ORIGINAL RESEARCH PAPER

Health Science

EFFECT OF AGEING ON NUMBER OF MITRAL CELLS AND GLOMERULI OF THE HUMAN OLFACTORY BULB

KEY WORDS: olfactory bulb, mitral cells, glomeruli

Dr Monali D Sonawane	PhD Scholar, [Anatomy] M.G.M Institute of Health Sciences, Navi Mumbai, Maharashtra, India-410209
Dr Pravin Raghunath Rathod*	PhD Scholar, [Anatomy] M.G.M Institute of Health Sciences, Navi Mumbai, Maharashtra, India-410209 *Corresponding Author
Dr Aruna Mukherjee	Professor Department of Anatomy M.G.M Medical College, Navi Mumbai, Maharashtra, India-410209

ABSTRACT

Aims of the study- To detect changes in mitral cells and glomeruli of the human olfactory bulb in different age groups. Material - 22 brain specimens were collected from cadavers from Anatomy department of MGM Medical College, Navi Mumbai and from National Institute of Mental Health and Sciences (NIMHANS), Bangalore.

Method- The study was carried out in 20 undemented specimen, divided into four groups (5 samples in each) according to age: group I (20-39yrs), group II(40-59yrs), group III (60-79yrs), group IV(80 yrs and above) and 2 specimen of Alzheimer's disease as a control group. Numbers of glomeruli and mitral cells of olfactory bulb were calculated from H &E and Luxol fast blue stained sections respectively.

Results- There was significant reduction in number of glomeruli and mitral cells in group III,IV and control group as compare to group I and II (p<0.001). Laminar architecture significantly lost in group III, IV and control group.

Conclusion- The number of mitral cells and glomeruli decreased with advancing age which may be responsible for olfactory dysfunction.

Introduction

A Histological Study The olfactory system has several interesting anatomical and physiological features although olfaction has remained a 'neglected sense'. The olfactory system consists of olfactory nerves, bulb, tract and the olfactory cortex. It is the most primitive sensory system.

The olfactory bulb is considered as the first synaptic station in the olfactory pathway. It receives axons of olfactory nerves and is continuous posteriorly with the olfactory tract, through which the output of the bulb passes directly to the olfactory cortex.[1]

From the surface to the centre core the olfactory bulb consists of following six layers: i) olfactory nerve ii) glomerular iii) external plexiform iv) mitral cell v) internal plexiform vi) granular cell layers.[2] Each glomerulus is the functional unit for processing sensory inputs and play basic role in smell perception. Mitral cells are principle cells of olfactory bulb. Their axons are connected with several synaptic glomeruli and help in the convergence of olfactory stimuli. The mitral cells detect the ratios of different odorants and carry all odor information from nose to the brain.

Disturbances of olfaction are common. Studies have shown that 3.8–5.8% of the general population have anosmia but the prevalence rates increases to 13.9% in individuals over 65 years, 50% between 65 to 80 years old and 80% in subjects >80 years.[3] Thus olfactory functioning may be a valid indicator of the ageing brain. Olfactory dysfunction manifests early in the neurodegenerative diseases such as Alzheimer's disease and may represent an important early clinical symptom suggestive of neurodegeneration.

There have been few structural studies of the olfactory bulb in the ageing brain in the Indian population. The present work is therefore designed to detect the age related structural changes in the olfactory bulb. This work will provide a baseline data for further correlative studies of functional modalities of olfaction.

Material and Method
Sample collection-

22 brain specimens were collected from cadavers from Anatomy department of MGM Medical College, Navi Mumbai and from National Institute of Mental Health and Sciences (NIMHANS), Bangalore after institutional ethical committee clearance and written consent from close relatives. Information regarding name, age, sex, marital status, occupation, place of residence, educational status, socioeconomic status, systemic illnesses (diabetes, hypertension), and treatment history were recorded.

Assessment of cognitive & functional status was obtained from interview of close relatives by using Clinical Dementia Rating (CDR). The exclusion criteria included history of any neurological disease, occupational exposure to potential neurotoxicants, nasal pathology, nasal surgery and head trauma.

Out of 22 brain specimen, 2 specimen were from known cases of Alzheimer's disease which were obtained from Human Brain Tissue Repository - National Institute of Mental Health and Neurosciences [HBTR-NIMHANS], Bangalore, The study was carried out in 20 undemented specimens, divided into four groups(5 samples in each) according to age: group I (20-39yrs), group II(40-59yrs), group III (60-79yrs), group IV(80 yrs and above) and 2 specimen of neurodegenerative diseases(Alzheimer's disease) as a control group.

Histological Methods-

After checking weight, brain specimens were preserved in 10% buffered formalin for 4 weeks with neutral pH.

Olfactory bulb and tract were taken out from the olfactory sulcus which is situated at the orbital surface of the frontal lobe.

Samples were processed for paraffin embedding, block making, sectioning and staining.

The specimens were properly oriented in paraffin blocks and longitudinal sections of 9µm thick will be obtained from olfactory bulb.

Histological evaluation were done with following stains: I) Haematoxylin and Eosin stain to study laminar architecture(layers) of olfactory bulb and to count glomeruli ii) Luxol fast blue stain to count mitral cells

The number of glomeruli was counted at 40X objective with the help of $1mm^2$ reticule with H and E stain. The mean numbers of glomeruli were obtained by counting five randomly selected fields

per section. Similarly the mean number of mitral cells of olfactory bulbs was calculated per mm^2 area from 9µm thick Luxol Fast Blue stained sections. Mitral cells were identified by their large size, prominent nucleoli and their presence between internal and external plexiform layers.

Statistical analysis-

SPSS 22 software was used for stastical analysis. **Chi-square test** was used as test of significance for qualitative data to compare the possible difference between groups. **ANOVA (Analysis of Variance)** will be the test of significance to identify the mean difference between more than two groups for quantitative and qualitative data respectively.

Observations and results-

Table and chart no.1: HE Stain of olfactory bulb to demonstrate laminar architecture between five groups
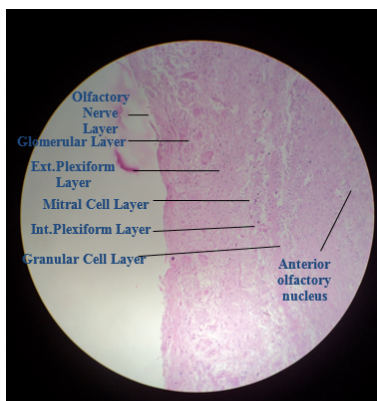
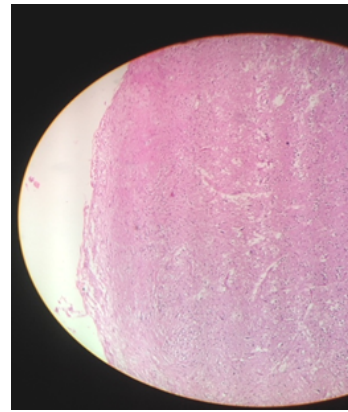| Group	Laminar Architecture			
	Lost		Normal	
	No.of specimen	%	No.of specimen	%
I (20 to 39 years)	0	0%	5	100%
II(40 to 59 years)	1	20%	4	80%
III(60 to 79 years)	2	40%	3	60%
IV(>80 years)	3	60%	2	40%
V(Controls)	2	100%	0	0.0%
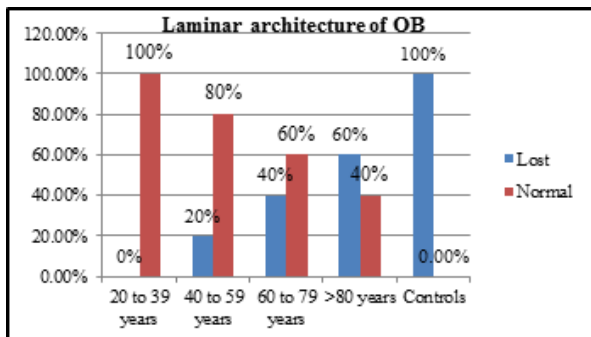
$\chi^2 = 9.565$, df = 4, p = 0.048*

In group 1, 100% had normal laminar architecture. In group 2, 80% had normal and 20% lost laminar architecture. In group 3, 60% had normal and 40% had lost laminar architecture, in Group 4, 40% had normal and 60% had lost laminar architecture and in Controls 100% of them had lost laminar architecture.
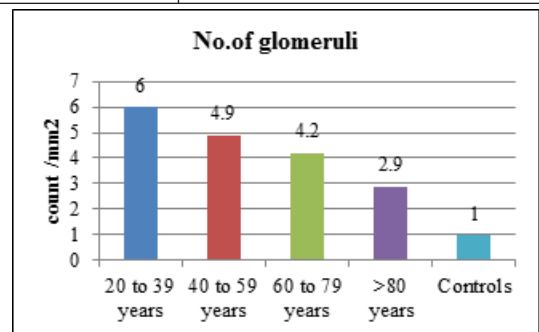
Fig no.1: Olfactory Bulb-H & E (10X)

a. Normal laminar architecture

b.Laminar architecture lost

Table and Chart 2: H& E Stain of olfactory bulb to demonstrate no. of glomeruli (Count/mm2) in five groups

Group	No.of glomeruli (Count/mm2)	
	Mean	SD
I (20 to 39 years)	6.0	0.3
II(40 to 59 years)	4.9	0.9
III (60 to 79 years)	4.2	0.4
IV(>80 years)	2.9	0.6
V(Controls)	1.0	0.2
P value	<0.001*	

In the study mean no. of glomeruli was highest in Group I (20 to 39 years) i.e. 6 ± .3 mm and lowest in Control group i.e. 1 ± 0.2 mm. With increase in age there was decrease in no of glomeruli in olfactory bulb. This difference in mean no. of glomeruli in olfactory bulb was statistically significant.

Table and Chart no.3: LFB Stain of olfactory bulb to demonstrate no. of mitral cells (Count/mm2) in five groups

Group	No. of Mitral Cells (Count/mm2)	
	Mean	SD
I (20 to 39 years)	6.9	0.2
II(40 to 59 years)	6.1	0.6
III(60 to 79 years)	4.7	1.0
IV(>80 years)	3.5	1.2
V(Controls)	1.9	0.1
P value	<0.001*	
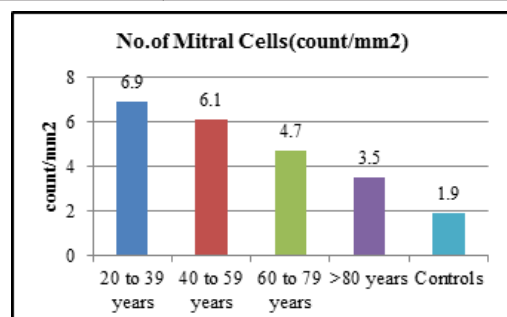
In the study mean no. of mitral cells was highest in group I (20 to 39 years) i.e. 6.9 ± .2 mm and lowest in control group i.e. 1.9 ± 0.1 mm. With increase in age there was decrease in no of mitral cells in olfactory bulb. This difference in mean no. of mitral cells in olfactory bulb was statistically significant.
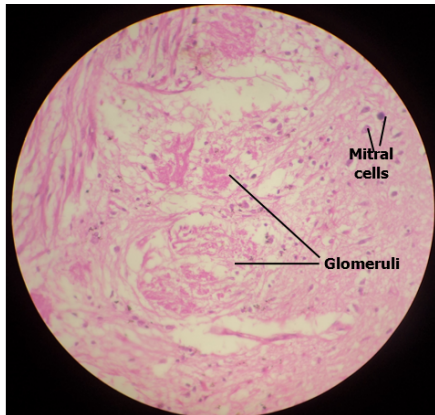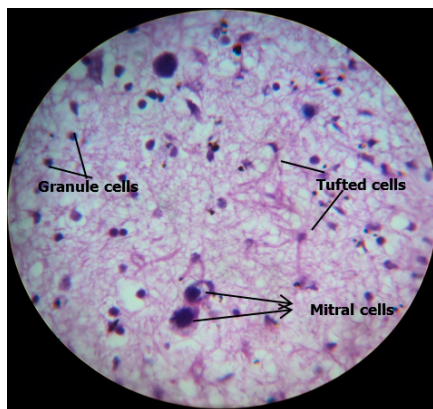

Fig.no.2- a. Olfactory Bulb-H & E (40X)

b. Olfactory Bulb-H & E (100X)

Discussion-

Earlier histological studies have shown that there was an age-related decline in the size of the olfactory bulb, a number of its laminae, number of mitral cells and glomeruli olfactory bulb neurons.[5,6] . Liss and Gomez [7] reported that the olfactory bulbs and tracts from persons over 70 years of age exhibited moderate loss of neurons and nerve fibers.

Other studies showed marked age-related decrease in the mean number and diameter of glomeruli and mitral cells with advancing age in both male and female persons and the difference was found to be statistically significant[2,3]

Similarly, in the present study, the number of mitral cells and glomeruli was decreased with age. In group III, mean number of the glomeruli per mm^2 was 4.2 and mean number of the mitral cells per mm^2 was 4.7. In group IV, mean number of the glomeruli per mm^2 was 2.9 and mean number of the mitral cells per mm^2 was 3.5. In control group, mean number of the glomeruli per mm^2 was 1 and mean number of the mitral cells per mm^2 was 1.9.

The organization of the complex neuronal structure of the olfactory bulb is damaged during the course of Alzheimer's disease.[8] In the present study, laminar architecture of the olfactory bulb was lost in 40% specimen of group III, 60% cases of group IV and 100% cases of control group.

Conclusion

The number of mitral cells and glomeruli decreased with advancing age which may be responsible for olfactory dysfunction.

References

1. Crossman, A.R., Special senses. In: Standring S, (Edi.).(2005). Gray's anatomy: the anatomical basis of clinical practice. Edinburgh: Elsevier Churchill Livingstone, 431-432.
2. Samaulhaq, M.T., & Khalid, P.L. (2008). Age and Gender Related Differences in Olfactory Bulb Glomeruli in Human.Biomedica, 24, 12-16.
3. Samaulhaq, M.T.,&Khalid P.L.(2008).Age and Gender Related Differences in Mitral Cells of Olfactory bulb.Journal of The Collegeof Physians and Surgeons Pakistan,18,669-673.
4. Attems, J.,Walker, L.,Jellinger, K.A.(2015).Olfaction and Ageing:A Mini Review. Gerontology, 21-5.
5. Bhatnagar, K. P., Kennedy, R. C., Baron, G., Greenberg, R. A. (1987). Number of mitral cells and the bulb volume in the aging human olfactory bulb: A quantitative morphological study. Anat. Rec, 218, 73-87.
6. Meisami, E., Mikhail, L.,Baim, D., Bhatnagar, K. P.(1998). Human olfactory bulb:Aging of glomeruli and mitral cells and a search for the accessory olfactory bulb. Ann. N. Y. Acad., Sci , 855,708-715.
7. Liss, L., & Gomez, F. (1958).The nature of senile changes of the human olfactory bulb and tract. AMA. Arch. Otolaryngol, 67, 167-171.
8. Kovács, T.(2015).The olfactory system in alzheimer's disease: pathology, pathophysiology and pathway for therapy. Translational Neuroscience, 4(1),34-45.