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Immunolocalization of the calcium binding S100 protein in the Purkinje cell, astrocytes and neurons of young broiler birds

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Abstract
S-100 protein family is a multigenic group of non-ubiquitous cytoplasmic EF-hand calcium (Ca^{2+})-binding proteins with a regulatory role in a variety of cellular processes. The present study reports the Immunolocalisation of calcium binding S-100 protein in the brain of In bro brown layers (IBL)-80 poultry birds. S-100 protein immunoreactivity was detected in Purkinje cells in cerebellum, astrocytes and its foot process, small neurons and fibers in brain with different pattern of distribution by immunohistochemistry. The results suggest that S-100 may play a key role in the maintenance and development of the central nervous system of the poultry birds.

Keywords: Calcium binding S-100 proteins, Brain, IBL-80 poultry birds, Immunohistochemistry, Purkinje cells

1. Introduction
S-100 proteins are non-ubiquitous, small, acidic proteins of 10-12 kDa and contain two distinct EF-hands. S-100 proteins belong to a family of calcium binding proteins of 20 members. S-100 proteins exhibit a unique pattern of tissue/cell type specific expression [1]. Most S-100 proteins along with calmodulin and troponin-C are considered Ca^{2+}-signaling proteins and all have the conserved calcium-binding motif termed the EF-hand [2]. These proteins act as intracellular regulators and as extracellular signaling proteins. These proteins regulate cell proliferation, differentiation, apoptosis, Ca^{2+} homeostasis, energy metabolism, migration/ invasion, inflammation and tissue repair and/ or to exert antimicrobial activity. S-100 protein family is distributed in neuronal as well as non-neuronal cells. The association of several members of this family in the development and function of the nervous system has developed a new interest in these proteins. Certain S-100 proteins in serum and other biological fluids are used as biomarkers of brain injury [3].
S100 protein has been detected in the nervous system of invertebrates [4] and vertebrates *viz*., zebra fish [5], laboratory animals [6-8], avian brain [9-12], cat [9, 13], bovine [14] and human [15-17]. The aim of the present study was to examine the distribution of S-100 protein in the brain of IBL-80 broiler birds using immunohistochemical technique.

2. Materials and Methods
In the present study, brains from two IBL-80 poultry broiler birds of 1-6 weeks of age were collected in 10 % neutral buffered formalin. For histological studies, the samples were processed and paraffin blocks were prepared. 4-5µm tissue sections were cut and stained with routine haematoxylin and eosin (H & E) stain.
For immunohistochemical studies, 4-5µm sections were cut and mounted on Poly-L-Lysine coated slides. After Deparaffinization and rehydration, antigen retrieval of tissue sections was done in citrate buffer (pH 6). Non-specific binding blocking by normal horse serum and endogenous peroxidase blocking by 3% hydroden peroxide (H_2O_2) was followed by overnight incubation with primary antibody polyclonal rabbit anti-S-100 (Z-311, Dako, Denmark) in a humidified chamber at 4 °C. After primary antibody, the slides were incubated with secondary antibody (Vectastain Elite Kit, Vector, USA). Colour was developed with substrate Diamino benzidine (DAB) (Vector, USA) and counterstained with Mayer's hematoxylin. Omission of primary antibodies was used for negative control.

3. Results and Discussion

In the present study, the distribution of immunoreactivity for protein S-100 was investigated in the brain of an avian species, IBL-80 broiler chicken. Histology of the brain section showed normal histological features of the cerebellum part of the brain (Fig. 1). S-100 protein exists widely in the cytoplasm, nucleoplasm, cytoplasmic membrane, outer membranes of cell organelles and cell processes of glial cells specially the astrocytes and its foot process. In the cerebellum, positive staining for S-100 was observed in the Purkinje cells and their dendritic process (Fig. 2). High density cytoplasmic and nuclear staining of the Purkinje cells was observed along with neurons and glial elements throughout the brain. Similar results were observed in chicken Purkinje cells of the cerebellum [9] but contrast to the findings by Castagna and co-workers in quail [12]. Molecular and granular layer of cerebellum showed weak Immunostaining which is in contrast to high density immunostained cells in the granular layer of quail [12]. We also observed that the granular layer of cerebellum of adult white leghorn showed deep immunostaining (unpublished data).

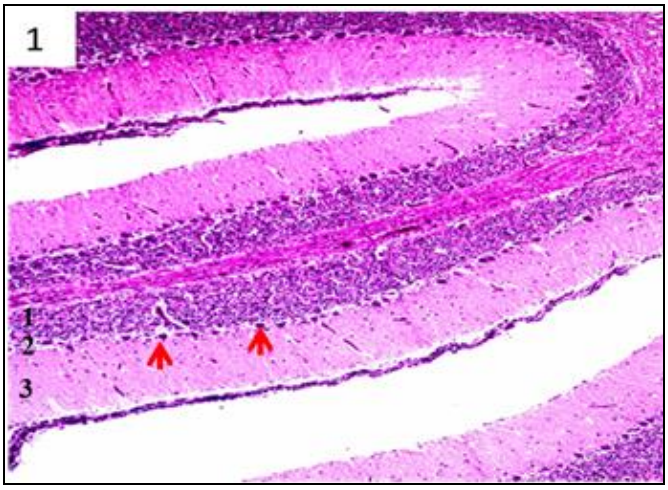

Fig 1: Representative photomicrograph of normal histology of the cerebellum of the brain showing three distinct layers: 1. the granular layer, 2. the ganglionic layer with purkinje cells (arrow) and 3. the molecular layer. 20X H&E

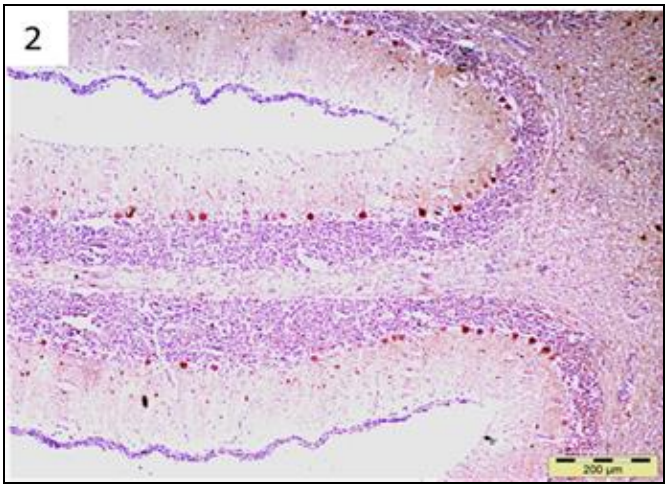

Fig 2: Representative photomicrograph of brain showing Immunolocalisation of S-100 (brown colour) in Purkinje cells of cerebellum. 20X (IHC)

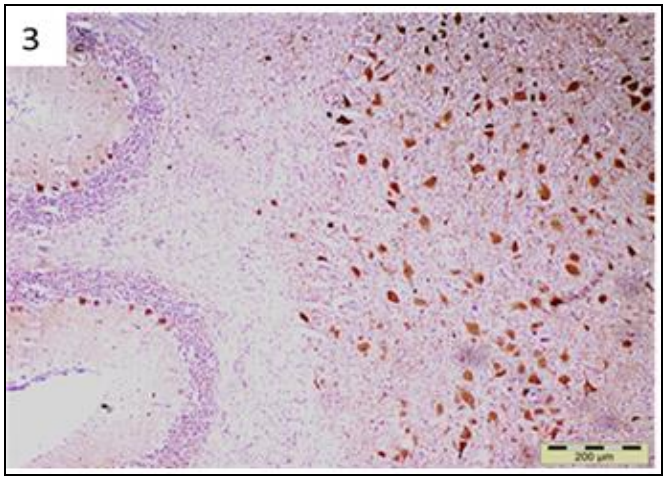

Fig 3: Representative photomicrograph of brain showing Immunolocalisation of S-100 (brown colour) in Purkinje cells of cerebellum as well as astrocytes. 20X (IHC)

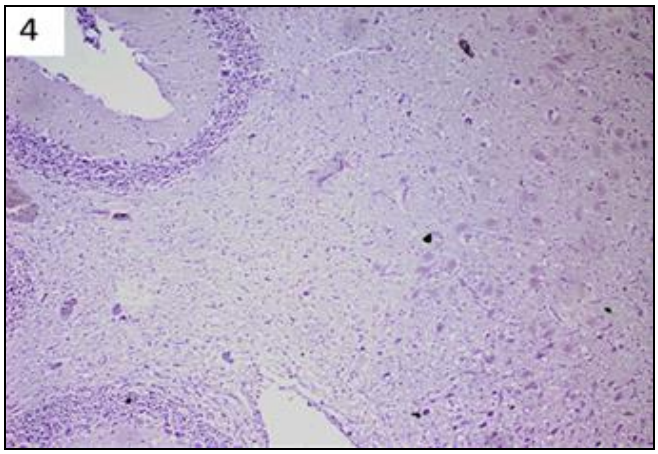

Fig 4: Representative photomicrograph of brain showing absence of brown colour in the tissue section. Negative control (20X, IHC)

The difference in the expression of S-100 in young and adult birds may suggest their contribution in the development and function of the brain. Astrocytes and its foot process (Fig. 3, 4) were highly Immunoreactive similar to that of other studies [9-12, 18]. During normal development, S-100 proteins may be differentially expressed in cells. In the central nervous system (CNS), the immunoreactivity has been found in astrocytes, oligodendrocytes, Bergman and ependymal cells types in rats and human [19, 20]. S-100 protein can be used as a sensitive and reliable marker for central nervous system injury because of its predominant location in astroglial cells. S-100 proteins have neurotrophic activity enhancing neurite outgrowth and survival of neurons during development [10]. Further, expression pattern of S100 proteins in brain could represents its functions in the maintenance of Ca^{2+} homeostasis, cellular structure, energy metabolism, intracellular signal transduction, cell cycle progression as well as synaptic plasticity [20].

4. Conclusion

Thus in the present study, the expression of S-100 proteins in the cerebellum, astrocytes and small neurons of the brain may conclude that of S-100 proteins are involved in the maintenance as well as development of central nervous system of IBL-80 chicken.

5. References

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