Original Research Article

Study of Cellular Morphometry in Colorectal Epithelial Lesions with Clinicopathological Correlation

Dr. Koushik Chakraborty¹, Dr. Sucharita Sarkar², Dr. Asim Kumar Manna³, Dr. Saswati Sengupta⁴, Dr. Mousumi Bag⁴

¹Consultant Pathologist, Mohan’s Diabetic Clinic, Kolkata, West Bengal  
²Demonstrator, R.G Kar Medical College & Hospital, Kolkata -700004, West Bengal  
³Professor, Institute of Post Graduate Medical Education and Research, Kolkata-700 020, West Bengal  
⁴Post Graduate Trainee, Dept of Pathology, Institute of Post Graduate Medical Education and Research, Kolkata-700 020, West Bengal

Corresponding Author: Dr. Sucharita Sarkar

ABSTRACT

Diseases of the colon and rectum v.i.z. different types of colitis, polyps and colorectal carcinoma are representing a wide spectrum of differential diagnosis. This study was expected to classify different colorectal epithelial lesion with the help of various cellular morphometry in different types of lesions. In the study, morphometric parameters including nuclear and cytoplasmic dimensions as well as nucleo/cytoplasmic ratio in different colonic and rectal epithelial lesions. Juvenile Polyp v/s Adenomatous Polyp showed significant difference between mean nuclear diameter (p value=0.0013), mean nuclear area (p value=0.0015) and mean nuclear perimeter (p value=0.0013) & nucleo-cytoplasmic ratio (p value=0.00003) Polyp. Adenomatous Polyp v/s Adeno Carcinoma showed the mean cytoplasmic diameters (p value=0.00002) and the nucleo-cytoplasmic ratio (p value=0.00003) did show immensely significant difference and so Adeno Carcinoma v/s Poorly differentiated Carcinoma (mean nuclear diameter (p value=0.006), mean nuclear area (p value=0.0023) and mean nuclear perimeter (p value=0.006) nucleo-cytoplasmic ratio (p value=0.00180). So, it was concluded that even with the advent and popularity of immunohistochemistry, morphometric study was also found reliable resolving diagnostic dilemmas between benign, premalignant and malignant colorectal epithelial lesions.

Keywords: Colorectal carcinoma, Polyp, Morphometry, Nuclear diameter

INTRODUCTION

Intestinal mucosa is susceptible to many pathological processes like autoimmune and malignant diseases. [¹] The intestinal mucosa contains two types of cells: - absorptive cells in luminal side and mucus-secreting Goblet cells predominate in the base of the Crypts. [², ³]

Infecctive colitis, Microscopic colitis, including the two diseases- collagenous colitis and lymphocytic colitis, Pseudomembranous Colitis, Ischaemic colitis, Colonic polyps, inflammatory polyps. Ulcerative colitis, Colitis cystica profunda and ultimately Carcinoma of colon are identified by well defined histomorphological changes.

Now the question is whether colon cancer arises as such, or is it the outcome of repeated genome alterations manifested by well defined histomorphological changes, known as the "Polyp-cancer sequence"?
Here lies the importance of morphometric measurements in distinguishing benign lesions from pre-malignant and malignant ones. The morphometric data on the human rectal mucosa are very infrequent but available studies of the human colon indicate that morphometry can be used successfully for separation of healthy, benign and malignant growths in adenomatous tissue (Kayser et al, 1985). A detailed cellular morphometry can be used for detection of mild abnormalities which otherwise may be overlooked.

Normal colorectal mucosa and adenocarcinoma can be morphometrically classified based on: - (1) nuclear size, nucleo cytoplasmic ratio and nuclear position within the cell; (2) the variability of nuclear size; (3) nuclear elongation and polarity; (4) nuclear shape and its variation. Scatter plots confirmed complete separation of normal mucosa from adenocarcinoma. Excellent reproducibility of nuclear morphology has been reported for patients with breast carcinoma previously. In a study conducted by Cagle et al. suggested that, to obtain high reproducibility and objectivity in morphometric analysis, it was essential to measure the large number of nuclei per case with a uniform technique.

Variations existing in the colorectal crypt at the time of cellular differentiation are detectable by using computer-aided morphometric techniques on routine H&E stained and semi-thin toluidine blue stained sections from colonic mucosa. Generally, most of the morphometric parameters including nuclear volume, nucleo volume weighted mean volume, cellular volume, cytoplasmic volume, mean nuclear diameter and nuclear maximum angle (Agmax) nuclear axial ratio were found increasing between basal and surface segments. But, the nuclear-cytoplasmic (N/C) ratio, nuclear shape factor (NSF) and nuclear circularity index (NCI) were decreasing between these segments. Epithelial cells in the basal segment had the highest N/C ratio and the lowest cell volume due to their low volumes of cytoplasm, then substantial increase of cytoplasmic volume occurred in the intermediate segment and further in basal segment cell. We studied morphometric parameters include nuclear and cytoplasmic dimensions as well as nucleo/cytoplasmic ratio in different colonic and rectal epithelial lesions.

Study on nuclear axial ratio, NSF, NCI and Agmax showed that epithelial nuclei were more ellipsoidal in shape and were aligned more perpendicular to the basement membrane as they reached the surface epithelium. Numerical densities per unit area or volume for epithelial cell nuclei were highest in the basal segment, suggesting that the basal segment was the active proliferating zone. Nuclear shape has been reported to be an important prognostic factor for patients with colorectal carcinoma.

**Aims and Objectives**

Role of morphometry in assessment of different colonic and rectal epithelial lesions with histopathological study and clinical correlation.

**MATERIALS AND METHODS**

A prospective study was conducted in the department of pathology of a tertiary care hospital over a period of two years with the patients with gastrointestinal problems attending the Out-patient Departments of Gastroenterology, General Medicine, and Paediatric Medicine and referred for further investigation including colonoscopic biopsy. 100 patients presenting with the complaints like per-rectal bleeding, pain abdomen, recurrent diarrhea, weight loss, anaemia etc were examined and investigated. This includes colonoscopy and biopsy for histopathological examination. The ones who refused to undergo colonoscopy were excluded from the study. 10 controls were taken from the normal/healthy area of the biopsy material adjacent to the pathological lesion.

Biopsy specimens were processed and paraffin blocks prepared, and then stained tissue sections were examined for...
histopathology and morphometry in colonic epithelial cells.

Clinical parameters like routine blood examinations, USG abdomen, colonoscopy, histopathological study of biopsy samples and special investigations like stool for Occult Blood Test were studied along with following morphometrical parameters:

Mean Nuclear Diameter (MND), Mean cytoplasmic diameter (MCD), Mean nuclear area (MNA), Mean Nuclear Perimeter (MNP), Nucleo-cytoplasmic ratio (N: C) etc.

Morphometric analysis was done on H & E stained histological sections compare these different types of benign, premalignant and malignant lesions with the aid of an ocular morphometer attached to the 10X eyepiece of a microscope using a 40X high power objective. The ocular morphometer was calibrated using a calibration slide or stage micrometer provided alongwith. One smallest division on that stage micrometer had been equated with 0.01 mm. One smallest division of ERMA ocular micrometer is equated with 2.5µ (micrometre). 100 random nuclei from the most atypical area of the sections were subjected to analysis.

The mean nuclear diameter (MND) and the mean cytoplasmic diameter (MCD) were measured directly while the other parameters were derived by calculation as follows. [6, 10]

Mean nuclear area (MNA) = $$\pi \left( \frac{MND}{2} \right)^2 / 4$$
Mean nuclear perimeter (MNP) = $$\pi \times MND$$
Nucleo-cytoplasmic ratio (N:C ratio) = $$\frac{MND}{MCD}$$

At least 10 measurements were taken for the parameters like Nuclear Diameter (ND) and Cytoplasmic diameter (CD) and an average value was taken to represent the Mean Nuclear Diameter (MND), Mean cytoplasmic diameter (MCD).

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.
Koushik Chakraborty et.al. Study of Cellular Morphometry in Colorectal Epithelial Lesions with Clinicopathological Correlation

FIGURE 4 (H&E×400) Adenocarcinoma through ocular morphometer showing nuclear atypia

FIGURE 5 (H&E×400) Poorly differentiated Carcinoma through ocular morphometer

Table 1 Chart showing the measured morphometric parameters of the ten control cases (10 Controls)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Endoscopic finding</th>
<th>Diagnosis</th>
<th>Morphometric Measurements</th>
<th>N.C. Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mND (µ)</td>
<td>mCD (µ)</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
<td>7.87</td>
<td>24.27</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>Normal</td>
<td>5.54</td>
<td>18.79</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>Normal</td>
<td>7.59</td>
<td>22.92</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>Normal</td>
<td>6.78</td>
<td>21.07</td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>Normal</td>
<td>7.55</td>
<td>22.88</td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>Normal</td>
<td>6.69</td>
<td>26.68</td>
</tr>
<tr>
<td>7</td>
<td>Normal</td>
<td>Normal</td>
<td>7.54</td>
<td>22.83</td>
</tr>
<tr>
<td>8</td>
<td>Normal</td>
<td>Normal</td>
<td>6.98</td>
<td>23.17</td>
</tr>
<tr>
<td>9</td>
<td>Normal</td>
<td>Normal</td>
<td>5.96</td>
<td>19.83</td>
</tr>
<tr>
<td>10</td>
<td>Normal</td>
<td>Normal</td>
<td>5.29</td>
<td>33.85</td>
</tr>
</tbody>
</table>

These measurements were taken from normal looking areas of the biopsy specimens and formed the reference values for comparison of different lesions. These control cases were compared with the most benign lesions and subsequently the benign lesions were compared between themselves. Then, the premalignant lesions were compared with the benign ones. And finally, the malignant lesions were compared with the premalignant lesion as well as between themselves.
RESULT AND ANALYSIS

Pie diagram 1 demonstrate that in this study, the most frequently encountered cases were of Inflammatory Bowel Disease (23%) closely followed by Non-specific Colitis (19%). Different inflammatory diseases (Acute Inflammatory colitis, Non-specific Colitis, Inflammatory Bowel Disease, Granuloma/Tuberculosis) accounted for 56% cases. Different forms of Polyps made up 29% cases. Among the polyps, the most common was Juvenile Polyp (15%) and was third common amongst the total cases.

Pie diagram 2 shows that 7% of cases under study are malignant lesion, 15% premalignant and rest 78% are benign.

Table 1 showing the measured morphometric parameters of the ten control cases.

Table 2 demonstrates -Value Chart comparing the morphometric parameters of the different lesions.

Control v/s Acute Inflammatory colitis:-

Significant difference (p value ≤0.05) was noted only between the mean cytoplasmic diameters (p value=0.0015) and consequently in the nucleo-cytoplasmic ratio (p value=0.0016) also. There was no significant difference between the nuclear characters like mean nuclear diameter, mean nuclear area and mean nuclear perimeter. (p value=0.29, 0.271 and 0.296 respectively)

Acute Inflammatory colitis v/s Non-specific Colitis:-

Significant difference (p value ≤0.05) was noted only between the mean cytoplasmic diameters (p value=0.018) and consequently in the nucleo-cytoplasmic ratio (p value=0.0009) also. There was no significant difference between the nuclear characters like mean nuclear diameter, mean nuclear area and mean nuclear perimeter. (p value=0.806, 0.771 and 0.804 respectively)

Non-specific Colitis v/s Inflammatory Bowel Disease (Figure 1):-

There was no significant difference between the nuclear characters like mean nuclear diameter (p value=0.663), mean nuclear area (p value=0.591) and mean nuclear perimeter (p value=0.664). The mean cytoplasmic diameters (p value=0.808) and the nucleo-cytoplasmic ratio(p value=0.960) also failed to show any significant difference in morphometric measurements.

Inflammatory Bowel Disease v/s Granuloma:-

There was highly significant difference between the nuclear characters like mean nuclear diameter (p value=0.002), mean nuclear area (p value=0.004) and mean nuclear perimeter (p value=0.003). But the mean cytoplasmic diameters (p value=0.080) and the nucleo-cytoplasmic ratio (p value=0.17) failed to show any significant difference in morphometric measurements.

This significant difference in the nuclear characters can probably be attributed to the presence of Granuloma in which the cells are clustered together and there are giant cell formations. Granuloma formation can occur in Inflammatory Bowel Disease also but the giant cells are typically lacking.
Granuloma v/s Hamartomatous Colonic Polyp:
There was highly significant difference between the nuclear characters like mean nuclear diameter (p value=0.0006), mean nuclear area (p value=0.0008) and mean nuclear perimeter (p value=0.0006) as well as the mean cytoplasmic diameters (p value=0.0004). The nucleo-cytoplasmic ratio (p value=0.297) however failed to show any significant difference in morphometric measurements. This significant difference, mainly in the nuclear characters can probably be attributed to the presence of Granuloma in which the cells are clustered together and there are giant cell formations.

Hamartomatous Colonic Polyp v/s Hyperplastic Polyp (Figure 2):
There was no significant difference between the nuclear characters like mean nuclear diameter (p value=0.94), mean nuclear area (p value=0.776) and mean nuclear perimeter (p value=0.940). The mean cytoplasmic diameters (p value=0.539) and the nucleo-cytoplasmic ratio (p value=0.401) also failed to show any significant difference in morphometric measurements.

Hyperplastic Polyp v/s Juvenile Polyp:
There was no significant difference between the nuclear characters like mean nuclear diameter (p value=0.06), mean nuclear area (p value=0.053) and mean nuclear perimeter (p value=0.054). The nucleo-cytoplasmic (p value=0.465) ratio also failed to show any significant difference in morphometric measurements. The mean cytoplasmic diameters (p value=0.0004) however reported a significant difference which can probably be attributed to the average high cytoplasmic diameters recorded in the cases of Hyperplastic Polyp studied. However, only 2 cases of Hyperplastic Polyp were found in the study population while there were 15 cases of juvenile Polyp for comparison and hence this difference cannot claim significance.

Juvenile Polyp v/s Adenomatous Polyp (Figure 3):
There was highly significant difference between the nuclear characters like mean nuclear diameter (p value=0.0013), mean nuclear area (p value=0.0015) and mean nuclear perimeter(p value=0.0013). The nucleo-cytoplasmic ratio (p value=0.00003) also showed a significant difference. The mean cytoplasmic diameters (p value=0.437) however failed to show any significant difference in morphometric measurements.

This can well be attributed to the fact that Adenomatous Polyps are premalignant lesions and they are typically characterized by Dysplastic changes. It is a point to be noted that these Dysplastic changes occur in the nucleus and hence we can see the significant difference between nuclear measurements when this Dysplastic nuclei of the Adenomatous Polyps are compared against the benign condition Juvenile Polyp. This nuclear Dysplasia also leads to appreciable change in the nuclear size resulting in a significant difference between the nucleo-cytoplasmic ratio of the Adenomatous Polyps when compared against the Juvenile Polyps, the latter being a benign condition. At the same time, we must consider the fact that these Dysplastic changes are associated with the nucleus only and the cytoplasm is yet to attain those changes in a premalignant lesion like Adenomatous Polyp. So, it is not surprising that the mean cytoplasmic diameter of these two lesions failed to show any significant difference.

Adenomatous Polyp v/s Adeno Carcinoma (Figure 4):
There was no significant difference between the nuclear characters like mean nuclear diameter (p value=0.84), mean nuclear area (p value=0.067) and mean nuclear perimeter(p value=0.084). The mean cytoplasmic diameters (p value=0.0002) and the nucleo-cytoplasmic ratio (p value=0.00003) did show immensely significant difference in morphometric measurements.

As already discussed in the context of Adenomatous Polyp v/s Juvenile Polyp, the
nuclear dysplasia occurring in Adenomatous Polyps are precedent of the nuclear changes in Adenocarcinoma. Hence their nuclear measurements are closer to malignant lesions like Adenocarcinoma and therefore fail to show any significant difference when compared to the latter. However in malignancies like Adenocarcinoma, the entire cell attains malignant characters but the cytoplasm of premalignant lesions like Adenomatous Polyps are of rather benign proportions. Hence we do find a significant difference between the cytoplasmic measurements between these two lesions.

**Adeno Carcinoma v/s Poorly-differentiated Carcinoma (Figure 5):**

There was a highly significant difference between the nuclear characters like mean nuclear diameter (p value=0.006), mean nuclear area (p value=0.0023) and mean nuclear perimeter (p value=0.006) and also between the nucleo-cytoplasmic ratio(p value=0.0018). The mean cytoplasmic diameters (p value=0.928) however failed to show any significant difference in morphometric measurements.

This can well be explained by the fact that cellular characters of Poorly-differentiated Carcinoma are very much different from those of Adenocarcinoma and it is this difference that makes it an altogether different entity. Such Poorly-differentiated Carcinomas might have risen from Adenocarcinomas or else have appeared de novo; but even in the former situation, a tumour to be labeled as Poorly-differentiated Carcinoma, has to lose all the nuclear and cytoplasmic characters of a typical Adenocarcinoma.

**DISCUSSION**

This study was done to assess the role of morphometry in classifying colorectal epithelial lesion lying in the borderline between benign and malignant imposing diagnostic difficulty. Nuclear morphometry is an objective method that can rule out minute variation of nuclear size and shape. Changes in morphometric value have been shown to be associated with progression from normal colonic mucosa to polyps and cancer.

With In these study one hundred cases benign, malignant as well as premalignant colorectal epithelial lesions were considered. The benign lesions included Acute Inflammatory colitis, Non-specific Colitis, Inflammatory Bowel Disease, Granuloma of Tuberculosis, Hamartomatous Colonic Polyp, Hyperplastic Polyp, and Juvenile Polyp. Adenomatous Polyp showing dysplasic changes is considered premalignant, while Adenocarcinoma and Poorly-differentiated Carcinoma are malignant by definition.

However, the benign lesions were encountered more frequently and comprised 78% of the studied cases. Malignant lesions made up 15% of the cases while the rest 7% were premalignant lesions.

In this study, the most frequently encountered cases were of Inflammatory Bowel Disease (23%) closely followed by Non-specific Colitis (19%). Different inflammatory diseases (Acute Inflammatory colitis, Non-specific Colitis, Inflammatory Bowel Disease, Granuloma/Tuberculosis) accounted for 56% cases. Different forms of Polyps made up 29% cases. Among the polyps, the most common was Juvenile Polyp (15%) and was third common amongst the total cases.

A vast majority of the cases were between 21 to 60 years (62%) and one fifth of the study population was within 20 years of age. The aged (>60 years) had only 18% representation in this study. But among these aged patients, there was a very poor female representation (6%).

Females were a minority in this study population (41%) and among them, about three-fourth came with different inflammatory lesions. Only meager 3% females came with carcinomas. But for the majority male population (59%), nearly one fourth of the cases (24%) reported malignancy. Polyps were reported more among the males (32%) but they were still second to the various inflammatory lesions which made up 44% of male cases.
Morphometric measurements revealed minor differences between the benign lesions, but cases of Tuberculosis with Granuloma had significant differences with other benign lesions on the basis of nuclear features mainly because of the Giant cell formation. Adenomatous Polyp, a premalignant lesion, showed marked difference in nuclear characters from other benign polyps.

However the aforesaid entity differed from Adenocarcinoma only on cytoplasmic measurements. Adenocarcinoma and poorly differentiated carcinoma differed from each other significantly not only on nuclear characters but also on nucleo-cytoplasmic ratio.

Thus, morphometric analysis proved to be very useful in distinguishing benign lesions from premalignant ones as well as premalignant lesions from malignant ones. Additionally, morphometric parameters could also distinguish Tubercular Granuloma from other benign lesions including granuloma forming Inflammatory Bowel Disease.

Dragan Mihailovic et al showed in his study showed mean volume-weighted nuclear volume, nuclear area and perimeter of epithelial cells surrounding carcinoma was significantly higher than in adenoma with low-grade dysplasia or chronic colitis with the mean volume weighted nuclear volume had a sensitivity and specificity of 90.5% and 92.7%, respectively.

PW Hamilton et al had conducted discriminant analysis with different parameters including variability of nuclear size, shape and nucleo-cytoplasmic ratio of colonic epithelial cells. Their study had successfully illustrated that morphometric measurements could help to differentiate Adenocarcinoma of colon from normal colonic mucosa as well as from benign lesions like Ulcerative Colitis. Our findings are very much in keeping with their findings and inference.

Sato E et al had used electron microscopy with image analyzer to compare the nuclear areas, perimeter, diameter and form factor of different colonic epithelial lesions including normal epithelium, Peutz-Jeghers polyp, adenoma, dysplasia and carcinoma and demonstrated that the area of nuclei was significantly larger in dysplasia and carcinoma than the normal. That study suggested the possibility of quantitative diagnosis of epithelial lesions by measuring the area and perimeter of some 50 cells of each lesion. Our study had been conducted with measurement of morphometric parameters of 10 cells of each lesion and we could arrive at similar inference with statistical significance.

Astrid Richter et al had used morphometry as an effective tool to differentiate colonic epithelial cells of normal subjects from those of precancerous lesions in HNPCC (Hereditary non polyposis colon cancer) individuals. In our study also, we have been able to discriminate premalignant lesions from malignancies by the use of morphometry.

Ikeguchi et al in 1994 conducted a study on morphometric nuclear features (nuclear area, perimeter, and shape) were analyzed in 343 patients with colorectal carcinoma and in 57 patients with colorectal adenoma .The mean nuclear area (NA) was found to be enlarged in adenoma and carcinoma (normal mucosa: n 5 343, mean NA 5 19 mm2; adenoma: mean NA 5 34mm2; mucosal carcinoma: n 5 15, mean NA 5 45 mm2; P =0.001). Among 343 colorectal carcinomas, NAs of cancer cells in tumors with lymphatic invasion, venous invasion, lymph node metastasis, or hepatic metastasis were significantly larger than those of cancer cells in tumors without such factors. Moreover, the large nuclear area of cancer cells was recognized as one of the risk factors of metachronous hematogenic metastasis in patients after curative surgery.

Fernández-López F et al investigated relationships postsurgery survival and nucleus morphometry in 90 patients of colorectal tumor. The nucleus-size variables like maximum diameter, minimum diameter, perimeter, area (means
for 100 nuclei from each patient were used in all cases).showed that patients with large maximum nucleus diameter (where large = greater than the first quartile) had significantly worse survival than patients with smaller maximum nucleus diameter (mean survival, 28 vs. 43 months). Similar results were found for the other nucleus-size variables.

Nuclear atypia was independently and significantly associated with lymph node metastasis by multivariate analysis in a study conducted by Kojima M et al. [18]

James W Bacus et al [11] had used image morphometric method to detect a systematic effect of lowered mean nuclear grade and a decrease in the variability of nuclear grade expression in a chemoprevention setting. They demonstrated how morphometry could be very useful in supplementing the pathologist’s histopathological grading by providing objective, quantitative assessments.

However, only colonoscopic biopsy specimens were considered in our study. Hence the extent of lesion could not be assessed properly as in basement membrane breach. So this study cannot throw light on means to differentiate carcinoma in situ from invasive neoplasms.

CONCLUSION

We can herein arrive at the inference that even with the advent and popularity of immunohistochemistry, morphometric study can come in handy in reliably resolving diagnostic dilemmas between benign, premalignant and malignant colorectal epithelial lesions in conjugation with histopathology.

REFERENCES
13. Dragan Mihalovic Karyometric Analysis of Precancerous Lesions of Human Colorectal
Mucosa Microscopy and Analysis (UK), 2002 91, 27-29

How to cite this article: Chakraborty K, Sarkar S, Manna AK et al. Study of cellular morphometry in colorectal epithelial lesions with clinicopathological correlation. International Journal of Research and Review. 2019; 6(7):1-10.

*****