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Original Research Article

Morphometric and Histopathological Study of Gastric Epithelial Lesions with special reference to proliferative activity

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Abstract: Disorders of the gastric epithelium are frequent cause of clinical disease with inflammatory and neoplastic lesions being particularly common. Adenocarcinoma is the commonest gastric malignancy, commonly arising from the antrum or lesser curvature. Morphometry and immuno staining help in classifying different lesions of stomach, specially when there is dilemma in concluding a lesion either benign, premalignant or malignant particularly in small biopsy specimens.The aim is to assess the role of morphometry and proliferative markers in diagnosis of gastric epithelial lesions.In this study, total 100 gastric biopsy specimens from 100 patients were included and analysed by H&E stained sections using morphometric parameters as well as proliferative markers like Ki-67 and proliferating cell nuclear antigen (PCNA).Statistically significant differences found in between the gastric premalignant and malignant epithelial lesions in terms of morphometric parameters and in expression of proliferative markers.Morphometry and immunohistochemistry help in the proper diagnosis of different gastric epithelial lesions particularly those lying in the grey zone on routine histopathological sections.
Keywords: Gastric epithelial lesions, morphometry, proliferative activity, Ki-67

INTRODUCTION:

Disorders of the gastric epithelium are frequent cause of clinical disease with inflammatory and neoplastic lesions being particularly common [1]. The risk factors associated with gastric epithelial lesions include infection by bacteria (H. pylori, H.heilmannii, Mycobacteria etc), virus (CMV, HSV, VZV) fungus (Candida, Histoplasma), therapy with some drugs (aspirin, alcohol, NSAIDs, iron, aluminium containing antacids), radiation and chemotherapy (with 5-fluorouracil), caustic injury [2]. Less profound risk factors are pernicious anaemia, Menetriar disease, gastric polyps particularly gastric adenoma [3] and blood group A [4]. Adenocarcinoma is the commonest malignant tumour of stomach, commonly arising in the antrum and lesser curvature. Males are affected more than females. It is very rare in children and adolescent [5]. Gastric dysplasia is believed to be the penultimate stage of gastric carcinogenesis [6] and usually occurs against a background of long standing chronic gastritis [7].On routine histopathology examination, it is sometimes difficult to differentiate between the benign epithelial lesions of stomach (like gastric adenomas, polyps, gastric dysplasia) and gastric carcinoma. Morphometric analysis helps us in this grey zone. Morphometry is the quantitative description of the biological structure. To improve the clinical value of malignancy grading, it has been suggested to quantify nuclear pleomorphism by measuring nuclear features such as nuclear area, perimeter and diameter [8]. Morphometrical assessment study in classification of different grades of gastric dysplasia and malignancy by Shaol L , using nuclear area and nuclear perimeter as discriminating variables showed good results for the prediction of different individual case [9]. Chronic gastritis is one of the most frequent gastric diseases. The morphometry in diagnosis of chronic gastritis can as appear to be a potentially significant tool [10].

The Ki-67 antigen was discovered as a nuclear antigen that was expressed in proliferating cells by Gerdes *et al.;* [11] in 1983. It is also a gene marker of nuclear proliferation which exists in all stages of the cell cycle except at stage G_o. It is expressed in stage G_1 in the cell cycle and

increased in stage S and stage G_2, reaching the peak in stage M and disappearing rapidly at the late stage of division. Since its half life calculation is short, it degrades quickly after breaking away from the cell cycle. Ki-67 has become the most reliable marker to determine the proliferating activity of tumor cells [11,12]. Proliferating cell nuclear antigen (PCNA) is a 36kd non-histone nuclear protein. Expression of PCNA becomes maximal during S-phase and declines again during G2 and M phases. Therefore, its level correlates directly with rates of cellular proliferation and DNA synthesis [13].

Aims of this study was to

1. Diagnose different gastric epithelial lesions in biopsy specimen.
2. Assess the morphometric parameters of benign and malignant epithelial lesions.
3. Assess the proliferative activity in different lesions with the help of Ki-67 and PCNA.

MATERIALS AND METHODS:

The study was performed in the Department of Pathology in collaboration with the Department of Gastroenterology over a period of two years (2011-2013). The specimens were taken from endoscopic biopsy of gastric epithelium. Detailed history, clinical findings were noted from the patients. Total 100 biopsy samples from 100 patients and 10 controls (from the normal gastric biopsy specimens) were included in this study. The specimens were fixed in formalin, processedand embedded in paraffin block. Sections of 3 micron thickness were affixed on egg albumin coated slides and poly-l-lysine coated slides. The former were stained by Haematoxylin and Eosin (H&E) stains and the later group were for use in Ki-67 and PCNA index study [14, 15]. After proper fixation sections were also stained by Periodic acid-Schiff (PAS) technique for demonstration of mucin. H&E and PAS stained slides were examined thoroughly by three pathologists and a provisional diagnosis of each case was given. Morphometric analysis were done on H & E stained histological sections with the aid of an ocular micrometer attached to the 10X eyepiece of a microscope using a 40X high power objective. One smallest division of ERMA ocular micrometer is equated with 2.5µm. 50 random nuclei from the most atypical area of the sections were analyzed. Morphometric analysis was performed in terms of mean nuclear diameter (MND), mean nuclear area (MNA), mean cell diameter (MCD), mean nucleo-cytoplasmic ratio (N/C ratio) [16] as well as Mean MND(MMND), Mean MCD(MMCD), Mean MNA(MMNA), Mean Mean Nuclear Perimeter (MMNP) etc. For calculation of PCNA labelling index (PCNA LI%) at least 1000 nuclei were counted under 1000× magnification . Finally a grand chart was produced tabulating histological diagnosis, morphometric findings, interpretation of Ki-67 score (%) and PCNA LI(%). The results obtained were then analysed. The measure of central tendencies like mean of the data was considered. Subsequently Unpaired student t-test was used to reveal whether any significant difference is present between lesions with different diagnosis. Total 100 cases were distributed along various age groups according to histopathological diagnosis (Table 1). Maximum cases of superficial gastritis with or without regenerative hyperplasia (SG±RHP)(56%) (Figure 1A) were found between 20 and 39 years of age. Out of 12 cases of gastric dysplasia (GD) (Figure 1B), 8 cases (66.7%) were in 40 years to 59 years. Adenocarcinoma of intestinal (ADC-I) and diffuse (ADC-D) type (Figure 1C and Figure 1D) predominated beyond 60 years and 40 years of age respectively. Table 2 shows morphometric measurements of different lesions in H & E stained sections in respect to controls. Table 3 showing Ki-67 score and PCNA labelling index in percentage. The range of Ki-67 score in this study was 4-65%. Mean Ki-67 expression of SG±RHP, GD, ADC-I and ADC-D were 7.37%, 37.50%, 46.85% and 42.50% respectively. Mean PCNA labelling index were 7.04%, 35.25%, 43.16% and 39.37% for SG±RHP, GD, ADC-I and ADC-D respectively in respect to a mean control value of 6.60%. When comparing the P values of different lesions significant differences were found between benign, premalignant and malignant cases (Table 4).

DISCUSSION:

The study was done to assess the role of morphometry in this modern era of immunohistochemistry. In routine day to day practice, it is very common to encounter difficulties in diagnosing an epithelial lesion of stomach lying in the borderline between benign and malignant. As depicted in table-1, the commonest diagnosis was superficial gastritis with or without regenerative hyperplasia (SG±RHP) (Figure 1A) followed by adenocarcinoma of intestinal type (ADC-I), gastric dysplasia (GD) (Figure 1B) and adenocarcinoma of diffuse type (ADC-D) (Figure 1C and Figure 1D). SG±RHP was most prevalent in the age group of 20-39 years. Out of 4 cases of superficial gastritis of young age (<20 years) , 2 cases were diagnosed as granulomatous gastritis. One of them had previous history of pulmonary tuberculosis and responded to anti tubercular therapy and the other patient had Crohns disease. Features of active inflammation (like polymorphonuclear leucocytes in surface and foveolar epithelium leading to pit abscess)[17] were present in 30 cases of superficial gastritis with regenerative hyperplasia,

out of which 27 cases (90%) proved to be positive for H. Pylori. This finding corroborated with the study of Selvi Thirumurthi & David Y. Graham who showed that the prevalence of H. pylori in the Indian subcontinent could be as high as 80 per cent or more in rural areas [18]. ADC-D cases showed younger age preferencethan ADC-I cases. Only 4 cases of ADC-I and 2 cases of ADC-D were found beyond 80 years of age.

Table-1: Age distribution of cases (n=100)

Histopathological Diagnosis(H &E)	No.of cases	Age in years				
		<20	20-39	40-59	60-79	>80
Superficial gastritis with or without regenerative hyperplasia(SG ± RHP)	50	4	28	12	6	0
Gastric Dysplasia(GD)	12	0	4	8	0	0
Gastric Adenocarcinoma of intestinal type (ADC-I)	30	0	2	10	14	4
Gastric adenocarcinoma of diffuse type (ADC-D)	8	0	2	4	0	2

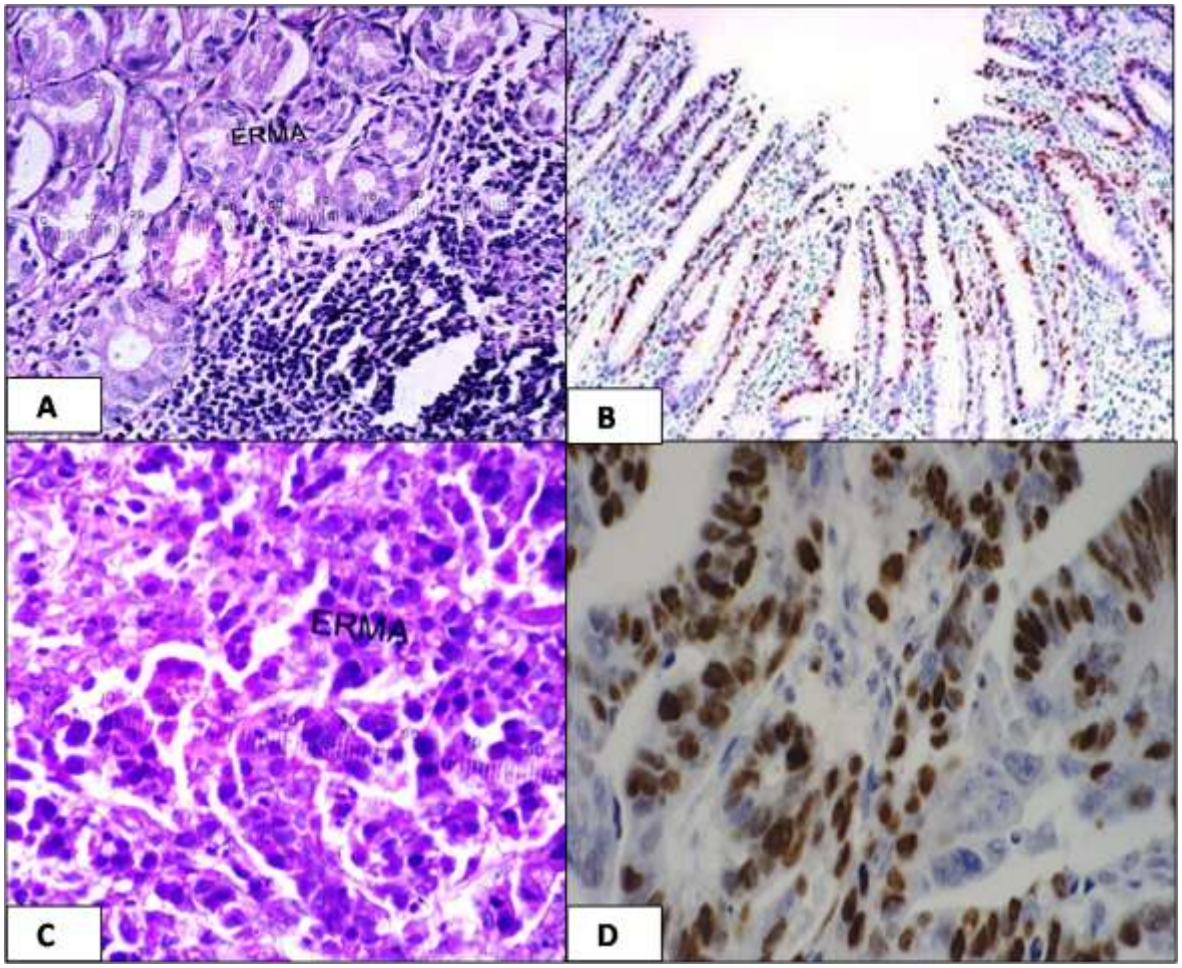

Fig 1A: Photomicrograph showing histology of superficial gastritis (HE x400)
Fig 1B: Photomicrograph showing histology of gastric dysplasia (Monoclonal antibody against PCNA x400)
Fig 1C: Photomicrograph showing histology of gastric adenocarcinoma of diffuse type (HE x 400)
Fig 1D: Photomicrograph showing histology of gastric adenocarcinoma (Monoclonal antibody against Ki-67 x400).

In this study Mean Mean Nuclear Diameter (MMND) of different benign gastric epithelial lesions with or without regenerative hyperplasia , borderline lesions like gastric dysplasia and

malignant lesions like adenocarcinoma of intestinal and diffuse type were 4.51µ, 7.79µ,7.81µ and 9.13µ respectively in relation to control value of 4.21µ (Table 2). Mean Mean Nuclear Area (MMNA) were 17.71μ^2 for SG±RHP, 55.89μ^2 for GD, 56.58μ^2 for ADC-I and 76.15μ^2 for ADC-D (Table 2). Enlargement of nucleus was seen in GD (minimum N:C ratio among 12 cases was 0.54), ADC-I (minimum N:C 0.54) in relation to the maximum mean N:C ratio (0.5) in case of regenerative hyperplasia with reactive atypia was seen in this study.

Table 2: Results of Morphometric study (on H & E stained sections)

Histopathological Diagnosis	MMND(µ)	MCD(µ)	MM(N:C)	MMNA(μ^2)	MMNP(µ)
Control	4.21	15.15	0.41	15.9	15.28
SG ± RHP	4.51	13.65	0.35	17.71	14.45
GD	7.79	14.23	0.67	55.89	25.54
ADC-I	7.81	13.63	0.69	56.58	26.13
ADC-D	9.13	11.54	0.74	76.15	28.66

From these observations, we can conclude that MNA , MND and N:C ratio can be used as important morphometric parameters to differentiate normal epithelial cells (controls) and various benign gastric lesions from borderline and malignant one. The p values were found to be highly significant in differentiating controls as well as various benign from borderline and malignant lesions (p value <0.05). These findings corroborated with the study of Enchev V and Rigaut JP, who noted that the highest values for all nuclear dimensions were observed in primary gastric carcinoma [19].

Proliferative activity of various gastric epithelial lesions were studied with the help of monoclonal antibody against Ki-67 and PCNA. Table 3 show Ki-67 score (%) and PCNA labelling index (%) of controls and that of various gastric epithelial lesions respectively. The range of Ki-67 score was 4-65 %. In controls it was between 4-9% with a mean of 5.6 %. Benign lesions showed significant differences ($p<0.05$) in respect to gastric dysplasia and adenocarcinoma (Figure 1D) but there was no significant difference in Ki-67 expression in comparison to control (Table 4). J Yu and W K Leung *et al.;* also found increased Ki-67 score with increased epithelial cell turnover [20].

Table 3: Ki-67 score (%) and PCNA Labelling index (%) of gastric epithelial lesions.

Histopathological diagnosis	Immuno staining done (n=100)		Range (%)		Mean (%)	
	Ki-67	PCNA	Ki-67	PCNA	Ki-67	PCNA
Control	10	10	4-9	3-10	5.60	6.60
SG ± RHP	50	50	4-20	5-12	7.37	7.04
GD	12	12	25-50	25-50	37.50	35.25
ADC-I	30	30	30-60	25-60	46.85	43.16
ADC-D	08	08	25-65	30-55	42.50	39.37

Table 4: P Values of different parameters

Morphometric parameters	CVs SG±RHP	SG±RHP Vs GD	GD Vs ADC-I	GD Vs ADC-D
MND	$5.1x10^{-1}$	$1.4x10^{-9*}$	$9.8x10^{-1}$	$1.2x10^{-1}$
MNA	$6.2x10^{-1}$	$1.4x10^{-12*}$	$9.0x10^{-1}$	$1.1x10^{-1}$
MCD	$3.3x10^{-1}$	$6.9x10^{-1}$	$6.6x10^{-1}$	$7.4x10^{-1}$
M (N:C)	$6.9x10^{-2}$	$1.0x10^{-13*}$	$9.6x10^{-1}$	$2.2x10^{-1}$
MNP	$5.4x10^{-1}$	$1.4x10^{-10*}$	$7.2x10^{-1}$	$2.9x10^{-1}$
Ki-67 Score (%)	$6.4x10^{-2}$	$1.8x10^{-3*}$	$1.8x10^{-3*}$	$3.2x10^{-1}$
PCNA LI (%)	$5.2x10^{-1}$	$2.1x10^{-24*}$	$2.2x10^{-2*}$	$3.5x10^{-1}$

*Significant ($p<0.05$);

The range of PCNA LI values in our study was 5-60%. In controls it was found to be in the range of 3-10%. In adenocarcinoma, PCNA LI range from 25-60% (Table 3). Using unpaired Student's t-Test a significant lower value of PCNA LI was found in controls as well as in the benign lesions as compared to that of GD (Figure 1B) and adenocarcinoma (Table 4). Higher values of Ki-67 (15-20%) and PCNA (10-12%)

expression were found in some benign cases and it was due to the increased mitotic activity as a result of active inflammation and regeneration in those lesions. Adenocarcinoma showing strong (50%-60%) expression of proliferative markers were clinically aggressive tumour and prognosis was worse. This finding was corroborative with the study of S Jain and M I Philipe *et al.;* [21] and Maeda K and Cheung YS *et al.;*[22].

Morphometric parameters failed to differentiate between gastric dysplasia of high grade/ carcinoma in situ and invasive adenocarcinoma because morphometry only provides information on cytological features and not on basement membrane breakage.

Some cases of intestinal adenocarcinoma with intracellular mucin and signet ring appearance showed no significant difference in morphometric parameters in respect to benign lesions and confirmed by mucin stain (PAS) by identifying infiltrative foci in lamina propria or submucosa.

So, from this study, it is concluded that morphometry and immunohistochemistry side by side help in the proper diagnosis of different gastric epithelial lesions which lie in the grey zone on routine histopathology.

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