Fate and ultra-structural features of chicken skin mucosa around juvenile polyps

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Abstract

Objective : **Chicken skin mucosa (CSM) is a common finding around juvenile polyps in children. Its ultrastructural features and fate after polypectomy are not yet clear. The aim was to study ultra-structural features and outcome of this CSM compared to that of the polyps and distant endoscopically normal mucosa.**

Material and Methods : **From 240 children with juvenile polyps, 45 needed a second colonoscopy. Thirty six patients showing CSM represented the cohort of this study. One polyp only was studied in each patient. The histologic features of the CSM were compared to normal and polyp mucosa. The fate of CSM was evaluated in the second colonoscopy.**

Results : **The mean numbers of intraepithelial lymphocytes, as well as *lamina propria* inflammatory cellular infiltrates were significantly higher in polyp mucosa than in CSM. Goblet cells were significantly higher in CSM compared to normal mucosa with marked depletion in the polyp mucosa. The *muscularis mucosae* thickness was significantly higher in CSM compared to polyps ($p < 0.0001$) and both showed higher values than the normal mucosa. The CSM almost disappeared within a month period following polypectomy.**

Conclusions : **The polyp showed the most intense mucosal inflammatory reaction. CSM with the unique thickening of *muscularis mucosae* especially around larger polyps almost disappeared after polypectomy. So these results suggest that CSM is a benign compensatory reaction induced by the mechanical effect of the polyp.** (Acta gastroenterol. belg., **2011**, 74, **17-21**).

Key words : chicken skin mucosa, histopathology, juvenile polyps.

Introduction

Juvenile polyps are the most common bowel polyps in children (1). They show surface erosions and reactive epithelial changes with diffuse inflammatory reaction in the *lamina propria* (2). Shatz *et al*. (3) first described chicken skin mucosa (CSM) as an endoscopic finding associated with adenomatous polyps and adenocarcinoma, suggesting a preneoplastic lesion. Later it was noticed during colonoscopic evaluation of colonic mucosa adjacent to juvenile polyps in 73% of children (4). Moreover, it was not found in association with familial adenomatous polyposis, Peutz-Jeghers syndrome or juvenile polyps with adenomatous changes in children (5). The detailed ultrastructural features of CSM in relation to the polyp mucosa and their fate after polypectomy are not well studied.

Hence, the aim of this work was to study the ultrastructural features of this particular colonic mucosa compared to that of the polyps and distant endoscopically normal mucosa and follow up their outcome after polypectomy.

Material and methods

After approval of the Institutional reviewing board of the Pediatrics Department, Faculty of Medicine, Ain Shams University, an informed consent was taken from parents of patients presenting to Gastroenterology Unit, Children's Hospital, Ain Shams University with suspected colonic polyps. The polypectomy was discussed as a therapeutic step but the use of the polyp, adjacent and distant mucosal biopsies for the sake of the study was clear and discussed as a research issue. From January, 2007 to December, 2009, among 520 patients presenting with bleeding *per rectum* (BPR) (502) or protruding mass from anal opening (18), 240 patients proved to have juvenile polyps. Forty five patients had indication of a second colonoscopy because of bad preparation hindering full colonoscopy. This second colonoscopy was planned within a month according to the time schedule of the unit. Out of the forty five patients, thirty six patients showed CSM at their first colonoscopic assessment and were considered the cohort of this study. In all of the 36 patients we were able to do polypectomy of one polyp during the first colonoscopy in addition to biopsies taken from CSM and normal mucosa.

Exclusion criteria :

1. Children who proved to have adenomatous polyps or Peutz-Jeghers syndrome.
2. Positive family history of juvenile polyps.
3. Children with ulcerative colitis with polypoid masses of the colon or other inflammatory polyps.

Each patient was subjected to medical history-taking with special emphasis on gastrointestinal symptoms.

Careful clinical examination was conducted with special emphasis on vital signs, abdominal examination and *per rectum* (PR) examination.

Basic laboratory assessment included complete blood count (CBC) by Coulter 1660, bleeding profile including bleeding time (BT), clotting time (CT), prothrombin time (PT) and partial thromboblastin time (PTT).

Colonoscopy was done using pediatric Pentax video colonoscope (PENYAX GmbH CO. Julius Vosseler

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Strasse 104, 22527 Hamburg, Germany). Mechanical bowel preparation was done using oral sodium pisocolate drops, clear fluid diet and enemas (6). All patients had light sedation by giving midazolam (dormicum-Roche) slow IV injection in a dose of 0.05-0.08 mg/kg while observing the patient's vital functions and level of consciousness in addition to electronic monitoring of vital data. Biopsies were taken from CSM (2 biopsies around one polyp in each patient, 1 cm from the base of the polyp) and from distant colonoscopically normal mucosa (2 from each) just before polypectomy. In cases with multiple polyps, the largest one was included in the study. Pedunculated polyps were removed with an electrocautery snare using voltage of 2-3 milliamperes (7). For light microscopic examination, the obtained specimens were fixed in 10% formalin and dehydrated in ethyl alcohol. Paraffin blocks were prepared ; 3-5 μm thick sections were cut using a Shandon AS 325 microtome. Sections were stained with hematoxylin and eosin. Measurements were done using an image analyzer (Leica Q500). The number of goblet cells, macrophages, eosinophils, neutrophils and lymphocytes were counted digitally in 6 serial sections at a magnification of 400. The thickness of muscularis mucosa was measured by drawing vertical lines from the inner limit to the outer limit of the muscle layer in 6 serial sections in each group.

Biopsy from CSM on second colonoscopy was done only when this endoscopic finding was still present at the same distance recognized in the first session.

Scanning electron microscopy was done for 12 patients (polyp, adjacent mucosa and distant mucosa for each patient). The specimens were put in buffered gluteraldehyde 2.5%. The specimens were rinsed 2 twice in phosphate buffer 15 minutes each. Rinse buffer was removed and replaced with post-fixative 1% OSo4 in 0.1 M phosphate buffer pH 7.2 for 2 hours. The tissue was dehydrated in ethanol and dried using critical point dryer.

Statistical methods

A standard computer program for windows, release 10.0 (SPSS, inc, USA) was used for data entry and analysis including description of quantitative variables in the form of mean, standard deviation (SD) and range, description of qualitative variables in the form of frequency and percentages and comparisons of different variables in various groups were done using Friedman Test, Wilcoxon, Signed Ranks Test, and Student T Test. P-Value was used to define degree of statistical significance ($P < 0.05$ was considered significant and $P < 0.01$ was considered highly significant).

Results

The patients of the study were 27 males and 9 females (male to female ratio of 3:1). The age ranged from 24 to 144 months and a mean age of 49.3 ± 20.2 months. All of them presented with BPR with a duration ranging between 45-364 days and a mean of 200.1 ± 134.0 days. Duration of last episode of BPR ranged from 4-90 days with a mean of 35.0 ± 25.9 days. The most common associated symptoms were abdominal pain in 50%, and diarrhea in 17%. History of previous episode was positive in 58%. Pallor was noticed in 20 patients (56%).

Colonoscopic evaluation of 240 patients with 380 juvenile polyps showed CSM around 220 polyps (58%). CSM was more commonly seen around larger polyps (> 2 cm) than smaller ones ($p < 0.001$). In the 45 patients who had underwent second colonoscopy there was 55 polyps with CSM around 40 of them (73%). The 36 patients of this study demonstrated 48 polyps with 40 of them showing CSM (83%). All the studied 36 polyps from the 36 patients concerned had had CSM.

Histologic parameters of the thirty six patients including normal mucosa, CSM and polyp mucosa were compared (Table I) with histopathologic pictures of CSM (Fig. 1) and polyps (Fig. 2) being illustrated. The mean numbers of intraepithelial lymphocytes as well as *lamina propria* infiltration with eosinophils, neutrophils, lymphocytes and macrophages (/high power field) were significantly higher in polyp mucosa than in the CSM. They were trivial in the normal mucosa. The number of goblet cells/HPF was significantly higher in the CSM compared to normal mucosa ; they were almost depleted in the polyp mucosa. Muscularis mucosa thickness (μm) was significantly higher in CSM than in the polyps and the normal mucosa. Lymphocyte aggregates, dilated congested blood vessels and hemorrhagic areas were seen significantly more frequent in polyp mucosa compared to CSM.

The thickness of the *muscularis mucosae* was significantly higher in CSM around larger polyps measuring more than 2 cm (92.5 ± 12.0 μm) compared to that around smaller ones measuring less than 2 cm (65.4 ± 11.7 μm) with a P value of 0.001.

Entameba histolytica was seen in 33.33% of the studied cohort. There was no significant relation between the ultrastructural features of the three zones and the presence of *Entameba histolytica*.

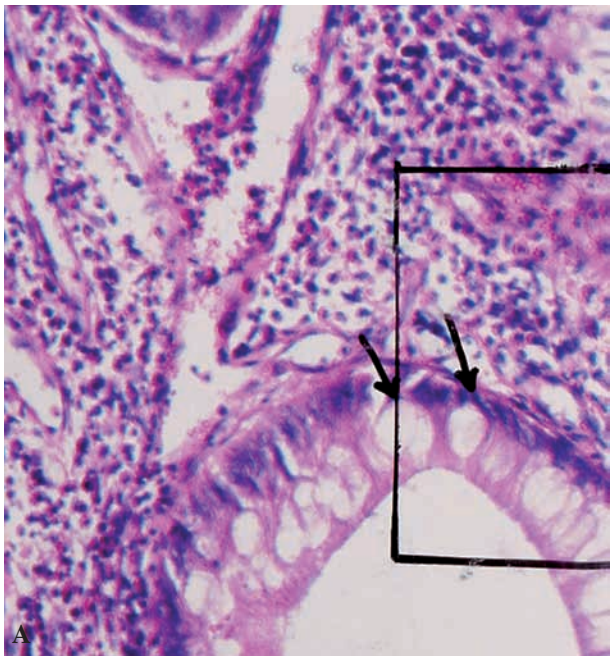
Using scanning electron microscopy, the normal colonic mucosa showed numerous of regular cryptal units which were seen to be delineated by narrow furrows. The colonic mucosa showed velvet appearance due to the microvilli of the columnar cells. Mucosal cells appeared as elevated cells separated from the nearby cells. The CSM (Fig. 3) showed smooth surface due to loss of microvilli. Accumulated mucus and cellular debris were seen in the furrows between the crypts. Biopsies from the mucosa of the polyps showed irregular appearance of mucosal surface. The cryptal units appeared to be separated by wide furrows. Mucus and cellular debris were seen to be accumulated as well.

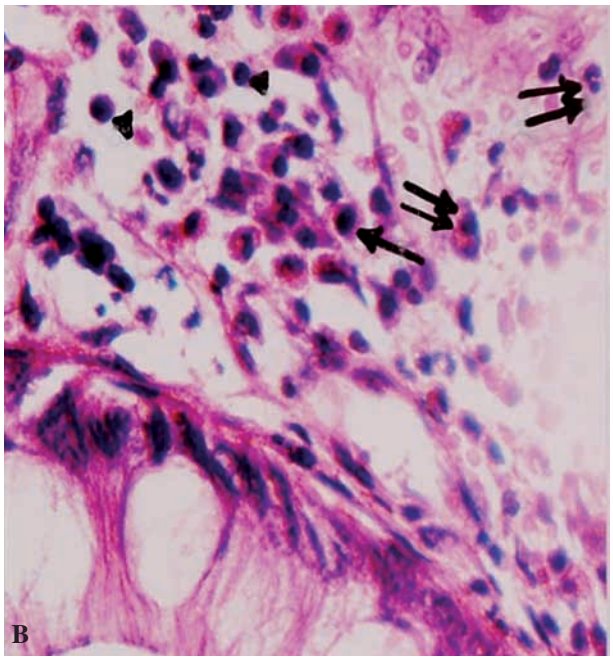
On second colonoscopy, CSM significantly disappeared in 28 patients and was sill present in 8 patients in a one month period following polypectomy (Table II).

Table I. — **Comparison of quantitative histologic findings of the polyps, chicken skin mucosa and normal mucosal biopsies**

	Polyps (I)	Chicken skin mucosa (II)	Normal mucosa (III)	P value (I Vs II)	P value (I Vs III)	P value (II Vs III)
Histologic findings	Mean ± SD	Mean ± SD	Mean ± SD			
Lymphocytes (cell/HPF)	239.33 ± 35.14	149.42 ± 31.74	60.75 ± 9.76	< 0.0001	< 0.0001	< 0.0001
Intraepithelial lymphocytes (cell/100 epithelial cells)	26.38 ± 5.87	10 ± 3.91	3.58 ± 1.44	< 0.0001	< 0.0001	< 0.0001
Macrophage (cell /HPF)	73.17 ± 11.95	51.92 ± 8.68	23.58 ± 8.65	< 0.0001	< 0.0001	< 0.0001
Eosinophils (cell/HPF)	35.00 ± 6.52	26.92 ± 3.63	3.5 ± 1.57	< 0.0001	< 0.0001	< 0.0001
Neutrophils (cell/HPF)	86.08 ± 27.44	21.67 ± 4.64	3.5 ± 1.51	< 0.0001	< 0.0001	< 0.0001
Muscle thickness (μm)	32.79 ± 6.08	86.52 ± 9.89	27.77 ± 4.87	< 0.0001	0.03	< 0.0001
Goblet cells cell/HPF	18.17 ± 3.24	72.92 ± 11.16	47.08 ± 5.32	< 0.0001	< 0.0001	< 0.0001

HPF : high power field.





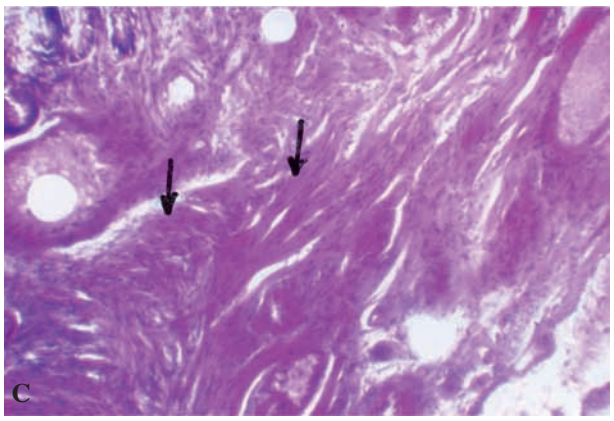


Fig. 1. — Sections in mucosa around polyps showing :
A) The crypts appeared lined by vacuolated cells, goblet cells (arrows) are numerous (H & E × 250).
B) The *lamina propria* is infiltrated by mononuclear cells (eosinophils) (H & E × 640).
C) Increased thickness of *muscularis mucosae* (arrow) (H & E × 640).

The histologic comparison of CSM before and after polypectomy showed marked decrease of their unique features with values being near to normal mucosal findings (Fig. 4).

Discussion

Colonic CSM around juvenile polyps was a common finding among patients undergoing second colonoscopy (72.73%). Nowicki *et al*. (5), found that CSM is associated with 35% of all polyps in general (75.6% of solitary juvenile polyps, and 35.9% of multiple juvenile polyps).

There is clear concentric distribution of mucosal inflammatory cells including lymphocytes, intraepithelial lymphocytes (IEL), macrophages, neutrophils and

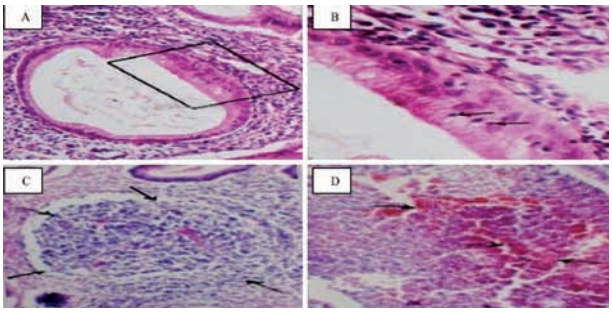


Fig. 2. — Sections in the polyp mucosa showing :
A) The cell lining a dilated transversely cut crypt, goblet cells are hardly seen and lymphocytic infiltration is clear in the *lamina propria*. The rectangle shows an area lined by high columnar cells with intraepithelial lymphocytes (H & E × 250).
B) A higher magnification demonstrating the *lamina propria* infiltrated by mononuclear cells & intra-epithelial lymphocytes appear between the surface columnar cells (H & E × 640).
C) *Lamina propria* in a polyp showing large lymphoid follicle (H & E × 250).
D) *Lamina propria* in a polyp showing hemorrhagic infiltration (H & E × 250).

eosinophils with polyp mucosa having the most intense infiltrate. This may reflect heterogeneity of the inciting factors whether infectious, inflammatory, traumatic or allergic ones.

Lymphocytic infiltration of colonic mucosa is seen in chronic colitis, mostly in inflammatory bowel disease (IBD), lymphocytic colitis, diversion colitis and with some chronic infections such as amebic dysentery (8-11). The *lamina propria* macrophages were found to be increased in IBD (12) and edges of the typhoid ulcer (13). The presence of focal infiltrates of a large number of eosinophils, is the characteristic microscopic feature of allergic colitis (14), primary eosinophilic gastroenteritis (15) pinworms infection, drug reactions, Churg-Strauss syndrome, hyper eosinophilic syndrome and IBD (16). Neutrophilic infiltration of colonic mucosa with edema, congestion and possible ulceration are the characteristic features of acute "active" colitis that is seen in acute self-limiting colitis caused by different organisms (17-21). Mild to moderate infiltration of neutrophils is observed with amebic colitis (11). However, in our present study there was no significant relation between inflammatory reaction and presence of *Entameba histolytica* infection.

The mean number of goblet cells, showed significantly higher values in CSM compared to the normal one. However, they were significantly depleted from the mucosa of the polyps. Colonic goblet cell hyperplasia is a striking pathological feature that was demonstrated in many experimental parasitic helminth infections (22-27) and in Crohn's disease (28).

The thickness of the muscularis mucosa was significantly higher in CSM compared to polyps but both were higher than in the normal mucosa. Thickening of the *muscularis mucosae* with smooth muscle extension into the *lamina propria* associated with hyperplastic or villiform appearance of the surface and edema with increased numbers of fibroblasts are recognized features of solitary rectal ulcer syndrome (29). Hypertrophy and disorientation of muscle fibers could be secondary to chronic mechanical and ischemic trauma and inflammation by hard stools, and intussusception of the rectal mucosa is the suggested mechanism (30). Hypertrophy of the *muscularis mucosae* and the *muscularis externa* can occur in diversion colitis, the degree of which was positively correlated with duration of diversion (31).

Table II. — **Second colonoscopy of 36 patients with chicken skin mucosa with previous polypectomy**

	Re-colonoscopy less than 2 weeks (16)	Re-colonoscopy more than 2 weeks (20)	X2	P
Persistent chicken skin mucosa	7	1	7.72	0.0055
Absent chicken skin mucosa	9	19		

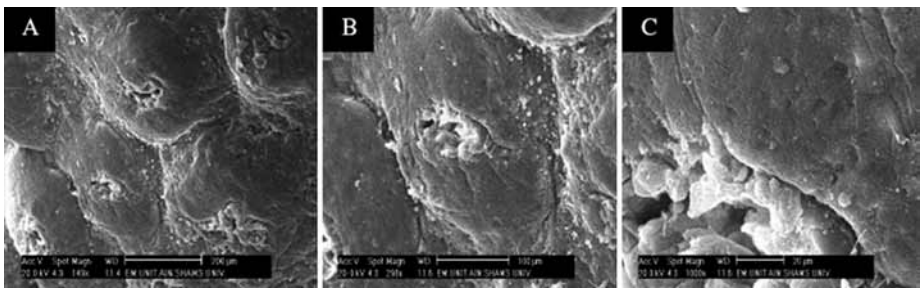


Fig. 3. — Scanning electron microscopic (SEM) photographs of chicken skin mucosa showing :
A) The cryptal units with smooth surface due to loss of microvilli (SEM × 75).
B) Accumulation of mucus and cellular debris in the mouth of crypts (SEM × 1298).
C) Accumulation of mucus closing the opening of goblet cells. Mucus and cellular debris are accumulated in the furrows between the crypts (SEM × 1000).

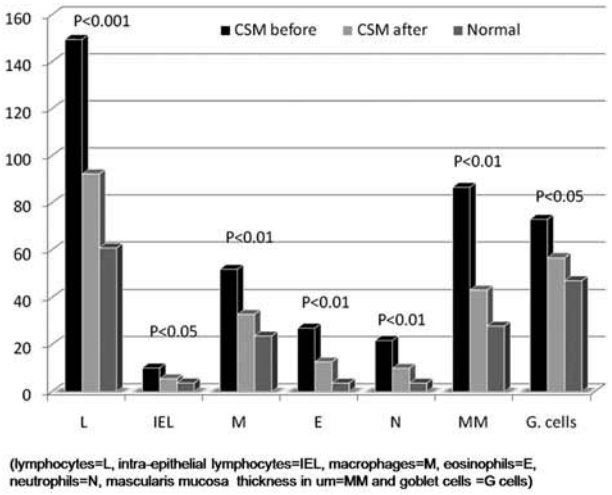


Fig. 4. — Bar chart of histologic findings in chicken skin mucosa before polypectomy, chicken skin mucosa after polypectomy and normal mucosa.

Although all inflammatory cells were more intense in polyp mucosa yet, CSM showed two unique histologic abnormalities ; namely high number of goblet cells and muscularis mucosal thickening especially around large polyps.

Following polypectomy, the thickness of *muscularis mucosae* and number of goblet cells were significantly decreased. Moreover, in most of the cases, CSM disappeared within a month period following polypectomy.

The marked decrease of these two features after polypectomy and the disappearance of CSM are clues to the compensatory benign nature of this colonic lesion.

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