

Gastric enzymes as a screening test for gastric cancer

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SUMMARY Levels of lactic dehydrogenase and beta-glucuronidase were measured in gastric wash samples obtained from 445 patients over 40 years old attending a routine diagnostic endoscopy clinic. An index was derived from the two levels and used as a test for the presence of gastric cancer. Of the 24 patients with an endoscopic diagnosis of gastric cancer, 21 (91.3%) proved positive on this test, including all four cases of early gastric cancer, which were found at repeat endoscopy. The specificity of the test for gastric cancer was 81.3%. Among those patients with false positive results who had endoscopic biopsy were four out of the five cases of severe dysplasia, and four out of the 13 cases of type 2B intestinal metaplasia. Atrophic gastritis alone or with intestinal metaplasia was found in 95.9% of the false positives who were biopsied.

Gastric cancer, the fourth most common malignancy in this country, has an appalling prognosis (4.7% five year survival in the UK¹). Earlier diagnosis at the stage of 'early gastric cancer' (carcinoma in which the depth of invasion is limited to the submucosal layer of the stomach on histological examination²) has a much improved survival (70.4% at five years¹). This has led to considerable interest in detecting early gastric cancer and any possible 'pre-malignant' gastric mucosal changes such as severe dysplasia and type 2B intestinal metaplasia (IM). Some means of improving the detection rate at routine endoscopy is thus desirable.

Confirming earlier studies,^{3,4} a recent report⁵ showed that the levels of the enzymes lactic dehydrogenase and beta-glucuronidase in gastric juice were raised in patients with gastric cancer, including early gastric cancer. The study population was, however, a highly selected group of patients before surgery with a high proportion of cancer cases (42 out of 113 tested).

We have extended our study to an unselected group consisting of those patients presenting for routine diagnostic upper gastrointestinal endoscopy over a period of two years. The aim of the study was to assess if measurement of intragastric lactic

dehydrogenase and beta-glucuronidase might prove a useful adjunct to upper gastrointestinal endoscopy in detecting both early cancers and pre-malignant mucosal changes.

Methods

PATIENTS

The study population consisted of a prospective group of all patients over the age of 40 years presenting for routine diagnostic upper gastrointestinal endoscopy to our clinic. The presence of blood or food in the stomach was the only exclusion.

ENDOSCOPY

During the endoscopy, resting juice was aspirated and discarded. Before entering the duodenum (to minimise duodenogastric reflux), 100 ml Sorensen's buffer (67 mmol phosphate pH 7.5) containing 4 g/l xylose, warmed to body temperature, was sprayed *via* the biopsy channel of the endoscope upon the antral mucosa. The buffer ran down over the mucosa, washing it, bearing away the adherent juice and pooling in the fundus, where it was collected by aspiration during retroversion of the endoscope. This procedure added only a short time to the duration of the endoscopy.

Patients were grouped according to the endoscopic diagnosis to enable between group comparisons of enzyme level. The groupings used were: (1) Normal

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endoscopy, (2) Oesophagitis or oesophageal ulceration, (3) Duodenitis or duodenal ulceration, (4) Acute erosive gastritis (usually NSAID related), (5) Chronic gastritis, (6) Gastric ulceration, (7) Isolated gastric polyp, (8) Gastric carcinoma.

Chronic gastritis (5) was recognised by erythema, granularity, superficial ulceration or haemorrhage, chronic erosions, mucosal atrophy, visible areae gastricae or intestinal metaplasia.

HISTOLOGY

An average of three biopsies were taken of any suspicious lesion or area of gastritis and examined by routine histology. Patients were also grouped on the basis of the worst histological feature present. The groupings were: (1) No biopsy, (2) Normal histology, (3) Superficial gastritis, (4) Atrophic gastritis, (5) Gastritis with intestinal metaplasia, (6) Adenocarcinoma.

The 'no biopsy' group either had endoscopically normal stomachs or mild gastritis and other pathology to explain their symptoms, hence no biopsy was taken.

Histological sections from 83 consecutive patients without carcinoma undergoing biopsy were also examined after staining by the high-iron diamine/Alcian blue method⁶ for the presence of sialomucin and sulphomucin containing intestinal metaplasia.

ENZYMES

The gastric washings were analysed for lactic dehydrogenase (after the method of Henry⁷ adapted for multiple analysis) and for beta-glucuronidase (Sigma kit 325), within one week, having been stored in small aliquots at -60°C .

STATISTICAL ANALYSIS

One way analysis of variance was used to assess between group differences in enzyme level utilising data transformed to the cubed root to normalise the distribution.

Results

There were 30 cases of obvious advanced gastric cancer which were excluded because of the presence of large amounts of blood or food in the stomach. The mean age of the 445 patients (215 men) who were tested was 67.1 years (SD 11.6). The levels of both lactic dehydrogenase and beta-glucuronidase showed a marked positive skew in their distributions. In order to normalise distributions and also to make results comparable with those in the earlier study,⁵ the sum of the cubed root of the lactic dehydrogenase level in IU/l, and the cubed root of the beta-glucuronidase level in IU/ml, was calculated to form an index which could be used as a test for gastric cancer. The mean index for patients with endoscopically normal stomachs ($n=131$) was 3.4 (SD 1.6). A level of 5.8 and greater was taken as positive, this figure representing 93% of a normal distribution (mean plus 1.5 times standard deviation) based on those patients with endoscopically normal stomachs (normal endoscopy, oesophageal or duodenal pathology).

ENDOSCOPIC DIAGNOSIS

The number of patients, and the positive test results – that is, an index greater than or equal to 5.8, for each endoscopic group are seen in Table 1. Of the 24 carcinoma cases, 21 (87.5%) were positive but one of

Table 1 Endoscopic diagnosis

	NO	OE	DU	AG	CG	GU	PO	CA																																				
No.	62	31	38	12	190	81	7	24*																																				
Age	64.0	64.1	63.3	71.1	67.8	67.6	78.1†	71.8†																																				
SD	13.5	10.8	10.1	9.5	11.5	10.9	7.4	10.1																																				
Positive	8	1	1	0	48	18	3	21																																				
% pos.	12.9	3.2	2.6	0	25.3	22.2	42.9	87.5																																				
Index	3.918	3.246	2.692	3.107	4.412	4.261	4.711	7.459																																				
SD	1.739	1.475	1.259	1.414	2.042	1.698	2.152	2.351																																				
	$F_{437} 15.8; p<0.01$																																											
Between group differences:	<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:25%;"></td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> <td>p<0.05</td> <td></td> <td></td> <td></td> <td>p<0.05</td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> <td></td> <td>p<0.05</td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td>p<0.05</td> <td></td> <td></td> <td></td> <td>p<0.05</td> <td></td> <td></td> <td></td> </tr> </table>																					p<0.05				p<0.05						p<0.05					p<0.05				p<0.05			
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Number of patients and mean age and SD, number with index ≥ 5.8 and % of group positive, mean index and SD, and analysis of variance, for patients grouped according to endoscopic diagnosis. (NO normal; OE oesophageal pathology; DU duodenal pathology; AG acute erosive gastritis; CG chronic gastritis; GU gastric ulcer; PO isolated polyp; CA gastric carcinoma.)

*Includes one case of pancreatic cancer mistakenly diagnosed as gastric cancer; †Significantly different from the other groups ($p<0.01$).

Table 2 with analysis of variance. The mean index in the carcinoma group was significantly higher than all other groups. The mean index in the intestinal metaplasia group was significantly higher than that in the no biopsy, normal and superficial gastritis groups. The mean index in the atrophic gastritis group was significantly higher than that in the no biopsy and superficial gastritis groups.

MUCIN HISTOCHEMISTRY

Biopsies from 83 consecutive patients were classified according to the mucin histochemistry. The histological findings on these biopsies were normal (nine), superficial gastritis (12), chronic gastritis (62) and intestinal metaplasia. Most cases of sulphomucin bearing intestinal metaplasia also had sialomucin but were grouped separately. Forty one sections showed neither sialomucin nor sulphomucin intestinal metaplasia and of these, seven cases (17%) were associated with a positive index. Twenty nine sections showed only sialomucin intestinal metaplasia and of these, three cases (10.3%) had positive enzymes. Thirteen sections showed sulphomucin intestinal metaplasia and of these, four cases (30.8%) had positive enzymes.

Discussion

Gastric wash enzymes were used as a screening test for gastric cancer in a general endoscopy population. The test is quick to do and does not add further to the inconvenience suffered by the patient from the endoscopy. Furthermore the enzyme assay method is simple, cheap, and already available in many laboratories, unlike other proposed markers of gastric malignancy such as foetal sulphoglycoprotein antigen (FSA).⁸ Unfortunately there are no satisfactory serum markers that could be used in screening for gastric cancer.

Not all cases of gastric cancer were positive and the sensitivity of the test was 91.3%. All four cases of early gastric cancer, and one case of linitus plastica, however, were positive. While the specificity of the test (81.3%) was low, this has to be viewed in terms of screening rather than diagnosis. The predictive power of the test (72%) is higher than that of FSA (70%).⁸ The possibility that an early gastric cancer was missed may account for some of the false positives. Certainly four of our patients who had positive enzymes were subjected to repeat endoscopy and were subsequently found to have recognisable early gastric cancer.

We have shown that 95.9% of the false positives had some degree of atrophic gastritis with or without

intestinal metaplasia. There is growing acceptance of the concept of intestinal metaplasia, especially the sulphomucin variant, as a premalignant lesion:⁹ four of the 13 cases in our study with sulphomucin intestinal metaplasia were positive on this test. A more important premalignant lesion in the stomach is severe dysplasia,¹⁰ and four of the five cases with severe dysplasia in our study were positive. An earlier study¹¹ found severe dysplasia in three out of 17 false positives, but not at all in 17 matched negatives. In the same study, sulphomucin intestinal metaplasia was found in eight of 17 false positives but only one of 17 matched negatives.

These findings suggest that the false positive group may be at increased risk of developing gastric cancer in the future. We propose to follow up this false positive group with periodic endoscopic surveillance, together with a matched group of negatives, when the true specificity of this test may increase.

In conclusion, the measurement of the enzymes lactic dehydrogenase and beta-glucuronidase in gastric wash samples could be a useful screening test for early gastric cancer in an endoscopy population.

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