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Morphometrical Analysis of Structural Abnormality of Tubular Tumors of the Stomach with Image Processing

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Four indices, index of tubular density, index of dispersion of tubular size, degree of complexity of tubular shape and frequency of complex-shaped tubuli, were defined to quantify the structural abnormalities of gastric tumors in morphometrical analysis by image processing. The values of each index corresponded well with the degree of each structural abnormality found pathologically. These indices were considered to be valid for representing their respective structural factors. There were significant differences among the mean values of the scores calculated by a formula using all 4 indices of benign tubular adenoma, borderline lesion and well-differentiated type tubular adenocarcinoma ($P<0.05$). Therefore, the discriminant formula was considered to be valid for integrating these indices and for representing structural abnormality of gastric tubular tumors.

Key words: Gastric tumor — Morphometry — Structural abnormality

Histological differential diagnosis between malignant and benign lesions of gastric tumors is based on so-called pattern recognition of the complex histological picture. In order to develop standardized histological criteria, the structural and cellular features to be evaluated must be quantified. There have been many studies quantifying structural and cellular abnormalities in many organs.[1–13] Jarvis and Whitehead[8] and Tosi *et al.*[12,13] morphometrically studied gastric dysplasia using stereology. However, stereology cannot analyze irregularities in shape and size of tubuli. Moreover, theoretically it cannot be applied in the cases of gastric tumors, because their tubular structure has polarity and does not have a three-dimensionally random structure.[14,15] Three-dimensional reconstruction study was reported to be useful for discriminating structural change in dysplasia from that in carcinoma of the stomach.[16,17] However, it is not generally applicable because it is very time-consuming.

In the present morphomertrical study by the use of a computer image analyzer, four indices defined to quantify structural factors were assessed for validity. They were also integrated by applying a discriminant function in order to represent the overall structural abnormalities.

MATERIALS AND METHODS

Materials Ninety-five surgically resected gastric tubular tumors of intestinal type were examined histologically. According to histopathological criteria differentiating malignant from benign lesions in The General Rules for the Gastric Cancer Study,[18] these cases were diagnosed as follows; 18 cases of benign tubular adenoma, 24 cases of borderline lesion, 53 cases of tubular adenocarcinoma, well-differentiated type (36 cases were intramucosal and 17 cases were submucosally infiltrative). Intestinal metaplastic mucosae of 16 cases were studied as controls. The typical histological picture of each group is exemplified in Fig. 1a–1c.

Sampling for morphometrical measurement Mucosal lesions were selected for examination by excluding submucosally invasive lesions of infiltrative carcinoma, because the structural abnormalities of invasive lesions were quite different from those of the mucosal lesions. Tubuli which appeared to be destroyed or deformed due to the preparation process of the specimens were also excluded from morphometrical analysis. Two or three histological images magnified 40 times (objective 10 times and TV camera 4 times) were sampled at random in each case. The histological images observed with a microscope (Olympus, New Vanox-S) were input to a color image analyzer (Olympus CIA system) via a TV camera (Ikegami ITC-370M) (Fig. 2a). The outlines of tubuli were traced manually with a digitizer (Fig. 2b). The processed images of the tubuli were revealed with the image analyzer (Fig. 2c).

Area, perimeter, maximum diameter and maximum width were calculated for each of the tubuli showing full outlines within the margin of the TV monitor.

Index of tubular density (ITD): ITD, defined as the area ratio of tubuli to (tubuli+stroma), was calculated as follows[7]: ITD = (area of tubuli/area of (tubuli + stroma)) in an image magnified 40 times × 100.

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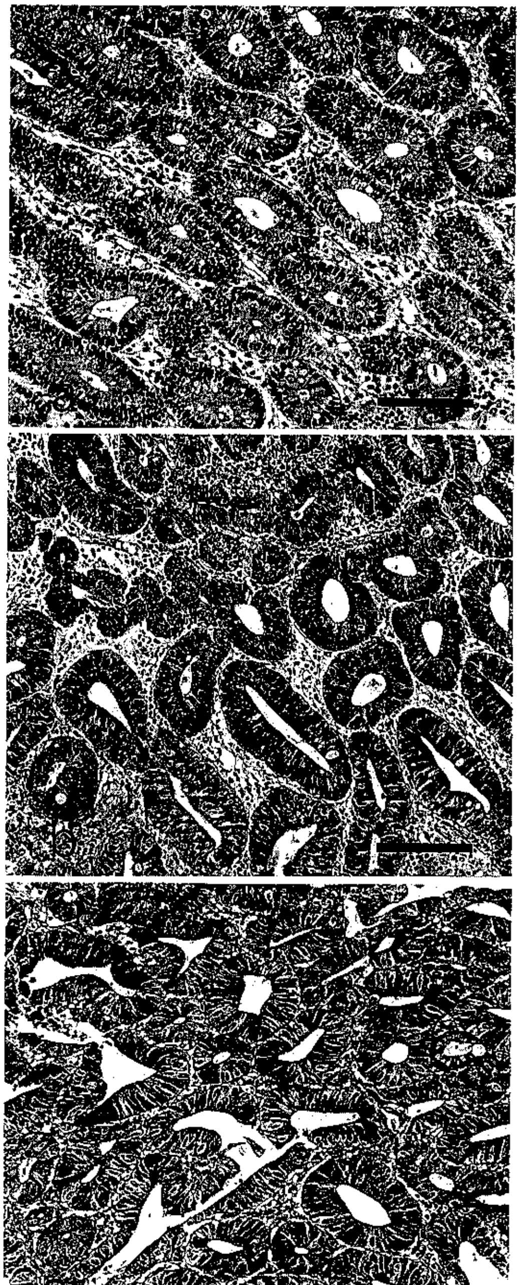

Fig. 1. Histological pictures of tubular tumors of the stomach. a: Benign tubular adenoma (H-E staining, ×160, bar; 100 μm). Spindle-shaped nuclei are regularly arranged on the basement membrane side. Many tubuli are of round or oval shape and of almost constant size. b: Borderline lesion (H-E staining, ×160, bar; 100 μm). Nuclei show moderate pleomorphism and moderately irregular arrangement. Nucleoli are relatively prominent. Tubuli are of irregular size and some of them are of irregular shape. c: Well-differentiated type tubular adenocarcinoma (H-E staining, ×160, bar; 100 μm). Nuclei show marked pleomorphism and markedly irregular arrangement. Nucleoli are prominent. Tubuli are of irregular size and many of them are of irregular shape.

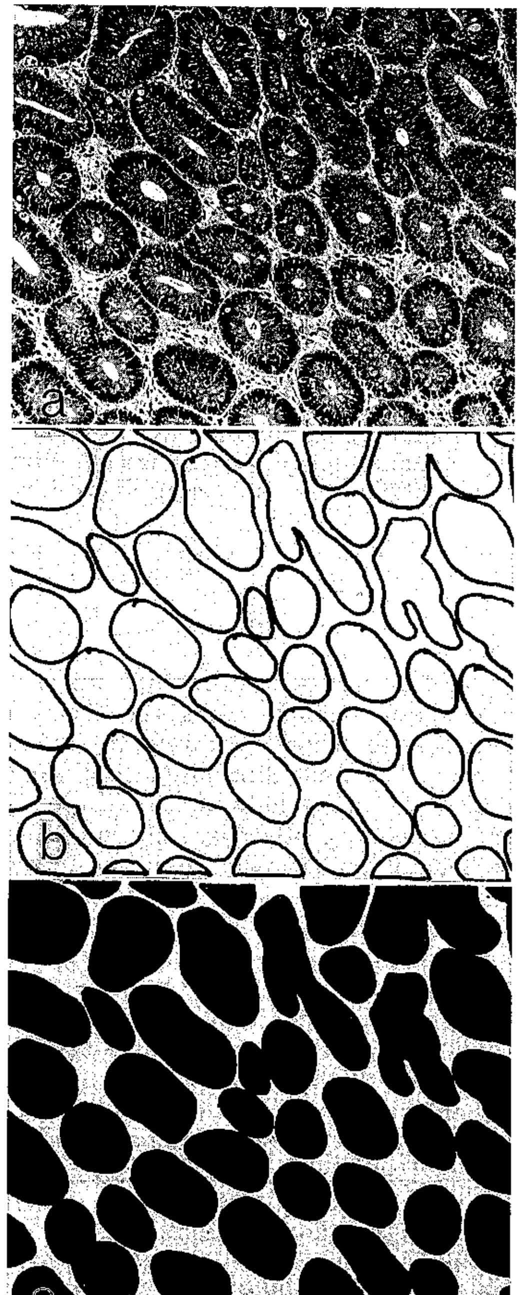

Fig. 2. Original histological picture (×205 by original magnification) (a), outline of tubuli drawn by hand on the image analyzer (b) and figures of tubuli processed with image processing (c).

Index of dispersion of tubular size (IDS): IDS was defined and calculated as follows; IDS=(standard deviation of maximum width of tubuli/average of maximum width of tubuli) in an image magnified 40 times ×100.

Degree of complexity of tubular shape (DCT): DCT was defined and calculated as follows; DCT=round factor of a measured tubulus/round factor of a corresponding oval with the same aspect ratio as that of the measured tubulus.

Frequency of complex shaped tubuli (FCT): FCT was defined and calculated as follows; FCT=(number of tubuli showing DCT value of 1.5 or higher/total number of tubuli) in an image magnified 40 times ×100.

Statistical discrimination of the structural abnormalities of gastric tumors A linear discriminant function employing Mahalanobis' generalized distance was used to discriminate the structural abnormalities of well-differentiated type tubular adenocarcinoma from those of benign tubular adenoma.

Statistical analysis Student's t test was performed for each index of ITD, IDS and FCT on the mean values of the groups classified according to pathological diagnosis, in order to find statistically significant differences between these groups ($P<0.05$, in a one-sided test).[19] The linear discriminant function was calculated using Statistical Library Software (IBC Corporation, Miyazaki).

RESULTS

The errors of measurement of area, perimeter, maximum diameter and maximum width were examined by calculating variability in terms of coefficient of variation. The variability that might be produced in the input process of the histological images was found to be less than 5%. This is within a permissible range.

The mean ITD value of each group tended to increase with the severity of histological abnormalities (Table I). There were significant differences between the control and benign tubular adenoma and between benign tubular adenoma and intramucosal well-differentiated type tubular adenocarcinoma. On the other hand, no significant differences were observed between benign tubular adenoma and borderline lesion, between borderline lesion and intramucosal well-differentiated type tubular adenocarcinoma, and between intramucosal and submucosally infiltrative well-differentiated type tubular adenocarcinomas.

Figure 3 shows processed images of tubuli in four cases, showing different degrees of irregularity in tubular size. The most marked irregularity was found in Case 4 and Cases 3, 2 and 1 showed milder irregularity in that order. The IDS value for each was as follows: 29.13 in Case 1, 55.44 in Case 2, 75.31 in Case 3 and 93.12 in Case 4. These values corresponded well to the degree of irregularity in tubular size which we observed pathologically.

As shown in Table II, the mean IDS value increased with the severity of abnormalities. There were significant differences between the control and benign tubular adenoma, between benign tubular adenoma and borderline

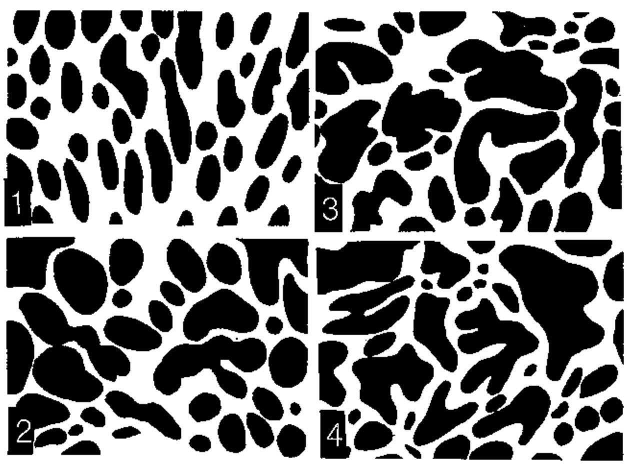

Fig. 3. Processed images of tubuli in Cases 1–4 showing different degrees of irregularity in tubular size. IDS value is 29.13 in Case 1, 55.44 in Case 2, 75.31 in Case 3 and 93.12 in Case 4.

Table I. ITD Values

Histological diagnosis	Mean±SD
Control	55.7±11.8[a)]
Benign tubular adenoma	69.4±7.2[b)]
Borderline lesion	71.4±8.8[c)]
Well-differentiated type tubular adenocarcinoma	
Intramucosal	73.9±8.7[d)]
Submucosally infiltrative	74.8±7.8[e)]

$P<0.05$: Student's t test [significant: *a)*:*b)*, *b)*:*d)*; not significant: *b)*:*c)*, *c)*:*d)*, *d)*:*e)*].

Table II. IDS Values

Histological diagnosis	Mean±SD
Control	26.2±7.3[a)]
Benign tubular adenoma	38.2±10.9[b)]
Borderline lesion	48.0±13.1[c)]
Well-differentiated type tubular adenocarcinoma	
Intramucosal	57.6±15.1[d)]
Submucosally infiltrative	58.7±16.0[e)]

$P<0.05$: Student's t test [significant: *a)*:*b)*, *b)*:*c)*, *c)*:*d)*; not significant: *d)*:*e)*].

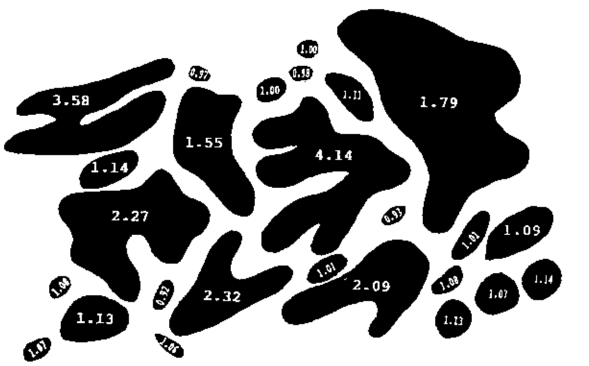

Fig. 4. DCT values in Case 4 of Fig. 3. DCT values are shown by the figures in the tubuli. Seven out of 26 tubuli show DCT values of 1.5 or higher. FCT value can be calculated to be 26.92 (7/26×100).

Table III. FCT Values

Histological diagnosis	Mean±SD
Control	6.7±7.2[a)]
Benign tubular adenoma	8.0±7.2[b)]
Borderline lesion	13.7±9.7[c)]
Well-differentiated type tubular adenocarcinoma	
Intramucosal	15.6±9.5[d)]
Submucosally infiltrative	20.2±14.1[e)]

$P<0.05$: Student's t test [significant: *b*):*c*), *d*):*e*); not significant: *a*):*b*), *c*):*d*)].

Table IV. Discrimination of the Structural Abnormality of the Gastric Tumors

Histological diagnosis	Discrimination		Total
	BTA	WDTA	
BTA	42 (79%)	11 (21%)	53 (100%)
BL	40 (46%)	47 (54%)	87 (100%)
WDTA	45 (29%)	107 (71%)	152 (100%)
Intramucosal	33 (30%)	77 (70%)	110
Submucosally infiltrative	12 (29%)	30 (71%)	42

BTA, benign tubular adenoma; BL, borderline lesion; WDTA, well-differentiated type tubular adenocarcinoma.

Table V. Discriminant Score of Each Group

Histological diagnosis	Discriminant scores (mean±SD)
Control	−1.844±0.537[a)]
Benign tubular adenoma	−0.912±0.802[b)]
Borderline lesion	−0.172±1.360[c)]
Well-differentiated type tubular adenocarcinoma	
Intramucosal	0.258±1.061[d)]
Submucosally infiltrative	0.387±1.050[e)]

$P<0.05$: Student's t test [significant: *a*):*b*), *b*):*c*), *c*):*d*); not significant: *d*):*e*)].

lesion, and between borderline lesion and intramucosal well-differentiated type tubular adenocarcinoma. On the other hand, there was no significant difference between intramucosal and submucosally infiltrative well-differentiated type tubular adenocarcinomas.

Figure 4 shows an image of tubuli from Case 4. The DCT value of each tubulus is also shown. DCT values of the circles and the ovals were almost 1.0. DCT values of complex tubuli were 1.5 or higher. The DCT values corresponded well to the degree of irregularity in tubular shape.

The mean values of FCT tended to increase with the severity of abnormalities (Table III). There were statistically significant differences in FCT values between benign tubular adenoma and borderline lesion and between intramucosal and infiltrative well-differentiated type tubular adenocarcinomas, but there were no significant differences between the control and benign tubular adenoma and between borderline lesion and intramucosal well-differentiated type tubular adenocarcinoma.

The discriminant formula and critical value to discriminate structural abnormalities of well-differentiated type tubular adenocarcinoma from those of benign tubular adenoma were obtained as follows:

Discriminant score

$$=0.0165067\times[\mathrm{ITD}]+0.0538823\times[\mathrm{IDS}]+0.0350919\times[\mathrm{FCT}]-4.38342$$

Critical value: -0.296867

As shown in Table IV, 42 lesions (79%) in benign tubular adenoma and 107 lesions (71%) in well-differentiated type tubular adenocarcinoma were discriminated correctly. In total, 73% (149 out of 205 lesions) of benign tubular adenoma and well-differentiated type tubular adenocarcinoma was discriminated correctly. Forty (46%) of the borderline lesions were classified into the benign tubular adenoma group and 47 (54%) were classified into the well-differentiated type tubular adenocarcinoma group.

The discriminant score of each group is demonstrated in Table V. There are significant differences of discriminant scores between the control and benign tubular adenoma, between benign tubular adenoma and borderline

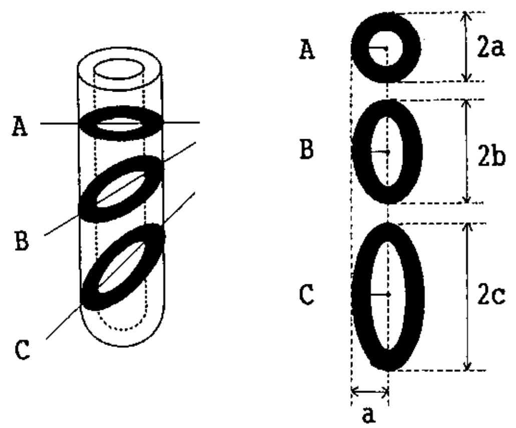

Fig. 5. Variation of histological figures of a tubulus depending on section. A is a figure sectioned vertically, B is that sectioned obliquely and C is that sectioned more obliquely.

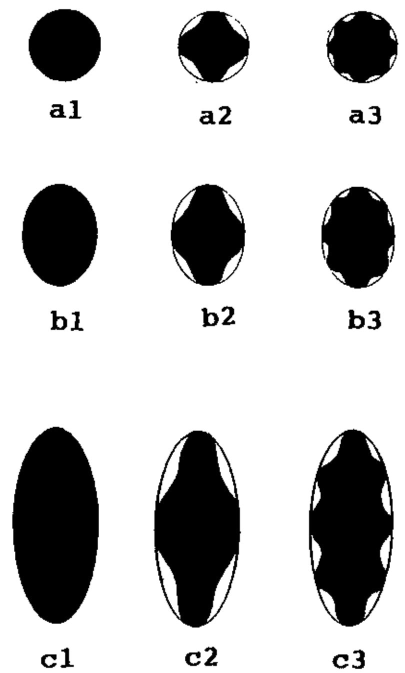

Fig. 6. Variation of irregular tubular shape obtained by three kinds of section; a1, a2, a3 obtained when tubuli are cut vertically, b1, b2 and b3 obtained when they are cut obliquely. c1, c2 and c3 obtained when they are cut more obliquely. a1, b1 and c1 are regular, a2, b2 and c2 are more irregular and a3, b3 and c3 are the most irregular in shape.

lesion and between borderline lesion and well-differentiated type tubular adenocarcinoma. On the other hand, there is no significant difference of discriminant scores between intramucosal and submucosally infiltrative well-differentiated type tubular adenocarcinomas.

DISCUSSION

When pathologists assess gastric structural abnormalities, they first observe many tubular factors, such as tubular density, irregularity of tubular shape and size and back-to-back arrangement, and then integrate these factors to evaluate the tubular abnormalities. These factors must be quantified and integrated statistically in order to assess the structural abnormalities objectively.

The present authors reported that ITD was useful for representing tubular density.[7] However, ITD alone was not sufficient to express the overall structural abnormalities. No other formulae have been reported that can represent the irregularity of size and complexity of shape as successfully as our IDS, DCT and FCT.

There are various measurements that can express tubular size, such as area, perimeter, maximum diameter and maximum width. However, the measurement results of the tubular cross sections on the histological specimen depend on how these tubuli are cut during the preparation of a specimen. Figure 5 shows various cut surfaces of a tubulus, assuming the tubulus is a cylinder. If the cylinder is cut vertically, a circular section can be obtained (A). If the cylinder is cut obliquely, an oval section can be obtained (B and C). Area, perimeter and maximum diameter can vary markedly depending on how the cylinder was cut, even though the size of cylinder is constant. On the other hand, maximum width is constant and independent of section. Therefore, maximum width could be considered the best parameter representing tubular size in histological specimens.

Standard deviation was used to represent irregularity of nuclear size in some studies.[1, 2, 12, 13] However, standard deviation greatly depends on the size of the mean value. The coefficient of variation (standard deviation/mean value $\times$ 100) is statistically useful for examining dispersion between samples showing different mean values.[19] Therefore, in the present study coefficient of variation was used instead of standard deviation to represent irregularity of tubular size. From the results shown in Fig. 3 and Table II, IDS can be considered a useful index to represent irregularity of tubular size, and corresponds well to the severity of abnormalities.

Circular or oval shaped tubuli are not considered to be complex in shape. On the other hand, a tubulus whose shape deviates from a circle or an oval is considered to be complex in shape (Figs. 4 and 6). Round factor ((perimeter)^2/(area$\times 4\pi$)) is useful for quantifying the deviation from a circle.[20] However, it is not useful to quantify the complexity of a tubular shape because it increases not only with the complexity of the tubular shape but also

Table VI. Relation between Tubular Irregularity, Aspect Ratio, Round Factor and DCT

No. of tubulus	Degree of irregularity	Aspect ratio	Round factor	DCT
a1		1.00	1.00	1.00
b1	−	1.50	1.07	1.00
c1		2.50	1.35	1.00
a2		1.00	1.33	1.33
b2	+	1.50	1.40	1.32
c2		2.50	1.82	1.34
a3		1.00	1.40	1.40
b3	++	1.50	1.48	1.39
c3		2.50	1.91	1.42

with the aspect ratio of the tubulus. Our DCT avoids the dependency on the aspect ratio. As shown in Fig. 6 and Table VI, aspect ratio and round factor did not correspond to the irregularity of tubulus, while DCT corresponded well to the irregularity of tubular shape. As shown in Fig. 4, tubuli which were histologically regarded as regular in shape showed DCT values below 1.5, and tubuli which were regarded as complex in shape showed values of 1.5 or higher. We therefore considered a DCT value of 1.5 or higher to indicate complexity of tubular shape. FCT values calculated using this criteria were found to correspond well to the severity of abnormalities (Table III).

Though these indices are useful for representing the respective structural factors, it is impossible to classify gastric tumors using only one index. These indices must be integrated statistically by multivariate analysis. The discriminant function is a method for integrating multivariate data and for classifying items into groups.[21] In this study the discriminant function was employed in order to integrate these structural factors and to discriminate the structural abnormality in benign tubular adenoma from that in well-differentiated type adenocarcinoma.

As shown in Table IV, 79% of lesions in benign tubular adenoma and 71% of lesions in well-differentiated type tubular adenocarcinoma were discriminated correctly by the discriminant scores calculated from ITD, IDS and FCT values. Moreover, there were significant differences of the mean values of the discriminant scores in benign tubular adenoma, borderline lesion and well-differentiated type tubular adenocarcinoma ($P<0.05$). Therefore, the integration of these indices can be considered to be useful for representing the overall structural abnormalities of the gastric tubular tumors.

We conclude from the present study that ITD, IDS, DCT and FCT are useful for representing structural factors and that the integration of these indices can represent the overall structural abnormality.

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