



Original Article

Histological Scoring of Rectal Mucosa by Digital Quantitative Method in Bangladeshi Subjects

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Abstract

Chronic inflammation is characterized by increased numbers of round cells in lamina propria, associated with irregularity of glandular system and increasing number of glands per area. But still there is no objective way to demarcate between normal and diseased mucosa by counting the number of inflammatory cells. Until date it is done arbitrarily which depends primarily upon the knowledge and experience of pathologist. So this study has been done with aims to score normal rectal mucosal histology using digital quantitative methods in Bangladeshi subjects. The variable recorded were the diameter of ten consecutive transversely cut glands, the space of lamina propria between glands and number of gland per high power field. Thirty patients with ankylosing spondylitis having normal rectal mucosa were enrolled for histological scoring by digital quantitative methods. In this study it is found that 90% of subjects have the score of 17 or less. This data obtained serve as a basis for demarcating the histological state of rectal mucosa from various chronic inflammatory condition of rectum.

TAJ 2009; 22(2): 190-193

Introduction

Conforming the normal architecture of alimentary canal, large gut consist of four layers. Unlike other part of alimentary canal, morphometry of large gut mucosa is influenced much by environmental factors. Enteric bacterial flora affects the structure of large gut mucosa by inciting physiological inflammation from neonatal period (1). As microflora in the gut and dietary factors affects the structure of colonic mucosa, there may be individual variation in the normal colonic mucosal histology. Chronic inflammation of the rectal mucosa is mainly characterized by

increased infiltration of round cells in lamina propria. Other histological characteristic are based on regularity of the glandular systems and number of mucosal glands per defined areas. Several authors have defined the histological characteristics of the normal rectal mucosa (2,3,4). but still there is no objective way to demarcate between normal and diseased mucosa by counting the number of inflammatory cells. Presently it is done arbitrarily which depends heavily upon the knowledge and experience of pathologist (5). Keeping this fact in mind Rubio et al devised an objective method to score normal rectal mucosal

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histology.(6).In this study we have tried to score the histology of normal rectal mucosa using digital scoring methods in Bangladeshi subjects.

Methods and Materials

38 patients with ankylosing spondylitis having no gastrointestinal symptoms were subjected to colonoscopy for evaluation of bowel pathology . The main purpose of examination was to see the colonic abnormalities in ankylosing patient without any gastrointestinal symptoms. Out of 38patients, 4 patients showed naked eye colorectal abnormalities. And remaining 34 patients had no visible colorectal lesion and four pieces of biopsy taken from rectal mucosa , placed in a fixative of 4% neutral formalin and send for histopathological study. The mucosa that did not show any inflammatory lesion microscopically, are considered normal and evaluated for histological scoring using digital scoring methods advocated by Rubio and Koch. Finally 30 patients were enrolled for this study.

This study was carried out in Rheumatology wing of department of medicine in collaboration with the department of gastroenterology and pathology , BSM medical university , Dhaka. The study period extended from Nov 2004 – Oct 2005. Four main histological features were considered which have been taken from the work done by Rubio and Koch (1981). One representative section were chosen to record the following items:

- 1) The diameter (minor axis) ten consecutive , transversely cut glands.
- 2) The linear distance along the lamina propria between ten consecutive glands.
- 3) The number of glands per area.
- 4) The number of nuclei in ten consecutive areas of lamina propria between glands.

The fourth items has not possible to used because of technical constraints. The diameter of the glands and the distance between them were measured by the aid of a calibrated ocular microscale. As regards the number of glands per area, the following procedure was applied : all entire glands found in the field and the gland cut by left side of the microscopic field (i.e, clockwise6 to 12 h) were counted but not those cut

by the right side of the field (i.e, Clockwise 12 to 6h). All measurement and observations were done at $\times 480$ magnification ($\times 10$ ocular and $\times 40$ objectives)and $\times 122$ magnification.

Data were analyzed with statistical package for social science (SPSS). Data were expressed as mean and standard deviation .Frequencies of data were calculated as percentages.

Results.

Thirty patients were studied for histological scoring of rectal mucosa for the purpose to demarcate between normal and inflamed mucosa. The age range of patients were 19-42 with mean age 26.78 ± 6.40 and male : female ratio 19:11. Table 1 showed the mean diameter of transversely cut rectal glands and measurement varies between $16-90 \mu\text{m}$. Majority of subjects the glandular diameter were within $46- 60 \mu\text{m}$. Interglandular linear distance have been displayed in table 2 . Here it is seen that the distance between glands were ranging from 4.6 to $22.5 \mu\text{m}$. In Most subjects 21(70%), it was 4.6 to $13.5 \mu\text{m}$. Regarding the number of gland per area, this study depicted the number of glands in defined areas were between 6-35 and majority(80%) of subjects it was 16-25per defined areas.

Total digital scoring i.e. the sum of values of above three criteria were estimated to simplify the definition of normalcy in individual patients and for future comparison of normal mucosa and inflamed mucosa. In this study total digital score were ranged from 14-18 with mean & SD (15.5 ± 1.35). In 80% cases total score was ≤ 16 and more than 16 in 6 (20%)cases (Table-4).

Table 1: The mean glandular (minor) diameter in 10 consecutive glands in rectal mucosa (n=30)

Glandular diameter in μm	No. of subjects	Digital code
0-15	00 (0%)	1
16-45	03 (10%)	2
46-60	18 (60%)	3
61-75	06 (20%)	4
76-90	03 (10%)	5
91-105	00 (0%)	6
106-120	00 (0%)	7

Table 2: The mean linear distance of lamina propria between 10 consecutive glands (n=30)

Distance between glands μm	No. of subjects	Digital code
0-4.5	00 (0%)	1
4.6-9.0	09 (30%)	2
9.1-13.5	12 (40%)	3
13.6- 18.0	06 (20%)	4
18.1-22.5	03 (10%)	5
22.6-27.0	00 (0%)	6
27.1- 31.5	00 (0%)	7
31.6-36.0	00 (0%)	8
>36	00 (0%)	9

Table 3: Number of glands in defined area (n=30)

No. of glands per area	No. of subjects	Digital code
≤ 5	00 (0%)	10
6-10	01 (3.3%)	9
11-15	02 (6.6%)	8
16-20	18 (60%)	7
21-25	06 (20%)	6
26-30	02 (6.6%)	5
31- 35	01 (3.3%)	4
36-40	00 (0%)	3
41-45	00 (0%)	2
>45	00 (0%)	1

Table 4: distribution of Total digital score (Sum of values from three characters)(n=30).

Total digital score from sum of values of three characters	No. of subjects
14	09(30%)
15	06(20%)
16	09(30%)
17	03(10%)
18	03(10%)

**Fig. 1 :** Microscopic specimen of histological stage-1: Rectal mucosa showing mild infiltration of mononuclear cells in lamina propria and in crypt base (H & E x 480).**Fig. 2:** Microscopic specimen of histological stage-1: Rectal mucosa showing mild infiltration of mononuclear cells in lamina propria and in crypt base (H & E x 122)

Discussion

Normal individual may contain inflammatory cells in lamina propria of colorectal mucosa and there is wide range of variation of such cells in normal subjects. As a result the interpretation on histological variation of normal limits is entirely depends on deep knowledge and long experiences of histological expertise. It is noteworthy that many works have been done on histological description of normal rectal mucosa(2,3,4 6,7). But few attempts have been made to quantitate the numbers of cells present in the normal mucosa. Korelitz and Sommers investigated the number of mucin producing cells in the glands and the type of inflammatory cells by differential cell counts(8). In fact they have not defined objectively the characteristics of the mucosa of individual patient but they describe only some features related to normalcy .In this study we have tried to score the histology of rectal mucosa by some characteristics devised by Rubio and Kock in 1981(6). There is a little limitations of this study that we could not use all four items for scoring. One have been omitted because of Instrumental constraint. Nevertheless sum of the three features analyzed separately for each subjects and it is observed that 80% of the patients have score of16(80%). So it is very much comparable to study done by Rubio and kock(6).Therefore we can come to conclusion from this study that the normal histological characteristics of rectal

mucosa in our population are almost similar with western individuals. Finally before making conclusion a large scale study using four characteristics aforementioned with colorectal biopsy from normal subjects could be attempted and that would be useful for expertise to demarcate normal mucosa from inflammatory mucosa in histopathological field of our country.

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