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Nuclear Morphological and Morphometric Features of Reactive versus Neoplastic Gastric Mucosa

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Introduction: Gastric carcinoma is the second most common tumor in the world. Highest incidence is found in Japan, Chile and Italy. In tumor progression model of intestinal type of gastric carcinoma, the cellular changes progress from initial inflammation and chronic gastritis to metaplasia, dysplasia and adenocarcinoma. Distinguishing regenerative atypia from dysplasia and carcinoma is the most daunting challenge for a pathologist. Focusing on cytological especially nuclear features can provide an opportunity for early diagnosis and may improve patient's survival.
Aim: The aim of this study was to compare the nuclear morphological and morphometric features of regenerative atypia, dysplasia and intestinal type of gastric adenocarcinoma and to identify the features potentially useful in diagnosing infiltrating carcinoma on nuclear changes.
Material and Methods: 50 gastric endoscopic biopsies or gastric resection specimens were evaluated at the Pathology Department Pakistan Institute of Medical Sciences between January 2008 and October 2009. The specimens were grouped as normal, reactive atypia, dysplasia and carcinoma. Using oil immersion microscopy, we studied nuclear features like number and location of nuclei (basal, mid or apical part of cell), nuclear size (area, major axis length and minor axis length), anisonucleosis, poikilonucleosis, nuclear/cytoplasmic (N/C) ratio (low, normal, or increased), chromatin (hyperchromaticity, chromatin distribution, chromatin quality), number of nucleoli, presence of bizarre nuclei, nuclear membrane folds and breaks, presence and location of mitoses and atypical mitoses. Morphometric measurement of nuclear area, major axis length and minor axis was done in each case.
Results: Marked nuclear enlargement and pleomorphism, frequent prominent nucleoli, irregularly thickened nuclear membranes with frequent folds and breaks, irregularly distributed clumpy chromatin, bizarre nuclei and high mitotic count especially atypical mitoses favour malignancy whereas mild nuclear enlargement and pleomorphism, occasional prominent nucleoli, regular nuclear membranes, uniform chromatin distribution and few mitotic figures are indicative of regenerative atypia. Mean nuclear area, major and minor axis length increase in a step ladder pattern in order of normal control, reactive atypia, dysplasia and carcinoma.
Conclusions: Nuclear or cytological classification schemes can be useful in early diagnosis of gastric carcinoma. Further research studies with long term follow up of the patients should be done.
Keywords: Reactive atypia, dysplasia, intestinal type of gastric carcinoma, nuclear morphology, morphometry.

Introduction

Neoplasia means the process of "new growth" and the resulting growth is called a neoplasm. According to Willis, "a neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change". This excessive, unregulated, autonomous proliferation results from heritable genetic alterations that are passed down to the progeny of tumor cells. Development of malignant tumors follows a gradual process of molecular and morphological events progressing from preneoplastic to neoplastic and ultimately invasive cancer.[1] In most cases of intestinal type of gastric carcinoma, the tumor progression model begins with chronic atrophic gastritis and intestinal metaplasia progressing through various stages of dysplasia, carcinoma in situ and superficial carcinoma.[2]

Dysplasia should be clearly separated from regenerative hyperplasia that is seen in areas of mucosal injury, such as gastritis[3] and peptic ulceration. Regenerative hyperplasia can be simple or atypical, the latter has been reported following

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chemoradiotherapy[2,4-6]. The fundamental problem of differentiating neoplastic precancerous and cancerous lesions from non neoplastic reactive or regenerative proliferation[7], is quite challenging issue for the pathologist, since atypical regenerative hyperplasia (ARH) of diverse epithelia can mimic neoplasms. Examples include ARH of gallbladder, oesophagus, stomach, bladder, prostate, and skin, simulating carcinoma.[8] Similarly, chemotherapy associated gastric ulcers and epithelial atypia may be misinterpeted as carcinoma.[4-6] It is known that reactive or regenerative atypia usually resolves with therapy while dysplasia may regress, persist, or progress to frank carcinoma. In one study, cancer was associated with 36% cases of moderate gastric epithelial dysplasia and with 80% of severe dysplasia.[9] Thus, accurate histopathological assessment of the gastric mucosa is a good predictor of cancer risk in an individual patient. Despite a steadily declining incidence over the past six decades, gastric cancer is still the second most common tumor in the world, accounting for approximately 2.5% of all cancer deaths in United States. It is also a leading cause of cancer deaths worldwide.[1] Highest incidence is found in Japan, Chile and Italy.[2] These figures provide a compelling evidence for researchers to devise newer means to detect the early, curable phase of gastric cancer and prevent its progression.

Although the diagnosis of non-invasive neoplastic (dysplastic) and frank carcinomas of stomach is based on three histological features: (i) cellular atypia, (ii) abnormal differentiation and (iii) disorganized mucosal architecture[10], the major features of malignancy are related to cell nuclei[11] and because currently, only few molecular markers are available to identify dysplasia, at present, we must still rely on cell morphology[12].

This is especially true of small endoscopic biopsies when architectural features are difficult to assess due to poor tissue orientation or scanty material. Previously, different morphological and morphometric variables in gastric dysplasia and carcinomas have been studied.[13-15] Enchev et al[16] compared some cytomorphological features in normal gastric mucosa, gastritis, gastric ulcer, polyps and carcinoma of the stomach. However oil immersion nuclear features of normal mucosa, reactive atypia, dysplasia and carcinoma have not been previously studied in detail. In this research, we have made an attempt to identify useful nuclear features that would differentiate reactive from neoplastic conditions and dysplasia from carcinoma.

Aim

The aim of the present study was to compare the nuclear morphological and morphometric features of regenerative atypia, dysplasia and intestinal type of gastric adenocarcinoma and to identify the potentially useful nuclear changes in diagnosing infiltrating carcinoma.

Materials and Methods

Forty gastric biopsy and surgical resection specimens we retrieved from record of surgical pathology department Pakistan Institute of Medical Sciences, between January 2008 and October 2009. Of these 10 cases were diagnosed as chronic gastritis or gastric ulcer with atypia, 15 as dysplasia and 15 as intestinal type of gastric adenocarcinoma. For each case, 10 random fields of paraffin embedded, Haematoxylin & Eosin stained sections with thickness of 4-5 micrometer were examined using oil immersion microscopy. A variety of nuclear morphological features were evaluated. These included the number and location of nuclei (basal, mid or apical part of cell), nuclear size (area, major axis length and minor axis length), anisonucleosis, poikilonucleosis, nuclear/cytoplasmic (N/C) ratio (low, normal, or increased), chromatin character (hyperchromaticity, chromatin distribution, chromatin quality), number of nucleoli, presence of bizarre nuclei, nuclear membrane folds and breaks, presence and location of mitoses and atypical mitoses.

For morphometric analysis, the digital images were generated by a video camera (Sony) using Zeiss microscope at a magnification of 400x. A total of hundred cells were randomly selected and parameters like nuclear area, major and minor axis length were measured in each case. Image processing was done by an automated image analysis software Cell Profiler. (available at http:/www.cellprofiler.org)

To study dysplasia, we examined sections from periphery of cancer lesion in gastrecomy specimens or endoscopic biopsies with no evidence of invasion. As controls, 10 biopsy or resection specimens of normal stomach were also evaluated for similar features.

The proportion of cases with each feature was compared among the study and control groups, and differences were statistically analyzed with chi-square test and t tests using SPSS package program (version 13). *P value* < 0.05 was defined as significant.

Results

The male/female ratio (M:F) of the study group was 3:2, and their mean age was 40 years (range: 18 years to 88 years). The M:F ratio of the control group was 1:1, and their mean age was 37.7 years (range: 2 years to 96 years).

In reactive atypia, the cells were mono or binucleated with 0-3 prominent nucleoli. Nuclei were mildly enlarged with mean area of 17.71 ± 4.2 μm (range 11.94 – 25.61 μm) as compared to a control value of 7.66 ± 2.3 μm. The nuclei were located in mid (70% cases) or basal region (30%), Aniso and poikilonucleosis was mild in 80% and moderate in 20%. Nuclear membrane breaks and folds were absent (100% and 90% cases respectively). The chromaticity ranged from hyperchromatic (40%) to euchromatic (40%) and hypochromatic (20%). Chromatin distribution was uniform and clumping was absent (100%).

In dysplasia group, the cells had 1-3 nuclei with 0-5 prominent nucleoli. Nuclei were moderately enlarged with mean area of 37.81 ± 5.34 μm (range 25.28 – 55.55 μm). The nuclei were either located in apical (66.7 % cases) or mid zone of the cell (33.3%). Anisonucleosis and poikilonucleosis was moderate in 60% cases. Nuclear membrane breaks and folds were present in 60% and 80% of cases respectively. The two features were more frequently seen in 33.3 % cases. The chromaticity ranged from hyperchromatic (53.3%) to euchromatic (40%) and hypochromatic (20%). Chromatin distribution was nonuniform (86.7%) and clumping was present (80%). Bizarre nuclear forms were seen in only 20% cases.

The adenocarcinoma group featured 1-5 nuclei per cell with 0-6 prominent nucleoli, marked nuclear enlargement with mean area of 49.46 ± 9.14 μm (range 35.28 – 64.17 μm). The nuclei were approaching apical region of the cell (100 % cases) and showing marked anisonucleosis (86.7%) and poikilonucleosis (93.3%). Nuclear membrane breaks and folds were present in 100% cases and frequent in 80% and 53.3% respectively.

The chromaticity ranged from hyperchromatic (60%) to euchromatic (6.7%) to vesicular (26.7) and hypochromatic (6.7%). Chromatin distribution was nonuniform (100%) and clumping was present (66.7%). Bizarre nuclear forms were seen in 86.7% cases.

A summary of pathologic features is presented in table 1.

Table 1: Comparison of nuclear features among study groups.

	Reactive atypia (Cases %)	Dysplasia (Cases %)	Carcinoma (Cases %)
Nuclear area	17.71 μm	37.81 μm	49.46 μm
Pleomorphism	Mild (80%)	Mild (6.7%) Moderate (60%) Marked (33.3%)	Moderate (6.7-13.3%) Marked (86-93%)
Chromatin distribution	Uniform (100%)	Irregular (86.7%)	Irregular (100%)
Nuclear membrane folds	Absent (90%)	Present (80%) Frequent (33.3%)	Present (100%) Frequent (53.5%)
Nuclear membrane breaks	Absent (100%)	Present (60%) Frequent (33%)	Present (100%) Frequent (80%)
Bizarre nuclear forms	Absent (100%)	Present (20%)	Present (80%)
Nucleoli	0-3/nucleus	0-5/nucleus	0-6/nucleus
Mitotic count	0-2/10hpf	0-5/10hpf	Upto 15/10hpf

Atypical mitoses	Absent	0-1/10hpf (6.7%)	0-5/10hpf in (73.3%)

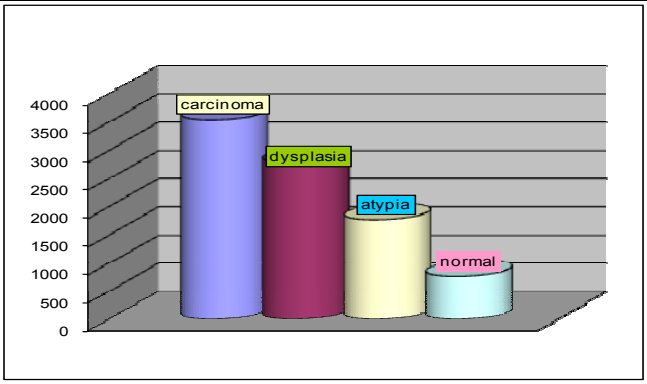

Figure 1: Bar graph showing a step ladder increase in mean nuclear area from normal to carcinoma group.

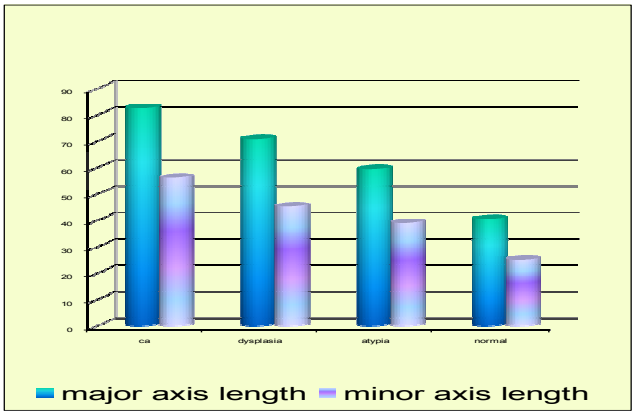

Figure 2: Bar graph showing an increasing tendency in major and minor axis length from normal to carcinoma group.

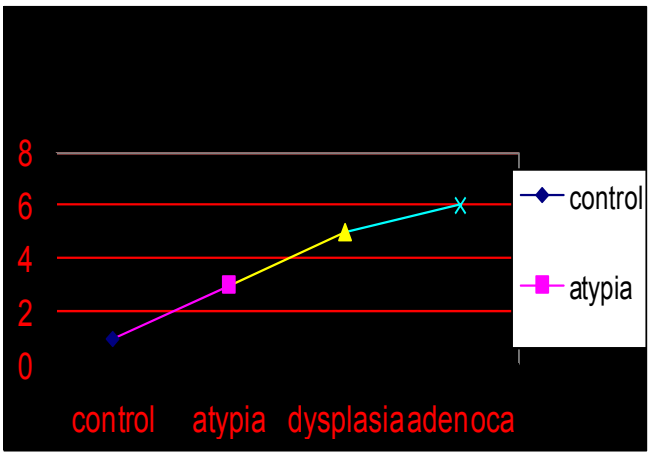

Figure 3: Line graph showing comparison of number of nucleoli among study and control groups.

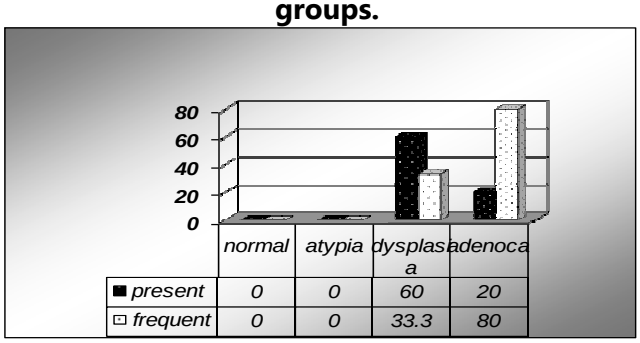

Figure 4: Bar graph showing distribution of nuclear membrane breaks among study and control groups.

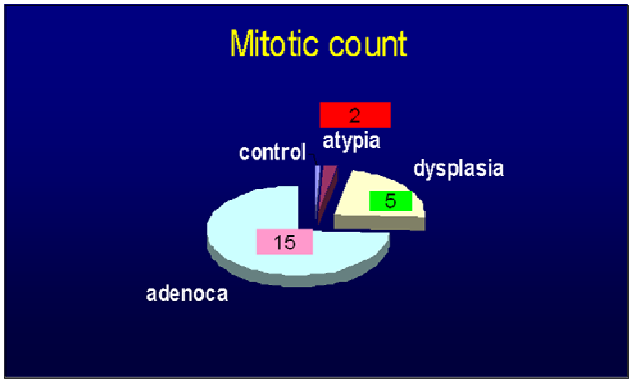

Figure 5: Pie chart showing comparison of mitotic count among study and control groups.

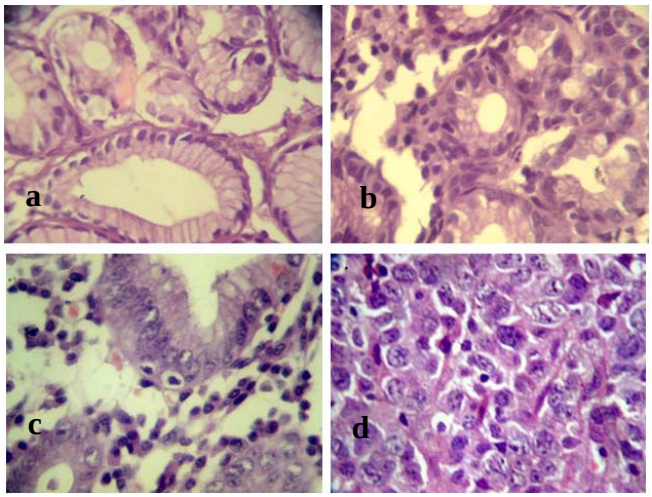

Figure 6 (a-d): Location of nuclei and pleomorphism. In normal gastric mucosa (a) nuclei are small, uniform and basally located. In reactive atypia (b) the nuclei are occupying basal and mid area of the cells and there is mild nuclear enlargement and pleomorphism. In dysplasia (c) and gastric adenocarcinoma (d) the nuclei show moderate to marked pleomorphism with significant enlargement and are occupying most of the cell area (H & E x 400).

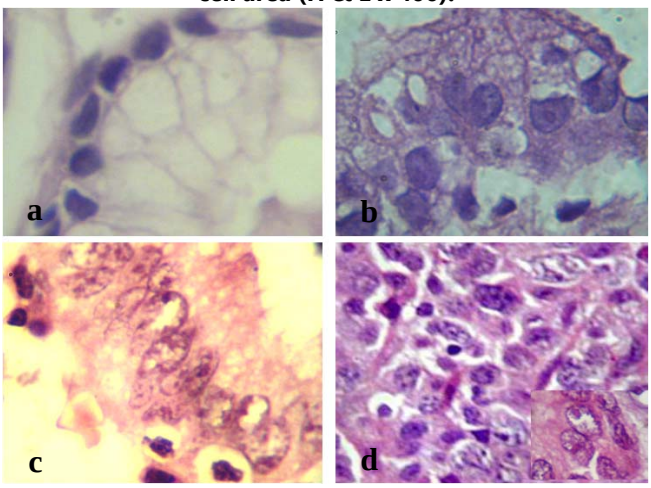

Fig 7 (a-d): Nuclear membrane & chromatin distribution among control and study groups. Normal gastric mucosa (a) shows uniform chromatin distribution and inconspicuous nuclear membranes. In reactive atypia (b) chromatin distribution is uniform and membranes are prominent. In dysplasia (c) and carcinoma (d) the membranes are irregularly thickened and chromatin is nonuniform, coarse and clumpy (H & E x 1000).

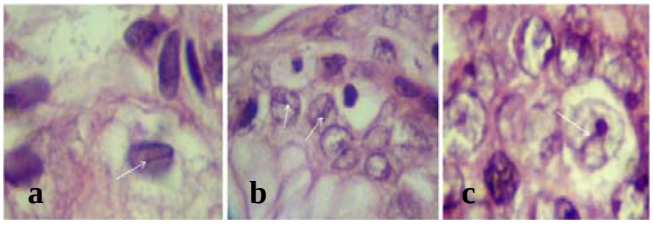

Figure 8(a-c): Nuclear membrane folds in reactive group (a), dysplasia (b) and adenocarcinoma (c). (H & E x 1000)

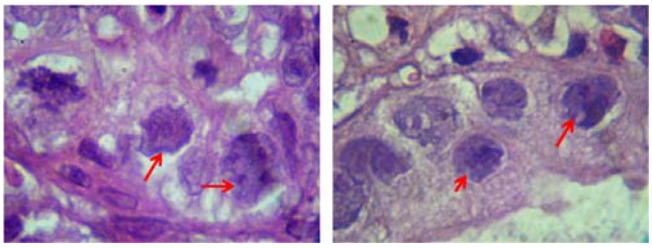

Figure 9: Nuclear membrane breaks and indentations in gastric adenocarcinoma (H & E x 1000)

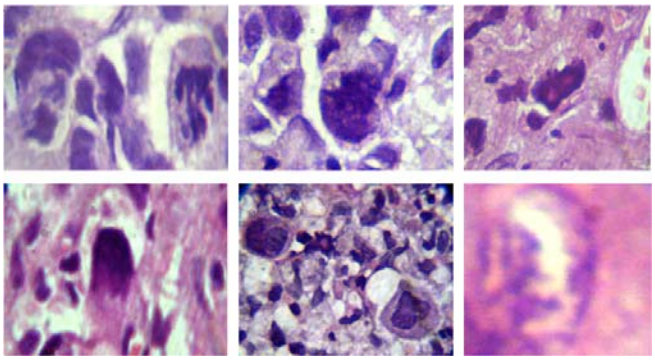

Figure 10: Bizarre nuclear forms in different cases of gastric adenocarcinoma (H & E x 1000)

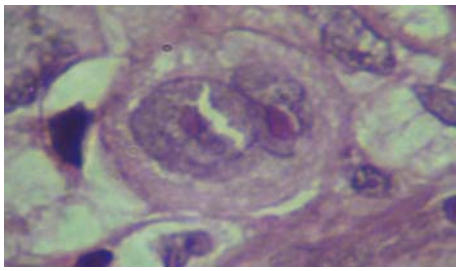

Figure 11: RS like cell in a case of gastric adenocarcinoma. (H & E x 1000)

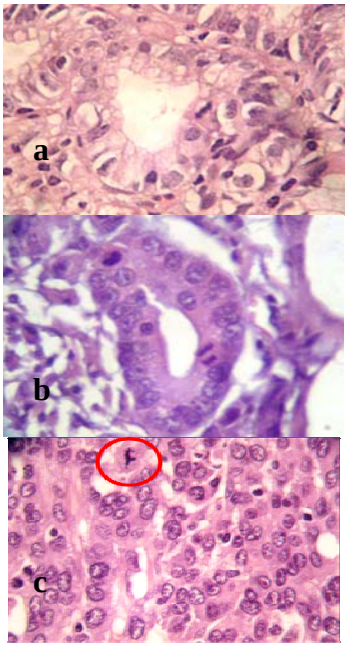

Figure 12 (a-c): Mitotic figures in reactive atypia (a) and intestinal type of gastric adenocarcinoma (b). Fig c shows atypical mitoses in carcinoma (H & E x 400).

Discussion

The association between chronic inflammation and cancer is now well established.[17] In intestinal type of gastric adenocarcinoma, the initial chronic inflammation is induced by a number of factors namely H. pylori, autoimmune gastritis, alcohol, cigarette smoking, antrectomy, gastric obstruction, radiations, granulomatous conditions, amyloidosis, graft versus host disease and uremia.[1] The inflamed epithelium undergoes repeated episodes of regeneration and the newly formed baby cells appear quite immature and atypical and must be differentiated form dysplasia. Since dysplasia is the neoplastic stage before cancer cells invade, the diagnosis of dysplasia is an area of major concern for the pathologist and the ability to diagnose dysplasia means the ability to distinguish neoplastic from nonneoplastic lesions.[12]

Time and again different attempts have been made to define the cytological as well as architectural criteria of dysplasia and invasive carcinoma. Unfortunately, the diagnostic criteria and grading schemes have evolved differently in different parts of the world. This has resulted in disagreement regarding differentiating features of both preinvasive and early gastric cancer between Western and Japanese histopathologists. Therapeutic guidelines are also controversial.[18]

Keeping in view the prime importance of early diagnosis, we have made an attempt to classify the regenerative and neoplastic lesions of gastric mucosa on the basis of nuclear features. We observed that marked nuclear enlargement and pleomorphism, frequent prominent nucleoli, irregularly thickened nuclear membranes with frequent folds and breaks, irregularly distributed clumpy chromatin, bizarre nuclear and high mitotic count especially atypical mitoses favour malignancy whereas mild nuclear enlargement and pleomorphism, occasional prominent nucleoli, regular nuclear membranes, uniform chromatin distribution and few mitotic figures are indicative of regenerative atypia. Hiroshi et al[13] described similar results in their study. They proposed that cytologic nuclear pleomorphism, distinct nuclear border, irregular thickness of the nuclear membrane, irregular chromatin clumping, prominent and distinct nucleoli were observed in carcinomas and latent stage before carcinoma.

In our study the morphometric analysis of mean nuclear area, major and minor axis length increased in a step ladder pattern in order of normal control, reactive atypia, dysplasia and carcinoma. By using this technique, accurate reproducible objective classification of these lesions can be easily obtained. Jarvis et al [14] also studied morphometric analysis of gastric dysplasia in which nuclear size proved to be the main discriminating variable. Enchev et al [16] investigated cytomorphometric features in normal gastric mucosa, gastritis, gastric ulcer, polyps and carcinoma of the stomach. They observed low number of cell groups and small nuclear size in normal mucosa and gastritis. In gastric ulcer, polyps and carcinoma the number of the cell groups was high and nuclear size was large. Shao et al [15] studied a number of morphometric variables in dysplasia and carcinoma of gastric mucosa. The results showed that index of structural atypism, gland area, gland perimeter, gland maximum diameter, nucleus-gland ratio index and nuclear area increased in intestinal metaplasia, mild dysplasia, moderate dysplasia, severe dysplasia and carcinoma.

In countries other than Japan, the vast majority of gastric carcinomas are diagnosed at advanced stages, with a five year survival rate lower than 20%. In Japan, approximately half of gastric carcinomas are detected at an early stage and surgical treatment results in 10 year survival rates higher than

85%. The Japanese experience demonstrates that the early detection of GC dramatically improves its prognosis.[19] We propose that nuclear and cytological features can help in this early detection.

Comments

As compared to western world, the incidence of gastric carcinoma in our country is much higher and diagnosis at an early stage can be done using nuclear or cytological classification schemes. However this may require further research studies with long term follow up of the patients before such schemes are implemented.

The use of nuclear morphometry for diagnosis of regenerative and neoplastic conditions of gastric mucosa provides accurate reproducible objective classification of these lesions.

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