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Morphometric aspects of the human hypoglossal nerve in adults and the elderly

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Key words: hypoglossal nerve, aging, dysphagia.

Summary

Aim: Perform a morphometric analysis of the myelinic fibers of the right hypoglossal nerve, in two age groups; to verify quantitative changes as a result of the aging process. **Study design:** anatomic. **Material and Method**: A 1cm fragment of the right hypoglossal nerve was collected from 12 male corpses without any medical history of diseases such as: diabetes, alcoholism, and malignant neoplasia. The sample was divided in two groups: group with six corpses under sixty years old (adult), and another group with six corpses sixty years old or above (elderly). The material was fixed at 2.5% glutaldehyde and 2% paraformaldehyde solution; post-fixed at 2% osmium tetroxide; dehydrated with increasing ethanol concentrations, and included in epoxy resin. Semi-thin sections of 0.3µm were obtaining, colored in 1% toluidine blue, and evaluated with light microscope combined with image analyzing system. The following morphometric data were quantified: intraperineural transversal section area, number, and diameter of the myelinic fibers. **Results**: The intraperineural area of the hypoglossal nerve was similar in both age groups (p=0.8691). The average area in the adult group was 1.697mm2 and in the elderly group it was 1.649mm2. The total number of myelinic fibers of the hypoglossal nerve was similar in both age groups (p=0.9018). The adult group presented an average of 10,286 ± 2,308 myelinic fibers, and the elderly group presented an average of 10,141 ± 1,590 myelinic fibers. A bimodal distribution of the myelinic fibers was observed, with a significant peak on the 9µm fibers, and another smaller peak on the 2µm fibers. **Conclusion**: The intraperineural area and the total number of myelinic fibers of the right hypoglossal nerve are similar in both age groups.

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INTRODUCTION

The aging process triggers modifications in the human body that are responsible for many different types of clinical manifestations, represented in the upper aerodigestive tract as vocal disorders and swallowing disorders. Oropharyngeal dysphagia is a frequent symptom in the elderly, especially in men aged over 60 years and it is normally associated with increase in duration of swallowing oropharyngeal phase.[1]

Many different authors have demonstrated that the aging process is also related with reduction of pharyngeal and supraglottic sensitivity [2, 3] and it is considered a factor responsible for the onset of dysphagia, aspiration and repetitive pneumonia in the elderly, owing to reduction of reflexes that protect the lower airways. Other modification observed in the elderly is the delay in opening of upper esophageal sphincter [1, 4, 5] and reduction of cricopharyngeal muscle tone [5].

Little has been studied about the action of aging in muscles [6, 7] and cranial nerves [8-12]. A study carried out with the genioglossus muscle of rats demonstrated that there is reduction in number of neuromuscle joints in older rats. However, there was no difference in the nerve area responsible for innervation of the muscle [7]. Research studies involving the laryngeal muscle system presented results that suggested numeric reduction of muscle fibers of thyroarytenoid muscle, especially slow contraction muscle fibers [13], reduction of proteins responsible for muscle contraction [14], and increase in connective tissue or endomise [15, 16]. Other authors, based on electromyography assessment, presented results that suggested denervation of axonal lesion involving the motor control of larynx in the elderly, with consequent affection to contraction of laryngeal muscles [17]. Morphometric studies of laryngeal nerves in humans demonstrated reduction of the number of myelin fibers, especially small diameter fibers.[10,12]

The purpose of the present study was to carry out morphometric analysis of the number and diameter of myelin fibers of the right hypoglossal nerve, in two age groups (adult and elderly), to check quantitative modifications resulting from the aging process.

MATERIAL AND METHOD

The research project was approved by the Research Ethics Committee, Hospital Sao Paulo/ Federal University of Sao Paulo (UNIFESP). We collected 1cm fragments of right hypoglossal nerve from 12 cadavers submitted to autopsy in the Service of Death Verification - University of Sao Paulo, between June 2003 and November 2004. Fragments were collected between 9 and 18 hours after the death and analyzed by the Center of Electron Microscopy, UNIFESP.

We selected male copses without past history of diabetes, alcohol abuse, malignant neoplasm or sudden weight loss [18-20]. The sample was divided into two age groups: adult group (age below 60 years), comprising six corpses, and elderly group (age over 60 years), also comprising six corpses.

The fragment of right hypoglossal nerve was collected from the region after the crossing of the nerve with the internal and external carotid artery, after the emergence of the hypoglossal loop. The fragments were obtained by transversal section (perpendicular to the hypoglossal long axis) to enable quantification of the following morphometric data: area of transversal intraperineural section (area representing the number of myelin fibers), number and diameter of myelin fibers [21]. Fragments were fixed in a solution at 2.5% glutaldehyde and 2% formaldehyde at buffer solution of sodium cacodilate 0.1 M, pH 7.4 (modified by Karnovsky, 1965),[22] post-fixed in osmium tetroxide at 2% in buffer solution of sodium cacodilate 0.1M, pH 7.4, dehydrated in increasing concentrations of ethanol and included in resin type Araldite 502®.

The material was sectioned with ultramicrotome with glass knives to obtain super thin sections of 0.3

(ANOVA). We adopted the level of significance of

the hypoglossus nerve after passing through the hypoglossus canal on the skull base.[29]

Despite the fact that we did not observe modification in total number and in distribution of myelin fibers between adult and elderly subjects, the literature reports that some diseases involve the lower motor neuron, such as amyotrophic lateral sclerosis, leading to reduction in number of medium diameter myelin fibers (5-10mm), which results in tongue atrophy; this is one of the main signs of advanced stage of the disease.[29]

Aging seems to determine a selective loss of myelin fibers, and it is quite common in elderly to have reduction of the number of smaller diameter fibers of laryngeal nerves that are responsible for sensitivity [10,12] and by the motor innervation of slow contraction muscle fibers.[30] Schwann cells, from the myelin sheath, are responsible for nourishing the axon, and the thicker the myelin sheath, the more protected the axon. Given that the tongue muscle is comprised mainly by fast contraction muscle fibers [31,32], innervated by medium diameter myelin fibers (5-10

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