EXPRESSION OF INTERSTITIAL CELLS OF CAJAL IN COLORECTUM OF PATIENTS WITH HIRSCHSPRUNG'S DISEASE

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Abstract

Background and Objectives: Precise mechanism underlying the development of Hirschsprung's disease (HD) is unknown. Interstitial cells of Cajal (ICC) acts as pacemaker and their alteration has been proposed to occur in HD. The aim/rationale of the study is to find out whether there is any difference in number of ICCs in HD and non-HD patients, which in turn will support the assessment of level of disturbed ICCs in the proximal normo-ganglionic segment of HD, in order to improve the post-operative management of HD patients.

Methods: The study comprised total of 55 clinically suspected cases of Hirschsprung's disease.

Results: Out of 55, 36 were proved to be HD and 19 composed the Non-Hirschsprung's disease (NHD) group on H & E examination. The number of ICCs/mm² in outer muscular layer showed significant (p = 0.033) decrease in HD as compared to NHD group whereas no significant decrease was seen in inner muscular layer. There was significant decrease in number of ICCs/mm² in outer muscular layer of HD as compared to NHD.

Conclusion: Expression of Interstitial cells of Cajal in outer muscular layer of colorectum of Hirschsprung's disease was significantly reduced.

Key words: Hirschsprung's disease, Interstitial cells of Cajal, enteric nervous system.

INTRODUCTION

Interstitial cells of Cajal (ICCs) have been the focus of lively debate for a number of years. It acts as pacemaker of enteric nervous system.[1] Injury to ICC in patients with dysmotility is always associated with injury to enteric nerves.[2,3] ICC are of non-neuronal, mesenchymal origin, sharing precursors with smooth muscle cells.[1,4] Antibody against Kit (CD117), the protooncogene that encodes the receptor tyrosine kinase, is used as a simple, efficient means of specifically labeling ICC and they consist of a fusiform cell body with a thin cytoplasm, a large oval nucleus and dendritic like processes.[5] ICC form distinct networks in the myenteric and submucosal regions. Loss or deficiency of ICCs networks have been reported to be associated with Hirschsprung's disease (HD). Myenteric ICCs were found to be markedly reduced not only in the aganglionic segment, but also in the transitional zone and ganglionic part of HD bowel.[6-8] Reduction of myenteric ICC in the normoganglionic sigmoid colon in HD may be the cause for the dysmotility disturbances seen in many patients after pull-through operation due to the defective transmission of electrical events between the enteric nervous system and adjacent smooth muscle.[6] Despite the widespread use of CD117 antibodies in this setting, there is no agreement on what constitutes a normal/abnormal number of ICC or what a normal/disrupted network of ICC is?

PATIENTS AND METHODS

Sample size of 55 cases was calculated with 95% confidence level, 13% margin of error and taking expected percentage of Hirschsprung's disease i.e., 38% in clinically suspected patients. Between March 2012 and August 2012, rectal biopsies from 55 patients who presented clinically with symptoms of Hirschsprung's disease were collected from patients from CH & ICH, Lahore based on inclusion criteria of (a) Clinically suspected cases of HD & (b) both sexes and less than 14 years of age. Submucosal and inadequate biopsies were excluded from the study. Routine processing and serial sectioning & staining with H & E followed by CD117 immunohistochemical staining were carried out at Histopathology department, Shaikh Zayed Hospital, Lahore. CD117 positive ICCs were counted in consecutive five high power fields x 400 in inner circular and outer longitudinal muscle coats separately by ocular graticule using light microscope (Leica, DM 1000) by the method described by Culling 1974. Data was entered and analyzed using SPSS version 18.0.

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RESULTS

Colorectal biopsies from total of 55 cases were processed and examined. After H & E examination of slides, there were 36 (65%) cases proved to be Hirschsprung's disease (aganglionic) and 19 (34%) cases were non-Hirschsprung's (ganglionic) group. Out of these 55 cases, 5 cases were excluded for immunostaining because whole of biopsy tissue was sacrificed in making the diagnosis. So, total of 50 cases were left for immunostaining with CD 117 for demonstration of ICCs.

In aganglionic group, 29 (81%) out of 55 were male and 7 (19%) were female (M:F= 4.1:1). In Hirschsprung's disease group, 22 patients {11 (30.5%) each less than 1 year & 1.1 to 2 years} were under the age of 2 years, 4 (11.1%) were 2.1 – 3 years, 6 (16.7%) were 3.1 – 4 years, 1 (2.8%) was 5 years, 7 (19.4) were 6.1 – 7 years, 1 (2.8%) was of 9 years and 1 (2.8%) was 10 years of age. Figure 1 showed the frequencies of major presenting complaints in both groups.

The number of ICCs/mm² in outer muscular layer in each section was 6.54 ± 7.5 showing significant (p = 0.033) decrease in HD as compared to NHD group. The number of ICCs/mm² in inner muscular layer in each section was 7.03 ± 9.6 showing no significant (p = 0.403) decrease in HD as compared to NHD group as shown in Table.

Table 1: *Shows the numbers of ICCs/mm² in outer and inner muscular layers, mean ± Standard deviation and p value in Hirschsprung's disease (HD) and Non-Hirschsprung's group (NHD).*

		N	*Mean*	*SD*	*p value*
ICCs/mm² in outer muscular layer	HD	32	6.54	7.5	p □ 0.05 p = 0.033
	NHD	18	11.57	8.2	
ICCs/mm² in inner muscular layer	HD	32	7.03	9.6	p = 0.403
	NHD	18	9.53	10.7	

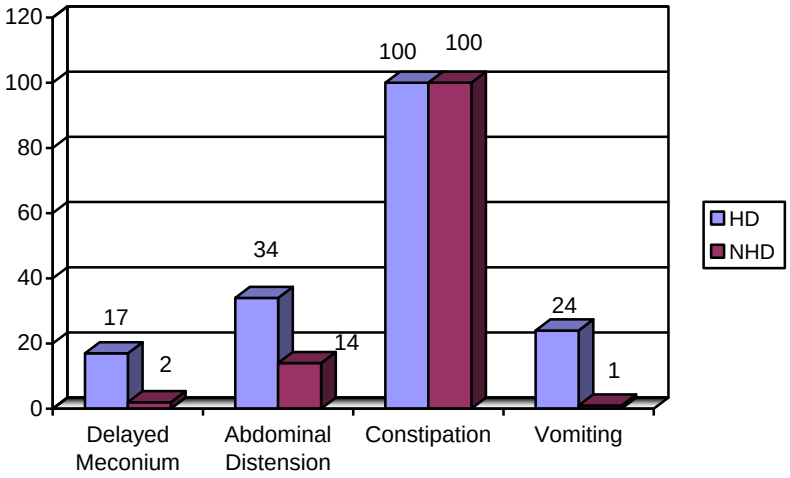

Fig. 1: *Shows the comparison of presenting complaints in HD and NHD in terms of percentages.*

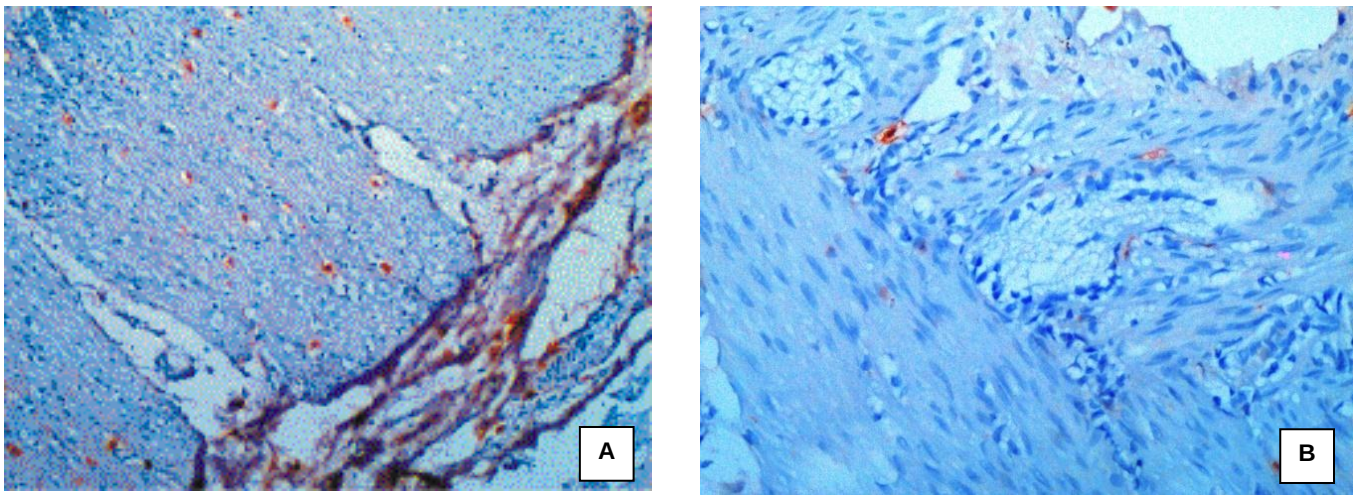

Fig. 2: *(A) Photomicrograph shows scattered c-kit positive ICCs in inner layer of muscularis externa in ganglionic biopsy (IHC ×200). (B) Photomicrograph shows very few scattered c-kit positive ICCs in inner and outer muscular layers in a case of HD (IHC ×200).*

DISCUSSION

Interstitial cells of Cajal are a group of cells interacting with enteric neurons and smooth muscle cells. They act as mediators of neurotransmission and pacemakers of peristaltic movement of the gut.[1] Experiments shows the physiological roles of ICC, which indicate that the-

se cells are involved in transmission and conduction of slow electrical waves and they act as neural mediators between enteric nervous system and enteric musculature. There are synaptic like contacts and gap junctions between nerve terminals and intramuscular ICCs. These intercellular interactions help ICCs to function as electrical conductors. On light microscopy, it is very difficult to study these cells. However, the distribution of these cells can be evaluated by transmission electron microscopy (EM). Unluckily, EM is not available in every centre for easy evaluation. Recognition of trans-membrane tyrosine kinase receptor, c-kit through conventional immunohistochemistry has been evolved as valuable adjunct to routine light microscopy for highlighting the ICCs distribution. [8] Knowing the physiological role of ICC, it can be obviously interpreted that abnormal distribution of ICCs may disrupt pacemaker activity of the gut resulting in disturbed peristalsis.

Wang et al. in 2009 conducted a study on aganglionic region of the colon of patients affected by HD.[9] They showed that the number of intramuscular ICCs was markedly reduced to complete lack of these cells.[9] Few studies showed reduced density of c-kit immunoreactivity in aganglionic segments of HD patients.[2] Anatol et al. in 2008 revealed in his study that 75% of HD patients had marked diminution of ICCs in aganglionic bowel, although 25% of ganglionic bowel also showed decrease positivity for ICCs.[8]

Altered distribution of ICCs in the internal anal sphincter in HD may contribute to motility dysfunction on these patients. In the muscle layer of normal IAS (internal anal sphincter), the number of ICCs (6.9 ± 2.1) was significantly higher than IAS of HD (1.1 ± 0.3) samples (p □ 0.5).[7] In the present study, the number of ICCs/mm^2 in outer muscular layer in each section was 6.54 ± 7.5 showing significant (p = 0.033) decrease in HD as compared to NHD group. The number of ICCs/mm^2 in inner muscular layer in each section was 7.03 ± 9.6 showing no significant (p= 0.403) decrease in HD as compared to NHD group.

A study carried out by Wedel et al. quoted ICC in circular muscle coat in control group as 24.6 ± 11.9 & 32.1 ± 13.7 and in longitudinal muscle coat 15.1 ± 7.9 & 3.6 ± 2.4 whereas in megacolon they quoted 10.0 ± 6.8 & 8.4 ± 7.5 in circular muscle and 4.8 ± 2.8 & 1.1 ± 1.0 in longitudinal muscle coat.[10] Their results showed obvious statistically decreased number of ICC in inner circular and outer longitudinal muscle coats when compared megacolon group with control group.[11] The proportions of mature ICC in the narrow segment (0.059 ± 0.099%) of HD colon were significantly reduced when compared to their proximal segment (1.144 ± 0.173) in adult normal colon.[12]

Like previous studies, in the present study, we also found the significant (p < 0.05) decrease in outer muscular layer of aganglionic segment of HD. However, decrease in ICC in inner muscular layer was not significantly (p = 0.323) reduced in HD comparing with ganglionic segments. As in this study we divided our patients in two groups i.e., aganglionic and ganglionic based on H & E examination of colorectal biopsies who was clinically suspected as HD. There was no problem/ controversy regarding diagnosis of aganglionosis but when we talk of ganglionic segments actually it may include the normal number of ganglion cells and allied HDs, which are hypoganglionosis, hyperganglionosis and immature ganglion cells. Allied HDs will also show normal looking ganglion cells on H & E examination. All of these diseases also present clinically as HD i.e., with clinical symptoms of intestinal obstruction during the course of disease. It may be postulated that these patients may or may not have associated altered ICCs. If they have associated ICC abnormality, then the results of ganglionic group may be disturbed as well, but we are not sure about it because of lack of significant literature data. In our study, the reason of no significant alternation of number of ICC/mm^2 in inner muscular layer may be that ganglionic group might include allied HDs.

We ***conclude*** from this study that expression of ICC in outer muscular layer of colorectum of Hirschsprung's disease is significantly reduced.

Limitation of the Study

Absolutely normal and healthy colorectal biopsies with normal ganglion cells and ICCs for precise comparison are difficult to obtain. In literature, it's hard to find out any definite objective criteria on light microscopy to filter out allied HDs as in the present study, so broadly they are divided into ganglionic and aganglionic groups.

Authors' Contributions

NH: Concept and methodology. AR: Preparation of manuscript. AHN: Supervision and guidance.

ACKNOWLEDGEMENTS

We are thankful to the administration of CH and ICH Lahore for allowing us to use the clinical material, and Lab. staff of Pathology Department Sheikh Zayed Hospital Lahore for technical help.

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