The impact of Chronic Rhinosinusitis on olfaction (immunohistochemical study)

Thesis

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List of Abbreviations

Abb.	Full term

- *CRS……..chronic rhinosinusitis.*
- *CRSsp……..chronic rhinosinusitis without polyp.*
- *CRSwp……chrinic rhinosinusitis with polyp.*
- *OSN……olfactory sensory neurons.*
- *OE……..olfactory epithelium.*
- *OM……..olfactory mucosa.*
- *DAB……diaminobenzidine.*
- *ARS……acute rhinosinusitis.*
- *LMS……Lund Mackay score.*
- *ORN……..olfactory receptor neurons.*

Introduction

Chronic rhinosinusitis (CRS) is defined as "presence of two or more symptoms one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip) +/- facial pain/pressure +/- reduction or loss of smell for >12 weeks" (Fokkens et al., 2012).

Olfactory epithelium has a laminar organization composed of an apical layer of olfactory supporting cells, several layers of mature and immature olfactory sensory neurons underneath, and one or more lower layers composed of proliferating basal cells. Changes in the olfactory mucosa demonstrate an influx of chronic inflammatory cells with fibrosis and thickening of the lamina propria, normal pseudostratified, goblet cell hyperplasia, squamous metaplasia, and erosion (Karen K.et al.,2009).

CRS considered to be the most common cause of olfactory dysfunction and accounts for 14-30% of the cases . The incidence of olfactory dysfunction among the population is still a matter of debate. Some studies report an incidence of 1-3% of dysfunction among population (Caroline H et al., 2011).

On studying the pathogenesis of olfactory dysfunction in cases of CRS, some studies explained it by a conductive olfactory loss, caused by swollen or hypertrophic nasal mucosa or nasal polyps, inducing an impaired access of odorants to the olfactory cleft but have failed to prove this hypothesis, as there is only little correlation between nasal resistance and the degree of olfactory dysfunction (Cowart et al., 1992). In addition, results of surgical therapy, although improving the nasal patency, are sometimes uncertain when considering the olfactory dysfunction.

While some other studies have shown that the olfactory disturbance might also be explained by inflammatory process in the olfactory cleft . Biopsies of the olfactory neuroepithelium in patients suffering from CRS revealed inflammatory changes in the nasal mucosa (Hellings and Rombaux, 2009) (Konstantinidis et al., 2007). These biopsies have been performed using light and electron microscopy , as well as an immunohistochemical method (Yamagishi M. et al.,1988).

The human olfactory neuroepithelium is a region of the nervous system that supports ongoing neurogenesis. Nestin is an intermediate filament protein present in the basal region of the adult olfactory neuroepithelium in the zone that supports ongoing neurogenesis in the adult. It is expressed by various cell types during development. However, its expression is usually transient and does not persist into adulthood, except for neural precursors of the central nervous system so Nestin has been widely used as a marker for proliferating neural progenitor cells in the nervous system (Doyle et. al.,2001).

Studies revealed that nestin expression is not dependent on age, but rather appears to be related to the olfactory function, as a comparison with specimens obtained from patients suffering either from persistent anosmia or hyposmia suggests (Minovi et al.,2010).

AIM OF THE WORK

The aim of this study is to determine the impact of chronic rhinosinusitis on olfactory function based on clinical, radiological, histopathological and immunohistopathological studies.

Anatomy and Physiology

Anatomy of paranasal sinuses and nasal cavity:

The knowledge of the normal anatomy and physiology of the nasal cavity and paranasal sinuses is so important to understand the pathogenesis of any sinus disease. There are four pairs of sinuses named after the bones of the skull they pneumatize. They are the maxillary, ethmoid, frontal, and sphenoid sinuses (Arash et al, 2007).

The structure of the nose is designed to direct inspired air toward the olfactory epithelium, located superiorly and posteriorly within the nasal cavity. The paired nasal passages are divided in the midline by the nasal septum. Each lateral nasal wall is formed by up to four bony outgrowths or turbinates (generally inferior, middle, superior, and in some cases supreme). The nasal valve lies anteriorly at the vestibule of the nose and is formed by the lower border of the upper lateral cartilage, the septum, and the anterior portion of the inferior turbinate; this cross sectional area is the point of highest resistance of the respiratory tract. Airflow patterns in the nose are affected by anatomic and physiologic factors that may modify these structures. Alteration of the normal laminar airflow through the nose results in turbulence that may impair the direction of air superiorly toward the olfactory epithelium (Fig.1) (Patel and Pinto, 2014).

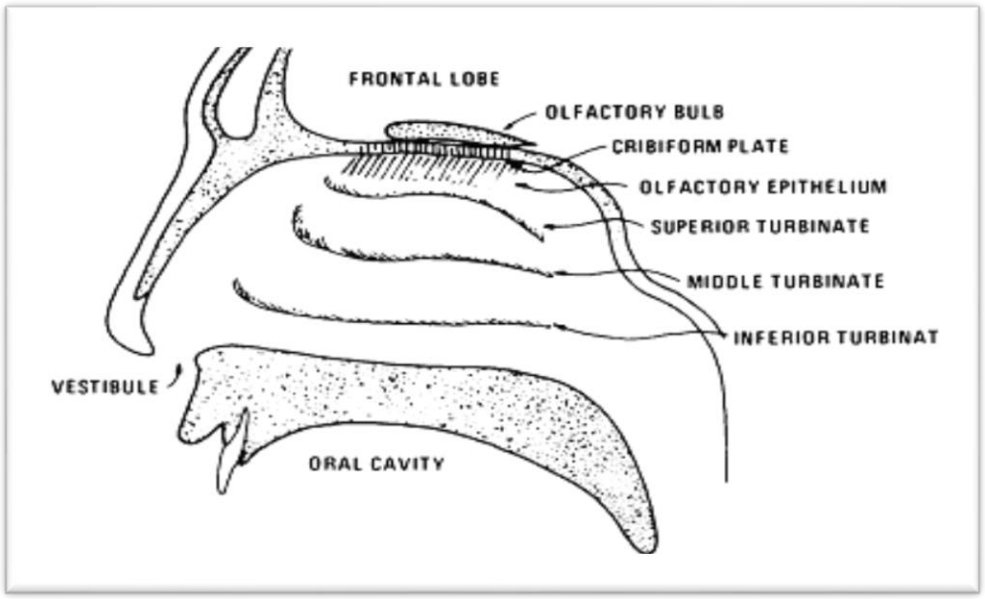

Fig 1. Midsagittal view of nasal cavity, showing location of olfactory structures and turbinates (Benignus and Prah., 1982).

The olfactory region consists of cilia projecting down out of the olfactory epithelium into a layer of mucous which is about 60 microns thick. This mucous layer is a lipid-rich secretion that bathes the surface of the receptors at the epithelium surface. The mucous layer is produced by the Bowman's glands which reside in the olfactory epithelium. The mucous lipids assist in transporting the odorant molecules as only volatile materials that are soluble in the mucous can interact with the olfactory receptors and produce the signals that our brain interprets as odor. Each olfactory receptor neuron has 8-20 cilia that are whip-like extensions 30-200 microns in length. The olfactory cilia are the sites where molecular reception with the odorant occurs and sensory transduction (i.e., transmission) starts (Fig.2) (Leffingwell, 2001).

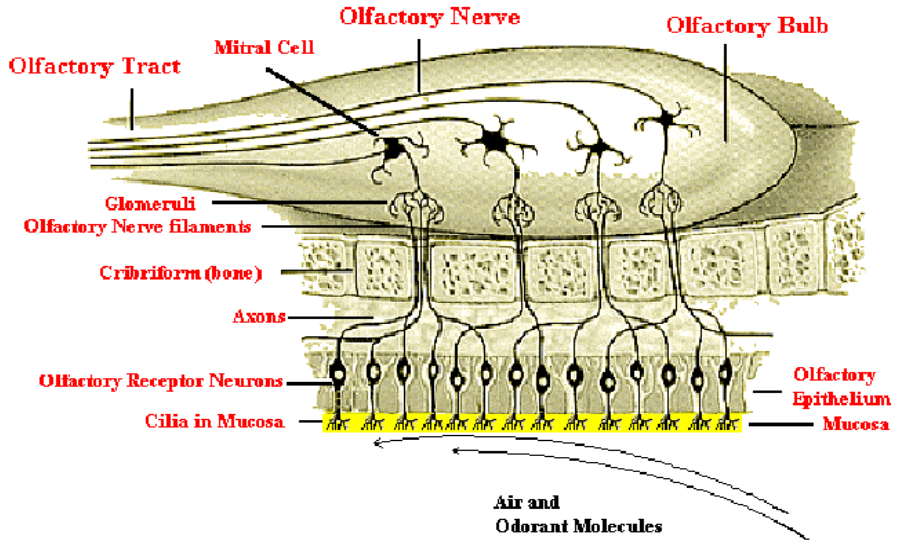

Fig 2: Olfactory mucosa (Leffingwell, 2001).

Olfactory epithelium has been thought to be a small area of about 2.5 square centimeters along the cribriform plate at the superior-most portion of the nose, medial to the superior turbinate and along this turbinate itself. However, more recent studies have revealed a more extensive distribution that extends further down the nose as far as the anterolateral middle turbinate and also inferiorly from the cribriform plate down to upper part of the septum (Kern, 2000).

Olfactory epithelium is much thicker (60-70 microm) than the surrounding respiratory epithelium (20-30 microm). The epithelium is pseudostratified columnar, and it rests on a vascular lamina propria (Leffingwell, 2001).

Approximately 10 to 20 million olfactory neurons within the olfactory epithelium are located among a variety of supportive cells (Fig.3). This pseudostratified columnar epithelium includes basal cells (that have been shown to function as stem cells that can give rise to all components of the

epithelium), Bowman's glands, microvillar cells, and sustentacular cells, which are thought to support olfactory neuron function. In rodents, at least two populations of basal cells have been morphologically defined which reside in the olfactory progenitor layer: the globose basal cells (GBCs) and the horizontal basal cells (HBCs). Contrary to rodents, in the human OE a morphological discrimination among basal cells is not possible. Several lines of evidence have demonstrated that it is Globose basal cells that proliferate to give rise to neurons in normal neuroepithelium (Minovi et al., 2010).

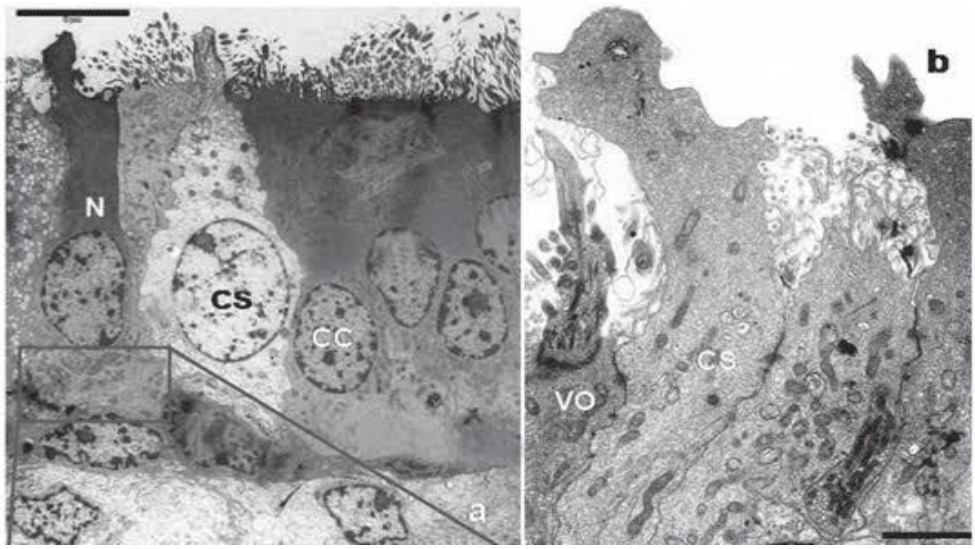

Fig 3. Electron microscopy of olfactory mucosa (a) olfactory neuron (N) sustentacular (CS) and ciliated (CC) cells in columnar distribution (Becker,1994).

Bowman's acini are exocrine and produce substances that are essential for olfaction. Key components of olfactory mucus are called odorant binding proteins that act to facilitate odorant receptor interaction (Pinto,2011).

Olfactory supporting cells and serous cells of the mucus glands were cytochrome 2A5-immunoreactive (cyp2A5-ir). Cyp2A5 plays an important role in the metabolism of airborne compounds found in the environment and its localization in the olfactory supporting cells provides protection for the underlying olfactory sensory neurons. Olfactory supporting cells were absent in eroded olfactory epithelium and absent in squamous-like olfactory epithelium, potentially leaving the underlying neuronal cell layers and lamina propria vulnerable to airborne toxicants and environmental insults. In addition, there is growing evidence that olfactory supporting cells play a role in the maintenance of the olfactory neurons (Chen et al.,2003).

It was suggested that olfactory supporting cells play a role in olfactory sensory neuron proliferation by ATP-induced neutrotrophic factor mediation (Jia & Hegg, 2008).

Olfactory marker protein-immunoreactive (OMP) mature olfactory sensory neurons were observed in the normal OM pattern. These cells exhibited a typical morphology with dendritic processes, cell bodies located below the olfactory supporting cells and axonal processes projecting through the basement membrane and into the lamina propria. Similar cellular morphologies were observed in goblet cell hyperplasia. In squamous metaplasia, OMP olfactory sensory neurons had abnormal morphology in which the olfactory sensory neurons lacked dendritic processes and were localized just above the basement membrane and/or in the apical squamous layers (Yee et al.,2009).