Comparison of fractional anisotropy and apparent diffusion coefficient among hypoxic ischemic encephalopathy stages 1, 2, and 3 and with nonasphyxiated newborns in 18 areas of brain

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Abstract

Purpose: To determine the area and extent of injury in hypoxic encephalopathy stages by diffusion tensor imaging (DTI) using parameters apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values and their comparison with controls without any evidence of asphyxia. To correlate the outcome of hypoxia severity clinically and significant changes on DTI parameter.

Materials and Methods: DTI was done in 50 cases at median age of 12 and 20 controls at median age of 7 days. FA and apparent diffusion coefficient (ADC) were measured in several regions of interest (ROI). Continuous variables were analyzed using Student’s t‑test. Categorical variables were compared by Fisher’s exact test. Comparison among multiple groups was done using analysis of variance (ANOVA) and post hoc Bonferroni test. Results: Abnormalities were more easily and accurately determined in ROI with the help of FA and ADC values. When compared with controls FA values were significantly decreased and ADC values were significantly increased in cases, in ROI including both right and left side of thalamus, basal ganglia, posterior limb of internal capsule, cerebral peduncle, corticospinal tracts, frontal, parietal, temporal, occipital with P value < 0.05. The extent of injury was maximum in stage‑III. There was no significant difference among males and females. Conclusion: Compared to conventional magnetic resonance imaging (MRI), the evaluation of FA and ADC values using DTI can determine the extent and severity of injury in hypoxic encephalopathy. It can be used for early determination of brain injury in these patients.

Key words: Apparent diffusion coefficient; diffusion tensor imaging; fractional anisotropy; hypoxic ischemic encephalopathy

Introduction

Neonatal encephalopathy associated with perinatal hypoxia‑ischemia has been one of the leading causes of neonatal mortality and permanent neurological disability worldwide. Moderate to severe hypoxic ischemic encephalopathy (HIE) occurs at a rate of approximately

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with a total HIE incidence of 3–5 cases per 1,000 term live births. Lawn et al., 2005 found the incidence is up to 10-fold higher in developing countries and globally, 23% of the 4 million annual neonatal deaths are attributed to birth asphyxia. Perinatal asphyxia is believed to account for 10–20% of cases of cerebral palsy and 30% risk of disabilities including blindness, deafness, autism, epilepsy, global developmental delay, and problems with cognition, memory, fine motor skills, and behavior.

Various neuroimaging studies, i.e., magnetic resonance imaging (MRIs) are well-known to know the extent of injury in HIE. Recently, newer techniques such as diffusion-weighted imaging (DWI) and diffusion tensor MRI (DTMRI) have been proved to be more sensitive than conventional MRI to explore brain development and white matter (WM) fibers density and maturation. Tractography is performed in DTMRI and the findings are expressed in colored 3D shape. In normal brain, free diffusion of water occurs equally in all directions, termed as “isotropic” diffusion. If the water diffuses in a medium with barriers, the diffusion becomes uneven, which is termed “anisotropic” diffusion. Anisotropy is measured in several ways. One way is by a ratio called fractional anisotropy (FA).

Dynamic changes of FA and trace may be explained by concomitant maturation-induced changes in tissue microstructure, such as reduction in water content, greater cohesiveness of fiber tracts or fiber organization, maturation of axons, and myelination. Conditions where the myelin or the structure of the axon are disrupted, such as trauma, tumors, and inflammation are known to reduce anisotropy, as the barriers are affected by destruction or disorganization. Apparent diffusion coefficient (ADC) is defined as the ratio of mean-squared displacement and the diffusion time. Both of these values are important to assess brain damage. Therefore, it is also useful to identify a noninvasive method to detect and monitor the evolution of HI-induced damage for treatment selection and to determine the effectiveness of treatment.

There is limited experience of using DTMRI in different areas of brain in perinatal asphyxia, though several studies have shown changes in HIE. DWI is a better modality to correlate the outcome of asphyxiated babies. This study was conducted to determine the area and extent of injury in hypoxic encephalopathy stages by diffusion tensor imaging (DTI) using parameters apparent diffusion coefficient (ADC) and FA values and their comparison with controls without any evidence of asphyxia. Outcome of hypoxia severity clinically and significant changes on DTI parameter was also determined.

Materials and Methods

This prospective observational study was conducted in the Department of Pediatrics and the Department of Radiology, Institute of Medical Sciences, Banaras Hindu University. The period of study extended from December 2011 to July 2013. Informed consent was taken from all parents before inclusion in the study. The study was approved by the Institute Ethics Committee.

Inclusion criteria

The study population comprised of term newborns (37–41 weeks) who suffered perinatal asphyxia. Perinatal asphyxia was defined as the need for delivery room resuscitation and development of clinical manifestations suggestive of HIE. Both inborn and outborn babies were included. Control group comprised of newborns were evaluated for possible sepsis, but had negative laboratory work up including blood culture.

Exclusion criteria

Newborns with sepsis, respiratory distress, metabolic disorders, and major congenital malformations were excluded from the study.

Newborns were managed as per unit protocol. Progression of HIE into different stages was categorized according to the classification of Fenichel. DTMRI was done using 1.5 Tesla MRI Magnetom Avanto (Version; Bv-I7A) Siemens medical system, Erlangen, Germany, between D5 and D20 of life in those who survived. Straps and bolsters were used to help the infants stay still and maintain the correct position during imaging. Lorazepam at 0.05 mg/kg/dose was used to sedate the infants. The protocol consisted of high resolution anatomical images acquired with a T1-weighted sagittal three-dimension (3D) magnetization-prepared rapid gradient-echo (MPRAGE) sequence [TR 7.1, TE 3.45ms, TI 100Oms, and flip angle 7 degrees, field of view (FOV) 256 mm × 256 mm, and slab thickness 150 mm]. The acquisition matrix was 256 × 192 × 128, reconstructed voxel resolution of 1.0 mm × 1.0 mm × 1.33 mm. The DTI sequence was a single shot balanced echo planar imaging (EPI) sequence with timing parameters of TR 6000 ms and TE 97 ms. The 20 contiguous transverse slices with a slice thickness of 5 mm were aligned parallel to the anterior commissure and posterior commissure plane and covered all, but the topmost part of the brain. The FOV was 128 mm × 128 mm, acquisition matrix 96 × 128, reconstructed to 128 × 128, giving a reconstructed in-plane resolution of 1.78 mm × 1.78 mm. For each slice, one image without diffusion weighting (b = 0s/mm square) and six images with diffusion weighting (b0 = 1000s/mm square) applied along six non-collinear directions were acquired. The six DTI acquisitions for each subject were registered using a mutual information cost function and a 12 parameter affine transformation with the first b = 0s/mm square volume reference.

Data post-processing was done using the automated Neuro-3D software (Siemens Medical System, Erlangen, Germany).
Germany) on the offline workstation. Quantitative analysis was done by drawing region of interest (ROI) following a pre-standardized protocol in the interactive maps generated by the software to include the WM tracts emanating from most functionally active areas in the brain. FA and ADC were measured in several ROI in brain including both right and left side of thalamus, basal ganglia, posterior limb of internal capsule, cerebral peduncle, corticospinal tracts, frontal, parietal, temporal, and occipital.

**Results**

The study population comprised of 50 term newborns with HIE as cases and 20 term newborns as controls. Among cases, 8 (16%) neonates progressed up to HIE stage I, 6 (12%) up to stage HIE II, and 36 (72%) up to stage HIE III. During hospital stay, 16 (32%) neonates with HIE expired. Among cases, the incidence of HIE stage III was significantly higher in unbooked mothers ($P < 0.01$) and in extramural deliveries ($P < 0.01$). There was no statistical difference in gravidity, parity, gestation, birth weight, sex, mode of delivery, and birth through meconium stained amniotic fluid (MSAF) among three stages of HIE [Table 1]. Neuroimaging findings were suggestive of more damage in higher stages of HIE [Figure 1]. Figure 2 is suggestive of diffuse damage to brain with abnormal diffusion and abnormal myelination. FA was significantly reduced and ADC was significantly increased in HIE in all areas (ROI) compared to controls [Table 2]. In boxplot number 1–18 denotes different areas respectively as follows: 1. Right thalamus 2. Left thalamus 3. Right basal ganglia 4. Left basal ganglia 5. Right posterior limb of internal capsule 6. Left posterior limb of internal capsule 7. Right cerebral peduncle 8. Left cerebral peduncle 9. Right corticospinal tracts 10. Left corticospinal tracts 11. Right frontal 12. Left frontal 13. Right parietal 14. Left parietal 15. Right temporal 16. Left temporal 17. Right occipital 18. Left occipital. Boxplot 1 and 2 is suggestive of that FA was low in cases as compared to controls and there was a variation of FA in cases was due to varying ischemic insult among HIE. Boxplot 3 and 4 suggested that ADC was comparatively higher in cases as compared to controls.

Statistical analysis was done using the SPSS version 16.0 (NY, USA). Data was expressed as mean ± standard deviation for continuous variables and percentage for categorical variables. Continuous variables were analyzed using Student’s $t$-test or Mann-Whitney U-test as applicable. Categorical variables were compared by Fisher’s exact test.

![Figure 1: An incidence of abnormal DTMRI in HIE compared to controls](image-url)

**Table 1: Baseline characteristics of the study population**

| Parameters          | Case ($n=50$) | Control ($n=20$) | Comparison between cases and controls ($P$) |
|---------------------|---------------|-----------------|------------------------------------------|
| Maternal            |               |                 | 0.012                                     |
| Booked              | 3 (37.5%)     | 6 (100%)        | 12 (60%)                                 |
| Unbooked            | 5 (62.5%)     | 0 (0%)          | 8 (40%)                                  |
| Gravida Median      | 1             | 1               | 1                                        |
| Parity Median       | 1             | 1               | 1                                        |
| Birth weight (g)    | 2800 ± 460    | 2863 ± 365      | 2788 ± 342                               |
| GA (wk)             | 37.5 ± 1.1    | 37.8 ± 1.2      | 37.8 ± 1.3                               |
| Delivery            |               |                 | 0.139 (NS)                               |
| Intramural          | 4 (50%)       | 1 (16.67%)      | 2 (5.56%)                                |
| Extramural          | 4 (50%)       | 5 (83.33%)      | 34 (94.44%)                              |
| Mode of delivery    |               |                 | 0.024                                    |
| SVD                 | 6 (75%)       | 5 (83.33%)      | 29 (80.56%)                              |
| Cesarean section    | 2 (25%)       | 1 (16.67%)      | 7 (19.44%)                               |
| Sex                 |               |                 | 0.778 (NS)                               |
| Male                | 6 (75%)       | 5 (83.33%)      | 24 (66.67%)                              |
| Female              | 2 (25%)       | 1 (16.67%)      | 12 (33.33%)                              |

SVD: Spontaneous vaginal delivery.
Kushwah, et al.: Fractional anisotropy and apparent diffusion coefficient in HIE

Comparison among multiple groups was done using analysis of variance (ANOVA) and post hoc Bonferroni test. Pearson correlation coefficient was calculated for different variables. P value of <0.05 was considered statistically significant. There was statistically significant difference in FA between controls and cases, except in left basal ganglia, left cerebral peduncle, left parietal, right occipital and ADC, except in left thalamus and right posterior limb of internal capsule [Table 3]. The extent of neuronal injury was maximum in stage III HIE. When compared with controls significant decrease in FA was observed in right

**Table 2: Diffusion tensor magnetic resonance imaging in study groups**

| Parameter                  | Cases (n=29) | Controls (n=17) | P     |
|----------------------------|--------------|-----------------|-------|
| **Thalamus**               |              |                 |       |
| Right Fractional anisotropy| 201.55±64.39 | 262.49±51.31    | P<0.05|
| Apparent diffusion coefficient | 979.60±206.29 | 842.81±61.31    | P<0.05|
| Left Fractional anisotropy  | 194.27±56.52 | 279.89±64.03    | P<0.001|
| Apparent diffusion coefficient | 1023.71±121.35 | 838.81±50.64    | P<0.001|
| **Basal ganglia**          |              |                 |       |
| Right Fractional anisotropy| 145.30±48.06 | 179.96±67.61    | P<0.05|
| Apparent diffusion coefficient | 1070.41±140.00 | 849.18±84.28    | P<0.001|
| Left Fractional anisotropy  | 153.04±47.70 | 192.46±69.00    | P<0.05|
| Apparent diffusion coefficient | 1070.46±149.02 | 854.42±61.68    | P<0.001|
| **Posterior limb of internal capsule** | | | |
| Right Fractional anisotropy| 398.28±151.70 | 590.89±108.42   | P<0.001|
| Apparent diffusion coefficient | 979.60±206.29 | 842.81±61.31    | P<0.001|
| Left Fractional anisotropy  | 433.10±120.69 | 621.62±115.47   | P<0.001|
| Apparent diffusion coefficient | 989.71±129.39 | 854.42±61.68    | P<0.001|
| **Cerebral peduncle**      |              |                 |       |
| Right Fractional anisotropy| 220.29±57.48 | 363.54±121.78   | P<0.001|
| Apparent diffusion coefficient | 986.45±120.45 | 809.45±175.62   | P<0.001|
| Left Fractional anisotropy  | 234.95±57.86 | 320.84±113.35   | P<0.05|
| Apparent diffusion coefficient | 997.48±99.42  | 814.56±99.85    | P<0.001|
| **Corticospinal tracts**   |              |                 |       |
| Right Fractional anisotropy| 257.61±107.56 | 381.82±132.92   | P<0.05|
| Apparent diffusion coefficient | 1340.50±235.29 | 920.45±111.97   | P<0.001|
| Left Fractional anisotropy  | 263.18±120.66 | 400.97±134.47   | P<0.05|
| Apparent diffusion coefficient | 1306.26±184.26 | 906.46±92.99    | P<0.001|
| **Frontal**                |              |                 |       |
| Right Fractional anisotropy| 180.27±81.63 | 335.95±142.75   | P<0.001|
| Apparent diffusion coefficient | 1393.17±469.95 | 877.35±148.80   | P<0.001|
| Left Fractional anisotropy  | 168.28±90.49 | 314.40±103.59   | P<0.001|
| Apparent diffusion coefficient | 1254.21±295.94 | 825.66±94.18    | P<0.001|
| **Parietal**               |              |                 |       |
| Right Fractional anisotropy| 163.50±51.43 | 232.17±78.06    | P<0.05|
| Apparent diffusion coefficient | 1316.00±313.61 | 881.89±99.15    | P<0.001|
| Left Fractional anisotropy  | 152.44±69.71 | 249.95±71.12    | P<0.001|
| Apparent diffusion coefficient | 1304.19±223.35 | 875.05±131.47   | P<0.001|

**Table 2: Contd...**

| Parameter                  | Cases (n=29) | Controls (n=17) | P     |
|----------------------------|--------------|-----------------|-------|
| **Temporal**               |              |                 |       |
| Right Fractional anisotropy| 140.07±78.40 | 214.78±51.52    | P<0.05|
| Apparent diffusion coefficient | 1254.61±321.33 | 892.59±63.16    | P<0.001|
| Left Fractional anisotropy  | 151.89±60.69 | 220.72±57.99    | P<0.001|
| Apparent diffusion coefficient | 1353.31±281.73 | 889.62±59.95    | P<0.001|
| **Occipital**              |              |                 |       |
| Right Fractional anisotropy| 217.31±100.39 | 449.24±457.12   | P<0.05|
| Apparent diffusion coefficient | 1306.91±240.08 | 848.16±126.46   | P<0.001|
| Left Fractional anisotropy  | 209.37±57.75 | 329.85±94.07    | P<0.001|
| Apparent diffusion coefficient | 1297.22±229.00 | 913.65±78.52    | P<0.001|

**Figure 2:** Baseline characteristics of the study population.

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thalamus, right basal ganglia, bilateral posterior limb of internal capsule, cerebral peduncle, corticospinal tracts, frontal, right parietal, and right temporal region. ADC values were significantly higher in left thalamus, bilateral basal ganglia, right corticospinal tracts, bilateral frontal, parietal, temporal, and occipital areas. Between controls and stage I, FA was significantly reduced in right posterior limb of internal capsule and right cerebral peduncle. Between controls and stage II, significant increase in ADC was seen in corticospinal tracts, temporal and occipital of both sides. Thus, we also found difference in neuronal injury in left and right side. Stage III showed maximum extent of injury. When compared with controls, significant decrease in FA was observed in right thalamus, right basal ganglia, bilateral posterior limb of internal capsule, cerebral peduncle, corticospinal tracts, frontal, right parietal, and right temporal region, whereas significantly higher ADC values were observed in left thalamus, bilateral basal ganglia, right corticospinal tracts, bilateral frontal, parietal, temporal, and occipital areas.

When compared between stage I and stage III, significant difference was observed in FA in left thalamus, bilateral temporal and ADC in right corticospinal tracts, bilateral parietal, temporal, and occipital areas.

Brain WM myelination is a long process which starts well before birth and continues until adulthood. Postmortem studies have shown that myelination progresses in a caudo-rostral way, at different rates depending on location, with earlier maturation of motor and sensory tracts in comparison with cortico-tracts association fibers.
### Table 3: Comparison of fractional anisotropy and apparent diffusion coefficient among controls and study groups

| Parameter                        | Controls (n=17) | HIE Stage I (n=7) | HIE Stage II (n=5) | HIE Stage III (n=17) | ANOVA |
|----------------------------------|----------------|------------------|-------------------|----------------------|-------|
|                                  |                |                  |                   |                      | F     |
| Thalamus                         |                |                  |                   |                      | P     |
| Right                            |                |                  |                   |                      |       |
| Fractional anisotropy            | 262.49±51.31   | 244.1±70.79      | 209.78±57.13      | 186.08±62.19         | 5.08  |
| Apparent diffusion coefficient   | 842.81±61.31   | 814.9±369.55     | 1064.7±120.9      | 944.24±236.29        | 2.10  |
| Left                             |                |                  |                   |                      |       |
| Fractional anisotropy            | 279.89±64.03   | 339.44±259.28    | 195.5±21.98       | 181.06±51.98         | 4.49  |
| Apparent diffusion coefficient   | 838.81±50.64   | 826.89±376.86    | 1056.54±179.77    | 1018.85±92.49        | 5.19  |
| Basal ganglia                    |                |                  |                   |                      |       |
| Right                            |                |                  |                   |                      |       |
| Fractional anisotropy            | 179.96±67.61   | 154.86±19.59     | 170.34±35.04      | 121.96±29.39         | 4.50  |
| Apparent diffusion coefficient   | 849.18±84.28   | 891.63±387.32    | 1074.22±156.82    | 1081.19±151.48       | 5.25  |
| Left                             |                |                  |                   |                      |       |
| Fractional anisotropy            | 192.46±69.00   | 256.23±252.45    | 145.12±26.4       | 141.36±39.89         | 2.15  |
| Apparent diffusion coefficient   | 854.42±61.68   | 884.59±421.74    | 1000.04±143.5     | 1104.26±131.49       | 5.54  |
| Posterior limb of internal capsule |            |                  |                   |                      |       |
| Right                            |                |                  |                   |                      |       |
| Fractional anisotropy            | 590.89±108.42  | 365.69±143.42    | 521.76±114.44     | 371.39±138.09        | 10.53 |
| Apparent diffusion coefficient   | 835.66±62.43   | 845.31±380.03    | 1000.26±104.67    | 953.8±138.82         | 2.08  |
| Left                             |                |                  |                   |                      |       |
| Fractional anisotropy            | 621.62±115.47  | 501.01±210.22    | 497.06±125.73     | 417.86±100.62        | 7.10  |
| Apparent diffusion coefficient   | 824.69±65.96   | 827.51±411.19    | 995.6±100.48      | 987.95±85.38         | 3.49  |
| Cerebral peduncle                |                |                  |                   |                      |       |
| Right                            |                |                  |                   |                      |       |
| Fractional anisotropy            | 363.54±121.78  | 229.86±50.08     | 256.3±69.8        | 203.81±44.18         | 10.43 |
| Apparent diffusion coefficient   | 809.45±175.62  | 838.24±385.86    | 1035.1±76.55      | 971.71±130.46        | 2.87  |
| Left                             |                |                  |                   |                      |       |
| Fractional anisotropy            | 320.84±113.35  | 316.03±223.79    | 290.28±67.23      | 213.52±49.63         | 2.80  |
| Apparent diffusion coefficient   | 814.56±99.85   | 837.87±413.55    | 1012.16±47.03     | 977.84±82.42         | 3.41  |
| Corticospinal tracts             |                |                  |                   |                      |       |
| Right                            |                |                  |                   |                      |       |
| Fractional anisotropy            | 381.82±132.92  | 306.74±163.61    | 315.88±135.18     | 236.74±84.92         | 3.97  |
| Apparent diffusion coefficient   | 920.45±111.97  | 1044.46±483.13   | 1307.52±160.14    | 1390.26±238.27       | 11.14 |
| Left                             |                |                  |                   |                      |       |
| Fractional anisotropy            | 400.97±134.47  | 332.06±220.88    | 280.1±121.26      | 255.01±118.91        | 3.10  |
| Apparent diffusion coefficient   | 906.46±92.99   | 1056.61±534.58   | 1308.4±173.03     | 1327.14±152.08       | 10.20 |
| Frontal                          |                |                  |                   |                      |       |
| Right                            |                |                  |                   |                      |       |
| Fractional anisotropy            | 335.95±142.75  | 248.47±175.22    | 192.3±103.02      | 182.33±87.96         | 4.56  |
| Apparent diffusion coefficient   | 877.35±148.80  | 996.99±438.59    | 1085.72±120.55    | 1526.56±561.04       | 8.40  |
| Left                             |                |                  |                   |                      |       |
| Fractional anisotropy            | 314.40±103.59  | 257.56±241.46    | 197.88±81.46      | 168.44±108.67        | 3.65  |
| Apparent diffusion coefficient   | 825.66±94.18   | 1016.64±474.39   | 984.86±547.78     | 1318.38±189.92       | 9.03  |
| Parietal                         |                |                  |                   |                      |       |
| Right                            |                |                  |                   |                      |       |
| Fractional anisotropy            | 232.17±78.06   | 167.9±59.85      | 179.66±54.72      | 164.96±57.16         | 3.41  |
| Apparent diffusion coefficient   | 881.89±99.15   | 1000.74±436.14   | 1216.64±216.91    | 1386.56±350.22       | 9.41  |
| Left                             |                |                  |                   |                      |       |
| Fractional anisotropy            | 249.95±71.12   | 244.19±281.06    | 153.96±30.5       | 160.96±85.3          | 1.89  |
| Apparent diffusion coefficient   | 875.05±131.47  | 898.39±400.59    | 1241.4±272.81     | 1384.24±195.23       | 17.06 |

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Table 3: Contd...

| Parameter | Controls (n=17) | HIE Stage I (n=7) | HIE Stage II (n=5) | HIE Stage III (n=17) | ANOVA |
|-----------|----------------|-------------------|-------------------|---------------------|-------|
| Temporal  |                |                   |                   |                     |       |
| Right     |                |                   |                   |                     |       |
| Fractional anisotropy | 214.78±51.52 | 216±136.73 | 154.54±54.93 | 116.17±39.56 | 7.24  | P<0.05 |
| Apparent diffusion coefficient | 892.59±63.16 | 930.06±410.98 | 1304.04±306.4 | 1332.79±224.77 | 12.76 | P<0.001 |
| Left      |                |                   |                   |                     |       |
| Fractional anisotropy | 220.72±57.99 | 296.7±279.59 | 153.4±38.16 | 135.44±44.86 | 3.81  | P<0.05 |
| Apparent diffusion coefficient | 889.62±59.95 | 998.81±450.83 | 1311.86±399.03 | 1416.28±262.53 | 12.29 | P<0.001 |
| Occipital |                |                   |                   |                     |       |
| Right     |                |                   |                   |                     |       |
| Fractional anisotropy | 449.24±457.12 | 297.97±129.59 | 280.4±151.83 | 193.04±93.39 | 2.16  | NS     |
| Apparent diffusion coefficient | 848.16±126.46 | 973.46±461.67 | 1270.82±306.95 | 1365.92±213.52 | 13.45 | P<0.001 |
| Left      |                |                   |                   |                     |       |
| Fractional anisotropy | 329.85±94.07 | 328.14±315.42 | 241.42±50.96 | 199.31±60.76 | 3.01  | P<0.05 |
| Apparent diffusion coefficient | 913.65±78.52 | 944.14±460.96 | 1256.62±240.72 | 1373.14±195.1 | 13.41 | P<0.001 |

ANOVA: Analysis of variance, HIE: Hypoxic ischemic encephalopathy

Table 4: Comparison between controls and study groups

| Parameter                  | Control vs. Stage I | Control vs. Stage II | Control vs. Stage III | Stage I vs. Stage II | Stage I vs. Stage III | Stage II vs. Stage III |
|----------------------------|---------------------|----------------------|-----------------------|----------------------|-----------------------|------------------------|
| Thalamus                   |                     |                      |                       |                      |                       |                        |
| Right                      |                     |                      |                       |                      |                       |                        |
| Fractional anisotropy      | NS                  | NS                   | NS                    | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS              | NS                   | NS                    | NS                   | NS                    | NS                     |
| Left                       |                     |                      |                       |                      |                       |                        |
| Fractional anisotropy      | NS                  | NS                   | NS                    | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS              | NS                   | NS                    | NS                   | NS                    | NS                     |
| Basal ganglia              |                     |                      |                       |                      |                       |                        |
| Right                      |                     |                      |                       |                      |                       |                        |
| Fractional anisotropy      | NS                  | NS                   | NS                    | NS                   | P<0.05                | NS                     |
| Apparent diffusion coefficient | NS              | NS                   | NS                    | NS                   | NS                    | NS                     |
| Left                       |                     |                      |                       |                      |                       |                        |
| Fractional anisotropy      | NS                  | NS                   | NS                    | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS              | NS                   | NS                    | NS                   | NS                    | NS                     |
| Posterior limb of internal capsule |         |                      |                       |                      |                       |                        |
| Right                      |                     |                      |                       |                      |                       |                        |
| Fractional anisotropy      | NS                  | NS                   | NS                    | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS              | NS                   | NS                    | NS                   | NS                    | NS                     |
| Cerebral peduncle          |                     |                      |                       |                      |                       |                        |
| Right                      |                     |                      |                       |                      |                       |                        |
| Fractional anisotropy      | NS                  | NS                   | NS                    | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS              | NS                   | NS                    | NS                   | NS                    | NS                     |
| Corticospinal tracts       |                     |                      |                       |                      |                       |                        |
| Right                      |                     |                      |                       |                      |                       |                        |
| Fractional anisotropy      | NS                  | NS                   | NS                    | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS              | NS                   | NS                    | NS                   | NS                    | NS                     |

Contd...
Table 4: Contd...

| Parameter                  | Control vs. Stage I | Control vs. Stage II | Control vs. Stage III | Stage I vs. Stage II | Stage I vs. Stage III | Stage II vs. Stage III |
|----------------------------|---------------------|----------------------|-----------------------|----------------------|-----------------------|------------------------|
| Apparent diffusion coefficient | NS                  | $P<0.05$             | $P<0.001$             | NS                   | $P<0.05$             | NS                     |
| Left                       | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS                  | NS                   | $P<0.001$             | NS                   | NS                    | NS                     |
| Frontal Right              | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS                  | NS                   | $P<0.001$             | NS                   | NS                    | NS                     |
| Left                       | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS                  | NS                   | $P<0.001$             | NS                   | NS                    | NS                     |
| Parietal Right             | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Left                       | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | NS                   | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS                  | NS                   | $P<0.001$             | NS                   | NS                    | NS                     |
| Temporal Right             | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Apparent Diffusion Coefficient | NS                  | $P<0.05$             | $P<0.001$             | NS                   | $P<0.05$             | NS                     |
| Left                       | NS                  | NS                   | $P<0.05$              | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | NS                   | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS                  | $P<0.05$             | $P<0.001$             | NS                   | $P<0.05$             | NS                     |
| Occipital Right            | NS                  | NS                   | NS                   | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | NS                   | NS                   | NS                    | NS                     |
| Left                       | NS                  | NS                   | NS                   | NS                   | NS                    | NS                     |
| Fractional anisotropy      | NS                  | NS                   | NS                   | NS                   | NS                    | NS                     |
| Apparent diffusion coefficient | NS                  | $P<0.05$             | $P<0.001$             | NS                   | $P<0.05$             | NS                     |
| NS: Non-significant        |                     |                      |                      |                      |                      |                        |

Diffusion MRI permits biological tissue structure to be probed and imaged on a microscopic scale non-invasively. \cite{18} Because water diffuses more easily in the direction of the fibers than orthogonally, where it is hindered by myelin sheaths or axonal membranes, this technique has been used to study the organization of the adult WM in fiber bundles. \cite{19}

In DTI, the ADC and diffusion anisotropy indices such as FA are used to estimate the tissue integrity by analysis of the magnitude of the diffusion of water molecules and their mobility and their deviation from the isotropic diffusion. \cite{20,21} The ADC usually defined as the ratio of mean-squared displacement and the diffusion time. The ADC value can increase in some forms of pathology, particularly vasogenic edema or accumulation of cellular debris from axonal damage. Therefore, decrement of either FA value and increment of ADC value may indicate injury of the neural tract. Anisotropy values are reduced in WM damaged areas of brain. Disruptions of integrity for a neural tract also appear to indicate injury of the neural tract.

Changes in anisotropy involving both anisotropy measurements and vector maps will likely prove especially relevant in premature infants, who tend to sustain injury to WM. In the chronic stage of periventricular leukomalacia (PVL), reductions in relative anisotropy may be present, and vector maps may show disruption of WM tracts distant from the focal, cystic lesions detected by conventional imaging. In this case, changes in anisotropy are detectable not only near the site of primary injury, but also in the posterior limb of the internal capsule, indicating a disturbance of developing fibers which project through this area.

Previous studies have proved that early neurologic outcome in neonates with HIE is associated with lower FA.
and higher ADC values in specific areas of white or gray matter.\cite{20-23} During the first week of hypoxic injury, FA values will decrease with both severe and moderate injury as assessed by conventional imaging, whereas ADC values were reduced only in severe injury. Abnormal ADC values pseudo-normalized during the second week and increased after that in chronic phase, whereas FA values continued to decrease.\cite{24} ADC varies with age also in HIE. In a study of neonates who showed areas of reduced ADC were younger at the time imaging (median age 5.5 days, range 2–11 days). The median age was higher in subjects who had normal ADC values (median age 7 days, range, 4–10 days). Those with areas of increased ADC were even older (median age 9.3 days, range, 7–11 days). The age wise differences in ADC were not statistically significant.\cite{25} So, in previous few studies when the study was done early at day 4–7 after birth a significant decrease in ADC values was observed in asphyxia.\cite{26} The cause of raised ADC in our study could be due to higher mean age.

DTI can be a qualified biomarker for the early evaluation of neuroprotective interventions as well as for prognosis.\cite{29-33} Porter et al., (2010) performed DTMRI using 3T in eight healthy control infants, 10 untreated, and 10 hypothermia-treated infants with neonatal encephalopathy with median postnatal age at scan was 1 day (range 1–21) in the healthy infants, 6 days (range 4–20) in the cooled, and 7 days (range 4–18) in non-cooled infants. The authors found that FA was significantly reduced in several WM tracts, anterior and posterior limbs of the internal capsule, corpus callosum, and optic radiations not only in the noncooled infants, but also in the internal capsule in the cooled group. Noncooled infants had significantly lower FA than the cooled treated infants.\cite{34} Ancora et al., (2013) studied effect of brain cooling in moderate to severely affected hypoxic neonates and performed brain MRI and DTMRI to predict whether DTMRI is a better early predictor of brain damage. The authors found that the decrement of FA was maximum in the frontal and parietal WM, but they did not find any difference to predict early injury in brain using DTMRI.\cite{35} Lemmon et al. demonstrated early changes in DTI in cerebellar region in hypoxic newborn, which is a very late finding via conventional neuroimaging modalities.\cite{36} In a study conducted by ZHANG et al., demonstrated that DTI provides sensitive detection and early diagnosis of WM injuries in premature infants with HIE.\cite{37}

**Conclusion**

The extent of brain injury after perinatal asphyxia was measured by DTMRI. On DTMRI analysis, a decrease in FA and increase in ADC was observed in all ROI in HIE compared to controls. ADC was decreased in asphyxia in some studies also, but no definite values are known depending on the day and extent of injury. Further studies may be required to know the correlation of ADC and day of life in asphyxiated newborns. Till now, no studies have been done which compared different stages of asphyxia. In our study the differences were most marked in stage III HIE.

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**Conflicts of interest**

There are no conflicts of interest.

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