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THE QUANTITATIVE ANALYSIS OF MYENTERIC PLEXUS IN THE SIGMOID COLON OF THE LARGE INTESTINE OF THE HUMAN ABORTED FOETUS – A DESCRIPTIVE QUANTITATIVE STUDY.

Dr.Bhavani Prasad.G.[1] Dr. Chandra X. Phillip[2] Dr.Anita Ramdas[1] Dr.Valsa Diana. G.[3]

1. Dr.bhavani prasad. Goriparthi, research scholar, department of anatomy, Pondicherry institute of medical sciences, pondicherry.
2. Dr.chandra .x. Phillip (corresponding author), professor, department of anatomy, mahatma gandhi medical college and research center, sri balaji vidya peeth, pondicherry.
3. Dr. Anita ramdas, professor, department of pathology, pondicherry institute of medical sciences, pondicherry.
4. Dr.valsa diana.g., specialist(sag), rajiv gandhi govt.women and children's hospital, pondicherry.

Abstract:

Background: The large intestine is a derivative of the midgut and the hindgut. It is extending from the caecum to the anal canal. The quantitative values of biopsy segments of a particular part of the colon especially the rectum was published but the normal morphometric values of sigmoid colon studies were minimal.

Aim: The present study was designed to determine the quantitative analysis of the myenteric plexus of the sigmoid segment of the large intestine.

Materials and Methods: A Descriptive study was carried out at Pondicherry Institute of Medical Sciences, Pondicherry from the year 2014 to 2022 and the aborted human foetuses samples were collected from Rajiv Gandhi Govt. Women and Children Hospital, Pondicherry, and from Obstetrics and Gynaecology department, Pondicherry Institute of Medical Sciences, Pondicherry. The total sample size was 50 human aborted foetuses. The age of aborted human foetuses was confined to Biparietal diameter, Crown-rump length and correlated with clinical history The aborted human fetuses were assigned into two groups. Group A was from 13 weeks of gestation to 20 weeks of gestation. Group B was from above 20 weeks of gestation to 36 weeks of gestation. The NADPH diaphorase histochemistry, an enzyme histochemistry technique was used to study the tissue preparations. A para median incision over the abdomen was given and exposed the entire large intestine. The entire large intestine was divided into seven segments i.e. A1- caecum, A2- ascending colon, A3- transverse colon, A4 – descending colon, A5- sigmoid colon, A6- rectum and A7 -anal canal. The aborted human foetuses were assigned into two groups (group A (n = 29), ≤ 20 Weeks of Gestation and group B (n= 21) ≥ 20 Weeks of Gestation.

Statistical analysis: The images were captured by using camera attached binocular microscope and the images were analyzed for various parameters of the myenteric plexus of the sigmoid colon by using Image J software. The data were expressed as mean± standard deviation (SD). The independent sample t-test was done for parametric analysis. The SPSS software package version 20.0 was used for statistical analysis.

Results: The foetuses were assigned into two groups. Group A (n = 29), ≤ 20 WG and Group B (n= 21) ≥ 20 WG. The present study reported that the number of nerve cells in a ganglion and the area occupied by nerve cells was increased in group A than the group B.
Conclusion: The total area, perimeter, diameter, muscular area, inter ganglion distance, neurons per ganglion, myenteric fraction and numerical density were compared between the two groups. The normal measurements of the myenteric plexus of the sigmoid colon will be suggestive for denoting the pathological stage of the sigmoid colon in motility disorders.
Keywords: Gestation, Myenteric fraction, Numerical density, Sigmoid colon, Morphometry, Muscular area.

Introduction:

The large intestine is a derivative of the midgut and the hindgut. It is extending from the caecum to the anal canal. the mucosa is derived from the endoderm and the muscularis externa from the mesoderm. the innervation of these muscles has a major role in the motility of the large intestine (1,2). The enteric nervous system contributes to the innervation of the gut. It is considered the second brain in the gut (3). It is a part of the peripheral nervous system. The enteric nervous system is derived from the neural crest cells (4,5,6). At 12 weeks of gestation, the incorporation of neuroblast cells was finished at the distal end of the rectum. If this process was stopped or ceased at any stage, on or before 12 weeks of gestation, leads to the disappearance of the intramural plexus at any segment of the gastrointestinal tract [7]. NADPH diaphorase enzyme activity was identical to nitric oxide synthase activity in neurons [8]. The number of ganglia and ganglion cells in the enteric nervous system is not the same and varies in different locations of the gastrointestinal tract [9]. Morphometric measurements of enteric nervous system components were very useful as a diagnostic tool to identify the disorders related to the innervation of the gut [10,11]. The morphological and neurochemical properties of the human enteric nervous system are different from other species, even during development, therefore an in-depth investigation of the human enteric nervous system is necessary [12]. The quantitative values of biopsy segments of the particular part of the colon especially the rectum were published but the normal morphometric values of other segments of the large intestine including the sigmoid colon were minimal [12]. It is very important to be known the normal quantitative values of the myenteric plexus of any segment of the gut in the clinically advanced era. So, the present study was designed to determine the normal quantitative values of the myenteric plexus in sigmoid segments of the large intestine of aborted foetuses.

Materials and Methods:

A cross-sectional descriptive study was carried out at Pondicherry Institute of Medical Sciences, Pondicherry from October 2014 to May 2022 and the aborted human fetuses samples were collected from Rajiv Gandhi Govt. Women and Children Hospital, Pondicherry, and from Obstetrics and Gynaecology department, Pondicherry Institute of Medical Sciences, Pondicherry. The total sample size was 50. All human foetuses were collected according to the protocol approved by the Ethics Committee, Pondicherry Institute of Medical Sciences, Pondicherry and Rajiv Gandhi government Women and Children's Hospital, Pondicherry.

The foetuses were assigned into two groups. Group A [n = 29], ≤ 20 WG and Group B [n= 21] ≥ 20 WG. The foetal age was determined by measuring crown-rump length, and biparietal diameter, correlated with clinical history. Most of the foetuses used in the present study were obtained within 2 hours after delivery. A para median incision in the abdomen was given and the large intestine was exposed and immersed in 4% buffered paraformaldehyde for proper fixation. The fixed specimen was preserved at 4° C to minimize the post-mortem changes.

NADPH diaphorase histochemistry an enzyme histo chemistry technique was used to study the tissue preparations [12]. The entire large intestine was divided into seven segments i.e. A1- caecum, A2- ascending colon, A3- transverse colon, A4 – descending colon, A5- sigmoid colon, A6- rectum and A7-anal canal. the colonic tissue was fixed in fresh 4% buffered paraformaldehyde for 2 hours at 4°C. they were washed thoroughly after fixation in chilled 0.1M phosphate buffer. The samples were kept at 15% and 30% sucrose solution for 3 hours and 8 hours at 4° C for cryoprotection. The samples were then frozen in an optimum cutting temperature compound. 20 µm thick sections were cut by using a Leica cryostat. The frozen sections were mounted on 1% gelatine-coated slides. The slides were kept at -20°C for enzyme histochemistry. Cryostat sections on the glass slide were washed several times with 0.1 M phosphate buffer. NADPH diaphorase activity was visible by incubating the sections in 10 ml of 0.1 M tris-Cl buffer containing 10mg NADPH,1 mg of Nitro blue tetrazolium NBT and 0.3% triton X100 at 37° C for 45 minutes to 1 hour in the incubator in dark. The reaction was monitored under a dissecting microscope and was terminated by washing the tissues gently with chilled 0.1 M phosphate buffer when the stain was sufficiently intense. The sections were mounted in a mixture of glycerol and phosphate buffer.
The stained sections were examined under a microscope and images were captured using a camera-connected binocular microscope. The images were saved in JPEG format with minimum compression and maximum quality [table/fig.1]. The images were taken under 40x magnification [table/fig.2]were analysed by using Image J software version 1.53t. [developed at the US National Institute of Health, available at https://imagej.nih.gov/ij/] [13]. Before making measurements, the system was calibrated using a micrometre scale for the magnification at which the images were acquired. The parameters like neuronal cell area, length, myenteric fraction, inter ganglion distance, total muscular area, numerical density and number of neurons per ganglion were used for analysis.

Inclusion and Exclusion Criteria:
All the mothers who had no medical illness during the pregnancy and none of the foetuses had any congenital anomalies were included. The foetuses with neural tube defects were excluded from the study sample.

Statistical Analysis :
Statistical significance was determined using SPSS ver.20.0[IBM Corp., Armonk, N.Y., USA]. The data were expressed as mean± standard deviation. The independent sample ‘t-test was done for parametric analysis. P- value of less than 0.05 was regarded as statistically significant.

Results:

The neuronal cells and their process were stained in the myenteric plexus of the large intestine by NADPH diaphorase histochemistry. The total area, perimeter, diameter muscular area, inter ganglion distance (table/fig.3), neurons per ganglion, myenteric fraction and numerical density were compared between the two groups. The mean value in the number of neurons per ganglion in the ≤ 20 WG was 32.13 and in the ≥ 20 WG was 28.96. The inter ganglion distance in ≤ 20 WG was 70.31 and in the ≥ 20 WG was 93.27. the p value was not significant but there was an increase in group B than in group A. The numerical density was 5.29 in group A and 5.07 in group B.

Table/ fig.4: shows the various parameters related to the myenteric plexus of the sigmoid colon of the large intestine.

Sigmoid colon	Area (μm^2)	Perimeter (μm)	Diameter (μm)	Muscular area (μm^2)	Inter ganglion distance (μm)	Neurons per ganglion	Myenteric fraction	Numerical density
Group A (N=29) ≤ 20wG	45.86 ± 9.22	394.35 ± 75.86	173.45± 33.50	61.00 ± 9.68	70.31 ± 20.10	32.13 ± 8.73	71.962 ± 7.62	5.29 ± 0.85
Group B (N=21) ≥ 20WG	36.88 ± 8.03	337.46 ± 73.51	146.59 ± 34.83	56.12 ± 12.78	93.27 ± 31.83	28.96 ± 7.85	65.482 ± 8.76	5.07 ± 0.76
P value	0.633	0.952	0.631	0.039*	0.155	0.945	0.826	0.921

Discussion:

The development of the enteric nervous system in the human fetal gut is a topic of interest being investigated by many researchers worldwide. Studies have been done on animals but the data have been minimal on human specimens [14]. To the best of our knowledge, this study was one of the few studies reported on morphometric analysis of the sigmoid colon in aborted human foetuses. The neuronal cell profile in the myenteric plexus in the sigmoid colon of the large intestine was analysed and assessed in two groups for establishing normal morphometric values as the gestational age advanced.

S.singh et al [12] observed that the neuronal cell size increased gradually from 14 to 23 WG. Our study reveals that the nerve cell size was increasing constantly up to 20 WG. Hitchcock et al [8] observed that the nerve cells and nerve density peaked at 16 -20 weeks gestation and there was a fall in these values towards adult life. The present study reported that the number of nerve

cells in a ganglion and the area occupied by nerve cells was increased in group A i.e. ≤ 20 WG and these values were decreased in group B i.e. ≥ 20 WG.

Hitchcock et al [8] observed in the human foetal oesophagus that the myenteric fraction peaked at 16-20 WG and the myenteric fraction fell during the late second trimester and became constant from 30 WG. The present study also reveals that these values were increased up to 20 WG and decreased above 20 WG.

Subramanyam et al [15] reported in the normal large intestine that the inter ganglion distance showed an average value of 136.49 ± 39.0 μm with statistically significant ($P < 0.005$). The present study also revealed that the inter ganglion distance was 93.27 ± 31.83 μm in group B (≥ 20 WG) and the inter ganglion distance was 70.31 ± 20.10 μm in group A (≤ 20 WG).

Bhukya S et al [14] reported that the thickness of the circular muscle increased from 11WG to 19WG, but it was static until 26WG in the ascending and sigmoid colon. The thickness of the circular muscle in the oesophagus showed it increased continuously from 11 to 26 WG. The development of longitudinal muscle showed a pattern of continuous, uniform thickness increases in all segments from 13 to 26 WG. The circular muscle thickness was static up to 26 WG and longitudinal muscle thickness was slightly increasing up to 26 WG. The overall thickness was decreased when compared to the previous group of ≤ 19WG where both values were increasing. The present study revealed that the total muscular area increased up to 20 WG and decreased after 20 WG. It is statistically significant.

Limitations: the sample size was small. the availability and procurement of third-trimester samples were difficult.

Conclusion:

There are few studies for the quantification of the myenteric plexus in the sigmoid colon of aborted foetuses. The normal values will help accurately diagnose and determine the pathological stage of the sigmoid colon in gut motility disorders.

References:

1. Costa M, Brookes SJ. The enteric nervous system. Am J Gastroenterol 1994;89:S129-37.
2. Le Douarin NM. The neural crest. Cambridge: Cambridge University Press; 1982
3. Gershon MD. The second brain: the scientific basis of gut instinct and a ground breaking new understanding of nervous disorders of the stomach and intestines. New York: Harper Collins; 1998.
4. Furness JB, Costa M. The enteric nervous system. Edinburg: Churchill Livingstone; 1987.

5. Burns AJ, Douarin NM. The sacral neural crest contributes neurons and glia to the post-umbilical gut: spatiotemporal analysis of the development of the enteric nervous system. Development 1998;125:4335-47.
6. Serbedzija GN, Burgan S, Fraser SE, Bronner-Fraser M. Vital dye labelling demonstrates a sacral neural crest contribution to the enteric nervous system of chick and mouse embryos. Development 1991;111:857-66.
7. Okamoto E, Ueda T. Embryogenesis of intramural ganglia of the gut and its relation to Hirschprung's disease. J Pediatr Surg 1967;2:437-43.
8. Hitchcock RJ, Pemble MJ, Bishop AE, Spitz L, Polak JM. Quantitative study of the development and maturation of human oesophageal innervation. J Anat 1992;180(Pt 1):175-83.
9. Bernardini N, Segnani C, Ippolito C, De Giorgio R, Colucci R, Faussone-Pellegrini MS, et al. Immunohistochemical analysis of myenteric ganglia and interstitial cells of Cajal in ulcerative colitis. J Cell Mol Med . 2012;16(2):318–27.
10. Kim HK, Cheong H, Kang H, Bae JY, Song DE, Cho MS, et al. Histopathological evaluation of pediatric intestinal pseudo-obstruction: Quantitative morphometric analysis of pathological changes in the Enteric nervous system. Korean J Pathol. 2010;44(2):162
11. Boschetti E, Malagelada C, Accarino A, Malagelada JR, Cogliandro RF, Gori A, et al. Enteric neuron density correlates with clinical features of severe gut dysmotility. Am J Physiol Gastrointest Liver Physiol. 2019;317(6):G793–801.
12. Singh S, Shariff A, Roy TS, Kumar H. Prenatal development of the myenteric plexus in human sigmoid colon. J Morphol Sci 2013;30:156-66.
13. Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018
14. Bhukya S, S S, S S, J A Q, A A, Rani N, K D, A D, Nag TC, A S. Morphological changes of the myenteric plexus at different gut segments of human fetuses. J Histotechnol. 2021 Sep;44(3):150-159.
15. Subramanian H, Badhe BA, Toi PC, Sambandan K. Morphometric profile of large intestinal neuronal plexuses in normal perinatal autopsies and Hirschsprung disease. Neurogastroenterol Motil. 2017 Mar;29(3).

Fig 1 : shows the myenteric plexus between longitudinal and circular muscle layer under 10X magnification.

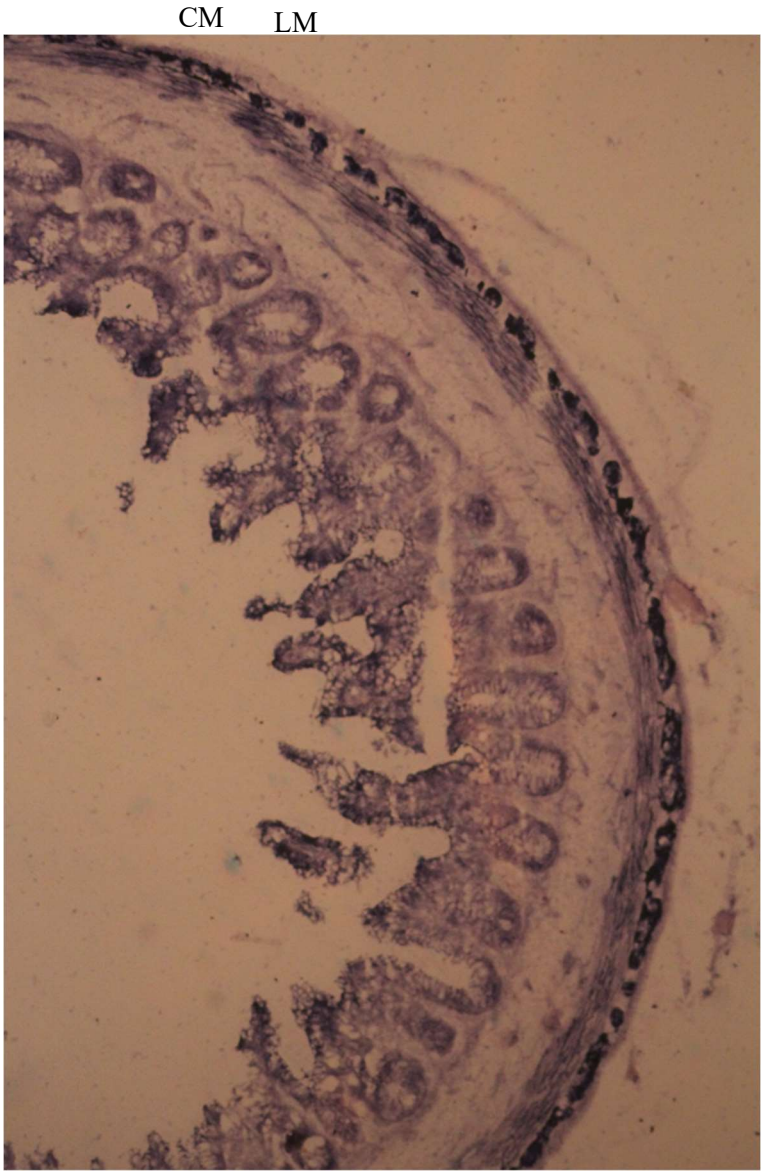

Fig. 2: Long arrow shows myenteric plexus between longitudinal muscle (LM) and circular muscle(CM) and Short arrow shows the sub mucous plexus of sigmoid colon (40 X)

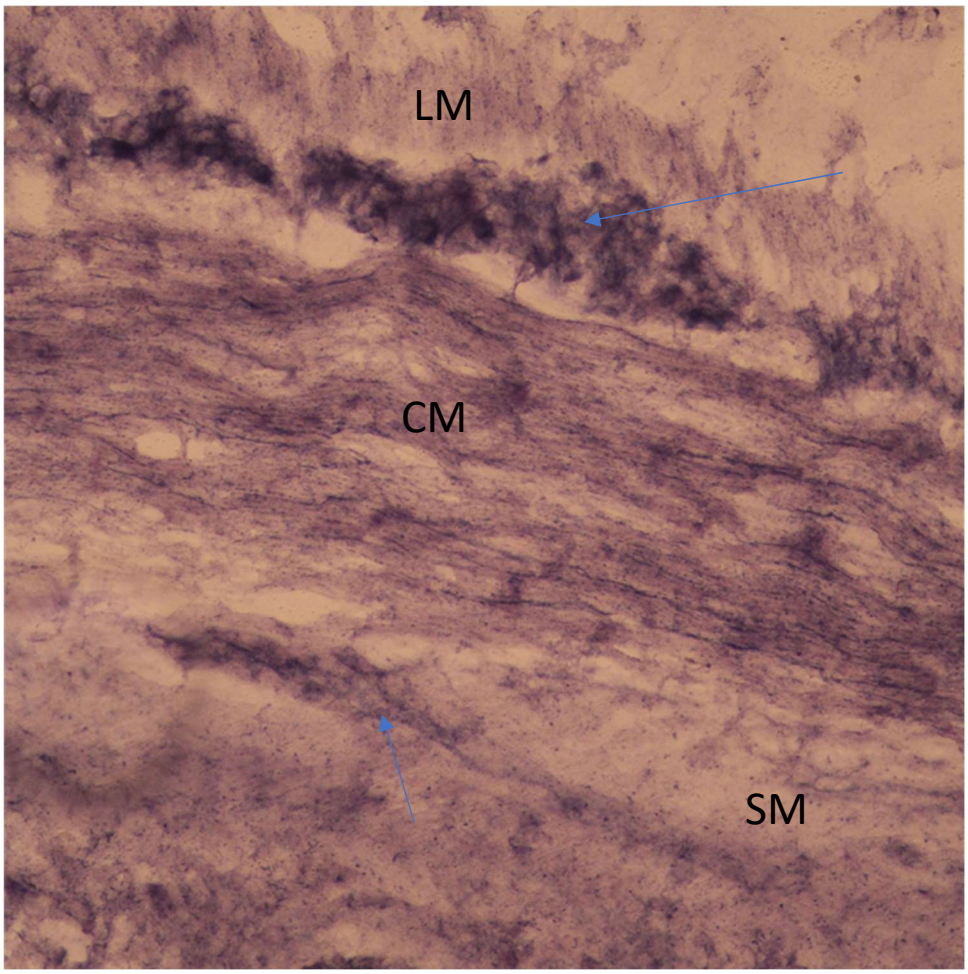

Fig. 3: arrows shows a short interganglion distance in the sigmoid colon (40X)