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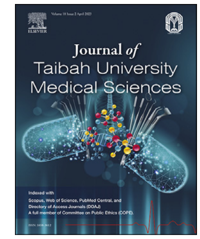

Original Article

# Histomorphometry of the cortical layers and the dentate nucleus of the human fetal cerebellum

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**الملخص**

**أهداف البحث:** هدفت هذه الدراسة إلى إيجاد القياس النسيجي للصفيحة القشرية المخيخية والنواة المسننة لمخيخ الجنين البشري وأيضا دراسة عدد الخلايا العصبية وشكلها، وعمر الحمل لظهور الورقات المخيخية، والمادة البيضاء، وشجرة الحياة المخيخية.

**طريقة البحث:** تمت دراسة المقاطع المجهرية من مخيخ الجنين البشري المصبوغة بالهيماتوكسيلين والأيوزين وبصبغة بيلشوفسكي.

**النتائج:** تباينت سماكة الصفيحة القشرية لمخيخ الجنين البشري بين الطبقة الحبيبية الخارجية- 36.06 ± 9.36 إلى 50.05 ± 34.06 ميكرومتر ، الطبقة الجزيئية- 32.76 ± 17.16 إلى 52 ± 28.6 ميكرومتر، طبقة خلايا بوركيني 9.36 ± 6.8 إلى 15.6 ± 4.68 ميكرومتر وطبقة حبيبية داخلية- 66.65 ± 24.42 إلى 146.63 ± 47.79 ميكرومتر في أسابيع الحمل المختلفة. وبالمثل، فإن عدد الخلايا العصبية لكل مجال رؤية عند قوة 1000 من المجهر المركب يختلف بين الطبقة الحبيبية الخارجية- 89.92 ± 42 إلى 142.84 ± 50 ، الطبقة الجزيئية- 15 ± 12.5 إلى 25 ± 8.25 ، طبقة خلايا بوركيني 3.5 ± 1 إلى 5 ± 2.5 وطبقة حبيبية داخلية- 98.5 ± 69.75 إلى 224 ± 47 في أسابيع الحمل المختلفة. كانت المادة البيضاء في مخيخ الجنين موجودة بالفعل في عمر الأسبوع الثاني عشر من الحمل بينما ظهرت الورقات المخيخية في الأسبوع 16–20 من الحمل. أصبحت شجرة الحياة المخيخية والنواة المسننة واضحة بعد الأسبوع العشرين من الحمل. كانت الخلايا العصبية الجنينية مستديرة باستثناء خلية بوركيني.

**الاستنتاجات:** تختلف سماكة وتعداد الخلايا العصبية للطبقات القشرية المخيخية للجنين البشري وقياسات النواة المسننة جنبًا إلى جنب مع السمات النسيجية الأخرى مع تقدم عمر الحمل من الأسبوع الثاني عشر من الحمل حتى الولادة.

**الكلمات المفتاحية:** المخيخ؛ الطبقات القشرية؛ نواة مسننة؛ قياس النسيج؛ جنين بشري؛ الخلايا العصبية

**Abstract**

**Objectives:** This study was aimed at determining the histomorphometry of the cerebellar cortical laminae and the dentate nucleus of the human fetal cerebellum; the number and shape of the neurons; and the gestational age of appearance of the cerebellar folia, white matter and arbor vitae cerebelli.

**Methods:** Microscopic sections of the human fetal cerebellum stained with hematoxylin and eosin and Bielschowsky silver stain were studied.

**Results:** The thickness of the cortical laminae of the human fetal cerebellum varied among gestational weeks as follows: external granular layer: 36.06 ± 9.36 −50.05 ± 34.06 μm, molecular layer: 32.76 ± 17.16 −52 ± 28.6 μm, Purkinje cell layer: 9.36 ± 6.8 −15.6 ± 4.68 μm and internal granular layer: 66.65 ± 24.42 −146.63 ± 47.79 μm. Similarly, the number of neurons per field of view at 1000X under a compound microscope varied among gestational weeks as follows: external granular layer: 89.92 ± 42−142.84 ± 50, molecular layer: 15 ± 12.5−25 ± 8.25, Purkinje cell layer: 3.5 ± 1−5 ± 2.5 and internal granular layer: 98.5 ± 69.75−224 ± 47.

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White matter in the fetal cerebellum was already present at the age of 12th gestational week, whereas cerebellar folia appeared at 16–20 gestational weeks. Arbor vitae cerebelli and the dentate nucleus became conspicuous after the 20th gestational week. Fetal neurons were round except for Purkinje cells.

**Conclusions:** The thickness and neuronal counts of the human fetal cerebellar cortical layers and the measurements of the dentate nucleus along with other histomorphological features varied with gestational age from the 12th week of gestation until birth.

**Keywords:** Cerebellum; Cortical layers; Dentate nucleus; Histomorphometry; Human fetus; Neuron



## Introduction

The cerebellum is a part of the hindbrain essential for the coordination of movements, balance and posture.[1] The major stages of cerebellar development occur from the early embryonic stage to the first postnatal years. The cerebellum develops from the thickening of the dorsal parts of the alar plates of the metencephalon. Two germinative zones, the ventricular zone, and the rhombic lips, are established in the cerebellar primordium.[2–4] Rhombic lips compress cephalocaudally, thus forming the cerebellar plate in the 12th week of gestation for the development of the cerebellum.[2]

Four cortical laminae exist in the fetal cerebellum, whereas three exist postnatally. The external granular layer is the outermost lamina[5,6] formed by the migration of cells from the rhombic lips.[7,8] Inward migration of the cells from the external granular layer forms the internal granular layer, which becomes the granular layer postnatally.[9,10] Stellate, basket and Purkinje cells are derived from the proliferation of cells from the ventricular zone, whereas granule cells are derived from the proliferation of the cells from the rhombic lips.[2–4,11,12] Deep cerebellar nuclei including the dentate nucleus develop from the precursor cells that migrate from the rhombic lips.[13,14] Complete measurements of the four cortical layers and the dentate nucleus of the human fetal cerebellum are lacking in the literature. Thus, this study quantitatively and qualitatively characterized the developmental changes in the cerebellar cortex and the dentate nucleus in human fetuses.

## Materials and Methods

### *Study design and setting*

This was a descriptive observational study. Spontaneously aborted human fetuses of 12–39 weeks gestational age were collected. Fetuses with neural tube defects and any degree of autolysis were excluded from the study. A total of 90 (46 male and 44 female) fetuses were included and divided into seven groups (12–16, 16–20, 20–24, 24–28, 28–32, 32–36 and > 36 weeks) according to Sturges' rule: $k = 1 + 3.322 \log_{10}N$.

### *Laboratory procedures and observations*

Collected fetuses were fixed in 10% buffered formalin[15] by opening of the posterior cranial fossa (Figure 1a). Paraffin embedded blocks of cerebellar tissue were sectioned into 5 and 10 μm, and stained with hematoxylin and eosin (H&E) (Figure 2a, b) and Bielschowsky silver stain (Figure 3a, b), respectively.

An ocular micrometer, calibrated as shown in Table 1,[16] was used for the measurement of the cortical layers and dentate nucleus. A Labomed compound microscope with eyepiece 10x, field number of 18, and objectives 40x or 100x were used for counting the neurons. Neurons were counted manually by two observers blinded to sample identity for each field of view (FOV) without use of a template. The field area was calculated by measurement of the field diameter. The field area of the section per FOV at 400X was 1.58 $mm^2$, and that at 1000X was 254.34 $\mu m^2$. X represents the final/total magnification, and x represents either the eyepiece or objective lens magnification.

### *Statistical analysis*

Kruskal Wallis and one way ANOVA tests were performed for the analysis of all numerical variables after the normality of data was assessed with the Shapiro–Wilk test. The number of neurons counted in H&E stained sections was validated through comparison with the neurons counted in Bielschowsky silver stained sections in paired samples T test (Graph 1). All nominal variables were calculated as percentages, and their associations were verified with Chi-square test. Descriptive statistics are presented as median ± interquartile range and mean ± standard deviation for nonnormally and normally distributed data, respectively. All statistical tests were measured at a 95% confidence interval with a 5% level of significance in SPSS 16.0 for Windows. Histomorphometry of the dentate nucleus is presented in box and whisker plots constructed in Jamovi 2.3.13 for Windows.

## Results

### *Histomorphometry and histomorphology of the cortical layers of the human fetal cerebellar hemisphere*

From 24 to >36 gestational weeks, the thickness of the external granular layer decreased from 50.05 ± 34.06 to 36.06 ± 9.36 μm ($p = 0.41$). From 24 to 36 gestational weeks, the thickness of the molecular layer increased from 35.36 ± 12.48 to 52 ± 28.6 μm ($p = 0.01$). The Purkinje cell layer began to appear after the 20th gestational week, and its thickness increased from 9.36 ± 6.8 to 15.6 ± 4.68 μm ($p = 0.09$) from 20 to >36 gestational weeks. Similarly, at the same age, the thickness of the internal granular layer increased from 66.65 ± 24.42 to 146.63 ± 47.79 μm ($p = 0.00$) (Table 2, Figures 2a and 3a).

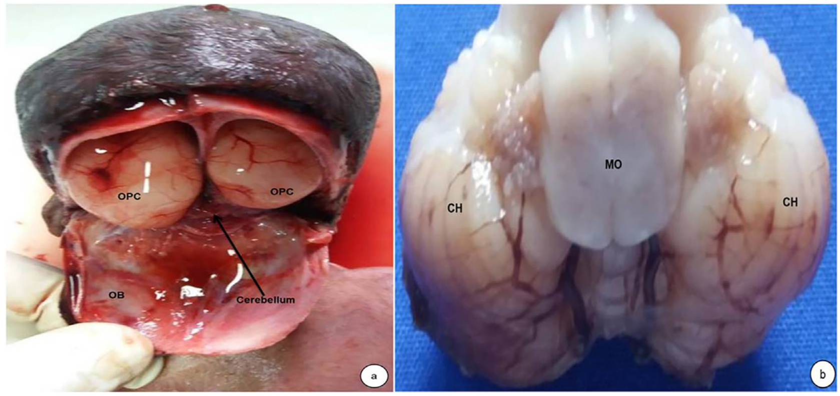



**Figure 1:** Human fetal cerebellum at 27 weeks 4 days. a. Opening of the posterior cranial fossa for the removal of the cerebellum. In the figure, an arrow indicates the cerebellum. b. Human fetal cerebellum with medulla oblongata. OB: occipital bone reflected after dissection, OPC: occipital pole of cerebrum, CH: cerebellar hemisphere, MO: medulla oblongata.

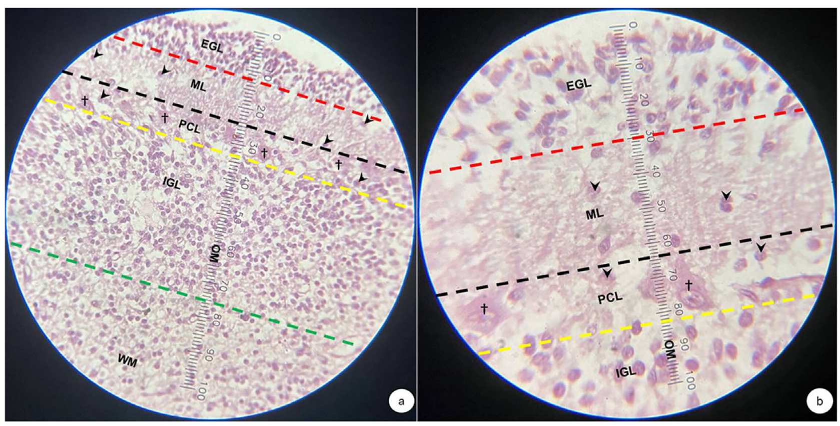



**Figure 2:** Hematoxylin and eosin (H&E) stained sections of the human fetal cerebellar hemisphere at a gestational age of 34 weeks 1 day a. Cortical layers and white matter are shown at 400X. b. Cortical layers are shown at 1000X. Arrowheads represent the migrating neurons, and † represents the Purkinje cells. Each mark of the ocular micrometer equals 3.12 μm at 400X and 1.28 μm at 1000X. EGL: external granular layer, IGL: internal granular layer, ML: molecular layer, OM: ocular micrometer, PCL: Purkinje cell layer, WM: white matter, X: final magnification.

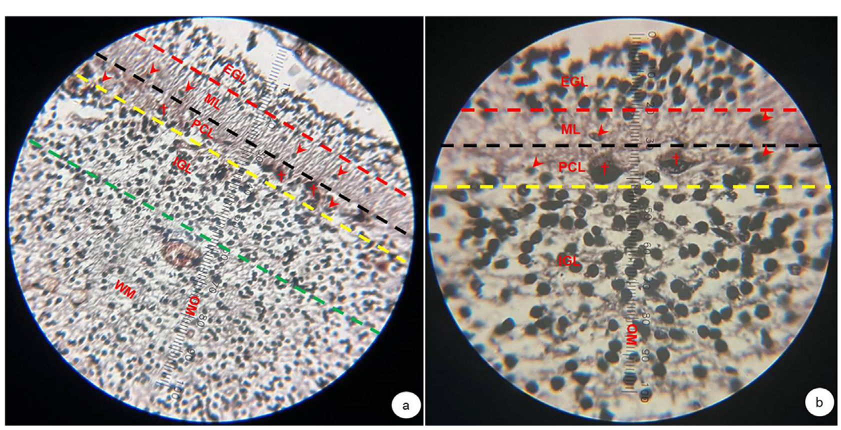
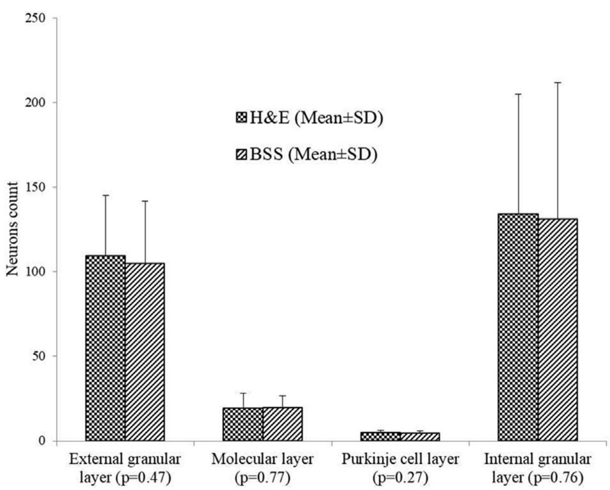



**Figure 3:** Bielschowsky silver stained sections of the human fetal cerebellar hemisphere at a gestational age of 34 weeks 1 day a. Cortical layers and white matter are shown at 400X. b. Cortical layers are shown at 1000X. Arrowheads represent the migrating neurons, and † represents the Purkinje cells. Each mark of the ocular micrometer equals 3.12 μm at 400X and 1.28 μm at 1000X. EGL: external granular layer, IGL: internal granular layer, ML: molecular layer, OM: ocular micrometer, PCL: Purkinje cell layer, WM: white matter, X: final magnification.

**Table 1: Calibration of the ocular and stage micrometers. One ocular mark equals y stage marks. y changes with the power of the objective lens and was calculated by dividing the stage micrometer marks by the ocular micrometer marks. One stage mark represents 10 µm; therefore, one ocular mark equals the number of stage marks multiplied by 10 µm.**

| Objective lens | Ocular micrometer marks | Stage micrometer marks | One ocular mark equals y stage marks | One ocular mark equals z µm |
|---|---|---|---|---|
| 4x | 16 | 50 | 3.125 marks | 31.25 µm |
| 10x | 15 | 20 | 1.333 marks | 13.33 µm |
| 40x | 16 | 5 | 0.312 marks | 3.12 µm |
| 100x | 39 | 5 | 0.128 marks | 1.28 µm |




**Graph 1:** Comparison between the number of neurons counted in hematoxylin and eosin (H&E) and Bielschowsky silver stained sections ($n = 90$) per FOV at 1000X ($p > 0.05$ between two groups in all four cortical layers). Error bars indicate the standard deviation (SD).

The number of neurons was counted per FOV at 1000X. From 16 to 36 gestational weeks, the number increased from 89.92 ± 42 to 142.84 ± 50 ($p = 0.00$) in the external granular layer but decreased from 25 ± 8.25 to 15 ± 12.5 ($p = 0.00$) in the molecular layer. Purkinje neurons decreased from 5 ± 2.5 to 3.5 ± 1 with the increase in gestational age from 24 to >36 weeks ($p = 0.00$). Granule cells in the internal granular layer decreased from 12 to 28 gestational weeks, then began to increase until 36 gestational weeks ($p = 0.00$) (Table 3).

Fetal neurons were round except for Purkinje cells. Purkinje cells changed from round to oval to flask shaped with the progression of fetal age ($p = 0.00$) (Table 5), and had a longitudinal diameter between 9.36 ± 6.8 and 15.6 ± 4.68 µm (Table 2).

*Histomorphometry of the dentate nucleus of the human fetal cerebellum*

The dentate nucleus was developed after the 20th gestational week (Table 4, Figure 4a, b). Insignificant differences were observed in the average values of the length ($p = 0.78$), breadth ($p = 0.32$) and thickness ($p = 0.54$) of the dentate nucleus and the number of neurons present within it ($p = 0.53$) with the progression of fetal age from 20 to >36 gestational weeks (Graphs 2–5).

*Gestational age of appearance of the cerebellar folia, white matter, arbor vitae cerebelli and dentate nucleus*

Cerebellar folia began to appear after the 16th gestational week ($p = 0.00$). White matter was present at the 12th week ($p = 1.00$). Arbor vitae cerebelli and the dentate nucleus became visible after the 20th week ($p = 0.00$) (Table 4).

## Discussion

*External granular layer*

The external granular layer was already present at the age of 12th gestational week, as previously described by Bell et al.[17] According to Rakic et al., this layer appears between 10

**Table 2: Measurement of the thickness of the cortical layers of the human fetal cerebellar hemisphere. Data expressed in median ± interquartile range (IQR). The statistical test performed was Kruskal Wallis test. Asterisk indicates the statistically significant value ($p < 0.05$) between the gestational age groups.**

| Gestational age (n) | Thickness of cortical layers Median ± IQR (µm) | | | |
|---|---|---|---|---|
| | External granular layer | Molecular layer | Purkinje cell layer | Internal granular layer |
| 12–16 weeks (14) | 40.56 ± 35.45 | 50.06 ± 22.62 | Not observed | 109.2 ± 76.43 |
| 16–20 weeks (14) | 37.44 ± 11.57 | 32.76 ± 17.16 | Not observed | 90.48 ± 72.68 |
| 20–24 weeks (13) | 41.6 ± 31.72 | 46.8 ± 32.8 | 9.36 ± 6.8 | 66.65 ± 24.42 |
| 24–28 weeks (12) | 50.05 ± 34.06 | 35.36 ± 12.48 | 12.48 ± 4.16 | 70.2 ± 30.98 |
| 28–32 weeks (10) | 41.95 ± 18.72 | 49.4 ± 9.57 | 12.48 ± 5.68 | 97.75 ± 64.82 |
| 32–36 weeks (13) | 37.44 ± 12.48 | 52 ± 28.6 | 12.48 ± 3.12 | 109.2 ± 67.67 |
| >36 weeks (14) | 36.06 ± 9.36 | 45.24 ± 18.72 | 15.6 ± 4.68 | 146.63 ± 47.79 |
| p-value | 0.41 | 0.01* | 0.09 | 0.00* |

**Table 3: Number of neurons in the cortical layers of the human fetal cerebellar hemisphere counted per FOV at 1000X. Data are expressed as median ± interquartile range (IQR) for external granular, molecular and Purkinje cell layers, and Kruskal Wallis test was performed. For internal granular layer data expressed as mean ± SD, one way ANOVA was performed. Asterisk indicates statistically significant (p < 0.05) difference between gestational age groups.**

| Gestational age (n) | Number of neurons Median/mean ± IQR/SD | | | |
|---|---|---|---|---|
| | External granular layer | Molecular layer | Purkinje cell layer | Internal granular layer |
| 12−16 weeks (14) | 98 ± 20 | 25 ± 8 | Not observed | 154 ± 31.5 |
| 16−20 weeks (14) | 89.92 ± 42 | 25 ± 8.25 | Not observed | 131.5 ± 34.5 |
| 20−24 weeks (13) | 104.3 ± 44.5 | 24 ± 24.5 | 4 ± 1 | 122 ± 55.5 |
| 24−28 weeks (12) | 107.75 ± 31.5 | 18 ± 3.75 | 5 ± 0.5 | 98.5 ± 69.75 |
| 28−32 weeks (10) | 116.5 ± 37.25 | 17 ± 11.5 | 5 ± 2.5 | 154 ± 77.25 |
| 32−36 weeks (13) | 142.84 ± 50 | 15 ± 12.5 | 4 ± 1.5 | 224 ± 47 |
| >36 weeks (14) | 112 ± 39.5 | 15.5 ± 8.5 | 3.5 ± 1 | 193 ± 49.5 |
| p-value | 0.00* | 0.00* | 0.00* | 0.00* |

**Table 4: Gestational age of appearance of cerebellar folia, white matter, arbor vitae cerebelli and the dentate nucleus in different gestational age groups. Asterisk denotes a statistically significant p-value (p < 0.05; chi-square test) between gestational age groups.**

| Variables | Observations | Gestational age groups of the human fetuses | | | | | | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | | 12−16 weeks (14) | 16−20 weeks (14) | 20−24 weeks (13) | 24−28 weeks (12) | 28−32 weeks (10) | 32−36 weeks (13) | >36 weeks (14) | |
| Cerebellar folia | Appeared n (%) | 0 (0%) | 12 (85.7%) | 13 (100%) | 12 (100%) | 10 (100%) | 13 (100%) | 14 (100%) | 0.00* |
| | Not appeared n (%) | 14 (100%) | 2 (14.3%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | |
| White matter | Appeared n (%) | 13 (92.9%) | 14 (100%) | 13 (100%) | 12 (100%) | 10 (100%) | 13 (100%) | 14 (100%) | 1.00 |
| | Not appeared n (%) | 1 (7.1%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | |
| Arbor vitae cerebelli | Appeared n (%) | 0 (0%) | 0 (0%) | 2 (15.4%) | 2 (16.7%) | 5 (50%) | 11 (84.6%) | 13 (92.9%) | 0.00* |
| | Not appeared n (%) | 14 (100%) | 14 (100%) | 11 (84.6%) | 10 (83.3%) | 5 (50%) | 2 (15.4%) | 1 (7.1%) | |
| Dentate nucleus | Appeared n (%) | 0 (0%) | 0 (0%) | 8 (61.5%) | 7 (58.3%) | 8 (80%) | 6 (46.2%) | 6 (42.9%) | 0.00* |
| | Not appeared n (%) | 14 (100%) | 14 (100%) | 5 (38.5%) | 5 (41.7%) | 2 (20%) | 7 (53.8%) | 8 (57.1%) | |

and 11 gestational weeks.[18] Its thickness started to increase from 16 to 28 gestational weeks, and reached maximum 50.05 ± 34.06 μm at 24−28 gestational weeks. Then, it started to decrease and reached minimum 36.06 ± 9.36 μm at >36 gestational weeks (Table 2). Although Rakic et al. have reported that the thickness remains almost constant at 30−40 μm at 9−32 gestational weeks,[18] Yamaguchi et al. have reported that the change in the thickness of the external granular layer occurs with the progression of fetal age,[5] similarly to our findings.

*Molecular layer*

Our findings indicated that the molecular layer had already formed at the age of 12th gestational week, as described by Bell et al.[17] We observed an increase (p = 0.01) in the thickness

**Table 5: Observation of neuron shape in the cortical layers of the human fetal cerebellar hemisphere in different gestational age groups. Asterisk denotes a statistically significant p-value (p < 0.05; chi-square test) between gestational age groups. NA indicates that the p-value is not applicable.**

| Gestational age groups (n) | Shape of neurons | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | External granular layer | Molecular layer | Purkinje cell layer | | | | | | | Internal granular layer |
| | Round n (%) | Round n (%) | Round n (%) | Oval n (%) | Flask n (%) | Round and oval n (%) | Round and flask n (%) | Oval and flask n (%) | Not observed n (%) | Round n (%) |
| 12−16 weeks (14) | 14 (100%) | 14 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 14 (100%) | 14 (100%) |
| 16−20 weeks (14) | 14 (100%) | 14 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (7.1%) | 0 (0%) | 0 (0%) | 13 (92.9%) | 14 (100%) |
| 20−24 weeks (13) | 13 (100%) | 13 (100%) | 1 (7.7%) | 4 (30.8%) | 0 (0%) | 4 (30.8%) | 1 (7.7%) | 0 (0%) | 2 (15.4%) | 13 (100%) |
| 24−28 weeks (12) | 12 (100%) | 12 (100%) | 0 (0%) | 4 (33.3%) | 0 (0%) | 2 (16.7%) | 0 (0%) | 3 (25%) | 3 (25%) | 12 (100%) |
| 28−32 weeks (10) | 10 (100%) | 10 (100%) | 0 (0%) | 2 (20%) | 4 (40%) | 1 (10%) | 0 (0%) | 2 (20%) | 1 (10%) | 10 (100%) |
| 32−36 weeks (13) | 13 (100%) | 13 (100%) | 2 (15.4%) | 1 (7.7%) | 8 (61.5%) | 2 (15.4%) | 2 (15.4%) | 0 (0%) | 0 (0%) | 13 (100%) |
| >36 weeks (14) | 14 (100%) | 14 (100%) | 3 (21.4%) | 0 (0%) | 5 (35.7%) | 3 (21.4%) | 1 (7.1%) | 4 (28.6%) | 0 (0%) | 14 (100%) |
| p-value | NA | NA | 0.00* | | | | | | | NA |

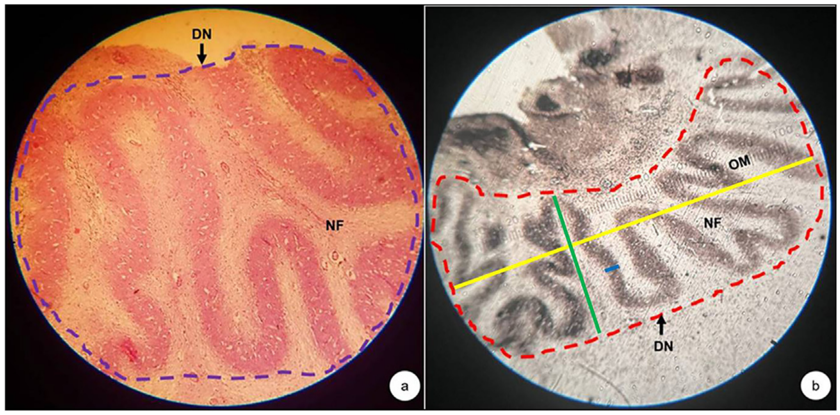
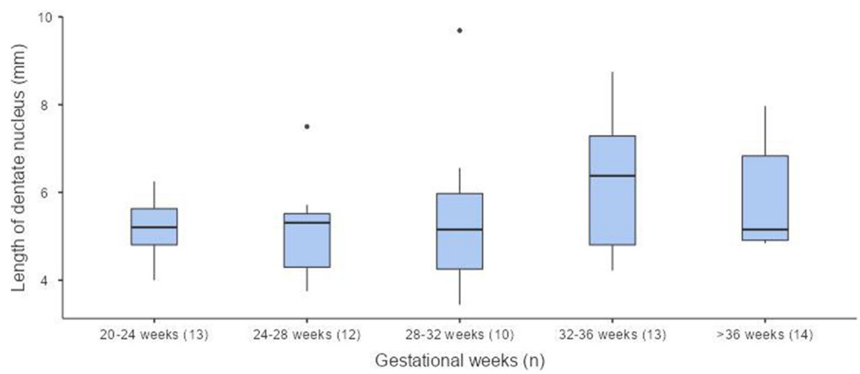



**Figure 4:** Human fetal dentate nucleus at a gestational age of 29 weeks 2 days. a. Hematoxylin and eosin (H&E) stained section at 100X. b. Bielschowsky silver stained section at 40X. In b, the yellow line represents length, the green line represents breadth, and the blue line represents the thickness of the dentate nucleus. DN: dentate nucleus, NF: afferent and efferent nerve fibers of the dentate nucleus, X: final magnification.

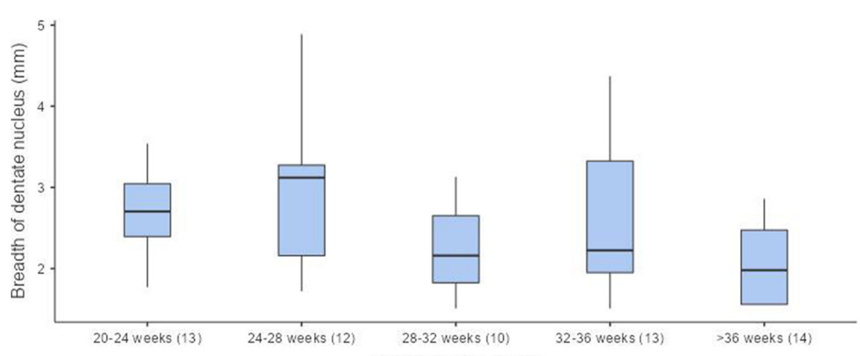



**Graph 2:** Length of the dentate nucleus in different gestational age groups. The horizontal line inside the box is the median length. Lower and upper boundaries of the box indicate the first and third quartiles, respectively. Lower and upper ends of the whiskers indicate the minimum and maximum values, respectively. Dots represent the outliers.

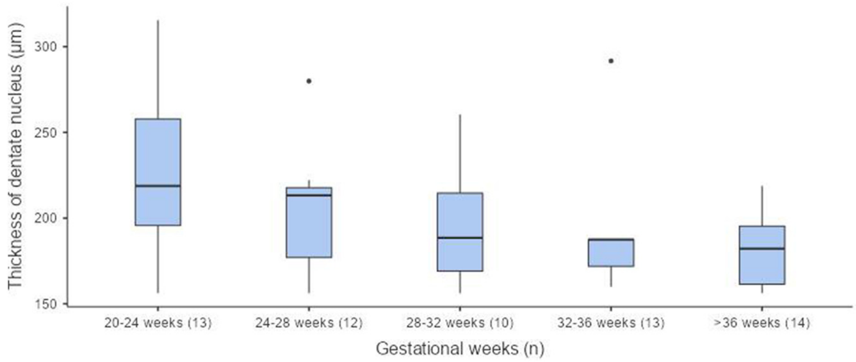
of the molecular layer from 24 to 36 gestational weeks. The thickness varied between 32.76 ± 17.16 μm at 16–20 gestational weeks and 52 ± 28.6 μm at 32–36 gestational weeks (Table 2). Likewise, postnatal and adult studies have shown an increase in thickness between 240 and 500 μm.[19]

*Purkinje cell layer*

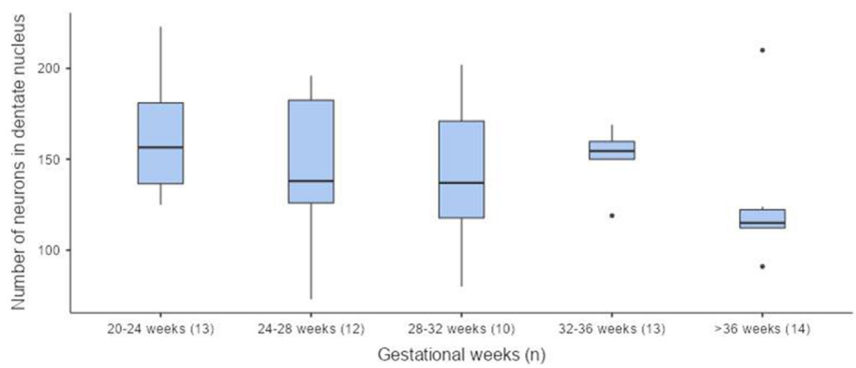
Purkinje cells are observed in the human fetal cerebellum by the ages of 13[18] or 28[17] gestational weeks. At 12–16 weeks, they are deeply distributed in the cerebellar plate, and at 16–




**Graph 3:** Breadth of the dentate nucleus in different gestational age groups. The horizontal line inside the box is the median breadth. Lower and upper boundaries of the box indicate the first and third quartiles, respectively. Lower and upper ends of the whiskers indicate the minimum and maximum values, respectively. Dots represent the outliers.




**Graph 4:** Thickness of the dentate nucleus in different gestational age groups. The horizontal line inside the box is the median thickness. Lower and upper boundaries of the box indicate the first and third quartiles, respectively. Lower and upper ends of the whiskers indicate the minimum and maximum values, respectively. Dots represent the outliers.




**Graph 5:** Neurons counted in the dentate nucleus in different gestational age groups. The horizontal line inside the box is the median number of neurons. Lower and upper boundaries of the box indicate the first and third quartiles, respectively. Lower and upper ends of the whiskers indicate the minimum and maximum values, respectively. Dots represent the outliers.

28 weeks, they are gradually organized into a single row deeper than the molecular layer.[20] However, our study indicated that Purkinje cells began to be well defined as a fixed layer between the molecular and internal granular layers by the age of 20th gestational week. Furthermore, the longitudinal diameter of the Purkinje cells increased from 20 to >36 gestational weeks, and measurements varied between 9.36 ± 6.8 and 15.6 ± 4.68 μm (p = 0.09) (Table 2). A study in rats has indicated that Purkinje cell size increases postnatally up to 23 μm.[21] Moreover, Purkinje cells have been found to be small in neurologic and psychiatric diseases such as autism[22] and schizophrenia.[23]

*Internal granular layer*

Three stages have been observed in the fetal development of the internal granular layer. In the undifferentiated stage, before 18 gestational weeks, the internal granular layer is scarcely distinguishable from the Purkinje cell layer. In the intermediate stage, at 18–30/35 gestational weeks, the internal granular layer is visible and shows essentially stable thickness. At 30/35–40 gestational weeks, a marked increase in thickness is observed, with the formation of cerebellar folia.[5] In our study, the internal granular layer was already present at the age of 12th gestational week, as described by Bell et al.[17] We observed an increase in its thickness from 20 to >36 gestational weeks, from 66.65 ± 24.42 to 146.63 ± 47.79 μm (p = 0.00) (Table 2). Furthermore, a study in adults has indicated that its thickness varies between 240 and 300 μm.[19] The average thickness of the internal granular layer was greater than that of other cortical layers (Table 2), owing to the profuse influx and the active proliferation of the cells in this layer.

*Number of neurons*

We observed that the number of neurons in the external granular layer increased with the progression of fetal age from 16 to 36 weeks, and varied between 89.92 ± 42 and 142.84 ± 50 per FOV at 1000X (p = 0.00). In the molecular layer, the number decreased with the progression of fetal age from 16 to 36 weeks, and varied between 15 ± 12.5 and 25 ± 8.25 (p = 0.00). Purkinje cells were recognized at the 20th gestational week, and their average count varied between 3.5 ± 1 and 5 ± 2.5 (p = 0.00). The number of neurons in the internal granular layer decreased from 12 to 28 gestational weeks, then began to increase in some groups (p = 0.00), with an average count between 98.5 ± 69.75 and 224 ± 47 (Table 3). The increase in the total number of neurons is also observed postnatally.[24] According to a prior study, the total number of Purkinje cells in the human adult cerebellum is approximately 30 million.[25] After birth, the internal granular layer becomes the granular layer, and consists of approximately 101 billion granule cells[25,26] with maximum cellularity in the human brain.[27] The focus of this study was to determine the number of neurons present per FOV to advance understanding of the comparative morphological changes occurring in the four cerebellar cortical laminae during development and to shed light on clinical conditions associated with changes in laminar cell density. For example, Purkinje cells are diminished by 24% in autism.[28]

### *Dentate nucleus*

Various studies have reported that the human dentate nucleus is developed at 12.5[29] or 20[30] gestational weeks. It initially has a 600−700 μm width, which decreases gradually to a final width of 150−250 μm at the 24th gestational week, owing to stretching with the growth of the cerebellum. The rapid growth of the dentate nucleus occurs in gestational weeks 20−25, and the vulnerable period may exist at 20−30 gestational weeks.[29,30] In our study, the dentate nucleus was conspicuous by the age of 20th gestational week ($p = 0.00$) (Table 4). We observed a slight increase in the length of the dentate nucleus with increasing fetal age from 20 to 36 gestational weeks (except at 28−32 weeks). The median value of the length varied between $5.21 \pm 0.82$ and $6.38 \pm 2.48$ mm ($p = 0.78$), whereas the median breadth varied between $1.98 \pm 0.91$ and $3.12 \pm 1.12$ mm ($p = 0.32$). The thickness of the dentate nucleus decreased from 20 to >36 gestational weeks, and the median value varied between $219 \pm 62.1$ and $182 \pm 33.9$ μm ($p = 0.54$) (Graphs 2−4).

The average number of large neurons within the dentate nucleus varied between $127.83 \pm 41.73$ and $162.87 \pm 34.05$ per FOV at 400X, and showed a decreasing pattern (except at 32−36 weeks) with the progression of gestational age from 20 to >36 weeks ($p = 0.53$) (Graph 5). This observation was supported by prior findings indicating a decreased number of neurons in the dentate nucleus with fetal age,[30−32] owing to an increase in neuronal volume and cell death.[31]

### *Shape of neurons*

Animal studies have indicated that the somata of the mature granule, stellate and basket cells are round or oval. Purkinje cells are piriform; Golgi cells are irregular stellate, triangular or polygonal; and Lugaro cells are fusiform.[33] Our fetal study indicated that immature neurons were round, except for Purkinje cells. Similarly to the findings of Zecevic et al.[20] we observed that Purkinje cells changed from round to oval and then to flask shaped with the progression of fetal age from 20 to >36 gestational weeks ($p = 0.00$) (Table 5), and were the largest neurons in the cerebellum, with a longitudinal diameter of $15.6 \pm 4.68$ μm during fetal life (Table 2).

### *Cerebellar folia, white matter and arbor vitae cerebelli*

Limited studies have examined the gestational age of appearance of the cerebellar folia, white matter and arbor vitae cerebelli in the human fetal cerebellum. Rakic et al. have described that cerebellar folia begin to appear by the age of 13th gestational week.[18] However, we observed that the cerebellar folia started to develop after the 16th gestational week, the white matter was already distinct at the age of 12th gestational week, and the arbor vitae cerebelli began to appear after the 20th gestational week (Table 4).

## Conclusions

The thickness of the cerebellar cortical layers, and the measurements of the dentate nucleus and the number of neurons within them, varied with the progression of fetal age. Among the four layers, the internal granular was the thickest and densest. Most fetal neurons were round. White matter was already distinct by the age of 12th gestational week, whereas the folia appeared after the 16th gestational week. The arbor vitae cerebelli and dentate nucleus were conspicuous after the 20th gestational week.

## Recommendations

The findings of this research are important for comparison of normal and neurologic, psychiatric and pathological fetal cerebellar histomorphology. These measurements may be used as developmental markers to analyze the in vivo neuroimages of the human fetal cerebellum.

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## Conflict of interest

The authors have no conflict of interest to declare.

## Ethical approval

Ethical approval was obtained from the institutional review committee (IRC) of Manipal College of Medical Sciences, Pokhara, with registration code MEMG/IRC/GA, 2017, and the institutional ethics committee (IEC) of Kasturba Medical College & Kasturba Hospital (KMC & KH), Manipal, with registration number IEC 192/2020.

## Authors contributions

PPP conceived and designed the study, conducted research, provided research material, collected and organized data, and wrote an initial draft of the article. CB and AG analyzed and interpreted data. SGK wrote the final drafts of the manuscript and critically reviewed it, and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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