Histochemical and immunohistochemical techniques in ulcerative colitis

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ABSTRACT

Objective: To demonstrate the mucin changes in Ulcerative Colitis, to evaluate the benefit of the staining method and to demonstrate the CEA staining pattern in dysplasia complicating ulcerative colitis (U.C).

Methods: Colorectal biopsies were examined for changes of U.C for which a combined PAS/Alcian blue stain was applied. CEA immunohistochemical stain was used for cases of dysplasia complicating ulcerative colitis.

Results: One hundred colorectal biopsies were examined, U.C. was diagnosed in (22%) of cases, the mean age was (36.68) years, (13) were males, and (9) were females. The prevalence of dysplastic changes complicating U.C were detected in (22.7%). The rectum was the most frequent site of dysplasia complicating, U.C.. Two of the cases showed mild dysplastic changes; two showed moderate dysplasia, and only one showed severe dysplasia. The secretory activity of mucin in colorectal mucosa was tow in surface epithelium and varied in the crypts from absent or weak to moderate reduction.

The site of CEA distribution seemed to be affected mainly by degree of dysplasia, it was predominantly along the apical surface of the cells and also in the cytoplasm in case of mild dysplasia, while in moderate dysplasia it was of cytoplasmic distribution, and in severe dysplasia there was intensive cytoplasmic distribution.

Conclusion: There was an increase in relative frequency of U.C. Immunohistochemical study of CEA localization in dysplastic gland is helpful in detection of early malignant change in U.C.

الخلاصة:

الهدف: توضيح تغيرات المخاط في مرض التهاب القولون التقرحي وتقييم جدوى الطريقة لإيضاح المظهر الصبغي لـ CEA في مصاعف الحال لالتهاب القولون التقرحي. الطرق: أجرى فحص لخزعات من القولون ومن المستقيم بصدد تغيرات التهاب القولون التقرحي بصورة

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Icerative colitis is a chronic inflammatory disease of the rectum and colon \cite{1,2,7}, and usually affects patients between (20-30) years, but may occur in younger and older individuals \cite{1,2,6,8,9}. It is characteristically a left-sided disease that usually begins in the rectosigmoid area, in some cases it remains localized to the rectum (ulcerative proctitis), but in most instances, it spreads proximally and sometimes involves the entire colon (pancolitis)\cite{6-8}.

Mucin changes in icerative colitis: In fulminant and acute active cases of short duration, the decrease in mucosubstance is slight or moderate, while in chronic active cases it is moderate or severe and these changes tend to revert again towards normal in quiescent phase \cite{10}.

Dysplasia: It is an unequivocally neoplastic but non-invasive epithelial proliferation of the colonic epithelium \cite{11,12}.

Riddel et al. (1983) Classified dysplasia into \cite{11}:
1- Negative for dysplasia: which includes all inflammatory and regenerative lesions.
2- Indefinite for dysplasia; is applied to epithelial changes that appear to exceed the limits of ordinary regeneration.
3- Positive for dysplasia: which includes only cases with unequivocally neoplastic mucosa \cite{11}.
4- Microscopically, dysplasia is identified on the basis of combination of microscopic features, which include;
5-1- Architectural alteration exceeding that resulting from repair in chronic colitis.
2- Cytological abnormality principally cellular and nuclear pleomorphism, nuclear hyperchromatism, loss of nuclear polarity, mitosis, marked stratification of nuclei and variable mucin depletion\textsuperscript{(11,12)}.
6-The distribution of CEA in normal epithelium is limited to the apical surface, while in dysplasia and carcinoma it is abundantly present in both cytoplasm and the lumen\textsuperscript{(8,13,14)}.

Normally Colorectal goblet cells are containing mainly acid mucin with small amount of neutral mucin \textsuperscript{(15)}. The composition of the goblet cell mucin varies within their level in the crypt and in different segments of colon; in the left colon sulphamucin predominates in the lower half of the crypt, whereas in the upper crypt and surface epithelium a variable proportion of sulphamucin and sialomucin are often demonstrated.

CEA is a glycoprotein of heterogeneous composition (MW 200,000), detected in small amounts in normal adult cells and benign colorectal tumors, but is present in large amounts in carcinoma \textsuperscript{(8)}. The immunocytochemical localization of CEA in colorectal tissue may have a potential value in the diagnosis of premalignant and malignant lesions \textsuperscript{(14)}.

**Patients and methods**

Colorectal biopsies were collected prospectively from (100) non selected patients with symptoms of large bowel disorders examined at the endoscopic units of AL-Jamhouri Teaching Hospital, AL-Salam Hospital, and private hospitals, in the period from October 2001 to July 2002. The age ranged from (2) months to (80) years. Medical history that included; age, sex and main presenting symptoms were recorded. All biopsies were fixed immediately in (10\%) formaldehyde for (24) hours, then the samples were processed routinely and embedded in paraffin blocks and stained with Haematoxylin and Eosin (H&E) stain.

1. 1-A combined PAS-Alcian blue (pH.2.5) staining for neutral and acidic mucin were done respectively.
2. According to (Riddel et al., 1983) criteria \textsuperscript{(11)}. Immunohistochemical staining for CEA was performed in cases of U.C to detect dysplastic changes by using an improved Biotin- streptavidin Amplified (BSA) detection system. The staining procedure was done according to the manufacturer instructions of staining protocol \textsuperscript{(16)}.

**Results**

Ulcerative colitis was diagnosed in (22\%) of cases, in which different forms of disease activity were seen, table (1). The mean age was (36.68) years, ranging from (12 to 60) years, (13) were males, and (9) were females, tables (2,3). The prevalence of dysplastic changes complicating U.C were detected in (22.7\%) with mean age (41.8) years ranging from (29 to 60) years, three of them were females and two of
them were males, table (3). The sex
distribution was statistically insignificant.
The rectum was the most frequent site
showing dysplasia complicating, U.C. 3/5
compared to other sites. Two of the cases
showed mild dysplastic changes; two with
moderate dysplasia, and only one had
severe dysplasia, table (4).

Mucin changes in U.C: The result of
PAS/Alcian blue techniques for the
demonstration of mucin, failed to detect
any qualitative difference in reactivity in
U.C. The secretory activity in colorectal
mucosa was diminished in surface
epithelium and varied in the crypts from
absent or weak to moderate reduction,
table(1).

Distribution of CEA immunohistochemical
staining in dysplasia complicating U.C: In
mild dysplasia, CEA distribution was
demonstrated predominantly along the
apical surface of the cells, while in
moderate dysplasia and in severe
dysplasia there was intensive cytoplasmic
staining.

Table 1: Mucinae changes in U.C. and dysplasia.

<table>
<thead>
<tr>
<th>Pathological Diagnosis of U.C.</th>
<th>No of cases</th>
<th>PAS score</th>
<th>Alcian blue score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>10</td>
<td>3 ++</td>
<td>3 ++</td>
</tr>
<tr>
<td>Inactive</td>
<td>7</td>
<td>1 +</td>
<td>2 +</td>
</tr>
<tr>
<td>Dysplasia: mild</td>
<td>2</td>
<td>1 ±</td>
<td>1 ±</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>1 ±</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>2 ± 8</td>
<td>1 ± 5</td>
</tr>
</tbody>
</table>

Table 2: Sex distribution of U.C. with and without dysplasia.

<table>
<thead>
<tr>
<th>sex</th>
<th>Ulcerative colitis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With dysplasia</td>
<td>Without dysplasia</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>total</td>
<td>5</td>
<td>17</td>
</tr>
</tbody>
</table>

(P value using Chi-square test=0.323). copic findings in U.C with dysplasia.
Table 3:

<table>
<thead>
<tr>
<th>Age (Year)</th>
<th>Sex</th>
<th>Site</th>
<th>Endoscopy</th>
<th>Symptom</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>Female</td>
<td>Descending colon</td>
<td>Ulceration</td>
<td>Bleeding per rectum</td>
<td>Mild</td>
</tr>
<tr>
<td>31</td>
<td>Female</td>
<td>Rectum</td>
<td>Thickening</td>
<td>Bleeding per rectum</td>
<td>Moderato</td>
</tr>
<tr>
<td>38</td>
<td>Male</td>
<td>Sigmoid</td>
<td>Polyp</td>
<td>Bleeding per rectum</td>
<td>Moderate</td>
</tr>
<tr>
<td>50</td>
<td>Female</td>
<td>Rectum</td>
<td>Ulceration</td>
<td>Bleeding per rectum</td>
<td>Mild</td>
</tr>
<tr>
<td>60</td>
<td>Male</td>
<td>Rectum</td>
<td>Ulceration</td>
<td>Bleeding per rectum</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Table 4: Predominance of CEA distribution in colonic biopsies of U.C with dysplasia

<table>
<thead>
<tr>
<th>Grade</th>
<th>Apical</th>
<th>Cytoplasmic</th>
<th>Basolateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>+</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Moderate</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Severe</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Discussion

Ulcerative colitis constituted (22%) of the cases, which is higher than (18%) reported by Saadi (1989) (18). This may be due to:
1. wider use of sigmoidoscopy.
2. our physicians now have high index of suspicion for U.C.

In Europe and North America, higher figures were also reported (36%) (5,19). The age ranged from (22 to 64) years with peak age distribution at 3rd decade, this more or less is similar to the studies of others (15-25) (19,20,21). Some reported higher figures(38.1)years (22,23). Regarding sex distribution, males were affected more than females, while (Al-Nakib et al., 1984) found a nearly equal sex distribution (F/M=21 /22)(2).

Mucin Pattern in U.C.

The study of mucin pattern in U.C. showed that, both types of mucin were detected but in decreased amount indicating that, the change in mucin is quantitative rather than qualitative; this finding is similar to that observed by Nazar (24). The amount of mucin decreased when the activity of disease is increased, this observation is in agreement with other workers (25,26,27). In our study, all types of mucin present in an inactive colitis were higher in amount than that found in active colitis, this is consistent with observations of other workers (28,29).

Special attention has been focused on pre-carcinomatous changes (dysplasia) in patients with U.C. which is considered as a histological marker for increased cancer risk, and thus, as a potential indication for colectomy in patients with U.C. In the present study, (22.7%) of cases of U.C. had dysplastic changes and this is more or less similar to the some studies (21 %)(12,30), and higher than that reported , by (Katran), and (Subbar), (14.7) and (6.5)
respectively \(^{(25,31)}\).

The frequency of the grade of dysplasia was (40%) mild dysplasia, (40%) moderate dysplasia, while severe dysplasia constituted (20%). These figures are more or less similar to those studies (18% mild, 35% moderate, 47% severe)\(^{(25,32,33)}\).

Statistically, it was found that there was no significant relationship between dysplasia complicating U.C. and sex distribution, which is in agreement with another study done by (Katran, 2001)\(^{(25)}\).

CEA Immunohistochemical Study:

We found a good correlation of CEA localization and Severity of dysplasia; this also was observed by others\(^{(17,25)}\). So this marker is considered as reliable indicator for pre-malignant changes in U.C.

References

15. Sugihara K., Jass J.R., and Colorectal


