

Evaluation of Immunohistochemistry (IHC) in Children with Chronic Constipation

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Abstract

Introduction

Constipation is one of the most common causes of referral to pediatric gastroenterology clinics. The histopathology and Immunohistochemistry (IHC) studies indicated that several neurons are currently detectable in motility of the gut. We aimed to evaluate calretinin in the children with chronic constipation.

Materials and Methods

In this cohort study, 40 children with chronic constipation, 28 boys (70%) and 12 girls (30%) referring to pediatric surgery ward in Shahid Mohammadi Hospital, Bandar Abbas-Iran, between January 2010 to February 2012, were recruited. Then, full rectal biopsy 1.5 cm above dentate line was performed for all children and calretinin immunoreactivity and pattern of staining for ganglion cells (nuclear and cytoplasmic) and also nerve fibers in different layers of the bowel (lamina propria, muscularis mucosa, submucosa, and muscularis propria) were measured. According to their pathologic results, the children were assigned in two groups for treatment: 1- Those with aganglioneurosis underwent pull-through operation, and 2- Patients with ganglion but abnormality in their immunohistochemistry received botulinum toxin.

Results

In this study, 9 (23.9%) aganglionic patients with the mean age of 3.6 ± 1.7 years compared with 31 (76.1%) hypoganglioneurosis patients with the mean age of 3.2 ± 1.2 years were examined. Pull-through operations were carried out for all patients in the aganglionic group, and for 6 (19.4%) patients in the hypoganglioneurosis group. Postoperative manometry was significantly better in both groups, but monomeric change was not significant between the two groups.

Conclusion

Our results revealed that calretinin is absent in aganglionic children, moreover we indicated, calretinin IHC is a very useful and valuable technique for detecting aganglioneurosis in patients with chronic constipation.

Keywords: Aganglionic, Calretinin IHC, Chronic Constipation.

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Introduction

Constipation is a common disorder in children and is diagnosed in about three percent of children referring to pediatric clinics. It is a serious problem in pediatric gastrointestinal practice and causes distress to the children and their families (1). However, the exact reason of constipation in children is not fully understood yet (2). According to North American Society of Gastroenterology, Hepatology, and Nutrition (NASPGHAN) definition, the constipation in children is a condition with delay or difficulty in defecation for 2 or more weeks accompanies with distress (3). It is classified as organic and functional constipation. Functional constipation and acquired megacolon are the most common causes of constipation in children. In these children, the defecation is painful so the pain related to defecation, result in withholding stool and if stool withholding continues, the urge for defecation slowly will disappear and worsen the stool retention, so, stool progressively will be hard and the spasm of anal sphincter will increase. If this condition becomes chronic, the rectum will be distended with very low sensitivity to urge to defecation and to the accumulation of stool that leads to fecal incontinence in children (4-6). Organic constipation including anatomic malformations, metabolic and gastrointestinal disorders, neurologic conditions, abnormal abdominal musculature and connective tissue disorders is diagnosed in 5% of children (5). The histopathology and IHC studies reported that several neurons are currently detectable in the gut musculature including primary afferent neurons, motor neurons, inter-neurons, secretomotor and vasomotor neurons. It is now possible to mark every individual nerve cell, which provides a foundation for the systematic analysis of how the enteric nervous system normally works and how it goes wrong in various clinically important motility disorders (7). The intricate system of gut neuron includes

ganglia, neural cell, Immunocytochemistry (ICC), and pacemaker cells connecting enteric nerves and intestinal smooth muscle and interneurons with many thousand secreted substances in distal bowel are important contributing factors to normal functioning and absence or dysfunction, which might have caused the clinical picture of constipation (8, 9). Considering such complex system requires precise knowledge of what is happening in order to enable us to design an adequate plan of treatment that can be denoted as pathologic mapping of the enteric nervous system. This may imply using calretinin as a marker for well-functioning ganglia (10, 11). Calretinin is a vitamin D- dependent calcium binding protein and is claimed that its immunostaining in the nerve fibers is absent in patients with chronic constipation (12), therefore, we conducted this cohort to evaluate children who are chronically constipated and were candidates for rectal biopsy and their enteric nervous system were mapped to show dysfunctional elements in order to recommend a new strategy in research and treatment of children's constipation based on this pilot finding.

Methods and Materials

In this cohort study, 40 children, 28 (70%) boys and 12(30%) girls referring to pediatric surgery ward in Shahid Mohammadi hospital, Banda Abbas, Southern Coast of Iran, between January 2010 to February 2012, were recruited.

Inclusion criteria were:

- History of chronic constipation >6 months on adequate medication;
- History of acute obstruction;
- Soilage;
- History of delayed meconium passage;
- Abnormal anorectal manometry, and
- Abnormal barium enema.

The study procedure was explained for parents of children and informed written consent was obtained. The Ethical Committee of Hormozgan University of Medical Sciences, approval did not need because the procedure was routine in Shahid Mohammadi hospital. Then, full rectal biopsy 1.5 cm above dentate line was performed, and full thickness paraffin block was prepared for identification of ganglion cells and calretinin, using Hematoxylin and Eosin (H&E) staining. Immunostaining was performed on paraffin-embedded archival tissues following the Avidin–Biotin peroxidase technique.

Then, the slides were incubated with the diluted primary antibody (Clone: DAK-Calret 1, monoclonal mouse anti-human, Dako, Denmark, 1/100 dilution) at 40°C in a humidified chamber for 60 min. Calretinin immunoreactivity and pattern of staining for ganglion cells (nuclear and cytoplasmic) and also nerve fibers in different layers of the bowel (lamina propria, muscularis mucosa, submucosa, and muscularis propria) were evaluated. Two pathologists independently reviewed the H&E and IHC stained slides and agreed on determined diagnoses. Then the children were assigned in two groups according to pathology results for treatment, 1) Those with aganglionosis underwent pull through operation, and 2) Patients with ganglion but abnormality in their immunohistochemistry received botulinum toxin (dysport, 500 unit, UK), nitroglycerin paste (Tolid Daru, Iran) for perianal application each night, domperidone (.5 mg/kg/d) tablet (Tolid Daru, 10 mg, Iran) daily according to weight for 6-12 month before enrolling for the operation. These drugs were used as domperidone for stimulation of ganglion, Botox (dysport 500 unit /UK) as nerve growth factor, and nitroglycerine for maturation of cajal cells according to findings of the biopsy results.

Data were analyzed using SPSS version 20. Categorical data are presented as numbers (%), and continuous data as mean \pm SD. We used the Chi² or Fisher's exact test to compare categorical variables and the Student's t-test, to compare continuous variables. $P < 0.05$ was considered significant.

Results

Nine aganglionic patients mean age 3.6 ± 1.7 years were compared with 31 hypoganglionosis patients mean age 3.2 ± 1.2 years. Pull-through operation was carried out for all patients in the aganglionic group. However, in the hypoganglionosis group, Pull through operation was done in 6 (19.4%) of the patients. (P-value < 0.001). Pathologic marker evaluation showed that calretinin marker was absent in the aganglionic group. Post operation manometry was significantly better in both groups, but the monomeric change was not significant between the two groups. In non-functional normal ganglionic group, only one conversion to pull-through operation occurred (one from nine patients); however, other parameters were the same as hypoganglionosis group. In our study the decisions of both pathologists showed perfect concordance

Discussion

Chronic constipation in children is associated with many enteric nervous system disorders and has been a matter of debate for many years (11, 13). The histopathology and immunohistochemistry (IHC) studies reported that several neurons are currently distinguishable in motility of the gut (14). Calretinin is a calcium binding protein in enteric neurons which innervate the mucosa (15). In this IHC study we evaluated calretinin in the children with chronic constipation and indicated that calretinin was absent in

aganglionic children. Our findings in current study were in line with previous practices that indicated calretinin-immunoreactive is absent in the aganglionic segment of Hirschsprung's disease (HD) (aganglionic megacolon)(16-17). Barshack et al. indicated the usefulness of IHC for calretinin in aganglionic patients for the first time (16) and several studies later proved that calretinin IHC was a useful ancillary technique in the pathological evaluation of patients with HD (17), moreover these trials emphasized that it is more accurate than AchE for the diagnosis or exclusion of HD (17-21). The usefulness of this technique is because, it simply can be stained on formalin fixed paraffin embedded tissue in fresh or archival specimens (17-21), moreover it is easy to interpret, available in the most of pathology department with IHC lab and do not need to expert staffs. (17-21).

More studies in this field indicated that calretinin IHC interpretation is very simple for inexperienced pathologists to diagnose aganglionosis. (15-17). Furthermore another study by Guinard-Samuel et al. revealed that interpretation of calretinin IHC is easier than AchE histochemistry for inexperienced pathologists (19). Another study by Hradfar et al. also indicated calretinin is a good marker for detection of ganglion cells, they emphasized that the specificity of calretinin IHC for diagnosing aganglionic sections was 100% (25). Baimbridge et al. indicated that in calretinin immunostaining there is positive control in the submucosal sections, for example some non neuronal cells including the histiocytes and mast cells showed cytoplasmic and nuclear immunoreactivity that can be considered as internal positive control. In this regard in segments with typical aganglionic, the immunoreactivity in nerve fibers or ganglion cells was absent but in mast cells

or histiocytes cytoplasm, weak to moderate staining was detected (26).

The main limitation of our study was the relatively small sample size that limits the ability to generalize the result of our practice, further experiences are recommended with larger series to validate the findings reported here.

Conclusion

Our results revealed that calretinin is absent in aganglionic children moreover we indicated, calretinin IHC is a very useful, valuable, for detecting aganglionosis in patients with chronic constipation.

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Conflict of interest: None.

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