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RESEARCH ARTICLE

Histological characteristics of colon and rectum of adults and neonate rats

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ABSTRACT

Background: The changes in histological picture of gut during the post-natal period are an important factor that may be responsible for changes in gut motility during the developmental process. **Aims and Objectives:** Histology of large gut is poorly understood, especially that of neonate. Therefore, the present study was undertaken to assess and confirm the histological differences in the neuronal and longitudinal muscle layers of colon and rectum of adult and neonate rats. **Materials and Methods:** In the present study, isolated large gut segments (colon and rectum) from adult and neonate albino rats (4 to 6 months) were used to examine the histological differences using hematoxylin and eosin staining. **Results:** The number of ganglion cells in the longitudinal muscle layer of adult colon was 16.31 ± 3.81 cells/mm^2 and 8.86 ± 1.79 cells/mm^2 in the rectum. In case of neonate, number of ganglion cells in the colon was 10.46 ± 1.82 cells/mm^2 and in the rectum 13.85 ± 2.82 cells/mm^2. Thus, no significant difference was observed in the number of ganglion cells in the colon and rectum of adult or neonate rat. Further, it was observed that the thickness of longitudinal muscle layer in adult rectum was more (108.75 ± 5.91 µm) as compared to colon (70 ± 16.83 µm). However, there was no difference in the thickness of colon (30 ± 2.89 µm) and rectum (27.5 ± 2.50 µm) neonates. Our data also indicated that the thickness of longitudinal muscle layer in colon and rectum increases with age. **Conclusion:** It may be concluded that number of ganglion cells in colon and rectum does not increase with age. However, thickness of smooth muscle in both colon and rectum, increase over time from neonate to adulthood and may have some relation with the contractile characteristics of colon and rectum. Increased thickness of smooth muscle in adult rectum as compared to colon may be implicated for more contractile force required in the rectum of adult rats for evacuation of fecal matter in rats.

KEY WORDS: Gut Motility; Histology; Ganglion Cell; Smooth Muscle

INTRODUCTION

Adequate contractile function of smooth muscle layers of the gut wall is pivotal for complete digestion process and finally to expel the undigested materials through the anal canal. During the post-natal period, changes in the histological picture of gut may be responsible for alteration in gut motility during developmental process. The principle changes have been observed in gut neuronal elements and smooth muscle.[1-5] The functions of gastrointestinal tract (GIT) is under control of an intricate network of neurons embedded in the wall of the GIT known as enteric nervous system (ENS). It has been reported that there are more neurons in the gut than present in the spinal cord.[6] The ENS is composed of an inner plexus known as Meissner's or submucosal plexus located in the submucosal layer, mainly implicated in regulation of secretion, and an outer plexus known as Auerbach's or myenteric plexus located between the intestinal muscular layers and involved in regulation of smooth muscle contractility.[7,8] Interactions between these plexuses in different parts of the gut help in

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motility. The ENS autonomously regulates various functions of the gut including motility, secretion, vascular tone, and release of hormones. However, the autonomic nervous system can modulate these functions. Thus, in humans and other mammalian species such as albino rats, specific contraction, and relaxation requires coordinated actions of smooth muscle, neurons of the ENS, and autonomic nervous system.

Various morphological or histological studies have been done to see the number of neurons in the plexus of the different parts of the GIT in different mammalian species including neonate.[1,9-11] Earlier studies in neonatal rat and in human newborn have reported reduced neuronal cell density.[1,5] Thus, during development, alteration in the post-natal ENS continues. The other factor that may affect the contractile function of gut is the muscular development. The thickness of smooth muscle is known to increase during post-natal development.[5] Further, as described in the previous studies, there is difference in the contractile activity of colon and rectum of adult and neonate rats. The thickness of smooth muscle is reported to increase during post-natal development.[12] However, reports of earlier studies about the differences in the number of ganglion cells and thickness of longitudinal layer in the colon and rectum are not consistent. Further, histology of the gut is poorly understood. Therefore, the present study was undertaken to assess and confirm the histological difference in neuronal and longitudinal muscle layers of colon and rectum of adult and neonate rats using hematoxylin and eosin staining.

MATERIAL AND METHODS

Animals

Adult albino rats of Charles Foster strain of 4-6 months and neonate of 10-16 days of same strain (n = 4 in each group) were used in this study. The animals were housed in a temperature, humidity and light-controlled room (12 h light and 12 h dark) with an *ad libitum* supply of food and water. The animal experiments were performed as per guideline of the Ethical Clearance Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Dissection of Animals

Adult rats were sacrificed by cervical dislocation and exsanguinations and neonate by decapitation. The abdomen was opened immediately by vertical incision and part of the gut containing colon and rectum was dissected out and cleaned by flushing out the gut contents and placed in a petridish containing chilled Krebs Ringer solution and pieces of colonic and rectal segments were prepared.

Experimental Protocol

The colonic and rectal segments were fixed in (10%) formalin solution. Then, tissues were processed in automatic histokinette machine (Sakura, Japan). First, tissues were dehydrated using increasing concentrations of alcohol, i.e., 70% for 2½ h, 90% for 2½ h, 95% for 2 h, and lastly 100% alcohol for 1 h. This was followed by 3 changes of copper alcohol (100% alcohol and anhydrous copper sulfate) of 1 h duration each. Consequently, the tissues were placed in 3 changes of xylene, each of 1 h duration and 3 changes of paraffin wax (melting point 60°C), each of 1½ h duration. Following this, paraffin blocks were prepared by instantly placing processed tissue in molten paraffin wax using L-shaped metallic molds. The block containing tissue with paraffin was allowed to cool. The solidified paraffin blocks were cut, trimmed, and prepared for tissue sectioning. Thin sections of longitudinal segments of colon and rectum (3 microns thick) were cut by rotator microtome. A ribbon of this section was taken and floated in water bath maintained at 56°C to make the sections wrinkle free. The ribbon was then cut into individual sections by a sharp knife. Sections were then placed on albumin pre-coated glass slide, wherein albumin acts as adhering medium for tissue sections. Then, the glass slide containing tissue section was placed over metallic hot plate at 60°C for at least 1 h. This allows proper adherence of section over glass slide. The paraffin of tissue section was then dissolved by keeping slides in xylene for 20 min. The excess xylene over glass slide was bloated on tissue paper and the slides were then processed under descending concentrations of 100%, 90%, and 70% alcohol, each for 5 min duration and lastly to water. For staining, the slides were kept in Harris' hematoxylin for 2 min, rinsed in running tap water for 1 min, briefly differentiated in 1% acid alcohol, rinsed in running tap water for 5 min, stained with 1% aqueous eosin for 20 s, and again rinsed in running water for 5 min. The slides were then treated with ascending grades of alcohol to remove water, i.e., 70%, 80%, and 90% alcohol, each for 1 min duration. Slides were then air-dried to remove water completely. The air-dried slides were placed in xylene for cleaning and mounted with DPX mountant and glass cover slip. The above procedure for tissue processing and staining was adapted from standard established method.[13] The stained and mounted sections were viewed under digital microscope (Nikon, Japan) and photomicrographs were stored for future analysis.

Statistical Analysis

Number of ganglion cells in the myenteric plexus was counted between longitudinal and circular muscle layer of both colon and rectum of adult and neonate rats. Number of ganglion cells is expressed in term of cells per mm^2 and width of longitudinal muscle layer were also calculated and expressed in μm. Student's t-test was applied wherever required. A value of $P \leq 0.05$ was considered as significant.

RESULTS

Number of Ganglion Cells/mm^2

Overall number of ganglion cells in the myenteric plexus between longitudinal and circular muscle layer of adult

colon was 16.31 ± 3.81 ganglion cells/mm^2 and 8.86 ± 1.79 ganglion cells/mm^2 in its rectum. Further, there was no difference in the number of ganglion cells between colon and rectum of adult rats ($P > 0.05$, Student's *t*-test for unpaired observations, $n = 4$, Figures 1 and 2). In case of neonate, number of ganglion cells was 10.46 ± 1.82 ganglion cells/mm^2 and 13.85 ± 2.82 ganglion cells/mm^2 in colon and rectum, respectively, and there was no statistically significant difference in the number of ganglion cells between neonate colon and rectum ($P > 0.05$, student's *t*-test for unpaired observations, $n = 4$, Figures 3 and 4).

Thickness of Longitudinal Muscle Layer

Muscle thickness of longitudinal muscle layer in adult was 70 ± 16.83 µm and 108.75 ± 5.91 µm in colon and rectum, respectively, and thickness was more in rectum than colon ($P < 0.05$, Student's *t*-test for unpaired observations, $n = 4$, Figures 1 and 2). On the other hand in neonate, thickness of longitudinal muscle layer was colon 30 ± 2.89 µm and 27.5 ± 2.50 µm in colon and rectum, respectively, and no statistically significant difference was observed in muscle thickness between colon and rectum of neonate ($P > 0.05$, Student's *t*-test for unpaired observations, $n = 4$, Figures 3 and 4).

DISCUSSION

In the present study, the thickness of longitudinal muscle layer in both colon and rectum was significantly increased in adults as compared to neonate. Further, it was observed that thickness of longitudinal muscle layer in the adult rectum was more as compared to its colon (Figures 1 and 2). Our data also indicated that the number of ganglion cells in neonate was not different from the number found in adults.

Development in the ENS is an ongoing process in the post-natal period. The changes in the morphology and functions of

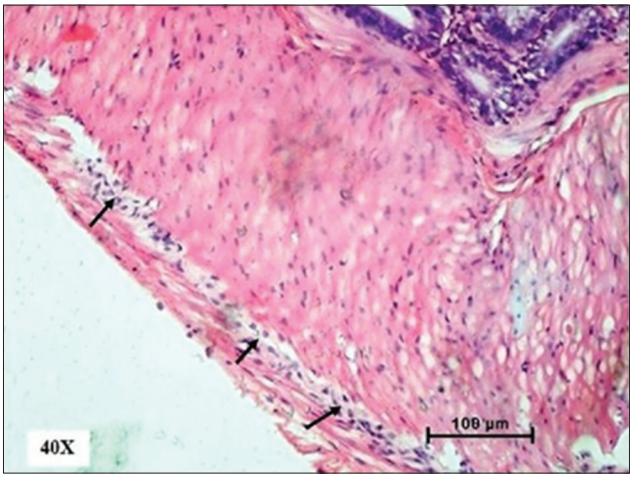

Figure 1: Photomicrograph of H- and E-stained adult rat colon. The arrow in picture indicates ganglion cells between longitudinal and circular muscle layers (×40 magnification)

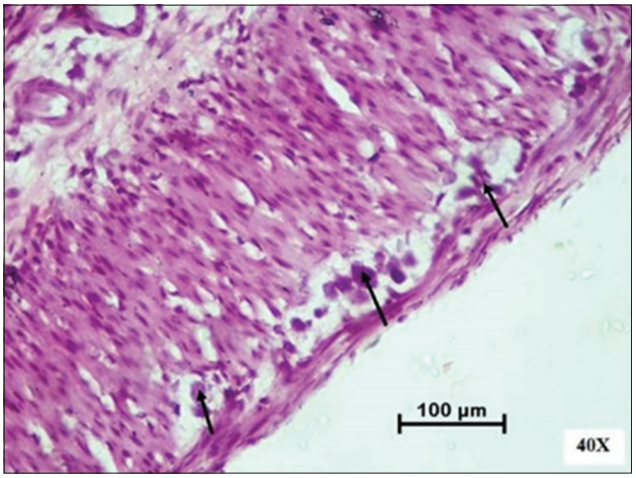

Figure 3: Photomicrograph of H- and E-stained neonate rat colon. The arrow in picture indicates ganglion cells between longitudinal and circular muscle layers (×40 magnification)

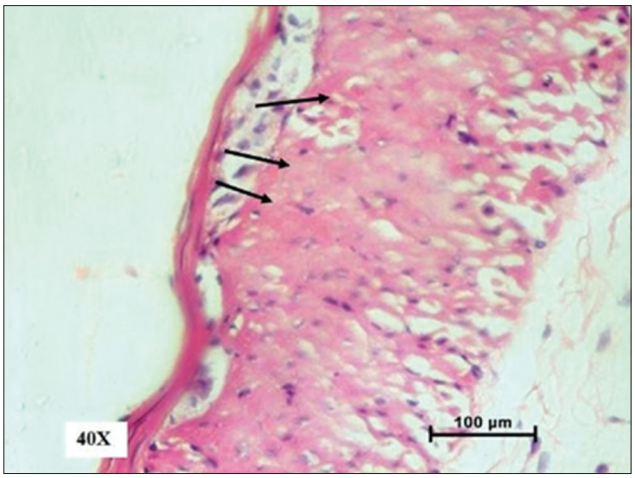

Figure 2: Photomicrograph of H- and E-stained adult rat rectum. The arrow in picture indicates ganglion cells between longitudinal and circular muscle layers (×40 magnification)

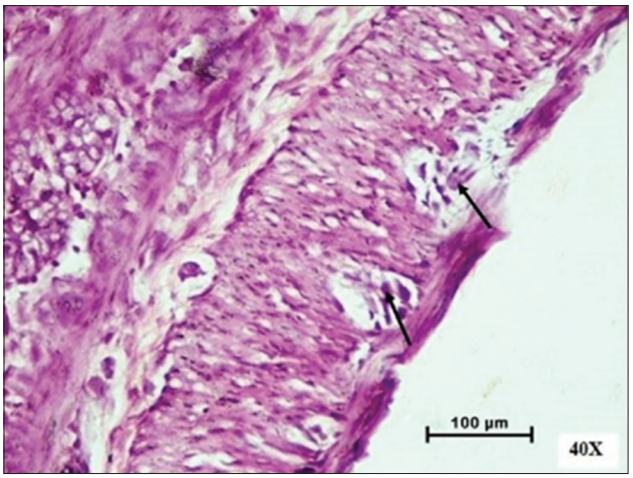

Figure 4: Photomicrographs of H- and E-stained neonate rat rectum. The arrow in picture indicates ganglion cells between longitudinal and circular muscle layers (×40 magnification)

the GIT begin in the fetal period and continue in the post-natal period. This study was performed to see whether the number of ganglion cell and thickness of longitudinal muscle layer in the colon and rectum of rats change over time from neonatal period to adulthood or not because, some of the contractile properties of gut may have correlation with it. Results of our study showed that there was no significant difference in the number of ganglion cells in the longitudinal muscle layer of colon and rectum of adult and neonate rats. In another study, it was reported that the number of neurons in the small intestine of newborn was more as compared to adult rats.[9] It may be due to difference in age of rats used, as in the present study, age of neonate rats was 10-16 days while in the earlier study, newborn rats aged 1-16 h were used. Further, alteration in neuronal density was also reported in other studies in neonate rat and human.[1,5,14]

In the present investigation, thickness of longitudinal muscle layer in both colon and rectum was significantly increased in adults as compared to neonate. Further, our data indicated that thickness in the adult rectum was more as compared to its colon (Figures 1 and 2). The difference in thickness may be implicated for the higher contractile force required in the rectum for evacuation of fecal matter in adult rats. Need of increased contractile force is probably because of change in the form of diet (mainly in the solid form) taken at this age. On the other hand, in neonate, the diet is mainly in the liquid form and increased contractile force in the rectum is not required in neonates. This is supported by our results which indicate no difference in thickness of longitudinal muscle layer between colon and rectum in neonates (Figures 3 and 4).

More number of experiments and histological study using specific stain for the neuronal cells will further help enlighten the structure-function relationship in large gut.

Thus, this study indicated that the thickness of longitudinal muscle layer in colon and rectum increases during development process from neonate to adult. Increase in thickness of longitudinal muscle in rectum was more as compared to colon.

CONCLUSION

It may be concluded that number of ganglion cells in neonate may not differ from the number found in adults. However, thickness of longitudinal muscle in adult rectum was more as compared to neonate probably to meet with the increased contractile force required at this stage to expel the undigested material from the anal canal. Thus, the contractile characteristics of colon and rectum in neonate and adult may have some relation with the muscle thickness.

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REFERENCES

1. Wester T, O'Briain DS, Puri P. Notable postnatal alterations in the myenteric plexus of normal human bowel. Gut. 1999;44:666-74.
2. Fu M, Tam PK, Sham MH, Lui VC. Embryonic development of the ganglion plexuses and the concentric layer structure of human gut: A topographical study. Anat Embryol. 2004;208(1):33-41.
3. Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res 2005;319(3):367-82.
4. Burns AJ, Roberts RR, Bornstein JC, Young MH. Development of the enteric nervous system and its role in intestinal motility during fetal and early postnatal stages. Semin Pediatr Surg. 2009;18(4):196-205.
5. de Vries P, Soret R, Suply E, Heloury Y, Neunlist M. Postnatal development of myenteric neurochemical phenotype and impact on neuromuscular transmission in the rat colon. Am J Physiol Gastrointest Liver Physiol 2010;299(2):G539-47.
6. Gershon MD, Chalazonitis A, Rothman TP. From neural crest to bowel: Development of the enteric nervous system. J Neurobiol 1993;24(2):199-214.
7. Wedel T, Roblick U, Gleiss J, Schiedeck T, Bruch HP, Kuhnel W, Krammer HJ. Organization of the enteric nervous system in the human colon demonstrated by wholemount immune histochemistry with special reference to the submucous plexus. Ann Anat. 1999;181(4):327-37.
8. Furness JB. The Enteric Nervous System. Malden, UK: Blackwell; 2006.
9. Gabella G. Neuron size and number in the myenteric plexus of the newborn and adult rat. J Anat 1971;109:81-95.
10. Gabella G. The number of neurones in the small intestine of mice, guinea-pigs and sheep. Neuroscience. 1987;22(2):737-52.
11. Maifrino LB, Liberti EA, De Souza RR. Morphological and quantitative study of the myenteric plexus of the mouse colon. Braz J Morphol Sci. 2007;24(3);192-5.
12. De Vries P, Soret R, Suply E, Heloury Y and Neunlist M. Postnatal development of myenteric neurochemical phenotype and impact on neuromuscular transmission in rat colon. Am J Physiol Gastrointest Liver Physiol. 2010;299(2):G539-47.
13. Culling CF. Handbook of Histopathological Techniques. 2nd ed. London: Butterworths; 1963.
14. Schäfer KH, Hänsgen A, Mestres P. Morphological changes of the myenteric plexus during early postnatal development of the rat. Anat Rec. 1999;256(1):20-8.

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