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. Injury to ICC in patients with dysmotility is always associated with injury to enteric nerves. ICC are of non-neuronal, mesenchymal origin, sharing precursors with smooth muscle cells. 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. 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. 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. 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.
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.

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<th>N</th>
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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. Experiments shows the physiological roles of ICC, which indicate that the-

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).
INTERSTITIAL CELLS OF CAJAL IN COLON OF PATIENTS WITH HIRSCHSPRUNG’S DISEASE

Intestinal interstitial cells of Cajal (ICC) play an important role in the regulation of motility and electrical activity in the gastrointestinal tract. They are involved in the pacemaker activity of the gut, the intercellular interaction of nerve terminals, and the release of mediators that control neurotransmission.

Wang et al. (2009) conducted a study on aganglionic region of the colon of patients affected by Hirschsprung’s disease (HD). They found that the number of intramuscular ICCs was markedly reduced in patients with HD compared to normal controls. However, ICCs were present but reduced in patients with aganglionic bowel.

Anatol et al. (2008) revealed in their study that 75% of HD patients had a significant diminution of ICCs in aganglionic bowel, although 25% of ganglionic bowel also showed a decrease in ICC density.

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 anal sphincter (IAS), the number of ICCs was significantly higher than in HD (1.1 ± 0.3) (Kim et al., 2007). In the present study, 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.

The study conducted by Wedel et al. (2009) 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. Their results showed obvious statistically decreased number of ICC in inner circular and outer longitudinal muscle coats when compared to normal muscle group.

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.

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, a 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/mm² 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.

AUTHOR’S CONTRIBUTIONS

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

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