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Original Article

Anatomy Section

Expression of Brain Derived Growth Factor in Hippocampus of Mid Gestational Human Fetuses

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ABSTRACT

Introduction: Brain Derived Growth Factor (BDNF) is a sub member of neurotrophin family and is a critical regulator of formation and plasticity of neuronal networks in hippocampal formation. It acts in an activity dependent manner and its expression is highly sensitive to developmental and environmental factors.

Aim: To observe the expression of BDNF in the developing hippocampus of mid gestational aborted human fetuses.

Materials and Methods: In the present study 15 aborted fetuses from 14 to 30 weeks of gestation, were procured from the department of Obstetrics and Gynaecology, LN hospital after obtaining ethical clearance. For each gestational age the tissue was stained with cresylviolet and H&E to see the general morphology of hippocampus and immunostaining of the sections was done for the expression of BDNF.

Results: Subparts of hippocampus including Ammons horn, subiculum and dentate gyrus were identified in all age groups. Immunostaining was detected in both cell bodies and fibers. Expression of BDNF was more marked in the pyramidal cells of hippocampus and granule cell layer of the dentate gyrus of higher gestational age groups as compared to lower ones.

Conclusion: There is gradual increase in the BDNF expression as fetal age advances. Increased expression of BDNF in higher gestational age groups showed that neurotrophins like BDNF influence the neuronal differentiation and is important to neuronal survival.

Keywords: Granule cells, Hippocampal plate, Intermediate zone, Marginal zone, Pyramidal cells, Ventricle zone

INTRODUCTION

The BDNF is a polypeptide growth factor. It activates high-affinity Tropomyosin receptor Kinase (Trk) as well as low affinity p75 neurotrophin receptor (p75NTR) [1,2]. Neurotrophins like BDNF direct growth and differentiation in the developing nervous system and helps in synaptogenesis, neuronal survival and promotes Long Term Potentiation (LTP) by modulating NMDA receptor subunit [3-6]. Its expression is highly sensitive to developmental and environmental factors. BDNF signaling enhances neurogenesis and electrophysiological activity reflective of general enhancement of hippocampal function [7]. Hippocampus is a part of limbic lobe emerging from the medial wall of temporal lobe and is associated with procuring of food, eating and emotional behaviour of the being. During development four fundamental embryonic zones i.e., Ventricle Zone (VZ), Intermediate Zone (IZ), Hippocampal Plate (HP) and Marginal Zone (MZ) has been identified as early as 9th week of gestation in hippocampus proper. Granule Cell Layer (GCL) is characteristic of dentategyrus [8-10].

This region has gained significance recently due to involvement of BDNF in development of hippocampus which is important for specific learning and memory process. Abnormal expression of BDNF is associated with various neuropathological disorders like schizophrenia, Alzheimer's and depression [11-13]. The present study aimed to observe the expression of BDNF in the developing hippocampus of mid gestational human fetuses.

MATERIALS AND METHODS

Fifteen aborted fetuses from 14-30 weeks of gestation were procured from the Department of Obstetrics and Gynecology, LN hospital after obtaining Ethical clearance (F.1/IEC/MAMC/(40)/6/2013/No:06) and written informed consent, in January 2014.

Gestational ages were determined by measuring various parameters, such as Crown Rump Length, Bi parietal Diameter and Foot Length and correlated with hospital data.

After procuring the fetus an incision was given on the scalp from the bregma along the sagittal suture for immediate fixation of the brain. The fetus was then immersion fixed in 4% paraformaldehyde. The brain was then removed from the cranial cavity after 24-72 hours and preserved in fresh fixative for 1-2 weeks. Brains that showed any degree of autolysis were not considered for the study. Slices of hippocampal area were dissected out. These were preserved in the fixative for 48 hours. Slices were labeled and processed for paraffin embedding. Seven micron thick serial sections were generated on a rotary microtome and every 3rd section was stained with Haematoxylin-Eosin stain. In each gestational age the 4th section and 5th section were processed for Nissl's stain and BDNF, respectively. Sections after deparafinisation were treated with methanol and 1% H_2O_2 for blocking the endogenous peroxidase activity. After washing with distill water antigen retrieval (unmasking) was carried out by putting the slides for 7-8 minutes in a microwave for sections to be treated with primary antibody BDNF. Slides were completely immersed in the citrate buffer during this step. After washing with phosphate buffer slides were treated with normal horse serum for 2 hours for blocking non specific antigen. Following this, without washing, the sections were incubated with monoclonal antibody for BDNF at a dilution of 1:200. The reaction was visualised by biotinylated mouse secondary antibody and using DiaminoBenzidine as a chromagen. The sections were then examined under a BX61 motorised microscope and the images were captured with Olympus DP71 camera. Processing of images was done with ImagePro plus MC 6 software. Expression of BDNF was observed and analysed.

RESULTS

The fetal measurements are shown in [Table/Fig-1] and the various fetal zones of hippocampus where the BDNF expressions were marked is shown in [Table/Fig-2].

Age (in weeks)	CRL (in cm)	BPD (in cm)	FL (in cm)	Number (Collected)	Gender (M/F)
18	18-19	4.6-5.0	2.6-3.0	4	3-M
					1-F
20	19-20	5.2-5.6	3.3-3.7	3	2-F
					1-M
22	20-22	5.6-5.8	3.7-3.9	3	2-M
					1-F
24	23-24	5.9-6.0	4.0-4.2	3	2-M
					1-F
28	26-28	6.0-6.2	4.5-4.7	2	1-M
					1-F

[Table/Fig-1]: Fetal measurements and collection data.

24th Gestational Weeks

Hippocampus attained more mature appearance developing inside the inferior horn of lateral ventricle cavity. Expression of BDNF was seen in both cell bodies and fibers and was more marked in HP and GCL of dentate gyrus as compared to earlier gestational age groups [Table/Fig-6a,b].

28th Gestational Weeks

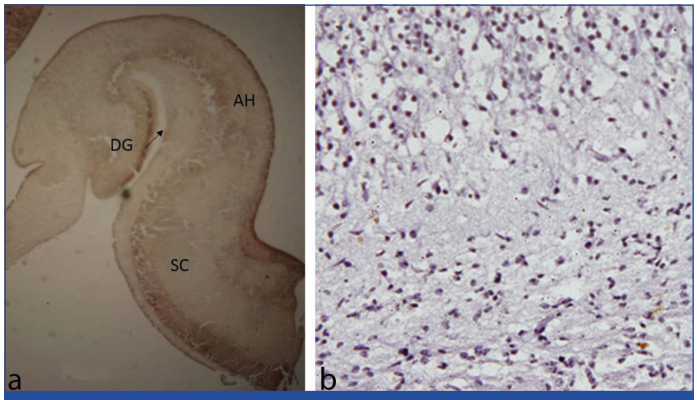
Hippocampus attained almost mature appearance. Subparts of hippocampus were clearly identified following the curve of hippocampal fissure. Hippocampal fissure had started to fuse with the main body of hippocampus. Maturation of cells was in more advanced stages. Expression of BDNF was seen more intense in cell bodies and fibers of VZ and Granule cell layer of DG as compared to earlier gestational age groups.

Fetal zone		18 wks	20 wks	22 wks	24 wks	28 wks
Ventricular Zone (VZ)	Neurons	Neuroblasts	Immature neuronal cells	Mature neuronal cells	More differentiated neuronal cells	Well differentiated neuronal cells
	BDNF expression	+	+	++	+++	+++
Intermediate Zone (IZ)	Neurons	Immature neurons	Immature neurons	Mature neuronal cells	More differentiated neuronal cells	Well differentiated neuronal cells
	BDNF expression	+	+	+	+	+
Hippocampal Plate (HP)	Neurons	Immature neurons	More differentiated neuronal cells	Few pyramidal cells	More differentiated pyramidal cells	Well differentiated pyramidal cells
	BDNF expression	+	+	++	+++	+++
Dentate Gyrus (DG)	Neurons	Immature neurons	Immature neurons	Mature neuronal cells	More differentiated granular cells	Well differentiated granular cells
	BDNF expression	+	+	++	+++	+++

[Table/Fig-2]: Expression of BDNF in various fetal zones of hippocampus in different gestational age groups.

18th Gestational weeks

Hippocampus was clearly identified developing inside the cavity of inferior horn of lateral ventricle. The fetal zones VZ, IZ, HP and MZ were seen. BDNF expression was detected in both cell bodies and fibers and was more marked in VZ and GCL of dentate gyrus as compared to IZ and MZ [Table/Fig-3a,b].



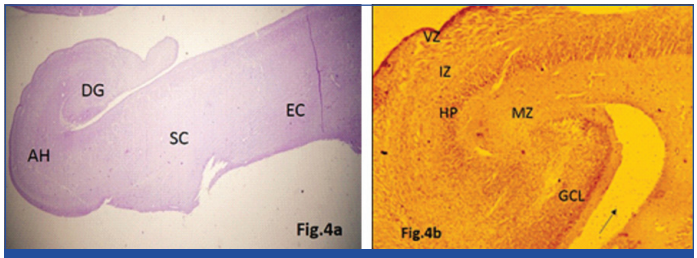
[Table/Fig-3]: a) Hippocampus showing Dentate Gyrus (DG), Ammon's Horn (AH), Subicular Complex (SC) and Entorhinal Cortex (EC) around the hippocampal fissure (arrow) (18 week). b) Neuronal cells along with cell processes showing BDNF expression (18 week) (IHC stain, 40x).

20th Gestational Weeks

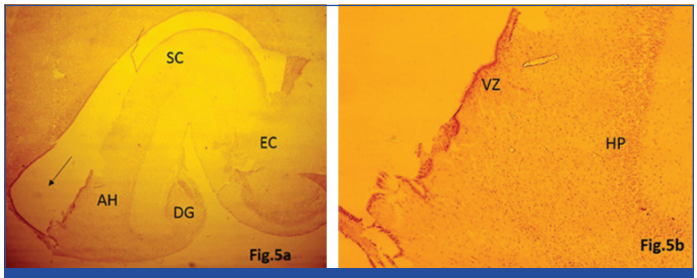
The subparts of hippocampus (Ammon's horn, subicular complex and dentate gyrus) were identified following the curve of hippocampal fissure. Fimbria was seen extending just above the dentate gyrus. BDNF expression was more marked in VZ, HP and granule cell layer of dentate gyrus (DG) as compared to IZ and MZ [Table/Fig-4a,b].

22nd Gestational Weeks

The hippocampus became more curved and mature in appearance. Subparts of hippocampus were clearly identified. BDNF expression was seen in all the fetal zones, though staining intensity was more marked in VZ and HP as compared to earlier gestational age groups [Table/Fig-5a,b].



[Table/Fig-4]: a) Hippocampus showing Dentate Gyrus (DG), Ammon's Horn (AH), Subicular Complex (SC) and Entorhinal Cortex (EC) around the hippocampal fissure (arrow) (20 week) (H&E stain, 4x). b) BDNF expression seen in Ventricular Zone (VZ), Hippocampal Plate (HP), and Granule Cell Layer (GCL) of Dentate Gyrus (DG) around hippocampal fissure (arrow) (20 week) (IHC stain, 4x).

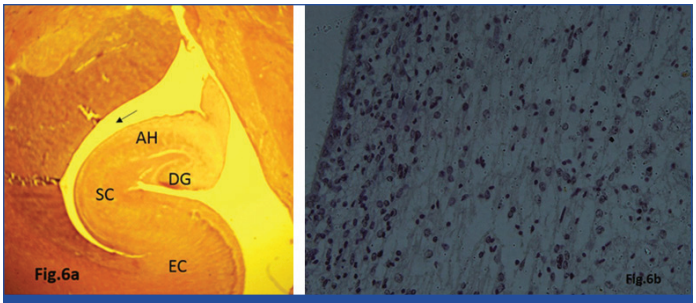


[Table/Fig-5]: a) Expression of BDNF in Hippocampus showing Dentate Gyrus (DG), Ammon's Horn (AH), Subicular Complex (SC) and Entorhinal Cortex (EC) developing inside the inferior horn of lateral ventricular cavity (arrow) (22 week) (IHC stain, 4x). b) Neuronal cells along with cell processes showing BDNF expression in Ventricular Zone (VZ) and Hippocampal Plate (HP) (22 week) (IHC stain, 4x).

Expression of immunohistochemical marker (BDNF) is more marked (+++) in higher gestational age groups as compared to lower ones (+) in various fetal zones of hippocampus.

DISCUSSION

The present study evaluated the expression of BDNF in the hippocampal formation of human fetuses ranging from 14th to 30th gestational weeks. This was a morphological study where we were able to observe the microscopic structure of hippocampal formation including dentate gyrus, cornuammonis and subicular complex



[Table/Fig-6]: a) Expression of BDNF in Hippocampus showing Dentate Gyrus (DG), Ammon's Horn (AH), Subicular Complex (SC) and Entorhinal Cortex (EC) inside the inferior horn of lateral ventricular cavity (arrow) (24 week). b) Neuronal cells along with cell processes showing BDNF expression (24 week)

and expression of BDNF was correlated. Neurotrophins like BDNF direct growth and differentiation in the developing nervous system. BDNF signaling enhances neurogenesis, neurite sprouting and electrophysiological activity [6,7].

The increase in BDNF expression in the temporal cortex of the neonates suggests that neurotrophin signaling is important in the early development of temporal cortex. BDNF and its receptors are expressed throughout the development and maturation of the human hippocampus [14].

BDNF regulates synaptic transmission and its release is activity dependent. BDNF is involved in trafficking NMDA receptor subunit to the plasma membrane of hippocampal neurons thereby increasing the potential for calcium influx. A sufficient local buildup of calcium causes postsynaptic BDNF release and promotes LTP, underlying mechanism for the retention of memory and learning process [15].

In the present study the lowest age of fetus included is 18 weeks where the primordial hippocampus was identified developing on the medial edge of inferior horn of lateral ventricle. Subparts of hippocampus were identified along the curve of hippocampal fissure in all the fetuses included in our study and four fundamental embryonic zones i.e., VZ, IZ, HP and MZ has been delineated. Expression of BDNF was detected in cell bodies and fibers of all age groups. BDNF expression was more marked in VZ, HP and GCL of dentate gyrus as compared to IZ and MZ. BDNF expression was more marked in the higher gestational age groups as compared to lower ones [Table/Fig-2]. Our findings corroborates with other studies [16-18] done on hippocampus of various animals for the expression of BDNF. BDNF mRNA is found to be lowest in the brains of early rats and increases into adulthood [16]. BDNF protein concentration increases over time during postnatal development of brain in albino rats and stabilises thereafter [17]. Infusion of BDNF into the adult brain in rats promotes neurogenesis and dendritic spine reorganisation in the rat hippocampal formation [18]. Increased expression of BDNF is seen in pyramidal layer of CA2 and CA3 in higher gestational age groups as compared to lower ones [19]. Decrease in expression of BDNF occurs in hippocampus during aging and in Alzheimer's disease [12,13]. During brain development, the BDNF is involved in various processes like neurogenesis, gliogenesis, synaptogenesis, regulation of cell death and elimination of improperly formed connections [20]. The concentration of BDNF pro-domain rises during adolescence and adulthood, following the increase of m-BDNF. It is released from the neurons after depolarisation with definitive physiological properties [21]. BDNF is highly expressed in limbic cortex and is a crucial factor in regulation of learning and memory [22]. Impaired regulation of BDNF expression is involved in various cognitive and neurodegenerative diseases like mood and anxiety disorders, Alzheimer's and Parkinson's disease [23,24].

Limitation(s)

It was an observational study where the expression of BDNF was observedvia immuno histochemical procedure. For better quantitative analysis stereological methods should be considered.

CONCLUSION(S)

Though extensive studies has been done on lower animals, human studies are limited. Thus, from the present study done on human hippocampus it is evident that there is a gradual increase in the BDNF expression as the fetal age increases.

Declaration: This study was presented in the 63rd NATCON, National Conference of Anatomical Society of India at King George's Medical University, Lucknow, India in 2015 as oral presentation.

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