Original Article

HISTOGENESIS OF HUMAN GASTRIC MUSCULATURE AND NERVE PLEXUSES

Dhanwate Anant Dattatray*, Mohammad Laeeque**, Shaikh Sanobar Istamullah***, Diwan Chhaya Vijay****

ABSTRACT

The knowledge of development of stomach including effective gastric emptying, and motor functions is essential for the provision of optimal nutritional care of the very preterm infant. The generation of functional neuromuscular activity within the pre-natal gastrointestinal tract requires the coordinated development of enteric neurons and glial cells and concentric layers of smooth muscle[1]. Very few documented studies have been done on developing gastric musculature and nerve plexuses. There has long been divergent and sometimes conflicting views expressed about various aspects of the structure of the pylorus, the pathology of which is implicated in Infantile Hypertrophic Pyloric Stenosis[2].

Hence, the microscopic structure and differentiation of human gastric musculature and nerve plexuses was studied in 53 aborted human fetuses of 10 to 31 weeks age under microscope and the differences between different age groups and with previous studies were compared. The study was conducted between 2008 to 2010, in the Department of Anatomy, Government Medical College Aurangabad. Tissues from body and pyloric parts of the stomach were collected, processed, stained with Haematoxylin and Eosin, Masson's Trichrome and Heidenhain's Iron Haematoxylin stains and studied under light microscope. At 10 weeks, all the four structural layers of stomach were discernible. Muscularis mucosae was observed as a thin condensed layer at 13 weeks becoming thicker and wavy in later weeks. The muscularis externa showed circular and longitudinal muscle layers from 10th week, oblique layer being added from 16th week. The muscularis externa was thicker in pyloric region than in the body part in all specimens. Ganglia of myenteric (Aurbach's) plexus were discernible since 13 weeks between circular and longitudinal muscle layers of muscularis externa with increase in their number, size and cellularity from 17 weeks onwards. However, no ganglion of submucosal (Meissener's) plexus was observed in any specimen of either fundic or pyloric part.

Key Words: Gastric musculature, muscularis externa, myenteric ganglia, Pylorus

INTRODUCTION

With the advent of newer diagnostic and therapeutic techniques in medical sciences it becomes imperative to have sound knowledge of the basic human morphology and developmental anatomy. The knowledge of structural and functional development of stomach including effective gastric emptying, and motor functions is essential for the provision of optimal nutritional care of the very preterm infant. Also the hypertrophy of pyloric musculature, deficiency of nerve terminals, ganglia and nerve fibers is implicated in the pathogenesis of Infantile Hypertrophic Pyloric Stenosis[3].

The earlier studies on the development of stomach were on its gross anatomical features and were mainly animal studies. Few human studies conducted on fetuses of western origin includes Keith and Jones (1902)[4], Welch (1921)[5], Fu et al (2003,04)[6,7], Wallace and Burns (2005)[1]. However, hardly any study has been done on Indian fetuses like Chimmalgi and Sant (2005)[8] which differ largely from their western counterparts in the rate of growth, differentiation and maturity. Hence, the microscopic structure and differentiation of human gastric musculature and nerve plexuses was studied

Aims and Objectives:

The objectives were:

1) To study the microscopic structure and differentiation of human gastric musculature and nerve plexuses in fetuses of different age.
2) To compare the differences between different age groups and with previous studies

MATERIALS AND METHODS

The study was conducted in the Department of Anatomy, Government Medical College and Hospital, Aurangabad between 2008 to 2010. After approval from the institutional ethical committee, 53 aborted human fetuses (34 males and 19 females) of 10 to 31 weeks of age were procured from this hospital for research work. Only the fetuses free from congenital anomalies were included in the study.

Fetuses were obtained within 1-2 hrs of abortions to avoid post-mortem changes and were immediately kept in 10% formalin. Gestational age of the fetus was calculated from the first day of last menstrual period (LMP). Fertilization age (actual age/conceptional age/developmental age) was obtained by subtracting two weeks from the LMP gestational age. Fertilization age (age in postconceptional weeks) was also determined from Crown Rump Length of the fetus and using table in the Moore and Persaud [9].

Stomach was dissected out within one hour of collection of fetus, samples of tissue 3-5mm in diameter were collected from body and pyloric parts and fixed in Bouin's fluid, processed to prepare paraffin embedded blocks and 5μ thick sections. The slides thus prepared were stained with Haematoxylin & Eosin (H&E), Masson's Trichrome(MT) and Heidenhain's Iron Haematoxylin stains and were studied under light microscope.

OBSERVATIONS

I. Microscopic observations in Fundic and Body **parts of the stomach:** (Colour Plates **I-II**)

10-12 weeks: All the four layers of stomach were clearly visible by 10th week with submucosa being the thickest layer. Muscularis mucosae was not demarcated. Muscularis externa showed well developed circular muscle layer. External to this, a thin layer of longitudinal muscle fibres was observed at places but was not uniformly distributed. No oblique muscle layer could be seen at this stage. Heidenhain's Iron Haematoxylin stain particularly demonstrates cell membranes, secretory granules and nuclear chromatin. All these structures and smooth muscles of muscularis externa were stained grey-black with it. Ganglia of neither myenteric nor submucosal plexus were discernible at this stage.

13-14 weeks: The muscularis mucosae made its appearance at this stage as a thin and interrupted layer of spindle shaped cells with elongated nuclei. However, it failed to take up specific muscle stain with Masson's Trichrome. Muscularis externa showed well developed circular and longitudinal muscle layers but oblique muscle layer was still not demarcated.

Very few, minute, round to oval, well circumscribed, pale stained structures were observed at the junction of outer longitudinal and inner circular layers of muscularis externa. Under high power, these were the clumps of ganglion cells (neurons) intermingled with supporting glial (satellite) cells marking the earliest appearance of parasympathetic ganglia of myenteric (Auerbach's) plexus. The ganglia, constituting neurons (with large nuclei) and satellite cells (with small nuclei) were circumscribed by connective tissue stained green by Masson's Trichrome stain. These structures were also well demarcated by Heidenhain's Iron Haematoxylin stain which stained cell membranes and nuclei of ganglion cells grey-black. No such ganglia were observed in the submucosa.

15-20 weeks: Muscularis mucosae was thicker and wavier layer and sent fine bundles of smooth muscle fibres between the fundic glands and into the meshwork of the lamina propria.. Muscularis externa showed additional inner oblique layer from 16 weeks.

The number and size of myenteric ganglia was significantly increased with more neurons and glia. However, ganglia were still not discernible in the submucosa.

25, 31 weeks: By twenty fifth week, mucosa and muscularis externa were the thickest layers. Muscularis mucosa was wavy and distinct. There was an increase in the thickness of muscularis externa, showing outer longitudinal, middle circular and inner oblique muscle layers distinctly. By 31st week, overall picture began to resemble that of an adult. The number, size and cellularity of myenteric ganglia were significantly increased; however, no ganglia were discernible in the submucosa.

II. Microscopic observations in Pyloric part of the stomach: (Colour Plate III-IV)

In the pyloric part, all the four layers were seen by 10th week as in the fundic part. Muscularis mucosae was seen as a condensed layer with H and E from 13th week and stained bright red with Masson's Trichrome from 15th week. From 13 weeks, mucosa and muscularis externa were the thickest layers. Muscularis externa was thicker than that of the body throughout the foetal period. It showed outer longitudinal and inner circular muscle layers from 10th week, oblique layer being added from 16th week. Circular muscle coat was thicker at all ages.

The myenteric ganglia of Auerbach's plexus, constituting neurons (with large nuclei) and satellite cells (with small nuclei) circumscribed by connective tissue, were discernible since 13 weeks. Their number, size and cellularity increased during subsequent weeks. They formed almost a continuous layer in the pylorus from 17th week. The number of these ganglia in pylorus was always more than that in the body part. However, no ganglion in the submucosa was observed in any specimen studied.

DISCUSSION

The microscopic structure and differentiation of gastric musculature and myenteric ganglia of body and pyloric part of the human stomach was studied in the fetuses of 10-31 weeks of age with following parameters: relative sizes of all the four layers; differentiation and growth of muscularis mucosae, muscularis externa and nerve plexuses.

A) Fundic and Body part of the stomach

1) Relative sizes of all the four layers:

All the four layers of stomach were clearly discernible from 10th week onwards. Submucosa was the thickest layer before 16 weeks. Mucosa, muscularis externa showed rapid growth from 17th week and were thickest layers from 25th week onwards.

In the fundus of a 3rd month fetus, Keith and Jones (1902)[4] observed four distinctly marked strata in its walls with submucosa being extremely thick and cellular. Chimmalgi and Sant (2005)[8] described that submucosa is the thickest layer at 15-20 and 25-27 gestational weeks while mucosa and muscularis externa are thicker at 21-24 and beyond 28 gestational weeks.

2) Muscularis mucosae:

In the present study, muscularis mucosae was demarcated as a thin and interrupted layer of spindle shaped cells by 13th week. However, it failed to take up stain for muscle with Masson's Trichrome at this stage and stained positively from 15 weeks. It became thicker and wavier in subsequent weeks. Welch (l921)[5] observed the muscularis mucosae at 65.0 mm CRL, although it was still incomplete at this stage. Arey (1974)[10] stated first appearance of muscularis mucosae at 160mm CRL stage (18 wks). Chimmalgi and Sant (2005)[8] demonstrated its appearance by the end of 22nd gestational week when it was seen only as condensation and failed to take up stain for muscle with Masson's Trichrome stain. Clearly discernible smooth muscle cells taking up special stain were seen only from 28th week. Variable staining of this layer in earlier weeks could be the reason why some workers quote its appearance at a later stage.

3) Muscularis externa:

At 10th week, muscularis externa showed well developed inner circular muscle layer and a thin external layer of longitudinal muscle fibres which appeared as discrete, scattered bundles. Longitudinal layer was seen as a well defined uniform layer from 13th week. Inner oblique layer was discernible from 16th week. Muscularis externa is relatively thicker between 17-18 weeks and from 25th week onwards.

Keith and Jones (1902)[4] stated that in a fundus of a 3rd month fetus, the muscular stratum consists almost entirely of circular muscle; there is then no evidence of a longitudinal coat, although this can be detected towards the pyloric end of the stomach. Welch (l921)[5] studied the development of stomach musculature in human fetuses. Circular muscle fibres differentiated at 17.0 mm CRL; became complete layer at 24.0 mm CRL and formed a coat of 0.01 mm thickness at 65.0 mm CRL (10th week).

The longitudinal coat was discrete at 17.0 mm stage, formed a layer at 65.0 mm stage (10th week); however, its development was not complete until the first year. The oblique fibres were discernible at 24.0 mm CRL and formed a band at 65.0 mm CRL (10th week).

Jirásek (2004)[11] stated that in the human stomach, muscularis externa consists of circular layer from 10th gestational week, longitudinal being added from 11th week. Wallace and Burns (2005)[1] found that, gut smooth muscle are matured in a rostrocaudal direction, from the 8th week. Circular muscle developed prior to longitudinal muscle. Chimmalgi and Sant (2005)[8] observed circular muscle layer from 15th gestational week, longitudinal layer being added by 28th week. Marciano and Wershil (2007)[12] stated that the muscular development of the stomach is complete by 7 months.

4) Myenteric and Submucosal Nerve plexus:

From 13 weeks onwards, parasympathetic ganglia of myenteric (Auerbach's) plexus were observed at the junction of longitudinal and circular muscle layers of muscularis externa as round to oval, well circumscribed, pale stained structures. These were clumps of ganglion cells (neurons) intermingled with supporting glial (satellite) cells circumscribed by connective tissue. Their number, size and cellularity increased during subsequent weeks and they formed almost a continuous layer in the pylorus from 17th week. The number of these ganglia in pylorus was always more than that in the body part. However, no ganglion in the submucosa was observed in any specimen studied.

Sanderson and Walker (2000)[13], Marciano and Wershil (2007)[12] observed neural precursors in the human fetal stomach at 7 weeks. Fu et al (2003)[6], Wallace and Burns (2005)[1] observed the migration of vagal level neural crest cells (NCCs) that colonize the entire gut in a rostrocaudal manner between 4 to 7 weeks. By 7 weeks, NCC colonization of the gut was complete with differentiation into neurons and glia which aggregated into ganglia of myenteric plexus in the foregut.

Furness (2006)[14] observed the neural crest cells in the developing stomach at 6 weeks, and the myenteric plexus by 8-9 weeks. By 12-14 weeks the gut has developed its adult form and pattern of enteric ganglia. The ganglionated submucosal plexus is present in the small and large intestines, but is absent from the oesophagus and contains only very few ganglia in the stomach. Johnson (2006)[15] also stated that the submucous plexus is absent or sparse in the esophagus and stomach.

The early detection of myenteric plexus ganglia in the aforesaid studies is because of use of immunohistochemical techniques. We concur with Furness (2006)[14] that by 12-14 weeks the gut has developed its adult form and pattern of enteric ganglia. Non-demarcation of submucosal plexus ganglia in any of the specimen studied is in accordance with Furness (2006)[14] and Johnson (2006)[15] that the ganglionated submucosal plexus is absent or sparse in the stomach.

Microscopic parameters in Pyloric part of the stomach:

1) Relative sizes of all the four layers:

By the 10th week, all four layers were seen in the pyloric part as in the fundic part with submucosa being the thickest layer. From 13 weeks onwards, mucosa and muscularis externa were the thickest layers. Muscularis externa was thicker than that of the body throughout the foetal period. These findings corroborate with those of Chimmalgi and Sant (2005)[8] who observed all the four layers by the 15th gestational week and at all stages, mucosa and muscularis externa layers were thicker than the similar layers in the fundus.

2) Muscularis mucosae:

Muscularis mucosae followed same pattern as in fundic and body part of the stomach.

3) Muscularis externa:

Muscularis externa was thicker than that of the body throughout the foetal period. It showed outer longitudinal and inner circular muscle layers from 10th week, oblique layer being added from 16th week. Circular muscle coat was thicker at all ages.

Welch (l921)[5] observed that circular muscle fibres differentiated at 17.0 mm CRL; became complete layer with definite thickening over the pylorus at 24.0 mm CRL and formed a coat of 0.03 cm thickness at the pyloric "sphincter" at 65.0 mm CRL (10th week). The longitudinal coat was discrete at 17.0 mm stage, became more apparent at the 41.0 mm stage with increase in the number and distribution of muscle fibres, formed a layer at 65.0 mm stage (10th week); however, its development was not complete until the first year.

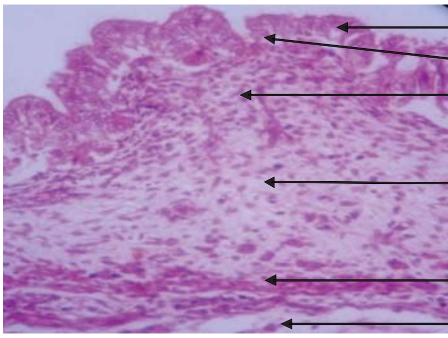
Fu et al (2004)[7] found that by the 7th week, the longitudinal and circular muscle layers started to differentiate in the foregut and by the 9th week, they are formed along the entire gut. Chimmalgi and Sant (2005)[8] stated that at all stages, both in body and pylorus, circular muscle layer was seen in the muscularis externa, the longitudinal layer being added only by the 28th gestational week.

4) Myenteric and Submucosal Nerve plexus:

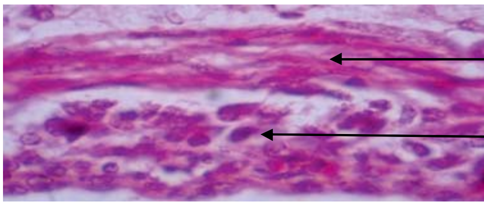
The myenteric ganglia of Auerbach's plexus, constituting neurons (with large nuclei) and satellite cells (with small nuclei) circumscribed by connective tissue were discernible since 13 weeks. Their number, size and cellularity increased during subsequent weeks and were always more than that in the body part. However, no ganglion in the submucosa is observed in any specimen studied.

The early detection of myenteric plexus ganglia in the studies mentioned earlier is because of use of immunohistochemical techniques. Friesen et al (1956)[16] studied the myenteric nerve plexuses in fetuses and infants using light microscope. At 12 weeks' gestation, the myenteric nerve layer of the pylorus appeared as an almost continuous layer of immature nerve cells with little, if any segmentation into nests or plexuses. At 14 to 16 weeks there was a tendency towards elongated plexuses of cells which were still undifferentiated. At 26 weeks the myenteric layer showed organization into definite plexuses which contained, in addition to the undifferentiated cells, some cells with vesicular nuclei. Shortly after birth more mature cells appeared in the plexuses.

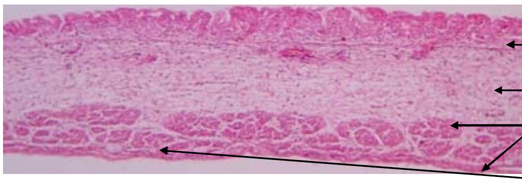
COLOUR PLATE I



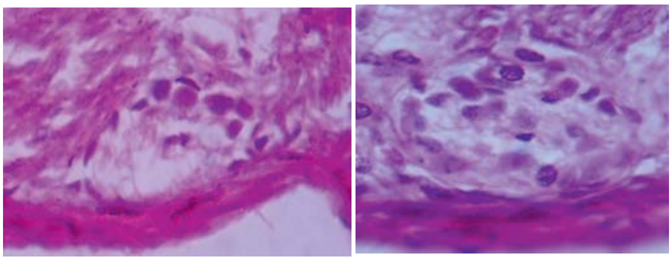
Stomach (Fundic); T.S; 10 weeks; H&E; 40X



Stomach (Fundic); T.S; 10 weeks; H&E; 100X; Musculoris Externa

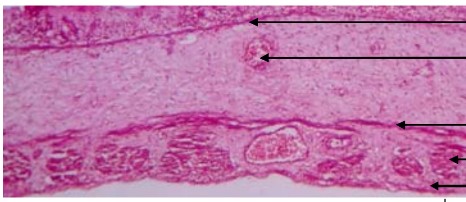


Stomach (Fundic); L.S; 13 weeks; H&E; 10X

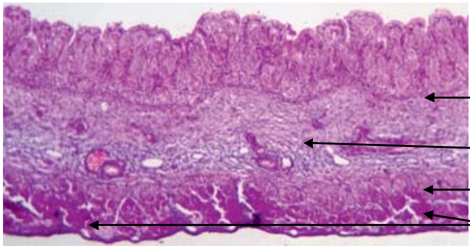


Stomach (Fundic); L.S; 13 weeks; H&E; 100X; Myenteric Ganglia

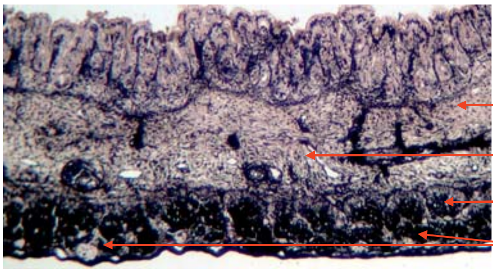
COLOUR PLATE I



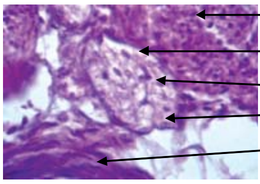
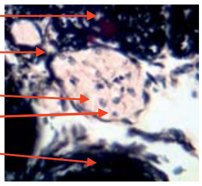
Stomach (Fundic); L.S; 16 weeks; H&E; 10X



Stomach (Fundic); L.S; 17 weeks; H&E; 10X



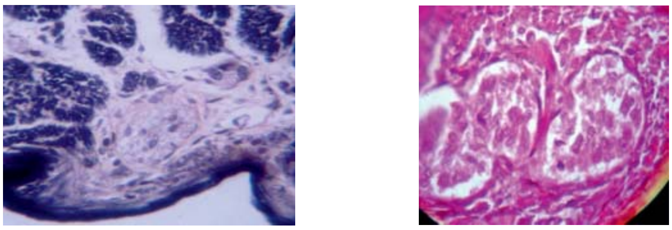
Stomach (Fundic); L.S; 17 weeks; IH; 10X

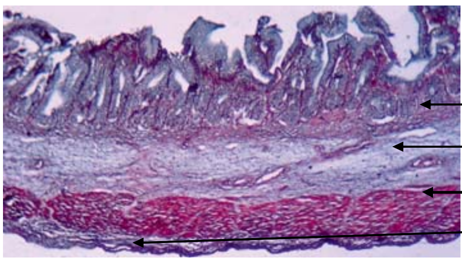
Stomach (Fundic); LS; 17 weeks;H&E; 100X

Stomach (Fundic); LS; 17 weeks; IH; 100X

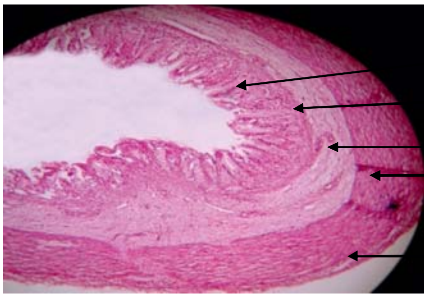
COLOUR PLATE III



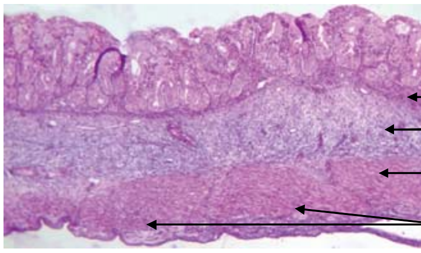
Myenteric Ganglia in Stomach(Fundic);L.S; 20 wks; IH; 40X and 31 weeks; H&E; 100X



Stomach (Pyloric); TS; 15 weeks; MT; 10X

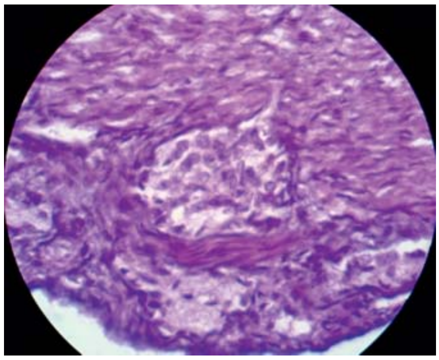


Stomach (Pyloric); TS; 16 weeks; H&E; 10X

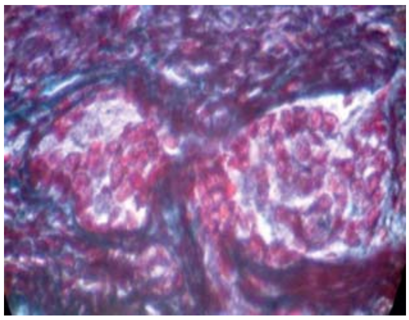


Stomach (Pyloric); L.S; 17 weeks; H&E; 10X

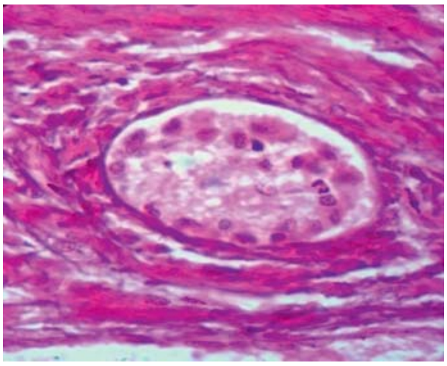
COLOUR PLATE IV



Myenteric Ganglia and Plexus in Stomach (Pyloric); 17 weeks; H&E; 100X



Myenteric Ganglia and Plexus in Stomach (Pyloric); 17 weeks; MT; 100X



Myenteric Ganglia in Stomach (Pyloric); 31 weeks; H&E; 100X

Observed Differentiation of Gastric musculature and Myenteric ganglia in our Study

Fertilization Age (weeks)	Fundic and Body part	Pyloric part
10	• All 4 layers present with submucosa being thickest • Inner circular and outer longitudinal layers of muscularis externa	• All 4 layers present with submucosa being thickest • Inner circular and outer longitudinal layers of muscularis externa
13	• Muscularis mucosae (MT negative) • Ganglia of Myenteric (Auerbach's) plexus	• Muscularis mucosae • Ganglia of Myenteric (Auerbach's) plexus
15	• Muscularis mucosae (MT positive)	• Mucosa and muscularis externa are the thickest layers
16	• Inner oblique layer of muscularis externa	• Inner oblique layer of muscularis externa
17-20	• Increased number, si ze and cellularity of myenteric ganglia	• Increased number, size and cellularity of myenteric ganglia
25-31	• Mucosa and muscularis externa are thickest layers	• Rapid increase in thickness of circular layer of muscularis externa

CONCLUSIONS

During development of human fetal stomach, cellular differentiation is followed by increase in size and organization of various layers. Muscularis mucosae is discernible from 13th week. Muscularis externa shows both circular and longitudinal muscle layers from 10th week, oblique layer being added from 16th week. Muscularis externa is thicker in the pyloric part at all weeks with circular layer being thickest. Parasympathetic ganglia of myenteric (Aurbach's) plexus are discernible since 13 weeks with increase in number, size and cellularity from 17 weeks onwards. They were densely situated in the pyloric part. However, no ganglion is observed in the submucosa indicating ganglionated submucosal plexus is absent or sparse in the stomach. These observations corroborate with most of the previous studies on western fetuses. The late demarcation of muscularis mucosae, muscularis externa in few of the earlier studies and Indian studies may be due to use of less sensitive stains. The early demarcation of myenteric ganglia in other studies is due use of more sensitive immunohistochemical stains.

REFERENCES

1. Wallace AS and Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res. 2005 Mar; 319(3):367-82
2. Keet AD. Introduction ; Infantile hypertrophic pyloric stenosis. In: Keet AD. The pyloric sphincteric cylinder in health and disease. Berlin Heidelberg: Springer-Verlag; 19931-2, 103-107.
3. Hernanz-Schulman M., Infantile Hypertrophic Pyloric Stenosis, Radiology. 2003, May;227(2):319-31
4. Keith A and Jones FW. A Note on the Development of the Fundus of the Human Stomach. Journal of Anatomy and Physiology. 1902; 36: XXXIV-XXXVIII
5. Welch EH. Development of the musculature of the stomach with special reference to its condition in the newborn child and the premature infant. Papers from Mayo Foundation and Medical School Univ Minnesota. 1921- 1922, 2, 3-23.
6. M. Fu, VCH Lui, MH Sham, ANY Cheung, P Kwong, H Tam. HOXB5 expression is spatially and temporarily regulated in human embryonic gut during neural crest cell colonization and differentiation of enteric neuroblasts. Developmental Dynamics. 2003; 228(1):1-10
7. M. Fu, P. K. H. Tam, M. H. Sham and V. C. H. Lui. Embryonic development of the ganglion plexuses and the concentric layer structure of human gut: a topographical study. Anatomy and Embryology. April 2004; 208(1): 33-41
8. Chimmalgi M and Sant SM. Study of fetal stomach under light microscope. J. Anat. Soc. India. 2005;54(2): 22-27
9. Moore KL, Persaud TVN. The developing human. 8th ed. Saunders-Elsevier. Philadelphia 2008: 96, 213-16

10. Arey LB. Developmental Anatomy- A textbook and laboratory manual of embryology. 6th ed. Philadelphia and London: WB Saunders company 1956: 243-44.
11. Jan E. Jirásek. An atlas of human prenatal developmental mechanics: anatomy and staging. Illustrated edition, Informa Health Care Publisher, 2004: 53
12. Marciano T and Wershil BK. The Ontogeny and Developmental Physiology of Gastric Acid Secretion. Current Gastroenterology Reports. 2007; 9: 479–81
13. Sanderson IR, Walker WA. Development of the gastrointestinal tract. PMPH-USA Publisher, 2000. Pages: 212-13.
14. John Barton Furness. The enteric nervous system. Illustrated Edition. Wiley-Blackwell Publisher, 2006 :25, 26, 28
15. Leonard R. Johnson. Physiology of the gastrointestinal tract. In: Kim E. Barrett, Leonard R. Johnson, Fayez K. Ghishan. 4th ed. Academic Press Publisher, 2006,(2): 578, 595
16. Friesen SR, Boley JO, Miller DR. The myenteric plexus of the pylorus: its early normal development and its changes in hypertrophic pyloric stenosis. Surgery I956, 39, 21-29.
17. Carleton HM and Drury RAB. Histological Techniqe for normal and pathological tissues and the identification of parasites. 3rd ed. London, New York, Toronto: Oxford university press 1957:11-115

* Corresponding Author
Dr. Dhanwate Anant Dattatray, MD
Assistant Professor,
Department of Anatomy,
Government Medical College,
Aurangabad, Maharashtra, India

** **Dr. Mohammad Laeeque,** MS
Assistant Professor,
Department of Anatomy,
Government Medical College,
Aurangabad, Maharashtra, India

**** **Dr. Shaikh Sanobar Istamullah,** MS
Associate Professor,
Department of Anatomy,
Government Medical College,
Aurangabad, Maharashtra, India

**** **Dr. Diwan Chhaya Vijay,** MS
Professor and Head,
Department of Anatomy,
Government Medical College,
Aurangabad, Maharashtra, India