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# Morphometrical Analysis of Nuclear Abnormality of Tubular Tumors of the Stomach with Image Processing

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**Nuclear size and shape on histological specimens were used to quantify nuclear abnormality in gastric tubular tumors. A valid technique for this morphometrical analysis by image processing was developed. Furthermore, the present study examined the nuclear size and shape to determine if they were reliable criteria to be used for differential diagnosis. Nuclear area, maximum diameter and maximum width on the histological specimens were defined as factors to represent nuclear size. Nuclear aspect ratio represented nuclear shape. Histological features revealed varied degrees of condensation of nuclei; this was due to different protocols for preparing the histological specimens. This finding indicated that the size of intermediate lymphocytes on the same specimens should be measured as a control in order to estimate the degree of nuclear condensation. The present study also demonstrated that at least 200 nuclei should be measured on the histological specimens to obtain accurate nuclear size and shape. Histologically, the nuclei in benign tubular adenoma and borderline lesions tended to be spindle-shaped, while those in well-differentiated type tubular adenocarcinoma tended to be round and swollen. The nuclei in borderline lesions were larger than those in benign tubular adenoma and smaller than those in well-differentiated type tubular adenocarcinoma. Our results for the above 4 factors corresponded well with these histological findings. The nuclear size and shape on histological specimens were concluded to be reliable criteria for assessing nuclear abnormality in gastric tubular tumors and useful ones for differential diagnosis.**

Key words: Gastric tumor — Morphometry — Nuclear abnormality

We reported that N/C ratio, tubular density, irregularity of tubular shape and irregularity of tubular size in tubular tumors of the stomach could be quantified with an image analyzer. Furthermore, 81% of benign tubular adenoma and well-differentiated type adenocarcinoma lesions could be classified correctly into benign and malignant status, respectively, with these factors.[1-3] In order to make a more reliable differential diagnosis, nuclear abnormalities must be quantified.

Quantification of nuclear abnormalities in various organs on histological specimens has been reported.[4-14] However, the measurement method was not sufficiently studied in those papers. For example, nuclear condensation due to preparation of the specimens was not fully discussed and the number of nuclei that must be measured for reliable quantification was not studied.

In the present study, we first examined the nuclear condensation caused by various protocols for preparing the histological specimens. An appropriate number of nuclei for measurement on the histological specimens was determined by analyzing data obtained from different total numbers of measured nuclei. We then examined whether nuclear size and shape in the histological specimens were useful to assess nuclear abnormalities and to classify gastric tubular tumors into benign tubular adenoma, borderline lesions and well-differentiated type tubular adenocarcinoma.

## MATERIALS AND METHODS

One hundred and thirty-one surgically resected cases were studied histopathologically. The following diagnosis was made according to the histopathological criteria in the General Rules for Gastric Cancer Study[15]: benign tubular adenoma in 20 cases; borderline lesions in 36 cases; and well-differentiated type tubular adenocarcinoma in 75 cases (intramucosal carcinoma in 55 cases and submucosally infiltrative carcinoma in 20 cases). Typical histological pictures of these 3 groups are demonstrated in Figs. 1, 2 and 3.

**Condensation of nuclei in the process of preparation** The following 3 factors in the process of preparing the specimens were evaluated: the duration from resection to fixation in formalin solution; the duration of fixation in formalin solution; and the concentration of formalin solution. The area of more than 250 lymphocytes was measured on each of the histological specimens prepared by various fixation protocols, in various sets of these 3 factors. The ratio of the measured area to the mean area was calculated for histological specimens prepared by means of the following protocol: the duration from resection to fixation, 0 h; the duration of fixation in formalin

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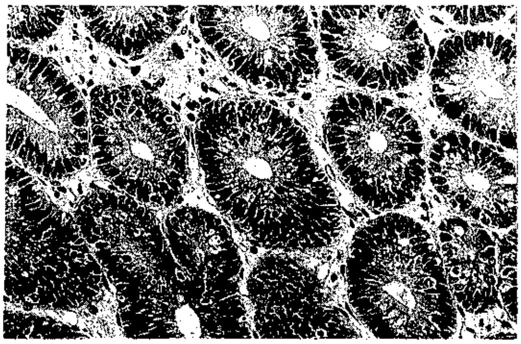

Fig. 1. Histological picture of benign tubular adenoma (H&E staining, ×330). The hyperchromatic nuclei are spindle-shaped and arranged regularly on the basement membrane side. Their size and shape are almost uniform.

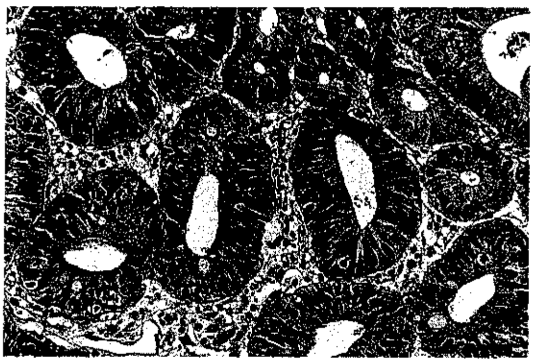

Fig. 2. Histological picture of borderline lesion (H&E staining, ×330). The nuclei are relatively large with relatively prominent nucleoli. Round nuclei can be observed among spindle-shaped nuclei. Some nuclei shift to the luminal side.

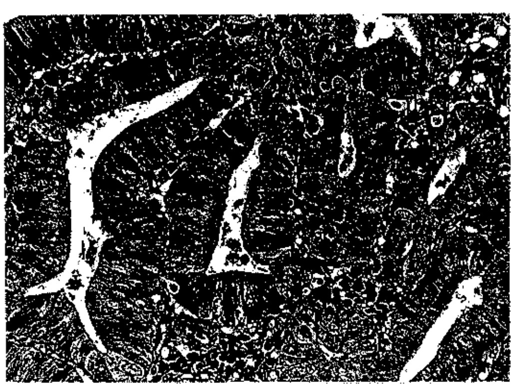

Fig. 3. Histological picture of well-differentiated type tubular adenocarcinoma (H&E staining, ×330). Nuclei are larger than those in Fig. 2 and round in shape with more prominent nucleoli than in Fig. 2 and with coarse chromatin pattern. The arrangement of nuclei is markedly irregular.

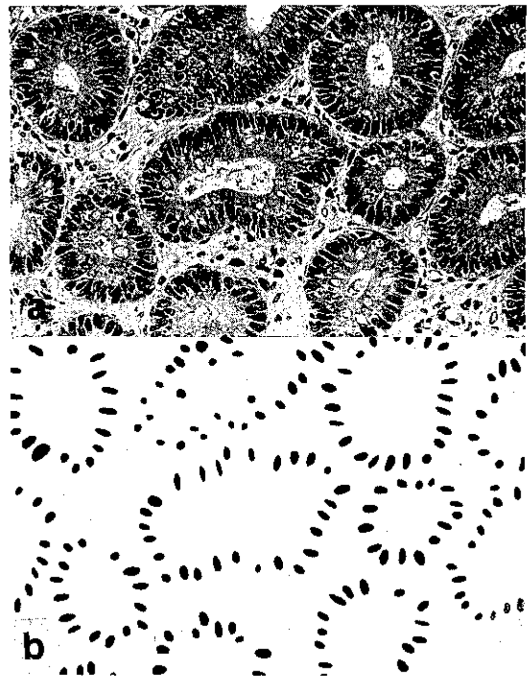


Fig. 4. Original picture of the tubular tumor of the stomach (H&E staining, ×330) (a) and figures of nuclei processed by image processing (b).

solution, 1 day; and the concentration of formalin solution, 20%.

**Examination of the number of measured nuclei** Seventy to 1,000 nuclei were selected at random for measurement from a 875×550 $\mu m^2$ sized image of a gastric tubular-tumor lesion. Area, perimeter, maximum diameter, maximum width and aspect ratio of each nucleus were measured and the mean value and the standard deviation were calculated.

**Measurement of nuclear size and shape** Paraffin-embedded tissue was cut in 2-$\mu$m-thick sections and stained with hematoxylin and eosin. Images sized 875×550 $\mu m^2$ were selected at random. The histological picture was input into the image analyzer (Olympus, SP-500) (Fig. 4a) and the outlines of nuclei were traced manually with digitizer on the TV monitor. Nuclear features were obtained by image processing (Fig. 4b). More than 200 nuclei per image were selected at random. The area, the maximum diameter, the maximum width and the aspect ratio of each nucleus were calculated. One image obtained with the image analyzer consisted of 245,760 (512×480) pixels and one pixel was 1.958 $\mu m^2$.

**Statistical analysis** Student's $t$ test was performed for analysis on the difference between the groups ($P<0.05$, in a one-sided test).

RESULTS

The condensation of nuclei and the fixation protocols are shown in Table I. The size of lymphocytes was not significantly different on the specimens prepared with 10% and 20% formalin solution. However, significant condensation was observed in those prepared with over-30% formalin solution. Significant condensation was observed on the specimens prepared by fixing materials more than 12 h after resection. The lymphocytes tended to become smaller with longer duration of fixation in formalin solution.

Table I. Condensation of Lymphocytes Caused by Various Protocols for Preparation of the Histological Specimens

| Concentration of formalin (%) | Duration from resection to fixation (h) | Duration of formalin fixation (day) | Size of lymphocytes ($\mu m^2$) |
|---|---|---|---|
| 10 | 0 | 1 | 19.63 (100.5%) |
| 20 | 〃 | 〃 | 19.53 (100.0%) |
| 30 | 〃 | 〃 | 18.70 (95.7%) |
| 100 | 〃 | 〃 | 16.86 (86.3%) |
| 20 | 6 | 1 | 19.02 (97.4%) |
| 〃 | 12 | 〃 | 17.21 (88.4%) |
| 〃 | 24 | 〃 | 17.13 (87.7%) |
| 〃 | 72 | 〃 | 16.06 (82.2%) |
| 20 | 0 | 3 | 19.12 (97.7%) |
| 〃 | 〃 | 5 | 18.40 (94.2%) |
| 〃 | 〃 | 7 | 18.35 (94.0%) |

(%): Ratio of the measured area to that obtained from the histological specimen prepared by the 20%; 0 h; and 1 day protocol.

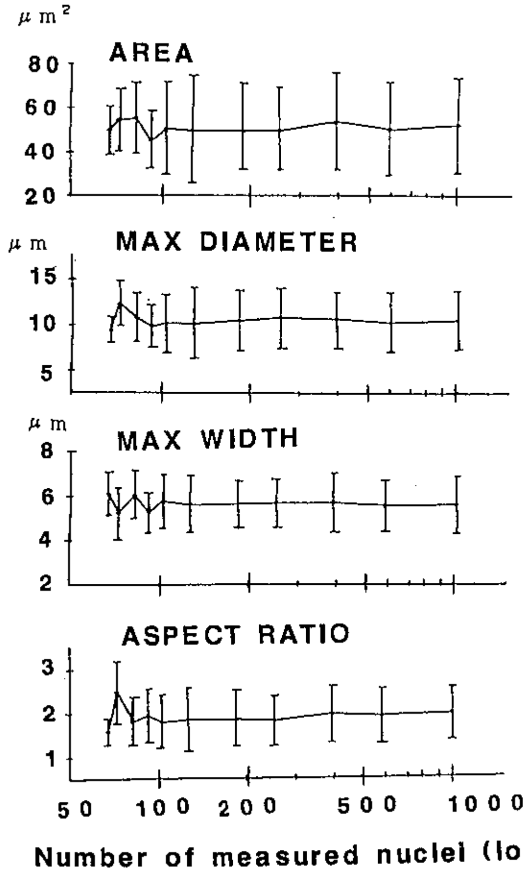


Fig. 6. Relation between the number of measured nuclei and the nuclear area, the maximum diameter, the maximum width and the aspect ratio. The transverse axis represents the number of measured nuclei and the perpendicular axis represents the nuclear area, the maximum diameter, the maximum width and the aspect ratio.

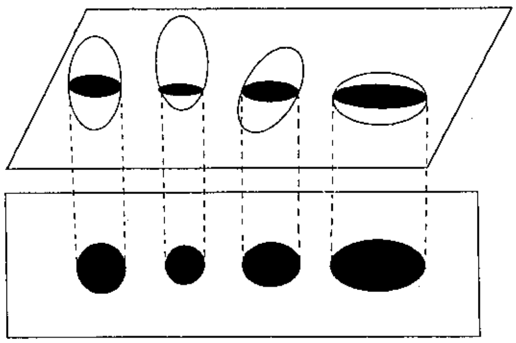

Fig. 5. Varied nuclear features dependent on section. The upper portion shows the three-dimensional distribution of 4 ovals and the bottom portion shows their two-dimensional figures. The shape and size of two-dimensional figures are different, though the 4 ovals are identical in size and shape.

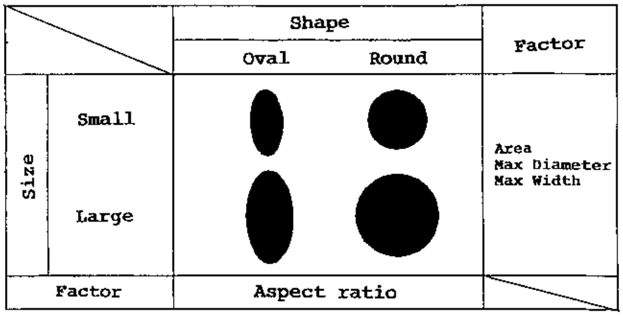


Fig. 7. Assessment of nuclear atypia. Nuclear atypia is assessed in terms of size and shape. The size can be represented by the area, the maximum diameter and the maximum width, and the shape can be represented by the aspect ratio.

The relation between three-dimensional and two-dimensional nuclear features is illustrated in Fig. 5. The upper portion shows the three-dimensional distribution of 4 ovals which are identical in size and shape, and the lower portion shows their two-dimensional features. These identical ovals appear different in size and shape in the two-dimensional representation. The relation between the number of measured nuclei and the area, the maximum diameter, the maximum width and the aspect ratio are demonstrated in Fig. 6. The mean value and standard deviation of each factor obtained from almost 200 nuclei were not significantly different from those obtained from 1,000 nuclei.

As shown Fig. 7, nuclear abnormality of gastric tubular tumors can be generally assessed with 2 factors: size (large or small) and shape (round or spindle-shaped). The area, the maximum diameter and the maximum width were taken as factors representing nuclear size, and the aspect ratio was taken as representing nuclear shape.

The nuclear area in each group of tubular tumors of the stomach is shown in Table II. There was no significant difference between the area of nuclei in intestinal metaplastic mucosa and that in benign tubular adenoma. There were significant differences between the area of nuclei of benign tubular adenoma and that in borderline lesions, and also between that in borderline lesions and in well-differentiated type tubular adenocarcinoma.

Table II. Area of Nuclei

| Histological diagnosis | Mean±SD ($\mu m^2$) |
|---|---|
| Control | 33.82±11.53[a)] |
| Benign tubular adenoma | 37.31±4.27[b)] |
| Borderline lesion | 44.70±8.14[c)] |
| Tubular adenocarcinoma, well-differentiated type | 52.26±11.53[d)] |

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

Table III. Maximum Diameter of Nuclei

| Histological diagnosis | Mean±SD ($\mu$m) |
|---|---|
| Control | 7.64±2.01[a)] |
| Benign tubular adenoma | 8.70±0.67[b)] |
| Borderline lesion | 9.84±1.37[c)] |
| Tubular adenocarcinoma, well-differentiated type | 9.90±1.23[d)] |

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

The maximum diameter of nuclei in each group is shown in Table III. The maximum diameter became larger in the order of intestinal metaplastic mucosa,

Table IV. Maximum Width of Nuclei

| Histological diagnosis | Mean±SD ($\mu$m) |
|---|---|
| Control | 5.11±0.76[a)] |
| Benign tubular adenoma | 4.98±0.41[b)] |
| Borderline lesion | 5.24±0.50[c)] |
| Tubular adenocarcinoma, well-differentiated type | 6.11±0.84[d)] |

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

Table V. Aspect Ratio of Nuclei

| Histological diagnosis | Mean±SD |
|---|---|
| Control | 1.50±0.39[a)] |
| Benign tubular adenoma | 1.81±0.19[b)] |
| Borderline lesion | 1.91±0.27[c)] |
| Tubular adenocarcinoma, well-differentiated type | 1.68±0.22[d)] |

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

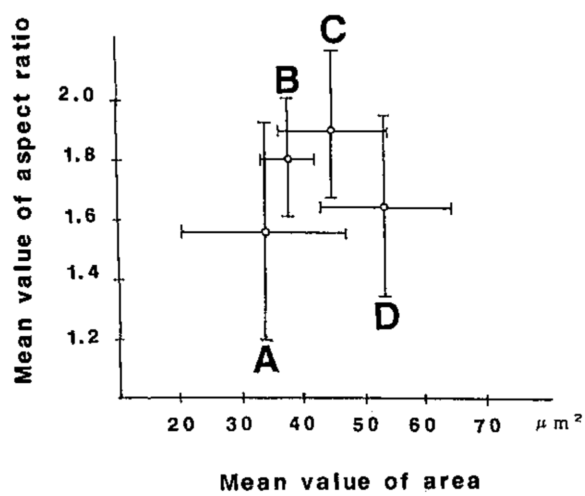


Fig. 8. Relation between size and shape of nuclei of tubular tumors of the stomach. The transverse axis represents mean value of the area, and the perpendicular axis, that of the aspect ratio. The open circle represents the mean values and the bar represents the range of ±1 SD. A, Control; B, benign tubular adenoma; C, borderline lesion; D, well-differentiated type tubular adenocarcinoma.

benign tubular adenoma and borderline lesions. There was no significant difference between the maximum diameter in borderline lesions and in well-differentiated type tubular adenocarcinoma.

The maximum width of nuclei in each group is shown in Table IV. There was no significant difference between the maximum width of intestinal metaplastic mucosa and in benign tubular adenoma. On the other hand, there were significant differences in the maximum width between benign tubular adenoma and borderline lesions, and also between borderline lesions and well-differentiated type tubular adenocarcinoma.

The aspect ratio of nuclei in each group is shown in Table V. There were significant differences between the aspect ratio of intestinal metaplastic mucosa and that in benign tubular adenoma, and also between borderline lesions and well-differentiated type tubular adenocarcinoma. On the other hand, there was no significant difference between that in benign tubular adenoma and that in borderline lesions.

The relation between one SD range of the nuclear area and that of the aspect ratio is illustrated in Fig. 8. One SD range in benign tubular adenoma did not overlap with that in well-differentiated type tubular adenocarcinoma. The area and the aspect ratio in borderline lesions were distributed midway between those in benign tubular adenoma and those in well-differentiated type tubular adenocarcinoma.

## DISCUSSION

Nuclear abnormality is one of the important findings for histological differential diagnosis between benign status and malignant status. Nuclear abnormality should be quantified to serve as a reliable histological criterion for diagnosis.

Jarvis and Whitehead and Tosi *et al.* measured nuclear size on histological specimens of gastric tumors and tried to differentiate malignant from benign tumors.[4-6] However, they did not validate their methods for measuring the nuclear size and shape on the histological specimens.

We should check for nuclear condensation during the preparation procedure of the histological specimens. As listed in Table I, we observed significant differences in the size of intermediate lymphocytes according to 3 factors in the preparation process: the concentration of formalin solution; the duration from resection to fixation in formalin solution; and the duration of fixation in formalin solution. The size of intermediate lymphocytes is considered to be almost uniform within each individual and also among different individuals. It appears that nuclear condensations in gastric tumors significantly depend on the 3 factors in the preparation process. Therefore, the area of intermediate lymphocytes on the same histological specimen should be measured as a control in order to estimate the condensation of nuclei in gastric tumors. In the present study, specimens on which the condensation ratio of intermediate lymphocytes was 10% or higher, were omitted from the analysis.

We should also pay attention to the appropriate number of nuclei to be measured. As shown in Fig. 5, the nuclear size and shape on histological specimens can vary significantly depending upon how the specimen is cut. In order to minimize the dependence on section, a sufficient number of nuclei must be measured. A Fig. 6 shows, the mean values and standard deviations of the nuclear area, the maximum diameter, the maximum width and the aspect ratio obtained from 200 nuclei were not significantly different from those obtained from 1,000 nuclei. It is considered that at least 200 nuclei must be measured on histological specimens.

As shown in Fig. 1, in a case in which benign tubular adenoma was histologically diagnosed, nuclei were small and spindle-shaped, and their size and shape were relatively uniform. In the present study, the maximum diameter and the aspect ratio of nuclei in benign tubular adenoma were significantly larger than those in intestinal metaplastic mucosa, while there were no significant differences in the area and the maximum width between them. This suggests that nuclei in benign tubular adenoma tend to be spindle-shaped without swelling.

As shown in Fig. 3, in a case in which well-differentiated type adenocarcinoma was diagnosed histologically, nuclei were swollen and round. In the present study, the area, the maximum diameter and the maximum width in well-differentiated type tubular adenocarcinoma were significantly larger than those in benign tubular adenoma. On the other hand, the aspect ratio was significantly smaller than that in benign tubular adenoma. This suggests that nuclei in well-differentiated type tubular adenocarcinoma tended to be swollen and round. In Fig. 2, the nuclei are relatively larger than those in benign tubular adenoma, but they do not appear to be round. Diagnosis of either benignancy or malignancy cannot be easily made for such cases and borderline lesions are diagnosed. In the present study, nuclei in borderline lesions were larger than those in adenoma and smaller than those in well-differentiated type tubular adenocarcinoma. The shape of nuclei was almost the same as that in benign tubular adenoma.

As shown in Fig. 8, the nuclei in intestinal metaplastic mucosa were distributed over a wide range. This was because nuclei at the bottom of the tubuli tended to be swollen and round and those at the upper part of the tubuli tended to be small and spindle-shaped. The distributions of one SD range of the area and the aspect ratio of each group corresponded well with our pathological findings. Therefore, nuclear abnormality represented by the area, the maximum diameter, the maximum width and the aspect ratio on the histological specimens can be considered to correspond well with the histological findings.

In conclusion, nuclear abnormality in gastric tubular tumors can be represented by the nuclear area, the maximum diameter, the maximum width and the aspect ratio measured on the histological specimens. Tubular tumors can be classified into benign, borderline and malignant groups in terms of these factors.

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