Key words:

- Chiari II malformation
- Brainstem
- Fetus
- Magnetic resonance imaging
- Diffusion tensor imaging

Key points:

- FA in the fetal midbrain is elevated in Chiari II malformations.
- FA is not elevated in hydrocephalus and mild ventriculomegaly without Chiari II.
- Measuring FA may help distinguish different causes for enlarged ventricles prenatally.
- Elevated FA may aid in the diagnosis of open neural tube defects.
- Elevated FA might contribute to stratification for prenatal surgery in Chiari II.

Abbreviations:

ADC – apparent diffusion coefficient

CNS – central nervous system

DTI - diffusion tensor imaging

FA – fractional anisotropy

GW – gestational weeks/ weeks of gestation

ROI(s) – region(s) of interest

SSFSE - single-shot fast spin-echo

TE – echo time

TR – repetition time

Introduction

The Chiari II malformation is still the most common form of congenital central nervous system (CNS) defect compatible with life [1], although its incidence has decreased to less than one in 1000 live births due to prenatal folic acid supplementation [2]. It is usually detected prenatally by measuring maternal serum alpha-fetoprotein, and, more importantly, by prenatal imaging [3; 4] as soon as during the first trimester. Chiari II malformations are almost always associated with open neural tube defects, mostly myelomeningoceles. This holds true for the vast majority of patients, except for very few cases where closed neural tube defects have been reported to be associated with Chiari II malformations [5-7]. It is thought that leakage of the cerebro-spinal fluid (CSF) through an open neural tube defect causes intracranial hypotension. A physiologic increase in intracranial hydrostatic pressure during early pregnancy is crucial for the consecutive growth of the bones forming the posterior cranial fossa [8-12]. Recently, genetic factors have also been suspected to play an important role in the pathogenesis of NTDs and Chiari II malformations, possibly leading to both posterior fossa malformations and histologic disruptions of the brainstem parenchyma [13-15]. In a Chiari II malformation, the posterior cranial fossa is too small for the normally sized brainstem and cerebellum. Crowding of the posterior fossa results in the compression of its content. Brainstem-related symptoms associated with Chiari II malformations frequently occur during infancy and comprise the following: dysphagia, possibly leading to aspiration pneumonitis and failure to thrive [16]; sleep-disordered breathing, such as central apnea and hypopnea [17]; inspiratory stridor [7; 18; 19]; and potentially lethal prolonged expiratory apnea with cyanosis often associated with experiences of pain or startling and presenting as cessation of expiratory effort and cyanosis [7]. These symptoms are thought to be due to compression and possibly dysgenesis of the brainstem that affect the respiratory nuclei and circuits, as well as the lower cranial nerves [14; 16; 18; 20]. Due to these potentially life-threatening consequences, surgical decompression of the posterior cranial fossa is often necessary [16; 19].

Prenatally, the Chiari II malformation is detected in over 88% of cases at a median time of 17 gestational weeks (GW) [21]. However, prenatal imaging findings on ultrasound and magnetic resonance imaging (MRI) are limited to the depiction of gross morphological alterations of the posterior fossa. Various imaging signs have been described, such as the banana sign [22-24], an effaced cisterna magna [25], the absence of the translucency of the fourth ventricle [4], the clivus-supraocciput angle (CSA) [25-27], a decrease in the volume of the posterior fossa [28], an increased sagittal diameter of the brainstem, and a decreased brainstem-to-occipital-bone diameter [29], and an intracranial translucency [4]. To date, no prenatal data on parenchymal changes within the fetal brainstem in this condition are available. Results of such studies could contribute to an optimized prenatal characterization

of Chiari II malformations, and shed light on the postnatal clinical severity of this condition. Moreover, these studies may also be helpful in the differentiation between open and closed neural tube defects.

Diffusion tensor imaging (DTI) is an MRI technique that measures the amount and directionality of proton motion within different tissues. It has successfully been used in vivo in fetal imaging [30], and has provided information about the external compression of neural structures in adults [31-35], and about the brainstem and cerebellum in pediatric patients with Chiari I malformations [36].

This study used DTI to detect and quantify structural changes of the brainstem parenchyma in fetuses with Chiari II malformations. The primary hypothesis was that fetal brainstem compression in Chiari II malformations results in higher fractional anisotropy (FA) values compared to those in fetuses with normal CNS development, mild ventriculomegaly, or hydrocephalus due to reasons other than Chiari II malformations.

Materials and Methods

The study protocol was approved by the Ethics Committee of the Medical University of Vienna, and the study was conducted according to the Declaration of Helsinki. The ethics committee waived written, informed consent due to the retrospective design of the study.

Patients

All patients included in this study were referred to our department for fetal MRI between 2007 and 2014 to rule out or to confirm findings on fetal ultrasound that were considered suspicious. The established gestational age was based on ultrasound examinations during the first trimester. The most common pathologies were open neural tube defect with Chiari II malformation, mild ventriculomegaly, hydrocephalus, gastroschisis, cleft lip and palate, congenital diaphragmatic hernia, and cystic adenomatoid malformation of the lung. Patient data were used for analyses in an anonymized and de-identified form.

Inclusion criteria

Fetuses with unequivocal imaging results with regard to the presence of a Chiari II malformation, together with an open neural tube defect (confirmed postnatally), mild ventriculomegaly, or hydrocephalus, and in whom a DTI sequence was acquired with the specifications described below, were included in our study. Fetuses with normal CNS development in whom a DTI sequence was acquired with the specifications described below, were also included in our study.

Exclusion criteria

T2-weighted and DTI sequences were excluded after meticulous visual inspection of three-dimensionally reformatted image stacks if fetal body or head motion was detected. The threshold for exclusion was low, as artifacts due to fetal body or head movements affect diffusion tensor metrics tremendously. Fetuses of multiple pregnancies, with intrauterine growth restriction, or with neural pathologies other than those related to neural tube defects of the spine, were excluded.

MRI

MRI examinations were performed on a 1.5 Tesla MR scanner (Philips Medical Systems, Best, The Netherlands) with a five-element, phased-array cardiac coil. During the examinations, neither contrast agents nor sedation were used.

The MRI protocol included an axial single-shot, fat-suppressed, echo planar DTI sequence (16 non-collinear diffusion gradient-encoding directions with b-values of 0 and 700 s/mm^2, TE 90 ms, TR shortest, flip angle 90°, field of view [FOV] 240, matrix 112 x 105, slice thickness 3 mm, acquisition time 1 min 16 sec) perpendicular to the long axis of the fetal brainstem, as previously described [37]. In addition, axial single-shot fast spin-echo (SSFSE) T2-weighted sequences were acquired (TR variable [14000 to 25000 ms], TE 100–140 ms, flip angle 90°, FOV 200 mm–230 mm, matrix 256 × 256, slice thickness 3 mm) [37]. The T2-weighted and the DTI sequences were acquired successively to minimize the chance of fetal head movement between the two sequences.

Image Evaluation

T2-weighted and DTI sequences were transferred to a Philips Achieva workstation (release 2.1.1.0). The images were postprocessed using the Diffusion Registration package (Philips, The Netherlands). The Diffusion Registration package uses 3D affine registration to match T2-weighted images with the b = 0 s/mm^2 volume of the DTI stack. Geometric coordinates were used to align the anatomical images with the DTI dataset.

Mean fractional anisotropy (FA) and apparent diffusion coefficient (ADC) values were determined in regions of interest (ROIs) placed on axial sequences of the fetal brainstem at the level of the midbrain. The midbrain was chosen as it is a cranial part of the brainstem expected to be affected by compression in a cranio-caudal direction, in case of hydrocephalus or mild ventriculomegaly, as well as by compression in the axial plane in case of a Chiari II malformation. Furthermore, it is easily identifiable on axial scans of the brain to guarantee the reproducibility of ROI drawings and measurements. Each ROI was drawn to cover the maximum of the transsectional plane of the fetal midbrain, but not to include the surrounding cerebrospinal fluid spaces. In each fetus, three different ROIs were drawn and the mean and standard deviation of the ADC and FA values were calculated. A schematic ROI is shown in Figure 1, drawn on an axial T2-weighted image co-registered with the DTI image.

A fetus was classified as having mild ventriculomegaly when the maximum width of the atria of the lateral ventricles was between 11 and 15 mm at least on one side, and as having hydrocephalus when at least one atrium measured 16mm or more [38].

Statistical Evaluation

Statistical planning and analysis were performed by a statistician (M.W.) using the IBM SPSS 21.0 software package for Microsoft Windows, SPSS, Chicago, Ill). The study cohort was divided into four groups: fetuses with normal CNS development; fetuses with Chiari II malformations; fetuses with mild ventriculomegaly; and fetuses with hydrocephalus. For each group of these fetuses, a group of age-matched control fetuses with normal CNS development was formed. For each of those fetuses with Chiari II malformation, mild ventriculomegaly, or hydrocephalus, an age-matched control fetus was selected from the group of fetuses with normal CNS development, with a maximum difference of +/- 3 days of gestation. To compare groups of fetuses with pathological CNS findings with each other, for each fetus with a Chiari II malformation, for example, another age-matched fetus with hydrocephalus was selected. The same was done for fetuses with other pathological CNS findings. If no age-matched fetus could be found for the comparison group, the fetus was excluded from this comparison. This resulted in a group of seven fetuses with Chiari II malformations, and a group of seven age-matched fetuses with hydrocephalus. A group of nine fetuses with Chiari II malformations was compared to nine fetuses with mild ventriculomegaly, and six fetuses with hydrocephalus were compared to six fetuses with mild ventriculomegaly. Age-based matching was performed because FA and ADC change with gestational age. Pearson correlation was used to calculate correlations of gestational age with ADC and FA. Student's t-tests for independent samples were used to calculate differences between groups of fetuses. A p-value ≤ 0.05 was considered statistically significant.

Results

Of 88 fetuses who had been imaged using DTI, between 2007 and 2014 at our institution, we included 46 fetuses with normal development of the CNS, 16 with Chiari II, 10 with hydrocephalus, and 16 with mild ventriculomegaly in our study. Twelve fetuses were excluded due to severe head motion during the acquisition of the DTI sequence (five fetuses with normal development of the CNS, one with Chiari II, two with hydrocephalus, and four with mild ventriculomegaly). Descriptive statistics for the subgroups of included fetuses with normal development of the CNS, Chiari II malformations, hydrocephalus, and mild ventriculomegaly are shown in Table 1. Statistics concerning atrial widths in the groups with Chiari II malformations, mild ventriculomegaly, and hydrocephalus are shown in Tables 2 and 3.

In all cases of Chiari II malformations, the prenatal diagnosis based on ultrasound and MRI was confirmed by postnatal surgery or postmortem examination. Pregnancies were terminated in 11 fetuses with Chiari II malformation and in six cases with hydrocephalus of other causes (intracranial supratentorial hemorrhages and aqueduct stenosis) between 18 and 29 GW. The terminations were based on the diagnoses established using fetal ultrasound and MRI, and, in cases of Chiari II malformations, also on maternal α-fetoprotein levels. Two fetuses with hydrocephalus of other causes, 12 fetuses with mild ventriculomegaly, and 41 fetuses with normal CNS were born alive after spontaneous birth or Caesarean section.

Among all 76 fetuses included in our study, ADC decreased significantly with gestational age ($r=-.466$; $p<.001$), whereas FA increased significantly ($r=.295$; $p=.010$) (Figure 2).

These correlations were particularly strong in fetuses with normal CNS (for GW and ADC, $r=-.572$; $p<.001$; for GW and FA, $r=.782$; $p<.001$) and weaker in the subgroup of fetuses with mild ventriculomegaly (for GW and ADC, $r=-.543$; $p=.068$; for GW and FA, $r=.621$; $p=.031$). In the other subgroups of fetuses (hydrocephalus, Chiari II) none of these correlations were significant (Chiari II, ADC: $r=.076$; $p=.787$; FA: $r=.219$; $p=.433$; hydrocephalus, ADC: $r=.486$; $p=0.229$; FA: $r=.129$; $p=.761$)

As a result of age-matching, gestational ages did not differ significantly between the groups of fetuses with pathologic CNS findings and their respective control fetuses with normal CNS development, or with a different type of pathologic CNS development (Table 4).

When comparing the 15 fetuses diagnosed with Chiari II malformations to those 15 age-matched control fetuses with normal CNS development, FA differed significantly ($p=.003$),

whereas ADC was not significantly different (p=.267). The comparison of 12 fetuses with mild ventriculomegaly with 12 age-matched control fetuses with normal CNS development did not reveal significant differences concerning ADC (p=.758) or FA (p=.205), nor did the comparison of eight fetuses with hydrocephalus with eight age-matched control fetuses with normal CNS development (ADC: p=.637; FA: p=.659). Comparisons of groups of fetuses with different types of pathologic CNS development with each other revealed no significant differences concerning ADC or FA. For more detailed descriptive statistics and p-values, see Table 4.

Discussion

Hypoplasia of the posterior fossa is a characteristic feature of the Chiari II malformation and can be readily visualized by prenatal ultrasound or fetal MRI. However, during prenatal life, the severity of brainstem compression in this condition is unknown. This study was able to demonstrate the potential of fetal MRI to identify changes in the DTI metrics of the brainstem in fetuses with Chiari II malformations. As brainstem pathology in Chiari II leads to various potentially life-threatening clinical symptoms [7; 16-19], the non-invasive quantitative imaging technique we present may have the potential to predict the postnatal clinical severity of this condition. Moreover, fetal DTI may help to optimize the inclusion criteria and outcome of recent prenatal myelomeningocele repair strategies [39-43]. Due to the diversity of posterior fossa morphology and grade of brainstem compression in open and closed neural tube defects [27], DTI may add further confidence to the prenatal differentiation between both conditions. Thus, fetal brainstem assessment by DTI may ultimately help to select the most promising candidates for fetal surgery.

By comparing fetuses with Chiari II malformations to normal controls and cases of ventriculomegaly, we were able to show a significantly higher midbrain FA in fetuses with Chiari II malformations. An increase in FA has been shown to occur in asymptomatically compressed nerve roots [32; 44]. Elevated FA in the midbrain in fetuses with Chiari II malformation can be explained by brainstem compression in the axial plane, limiting the diffusion of protons axially, but, to a lesser extent, cranio-caudally. Contrastingly, ADC as a measure of mean diffusivity, was not affected significantly in the midbrain of these fetuses. Therefore, the changes in FA cannot be explained by overall increased diffusivity, but are thought to be caused by changes in the internal architecture of the brainstem, supposedly resulting from external compression.

In fetuses with hydrocephalus due to reasons other than Chiari II malformations, the brainstem is compressed in a cranio-caudal direction, in contrast to the compression in the axial plane in fetuses with Chiari II malformations. This geometrically different compression in hydrocephalus without Chiari II malformations limits the directionality of the diffusion of water molecules in the brainstem less severely and might account for FA values that are not significantly different from those of normal fetuses.

Measuring FA in the brainstem of fetuses with hydrocephalus may aid in the differentiation of the pathogenesis of hydrocephalus. It is especially important when there is a neural tube defect evident, but differentiation between an open and closed subtype is difficult. It is known that Chiari II malformations are almost always associated with open neural tube defects, except for very few cases of closed neural tube defects associated with a Chiari II

malformation [5-7]. Therefore, if the FA in the fetal midbrain is elevated due to a Chiari II malformation, this may aid in the diagnosis of an open neural tube defect. It is a parameter that is uniquely intrinsic to the fetal brainstem, and stands in strong contrast to other parameters used to differentiate between open and closed neural tube defects prenatally, such as the clivus-supraocciput angle [25-27], the lemon and banana signs [22-24], and the effaced cisterna magna sign [25].

However, DTI metrics of the fetal brainstem are known to change with gestational age [45; 46]. In this study, we were able to confirm the results of these previous studies and found the FA values of the fetal midbrain to correlate, and ADC values to inversely correlate, with gestational age in fetuses with normal CNS development, both at a significant level. With regard to prenatal changes in diffusivity, it has been shown that the overall water content of the brain decreases during gestation, and therefore, those structures that impede water diffusion, such as cell membranes, increase in density [47]. Furthermore, myelination, as well as density and orientation of fibers, contribute to anisotropic diffusion pre- and postnatally [48]. Premyelination comprises an increase in the density of sodium channels and in the diameter of axons, among other changes in brain tissue, and has been shown to increase prenatal diffusion anisotropy prior to myelination [45; 49; 50]. Our data on fetuses with normal CNS development support these previous findings on prenatal CNS development, myelination, and premyelination. In addition, in fetuses with mild ventriculomegaly (the mildest form of CNS abnormality included in our study) FA also increases with gestational age, whereas ADC does not. In fetuses with Chiari II malformations or hydrocephalus, there were no significant correlations between gestational age and FA or ADC. In these cases, the disturbance of normal CNS development might affect some of those developmental changes mentioned above, which would eventually cause FA to increase and ADC to decrease. Alternatively, the overall increase of FA in fetuses with Chiari II malformations, due to compression of the midbrain in the axial plane, might have been too severe to allow the detection of physiologically increasing FA during maturation of the CNS in our group of 15 fetuses with Chiari II malformation.

Due to ongoing changes in diffusivity during gestation, fetuses with different types of pathologies and fetuses with normal CNS development can only be compared on an age-matched basis. Therefore, it is inevitable to select age-matched pairs of fetuses for comparison purposes. This is a limitation to our study and, generally, to this field of work. Only those fetuses for whom an adequately matched fetus for comparison can be found can be included in statistical analyses, while other fetuses have to be excluded due to the lack of an age-matched fetus for comparison.

Among the limitations of this study is, first, the high motion sensitivity of DTI and diffusion tensor metrics, such as FA and ADC. We addressed this issue by meticulous visual evaluation of T2-weighted and DTI sequences for motion artifacts. A very low threshold for excluding sequences depicting maternal, or fetal body or head motion, was used to avoid inaccurate measurements. Second, our measurements of FA and ADC revealed rather high standard deviations. Low acquisition times of MR sequences are necessary in fetal imaging due to maternal and fetal motion. Reducing acquisition time results in increased image noise and a decreased signal-to-noise ratio. The DTI sequence used in this retrospective study has been thoroughly evaluated in previous studies and has been shown to provide well-balanced acquisition times and image quality [51; 52]. Our repeated measurements of ADC and FA in each fetus also addressed this issue. In addition, the retrospective nature of this study is a limitation, but allows, for the first time, a comparison of diffusion tensor metrics of the brainstem in fetuses with Chiari II malformations, ventriculomegaly, hydrocephalus, and normal development. Finally, to date, there are no follow-up data available for the fetuses in this study who were born alive. Further evaluation of the postnatal outcome of patients in whom there were high FA values of the midbrain during gestation appears to be a promising field of investigation, and postnatal or postsurgical follow-up might reveal the functional correlates of altered diffusion parameters in the brainstem.

Conclusion

This is the first study to show that FA is significantly elevated in the midbrain of fetuses with Chiari II malformations when compared to FA in normally developing fetuses. Extrinsic compression of the fetal brainstem in the axial plane, which leads to impaired diffusion of water molecules in the axial plane, but not cranio-caudally, is supposedly the pathophysiological basis for this result. However, FA increases and ADC decreases significantly during gestation in normally developing fetuses. These results are in accordance with previously published data and most probably reflect maturational changes of brain tissue, such as myelination and premyelination. In fetuses with Chiari II malformation or hydrocephalus due to other causes, these maturational processes might be hindered or masked by pathologically increased FA (as shown in Chiari II malformations), and thus, do not enable the detection of a significant correlation of FA with gestational age.

Particularly for the differentiation of open and closed neural tube defects, measurements of the FA in the midbrain may be helpful, as a Chiari II malformation occurs almost exclusively in an open neural tube defect and shows increased FA, whereas hydrocephalus due to other causes does not.

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	n	GW				ADC		FA	
		min.	max.	mean	std. dev.	mean	std. dev.	mean	std. dev.
all fetuses	76	17.14	41	26.41	5.29	1.23	0.29	0.36	0.09
normal CNS	41	18.86	37.86	26.45	4.97	1.19	0.25	0.33	0.08
Chiari II	15	20.57	37.29	24.75	4.95	1.34	0.33	0.44	0.11
hydrocephalus	8	19.71	41	29.93	7.07	1.12	0.3	0.39	0.08
mild ventriculomegaly	12	17.14	34.14	25.98	5.02	1.28	0.33	0.35	0.05

Table 1 Descriptive statistics for all fetuses included in our study. n = number. GW = gestational weeks. min. = minimum. max. = maximum. std. dev. = standard deviation. ADC = apparent diffusion coefficient. FA = fractional anisotropy.

	n	Atrial width			
		min.	max.	mean	std. dev.
Chiari II	15	7	17	13.20	3.26
hydrocephalus	8	20	46	27.13	9.40
mild ventriculomegaly	12	12	14	13.17	0.72

Table 2 Atrial width of all fetuses with a Chiari II malformation, hydrocephalus due to other causes, and mild ventriculomegaly who were included in our study. n = number. min. = minimum. max. = maximum. std. dev. = standard deviation.

Pathology	*n*		Pathology	*n*	Atrial width p
normal CNS	*15*	**vs.**	Chiari II	*15*	**<.001****
normal CNS	*8*	**vs.**	hydrocephalus	*8*	**<.001****
normal CNS	*12*	**vs.**	mild ventriculomegaly	*12*	**<.001****
Chiari II	*7*	**vs.**	hydrocephalus	*7*	**.024***
Chiari II	*9*	**vs.**	mild ventriculomegaly	*9*	.702
hydrocephalus	*6*	**vs.**	mild ventriculomegaly	*6*	**<.001****

Table 3 Atrial width of fetuses for age-matched comparisons with Chiari II malformations, with hydrocephalus due to other causes, and with mild ventriculomegaly. n = number. min. = minimum. max. = maximum. std. dev. = standard deviation. * = significant at the level of p≤.05. ** = significant at the level of p≤.001.

Pathology	*n*	ADC		FA			Pathology	*n*	ADC			FA		
		mean	std. dev.	mean	std. dev.				mean	std. dev.	p	mean	std. dev.	
normal CNS	*15*	1.21	.27	.32	.08	**vs.**	Chiari II	*15*	1.34	.33	.267	.44	.11	.
normal CNS	*8*	1.05	.22	.37	.09	**vs.**	hydrocephalus	*8*	1.12	.30	.637	.39	.08	.
normal CNS	*12*	1.24	.27	.32	.06	**vs.**	mild ventriculomegaly	*12*	1.28	.33	.758	.35	.05	.
Chiari II	*7*	1.29	.31	.38	.12	**vs.**	hydrocephalus	*7*	1.13	.32	.368	.40	.09	.
Chiari II	*9*	1.40	.35	.42	.13	**vs.**	mild ventriculomegaly	*9*	1.25	.38	.399	.36	.04	.
hydrocephalus	*6*	1.17	.33	.41	.09	**vs.**	mild ventriculomegaly	*6*	1.24	.46	.596	.37	.04	.

Table 4 Comparison of age-matched groups of fetuses with different pathologies with regard to their apparent diffu
anisotropy (FA), and gestational weeks (GW). n = number. std.dev. = standard deviation.

Figure 1 ROI placement on DTI data of a normal brain at 32 GW (a-c) and of a brain at 37+3 GW with Chiari II malformation. a) and d) DTI data set in the axial view. b) and e) Overlay of axial T2-weighted sequence on an axial DTI data set. c) and f) Schematic drawings of the ROIs in the brainstem at the level of the midbrain on the overlay. FA and ADC were calculated from these ROIs.

Figure 2 Scatterplots indicating GW (gestational week) and ADC (apparent diffusion coefficient) or FA (fractional anisotropy) for each fetus. Regression analyses revealed a significant negative correlation between ADC and GW ($p<.001$) and a significant positive correlation between FA and GW ($p=.010$).

Figure 3 Scatterplots for each group of fetuses indicating GW (gestational week) and ADC (apparent diffusion coefficient) or FA (fractional anisotropy) for each fetus. Regression analyses in the group of fetuses with normal CNS revealed a significant negative correlation between ADC and GW ($p<.001$), and a significant positive correlation between FA and GW ($p=.010$). In the group of fetuses with mild ventriculomegaly, FA increased significantly with gestational age ($p=.031$).