A study on morpho-histogenesis of human foetal pons

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Abstract
Introduction: During the second month of intra uterine life, the metencephalon widens and the cells of the metencephalon present in the ependymal zone migrate into mantle and marginal zones and assume the adult position by 12th week of intrauterine life. The cellular collection constituting the various nuclei of tegmentum of pons is very much striking amidst reticular formation.
Materials and Methods: Out of 100 foetuses obtained, foetuses with a difference of two weeks gestation from first trimester to third trimester have been taken for the study (10wks,12wks, 14wks,16wks, 18wks, 20wks, 22wks, 24wks, 28wks, 32wks, 36wks and 40wks).
Results: The length of the pons increased from 3mm to 2cms and width increased from 5mm to 1.8 cm. The neurons in the earlier gestations are small and round. By the end of third trimester, they became large spindle (fusiform) shaped and multipolar.
Conclusion: Knowledge of the migration of neurons to form nuclear groups is essential for defects of the basal ventricular zone result in defects of specific cranial nerve nuclei such as the abducent and facial nerves and for any prenatal evaluation in cases of suspected of brain anomalies

Keywords: Abducent nucleus, Facial nucleus, Multipolar neurons, Spindle shaped neurons.

Introduction
Pons a bridge between medulla oblongata and mid brain is seen as early as 7th week of gestation as a derivative of metencephalon.[1] Studies on the morphometry and histogenesis of pons are meagrely available. During the second month of intra uterine life, the metencephalon widens and the cells of the metencephalon present in the ependymal zone migrate into mantle and marginal zones and assume the adult position by 12th week of intrauterine life. The cellular collections constituting the various nuclei of tegmentum of pons is very much striking amidst reticular formation. Many authors have studied the dimensions of human brain with references to age, sex, health and disease. The morphometric data and cytoarchitectural dimensions and structure of foetal brains are meagre. Hence, the present study is undertaken to make a detailed note and observation of cytoarchitectural components of foetal pons.

Materials and Methods
100 foetuses (10wks-2, 12wks-10, 14wks-8, 16wks-8, 18wks-7, 20wks- 11, 22wks-6, 24wks- 12, 28wks-8, 32wks-8, 36-10, 40wks- 10 in number) were obtained from general hospital, and local government and private hospitals (total 3 hospitals), after getting necessary permissions from the concerned hospital authorities and respective parents. These foetuses were well preserved and CR length measurements were noted and the gestational ages were computed (Hamilton, W. J., and Mossman 1972[2]). The earliest gestational age of the aborted foetuses was of 10 weeks. Beyond this one foetus for every 2 weeks gestation up to 24 weeks and one fetus for every four weeks until full term totalling 12 foetuses have constituted the study material.

The foetuses of earlier gestation could not be obtained for the study. For the detailed morphometric and cytoarchitecture study, the available 12 foetuses were grouped as follows: (Narasinga Rao B and Pramila Padmini M 2009[3])
Group I: up to12wks
Group II : 14-24 wks
Group III: 25-40wks

Length of the pons was measured by a digital verniercaliper from lower border to upper border of pons at the basilar sulcus. Breadth of the pons was measured just lateral to the attachment of trigeminal nerves as per the study of P. Chawla 1975.[4] The present study shows histogenesis of neurons in different nuclei of metencephalon. Transverse sections as a whole are studied at the level of facial colliculus from 20wks of gestation till 40wks. Complete hindbrain are fixed and processed for histological study prior to 20wks. 5 microns thick sections and every fifth section of the tissue taken was studied under 4x, 10x, 40x magnification (i.e., under 4x10= 40 times magnification, 10x10=100 times magnification, 40x10=400 times magnification) using a Labomed binocular microscope). Length and breadth of the neurons in the cytoarchitectural study has been measured by stage and ocular micrometer under high power by using H&E staining and Holmes silver nitrate. Study of fibres and tracts are not included in this article.

Results
Macroscopic Measurements
Ist trimester (up to 12wks) Breadth of the pons is more than the length. Length and breadth measured are 3mm and 5mm

IInd trimester (14 – 24wks) The length increased from 3mm to 6mm and the breadth increased from 5mm to 8mm (Table 1). By the end of II trimester there is an increase in breadth equal to the length which shows that the neurons continued to migrate and formed various nuclei in the tegmentum increasing the width of the pons.

IIIrd trimester: 28 – 40wks

The length reached a maximum of 2cm at 40 wks. It has increased by two times its length from starting of IIIrd trimester to the end of IIIrd trimester. The breadth increased from 1.2 cm to 1.8 cm from 28th to 40th week (Table 1). The length is more than the breadth which shows that there is growth along the cranio - caudal axis due to increase in the number of neurons and formation of various tracts.

Table 1: Showing the measurements of pons from 10-40wks

Age	Length	Breadth
10wks	3mm	5mm
12 wks	3mm	5mm
14 wks	3mm	5mm
16 wks	4mm	5mm
18 wks	4mm	6mm
20 wks	5mm	5mm
22 wks	5mm	6mm
24 wks	6mm	8mm
28 wks	1cm	1.2cm
32 wks	1cm	1.1cm
36 wks	1.5cm	1.3cm
40 wks	2cm	1.8cm

Microscopic Observations

At 10wks the metencephalon has widened and the cells of the metencephalon in the ependymal zone and marginal zone spanned out. The ventricular cavity is clearly distinct. During 12wks there is differentiation of basilar and tegmental part of pons (Fig. 1a). Cerebellum is seen to cover the dorsal surface of the pons. Entire tegementum seem to have rounded neuroblast cells. The nuclear groups are not distinct.

At 14wks there is further sharpening of the neuroblast cells. The basilar part of the pons has been identified with the migrating neurons that constitute nuclei pontisand are distributed in the white mater. Nuclei of abducent and facial nerves have been identified in the tegmentum of pons by 18 wks (Fig. 1b).

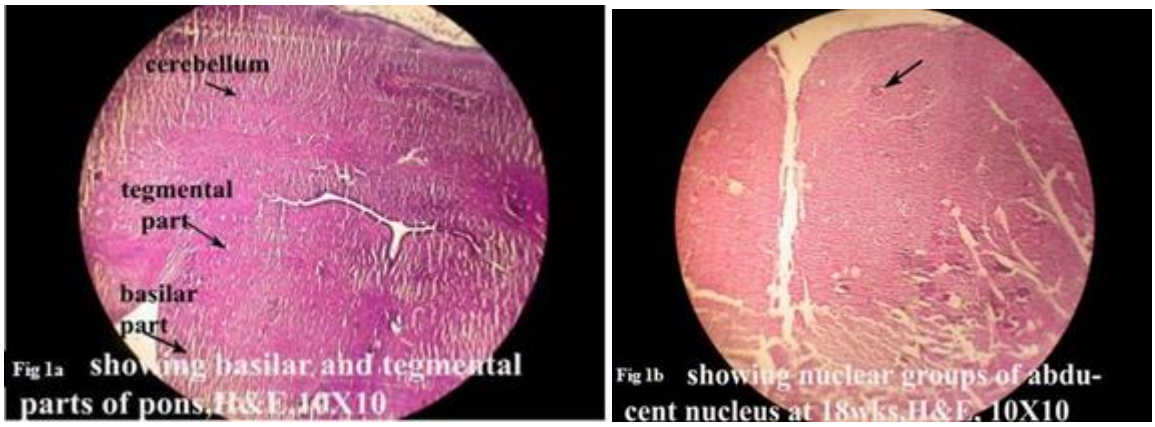

Fig. 1a: Showing basilar and tegmental parts of pons, H &E, 10X10; b: Showing nuclear groups of abducent nucleus at 18wks, H &E, 10X10

Cellular differentiation into multipolar cells, spindle shaped cells have been observed in the nucleus of abducent, facial and superior olivary nucleus (SOL). The SOL is arranged as many group of clusters at 28wks (Fig. 2a). By 32wks configuration of multipolar cells that constitute different nuclear groups of abducent, facial and superior olivary complex has been increased. At 36wks the neuronal size has increased. A prominent nucleus and nucleolus is clearly distinct. The cells are small, rounded and spindle shaped in abducent nucleus (Fig. 2b) and facial nucleus (Fig. 2c).

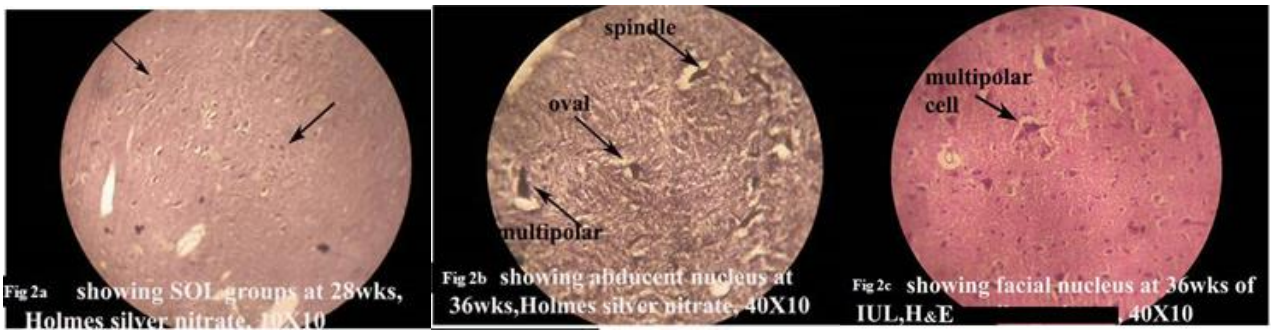

Fig. 2a: Showing SOI groups at 28wks, Holmes silver nitrate, 10X10; b: Showing abducent nucleus at 36wks, Holmes silver nitrate, 40X10; c: Showing facial nucleus at 36wks of IUL, H&E

Discussion

The abducent nucleus is a pontine nucleus directly involved in oculomotion through its connections with the lateral rectus muscle of the eye. The cytoarchitectural organization of the abducent nucleus in man showed that the nerve cell bodies were small, medium and large in size and polygonal, oval, round or spindle shaped. (R. Bianchi et al 1996[5]).

Cytoarchitectonic study revealed similarities between the facial motor nucleus of primates and the facial motor nucleus of other species, except for a mild rotation of the nucleus as also observed in humans (Van Buskirk C. 1945[6]). The components which are prominent of the superior olivary complex of primates were specifically studied, which are the medial (SOM) and lateral (SOL) superior olivary nuclei. Cell counts were done in human brainstems for these particular segments. The SOL appears somewhat inconspicuous in the human because it is organized into as many as six clusters of cells rather than forming as a circumferential configuration as in the monkey and cat (Strominger and Hurwitz 2004[7]). Neurons of the basilar pontine nuclei are derived from the pontobulbar portion of the rhombic lip and migrated circumferentially towards the ventral surface of the brain stem. They contribute to form mossy fibers to the maturing cerebellar cortex from 20 weeks of gestation onwards. The period of maturation of neurons in the metencephalon corresponds to the development of neuronal nuclear antigen, which begins to appear in pontine neurons about 14wks of gestation and is distinctly developed by 20wks of gestation (Sarnat et al., 1998[8]). In the present study the pontaine neuronal groups are distinct by 18wks gestation. Nozaki et al. 1992[9] have reported that after 27weeks, the pontine neurons are distinguishable from glial cells and the neuronal numbers remain relatively constant, and that the pontine neuronal numbers do not indicate the developmental stages. In the present study also the superior olivary nucleus has been identified as clusters of cells by 28wks.The cells of the abducent nucleus are small, round and spindle which is similar to the findings of R. Bianchi et al 1996.[5] Hatta T et al 2007[10] found that the ventral portion increased in size more rapidly than the dorsal portion. The proportion of the ventral portion in the total dorsoventral length was constitutively higher than that of the dorsal portion in the present range of CRL.

Knowledge of the migration of neurons to form nuclear groups is essential for an abnormal development of the superior rhombiclip which may cause diffuse granule cell hypoplasia while abnormal development of the cerebellar ventricular zone due to mutation of the *PTF1A* gene causes cerebellar (and pancreatic) agenesis (Sellick et al., 2004[11] Hoshino et al., 2005[12]) and defects of the basal ventricular zone result in defects of specific cranial nerve nuclei such as the abducens and facial nerves. (Al-Baradie et al., 2002,[13] Michielse et al., 2006[14]). Basic knowledge of the normal appearance and development of the fetal pons is essential for any prenatal evaluation in cases of suspected of brain anomalies, such as Dandy–Walker complex, pontocerebellar atrophy and rhombencephalosynapsis (de Souza N 1994,[15] Utsunomiya H 1998,[16] Litherland J 1993,[17] Barth PG 2000,[18] Chaves-Vischer V 2000,[19] Rudnik-Schoneborn S 2003[20]).

Conclusion

Migration of neurons and maturation of the neurons is important to know the consequences resulting from non-migration of neurons due to genetic mutations which may lead into defects of ventricular zone, pontocerebellar atrophy and rhombencephalosynapsis.

Note: As it is not possible to study all the nuclear groups at the same time and publish in the same article as some journals will accept only 5-6 figures, we the authors had study only about facial, abducent and superior olivary nucleus in the present article.

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Conflict of Interest: None.

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