

Localization and Distribution of Human Olfactory Mucosa in the Nasal Cavities

Localização e Distribuição da Mucosa Olfactiva Humana nas Fossas Nasais



Pedro ESCADA^{1,2}

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ABSTRACT

Introduction: The exact distribution of the human olfactory mucosa can only be determined in studies that evaluate the entire olfactory region. The purpose of this study is to determine the distribution of human olfactory mucosa in the nasal cavities, by performing the histological analysis, by light microscopy, of anatomical specimens of the olfactory region obtained from cadavers.

Material and Methods: The specimens were taken during the autopsy of fresh cadavers. In each of the specimens, the distance between the cribiform plate and the lower limit of the olfactory region was determined in three different locations of the septal and lateral walls.

Results: Of the 230 anatomical specimens available, 217 were excluded for medical or technical reasons. Morphometric studies were performed on 13 specimens (total 156 measurements). The lower limit of the olfactory mucosa in the nasal septum was 15.9 ± 3.2 mm, $15.3 \text{ mm} \pm 3$ and 16 ± 2.8 mm in the anterior, middle and posterior olfactory regions. The lower limit of the olfactory mucosa in the turbinate wall was 15.3 ± 2.4 mm, 14.8 ± 2.3 mm and 12.3 ± 1.9 mm in the equivalent regions. The minimum value observed was 12 mm.

Conclusions: The olfactory mucosa extends through the upper and middle turbinates and the confronting nasal septum in a minimum distance of 12 mm and that may exceed 16 mm. Knowing the exact distribution of the olfactory mucosa can guide the collection of this tissues in humans, for diagnostic or therapeutic purposes.

Keywords: Anthropometry; Cadaver; Nasal Septum; Olfactory Mucosa/anatomy and histology; Olfactory Receptor Neurons; Tissue and Organ Harvesting

RESUMO

Introdução: A distribuição da mucosa olfactiva humana só pode ser determinada em estudos que avaliem a totalidade da região olfactiva. O objectivo deste trabalho é determinar a distribuição da mucosa olfactiva humana a partir do estudo histológico, por microscopia óptica, de peças anatómicas da região olfactiva obtidas do cadáver.

Material e Métodos: Utilizaram-se peças anatómicas da região olfactiva colhidas durante a autópsia de cadáveres recentes. Em cada uma das peças foi determinada a distância entre a lâmina crivosa e o limite inferior da região olfactiva em três localizações diferentes da parede septal e da parede lateral.

Resultados: das 230 peças anatómicas disponíveis, 217 foram excluídas por razões clínicas ou técnicas. Realizaram-se estudos morfológicos em 13 peças num total de 156 medições. O limite inferior da mucosa olfactiva no septo nasal estava a $15,9 \pm 3,2$ mm, a $15,3 \pm 3$ mm e a $16 \pm 2,8$ mm nas porções anterior, média e posterior da região olfactiva. O limite inferior da mucosa olfactiva na parede turbinal estava a $15,3 \pm 2,4$ mm, a $14,8 \pm 2,3$ mm e a $12,3 \pm 1,9$ mm nas mesmas localizações. O valor mínimo observado foi de 12 mm.

Conclusões: A mucosa olfactiva estende-se pelo corneto superior e médio e pelo septo nasal confrontante numa distância que nunca é inferior a 12 mm e que pode ultrapassar os 16 mm. O conhecimento da distribuição exacta da mucosa olfactiva nas fossas nasais pode ser útil para orientar a colheita em seres humanos, com propósitos diagnósticos ou terapêuticos.

Palavras-chave: Antropometria; Cadaver; Colheita de Tecidos e Órgãos; Mucosa Olfactiva/ anatomia e histologia; Neurónios Receptores Olfactivos.

INTRODUCTION

Olfactory mucosa histology

The human olfactory mucosa is a pseudostratified columnar epithelium with a highly cellular lamina propria (Figure 1).¹

This epithelium has four different cell types: ciliated olfactory receptors, supporting cells, microvillar cells and basal cells.^{2,3}

Olfactory receptors are bipolar neurons from which a single dendrite extends to the olfactory neuroepithelium surface and one axon to the olfactory bulb. Bipolar neuron nuclei are located at all levels of the epithelium thickness,

the oldest cells' nuclei located closer to the epithelium surface. The nuclei variable configuration explains this typical pseudostratified pattern.

The bipolar neuron dendrite has a spindle-shaped tip, known as the olfactory vesicle. The olfactory vesicle extends towards the epithelial surface (Figures 2a and 2b), containing non-motile cilia with membrane receptors to which the odour molecules attach (this aspect is only observed through electron microscopy).

Each axon of the 6 to 20 million bipolar cells crosses the basement membrane towards the lamina propria, joins

1. Serviço de Otorrinolaringologia. Hospital de Egas Moniz. Lisboa. Portugal.

2. Faculdade de Ciências Médicas. Universidade Nova de Lisboa. Lisboa. Portugal.

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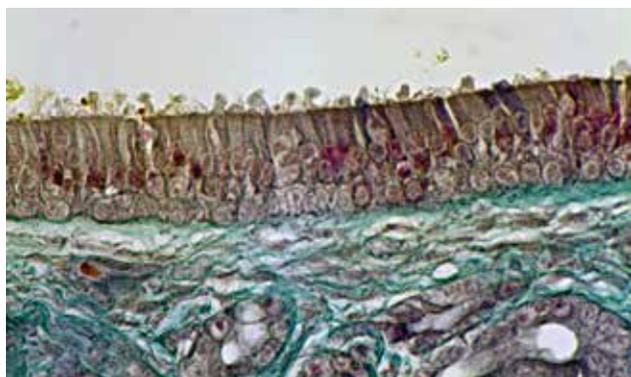
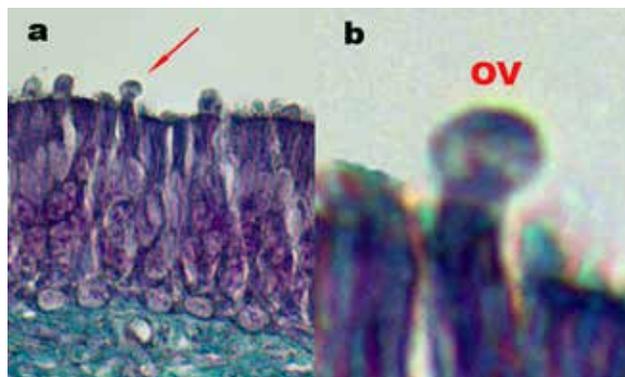


Figure 1 - Original optical microscopy image of the olfactory mucosa (cadaver specimen, Masson's trichrome, 200X original magnification). Pseudostratified epithelium. Thin basement membrane and cell-enriched lamina propria.



Figures 2a and 2b - Original optical microscopy image of the olfactory neuroepithelium (cadaver specimen, Masson's trichrome, 400X original magnification). The olfactory neuroepithelium surface is characterized by the presence of bipolar neurones with dendritic bulb-shaped projections, called the olfactory vesicles (b = detail of a; OV = olfactory vesicle).

together to form fascicles and nerves and passes through the 15 to 20 holes of each cribiform plate, forming a synapse within the olfactory bulb.³

The supporting cells, which are the prevailing cells, surround the bipolar cells contributing to the regulation and maintenance of the ionic environment and in this way favouring olfactory transduction.⁴

The microvillar cells, only identifiable by electron microscopy, were described for the first time in 1982 and were considered a second class of morphologically distinct receptor cells in the olfactory mucosa.⁵

The basal cells, the only kind of cells that do not extend towards the epithelial surface and lie over the basement membrane, are recognized as a different population of cells from the olfactory epithelium stem cells, with a capacity to continuously regenerate the affected olfactory cells throughout the life of the individual.⁶⁻⁸ They have auto-renovation and multipotent properties and are experimentally capable of differentiating into other cells, both neural and non-neural.^{1,9-11}

The lamina propria contains fascicles of axons, blood vessels, connective tissue and Bowman glands.¹² The axons of olfactory neurons are wrapped, in its transition from the olfactory neuroepithelium towards the olfactory bulb, by a unique glial cell lineage: the olfactory ensheathing cells.¹³

The Bowman's glands are tubuloalveolar glands, consisting of secretory acini arranged in circles and excreting its products through a duct that crosses towards the olfactory epithelium (Figures 3a and 3b).¹⁴ The products secreted by these glands towards the mucosal layer of the olfactory are essential for olfactory transduction.

Distribution of the olfactory mucosa in the nasal cavities

Human distribution of olfactory mucosa in nasal cavities remains to be adequately documented.^{1,15} The existing studies about olfactory mucosal structure are based on olfactory mucosa biopsies, are very limited in number and only performed for research purposes, aiming primarily to

establish the correlation between the observed structural and histopathological changes and the nature or degree of olfactory dysfunction.¹⁶ More recently, the olfactory mucosa has attracted researcher's attention, as it may be used as an early marker for neurodegenerative disorders and as a multipotent neural stem cell source, with application in regenerative medicine.¹⁷⁻²¹ We should remark, within this scope, the basic research and clinical research works of Portuguese researcher Carlos Lima regarding the use of olfactory mucosa in the treatment of chronic spinal cord traumatic lesions, in which the author of the present work participated,^{15,19-26} or the more recent animal research works in dogs, largely divulged in scientific community and in the media.²⁷

In 1892, von Brunn made measurements in the olfactory region in two post-mortem cases, showing areas of 307 and 238 mm² (Case 1: 133 mm² in nasal septum and 174 mm² in the lateral wall; Case 2: 99 mm² in nasal septum and 139 mm² in the lateral wall).²⁸ From the figure drawn by Lang, based on von Brunn measurements, we understand that the olfactory region extends to a position lower than the plane of the sphenoid sinus *ostium* and that it is distributed in areas of the middle turbinate,²⁹ contradicting the assumption sometimes made that the olfactory mucosa in the lateral wall would only involve the upper turbinate.

Many subsequent studies also confirm that the olfactory mucosa has a lower and more anterior distribution than that which is traditionally considered. In one of these studies, Leopold et al used electro-olfactogram (EOG) and localized biopsies to identify the presence of olfactory mucosa near the anterior attachment of the middle turbinate and in front of this insertion, in the medial wall, as well as in the lateral wall of the nasal cavity.³⁰ Restrepo et al collected olfactory receptor neurons in biopsies carried out in the nasal septum opposed to the upper portion of the middle turbinate,³¹ and Féron et al demonstrated the presence of olfactory epithelium distributed in regions of the middle turbinate in more than 50% of 71 biopsy specimens collected from healthy volunteers.³² Similar results have been obtained

by Rawson et al, who obtained morphologically identifiable olfactory neurons from the nasal septum opposed to the upper portion of the middle turbinate and in the middle turbinate itself.³³ The same authors observed that in the obtained biopsies, they were able to produce, in the most part of the specimens, olfactory neurons responsive to odorous substances.³⁴ Immunochemical studies carried out by Nibu et al have also confirmed that the olfactory mucosa is present in the lining epithelium of the lower portion of the middle turbinate.³⁵

Examination of the olfactory mucosa in nasal cavities

Most of the studies carried out to study and to identify the olfactory mucosa in humans were based on biopsies. Interestingly, the authors who developed the olfactory mucosa endoscopic biopsy technique recognized at the same time their insufficiency to achieve studies aiming to obtain mapping of the olfactory mucosa in the nasal cavities.³⁶ According to these authors, the exact distribution of the olfactory mucosa in nasal cavities could only be obtained in cadaver studies as the only method, by technical and ethical reasons, that allowed for the analysis of larger quantities of tissue or anatomical specimens that included the total olfactory region.

Until now, the only published studies in which more extensive cadaver anatomical specimens were used, were those by Paik et al and by Nibu et al.^{35,37} In both studies, anatomical sampling was carried out through an intracranial approach, upon craniotomy and brain and dura mater removal. The specimen included the cribriform plate, the nasal septum and upper and middle turbinates, removed as a whole using circular saws.

In the first study, 12 specimens were collected but in only three of them the epithelium was kept preserved and could be analysed. The donors were individuals with no past or present rhino-sinus pathology, with 34, 57 and 82 years old, the last one being a female. The technique used to visualize the olfactory mucosa (neuroepithelium) was optical microscopy of silver-stained samples by the Bodian

method. The criteria for identification were the presence of bipolar cell dendrites (olfactory vesicles) and the absence of respiratory cilia. The results were the identification of olfactory mucosa in all three specimens, in a medium vertical extension of 14.3 mm of the olfactory region (from the cribriform plate). The presence of respiratory epithelium areas within the olfactory neuroepithelium was identified, but only in a relevant form in the 82-year old patient.³⁷

In the second study, the two cadaver specimens used were obtained less than 24 hours after death. No demographic or clinical data from donors was mentioned. The identification of the olfactory mucosa was made using immunohistochemical techniques with antibodies that mark the olfactory neuroepithelium. The results showed that the olfactory mucosa extends more inferior than it was previously established, comprising the lower portion of the middle turbinate in its area of distribution.³⁵

Identification of the olfactory mucosa

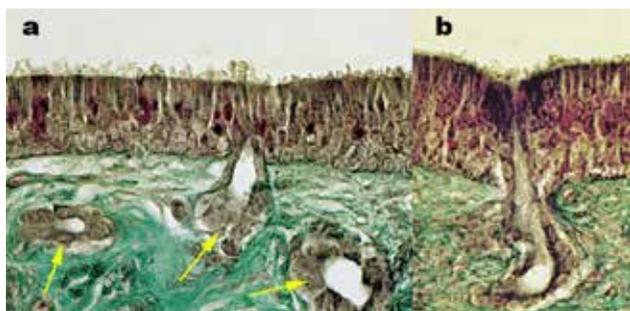
The different methods that have been used for human olfactory mucosa identification in specimens obtained from living donor biopsies include optical microscopy, transmission electron microscopy and immunohistochemical techniques.³⁸⁻⁴⁰ Nevertheless, electron microscopy and immunohistochemistry performed in a deceased body are affected by protein and neural tissue olfactory neuroepithelium degeneration occurring in the first hours after death.^{37,41,42} The study by Nibu et al using immunological markers used specimens from only two individuals and specimens were obtained less than 24 hours before death, a condition which is difficult to achieve in most of specimen collections from cadaver.

Due to all these reasons and until the present time, conventional optical microscopy is the most appropriate technique for identification of olfactory and respiratory mucosa in cadaveric specimens. The optical microscopy differentiation between olfactory and respiratory mucosa has been defined by Robert Kern in 2000.⁴³ According to this author, the criteria to distinguish olfactory from respiratory mucosa are as follows: both mucosa (olfactory and respiratory) consist of a pseudostratified epithelium; the olfactory mucosa has irregular cilia, no caliciform cells, a thin basement membrane and a cellular lamina propria and big and numerous nerve bundles; the respiratory mucosa has regular cilia, numerous caliciform cells, a thick basement membrane and a vascular lamina propria with scarce nerve bundles.

As described by Paik et al, normal olfactory mucosa identification must also be obtained through a positive identification of bipolar cells, which may be achieved by the presence of the olfactory vesicle; respiratory mucosa identification must be obtained by the identification of long, regular and uniformly distributed cilia in the cell epithelial surface.³⁷

Aim of the study

The aim of this work is to evaluate human olfactory



Figures 3a and 3b - Original optical microscopy image of the olfactory neuroepithelium (cadaver specimen, Masson's trichrome, 100X original magnification). a) Bowman's glands are spherical, with cells surrounding a central lumen (alveolar portion) a) and b) the Bowman's glands cross the olfactory neuroepithelium (tubular portion), opening in a gap located in its surface (arrows = alveolar portion of the Bowman's glands).

mucosa distribution in nasal cavities. This study was performed through histological examination, with optical microscopy, of anatomical parts from the olfactory region of human cadaver body parts.

MATERIAL AND METHODS

Obtaining anatomical specimens

Anatomical specimens of the olfactory region were selected from the human olfactory organs tissue bank at the Unidade Funcional de Neuropatologia - Hospital de Egas Moniz. Histopathological analysis was carried out at the Unidade de Microcirurgia – Centro Hospitalar Lisboa Ocidental.

Specimens were collected during clinical or forensic autopsy performed in recently deceased bodies. Specimen sampling was preceded by all necessary institutional authorizations, in accordance with current legislation and the present study was performed upon approval of the Ethics Committee of Centro Hospitalar de Lisboa Central. Most specimens were collected by Dr. Carlos Lima between 1987 and 1994.

The technique used for collecting anatomical specimens of the olfactory region was the intracranial approach. The upper surface of the anterior cranial fossa was exposed upon craniotomy and the brain and dura mater removed in addition to the olfactory bulb section. The specimens included the base of the skull and the nasal structures included in the cutting planes were obtained with the use of circular saws: on the foreground, sections were performed in front of the anterior limit of the cribiform plate and of the crista galli; on the background, sections were performed immediately behind the posterior limit of the cribiform plate at the anterior limit of the sphenoid planum; laterally, sections were performed one centimeter outside the lateral limit of the cribiform plate, in order to include, in the specimen, the full insertion of the middle and upper turbinates at the base of the skull.

Specimens were fixed in a 10% tamponated formalin solution and then placed in a decalcifying solution for 24 hours. After dehydration they were stored in paraffin. In addition to the archive of paraffinated blocks with the anatomical specimens, the anatomical specimen bank also keeps a record of donors characteristics, including demographic and clinically available data.

Selection of anatomical specimens

At first, the anatomical specimen donor clinical records were checked, in order to exclude those with any disorder inducing olfactory neuroepithelial pathological changes. Exclusion criteria were as follows: any known olfactory disorder, rhinitis and rhinosinusitis, nasal or skull injuries, nasal tumors or surgeries, neurovegetative disorders and specimens from individuals over 50 years of age 44- 47. The exclusion criteria based on age were established on the assumption and on the established evidence that with ageing, the olfactory mucosa is gradually replaced by respiratory mucosa.^{1,37,48,49}

Processing and observation of sections

The paraffinated blocks were cut in three different sections following the anterior, the middle and posterior portions of the specimen. All 3µm sections were performed following a cross-sectional direction of the block, and the sections followed a Hematoxylin-Eosine (H&E) and Masson's trichrome staining protocol.

The sections were observed using an optical microscope Olympus®, model CX40, with 40X, 100X, 200X, 400X and 600X magnifications. A Meiji® microscope, model MX5300H was used for the image record, with a connected digital camera Deltapix® model Infinity X equipped with the Deltapix Viewer Professional 1.7 software.

The first observation of the sections, performed in small magnification (40X), was intended to check the correct section orientation and specimen integrity. The quality of the histological technique was also evaluated, if necessary, with higher magnifications. All three sections of each anatomical specimen were observed.

Sections that allowed a positive identification of a bilateral olfactory region with an extension of at least 10 to 15 mm below the cribiform plate were selected. The olfactory region identification was based on the visualization of the most important anatomical features: cribiform plate; nasal septum and upper or middle turbinates. The histological technique quality was evaluated using parameters such as chromatin aggregation, cellular volume reduction, loss of intercellular contact and loss of microscopic resolution.⁵⁰

Olfactory region histological and morphometric analysis

Mucosa of both olfactory regions (right and left) in each section was observed at the microscope in all its length and was defined as olfactory or respiratory, according to the above mentioned criteria. The prevailing criteria in the differentiation between olfactory and respiratory mucosa were: the presence of the irregular cilia or of the dendritic ending (olfactory vesicle) of bipolar cells considered the specimen as olfactory mucosa while the uniform distribution cilia at cell's epithelial surface was indicative of the presence of respiratory mucosa.

In each observation, the distance between the cribiform plate and the lower limit of the olfactory mucosa (i.e. the transition from olfactory to respiratory mucosa) was determined and recorded (Figure 4) in both walls: middle (nasal septum) and lateral (middle or upper turbinate). The measurements were obtained with the help of a millimeter scale inserted in the slide and visualized in low magnification.

It must be mentioned that the different steps for tissue histological processing, in particular the paraffination process, reduces the size of structures when compared to their *in vivo* dimension.⁵⁰⁻⁵² In what concerns the olfactory region processing, this reduction has already been estimated at approximately 30% by other authors.³⁷ As such a possible error in our study would occur were we to estimate the olfactory region by default, which may be safer

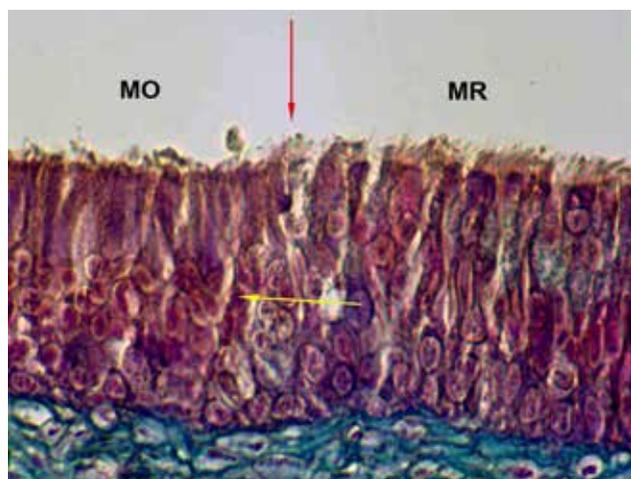


Figura 4 - Imagem original de microscopia óptica da mucosa da região olfactiva (amostra de cadáver, Tricrômico de Masson, ampliação original de 400X). Observa-se neste caso uma transição nítida entre a mucosa olfactiva e a mucosa respiratória (seta vermelha). O epitélio olfativo é identificável pela sua característica distintiva mais importante, a presença de cílios irregulares e vesículas dendríticas, contrastando com o epitélio respiratório, que apresenta maior densidade de cílios uniformes e paralelos. Outro aspecto muito característico na diferenciação entre a mucosa olfactiva e a mucosa respiratória é a membrana basal, muito espessa na mucosa respiratória (barra amarela) e fina na mucosa olfactiva (seta amarela) (MO = mucosa olfactiva; MR = mucosa respiratória).

as the results of the study are aimed to predict the most correct location for obtaining olfactory tissue in the living individual.

Used images

All images used in this work have been specifically obtained and prepared for the particular purpose of the present work and obtained from the existing specimens kept in the abovementioned human anatomical tissue bank.

RESULTS

Selection of anatomical specimens and positive identification of the olfactory region

Following the criteria described, 52 out of a total of 230 surveyed specimens were selected for further study. The exclusion criteria applied to the remaining 178 specimens are presented in Table 1. The excluded specimens presented in the age exclusion criteria row were specimens which were not excluded due to any other criteria.

From the 52 selected, sectioned and stained anatomical specimens, 39 further specimens were excluded after low magnification observation of the section and section repetition for reconfirmation of the exclusion criteria. The results of this phase of the study are presented in Table 1. In the end, only 13 anatomical specimens were selected, considered suitable for olfactory mucosa mapping in serial histological sections.

Histological/morphometric analysis of the olfactory region

Table 1 - Specimens excluded for clinical and technical reasons.

Absence of demographic or clinical data	18
Generalized CNS Infections (including HIV)	86
Neurodegenerative disorders	40
Over 50 years of age	21
Rhinitis or sinusitis	13
Insufficient size specimen	18
Incorrect section	16
Incorrect histological technique	5
Total	217

Table 2 summarizes all observations and measurements, indicating gender and age of the individual corresponding to the evaluated specimen. The measurements carried out in the middle and lateral walls were recorded separately. Two measurements were made for each section in each wall, as the same section included the olfactory regions of both nasal cavities.

In 59% of the performed measurements in the septal wall and in 62% of those performed in the lateral wall of the olfactory region, the limits of the observable specimen in the section consisted of olfactory mucosa and therefore did not include the transition from the olfactory to the respiratory mucosa. The maximum limit of the olfactory mucosa included in the specimen was recorded in these cases and these measurements were presented by its value preceded by the sign \geq .

Changes in the mucosa, particularly in the epithelium, were observed in 13% of the performed measurements in the septal wall and in 18% of those performed in the lateral wall of the olfactory region, preventing a conclusive identification of the lining mucosa. These cases are presented in the table with an empty cell (*missing value*). The real measurements of the histological transition between olfactory and respiratory mucosa have been used to calculate the average values of olfactory mucosa distribution which was calculated using the observable limit of the specimen that included olfactory mucosa in the remaining cases.

The lower limit of the olfactory mucosa in the nasal septum, determined by histology from the position of the cribriform plate, has been found, on average, at 15.9 ± 3.2 mm in the most anterior portion of the olfactory region, at 15.3 ± 3 mm in the middle portion and at 16 ± 2.8 mm in the most posterior portion. The minimum value of the distance between the base of the skull and the olfactory to respiratory mucosa transition (12 mm) has only been recorded in four observations (5% of the measurements).

The lower limit of the olfactory mucosa in the upper and middle turbinates, determined by histology from the cribriform plate position, was found on average at 15.3 ± 2.4 mm in the most anterior portion of the olfactory region, at 14.8 ± 2.3 mm in the middle portion and at 12.3 ± 1.9 mm in the most posterior portion.

Table 2 - Distance between the cribriform plate and the lower limit of the olfactory mucosa.

Gender and Age	Septal wall (nasal septum)						Lateral wall (turbinates)					
	Anterior region		Middle region		Posterior region		Anterior region		Middle region		Posterior region	
M 29	≥ 13	≥ 13	14	≥ 15	13	≥ 17	≥ 16	≥ 13	≥ 12	≥ 15	≥ 11	≥ 9
M 45	-	-	≥ 14	≥ 14	17	15	≥ 14	≥ 16	≥ 14	≥ 13	≥ 12	≥ 11
M 27	≥ 21	≥ 21	≥ 17	-	≥ 17	≥ 17	≥ 16	≥ 17	-	≥ 14		≥ 14
M 42	≥ 13	12	≥ 11	≥ 11	≥ 16	≥ 16	-	12	14	13	≥ 12	≥ 10
M 27	≥ 21	20	17	≥ 18	≥ 13	≥ 13	≥ 14	≥ 13	17	≥ 16	-	-
M 39	13	16	-	-	12	≥ 14	15	≥ 16	-	≥ 13	12	-
F 37	15	≥ 16	≥ 18	17	≥ 17	15	≥ 14	≥ 16	≥ 15	≥ 14	≥ 11	≥ 13
M 26	19	≥ 21	≥ 20	16	≥ 18	≥ 18	19	≥ 17	≥ 17	16	≥ 12	≥ 14
F 18	-	≥ 12	-	12	≥ 12	≥ 12	≥ 13	-	15	-	-	≥ 12
M 38	≥ 13	≥ 13	≥ 11	-	≥ 18	14	15	≥ 13	-	≥ 11	≥ 18	14
M 39	≥ 16	15	12	≥ 13	-	-	-	15	12	15	≥ 12	-
M 29	≥ 14	≥ 14	≥ 16	≥ 16	≥ 18	17	≥ 18	≥ 22	≥ 14	≥ 16	-	≥ 11
M 28	16	≥ 18	≥ 21	19	≥ 22	≥ 22	≥ 12	16	≥ 21	19	≥ 13	≥ 13
<i>n</i> :	23		21		24		23		22		19	
Average and standard deviation:	15.9 ± 3.2		15.3 ± 3		16 ± 2.8		15.3 ± 2.4		14.8 ± 2.3		12.3 ± 1.9	
Minimum:	12		≥ 11		12		12		≥ 11		≥ 9	
Maximum:	≥ 21		≥ 21		≥ 22		≥ 22		≥ 21		≥ 18	

DISCUSSION

Comprehensive summary of the results

The histological and morphometric studies performed demonstrate the following extension of olfactory mucosa in the olfactory region of nasal cavities: in the septal wall, the lower limit of the olfactory mucosa at the nasal septum was found at 15.9 ± 3.2 mm, 15.3 ± 3 mm and 16 ± 2.8 mm from the base of the skull and in the lateral wall, this limit was found at 15.3 ± 2.4 mm, 14.8 ± 2.3 mm and 12.3 ± 1.9 mm from the base of the skull, respectively at the anterior, middle and posterior portions of the olfactory region.

These values show that the olfactory mucosa extends to a lower level than the plane defined by the position of the sphenoid *ostium*, as this structure is located at 10.3 ± 4.3 mm from the base of the skull.⁵³

Limitations of the study

Despite the study design using cadaver anatomical specimens, aimed to overpass the limitations already mentioned by other authors,³⁶ some difficulties have arisen.

A relevant percentage of the anatomical specimens could not be used, either due to the existence of pathologies producing changes in the olfactory mucosa, or due to technical reasons: difficulty in obtaining sections with an anatomical orientation adjusted to the objective of the study and poor histological quality in some of the preparations.

In some cases, specimen collection did not preserve the lower portion of the olfactory region. This was due to the collection technique, which did not include the transnasal section of the base of the septum, a step which was already proposed by other authors for a human whole olfactory organ removal.^{35,54} Nevertheless, we should remark that the establishment of an anatomical specimens tissue bank preceded the publication of the abovementioned works.

Finally, the results of our study are still undervalued due to the fact that we admittedly did not apply the correction factor resulting from the need to compensate the effects of tissue histological processing when we performed morphometric studies.³⁷

Comparison of the obtained results with those from other studies

As we already mentioned, pioneer studies performed more than 100 years ago and several publications in particular over the last decades, described the olfactory mucosa area as extending lower than the sphenoid *ostium* plane and with a distribution to lower and more anterior regions than it was previously established, including lower regions of the middle turbinate, areas of the confronting nasal septum and even areas of the nasal septum located in front of the anterior insertion of the middle turbinate.^{3,16,18,28,30-35,37,55-60} The results that we present confirm these findings.

Clinical application of the study results

Nasal cavity biopsy has been increasingly used as a mean to obtain olfactory tissue for diagnostic purposes or neural multipotent stem cell collection. The correct knowledge of the exact distribution of the olfactory mucosa in the nasal cavities will facilitate clinical application of those techniques that require biopsy and collection of this tissue.¹

CONCLUSION

The results of this study confirm the presence of olfactory mucosa in a well identified area of the olfactory region, which may also be recognized through measurements or using endoscopic identifiable surgical references, as the sphenoid sinus *ostium*.

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CONFLICT OF INTERESTS

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