservative treatment and were correlated with the size of the gastric tumour. Patients with soluble E-cadherin concentrations higher than the 7025 ng/ml cut off value were more likely to have non-curative resection, possibly due to T4 invasion. In addition, these patients were more likely to have stage III or IV disease, although this was not statistically significant. However, this observation was biased by the fact that a large number of patients with advanced disease were inoperable and therefore their diseases could not be staged thus rendering the results less significant. Tumour size, depth of tumour invasion, and operability are important prognostic factors in patients with gastric carcinoma. Tumour size has been reported as a simple prognostic indicator for gastric carcinoma.³⁵ Soluble E-cadherin may originate from the rapid turnover of tumour cells. Therefore, the bigger the tumour size, the higher the soluble E-cadherin concentration. The increase in relative risk of metastases in patients with higher soluble E-cadherin concentrations reflected the role of E-cadherin as an “invasion suppressor molecule”.^{5 36}

Our data showed that serum soluble E-cadherin correlated with poor prognostic markers. High serum soluble E-cadherin predicted T4 invasion and palliative/conservative treatment. However, it was only elevated in a subgroup of patients. Therefore, further studies should aim at identifying the subgroup of gastric cancer patients who have elevated soluble E-cadherin concentrations, thereby increasing its sensitivity, comparing the prognostic value of soluble E-cadherin and conventional markers such as CEA in patients with gastric cancer, and also identifying other conditions that may affect soluble E-cadherin concentrations. In addition, prospective studies should also be carried out to investigate post-treatment soluble E-cadherin levels and

their role in therapeutic monitoring in patients with gastric cancer.

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