Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

NSE and GFAP expression in the brain of clinically normal ducks

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Article information

Article history:
Received July 1, 2025
Accepted October 30, 2025
Published July 5, 2026

Keywords:
NSE
GFAP
Brain
Neuronal Injury

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Abstract

The brain of ducks is highly susceptible to different health issues that might not show symptoms immediately. Both Neuron-Specific Enolase (NSE) and Glial Fibrillary Acidic Protein (GFAP) are considered as promising biomarkers for unmasking of neuronal diseases. Due to the lack of information about histopathological changes alongside NSE and GFAP expression in ducks during normal conditions, the current study's aim was to explore these potential changes in the brain tissue. Therefore, a total number of fifteen adult healthy ducks of both sexes were collected from local markets in Mosul city during the period from February to April 2024. Brain samples were collected and underwent to full post-mortem investigation, routine histological evaluation, and immunohistochemistry for protein expression of NSE and GFAP. Results showed absence of gross changes in the examined brain tissues. However, microscopic examination revealed diverse pathological changes at different severity scores in particular vacuolar degeneration, inflammation, edema, gliosis and Sarcocystis parasitic infection. Besides, both NSE and GFAP were expressed in different intensities in the brain tissue. Nonetheless, NSE severe expression was observed in seven samples (46.7%), followed by moderate expression in five samples (33.3%), and mild expression in only three samples (20%). Surprisingly, GFAP exhibited negative expression in only one brain sample (6.7%), followed by four samples (26.7%) which exhibited moderate expression. Notably, both mild and severe expressions were seen in the same percentage (33.3%). We conclude that healthy ducks can exhibit histopathological changes and NSE exhibited more intense expression than GFAP in the absence of induced-brain damage.

DOI: 10.33899/ijvs.2025.162243.4382,

Introduction

Research on the nervous system of avian species can encounter some difficulties due to the lack of knowledge regarding this vital system as a result of continuous scientific challenges, unavailability of specific laboratory tests, unspecified clinical signs and the data obtained are still limited in comparison with the massive data available from mammalian systems (1,2). The well-being of avian's brain can be impacted by internal and external conditions. Examples of internal conditions include infectious diseases, hereditary disorders, inadequate nutrition and metabolic disturbances (3,4). On the contrary, the external conditions involve all the conditions related to the surrounding environment of the bird as exposure to different types of contaminants (pesticides and fertilizers), long-term stress which has a negative effect on the immune system leading to increase the susceptibility to infections, conditions related to the birds' behaviour as extreme self-grooming. In addition, ingestion of contaminated food that can cause poisoning or enhancing the transmission of certain food-borne microorganisms (5,6).

The brain which is part of the central nervous system (CNS), is comparatively a complicated organ and is one of

the main target organs of many disorders that are caused by countless causative agents (7,8). Several elements constitute this sophisticated structure such as neurons, glial cells, and their fibers, controlling the regular activity of the body (9). Any injury to these crucial elements results in releasing of neurological protein biomarkers extracellularly. Measurement of these proteins is applicable in detection and assessment of the damage that occurs in the CNS. Numerous proteins are distinctive for a specific cell type and selectively expressed in the nervous system. Consequently, increased expression of these proteins gives an impression which cell type is affected by the disease process (10).

One of these neurological proteins is Neuron-Specific Enolase (NSE) which was identified by Moore and McGregor in 1965 (11). NSE is a glycolytic enzyme which can be measured in both the cerebrospinal fluid (CSF) and blood (12,13), and it is a beneficial biological marker expressed in neuronal bodies, axons, and neuroendocrine tissues. This protein plays a binary role in neurodegenerative disorders through promoting the inflammation and protection of neurons. High levels of NSE contribute in the degradation of extracellular matrix (ECM) and increase the number of inflammatory glial cells. Hence, influencing on the activated microglia and macrophages and stimulating their migration to the site of damage leading to necrosis of neurons (14).

Alternatively, astrocytes are a cluster of glial cells that have characteristic shapes and functions that can be different depending on their location in the brain. Some of the functions that are dedicated to these cells include formation of new neurons and nerve synapses, regulation of blood-brain barrier (BBB) permeability, preservation of extracellular homeostasis. Furthermore, astrocytes support neurons and have the ability to proliferate through the life-time regardless of the age status. This feature makes them an ideal goal for the treatment of CNS diseases (15). It's expected that dysfunctions of these cells can lead to a wide range of pathological conditions of neurons. Brain disorders are characterized by the effective inflammatory response of astrocytes, which is defined by the overexpression of Glial fibrillary acidic protein (GFAP) (16-18).

GFAP is an intermediate filament-III protein which was discovered in by Lawrence Eng and colleague1969 (19). It is exclusively found in astrocytes of the central nervous system, non-myelinated Schwann cells of the peripheral nervous system (PNS) and mature glial cells of the enteric nervous system (ENS) (20,21). The absolute function of GFAP is to stimulate the growth of neurons, myelination and maintain the mechanical strength of astrocytes (22). In addition, this protein plays a role in other functions related to astrocytes such as activity, plasticity, growth of axons, balance of ion and water transport and post-damage glial reaction (23). Expression of this gene has gained reasonable attention for many reasons. Firstly, it is considered as an indicator for astrocytes development. Secondly, the amplification of this gene is considered a marker for active gliosis. Also, the dominance of this gene in astrocytes serves as a tool for their genetic handling (24).

A great attention has been paid to avian neurological diseases these days with respect to expression of certain neurological biomarkers. Thus, it was decided in the current study to identify and describe all the possible histopathological changes that affect the brain tissue as well as the expression of NSE and GFAP in clinically normal ducks in Mosul city.

Materials and methods

Ethical approval

This research was undertaken in Veterinary Teaching Hospital, College of Veterinary Medicine, University of Mosul, Iraq. Institutional Animal Care and Use Committee approved the research proposal on 07/02/2024 which held the reference number UM.VET.2024.115.

Samples collection

A total of fifteen (n=15) adult local ducks from both sexes were purchased from local markets in Mosul city. All birds were clinically healthy and brain tissue samples were collected during February to April 2024. Brain samples were immediately collected after extermination and a full post-mortem inspection was carried out to uncover any macroscopic alterations. Grossly, all samples did not show any obvious changes.

Histopathological investigation

Brain tissue samples were harvested from the dissected birds and fixed in 10% neutral buffered formalin at room temperature for at least 72 hours before proceeding to the subsequent step. Dehydration of the samples was performed by immersing the tissues in ascending concentrations of ethyl alcohol (70%, 90% and 100%). After this, tissue samples were cleaned using xylene, embedded in paraffin wax, and then poured into blocks. After a period of time, a rotary microtome was used to make continuous slices in a thickness of 4-5µm. Then, these slices were accumulated on glass slides for the consequent staining step. Deparaffinization was done on several mounted slides which were stained with Harris Hematoxylin and Eosin (H and E) following basic protocols. At the same time other slides were employed for the immunohistochemistry procedure. Histopathological examination was conducted by two professional pathologists using a digital image camera (2.0 USB, Omax ToupView 9.0-Megapexil, China) which was supplied with image processing software. Calibration of the software was performed to all lenses of Microscope-Olympus-CX31 by the aid of 0.01mm stage micrometer (ESM-11/ Japan) (25-28).

Immunohistochemistry analysis

In order to detect NSE and GFAP solely in the brain tissue, immunohistochemistry technique (IHC) was employed. The immunostaining of NSE was carried out using a FLEX monoclonal NSE [Dako, Clone BBS/NC/VI-H14, Code: IR612], Ready-to-Use (Link). For GFAP detection, a FLEX polyclonal anti-GFAP [Code: IR524], Ready-to-Use (Link) was utilized in conjunction with the Dako EnVision FLEX detection system and autostainer Link. For deparaffinization and rehydration of tissue sections (pre-treatment step), the Dako PT Link machine was used for this purpose. Subsequently, heat-induced epitope retrieval (HIER) was performed by using the EnVisionTM FLEX target retrieval solution at high pH (Dako, DM828) for a duration of 20-40 minutes at 97°C. After removing the slides from the PT Link, washing with EnVisionTM FLEX wash buffer was performed for a duration of 1-5 minutes. Following this, slides were arranged in the pre-programmed Autostainer Link machine utilizing EnVisionTM FLEX DAB-Chromogen (Dako, DM827), and an aliquot of 100µ of primary antibodies (Ready-to-Use) was applied to the tissue sections. After washing with the buffer, an additional 100µ of EnVisionTM FLEX HRP conjugate (Dako, SM802) was introduced. Dehydration, clearing, and permanent mounting were conducted, in accordance with the manufacturer's recommendations.

Scoring of histopathological lesions

Histopathology scoring system provides a semi-quantitative framework to assess the severity and frequency of lesions observed in tissue samples based on the study data (15 brain samples with various histopathological lesions). Each lesion is scored based on the severity and distribution in the brain (29,30) (Tables 1).

Immunohistochemistry scoring system (Semi-quantitative)

IHC scoring system of NSE and GFAP was performed depending on widely used methods in experimental pathology. Evaluation of the staining is based on intensity and distribution (percentage of positive cells) as the categorical scoring from 0 to 3 (31) (Table 2).

Statistical analysis

IHC expression of NSE and GFAP in the brain tissues of examined ducks was scored on a four-point ordinal scale: negative (score 0), mild (score 1), moderate (score 2), and severe (score 3). Frequencies and percentages of ducks in each expression category were calculated for both markers. Comparative analysis between the distribution of NSE and GFAP expression scores was performed using Fisher's Exact Test which is appropriate for small sample sizes (n=15 per group) and categorical data with expected frequencies below five in some cells. Statistical testing was conducted using Sigma Plot software, version 12.5.

Table 1: Histopathology scoring system for duck's brain lesions as scoring criteria

Score	Severity	Description
0	Absent	No lesion detected
1	Mild	Focal involvement (1-2 foci) affecting <10% of tissue
2	Moderate	Multifocal involvement affecting 10–40% of tissue
3	Severe	Diffuse or extensive involvement affecting >40% of tissue

Table 2: IHC Scoring Scale for NSE and GFAP Expression

Score	Expression Level	Description
0	Negative	No detectable staining in tissue
1	Mild Positive	Weak staining in <25% of cells
2	Moderate Positive	Moderate staining in 25–50% of cells
3	Strong/Severe Positive	Strong staining in >50% of cells

Results

Histopathological changes

All the histopathological changes observed in this study are summarized in Table 3. Disturbances in cell metabolism and cell death exhibited high incidence rate for vacuolar degeneration which was 46.7% (Figure 1A-D) followed by apoptosis (40%) (Figure 3A), necrosis (26.7%) (Figure 2A and B, Figure 3B). However, the lowest percentage was for amyloid deposition (6.7%). In terms of cell adaptations, neuronal atrophy was observed only in two samples (13.3%), while neuronal hyperplasia and hypertrophy were obvious only in one sample for each lesion (6.7%).

On the other hand, circulatory disturbances revealed a wide range of differences wherein congestion (Figure 3D) and edema (Figure 1A-D and Figure 3A, C and D) were the highest in a percentage of 46.7%. Whereas the lowest percentage was for both hyperemia and hemorrhage (Figure 3C) (13.3% and 6.7, respectively). Injury of neurons was also noticed which was represented by satellitosis (Figure 1B) and neuronophagia in a percentage of 40% and 26.7%, respectively.

Several types of inflammations were detected in the examined tissues. For instance, chronic inflammations were seen in a percentage of 20% of the examined samples. Gliosis in a percentage of 13.3%, acute inflammation (Figure

2C and D) and viral inclusions in a percentage of 6.7% each. Surprisingly, bradyzoites of Sarcocystis were seen in seven samples (46.7%) (Figure 2A and B). For pigmentation disturbance, only one sample showed Lipofuscin pigmentation (6.7%).

Scoring of histopathology

The majority of samples showed mild lesions (33.3%) followed by moderate severity (26.7%). Severe lesions were seen in a percentage of 20%. Likewise, only 20% of the samples did not show any pathological changes (Table 4).

Table 3: Histopathological changes as pathological categorization, number of affected samples and rate of incidence in brain tissue of ducks

Pathological Category	Lesion	Number of affected samples	Incidence rate (%)
Disturbances in cell metabolism and cell death	Vacuolar degeneration	7	46.7
Cell adaptations	Apoptosis	6	40
	Necrosis	4	26.7
	Amyloid deposition	1	6.7
	Neuronal atrophy	2	13.3
	Neuronal hyperplasia	1	6.7
	Neuronal hypertrophy	1	6.7
	Congestion	7	46.7
	Edema	7	46.7
	Hyperemia	2	13.3
	Hemorrhage	1	6.7
Neuronal injury	Satellitosis	6	40
	Neuronophagia	4	26.7
	Chronic inflammation	3	20
	Gliosis	2	13.3
	Acute inflammation	1	6.7
	Viral inclusion bodies	1	6.7
	Granulomas	0	0
Parasitic infestation	Sarcocystis	7	46.7
Disturbances of pigmentation	Lipofuscin pigmentation	1	6.7

Total number of examined samples =15.

Table 4: Severity scores of the pathological changes in brain tissue of ducks

Severity Scores	No Lesions 0	Mild Lesions 1	Moderate Lesions 2	Severe Lesions 3
Sample 1		X		
Sample 2			X	
Sample 3				X
Sample 4				X
Sample 5		X		
Sample 6			X	
Sample 7			X	
Sample 8	X			
Sample 9				X
Sample 10		X		
Sample 11	X			
Sample 12		X		
Sample 13	X			
Sample 14			X	
Sample 15		X		
Total	3	5	4	3
Percentage (%)	20%	33.3%	26.7%	20%

Total number of examined samples =15.

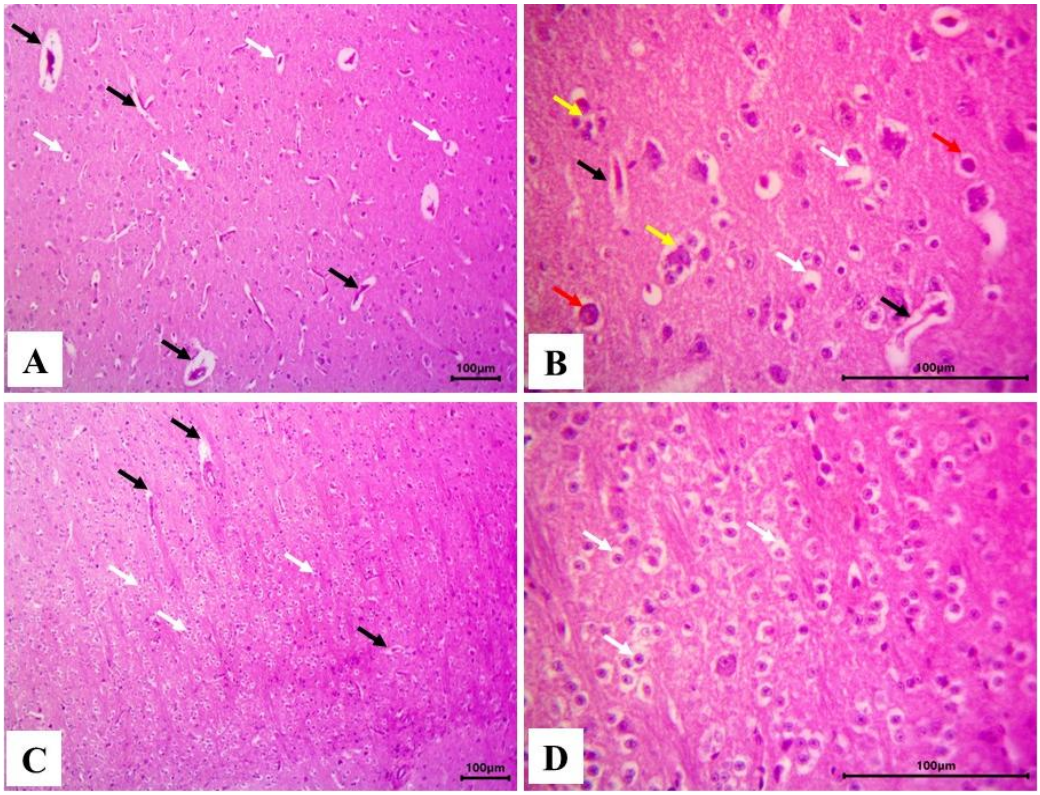

Figure 1: Histopathological findings in the brain tissue of examined ducks. A. Shows mild vacuolation (white arrows), moderate perivascular edema (black arrows). B. Shows moderate vacuolation (white arrows), mild perivascular edema (black arrows), mild cytogenic edema (red arrows) and mild satelliosis (yellow arrows). C. Shows severe vacuolation (white arrows), moderate perivascular edema (black arrows). D. Higher magnification of C. Stain: HE, scale bar=100µm.

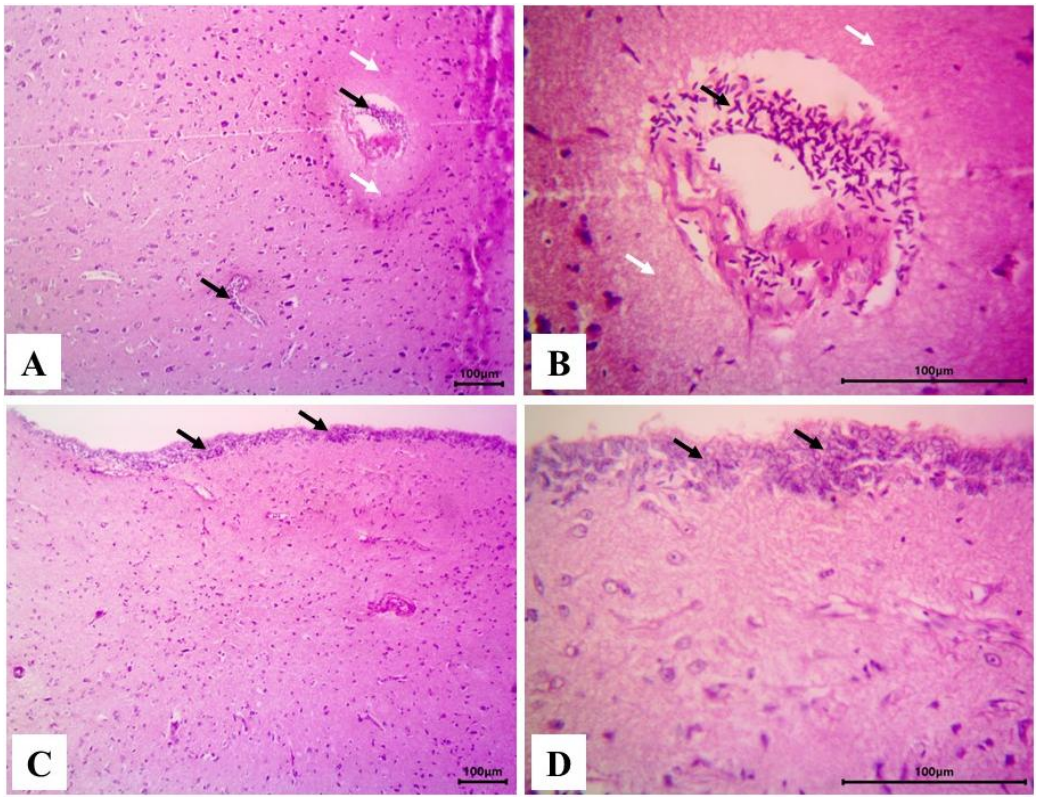

Figure 2: Histopathological findings in the brain tissue of examined ducks. A. Shows zone of liquefactive necrosis (white arrows) and parasitic infection with Sarcocystis (black arrows). B. Higher magnification of A. C. Shows meningitis represented by thickening and infiltration of inflammatory cells (black arrows). D. Higher magnification of C. Stain: HE, scale bar=100µm.

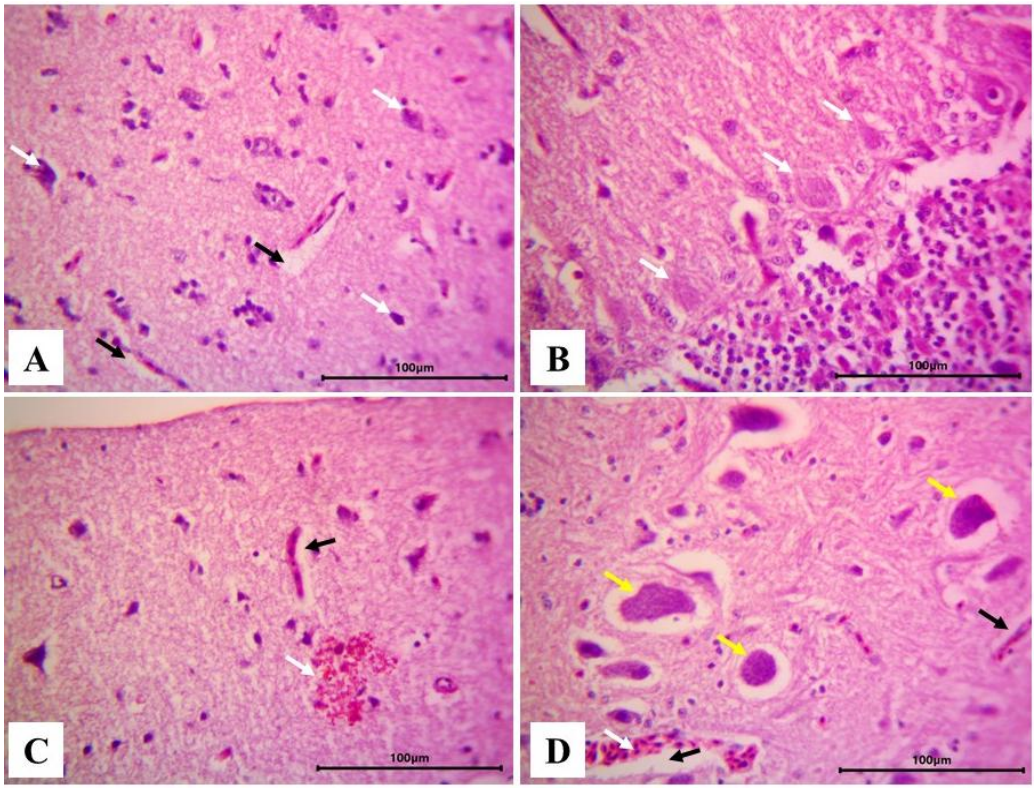

Figure 3: Histopathological findings in the brain tissue of examined ducks. A. Shows perivascular edema (black arrows) and apoptosis (white arrows). B. Shows necrosis of Purkinje cells (white arrows). C. Shows perivascular edema (black arrows) and hemorrhage in the cortex (white arrows). D. Shows perivascular edema (black arrows), congestion of blood vessel (white arrow) and cytogenic edema around ganglia (yellow arrows). Stain: HE, scale bar=100µm.

Expression of NSE and GFAP and scoring of immunohistochemistry in the brain tissue

IHC technique was performed to detect both NSE and GFAP expression in the brain tissue of examined ducks. All samples showed positive cytoplasmic expression for NSE in different scores and parts within the brain including the cerebral cortex and cerebellum. The highest score was for severe expression which was observed in seven samples (46.7%), followed by moderate expression which was seen in five samples (33.3%). For the mild expression, only three samples (20%) exhibited this type of score (Table 5), Figures 4 and 5.

Table 5: Scores of NSE expression in the brain tissue of examined ducks

Scores	IHC Marker	
	NSE	Percentage
Negative Expression 0	0	0%
Positive Mild Expression 1	3	20%
Positive Moderate Expression 2	5	33.3%
Positive Severe Expression 3	7	46.7%

On the other hand, GFAP expressed in the cytoplasm and in different intensities as well as in different parts through the brain tissue such as the cerebral cortex, neuropil, granular cell layer and Purkinje cells. Surprisingly, only one brain sample (6.7%) exhibited negative expression for GFAP, followed by four samples (26.7%) which exhibited moderate expression. Notably, both mild and severe expressions were seen in the same percentage (33.3%) in the examined tissue sections (Table 6), Figures 6 and 7.

Table 6: Scores of GFAP expression in the brain tissue of examined ducks

Scores	IHC Marker	
	GFAP	Percentage
Negative Expression 0	1	6.7%
Positive Mild Expression 1	5	33.3%
Positive Moderate Expression 2	4	26.7%
Positive Severe Expression 3	5	33.3%

For comparative analysis, Fisher's Exact Test was used to determine the difference in the expression intensity between NSE and GFAP. NSE demonstrated significant moderate and severe positive expressions in comparison with GFAP (33.3%) and (46.7%), respectively. On the other hand, GFAP exhibited significant negative and mild expressions compared with NSE (6.7%) and (33.3%), respectively (Table 7).

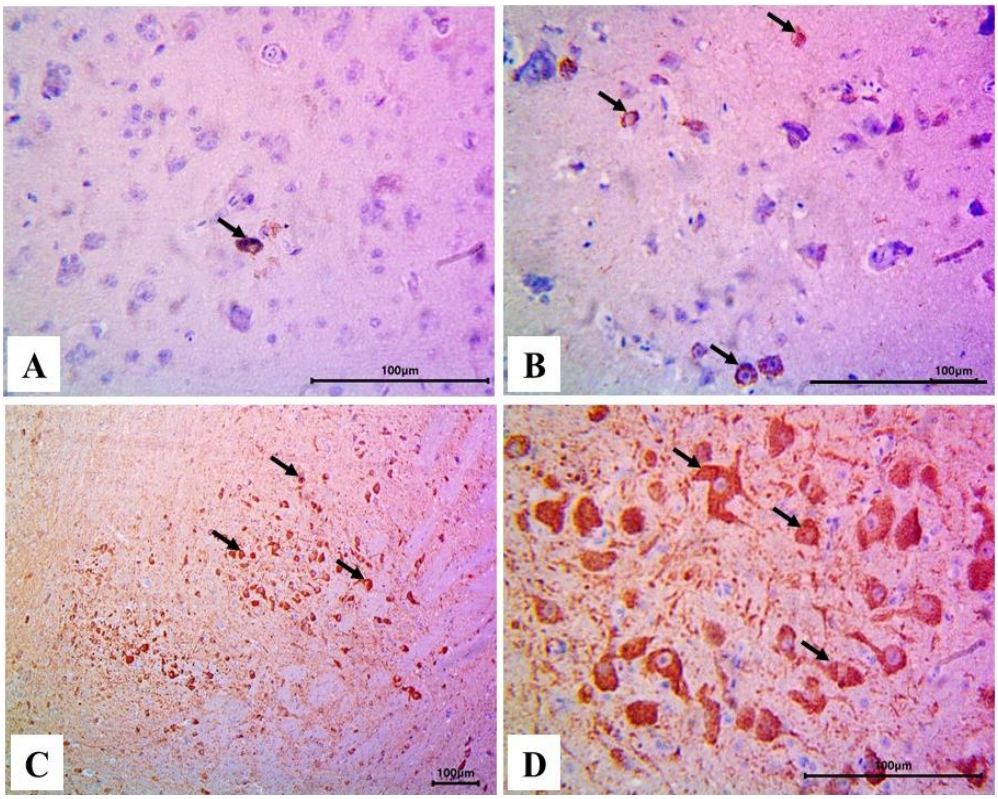

Figure 4: Immunohistochemical examination of NSE expression (cytoplasmic pattern) in the cerebral cortex of brain tissue of examined ducks. A. and B. Mild expression. C. Severe expression. D. High magnification of C. Black arrows indicate the positive expression, scale bar=100µm.

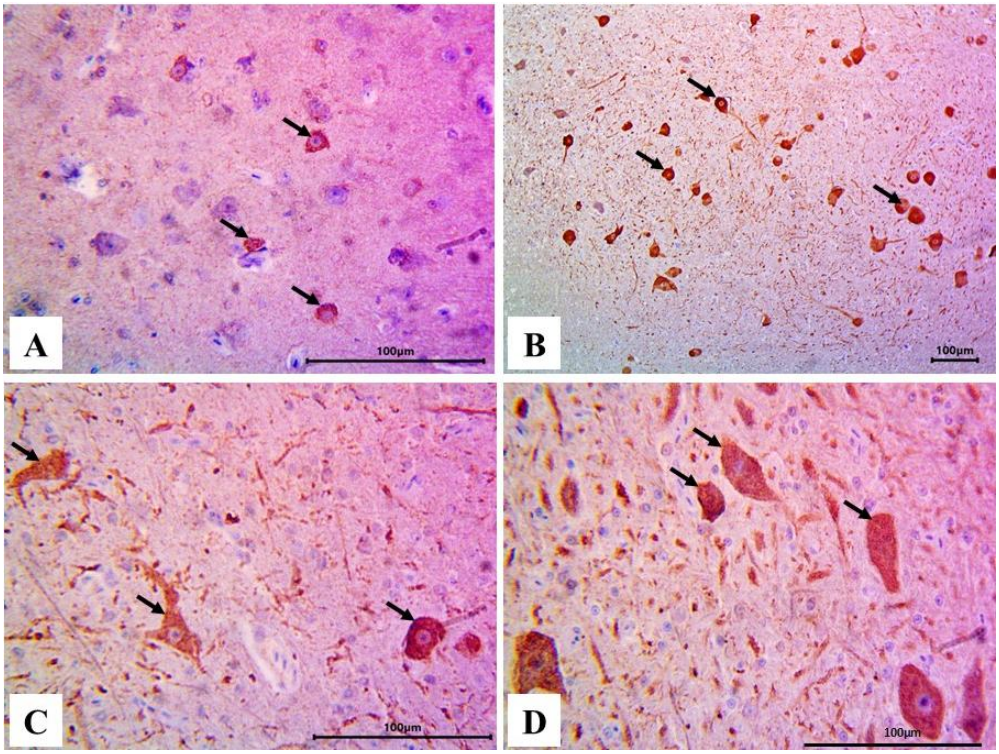

Figure 5: Immunohistochemical examination of NSE expression (cytoplasmic pattern) in different parts of the brain tissue of examined ducks. A. Mild expression in the cortex. B. Severe expression in the cerebellum. C. and D. Moderate expression in the cerebellum. Black arrows indicate the positive expression, scale bar=100µm.

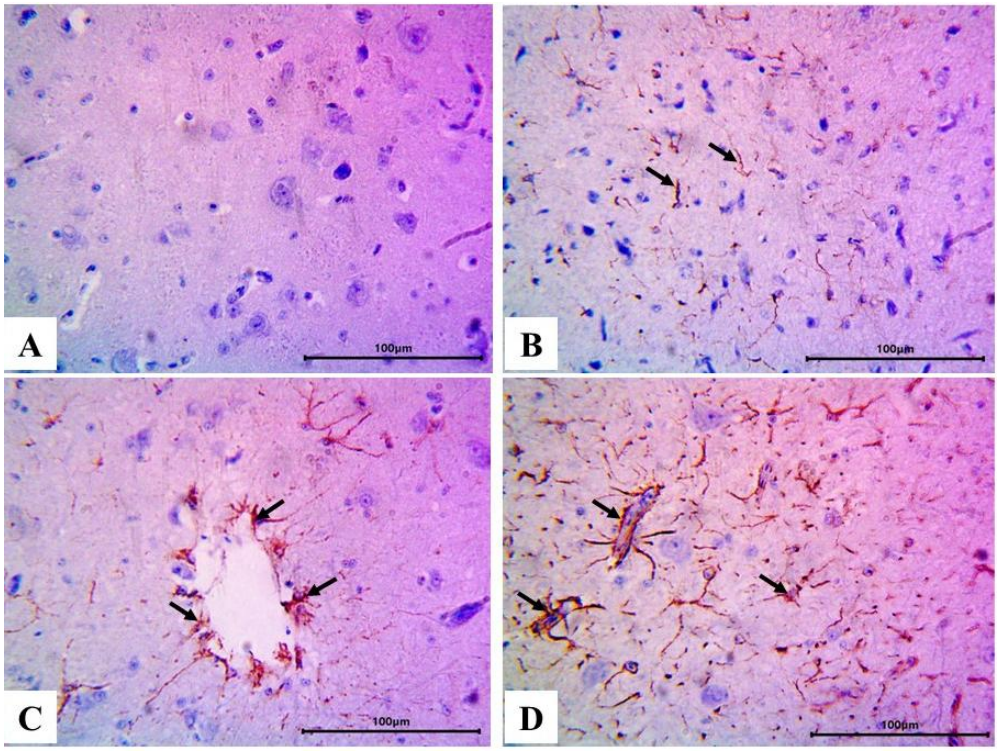

Figure 6: Immunohistochemical examination of GFAP expression (cytoplasmic pattern) in the cerebral cortex of brain tissue of examined ducks. A. Negative expression. B. Mild expression. C. Moderate expression. D. Severe expression. Black arrows indicate the positive expression, scale bar=100µm.

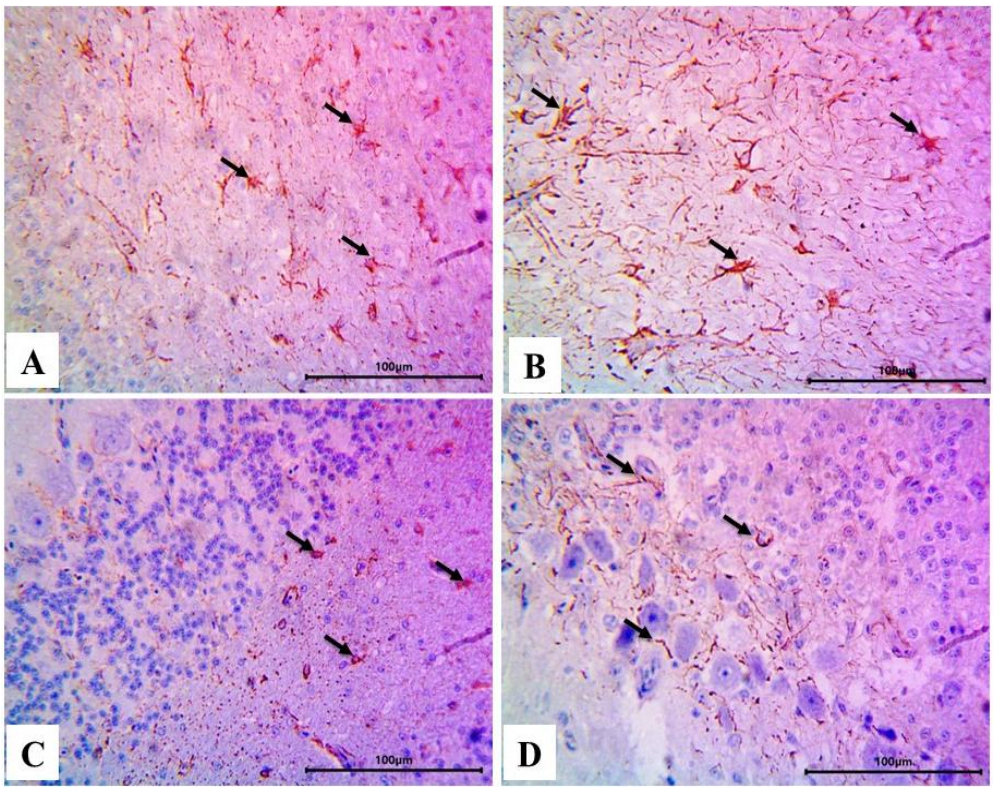

Figure 7: Immunohistochemical examination of GFAP expression (cytoplasmic pattern) in different parts of the brain tissue of examined ducks. A. Moderate expression in the cerebellum. B. Severe expression in neuropil. C. Mild expression in the granular cell layer. D. Moderate expression in Purkinje cells. Black arrows indicate the positive expression, scale bar=100µm.

Table 7: Comparison between scores of NSE and GFAP expression in the brain tissue of examined ducks

Expression Level	Score	NSE (No. & %)	GFAP (No. & %)	Difference (%)	P-value
Negative Expression	0	0 (0%)	1 (6.7%) *	6.7%	0.042
Positive Mild Expression	1	3 (20%)	5 (33.3%) *	13.3%	0.031
Positive Moderate Expression	2	5 (33.3%) *	4 (26.7%)	6.6%	0.048
Positive Severe Expression	3	7 (46.7%) *	5 (33.3%)	13.4%	0.037

Asterisks indicate significant differences for high value comparing to another marker.

Discussion

Central nervous system (CNS) is the main part of the nervous system in vertebrates. CNS is a complicated and crucial network in the living body controlling numerous key activities as receiving, processing and transmitting sensory signals throughout the body. This system consists of the brain and spinal cord. Moreover, it's supported by different kinds of cells as neurons and glial cells. The neuronal structures can be affected either by external factors or internal as inflammation or infection which subsequently lead to irreversible changes in this tissue or chronic lesions (32). Although ducks are less vulnerable to health disorders when they are compared to chickens which is due to their genetic resistance, this species is sensitive to different illnesses as mycotoxicosis, infectious agents and inadequate food (33).

Due to the lack of information about histopathological changes as well as NSE and GFAP expression in the brain tissue of ducks during normal conditions. Therefore, we aimed to conduct this study to uncover and describe these changes. In the present study, certain microscopic pathological changes were recognized in the examined brain tissue sections. One of these is vacuolar degeneration which was one of the remarkable pathological changes that exhibited higher percentage (46.7%) among the remaining changes of cell metabolism disturbances. It has been reported that this pathological condition does not occur naturally in healthy ducks and is also known as vacuolar myelinopathy (VM), which is a neurological disease caused by the neurotoxin of Cyanobacteria and cause nervous signs as weakness, incoordination and paralysis. It's manifested by diffuse vacuolization in the white matter of the brain and it is common in eagles, birds of prey and waterfowl (34). Another notable cause for these vacuoles in ducks is Tembusu virus (TMUV) which is capable to cross and destroy the BBB leading to non-pyogenic encephalitis and severe neurologic symptoms (35,36). However, identification of the causative agent was not a target as well as the examined ducks in the current work did not show any nervous signs which contradicts with the mentioned studies.

Other characteristic histopathological findings in the current work are neuronal satellitosis, gliosis and neuronophagia. Neuronal satellitosis can be defined as clustering of glial cells around neurons in the satellite space. (37) declared that neuronal satellitosis is a common finding in the brain of healthy wild and domestic birds and may not indicate any pathological process. However, other researchers reported that neuronal satellitosis can be more specific to neuronal injury and is characteristic to some avian viral infections. For instance, West Nile fever, Newcastle disease, and avian influenza (38-42). Moreover, gliosis refers to the proliferation and/or hypertrophy of glial cells in response to tissue injury, infections or neurodegenerative diseases and oxidative stress (43).

Numerous types of tissue injuries at different cellular levels were recorded during this survey with involvement of gliosis. One of these tissue injuries is parasitic infection represented by Sarcocystis which was seen in 7 samples (46.7%) of the total collected samples. This protozoan parasite is one of the food-borne diseases that infect many types of birds including ducks which are the intermediate host (44). It has been reported that gliosis is a common histologic finding in birds infected with this parasite (45,46). Surprisingly, our clinical ante-mortem examination of the birds did not show any clinical signs which agreed with that achieved by (47) who reported presence of the parasite in local ducks without any nervous signs. Moreover, neuronophagia was detected in (26.7%) of the examined samples. This process occurs when the glial cells engulf and degrade the neurons which is usually accompanied different viral diseases that attack the CNS. For instance, West Nile virus and avian influenza. These viruses initiate inflammatory responses that cause damage to the brain leading to the phagocytosis of damaged cells by neuronophagia to eliminate these debris (38,40,42).

Furthermore, apoptosis and necrosis were identified in the current study (40%, 26.7%), respectively. These two findings are both forms of cell death, but each process has its own mechanism. Apoptosis which is also known as programmed cell death, is an active physiological process that avoids inflammation and has several advantages such as tissue modeling during the embryonic life, upkeep of tissue balance in adults, and elimination of damaged or infected cells during pathological events. Various stimuli both physiological and pathological can activate apoptosis.

Examples of these stimuli include exposure to drugs, radiation, low oxygen levels, tumor necrosis factor receptors (TNF) or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and involvement of plasma-membrane-associated fas. On the other hand, necrosis is a passive pathological process accompanied by inflammatory response which is mediated by a set of caspases and triggered by toxins, heat stress, pathogens (48,49). Since these two processes are triggered and associated with certain physiological or pathological responses. As a consequence, more work is required to pinpoint the main causes in healthy ducks.

Although routine Hematoxylin and Eosin stain is a keystone and widely used in histopathology since decades that helps in identifying tissue changes during diseases and tumors, this technique has some limitations. In particular, it might be challenging to rely on this procedure due to the difficulty to distinguish between morphologically undamaged cells and the unviable ones. Hence, IHC is a fundamental and sensitive diagnostic approach nowadays that is able to detect and estimate the expression as well as the location of precise proteins in histological sections (50,51).

Two protein biomarkers of nervous system damage were used in the current work; NSE and GFAP to evaluate and compare their expression during steady conditions in adult ducks. Herein, we reported for the first time the expression of both proteins in different severities in the brain tissue of heathy ducks which were used in the current project. Biomarkers are crucial tools which are used in diagnosis and treatment of brain disease. These biomarkers can measure both the severity and evolution of acute injury and chronic diseases of the neurons such as Alzheimer's disease, Parkinson's disease, migraine, traumatic brain injury and other brain conditions (52). An example of these biomarkers is GFAP which is a 50 kilodalton (kDa) cytoskeletal protein mainly found and overexpressed by astrocytes in response to varied neurological conditions (22,53). These days, GFAP is considered as an astrocyte-specific marker. Therefore, the elevation in its level reflects presence of astrocytes activation which has been associated with multiple pathological conditions as traumatic brain injury (TBI), neuroinflammation and neurodegenerative disorders and brain metastasis (54). Additionally, it has been recorded that GFAP level in the serum is a beneficial indicator of astrogliosis in many neurodegenerative disorders and a promising diagnostic tool for Alzheimer's disease (53).

A recent study conducted by (55) in rats showed the potential use of both NSE and GFAP to monitor nervous system injuries induced by drug intoxication using several compounds. In the current study, GFAP expressed in different intensities and parts of the brain tissue as in the cerebral cortex, neuropil, granular cell layer and Purkinje cells. This was in agreement with (56) who conducted an in vivo experiment using 9a5b Newcastle disease virus to induce CNS damage. The results revealed that GFAP expressed positively in the control ducks particularly around Purkinje cell layer. On the other hand, a survey study conducted by (40) using wild birds of various species which were naturally infected with avian influenza virus H_5N_1 expressed GFAP. This was attributed to the neurotropism effect of the virus which caused severe tissue injury in the brain. Therefore, GFAP expression in healthy duck's brain tissue needs further investigation to outline the absolute underlying mechanisms of this expression. Today's data obtained from human research experiments showed the significant use of NSE and GFAP as valuable biomarkers in suicide attempters in a study conducted by (57). All participants were healthy who had no head injuries/trauma during the assessment. However, the level of NSE and GFAP were significantly higher in the suicide attempters than the healthy control individuals. Furthermore, (58) demonstrated that NSE can be used as a diagnostic marker using IHC technique for human neuroendocrine neoplasms (NENs) taken from different organs such as kidney, stomach, lung and breast. The study identified that NSE expression in all tumor samples was highest than the other markers used in the study.

In veterinary settings, NSE was used to determine its association with encephalitis caused by canine distemper virus in a study performed by (59). The researchers reported that dogs suffered from encephalitis expressed a significant increase in NSE values in serum comparing with the control animals. This investigation indicates the effectiveness of measuring NSE in serum for identification of encephalitis in dogs. NSE has been used as a precise biomarker of neuronal death by an in vivo experiment performed by (60) who showed the importance of immunostaining of NSE for neuronal integrity in different brain areas of rats treated with pentylenetetrazol (PTZ) to induce epileptic seizures. Additionally, (61) showed that NSE levels in serum increased significantly during acute brain damage-induced by cerebral ischemia in treated male rats in comparison with control group. All these experimental studies have shown the advantage of NSE and GFAP as neurologic biomarkers. In the current work, we showed that healthy ducks can have a wide range of histopathological changes in the brain. Also, NSE and GFAP expressed as a result of this tissue damage. For future work, further research is needed to illuminate and explore the association between NSE and GFAP expression in brain-induced injury in various animal models.

Conclusion

Although the ducks examined in the current study were clinically healthy, several histopathological changes at different severity scores were noticed particularly vacuolar degeneration, inflammation, edema and parasitic infection

represented by Sarcocystis. In addition, both NSE and GFAP were expressed in different intensities in the brain tissue and are significant neurological biomarkers for brain damage. However, NSE exhibited more intense expression than GFAP. To elucidate the exact role of both proteins, it is strongly recommended to study their role during experimental induced-neuronal injury or in the avian neuropathy.

Acknowledgment

The authors of this work would like to thank the staff of Pathology and Poultry Diseases Department, College of Veterinary Medicine, University of Mosul for their excellent support to accomplish the current study.

Conflict of interest

The authors declare there are no conflicts of interest regarding this study.

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التعبيرعن بروتين إنولاز الخاص بالخلايا العصبية والبروتين الحمضي الليفي الدبقي في دماغ البط السوي سريرياً

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الخلاصة

يُعد دماغ البط عرضة لأنواع متعددة من المشكلات الصحية التي قد لا تُظهر أعراضاً واضحة في الحال. يُعتبر كل من بروتين إنولاز الخاص بالخلايا العصبية والبروتين الحمضي الليفي الدبقي من المؤشرات الحيوية الواعدة للكشف عن الأمراض العصبية. ونظرًا لندرة المعلومات المتوفرة حول التغيرات المرضية النسيجية إلى جانب التعبير البروتيني في دماغ البط خلال الظروف الطبيعية، هدفت الدراسة الحالية إلى استكشاف هذه التغيرات المحتملة في نسيج الدماغ. لهذا السبب، تم جمع ١٥ بطة بالغة سليمة من كلا الجنسين من الأسواق المحلية في مدينة الموصل خلال الفترة من شباط إلى نيسان ٢٠٢٤. جُمعت عينات الدماغ وخضعت لفحص تشريحي شامل، تقييم نسيجي روتيني، وفحوصات الكيمياء النسيجية المناعية لتحديد تعبير البروتينات. أظهرت النتائج عدم وجود تغيرات عيانية في أنسجة الدماغ المفحوصة. إلا أن الفحص المجهري كشف عن تغيرات مرضية نسيجية متعددة و متفاوتة الشدة، شملت التنكس الفجوي، الالتهاب، الوذمة، الدباق والإصابة بطفيلي الحويصلات الصنوبرية. علاوة على ذلك، أظهر كل من بروتين إنولاز النوع العصبي والبروتين الحمضي الليفي الدبقي تعبيراً متفاوتاً في نسيج الدماغ. حيث سُجل أعلى مستوى من تعبير بروتين إنولاز النوع العصبي في سبع عينات (٤٦,٧%)، تلاها تعبير متوسط في خمس عينات (٣٣,٣%)، وتعبير خفيف في ثلاث عينات فقط (٢٠%). من المثير أن البروتين الحمضي الليفي الدبقي أظهر تعبيرًا سلبيًا في عينة واحدة فقط (٦,٧%)، في حين سُجل تعبير متوسط في أربع عينات (٢٦,٧%)، بينما تساوى كل من التعبير الخفيف والشديد بنسبة (٣٣,٣%). نستنتج أن البط السليم قد يُظهر تغيرات نسيجية مرضية وأن بروتين إنولاز الخاص بالخلايا العصبية أظهر تعبيراً أكثر شدة مقارنةً بالبروتين الحمضي الليفي الدبقي حتى في غياب إصابة دماغية مُحدّثة.