AGE AND GENDER RELATED DIFFERENCES IN OLFACTORY BULB GLOMERULI IN HUMAN

SAMAULHAQ, M. TAHIR AND KHALID P. LONE
Department of Anatomy, University of Health Sciences, Lahore –Pakistan

ABSTRACT
*The present study was designed to investigate the age and gender related differences in glomeruli of the human olfactory bulb in normal Pakistani population. The **s**tudy was conducted in the Department of Anatomy, University of Health Sciences, Lahore. The duration of the study was two years. Sixty olfactory bulbs, thirty each from male and female human cadavers (age 20-76 years) were collected from the mortuary of the King Edward Medical University, Lahore. Glomeruli were counted and their diameter was calculated from 10 µm thick H & E stained histological sections. Statistical analysis was done using ANOVA for age related differences and Independent "t" test for gender related differences. The results showed significant decrease in the number of glomeruli ($p < 0.001$) and their diameter ($p < 0.05$) with age. No gender related differences were observed. The number of glomeruli and their diameter both decreased with advancing age.*

INTRODUCTION

The sense of smell is an ancient sensory modality.[1] The olfactory pathway consists of following grossly identifiable structures: the olfactory mucosa, nerves, bulb, tract[2] and the olfactory cortex.[3] The olfactory bulb is the first relay station in the olfactory pathway[4] and receives axons of olfactory nerve and is continuous posteriorly with the olfactory tract through which the output of the bulb passes directly to the olfactory cortex.[5] The olfactory bulb is composed of the following layers from the surface inwards: (a) olfactory nerve, (b) glomerular, (c) external plexiform, (d) mitral cell, (e) internal plexiform and, (e) granular cell layers.[6] The major types of neurons in the olfactory bulb are, mitral, tufted, periglomerular, and granular cells.[7]

Glomeruli of the olfactory bulb are among the unique and highly interesting structures of the olfactory system.[8] Each glomerulus is a functional unit for processing sensory input.[9] Glomeruli are roughly spherical masses of dendritic processes.[10] Each glomerulus is a complex synaptic ball where the terminal arborization of the axon of the recaptor bipolar cell synapses with the dendritic arborization of the mitral and the tufted cells of the olfactory bulb.[6,11] The function of the glomerulus is to modify the response of the output neurons so that they are more sharply tuned to a given odour than those by the olfactory neurons conveying inputs to the glomerulus.[12]

A study conducted on female olfactory bulbs, between the ages 25-100 years in Canada, showed that structural changes occur with advancing age; the thickness of all the bulb layers decreased with age.[13] The peak performance in odour identification ability occurs in the 3rd through the 5th decades of life and it markedly declines after the 7th decade; generally females are reported to possess better sense of smell in all age groups.[14] The odour identification ability of women out performed men including olfactory tasks involving episodic recognition and identification of familiar odours.[15] Women discriminate among different body odours more easily than men.[16]

Most of the research on the olfactory bulb has been conducted in Europe and America. Age and gender are two important variables which have not yet been explored in Asian (especially among Pakistani). The present work was, therefore, designed to investigate the age and gender related changes in olfactory bulb in local Pakistani population by studying the histological structure of olfactory bulbs procured from male and female cadavers, dividing them into different age groups; it is hoped that the study will provide data for further correlative work with functional modalities of olfaction.

MATERIALS AND METHODS

Sixty cadaveric olfactory bulbs were obtained 3 to 7 hours after death from the mortuary of the King Edward Medical University, Lahore. Written consent from the relatives of the deceased was procured. The cadavers were kept in a cold room at 4°C soon after the death till the time of autopsy. The information regarding name, age, gender, marital status, occupation, places of residence, clinical history and cause of death were recorded. The study was carried out in six groups of varying ages for

males (groups I-III) and females (groups IV-VI) and ten olfactory bulbs were investigated in each group.

Group: I & IV 20 to 39 years of age
Group: II & V 40 to 59 years of age
Group: III & VI 60 years and above

The following exclusion criteria of Doty[17] *et al.* (1992) were adopted:

a. History of any neurological disease
b. Occupational exposure to potential neurotoxicants.
c. Nasal pathology.
d. Nasal surgery.
e. Head trauma.

Whole brain was removed along with its meninges; olfactory bulb and tract, situated at the orbital surface of the frontal lobe (Fig. 1) inside the meninges, were exposed; these were isolated from the olfactory sulcus carefully, removed and fixed in 10% formal saline for one week.

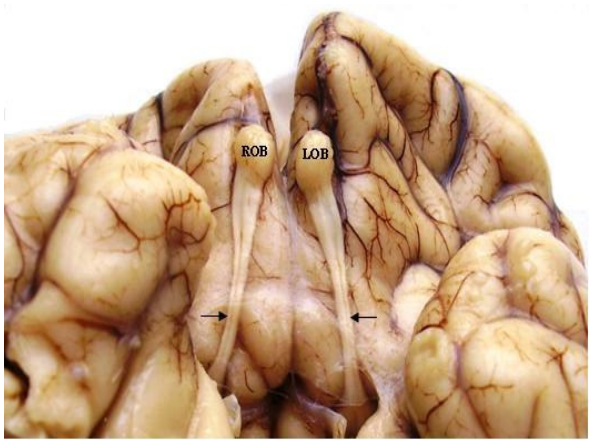

Fig. 1: *Photograph of the orbital surface of frontal lobe of human brain showing right (ROB) and left (LOB) olfactory bulbs. Right and left olfactory tracts (arrows).*

The schedule described by Bancroft and Gamble[18] (2002) was used for tissue processing; the specimen were properly oriented in the paraffin blocks; coronal sections, 10-µm thick,[19] were obtained from the anterior, middle and posterior parts of the bulb and stained with Hematoxylin and Eosin before examining them with the light microscope.

The number of glomeruli was counted at X 40 objective with the help of 1 mm² reticule which was calibrated with 1 mm stage micrometer scale. Only complete spherical or round shaped visible glomeruli were counted.[8] The number of glomeruli was manually counted from five randomly selected fields per section. Each count of the number of glomeruli per section was then added up with its succeeding count for obtaining the mean number of glomeruli per section. The sum total of three sections (anterior, middle and posterior) yielded the mean number of glomeruli to be used for statistical analysis. The diameter of the glomeruli was measured at X 40 objective using a precalibrated micrometer.[20] The maximum diameter of ten glomeruli was measured from different locations in the olfactory bulb for calculating the mean diameter of the glomeruli.

STATISTICAL ANALYSIS

The data regarding the number and diameter of glomeruli were analyzed by One way ANOVA followed by Tukey test. The significance was set at $p< 0.05$. The difference between genders for these parameters was analyzed by independent "t" test. The data was analyzed using SPSS version 13.0.

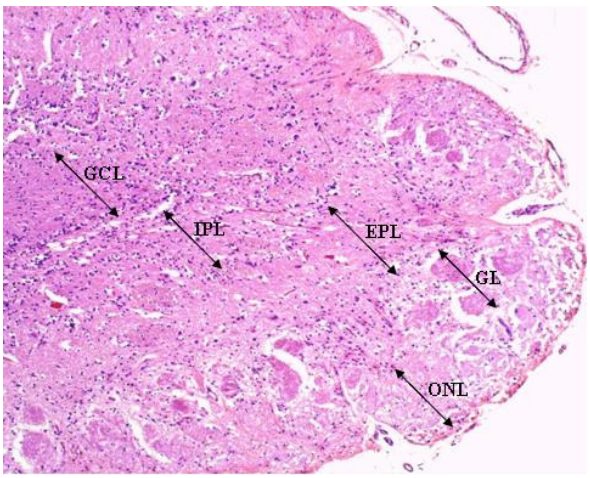

Fig. 2: *Human olfactory bulb (Group I) showing an overview of it's histological layers. Olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), internal plexiform layer (IPL), and granular cell layer (GCL). H and E. X 40.*

RESULTS

Light microscopy revealed that the human olfactory bulb consisted of six distinct layers. From surface inwards these were: olfactory nerve fiber, glomerular, external plexiform, mitral cell, internal plexiform and, granular cell layers. The glomeruli occupied a distinct position in the superficial layer of the olfactory bulb, forming one to three rows internal to the olfactory nerve layer (Fig. 2). Glomerular layer appeared to be the thickest layer of the olfactory bulb; glomeruli were round or oval masses of varying diameters. The olfactory nerve axons were seen terminating in the olfactory glomeruli (Fig. 3).

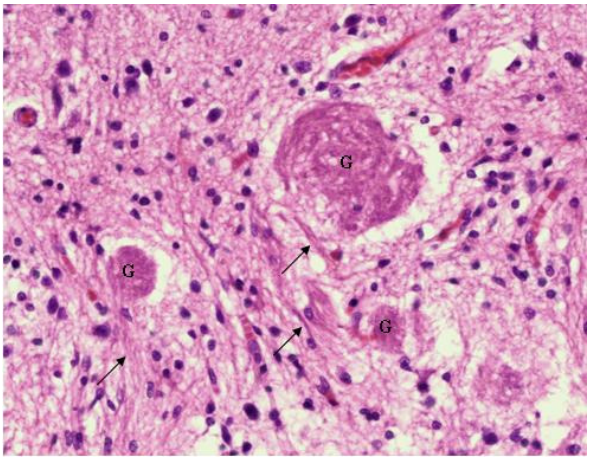

Fig. 3: *Human olfactory bulb (Group I) showing glomeruli (G) and olfactory nerve fibers (arrows). H and E. X 400.*

Age-related changes in number of glomeruli in males and females:

In males, mean number of the glomeruli in group III significantly decreased (Fig. 4) as compared to group I (Fig. 2). In few places the glomerular layer appeared very thin and lacked glomeruli (Fig. 5). In females groups IV, V and VI, no difference was observed histologically in the structure of the olfactory bulbs when compared to those in males. In group VI few glomeruli were observed in the glomerular layer.

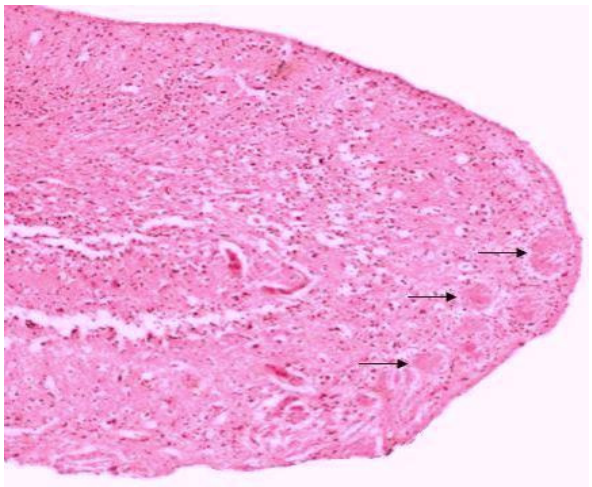

Fig. 4: *Human olfactory bulb (Group III) showing glomeruli (arrows) much less in number as compared with group I (Fig. 2). H and E. X 40.*

ANOVA followed by Tukey Post-Hoc test showed that there was significant ($p < 0.05$) age-related decrease in the mean number of glomeruli of olfactory bulb among groups I, II, and III of males (Fig. 6) and groups IV, V, and VI of females (Fig. 7).

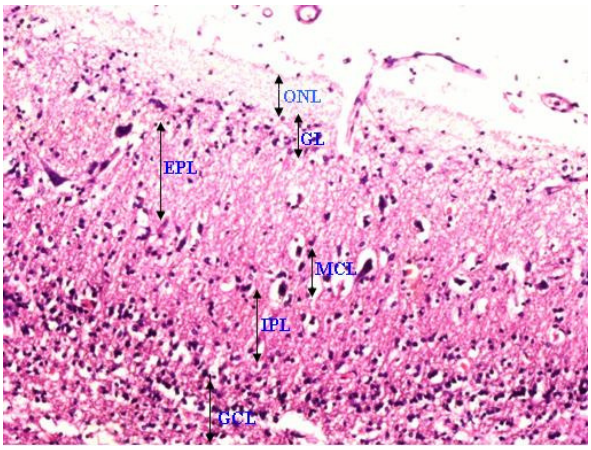

Fig. 5: *Human olfactory bulb ((Group III) showing olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), and granular cell layer (GCL). In glomerular layer glomeruli were not visible. H and E. X 200.*

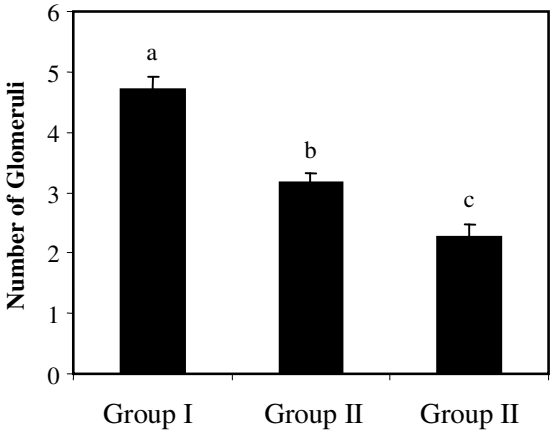

Fig. 6: *Mean number of the glomeruli in the males belonging to different age groups. The bars with different superscript are significantly different (ANOVA; Tukey) from each other.*

Diameter of the glomeruli in males and females:

ANOVA showed that there was significant age-related decrease in the mean diameter of the glomeruli among the groups I, II, and III. Multiple comparison between groups showed that the decrease in the mean diameter of the glomeruli was statistically significant ($p < 0.05$) between the groups I and III, and groups II and III (Fig. 8). In females, there was no difference between groups IV and V while between the groups IV and VI, and groups V and VI, the decrease in the mean diameter of the

glomeruli (Fig. 9) was statistically significant ($p < 0.05$).

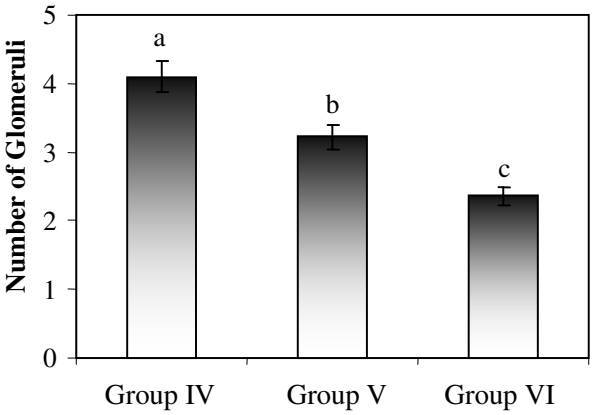

Fig. 7: Mean number of the glomeruli in the females belonging to different age groups. The bars with different superscript are significantly different (ANOVA; Tukey) from each other.

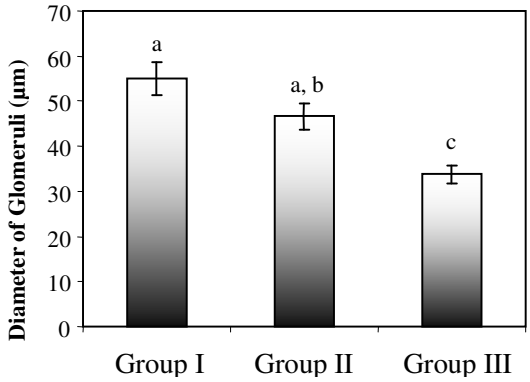

Fig. 8: *Mean diameter of the glomeruli in males belonging to different age groups. The bars with superscript "a" and "c" and, "b" and "c" are significantly different (ANOVA; Tukey) from each other.*

Gender related differences:
Number of glomeruli:

Comparison of age groups 20 to 39 years showed that the males had greater number of the glomeruli as compared to those in the females; the difference was, however, not statistically significant ($p > 0.05$). In the age group 40 to 59 years, and 60 years and above the females showed greater number of glomeruli, but the difference was also not statistically significant ($p > 0.05$).

Diameter of the glomeruli:

Comparison of age groups 20 to 39 years, 40-59 years and 60 years and above showed that the females had increased diameter of the glomeruli as compared with males, the difference was however, not statistically significant ($p > 0.05$).

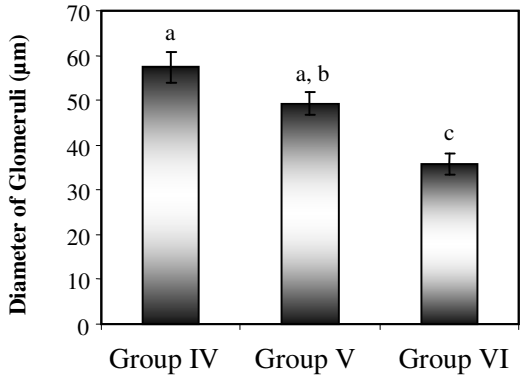

Fig. 9: *Mean diameter of the glomeruli in females belonging to different age groups. The bars with superscript "a" and "c" and, "b" and "c" are significantly different (ANOVA; Tukey) from each other.*

DISCUSSION

Earlier histological studies had shown that there was an age-related tendency to lose olfactory bulb neurons.[13,19] Women demonstrated better olfactory abilities than men and had less age-related decline in olfactory abilities.[21]

Liss[22] examined olfactory bulbs of humans ranging from 18 months to 80 years of age and concluded that no age-related differences were discernable. Liss and Gomez[23] reported that the olfactory bulbs and tracts from persons over 70 years of age exhibited moderate loss of neurons and nerve fibers. Hinds and McNelly[24] measured the volume of olfactory bulb layers in rats at 3, 12, 24, 27 and 30 months of age. A developmental increase in the volume of olfactory bulb layers was noted during the first 24 months. The period from 24 to 30 months, however, showed a decrease in the volume of all cell layers of the olfactory bulb.[24] Structural changes in the human olfactory bulb appear with advancing age. In general, the thickness of all the layers decreased with age**.** The largest decrease in the thickness was between the 3rd and 6th decades compared to the 6th versus 10th and 11th decades. Between the ages of 25 to 60 years and 60 to 90 years the decrease in the bulb volume was 13% and 15% respectively.[13]

Smith[25] examined the number of olfactory nerve fibers reaching the glomeruli in 205 human olfactory bulbs procured at autopsies from 121 individuals. He found a considerable age-related decrease in the number of fibers and alteration in the shape of glomeruli which, with the nerve fiber loss

became "motheaten" in appearance.[25] In an another study, the glomeruli Population declined at an approximate rate of about 10% per decade until only 25% of the glomeruli remained intact. Considerable loss of glomeruli (40% to 50%) occurred during the 4th to 6th decades of life.[19] Similarly, reduction in the number of the human olfactory glomeruli occurred with age.[26] Considering the fact that the glomeruli occupy a significant portion of olfactory bulb volume, it would be expected that the loss of a significant portion of glomeruli would appreciably reduce the volume of the olfactory bulbs as a whole with the advancing age.

The present study showed marked age-related decrease in the mean number of glomeruli and their diameter, occurring gradually with advancing age (Table 1) in both male and female persons and the difference was found to be statistically significant (Figs. 6-9).

Table 1: *Comparison of the number of glomeruli and their diameter (%) among different age groups.*

Sex	Groups Compared (Age in years)	Decrease in number of Glomeruli (%)	Decrease in Diameter of Glomeruli (%)
Male	I (20-39) to II (40-59)	48.73	17.89
	II (40-59) to III (60 and above)	39.82	37.93
	I (20-39) to III (60 and above)	107.96	62.62
Female	IV (20-39) to V (40-59)	27.32	16.58
	V (40-59) to VI (60 and above)	36.44	38.09
	IV (20-39) to VI (60 and above)	73.72	61

Microscopy of the human adult olfactory tissues revealed varying degrees of degeneration of the olfactory neuroepithelium. Complete depletion of the olfactory receptor cells were reported to occur frequently in the adult olfactory tissues.[27] The degeneration of the olfactory neuroepithelium is a usual phenomenon in humans and may occur with or without exposure to infection or toxic substances.[28] The number of olfactory fibers in the bulb was reported to decrease at an approximate rate of 1% per year throughout life.[25, 29] Although the causes of this change in olfactory bulb are not well understood, there is ample evidence that anatomical or functional changes in the olfactory epithelium can affect the olfactory bulb with reduction of glomeruli; this may, infact, be due to secondary degeneration of the olfactory receptor neurons in the olfactory neuroepithelium.[14,23] This is possibly the cause of age related attritional responses of olfactory receptor neurons to the odourants, altering olfactory performance.[30]

In the present study, no significant gender related differences in the olfactory bulb were observed. The gender related differences were calculated between the corresponding male and female groups according to age (ie., I and IV, II and V, and III and VI). Comparison between groups I and IV revealed that the males had greater number of glomeruli and less diameter of the glomeruli, as compared to the females. Comparison of groups II and V and, groups III and VI revealed that the males had less number and diameter of glomeruli as compared to the females. Smith[25] after studying 205 olfactory bulbs of males and females, concluded that sex differences were not apparent, a result corroborated by our data also.

It is **concluded** that the results of this study suggest that the glomeruli of the olfactory bulb decrease with age and there is no gender related difference in the population in the present study. Our findings have relevance in practical or applied settings as decline in number of glomeruli will decrease the olfactory ability, attenuating ability to detect olfactory stimuli, which in specific instances may produce serious consequences, i.e. exposure to toxic gases and fumes may be detected with difficulty.

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