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Cytological and Immunohistochemical Analysis of Human Fetal Brain across Different Gestational Ages

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ABSTRACT

Introduction: *Human fetal brain development involves complex processes of cellular proliferation, neuronal migration and differentiation. Although several studies have independently evaluated cytology or immunohistochemistry in fetal brain development, integrated analyses using both approaches remain limited.*

Methods: *This descriptive observational study was conducted on brain tissues obtained from nine aborted human fetuses of different gestational ages distributed across the first, second and third trimesters. Fetuses with visible congenital anomalies, autolysis, or damaged brain tissue were excluded. Histological evaluation was performed using Masson's trichrome and reticulin stains. Immunohistochemical analysis was carried out using Ki-67, c-myc and Ber-H2 (CD30) antibodies. Immunostaining intensity was graded semi-quantitatively on a scale from 0 to 4+ according to the percentage of immunoreactive cells.*

Results: *Developing neuronal cells and pyramidal neurons were observed predominantly in later gestational stages, along with scattered oligodendrocytes and reticular fibers. Immunopositive cells were mainly localized in the white matter and ventricular regions, whereas comparatively lower staining was observed in the granular layers. Progressive increases in immunoreactivity were observed with advancing gestational age, particularly in the ventricular zone, cortical plate and white matter. Among the markers studied, Ki-67 demonstrated the highest immunopositivity, followed by c-myc and Ber-H2 (CD30).*

Conclusion: *The present study demonstrates gestation-related variations in the immunohistochemical expression of Ki-67, c-myc and Ber-H2 (CD30) in the developing human fetal brain. These findings contribute baseline developmental data that may assist future studies investigating fetal neurodevelopment and neuropathological conditions.*

Keywords: fetal brain, immunohistochemistry, Ki-67, c-myc, CD30, neurodevelopment, neuronal migration.

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INTRODUCTION

The neuroepithelial lining of the ventricular cavities in the brain represents the major proliferation site of the central nervous system. Cells derived from this site develop into neurons and glia cells. Since new neurons arise outside the location where they will function, it is important that migration occurs for them to reach their final sites within the growing brain cortex. Migration involves moving postmitotic neuroblasts from the ventricular site to the final site along glial fibres extending in a radial direction to the surface of the brain (1).

There have been many studies involving the human fetal brain on cellular lineage and differentiation processes. According to Sakasi *et al* (1988), neurons differentiate prior to glial cells, implying that the two types of cells do not exist at the same time during the early stages of development within the ventricular zone (2). On the contrary, Stagaard *et al* (1989) indicated that neuron and glial lineages were arising together from similar precursor cells and that radial glial fibres mediated migration, a process that also occurs in other vertebrates (3).

Vascular microenvironment is of vital importance for neurodevelopment. According to Allop *et al* (1979), cerebral pericytes create intricate "peg-and-socket" type associations with vascular endothelial cells and are highly present in capillaries, whereas they are rare in the muscle tissue. Furthermore, cerebral capillaries are densely covered with neuropil in contrast to muscle tissue where there is only loose connective tissue (1).

Immuno-histochemical research also has contributed to our knowledge about developmental processes. According to Sasaki *et al* (1988), early vimentin immunoreactivity was identified in ventricular cells at seven weeks, neurofilament proteins in neuroblasts in an early period and NSE immunoreactivity was found since nine weeks. Glial fibrillary acidic protein (GFAP)-positive cells were detected at approximately nine weeks in the spinal cord and 15 weeks in the cerebrum; S-100 had wider glioblastic expression. At the initial stages, there were no myelin basic protein, which confirmed the idea of neuronal development being earlier than gliogenic one (2).

Further evidence by Stagaard *et al* (1989) showed vimentin-positive neuroepithelial cells between 4-16 weeks, with progressive lamination of the neural wall. Cells retaining vimentin beyond the ventricular zone were identified as radial glia and presumptive astrocytes, although vimentin alone was not a definitive lineage marker (3). Ullfig *et al* (1989) described region-specific patterns of vimentin-positive fibres, with dense bundles and radial extensions evident during mid-gestation and increasing organization by the eighth month, correlating with neuronal migration (4, 5).

Despite these advances, the precise relationship between neuronal and glial lineage development remains incompletely understood. Most studies have focused on markers such as vimentin, NFP, NSE, GFAP, S-100, Leu-7 and MBP. Therefore, the present study evaluates additional markers, including Ber-H2 (CD30), c-myc and Ki-67, to better characterize cellular proliferation, differentiation and developmental signaling in the human fetal brain across gestational stages. Since fetal brain tissue undergoes rapid growth and active neurodevelopment, we hypothesized that these markers may show physiological expression patterns in normal fetal brain tissue, thereby providing baseline developmental insights. ❑

MATERIALS AND METHODS

Study design and sample collection

This descriptive observational study was conducted in the Department of Anatomy in collaboration with the Department of Obstetrics and Gynaecology of a tertiary care teaching hospital between 2017 and 2019 after approval from the Institutional Ethics Committee (IEC) and Institutional Review Board (IRB).

Brain tissues were obtained from nine aborted human fetuses following therapeutic termination

TABLE 1. Distribution of fetal specimens according to gestational age

Specimen No.	Gestational age (weeks)	Trimester
1	8	First
2	10	First
3	12	First
4	16	Second
5	20	Second
6	24	Second
7	28	Third
8	32	Third
9	36	Third

of pregnancy after obtaining written informed consent from the legal guardians. The fetuses were categorized according to gestational age into first trimester (n=3), second trimester (n=3) and third trimester (n=3) groups (Table 1).

Eligibility criteria

Inclusion criteria comprised therapeutically aborted fetuses with preserved cranial structures; fetuses without gross congenital anomalies; and adequately preserved brain tissue suitable for histological examination.

Exclusion criteria were as follows: autolyzed specimens; fetuses with visible congenital malformations involving the central nervous system; mechanically damaged brain tissue; and inadequate tissue preservation.

Gestational age was determined from obstetric records and fetal biometric parameters.

Histological study

Brain tissue specimens measuring approximately 2 mm were dissected under a dissecting microscope and fixed in 10% formalin for 24 hours. Tissue processing and paraffin embedding were performed using standard histological procedures. Sections of 4–5 μm thickness were stained with Masson's trichrome and reticulin stains for cytological evaluation. Photomicrographs were obtained using a microscope-mounted digital camera.

Immunohistochemical study

For immunohistochemical analysis, 3 mm tissue sections were fixed in 10% neutral buffered formalin for 6–8 hours and processed routinely for paraffin embedding. Sections of 4 µm thickness were prepared on poly-L-lysine-coated slides.

Antigen retrieval was performed using heat-induced epitope retrieval in DAKO Target Retrieval Solution using a pressure cooker method for 20 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for five minutes. Primary antibodies were used as shown in Table 2.

TABLE 2. Primary antibodies used for immunohistochemical study

Antibody	Clone	Source	Dilution
Ki-67	MIB-1	DAKO	1:100
c-myc	Y69	DAKO	1:100
Ber-H2 (CD30)	Ber-H2	DAKO	1:50

Sections were incubated with primary antibodies followed by biotinylated secondary antibody and streptavidin–peroxidase complex using the DAKO Immunoperoxidase Kit on a DAKO AutoStainer platform. Visualization was performed using 3,3′-diaminobenzidine (DAB) chromogen, followed by hematoxylin counterstaining.

Controls

Reactive lymph node tissue served as positive control for CD30 and Ki-67 staining. Negative controls were prepared by omission of the primary antibody.

Immunohistochemical evaluation

Immunostaining was independently evaluated by two observers blinded to gestational age. Semi-quantitative grading was performed according to the percentage of immunoreactive cells:

- 0 = no staining
- 1+ = 1–25%
- 2+ = 26–50%
- 3+ = 51–75%
- 4+ = >75%

Areas evaluated included ventricular zone, cortical plate, intermediate zone, white matter, cerebellar cortex and ependymal lining.

Statistical analysis

As the study was exploratory in nature with a limited sample size, formal inferential statistical analysis was not performed. Observations were described qualitatively and semi-quantitatively. ❑

RESULTS

Histological findings

Masson's trichrome staining demonstrated developing neuronal cells and pyramidal neurons within the cerebral cortex, particularly in later gestational stages (Figure 1). Increasing cellular organization and cortical stratification with advancing gestational age were observed. Scattered oligodendrocytes were identified within the developing white matter.

Reticulin staining revealed sparse reticular fibers during early gestation, with comparatively increased distribution in later stages. Developing neuronal nuclei and immature neural elements were also observed (Figure 2).

Ki-67 immunostaining showed the highest degree of positivity among the markers studied, par-

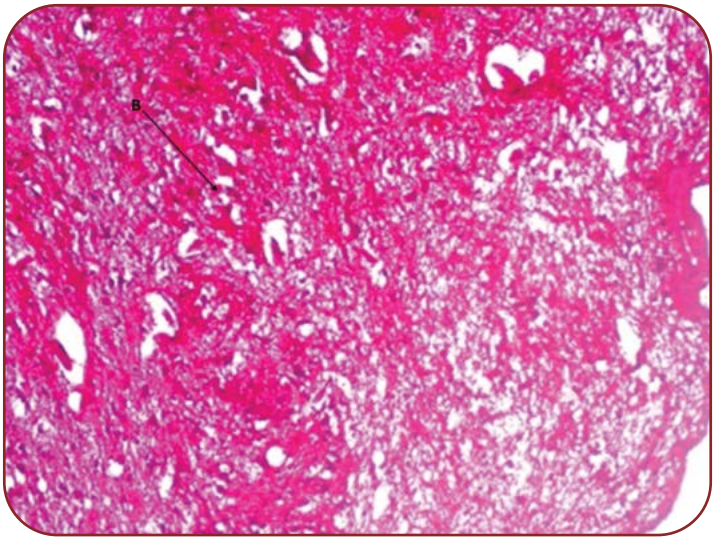

FIGURE 1. Masson's trichrome-stained section of developing cerebral cortex showing pyramidal neurons (arrow) in later gestational stages (20× magnification)

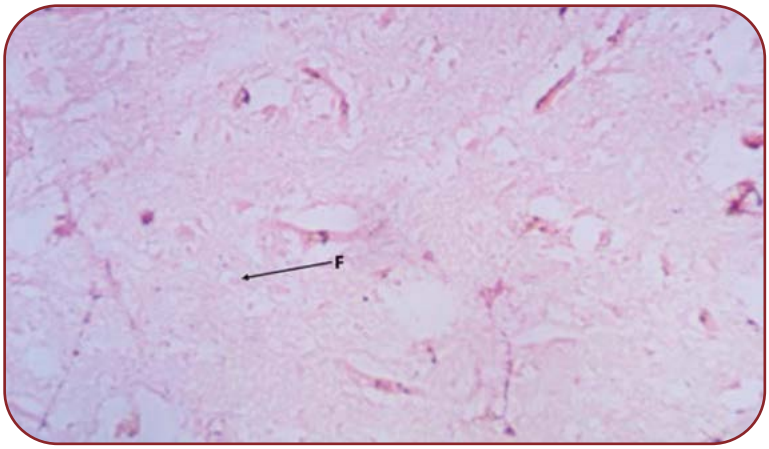

FIGURE 2. Reticulin-stained section showing developing neuronal nuclei and sparse reticular fibers during early gestation (20× magnification)

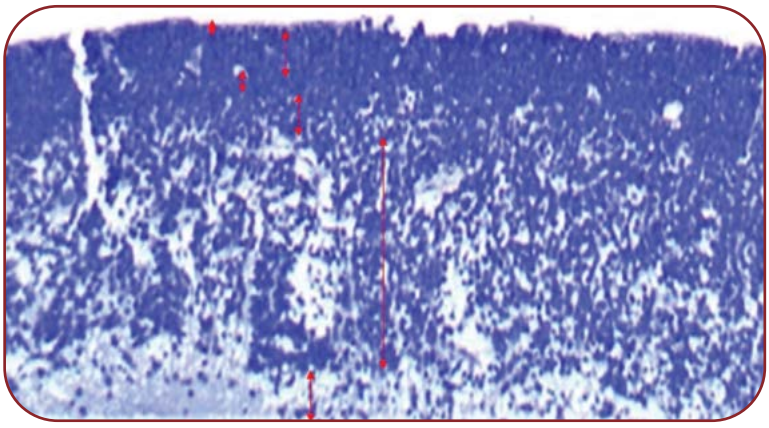

FIGURE 3. Immunohistochemical staining with c-myc antibody demonstrating cortical lamination and immunoreactive cells within the cerebral cortex (4× magnification)

ticularly within proliferative ventricular regions. Increased immunoreactivity was observed with advancing gestational age, especially in the white matter and subventricular zones (Figure 4).

The white matter demonstrated stronger immunopositivity than the granular layers across all gestational groups (Table 3). In the cerebellum, Purkinje cells demonstrated moderate staining intensity. ❑

DISCUSSION

The present study evaluated cytological and immunohistochemical changes in the human fetal brain across different gestational ages using Masson's trichrome staining, reticulin staining and immunohistochemical markers Ki-67, c-myc and Ber-H2 (CD30). Previous studies have demonstrated the importance of histological and immunohistochemical approaches in understanding fetal neurodevelopment and cellular differentiation processes (2, 3).

Histological observations in the present study demonstrated progressive maturation of cortical architecture with advancing gestation. Developing pyramidal neurons and increasing organization of cortical layers became more apparent in later gestational stages. Reticulin staining demonstrated relatively sparse reticular fibers in early gestation, which gradually increased during later developmental stages, suggesting progressive neural organization and maturation. Similar developmental progression of cortical lamination and neuroepithelial maturation has been described by Stagaard and Møllgård (3) and Ulfig *et al* (4).

Among the immunohistochemical markers studied, Ki-67 demonstrated the highest immunopositivity, particularly within ventricular and subventricular proliferative zones. Since Ki-67 is a well-established marker of cellular proliferation, its increased expression in these regions likely reflects active neurogenesis during fetal brain development. The observed increase in immunopositivity with advancing gestational age may correspond to increasing cellular complexity and regional differentiation within the developing brain. These findings are supported by previous developmental studies demonstrating active proliferative activity within fetal neuroepithelial regions during gestation (2, 3).

The expression of c-myc was comparatively moderate and was mainly observed in developing

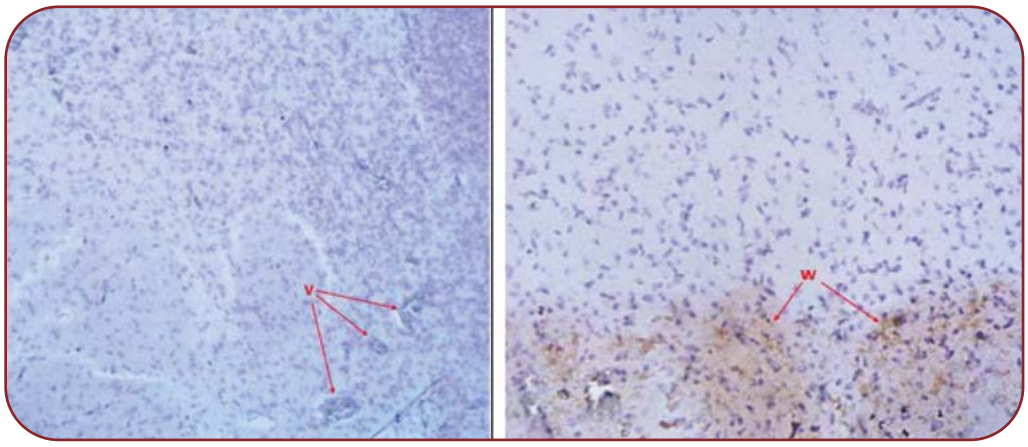

FIGURE 4. Representative photomicrographs showing increased immunopositivity with advancing gestational age, with brown staining indicating antigen expression and Ki-67 demonstrating greater immunoreactivity compared to c-myc and Ber-H2 (CD30)

Marker	First trimester	Second trimester	Third trimester
Ki-67	2+ to 3+	3+	3+ to 4+
c-myc	1+ to 2+	2+	2+ to 3+
Ber-H2 (CD30)	1+	1+ to 2+	2+

TABLE 3. Semi-quantitative immunohistochemical grading across gestational ages

cortical regions. c-myc is known to play an important role in cellular proliferation, differentiation, and developmental signaling. Its expression in the developing cerebral cortex in the present study supports its involvement in neuronal maturation and developmental regulation. Similar observations regarding molecular regulation during fetal brain maturation have been discussed in studies evaluating developmental protein expression in the human fetal brain (6, 7).

Ber-H2 (CD30) demonstrated comparatively lower immunoreactivity than Ki-67 and c-myc. The pattern observed in our study differs partially from the findings of Tamiolakis *et al* (8), who reported stronger CD30 immunoreactivity in earlier gestational stages. These differences may be related to variations in tissue sampling, gestational distribution, fixation protocols, or regional analysis of the fetal brain.

The present findings also demonstrated relatively stronger immunoreactivity within white matter regions compared to granular layers. This may reflect progressive maturation of glial and neuronal elements during fetal development. Previous studies have similarly emphasized the importance of white matter organization and progressive neurodevelopmental maturation during gestation (9).

Unlike previous studies that primarily focused on established developmental markers such as vimentin, GFAP and neurofilament proteins (2-4), the present study evaluated Ki-67, c-myc and Ber-H2 (CD30) together in human fetal brain tissue. Although limited by small sample size, the study provides preliminary baseline observations regarding the expression patterns of these markers during fetal neurodevelopment. ❑

CONCLUSION

The present study demonstrates progressive cytological and immunohistochemical changes in the developing human fetal brain across different gestational ages. Ki-67 showed the highest degree of immunoreactivity, indicating active proliferative activity within ventricular and subventricular regions during fetal neurodevelopment. c-myc and Ber-H2 (CD30) showed comparatively lower but detectable expression patterns.

These findings provide baseline developmental data regarding the expression of proliferation-associated markers in the fetal brain and may contribute to future research involving developmental neuropathology and neuro-oncology. Further studies with larger sample sizes and quantitative analysis are required to validate these observations. ❑

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Conflicts of interest: none declared.

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Ethical approval: Institutional ethical approval for the study protocol and participants' written informed consent were obtained.

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