A Histological and Histochemical Study on Olfactory Bulbs to Detection Amyloid Protein Depositions by Congo-Red and Routine Staining Techniques

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Abstract

In this study, histological and histochemical techniques were used to examine olfactory bulbs in the albino rat male. Thirty male albino rats were split into three age groups (10 animals each) in the current research: **Group I**: consider as a control group, including adult animals aged 3 months .**Group II:** include animals aged 6 months. **Group III**: include animals aged 12 months. The histological architecture of the layers of olfactory bulbs and their main cells was identified by using H & E staining techniques, meanwhile the composition of each layer in albino rat was evident. In the glomerular and mitral cell layer of group III, olfactory bulbs showed reduced neural density. Modified staining with Congo - red was conducted for histochemical studies. Compacted amyloid cores were found in group III animals' olfactory bulbs, while dispersed amyloid cores were found in group II&III olfactory bulbs' cortex. The present study adds to our knowledge of the impact of amyloid protein on olfactory bulbs and their prospective neurodegeneration involvement.

Key words: *Histology, Histochemistry, Congo red, Amyloid ,Granular cells, Mitral cells and olfactory bulbs.*

Introduction

In the olfactory signal conductive path, the olfactory bulbs are the station of relay. The OBs are the first chief relay in the processing of odor data process: it provides afferent input from olfactory sensory neurons (OSN) in the olfactory epithelium of the nasal cavity and responsible for the identification of odors in it: which is the only relay between the peripheral and central nervous system, it also processed the olfactory data [(5)]. The olfactory bulb's main histological architecture involve: olfactory nerve layer, glomerular olfactory layer, external plexiform layer, mitral cell layer, inner plexiform layer and granular cell layer [(8)]. The superficial olfactory nerve layer contains afferent axons from OSNs. OSN axons synapse with OB projections of neurons dendrites, mitral / cells, and sub aggregations of regional periglomerular cells (Pgm) [(13)]. These layers are arranged in the olfactory bulbs very obviously and regularly. The characteristics of olfactory bulbs structure facilitate the processing of data and also provide a scaffold for olfactory data spatial encoding [(5)].

The olfactory bulb consists of four types of cells: mitral cells, granular cells, tufted cells and short axon cells according to classical studies [(10; 5)]. One of these neurons, granular cells, have long been known to be morphologically uncommon because they don't have a standard axons, and recent studies in electron microscope have demonstrated that it participate in special reciprocal synaptic connections with mitral cells [(8)].Mitral cells are the biggest cells in the olfactory bulbs and as stated by electron and light microscope researches, moreover they are the main olfactory bulb efferent neuron [(6)]. The dendritic structures of mitral cells can be subdivided into primary and secondary dendrites, and both primary and secondary smooth dendrites pass through the surface of the outer plexiform layer and only the primary dendrites extend down to the olfactory glomerular layer [(18)].

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Within the glomerular layer the dendrites of mitral cells are in synaptic communication with olfactory nerves and periglomerular cells, but the only synapses on mitral cells elsewhere are the "reciprocal synapses'' with the granule cells [5]. Mitral cells, as the main olfactory bulbs efferent neurons, play a significant role in olfactory signal conduction and modification [9; 13].

Amyloids β (AB) are little pieces of a bigger proteins named "amyloid precursors protein" APP. While the ordinary function of APP has not yet been determined by researchers, they understand a lot about how it appears to operate [16]. In its full shape APP ranges from the inside of the brain cells to the outside through the fatty membrane around the cells. As APP is activated to perform its normal activities, other protein can cut it into different and smaller parts inside and outside cells. APP can be cut in several aspects: under some conditions, β-amyloid is one of the parts generated [3].

According to the hypothesis of amyloid, phases of beta-amyloid aggregations interrupt cell-to-cell communication and activate immune cells, these cells of immune system cause inflammation [12].

The process of neurodegeneration in diseases of cognition (Alzheimer's and Parkinson's diseases) may involve toxicity of β-amyloid (Aβ) that could be demonstrated in vitro and seems to be involving oxidative stress, this underlie the progression of neurodegeneration that consider as characteristic feature of AD [2].

It has been shown that the amyloid induces neuronal death, reduced plasticity of synapses, aberrant axons growth, tau hyper phosphorylation and chronic inflammation [7] . Aβ accumulation in the pathogenesis of AD is an early and essential case .First formation of temporal cortical regions, including the hippocampus, a memory-creating zone. Aβ aggregates have been indicated to form neurotoxic plaques that contribute to neurodegeneration accompanied by dementia [15].

Similarly, [11)) showed that capillaries, venioles and arterioles in the cerebral cortex also often have amyloid deposits. Congo red staining was considered as an approved histochemical marker for amyloid β-pleated-sheet [14]. Congo red: amyloid detection in tissue parts is significantly improved and verified by favorable Congo red staining. Thioflavin S and Congo red are represented the main histological stains that used for any type of amyloid [17].

Red stain is red-pink on its own .Under both light and polarized light microscopy, examination of tissue segments suspected of participation by amyloidosis must be carried out. Amyloidosis has a distinctive green apple birefringence when polarized[11].

In this study we analyzed the histological structure and histochemical (Congo red for amyloid protein) characteristics of OBs in order to evaluate the presence of amyloid depositions in the olfactory bulb layers. The aim of this research is to clarify the amyloid-histochemical and histological characteristics of olfactory bulbs in the rats of albino male in relation with aging.

Material and Methods

Experimental Animals:

Male albino rats aged between (3, 6, 12) months were obtained from the Animal House, Collage of Science, University of Babylon. The rats were housed in wire mess cages under standard condition with 12 hrs. Light and 12 hrs. dark cycle throughout the entire experimental period. Food and tap water supplied with libitum.

In the current research, thirty male albino rats will be split into three age groups (10 animals each), they are: Group I: considered as a control group, including adult animals aged 3 months. Group II: include animals aged 6 months. Group III: include animals aged 12 months.

Histological study:

The specimens of olfactory bulbs were taken from the brain of the albino rats, the samples were immersed in the solution of Bouins for two days. Tissue was dehydrated in graded ethanol and embedded in paraffin. 7μm horizontal sections of paraffin blocks were cut on a revolving microtome and installed on glass slides then, the following staining processes were completed [1].

Histochemical Study:

Selected sections have also been processed for histochemical amyloid protein demonstration. Sections have been deparaffinized through xylene and alcohol into

tap water. Thereafter, slides are immersed in alkaline sodium chloride. Twenty minutes later, they were immersed in alkaline solution of Congo red and then marked with alcoholic potassium. Thereafter, slides are countered with alum hematoxylin and dehydrated by xylene and ethanol [6].

Results

Histological Study:

In light microscopy, the main olfactory bulbs of the adult rat consisted of six concentrated layers: 1-The olfactory nerve layer (ONL), (fig.1A, and 1B). 2- The glomerular layer (GML). There were observation of periglomerular cell (Pgm) around the glomeruli (fig.1D, 1E). 3- The layer of the external plexiform layer (EPL), consists of fine nerve fibers and few granule cells, (fig.1A, 1B). 4- A layer of mitral cells (MCL) contained stomata of mitral cells in single row (fig.1A, 1B). They had an ovoid nuclei with single nucleolus deeply stained. Their cytoplasm include darkly stained basophilic granules (fig. 1C, 1E). 5-The inner plexiform layer (IPL), a thin layer of fine nerve fibers and some cells of granules (fig. 1A, 1B). 6- The layer of granule cells (GCL), contained a large amount of granule cells (fig. 1B).

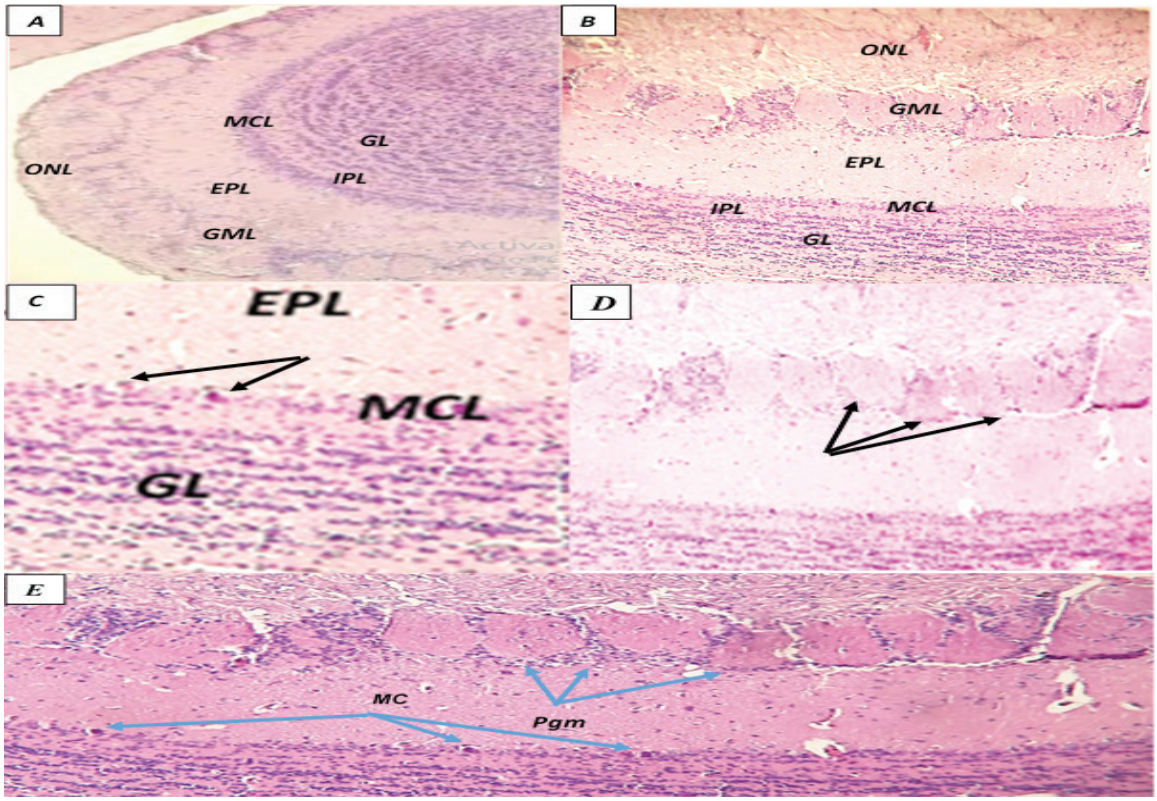
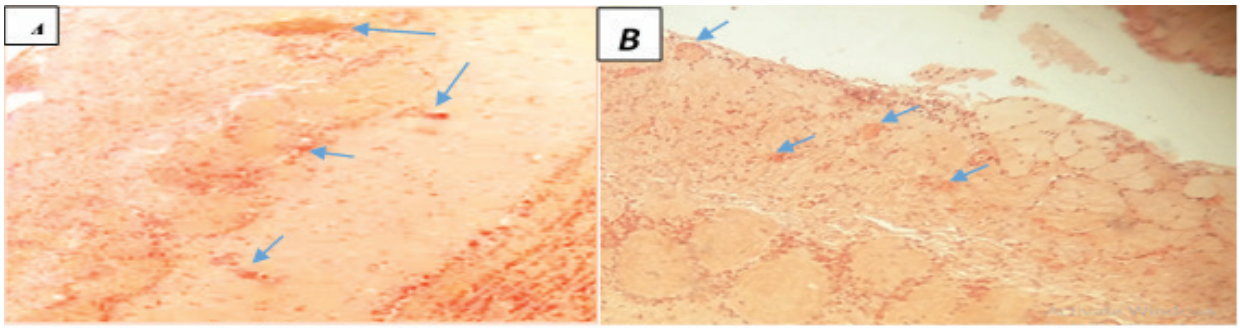


Fig.1: photomicrograph of histological section demonstrated to the concentric laminar organization of olfactory bulbs, H&E staining.4x, 10x, 40x.

The olfactory nerve fibers have been loosely segregated in group III rats. With a marked decrease in size, glomeruli were distorted in form (fig. 1). The EPL included a number of misplaced mitral cells' soma (fig.1B, 1E). Most mitral cells in MCL were decreased in size and generally present in a rounded, darkly stained nuclei with undefined nucleolus (fig.1C).

Histochemical Study:



Two forms of plaque of amyloid protein deposition were seen in the cortex of olfactory bulbs: diffuse plaques were shown in all layers of OBs of group III (fig. 2B). Compact amyloid plaques were found in the ONL, GML, MCL, and EPL layers of OBs of group III (fig. 2A, 2B). Amyloid angiophathy were detected in the different layers of OBs of group II and III, (fig.3).

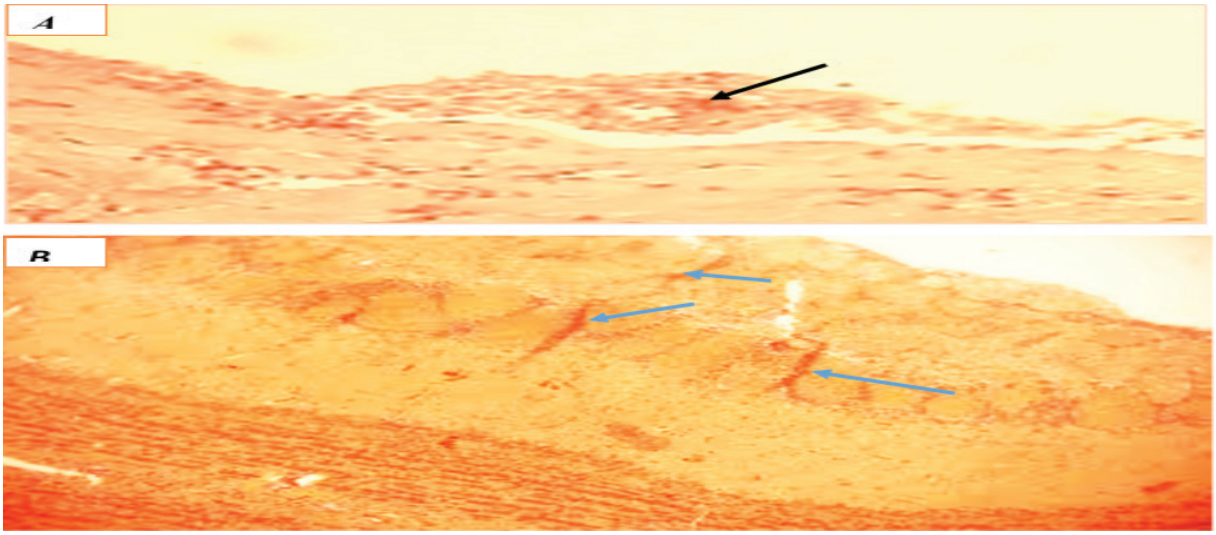


Fig.2: Photomicrograph of histological section showing plaque & diffuse staining for amyloid (AB, blue row) with Congo red –stain in olfactory tissue (2A, 2B), 10 x.

Fig.3 (A,B): Photomicrograph of histological section illustrated the Congo red stain highlighted the vascular amyloid depositions, note the staining of vessels walls (black & blue arrow), 40 x.

Discussion

In the pathway of the olfactory system, the olfactory bulbs are the essential components of olfactory system and relay station. We studied the histological architecture of the olfactory bulbs, according to the outcomes of our research, surveys were done to the structure of each layer of bulbs. The histological characteristics features of OBs constituent with the prior studies by Golgi [(10)]. Many periglomerular (Pgm) cells were found in the layer of glomeruli of olfactory bulbs. Two groups of neuronal cells are dispersed in the layer of mitral cells of olfactory bulbs, the cells with large cell bodies and cells with small cell bodies. The cells that characterized by large cell bodies seem to be correspond to the mitral cells as output neuronal cells, and the cells with small cell bodies may related to the tufted cells, that are well known as other kind of neurons of OBs of mammals, or the interneurons in the layer of mitral cells, but the correspondence could not be determined [(5)].

According to the finding of the present work, the histological characters among the bulbs of olfactory of albino rat are comparable in all groups (I, II, III), but the density of cells in each group was different. The complexity of the olfactory bulbs layers organization proportional to the olfactory bulbs information-processing capacity and represents the degree of

olfactory bulb development. The results of our outcomes showed that the morphology and amount of mitral cells in the group I &III were differed [18].

The results of this research are complementary and consistent with prior human OBs tissue reports that prevalent layers (NFL, GML, EPL, MCL, IPL, and GL) constitute the construction of all layers in the olfactory bulbs of albino rats. There was no distinction in olfactory bulb composition between group (I, II, III), except for cell density variations. In group I, in each layer, the density of cells was higher than in group II and III. No mechanism exited to explain this decline. The amount of granulated cells and mitral cells decreased but increased in size [11; 9]. The mitral cells considered as the largest neuronal cells in the olfactory bulbs, have primary and secondary dendrites, these processes oriented vertically or parallel to their soma, glial cells formed these dendrites of mitral cells. Axons of mitral cells converge in bundles of fibers and pass through the layer of granular cells [11].

[10] Demonstrated a "substantial layer-specific loose" of synapses ultra-structurally: synaptic density is decreased in the layer of glomerular cells but not the internal plexiform layer, leading to unbalance in circuitry of OBs. Our findings showed a decrease in GML and MCL density, consistent with [10] findings, these results indicate that decreased afferent synaptic input and local modulatory circuit synapses in OB glomeruli may contribute to particular age related changes in olfactory function.

In the current research, we showed diffusing and plaques of compact amyloid nuclei in the cortex of OBs by using modified Congo red staining. We also showed the enhanced amount of plaques and reduced neuronal populations in group III OBs compared to the olfactory cortex of group I& II, that showed typical dark orange colored patches under light microscope. It is well known that there are plenty of extracellular plaques of amyloid β peptide (*A*β) in the pathological marks of AD in the brain [16]. Amyloid has been discovered to be more localized in the neuronal processes in the current research, this finding was in agreement with the outcomes of other researchers who noted that in elderly people with and without Alzheimer's disease ,abnormality amyloid accumulate as neuropil threads [11]. The method of neurodegeneration in AD may involve toxicity of β-amyloid (*A*β). *A*β's neurotoxicity can be shown in vitro and seems to involve oxidative stress [2].

Our finding demonstrated that the blood vessels within the cortex of olfactory bulbs also lades depositions of amyloid that constituents with the results of [14]. Congophilic amyloid in blood vessels is called cerebral amyloid antipathy (CAA) [17].

Many researchers have shown that the deposition of *A*β peptide in the cerebral cortex leads in neural and morphological degeneration, cognitive loss, and modulation of enzyme markers such as acetylcholine esterase and choline acyltransferase; all of which are well-known symptoms of AD [19; 4].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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