Original Article

Amyloid Precursor Protein Immunohistochemical Changes in the Occipital Cerebral Cortex of Newborn Mice Affected by Prenatal Ketamine Exposure

Mohanad Suhail Najm[1], Nahidah Ibrahim Hammadi[2], Anas Hamed Musleh, Mahdi Salih Shalal[1]

[1]Department of Anatomy College of Medicine, University of Anbar, [2]Department of Laboratory Clinical Science, College of Pharmacy, University of Anbar, Anbar, Iraq

Abstract

Background: B-amyloid plaque is a lesion in the brain's gray matter, imaging studies show significant Ab aggregation in the occipital lobes. This inflammatory reaction causes neuronal impairment and neurotoxicity. Ketamine, an NMDA receptor blocker used in pediatrics, can cause neuronal death or neurodegeneration during embryonic phases, as animal trials have shown. **Objectives:** The study investigates the effects of therapeutic doses of ketamine on the occipital cerebral cortices of newborn mice to inform scientific decisions on its potential adverse effects. **Materials and Methods:** This experiment involved 60 pregnant female mice from a lab. The mice were divided into control and experimental groups. After birth, the newborn mice were preserved in formalin, and brain tissue was embedded. Immunohistochemical staining was performed on the brain tissue, and the pattern of anti-amyloid precursor protein expression was determined. The results were analyzed using a *t* test and SPSS version 26. **Results:** The study analyzed the immunohistochemical labeling patterns in the occipital cerebral cortex of neonate mice. The control group showed a negative pattern with no visible tissue or cell arrangements. The experimental group showed brown staining and immunohistochemical labeling with varying intensity and shapes. Brown DAP depositions were observed in both superficial and deep layers. The study found a significant difference ($P \leq 0.000$) in strongly positive pixels in the experimental group compared to the control group. **Conclusion:** The study reveals varying amyloid precursor protein reactivity and apoptosis in mice following prenatal ketamine therapy, with β-amyloid accumulation potentially contributing to these changes.

Keywords: Amyloid precursor protein, immunohistochemical, ketamine, occipital cortex

Introduction

B-amyloid (A) plaques are lesions in the brain's gray matter.[1] The accumulation of plaque and neuro-fibrillary tangles is a characteristic of Alzheimer's disease (AD).[2] Recent research suggests that epilepsy and cognitive deficits may also be linked to processes similar to AD.[3] In AD, Ab plaques accumulate in various brain areas, particularly in the cerebral cortices, such as the prefrontal, orbitofrontal, parietal, temporal, and cingulate cortices.[4,5] Imaging studies have shown a significant amount of Ab aggregation in the occipital lobes,[6,7] most likely due to pathological accumulation in the occipital cortices, which might have been interpreted as alterations in primary cortices such as the primary motor and sensory cortices, given the significant and perhaps underappreciated accumulation of Amyloid in the occipital lobes, as well as pathological data indicating the presence of Amyloid plaques in the occipital cortices.[6] The Aβ causes an inflammatory reaction, which most likely begins as a host defensive response to brain tissue breakdown and eventually adds to neuronal impairment. It is neurotoxic, inducing cell damage and death by hyperphosphorylation and significant alterations in tau protein.[8,9] Ketamine is an N-methyl-D-aspartate (NMDA) receptor blocker commonly used as an anesthetic, analgesic, and sedative in pediatric clinical practice. It is also classified as an unlawful substance in most countries.[10,11] The cerebral cortex contains a high concentration of NMDA receptors.[12]

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1750_23

Address for correspondence: Dr. Mohanad Suhail Najm, Department of Anatomy, College of Medicine, University of Anbar, Anbar, Iraq.
E-mail: mohanadsuhail@uoanbar.edu.iq

Submission: 01-Dec-2023 **Accepted:** 14-Feb-2024 **Published:** 30-Apr-2026

How to cite this article: Najm MS, Hammadi NI, Musleh AH, Shalal MS. Amyloid precursor protein immunohistochemical changes in the occipital cerebral cortex of newborn mice affected by prenatal ketamine exposure. Med J Babylon 2026;23:660-5.

Animal trials have demonstrated that exposure to anesthetic drugs during embryonic phases might result in neuronal death or neurodegeneration.[13] Theories of neurodegeneration in AD mostly focus on the toxic consequences of aggregated amyloid peptide oligomers formed by intramembranous proteolysis of the transmembrane protein Amyloid precursor protein (APP).[14]

The purpose of this study was to look at the immunohistochemical beta APP expression in newborn mice occipital cerebral cortices after prenatal exposure to therapeutic doses of ketamine to make scientific decisions about the effects of ketamine on developing brains, which may help to reduce adverse effects.

Materials and Methods

Grouping of animals

The animals involved in this experiment come from an institution that houses lab animals. In this experiment, 60 adult female mice (*Mus musculus*) who were pregnant and between 8 and 12 weeks old were employed. The animals ranged in weight from 20 to 40 g. explores the mouse ovulation cycle. After placing two females and one male in a cage, mating occurs. The day after mating, which was recognized as day 0 of pregnancy, vaginal plugs were discovered, confirming the pregnancy. The National Institutes of Health Guidelines for the Care and Use of Laboratory Animals were followed about all animals. The 60 pregnant mice were separated into control and experimental groups (30 mice in each group). On the (5th day, 10th day, 15th day, and 20 day) of pregnancy, the experimental group's pregnant mice received intraperitoneal injections of 50 mg/kg of ketamine hydrochloride. On the same gestational days, mice in the control group received an intraperitoneal injection of distilled water. Upon birth, neonates were chosen at random from each mother mouse's litter of six to ten young.

Sampling of tissues

The newborn decapitation of head after delivery in one day old by using a scalpel and removed the skin of head by using scissors and forceps. In 1 day old the skull shield is not removed.

Fixation, tissue processing, and sectioning

The newborn brains were fixed in 10% formalin, the fixed tissues were then passed for routine paraffin wax embedding process including dehydration, clearing, infiltration, and embedding.[15] On the surface of hot water (40°C), sections of brain tissue with a thickness of 5 mm were carefully placed. Following collection, the sections were mounted on crisp, powerful positive slides (AFCO).

Immunohistochemical staining

The immunohistochemical staining kits, provided by Abcam, contained rabbit polyclonal antibody to APP clone (ab15272) It is Synthetic peptide corresponding to Human APP aa44-62(N terminal), sequence: (HMNVQNGKWDSDPSGTKTC), and Rabbit specific DAB, (ab64261) detection kit from Abcam.

The sections were examined under light microscope, photographs were taken using a camera.

Statistical analysis

This analysis was done using the Statistical Package for the Social Sciences (SPSS) version 26 provided by IBM.[16] Outcomes are expressed as (mean, SD). An independent *t* test was used to analyze the mean values of a strong positive Pixel Count algorithm obtained by the software application Aperio Image Scope on APP immunohistochemical reactivity in the occipital cortex of neonate mice in both experimental and control groups. The *P* value was considered statistically significant.

Results

Immunohistochemical result of occipital cerebral cortex in control group

A negative pattern of immunohistochemical labeling was discovered during a light microscopic examination of the tissue at both low and high magnification. Unfortunately, the configuration of the occipital cortical tissues could not be seen in the immunohistochemical labeled paraffin sections, and the various forms and arrangements of the cortical cells were concealed as well. Although the hematoxyline dye did manage to successfully stain the nuclei of the cells in the occipital cortex, the cytoplasm, and cellular boundaries were not well identifiable, and the cortical tissue exhibited a homogenous staining intensity [Figure 1].

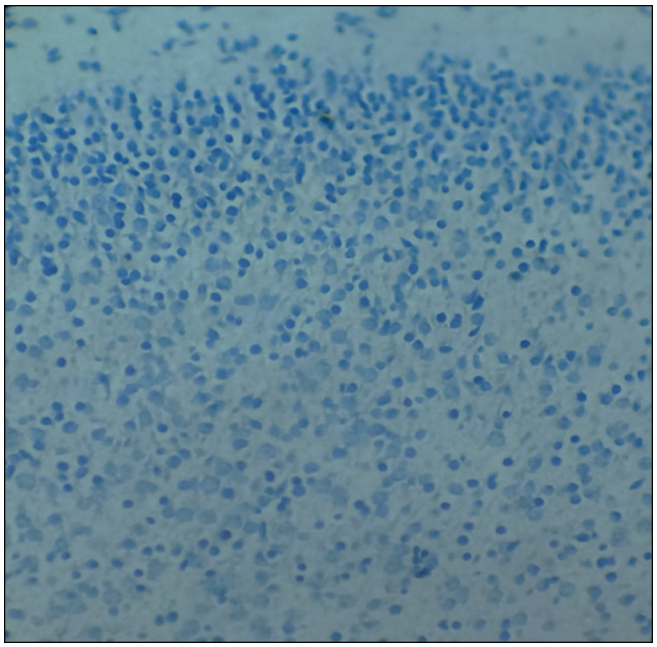

Figure 1: Sagittal paraffin section. Occipital cortex of the neonate mouse of the control group. Immunohistochemical Anti-APP staining (×400)

Immunohistochemical result of occipital cerebral cortex in experimental group

The immunohistochemical labeling and brown staining were observed in the occipital cortex. These brown stains were randomly distributed throughout the extracellular matrix, with varying intensities. The brown DAP depositions varied in shape and size, the accumulations seen in the superficial and deep layers of the occipital cortex. Light microscopic examination revealed a brownish hue throughout the occipital cortex, without any laminar discrimination of staining intensity [Figures 2 and 3].

Statistical analyses of APP immunohistochemical reaction between the occipital cortex of experimental and control group

The mean of strongly positive pixels in occipital cortices of the experimental group is 436,207 ± 282,613 and the mean of strongly positive pixels in the control group is 996.13 ± 823, according to a statistical analysis of the values of the strongly positive pixels obtained from the evaluation of APP immunohistochemical expression on neonate mice occipital cortices of experimental and control groups. There was a statistically significant difference ($P \leq 0.000$) [Table 1] and [Figures 4–6].

Table 1: The mean strong positive pixel obtained by the Aperio Image Scope analysis of the occipital cerebral cortex section of newborn mice

Group	Mean ± SD	*P* value
Experimental group	436,207 ± 282,613	0.000* (significant)
Control group	996.13 ± 823	

*P value ≤ 0.05 considered statistically significant

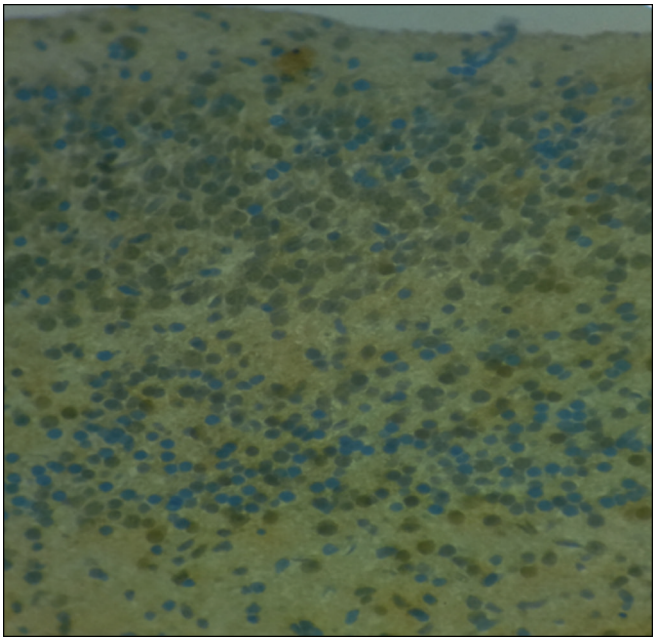

Figure 2: Sagittal paraffin section. Occipital cortex of the neonate mouse of the experimental group. Immunohistochemical Anti-APP staining (×400)

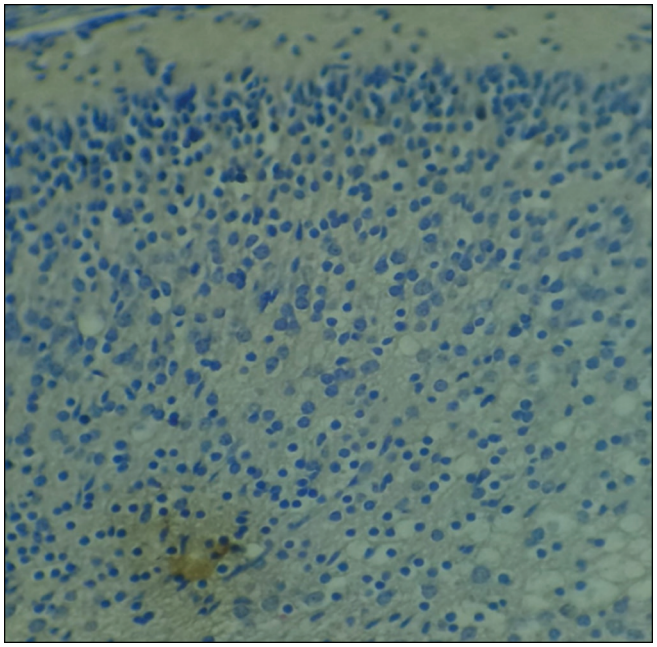

Figure 3: Sagittal paraffin section. Occipital cortex of the neonate mouse of the experimental group. Immunohistochemical Anti-APP staining (×400)

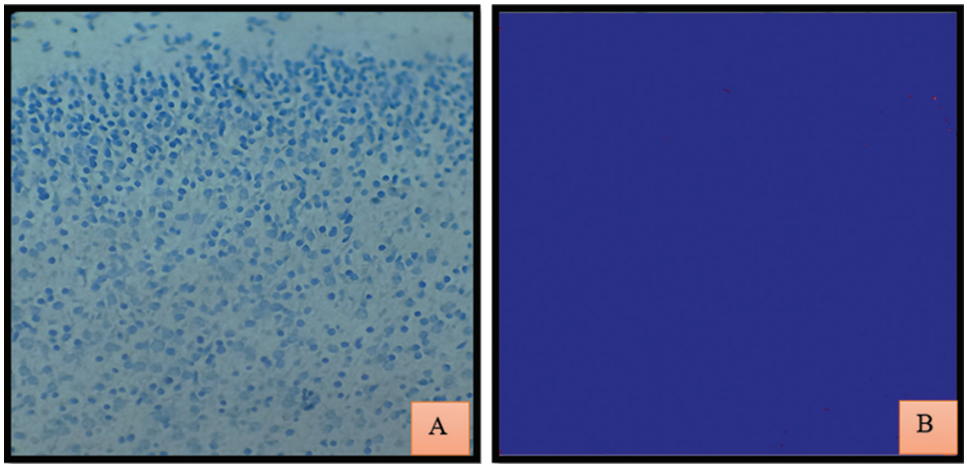

Figure 4: (A) APP reactivity in occipital cerebral cortex of neonate mice from control group. The APP negative stain is seen in all layer of occipital cerebral cortex. ×400. (B) The snap shoot as analyzed by Aperio positive pixel count algorithm

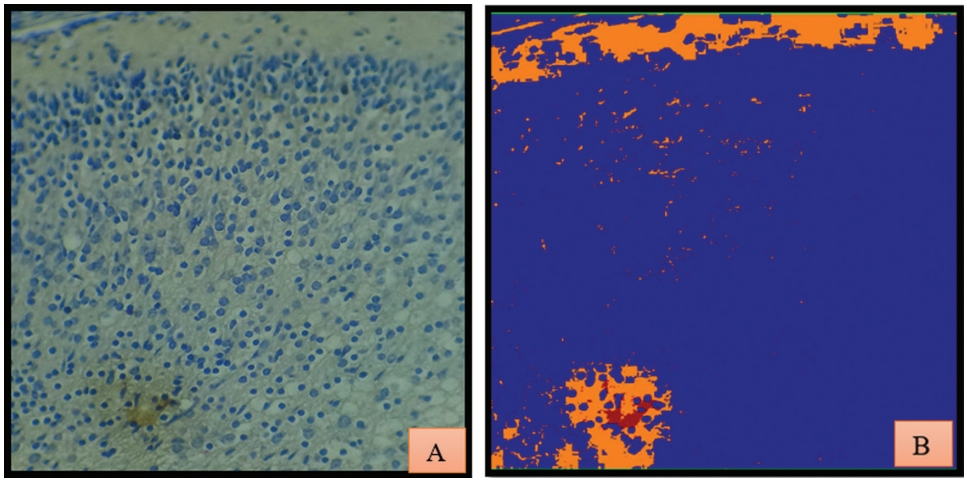

Figure 5: (A) APP reactivity in occipital cerebral cortex of neonate mice from experimental group. APP-positive stain is seen in all layer of occipital cerebral cortex. ×400. (B) The snap shoot as analyzed by Aperio positive pixel count algorithm

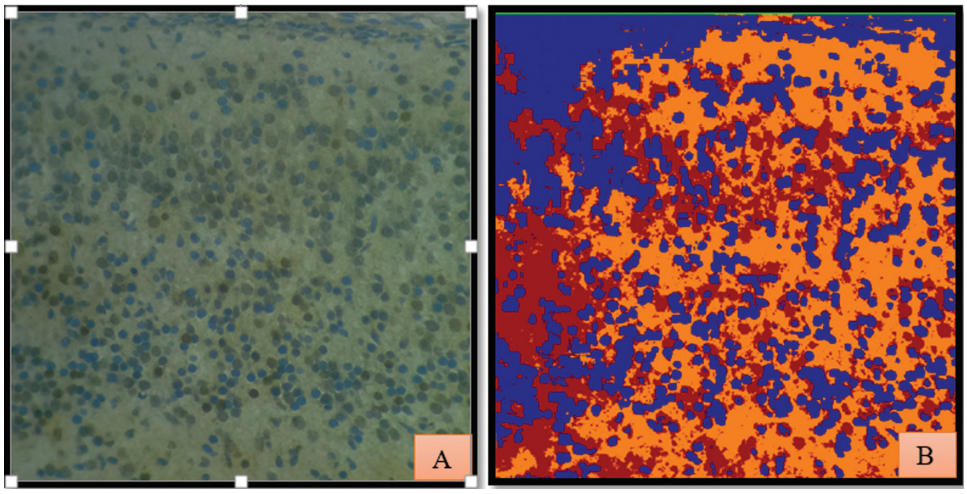

Figure 6: (A) APP reactivity in the occipital cerebral cortex of neonate mice from the experimental APP-positive stain is seen in the layers of occipital cerebral cortex. ×400. (B) The snap shoot as analyzed by Aperio positive pixel count algorithm

Discussion

Developing effective therapeutic techniques or eventually finding a preventative or cure for Alzheimer's disease necessitates a comprehensive strategy that takes into consideration multiple cause hypotheses. Although more study has to be done, a focus on numerous prospective strategies and topics has developed. According to the amyloid cascade theory, the formation of amyloid plaques in the brain is a key event in the etiology of Alzheimer's disease. As a result, contemporary treatment methods, such as the use of Beta-secretase and gamma-secretase inhibitors and immunotherapy targeting amyloid, try to prevent amyloid synthesis or increase amyloid clearance.[14] Another characteristic of Alzheimer's is abnormalities in the Tau protein, including irregular phosphorylation and aggregation. Research has looked at the harmful effects of ketamine on the developing brain, specifically in neonatal mice after prenatal exposure. Studies have shown abnormalities in neuronal apoptosis, synaptic transmission, plasticity, and learning-related activities, as well as visual impairment.[11] The objective of the study was to make scientific judgments to gain better knowledge of the effects of ketamine on the growing brain. This could help reduce negative consequences.

It is essential to note that ketamine toxic dosages had been researched using single repeated dose significance, and nontoxic intraperitoneal dosages were found to be up to twice as high as those used in this study.[17] The cerebral cortex undergoes development during both pregnancy and the early postnatal period. This investigation showed that therapeutic dosages of ketamine have harmful effects on the development of the cerebral cortex. Therefore, it is not recommended to use ketamine during pregnancy.[18] The research proposal has shown that therapeutic doses of ketamine can cause neuronal apoptosis, resulting in a gradual and permanent loss of sensory brain and

cerebellar neurons.[19] Single and short ketamine exposure has also been proven to induce a strong neurotoxic reaction in the developing brain.[20,21] Experimental studies on Ketamine have revealed evidence of its impact on cortical neurogenesis, particularly during the synaptogenesis period. The drug has been found to cause apoptosis, which is a widespread adverse effect observed in multiple brain regions of neonatal rats injected with ketamine. It has been documented that ketamine primarily mediates its apoptotic effects by blocking N-Methyl-D-aspartic acid (NMDA) receptors.[22] It has been reported that the distribution of neuroapoptosis is even in the variable regions of the neonate cerebral cortex when exposed to ketamine. It is more prominent in the temporal cortex (layers II and IV) and the primary visual cortex (layers II and V).[23]

The immunohistochemical APP reactivity showed different intensities in the occipital cortex of the animals involved in this study that were prominently noticeable compared to the control group. This conclusion has been confirmed by the statistical evaluations of the mean values of the strongly positive pixels algorithm found in this study. The animals in the control group displayed a normal and detailed histological architecture with negative and homogenous labeling in all layers of the cortex. However, the immunohistochemical reactivity in the occipital cortex of animals exposed to ketamine during pregnancy was significantly disrupted. The study also discovered a noticeable accumulation of APP in the superficial and deep lamina of the occipital cortex. This finding is consistent with a prior study that found greater tau binding in the superior and inferior occipital cortex, which is linked to visuospatial dysfunction.[24,25] APP is a membrane protein found in the synapses of neurons. It plays a crucial role in regulating synapse formation and repair, particularly in synaptic formation and repair.[26] Studies have shown that exposure to ketamine during the first 2 weeks after birth does not cause cell death in specific cortical layers.[22] This is attributed to the effect of ketamine on the NMDA-dependent maturation of interneurons.[27]

The breakdown of APP produces beta-amyloid (Aβ), which is the primary component of amyloid plaques found in the brains of individuals with Alzheimer's disease,[28] This suggests that additional brain pathology, such as tau accumulation, would be needed to impair visuoperceptual function in Alzheimer's Continuum.[29] It remains unclear whether prenatal exposure to ketamine could lead to pathological consequences from accumulated APP in the cerebral cortex during post-pubertal life.

This study suggests that the accumulation of β-amyloid in the cerebral cortex of mice can contribute to apoptotic manifestations in the cortex, potentially leading to neurodegenerative effects.[30] This accumulation is considered an early sign of Alzheimer's disease and is closely associated with visuospatial and verbal memory dysfunction in certain areas of the brain.[24] Alzheimer's disease is characterized by various histological abnormalities, including neuritic plaques and neuro-fibrillary tangles. The neurodegenerative development of Alzheimer's disease (AD) and other dementias usually happens in old age. The amyloid cascade hypothesis explains the biochemical mechanisms behind these diseases.[31] Some research also suggests that neuroinflammation may play a role in initiating pathology and disease progression.[32] During the cell death process, one stage involves the proteolysis of a membrane glycoprotein called APP, which results in the formation of B-amyloid (Ab). Ab was regarded as an independent factor that caused diseases as the primary toxic agent.[33]

This study found that immunohistochemistry APP reactivity revealed various intensities and morphology in the occipital cortex of the animals used in this investigation. In earlier research, significant A deposition was detected in the occipital lobes of AD patients.[6,34] This decision mimics the features of cerebral cortical apoptosis after prenatal ketamine treatment in mice, in which neurons of the occipital cortex revealed substantial apoptotic alterations linked with increased dendritic branching.[6,35] This study also discovered variations in APP accumulation on the occipital cortex about the susceptibility to ketamine's apoptogenic activity.[6,36] In our previous study, we found that younger patients had more diffuse accumulation and greater atrophy in the occipital area.[6] Findings that were comparable with those of early-onset occipital Amyloid accumulation in the present study.

Conclusion

The study found that the occipital cortex of the animals involved showed different levels of intensity and morphology in the APP reactivity. This was confirmed by statistical evaluations of the mean values of the strong positive pixels algorithm. The characteristics of cerebral cortical apoptosis in mice following prenatal ketamine therapy were also observed, where neurons of the occipital cortex showed significant apoptotic changes associated with increased dendritic branching. Additionally, the study identified differences in APP accumulation on the occipital cortex regarding vulnerability to the apoptogenic action of ketamine. The accumulation of β-amyloid in the cerebral cortex of mice was found to be a possible factor contributing to apoptotic manifestations in the cortex.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Elshazly AM, Sinanian MM, Elimam DM, Zakaria S. Overview of the molecular modalities and signaling pathways intersecting with β-amyloid and tau protein in Alzheimer's disease. Neuroglia 2023;4:191-208.
2. Lopez JAS, González HM, Léger GC. Alzheimer's disease. Handb Clin Neurol 2019;167:231-55.
3. Aroor A, Nguyen P, Li Y, Das R, Lugo JN, Brewster AL. Assessment of tau phosphorylation and β-amyloid pathology in human drug-resistant epilepsy. Epilepsia Open 2023;8:609-22.
4. Kurz C, Walker L, Rauchmann BS, Perneczky R. Dysfunction of the blood–brain barrier in Alzheimer's disease: Evidence from human studies. Neuropathol Appl Neurobiol 2022;48:e12782.
5. Therriault J, Lussier FZ, Tissot C, Chamoun M, Stevenson J, Rahmouni N, *et al*. Amyloid beta plaque accumulation with longitudinal [18F] AZD4694 PET. Alzheimers Dementia (Amst) 2023;15:e12391.
6. Hwang J, Kim CM, Kim JE, Oh M, Oh JS, Yoon YW, *et al*. Clinical implications of amyloid-beta accumulation in occipital lobes in Alzheimer's continuum. Brain Sci 2021;11:1232.
7. Agarwal A, Gupta V, Brahmbhatt P, Desai A, Vibhute P, Joseph-Mathurin N, *et al*. Amyloid-related imaging abnormalities in Alzheimer disease treated with anti–amyloid-β therapy. Radiographics 2023;43:e230009.
8. Ułamek-Kozioł M, Czuczwar SJ, Januszewski S, Pluta R. Proteomic and genomic changes in tau protein, which are associated with Alzheimer's disease after ischemia-reperfusion brain injury. Int J Mol Sci 2020;21:892.
9. Ariafar S, Makhdoomi S, Mohammadi M. Arsenic and Tau Phosphorylation: A mechanistic review. Biol Trace Elem Res 2023;201:5708-20.
10. Yuan J, Zhang Y, Yang H, Cao S, Luo Y, Yu T. Astrocytes are involved in the effects of ketamine on synaptic transmission in rat primary somatosensory cortex. J Integr Neurosci 2023;22:116.
11. Dong C, Anand K. Developmental neurotoxicity of ketamine in pediatric clinical use. Toxicol Lett 2013;220:53-60.
12. Suzuki Y, Nakamoto C, Watanabe-Iida I, Watanabe M, Takeuchi T, Sasaoka T, *et al*. Quantitative analysis of NMDA receptor subunits proteins in mouse brain. Neurochem Int 2023;165:105517.
13. Bleeser T, Brenders A, Hubble TR, Van de Velde M, Deprest J, Rex S, *et al*. Preclinical evidence for anaesthesia-induced neurotoxicity. Best Practice Res Clin Anaesthesiol 2023;37:16-27.
14. Nasb M, Tao W, Chen N. Alzheimer's disease puzzle: Delving into pathogenesis hypotheses. Aging Dis 2023;15:43-73.
15. Al-Alawchi MSSF, Alkafaji HAR. Evaluation of immunohistochemical expression of topoisomerase II alpha protein in patients with breast cancer and its correlation with different prognostic factors. Med J Babylon 2022;19:210-8.
16. Sadiq ZA, Hussein HK. Assessment of knowledge and attitudes among pregnant women's towards folic acid intake during pregnancy in a sample of women attending primary health care centers in Babylon province. Med J Babylon 2022;19:142-51.
17. Xu K, Lipsky RH. Repeated ketamine administration alters N-methyl-D-aspartic acid receptor subunit gene expression: Implication of genetic vulnerability for ketamine abuse and ketamine psychosis in humans. Exp Biol Med 2015;240:145-55.
18. Zhao T, Li Y, Wei W, Savage S, Zhou L, Ma D. Ketamine administered to pregnant rats in the second trimester causes long-lasting behavioral disorders in offspring. Neurobiol Dis 2014;68:145-55.
19. Rudin M, Ben-Abraham R, Gazit V, Tendler Y, Tashlykov V, Katz Y. Single-dose ketamine administration induces apoptosis in neonatal mouse brain. J Basic Clin Physiol Pharmacol 2005;16:231-43.
20. Sarić N, Hashimoto-Torii K, Jevtović-Todorović V, Ishibashi N. Nonapoptotic caspases in neural development and in anesthesia-induced neurotoxicity. Trends Neurosci 2022;45:446-58.
21. Straiko MM, Young C, Cattano D, Creeley CE, Wang H, Smith DJ *et al*. Lithium protects against anesthesia-induced developmental neuroapoptosis. Am Soc Anesthesiol 2009;110:862-8.
22. Najm MS, Mubarak HJ, Mohammed LH. Amyloid precursor protein immunohistochemical changes in the newborn mice frontal and parietal cerebral cortices affected by prenatal exposure to ketamine. Iraqi J Med Sci 2018;16:191-200.
23. Neudecker V, Xu J, Thomas MA, Penberthy KK, Kang E, Berg DA, *et al*. An update on preclinical research in anesthetic-induced developmental neurotoxicity in nonhuman primate and rodent models. J Neurosurg Anesthesiol 2023;35:104-13.
24. Cho H, Choi JY, Lee SH, Lee JH, Choi Y-C, Ryu YH, *et al*. Excessive tau accumulation in the parieto-occipital cortex characterizes early-onset Alzheimer's disease. Neurobiol Aging 2017;53:103-11.
25. Lin X, Fan Y, Zhang F, Lin Y. Cerebral microinfarct is emergency consequence of Alzheimer's disease: A new insight into development of neurodegenerative diseases. Int J Biol Sci 2022;18:1569-79.
26. Peppercorn K, Kleffmann T, Hughes SM, Tate WP. Secreted amyloid precursor protein alpha (sAPPα) regulates the cellular proteome and secretome of mouse primary astrocytes. Int J Mol Sci 2023;24:7165.
27. Furuie H, Yamada M. Neonatal blockade of NR2A-containing but not NR2B-containing NMDA receptor induces spatial working memory deficits in adult rats. Neurosci Res 2022;176:57-65.
28. O'brien RJ, Wong PC. Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci 2011;34:185-204.
29. Ossenkoppele R, Zwan MD, Tolboom N, van Assema DM, Adriaanse SF, Kloet RW, *et al*. Amyloid burden and metabolic function in early-onset Alzheimer's disease: Parietal lobe involvement. Brain 2012;135:2115-25.
30. Bok J, Ha J, Ahn BJ, Jang Y. Disease-modifying effects of non-invasive electroceuticals on β-amyloid Plaques and Tau Tangles for Alzheimer's disease. Int J Mol Sci 2022;24:679.
31. Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, *et al*. The amyloid-β pathway in Alzheimer's disease. Mol Psychiatry 2021;26:5481-503.
32. Zhang W, Xiao D, Mao Q, Xia H. Role of neuroinflammation in neurodegeneration development. Signal Transduct Target Ther 2023;8:267.
33. Larson ME, Lesné SE. Soluble Aβ oligomer production and toxicity. J Neurochem 2012;120:125-39.
34. JackCRJr, Lowe VJ, Senjem ML, Weigand SD, Kemp BJ, Shiung MM, *et al*. 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. Brain 2008;131:665-80.
35. Zhao T, Li C, Wei W, Zhang H, Ma D, Song X, *et al*. Prenatal ketamine exposure causes abnormal development of prefrontal cortex in rat. Sci Rep 2016;6:26865.
36. Shuping JL, Matthews DC, Adamczuk K, Scott D, Rowe CC, Kreisl WC, *et al*. Development, initial validation, and application of a visual read method for [18F] MK-6240 tau PET. Alzheimers Dement 2023;9:e12372.