DTI STUDIES WITH IMMUNOHISTOLOGICAL CORRELATION IN THE DEVELOPING HUMAN FRONTAL CEREBRUM

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Introduction: Microstructural growth of cerebrum is a complex but an orderly developmental process, beginning with the production of neuronal and glial precursors in the germinal zones. This is followed by the neuronal migration from the germinal zone to their eventual destination. The neurons finally organize into horizontal laminar aggregates and vertical columns, resulting in the production of normal cortical cytoarchitectonic patterns (1). The developing human cerebrum displays age specific changes in its pattern of lamination. A number of studies have been published that address the human brain development issues from the age of 9 weeks gestational age (GA) using MRI. Normal neuronal migration has been followed using T1-weighted images in formalin fixed specimens with histologic correlation (2). In contrast to conventional anatomical imaging, diffusion tensor imaging (DTI) has the potential to provide information about the microstructural organization within the neural tissue. Previous DTI study in human fetal brain has been shown gestational age related pattern of fractional anisotropy (FA) change in the developing human cerebrum (3). The aim of this study was to compare FA changes in the transient layers of frontal lobe with immunohistochemical analysis in human fetal brains between 15-37 weeks of GA.

Materials and methods: DTI was performed on 43 human fetuses with GA of 15–37 weeks. The younger fetuses were obtained after spontaneous abortion, whereas the older ones were obtained after medical abortion for incurable renal malformation, osseous dysplasia, or intrauterine death from unknown causes or were fresh still births. None of the fetuses had detectable central nervous system (CNS) malformations on antenatal ultrasound. All the MRI studies were performed on unfixed brains. The age of each fetus was based on a combination of postovulatory age and early ultrasonographic GA estimation.

Imaging: Whole-brain DTI data were acquired on a 1.5-T GE MRI scanner, with a standard quadrature knee coil for both transmission of radio frequency pulses and signal reception. DTI data were acquired by using a single-shot echo planar dual spin echo sequence with ramp sampling. The diffusion weighting b-factor was set to 700 sec mm^{-2}. The other acquisition parameters were TR = 8 sec, TE = 100 msec; number of axial sections = 30–34, slice thickness of 3 mm with no gap, FOV varying from 160 to 240 mm, and image matrix of 256 × 256 (following zero-filling). The field-of-view (FOV) varied between 160 and 240 mm depending on the size of the fetal head. A balanced and rotationally invariant dodecahedral diffusion encoding scheme with 10 uniformly distributed directions over the unit sphere was used for generating the DTI data. To enhance the SNR and reduce the phase fluctuations, the magnitude constructed images were repeated (NEX = 8) and temporally averaged on the scanner. The DTI data were processed using JAVA based software as described in detail elsewhere (4). For the purpose of DTI data quantitation elliptical regions of interest(s) (ROI) of 2 × 2 to 4 × 4 pixels were placed on cortical layer, subplate zone, intermediate zone, and subventricular zone of frontal lobe of the fetal brain.

Immunohistochemical analysis: Thirty-five out of 43 fetal brains were decapitated after imaging and were placed in 10 % formalin for fixation for extended period of time depending on the size of the fetal brain. After fixation brain was embedded in paraffin, and cut in series of 10-µm-thick sections, in axial plane for the purpose of comparison with DTI images. Sections were incubated overnight with primary antibodies glial fibrillary acid protein (GFAP), and neuron specific enolase (NSE) in a humid chamber. Secondary immunostaining was done by streptavidin biotin method (LSAB2 Kit Dakopatts, Denmark) and colour was developed with 3,3'diaminobenzidine tetrahydrochloride (DAB) . The sections were dehydrated, cleared, and mounted. Number of NSE positive cells in 9 fetal brains of different GA renging from 15-32 weeks of GA was counted in germinal matrix zone using Biovis image analysis software.

Results: Increase in cortical FA values was observed during the 15-26 weeks of GA, then after a progressive decline of cortical FA values was observed in frontal lobe (**Fig.1**). On immuno-histochemical analysis in cortical region maximal expression of GFAP was observed in sections of 26 weeks of GA fetus (**Fig.2B**). The FA values in the subplate zone showed an inverse correlation with GA (r = –0.56, P = 0.07), but this did not reach statistical significance (**Fig. 3**). Though we observed the increase in FA values as a function of GA statistically significant correlation was not observed between the FA values and GA in the intermediate zone. A decrease in FA in germinal matrix zone was observed as a function of time. A significant direct correlation between FA and number of NSE-positive cells (r=0.39, p=0.05) in germinal matrix was observed (Fig. 4)

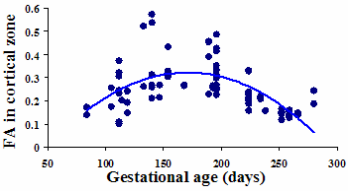

Figure 1

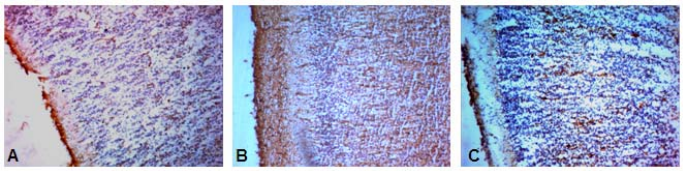

Figure 2

Fig 1: Variation of FA in the cortical zone with GA. **Fig. 2:** GFAP-immunostained sections showing orientation of radial glial fibres from frontal cortex of 16 weeks (A), 26 weeks (B), and 36 weeks (C) of GA. **Fig. 3**: Pattern of FA change in subplate (blue) and intermediate zone (pink) as a function of GA. **Fig. 4**: A plot between FA and number of NSE-immunostained positive cells in germinal matrix zone.

Discussion: In this study, using DTI and GFAP/NSE immuno-stain we provide the demonstration of temporal changes in laminar pattern of developing frontal cerebrum in human fetal brain. It is known that most of the neurons that form the cerebral cortex migrate to their destinations along the specialized radial glial fibers that span the entire thickness of the hemisphere from the ventricular surface to the pia (1). The observed increase in the FA value during the early GA (<26 weeks) is associated with this radial organization of the neuronal cells migrating from the germinal zone (fig. 2). After cessation of neuronal production, the radial glial cells gradually retract their ventricular and pial attachments and differentiate into astrocytes. The observed decline in the cortical FA after 26 weeks of GA (Fig. 1) reflects an alteration in the radial organization with neocortical maturation (Fig. 2C). The observed decline in FA values with GA probably represents a decrease in cellularity in the subplate zone with GA and is consistent with previous histology data (2). Significant direct correlation between FA values and NSE-positive cells in germinal matrix zone (Fig. 4) reflects that the population density of migrating neuronal cells (responsible for high FA during the early period of GA) gradually decreases over a period of time as the migration is completed. The ability to noninvasively monitor neuronal migration and maturation processes in vivo should greatly improve our understanding of the normal developmental pattern of the cerebrum in human fetal brain.

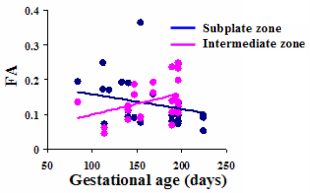

Figure 3

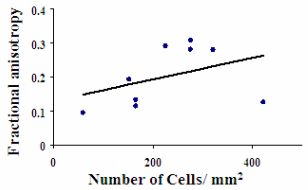

Figure 4

References: 1) Sidman RL, et al. ***Brain Res*** 1973;62:1–35; 2) Kostovic I, et al. Cereb Cortex 2002;12:536–544; 3) Gupta RK, et al. Journal of Neuroscience Research 2005;81:172-178; 4) Purwar A, et al. Proceedings of ESMRMB, September 21-23, 2006.