Childs Nerv Syst (2011) 27:723–728
DOI 10.1007/s00381-010-1329-1

ORIGINAL PAPER

Cognitive functions correlate with diffusion tensor imaging metrics in patients with spina bifida cystica

Manoj Kumar · Arti Srivastava · Shruti Agarwal · Sanjay Behari · Gyanendra K. Malik · Ram K. S. Rathore · Rakesh K. Gupta

Received: 2 June 2010 / Accepted: 26 October 2010 / Published online: 16 November 2010

Abstract
Purpose Spina bifida cystica (SBC) is a group of neurodevelopmental defects caused by improper neural tube closure, which may be responsible for deficits in cognitive functions. The purpose of this study was to examine changes in normal appearing deep gray and white matter brain regions in SBC patients compared with controls through diffusion tensor imaging (DTI) and correlate these changes with neuropsychometric tests.
Methods Conventional magnetic resonance imaging and neuropsychometric tests were performed on 13 patients and ten controls. DTI-derived fractional anisotropy (FA) and mean diffusivity (MD) were quantified in different brain regions in controls and patients.
Results Significantly decreased FA was observed in caudate nuclei, putamen, genu, splenium, and increased FA was found in middle cerebellar peduncle (MCP) in patients compared with controls. We observed significantly increased MD in genu and splenium. However, increased MD was found in fornix of patients compared with controls. Majority of neuropsychological tests were found to be significantly impaired and some of these showed significant correlation with DTI metrics in genu, splenium, and MCP in these patients.
Conclusions We conclude that DTI metrics are significantly abnormal in deep gray matter nuclei, genu, splenium, and MCP in SBC patients and may provide microstructural basis for neuropsychological abnormalities in these patients.

Keywords Congenital brain anomalies · Brain development · Neural tube defect · Cognitive function · Neuropsychological test

M. Kumar · A. Srivastava · R. K. Gupta (✉)
Department of Radiodiagnosis,
Sanjay Gandhi Postgraduate Institute of Medical Sciences,
Raebareli Road,
Lucknow, Uttar Pradesh, India 226014
e-mail: rgupta@sgpgi.ac.in

R. K. Gupta
e-mail: rakeshree1@gmail.com

S. Agarwal · R. K. S. Rathore
Department of Mathematics and Statistics,
Indian Institute of Technology,
Kanpur, Uttar Pradesh, India 208016

S. Behari
Department of Neurosurgery,
Sanjay Gandhi Post Graduate Institute of Medical Sciences,
Lucknow, Uttar Pradesh, India 226014

G. K. Malik
Department of Pediatrics,
Chhatrapati Shahuji Maharaj Medical University,
Lucknow, Uttar Pradesh, India 226003

Introduction

Brain development is a complex process linked with behavioral, emotional, and cognitive abilities that proceeds throughout the postnatal phase and continues into adulthood [1]. Formation of neural tube is a crucial event in the development of central nervous system (CNS). Proper closure of neural tube is extremely important because brain and spinal cord develops from the neural tube. The failure of neural tube closure results in congenital malformations that lead to spontaneous abortion, stillbirth, death in early infancy, or lifetime disability [2]. Spina bifida is the name given to any congenital gap in the vertebral column through which the contents of spinal cord may protrude. It is a group of neurodevelopmental defects caused by improper

neural tube closure during 3–4 weeks of gestation with a rate of 0.5–1 per 1,000 live births in the developed countries [3]. In spina bifida cystica (SBC), there is protrusion through the defect of the meninges (meningocele), spinal cord (myelocele), or both (meningomyelocele). Improper neural tube closure affects the normal brain development and may be responsible for deficits in cognitive and memory functions [4]. Cerebellar and corpus callosum abnormalities are part of the broad spectrum of deficits seen in neural migrations associated with antenatal development of children with neural tube defects [4]. Previous studies have reported cognitive dysfunction in children and adolescents with SBC [5].

Neuronal and axonal vulnerability have been demonstrated in children with meningomyelocele and shown to be associated with neurocognitive and behavioral deficits [6]. Corpus callosum is the largest commissural fiber bundle in the brain and plays an important role in inter-hemispheric functional integration. It has been reported that corpus callosum has a protracted growth pattern, with development continuing into adolescence, although the growth curve attenuates by adulthood [7]. In children with SBC, anomalies of corpus callosum have been found to be associated with dysgenesis of one or more structures [4].

There are only few quantitative conventional magnetic resonance imaging (MRI) studies describing abnormal development of the brain in SBC patients [4, 8, 9]. Reigel et al. have reported that region specific changes in gray and white matter (WM) in spina bifida occur as a result of congenital anomalies along with developmental reorganization in these patients [10]. A regional variation in lobar brain volume has also been reported in spina bifida [8].

Diffusion tensor imaging (DTI) has the potential to provide information about tissue microstructural organization, including indirect measures of myelination and axonal growth and is known to be more sensitive than conventional MRI [11]. The commonly used DTI metrics are fractional anisotropy (FA), which quantifies the preferential direction of water diffusion along WM tracts and mean diffusivity (MD), which measures the magnitude of diffusion. DTI along with cognitive measures have been shown useful in elucidating the relationship between the integrity of WM pathways and the efficiency of cognitive and neural processing during brain development [12, 13]. In a recent study, Hasan et al. quantified microstructural abnormalities in the major association pathways of the hydrocephalic brain in children with myelomeningocele using DTI and suggested age-related abnormal WM development [6].

However, a detailed study of abnormal deep gray and WM maturation in SBC patients using DTI and its correlation with neuropsychological (NP) test battery has not yet been performed. The aim of this study was to compare changes in DTI metrics quantified from deep gray and WM regions of the brain between SBC patients and controls. We have also attempted to correlate the changes in DTI metrics with a series of NP test battery using a broad range of cognitive, learning, and memory tasks related to different regions of the brain.

Materials and methods

Subjects

The present study was performed on 13 SBC patients (eight males and five females, aged 10–17 years with a mean age of 14) and 10 age/sex matched healthy controls (7 males and 3 females, aged 10–15 years with a mean age of 13). The patients either with pre operative SBC or who had surgical repair for SBC with normal brain imaging on conventional MRI were included in the study. The following number of SBC patients were included; lumbo-sacral meningomyelocele (n=2), lumbo-sacral meningocele (n=2), dorsal meningocele (n=6), and operated lumbar meningocele (n=3). These SBC patients underwent a set of NP test battery to assess cognitive functions. None of the patients had any history of seizure disorder. Patients with hydrocephalus or Chiari malformation were excluded from the study to overcome the confounding effect of these variables on DTI metrics. All subjects included in this study were right handed. Informed consent was obtained from parents of the patients after explaining the purpose of the study to perform MR imaging and NP tests. This study was performed within the guidelines of the Institutional ethics committee.

Conventional MRI protocol

Conventional MRI and DTI were acquired in the axial plane on 1.5 Tesla GE Signa LX MRI scanner (General Electric Medical System, Milwaukee, WI) using a standard quadrature birdcage receive and transmit radio-frequency head coil. Conventional MRI included T2-weighted fast spin echo (FSE) with repetition time (TR) (ms)/echo time (TE) (ms)/echo train length/no. of excitations (NEX)=6,000/85/16/4 and spin echo (SE) T1-weighted images with TR/TE/NEX=1,000/14/2. Both T1- and T2-weighted images were acquired from contiguous, 3 mm thick axial sections with 240×240 mm^2 field of view (FOV) and image matrix of 256×256.

DTI protocol

DTI data was acquired using a single-shot echo-planar dual SE sequence with ramp sampling [14]. Diffusion-weighted acquisition parameters were: b-factor=0 and 1,000 s/mm^2, slice thickness=3 mm with no interslice space, number of slices=34–38, FOV=240×240 mm^2, TR=8 s, TE=100 ms,

and NEX=8. The acquisition matrix was 128×80 and the homodyne algorithm was used to construct the k-space data to 128×128 and zero-filled to generate an image matrix of 256×256. A balanced and rotationally invariant diffusion-encoding scheme with ten non-collinear directions over the unit sphere was used for generating the DTI data [14].

DTI data was processed and evaluated using JAVA-based program as described elsewhere [15]. DTI-derived maps were displayed and overlaid on images with different contrasts to facilitate the region-of-interests (ROIs) placement. Threshold value of the color-coded FA map for display was kept at 0.2 above which the color-coded regions reflect the WM only. We have selected those brain regions which are known to influence neurocognitive deficits in these patients [12, 16–18]. Elliptical/or rectangular ROIs were placed in different regions of the brain including deep gray matter i.e. basal ganglia (caudate nuclei (CN), thalamus, and putamen at the level of third ventricle) and WM (cingulum (Cing) at the level of corona radiata, fornix, genu, and splenium at the level of massa intermedia) in SBC patients and controls (Fig. 1). We have also placed elliptical ROI on middle cerebellar peduncle (MCP) (Fig. 1) and the level was defined on the basis of the anatomic landmarks visible on DTI color maps. The size of ROI varied from 2×2 and 6×6 pixels.

Neuropsychological test

Neuropsychological tests were performed on controls and patients to assess the cognitive function, memory, and learning. The age/sex, education, and socio-economic status matched

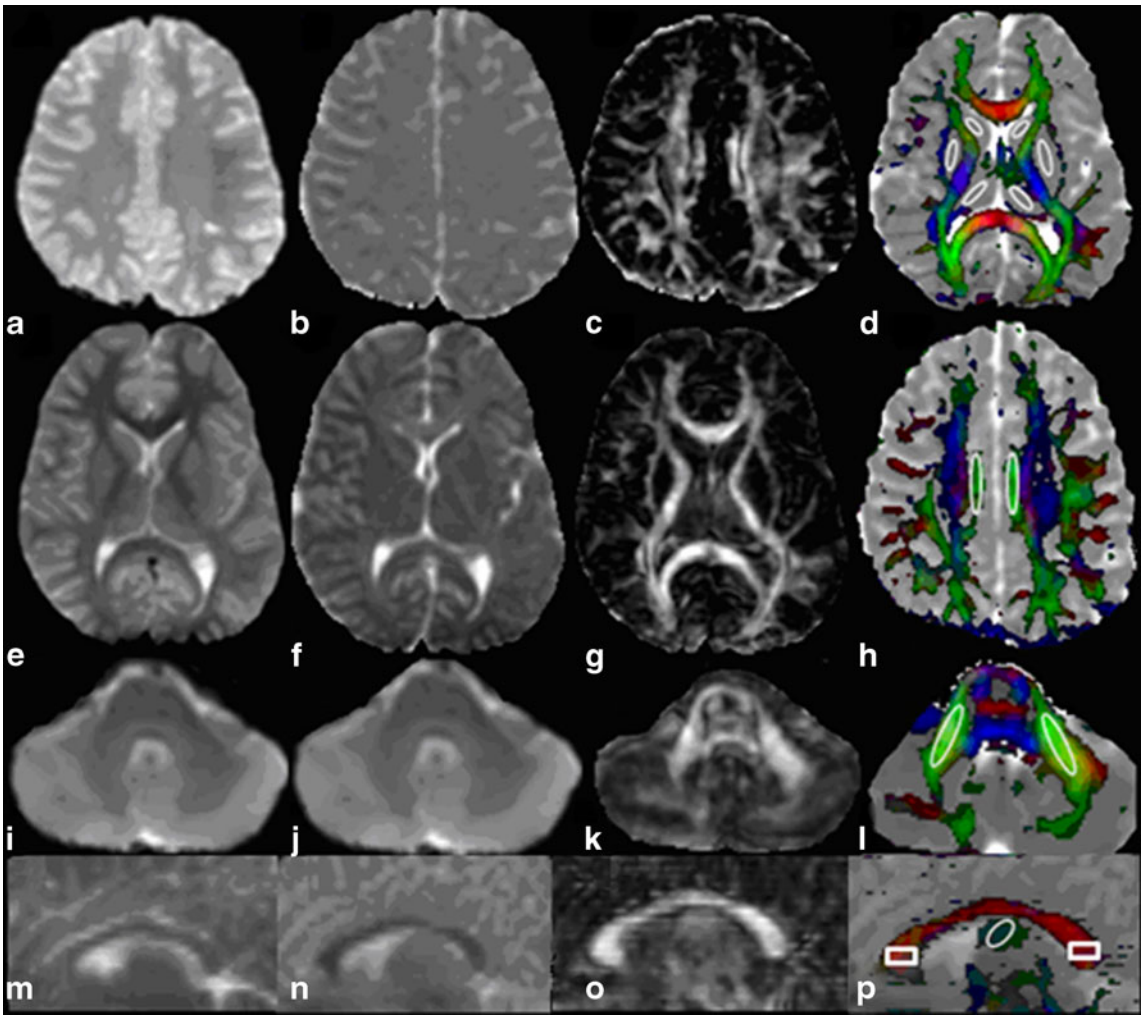

Fig. 1 Axial and sagittal images of a 14-year-old patient with spina bifida cystica showing normal appearing cerebral and cerebellar regions of the brain. T2-weighted image, mean diffusivity (MD), fractional anisotropy (FA), and color-coded FA maps at the level of third ventricle (**a**–**d**), corona radiata (**e**–**h**), pons (**i**–**l**), and massa intermedia (**m**–**p**) showing region-of-interest placed on color-coded FA maps on caudate nuclei, thalamus, and putamen, cingulum, middle cerebellar peduncles, genu, and splenium of corpus callosum and fornix, respectively. The threshold value of the color-coded FA map for display was kept at 0.2 above which the color-coded regions reflect the white matter only (*red* (right–left), *green* (*anterior–posterior*), and blue (*superior inferior*))

healthy controls were included in this study. NP tests included number connection test (NCT A and B) and figure connection test (FCT A) and subset of modified Wechsler Intelligence Scale for children–third edition (WISC-III; ages 6–17) [19]; five tests (picture completion test (PCT), digit-symbol test (DST), picture arrangement test (PAT), object assembly test (OAT), and block design test (BDT)). These tests have been validated and used in several other studies [19]. In NP tests, scores of ≤40, ≤70, and ≤40 were considered as normal limit for NCT A, B, and FCT respectively. Above these limits test were considered as abnormal. While in WISC-III (PCT, OAT, PAT, BDT, and DST) the scores ≥10 were considered as normal and below to this limit were abnormal. In NCT and FCT, lower scores represent better performance where as in WISC higher score represents better performance. Time duration for performing NP tests ranged from 55 to 60 min in patients and 40–45 min in controls.

Statistical analysis

We performed paired "*t*" test between values of DTI metrics in left and right regions of controls and did not find any statistically significant difference. For the purpose of statistical analysis, values from left and right measurements in all regions were pooled together in patients and controls. An independent Student's *t* test was performed to evaluate the differences in FA and MD values between patients and controls. Pearson's correlation coefficient was also used to determine the association between NP test scores and FA and MD values in SBC patients. A *p* value of less than 0.05 was considered to be statistically significant. All the statistical data computations were performed using statistical package for social sciences (SPSS, version 16.0 SPSS, Inc, Chicago, USA).

Results

On conventional MRI, no abnormality was observed in any region of the brain in these patients. The mean FA and MD values obtained from different regions of the brain in patients and controls are summarized in Table 1.

FA and MD values obtained from ROI analysis in deep gray and white matter regions

In CN and putamen, significantly decreased FA with no significant change in MD values was observed in patients compared with controls. However the changes in FA and MD values did not show any statistical significance in thalamus of patients compared with controls. In genu and splenium, significantly decreased FA with increased MD values was observed in patients compared with controls. In Cing and fornix, though FA showed an increasing trend in patients compared with controls, it did not reach the level of statistical significance. Significantly decreased MD values in patients were only found in fornix compared with controls. We observed significantly increased FA with no significant change in MD values in MCP in patients compared with controls (Table 1).

Neuropsychological tests

NCT A, NCT B, and FCT scores were significantly higher while PCT, OAT, PAT, BDT, and DST scores were significantly lower in patients as compared with controls (Fig. 2). We correlated FA and MD values with NP test scores in patients with SBC.

Correlation between DTI Metrics and NP Test scores

Significantly inverse correlation was found between NCT B scores and FA values obtained from genu ($r=-0.78$; $p=0.00$). FA values in MCP showed a significant positive correlation with NCT A ($r=0.75$; $p=0.01$) and a significant inverse correlation with PAT scores ($r=-0.72$; $p=0.02$).

A significantly positive correlation was observed between MD values and NCT A scores ($r=0.61$; $p=0.04$) in splenium.

Table 1 Comparison of fractional anisotropy and mean diffusivity in different regions of the brain between age/sex match healthy controls and spina bifida cystica patients

Regions	Fractional anisotropy			Mean diffusivity ($\times 10^{-3}$ mm^2/s)		
	Control	SBC	*p* value	Control	SBC	*p* value
Caudate nuclei	0.16±0.02	0.10±0.02	0.001	0.76±0.08	0.74±0.03	0.14
Putamen	0.12±0.03	0.10±0.03	0.03	0.71±0.02	0.71±0.08	0.14
Thalamus	0.15±0.05	0.15±0.05	0.75	0.80±0.12	0.77±0.12	0.47
Genu	0.56±0.03	0.52±0.05	0.04	0.71±0.04	0.76±0.06	0.04
Splenium	0.58±0.03	0.51±0.08	0.03	0.67±0.06	0.73±0.03	0.01
Fornix	0.36±0.06	0.39±0.05	0.16	0.87±0.07	0.76±0.07	0.02
Cingulum	0.38±0.07	0.44±0.08	0.07	0.75±0.02	0.74±0.03	0.41
MCP	0.59±0.04	0.63±0.04	0.03	0.74±0.07	0.76±0.05	0.37

MCP middle cerebellar peduncle, *SBC* spina bifida cystica

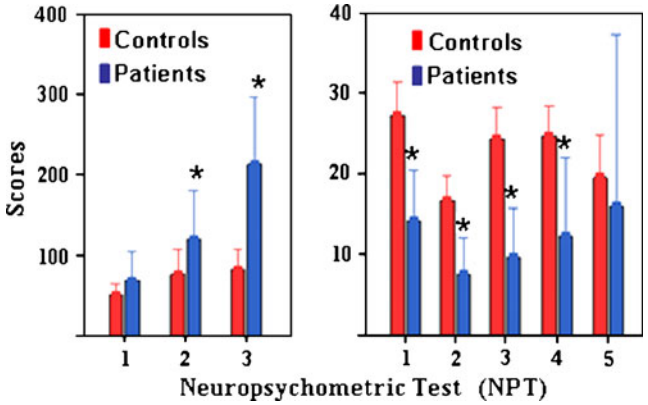

Fig. 2 Bar diagrams showing scores of various neuropsychometric tests (*NPT*) in normal as well as in SBC patients. Number and figure connection test (*1* number connection test A, *2* number connection test B and *3* figure connection test A) and WAISC (*1* picture connection test, *2* digit symbol test, *3* block design test, *4* picture arrangement test, and *5* object assembly test), respectively. *$p<0.05$ significant difference in NP test scores

Discussion

Present study demonstrates abnormal DTI metrics (FA and MD) in deep gray and white matter regions and NP test scores in SBC patients compared with controls. A significantly decreased FA was observed in CN and putamen in patients compared with controls. This study showed a significantly increased MD along with decreased FA in genu, splenium and increased FA in MCP. The NP test scores were found to be significantly impaired in patients and showed significant correlation with FA in the genu, splenium, and MCP in these patients.

It has been reported that posterior corpus callosum is selectively prone to injury in myelomeningocele due to diffuse CNS injury sustained in life or during gestation [7]. Reduced FA in corpus callosum in previous studies have been explained on the basis of WM damage due to increased cerebrospinal fluid (CSF) pressure secondary to acute hydrocephalous in patients with meningomyelocele [20, 21]. In the current study as none of the patient had hydrocephalous, the explanation of increased CSF pressure as a cause of decreased FA in corpus callosum in these patients appears to be unlikely. In a recent DTI study in patients with chiari II malformation, lower FA in corpus callosum has been reported in patients even in the absence of hydrocephalous [21]. A number of factors such as injury to corpus callosum during development, abnormal myelination, and degenerative changes secondary to abnormal periventricular WM development may be responsible for this reduced FA with increased MD in genu and splenium in this study.

There are a few DTI reports describing cerebellar WM maturation in normal children [22]; however, abnormal developmental studies in MCP have not been reported so far. A recent study has demonstrated increased FA along with decreased MD in MCP in normal children as they grow from birth to 11 years [22]. A previous MRI-based volumetric analysis has shown significant reductions in cerebellar volume in children with SBC [8]. In the current study, we observed significantly high FA in MCP in patients compared with controls and this may be explained in the light of previous imaging studies in patients with different neurodevelopmental anomalies like Williams's syndrome and Arnold Chiari malformation [23]. The cellular mechanisms behind increased FA are unknown. There are several factors which may be responsible for increased FA; such as increased myelination or microscopic deficits of axonal structures or decreased axonal diameter, packing density, branching, increased microfilament density, and reduced volume of cortical regions might contribute to high FA in certain regions in this study [23]. It is known that during brain development some axonal projections are established only transiently which subsequently get eliminated [23]. According to this theory it can be hypothesized that in case of SBC; elimination of such transient projections in the MCP gets disturbed and reflects in the form of abnormally high FA in these patients. However, this phenomenon has only been studied in animals at this point and it is not clear if it happen in humans as well.

The caudate nuclei plays a critical role in supporting, planning, and execution of strategies and behavior, required for achieving complex goals i.e. in action-outcome learning that subserves goal-directed action. This is in contrast to putamen, which appears to sub serve cognitive functions limited to stimulus–response [24]. Hasan et al. have reported increased FA in CN in patients of myelomeningocele with hydrocephalous and explained it on the basis of delayed synaptogenesis [13]. However, increased FA in the WM areas lateral to ventricles in patients with acute hydrocephalous was observed and has been explained on the basis of periventricular tissue compression secondary to the dilated ventricles [20]. In current study, we observed decreased FA and increased MD in CN of SBC patients. The difference in selection of patient population is probably the reason for discrepancy in the results of this study compared with Hasan et al. [13].

Limited data is available on neurocognitive and motor learning in SBC patients. In general, SBC patients commonly experience problem with a variety of nonverbal skills [6, 24]. It has been reported that SBC patients demonstrate lower levels of functioning in all brain regions, with impaired motor, perception, and visuospatial skills [25]. On neuropsychological studies, authors have shown differences in temperament, memory, and learning patterns in SBC patients even with average intelligence [18, 25]. In the current study we observed significantly impaired NP test scores in SBC patients compared with controls, even when their brain appeared normal on conventional imaging.

In the current study, SBC patients showed significantly poor performance in all components of NP tests. It has been reported that these connection tests reflect mental attention and visuo-spatial skills. For these skills, trans-hemispheric integration between primary and supplementary visual area of both hemisphere is required. A recent DTI study has reported reduced visuo-spatial skills and FA values in corpus callosum in the patients with Chiari II malformation even in the absence of hydrocephalous [21]. The observed significant inverse correlation between FA values in genu and MCP, with NCT B and NCT A, respectively, suggests that the control, organization, and quality of gross and fine motor movements of the upper extremities are mediated by the cerebellum and partly by visual cortex and parietal areas [25].

Small sample size and lack of histological confirmation of the changes seen on DTI are the limitations of this pilot study.

In conclusion, our study reveals abnormal DTI metrics in certain regions of the brain in SBC patients even on their normal presentation on conventional MRI. These DTI metrics show significant correlation with some of the NP test scores and may provide a microstructural basis for neuropsychological abnormalities in these patients.

Acknowledgment Manoj Kumar and Arti Srivastava acknowledge the financial assistance from the Indian Council of Medical Research and Life Sciences Research Board, DRDO, New Delhi, India, respectively.

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Lebel C, Walker L, Leemas A, Phillips L, Beaulieu (2008) Microstructural maturation of human brain from childhood to adulthood. Neuroimage 40:1044–1055
2. Gucciardi E, Pietrusiak MA, Reynolds DL, Rouleau J (2002) Incidence of neural tube defects in Ontario, 1986–1999. CMAJ 167:237–240
3. Williams LJ, Rasmussen SA, Flores A, Kirby RS, Edmonds LD (2005) Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995–2002. Pediatrics 116:580–586
4. Fletcher JM, Copeland K, Frederick JA, Blaser SE, Kramer LA, Northrup H, Hannay HJ, Brandt ME, Francis DJ, Villarreal G, Drake JM, Laurent JP, Townsend I, Inwood S, Boudousquie A, Dennis M (2005) Spinal lesion level in spina bifida: a source of neural and cognitive heterogeneity. J Neurosurg 102:268–279
5. Snow JH, Prince M, Souheaver G, Ashcraft E, Stefans V, Edmonds J (1994) Neuropsychological patterns of adolescents and young adults with spina bifida. Arch Clin Neuropsychol 9:277–287
6. Hasan KM, Sankar A, Halphen C, Kramer LA, Ewing-Cobbs L, Dennis M, Fletcher JM (2008) Quantitative diffusion tensor imaging and intellectual outcomes in spina bifida: laboratory investigation. J Neurosurg Pediatr 2:75–82
7. Fryer SL, Frank LR, Spadoni AD, Theilmann RJ, Nagel BJ, Shweinsburg AD, Tapert SF (2008) Microstructural integrity of corpus callosum with neuropsychological performance in adolescents. Brain Cogn 67:225–233
8. Juranek J, Fletcher JM, Hasan KM, Breier JI, Cirino PT, Pazo-Alvarez P, Diaz JD, Cobbs LE, Dennis M, Papanicolaoua AC (2008) Neocortical reorganization in spina bifida. Neuroimage 40:1516–1522
9. Kumar M, Gupta RK, Saksena S, Behari S, Malik GK, Kureel SN, Pandey CM, Rathore RKS (2010) Deep gray and white matter brain maturation differences in patients with spina bifida cystica from controls: a diffusion tensor imaging study. J Clin Neurosci 17:879–885
10. Reigel D, Rotenstein D (1994) Spina bifida. In: Check W (ed) Pediatric neurosurgery. WB Saunders, Philadelphia, pp 51–76
11. Mori S, Zhang J (2006) Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 51:527–539
12. Munakata Y, Casey BJ, Diamond A (2004) Developmental cognitive neuroscience: progress and potential. Trends Cogn Sci 8:122–128
13. Hasan KM, Eluvathingal TJ, Kramer LA, Cobbs LE, Dennis M, Fletcher JM (2008) White matter microstructural abnormalities in children with spina bifida myelomeningocele and hydrocephalus: a diffusion tensor tractography study of the association pathways. J Magn Reson Imaging 27:700–709
14. Le Bihan D (1995) Diffusion and perfusion MRI—applications to functional MRI. Raven Press Ltd, New York, pp 35–400
15. Nath K, Saraswat VA, Krishna YR, Thomas MA, Rathore RKS, Pandey CM, Gupta RK (2008) Quantification of cerebral edema on diffusion tensor imaging in acute-on-chronic liver failure. NMR Biomed 21:713–722
16. Brown LL, Schneider JS, Theodore IL (1997) Sensory and cognitive functions of basal ganglia. Curr Opin Neurobiol 7:157–163
17. Casey BJ, Giedd JN, Thomas KM (2000) Structural and functional brain development and its relation to cognitive development. Biol Psychol 54:241–257
18. Vachha B, Adams RC, Rollins NK (2006) Limbic tract anomalies in pediatric myelomeningocele and chiari II malformation: anatomic correlations with memory and learning—initial investigation. Radiology 240:194–202
19. Das A, Dhiman RK, Saraswat VA, Verma M, Naik SR (2001) Prevalence and natural history of sub clinical hepatic encephalopathy in cirrhosis. J Gastroenterol Hepatol 16:531–535
20. Assaf Y, Ben-sira L, Constantini S, Chang LC, Beni-adani L (2006) Diffusion tensor imaging in hydrocephalus: initial experience. Am J Neuroradiol 27:1717–1724
21. Herweh C, Akbar M, Wengenroth M, Blatow M, Mair-Waither J, Renbhein N, Nennig E, Shenk JP, Heildand S, Stippich C (2009) DTI of commissural fibers in patients with Chiari-malformation. Neuroimage 44:306–311
22. Saksena S, Husain N, Malik GK, Trivedi R, Sarma M, Rathore RKS, Pandey CM, Gupta RK (2008) Comparative evaluation of the cerebral and cerebellar white matter development in pediatric age group using quantitative diffusion tensor imaging. Cerebellum 7:392–400
23. Hoeft F, Naama BG, Haas BW, Golarai G, Ng D, Mills D, Korenberg J, Bellugi U, Galaburda A, Reiss A (2007) More is not always better: increased fractional anisotropy of superior longitudinal fasciculus associated with poor visuospatial abilities in Williams Syndrome. J Neurosci 27:11960–11965
24. Wills SK (1993) Neuropsychological functioning in children with spina bifida and/or hydrocephalus. J Clin Child Psychol 22:247–265
25. Lomax-Bream LE, Barnes M, Copeland K, Taylor HB, Landry SH (2007) The impact of spina bifida on development across the first 3 years. Dev Neuropsychol 31:1–20