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Quantitative analysis of the terminal branches of facial nerve in fresh frozen head and neck specimens

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Background: The first aim of this study was the quantification of nerve fibres found in terminal branches of facial nerve and the second aim was the ultrastructural analysis of these terminal branches in order to observe their ultrastructural differences, if present. In the examination of literature; we could not find any studies related to this subject.

Materials and methods: Four fresh frozen head and neck specimens were used and the dissections were done bilaterally. Therefore; totally 8 samples were examined. The samples were prepared according to routine transmission electron microscopic tissue preparation technique. The semi-thin sections were examined under light microscope by camera lucida. In every sample, the quantitative analysis was performed in 5 different areas in an area of 0.01 mm^2 and statistical analysis was done. Secondly; the ultrastructural appearance of these terminal branches were examined under transmission electron microscope.

Results: In the quantitative analysis of terminal branches of facial nerve in an area of 0.01 mm^2; the least number of nerve fibres were found in temporal branches and the highest number were detected in cervical branches. In transmission electron microscopic examination, no significant difference was found in between these branches. In the statistical analysis; statistically significant differences were obtained in between the temporal and buccal, marginal mandibular, cervical branches; zygomatic and marginal mandibular, cervical branches; buccal and marginal mandibular, cervical branches; marginal mandibular and cervical branches.

Conclusions: These numerical data will have an importance during the nerve repair process of terminal branches of facial nerve in various injuries. (Folia Morphol 2014; 73, 1: 24–29)

Key words: facial nerve, quantitative analysis, ultrastructure, fresh frozen, human, head, neck

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This study was presented in the XIIth European Joint Congress of Clinical Anatomy in Lisbon, (June 26 – 29, 2013) as an oral presentation.

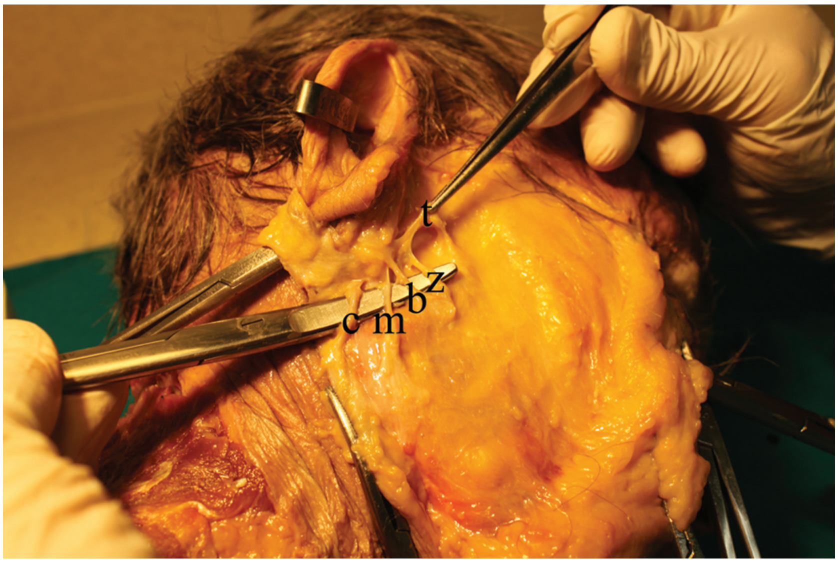

Figure 1. Photograph showing temporal (t), zygomatic (z), buccal (b), marginal mandibular (m) and cervical (c) branches of facial nerve.

INTRODUCTION

The facial nerve emerges from the base of the skull at the stylomastoid foramen and then enters the parotid gland. Within the substance of the gland; it branches into the temporofacial and cervicofacial trunks just behind the retromandibular vein. The trunks branch further to form a parotid plexus. Five main terminal branches which are the temporal, zygomatic, buccal, marginal mandibular and cervical rami arise from the plexus and diverge within the gland. These terminal branches leave the parotid gland by its anteromedial surface and they supply the muscles of facial expression [4, 17].

The terminal branches of the facial nerve are very thin and in formalin fixed cadavers, it is very difficult to find them. Secondly; for transmission electron microscopic examination of these branches; fresh or fresh frozen human samples are necessary. Therefore; in this study, they were examined in fresh frozen head and neck specimens. The first aim of this study was the quantification of nerve fibres found in the terminal branches of facial nerve and the second aim was the ultrastructural analysis of these terminal branches in order to observe their ultrastructural differences, if present. In the examination of literature; we could not find any studies related to this subject.

MATERIALS AND METHODS

In the study, 4 fresh frozen head and neck specimens were used and the dissections were done bilaterally. Therefore; totally 8 samples were examined from each terminal branch. Two of the head and neck specimens were male (ages 70 and 81 years) and 2 of them were female (ages 68 and 70 years). All procedures were approved by the Ethical Committee of Hacettepe University, Faculty of Medicine and the study was conducted in the gross anatomy dissection laboratory of Department of Anatomy, Faculty of Medicine, Hacettepe University.

Dissection

At the beginning of the dissection; a lazy S incision was made in the preauricular area. Following the incision; the skin and soft tissues were removed and the facial nerve trunk was observed. Then; the nerve trunk was followed and 5 terminal branches (temporal, zygomatic, buccal, marginal mandibular and cervical rami) were found when they were leaving the parotid gland by its anteromedial surface (Fig. 1). From this region; tissue samples were taken for quantitative analysis and transmission electron microscopic examination.

Tissue preparation

The facial nerve terminal branch samples were put into 2.5% glutaraldehyde for 24 h for primary fixation. Then, these samples were washed with Sorenson's Phosphate Buffer solution (pH: 7.4) and they were post-fixed in 1% osmium tetroxide for 2 h. After post-fixation, they were washed with the same buffer. Following this procedure; the tissue samples were dehydrated in increasing concentrations of alcohol

Table 1. The mean number of myelinated nerve fibres of temporal, zygomatic, buccal, marginal mandibular and cervical branches of facial nerve counted under 200 × magnification, in a constant area of 0.01 mm^2 using camera lucida (The myelinated nerve fibres were counted in 5 different areas in an area of 0.01 mm^2 during the quantitative analysis of each specimen and the mean values for each specimen were given)

Specimen no.	Temporal branch	Zygomatic branch	Buccal branch	Marginal mandibular branch	Cervical branch
1	37.4	37.6	39.6	45.2	50.6
2	38.4	39.4	40.0	44.4	49.8
3	38.6	37.2	39.8	46.0	50.0
4	38.6	39.8	39.8	40.4	52.6
5	38.6	38.2	38.8	45.6	49.8
6	38.2	39.4	38.6	43.8	49.4
7	35.8	36.6	39.8	45.0	49.4
8	37.8	39.2	39.6	41.0	51.6
Mean	37.93 ± 0.34	38.43 ± 0.42	39.50 ± 0.18	43.93 ± 0.75	50.40 ± 0.41

series. After dehydration, the tissues were washed with propylene oxide and embedded in epoxy resin embedding media.

Quantitative analysis

The semi-thin sections, about 2 μm in thickness, were cut with a glass knife on a LKB-Nova ultramicrotome (LKB-Produkter AB, Bromma, Sweden). These semi-thin sections were stained with methylene blue and they were examined under the camera lucida of a Nikon Optiphot (Nikon Corporation, Tokyo, Japan) light microscope by the same researcher for quantitative analysis.

The myelinated nerve fibres of the temporal, zygomatic, buccal, marginal mandibular and cervical rami were counted in areas of 0.01 mm^2 under 200× magnification by using a camera lucida. Parts of myelinated nerve fibres located at the lower and left hand edges were included in the counting, whereas those at the upper and right hand edges were not recorded [12–14]. From every specimen, one semi-thin section was taken by the ultramicrotome. In each semi-thin section, the myelinated nerve fibres were counted in 5 different areas in an area of 0.01 mm^2 during the quantitative analysis (Table 1). The statistical analyses were done by using Repeated Measures Analysis of Variance. Secondly, the ultrastructural appearance of these terminal branches were examined under transmission electron microscope.

Transmission electron microscopic examination

For transmission electron microscopic examination; trimming was done to the tissue blocks and their ultrathin sections which were about 60 nm in thickness were taken by the same ultramicrotome. These ultra-thin sections were stained with uranyl acetate and lead citrate and they were examined under Jeol JEM 1200 EX (Tokyo, Japan) transmission electron microscope. The electron micrographs of the specimens were taken by the same microscope.

RESULTS

In the study, the terminal branches of facial nerve were examined in 8 specimens obtained from 4 fresh frozen human head and necks bilaterally (Figs. 2A–E). From every specimen, 1 semi-thin section was taken by the ultramicrotome and the myelinated nerve fibres were counted in 5 different areas in an area of 0.01 mm^2 during the quantitative analysis of each specimen. The mean numbers of myelinated nerve fibres of temporal, zygomatic, buccal, marginal mandibular and cervical branches of facial nerve counted under 200× magnification, in a constant area of 0.01 mm^2 using camera lucida are given in Table 1.

In the quantitative analysis of these terminal branches, the least number of myelinated nerve fibres in an area of 0.01 mm^2 were found in temporal branches and the highest number were detected in cervical branches (Table 1). Statistical analyses using Repeated Measures Analysis of Variance revealed that the cervical branch had the highest number of nerve fibres followed by marginal mandibular branch with statistically significant differences (all $p < 0.001$). Also, the buccal branch had more fibres than the temporal branch ($p = 0.008$). In the statistical analyses, there were significant differences between the

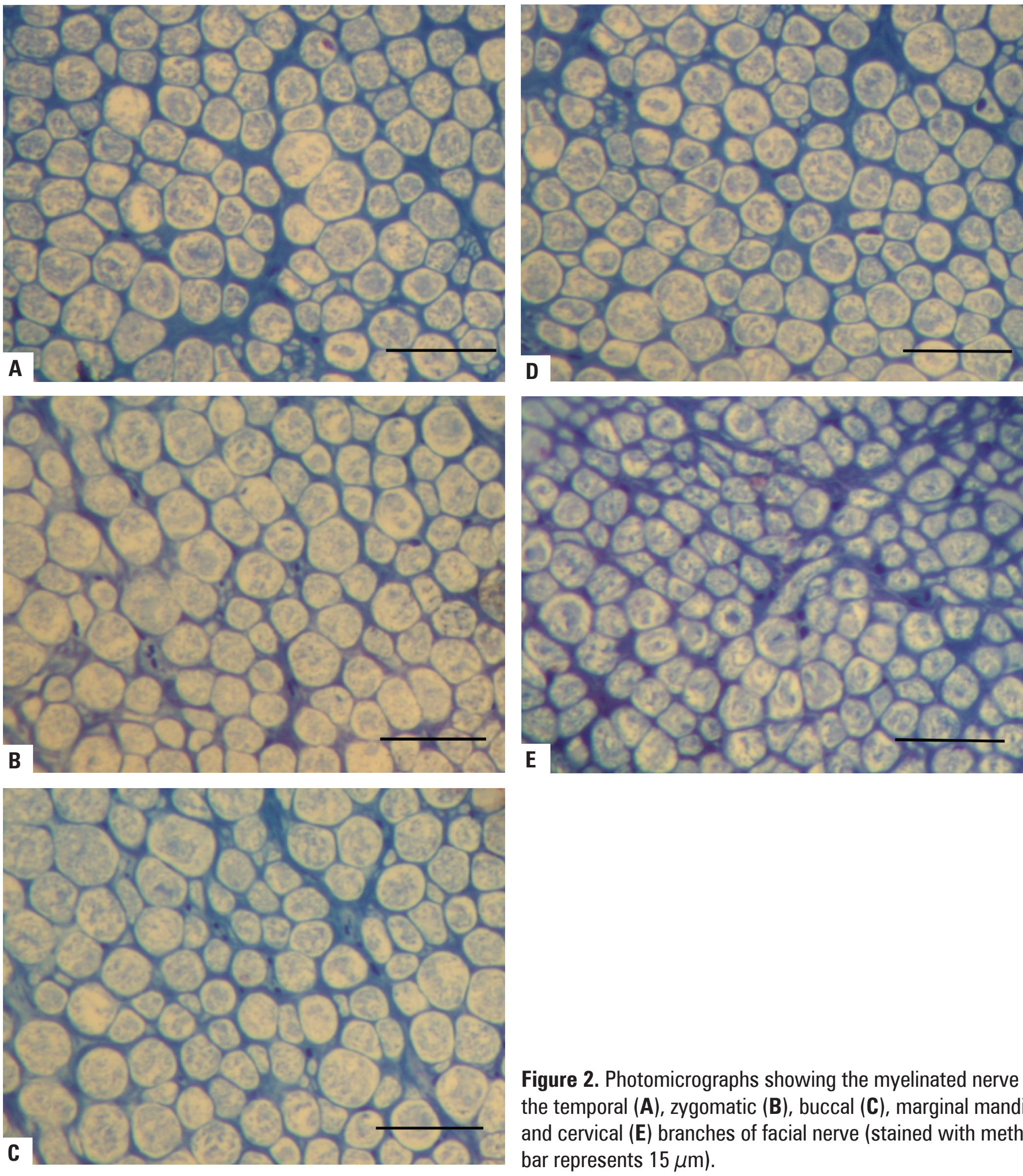

Figure 2. Photomicrographs showing the myelinated nerve fibres in the temporal (**A**), zygomatic (**B**), buccal (**C**), marginal mandibular (**D**) and cervical (**E**) branches of facial nerve (stained with methylene blue, bar represents 15 μm).

temporal and buccal, marginal mandibular, cervical branches; zygomatic and marginal mandibular, cervical branches; buccal and marginal mandibular, cervical branches; marginal mandibular and cervical branches.

In the transmission electron microscopic examination of the terminal branches of facial nerve, no ultrastructural differences were detected. The sizes, shapes and ultrastructural appearances of these myelinated nerve fibres did not show any marked difference between the groups (Figs. 3A, B).

DISCUSSION

Facial expression is an exclusively controlled trait and dysfunction of this nerve may lead to physical and psychological disorders. Additionally, the anatomy of facial nerve is very complicated and may show variations among ethnic groups. This variance may explain the diverse facial expressions and animations between these groups [6, 10]. These variations in facial nerve anatomy are well documented in the literature. Katz and Catalano [7] performed 100 parotidectomies and classified the terminal branching pattern of facial nerve into 5 groups. In all of the cases, the main trunk was divided into a larger frontozygomatic and smaller cervicofacial sections. Tzafetta and Terzis [19] made 10 fresh cadaveric hemifacial dissections and demonstrated great variability in branching pattern of the facial nerve and innervation of the mimetic muscles by facial nerve. Davis et al. [3] studied the

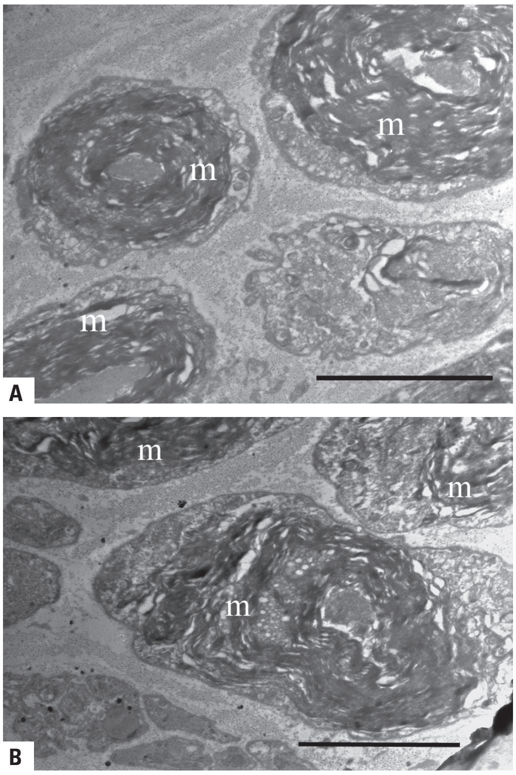

Figure 3. Electron micrographs showing the myelinated nerve fibres (m) in the buccal (**A**) and cervical (**B**) branches of facial nerve. No ultrastructural differences were detected in between the terminal branches of facial nerve (stained with uranyl acetate and lead citrate, bar represents 5 μm).

branching patterns of facial nerve and described 6 different patterns in 350 hemifacial dissections. The most common type was a single connection between the frontozygomatic and cervicofacial divisions, with a connecting loop between the zygomatic and buccal branches. Farahvash et al. [6] examined the extratemporal facial nerve and its branches in 42 hemifacial dissections in fresh cadavers. The results of their study confirmed the variable branching pattern of the extratemporal facial nerve. Erbil et al. [5] studied the close relationship between the parotid duct and the buccal and zygomatic branches of facial nerve in 10 cadavers. During their morphometric measurements, the reference points used for surgery of this region were taken into consideration as the landmarks for these measurements.

In the literature; there are many studies related to the number of terminal branches of facial nerve. Baker and Conley [2] observed that the cervical division of the extratemporal facial nerve had three to 5 branches. From these branches; 1 of them was buccal, 3 of them were marginal mandibular and 1 of them was cervical. Kim et al. [8] and Lineweaver et al. [9] have reported a high percentage of 2 and 3 branched marginal mandibular nerves. In the study of Farahvash et al. [6], a single marginal mandibular branch was seen in 95.7% of the cadavers. Al-Hayani [1] studied the marginal mandibular branch of facial nerve post mortem in 50 human subjects. This branch was found to be presented by 1 (32%) branch, 2 (40%) branches and 3 (28%) branches. Mizia et al. [11] measured the histological structure of human median nerve by using the computer-assisted image analysis method. The number of nerve bundles in this nerve varied from 13 to 38 and in the motor branch of the median nerve from 4 to 14. The mean cross-sectional area of the median nerve at the level of Carpal tunnel was found to be 0.19 cm^2.

There are only a very few studies related to the quantitative analysis of facial nerve especially in humans. Shimozawa [15] performed a fibre count analysis on the motor root of the mouse facial nerve with electron microscope in 6 mice. On an average, 84.9% of the total nerve fibres were myelinated and 14.6% were unmyelinated. In another study, Shimozawa [16] made a fibre count analysis on the facial nerve trunk distal to the geniculate ganglion in 7 mice. On an average, 93% of the total nerve fibres were myelinated and 6.3% were unmyelinated. A quantitative study of nerve fibres in the human facial nerve was done by Thurner et al. [18] in 7 patients. In 2 of these cases, the facial nerve fibres were counted at 5 different levels. The total number of myelinated nerve fibres in the facial nerve varied from 7500 to 9370 and the total number of myelinated nerve fibres in the intermediate nerve varied between 3120 and 5360.

CONCLUSIONS

According to our knowledge, there is no study in the literature examining the quantification of nerve fibres found in terminal branches of facial nerve. Secondly, we also performed ultrastructural analysis in these terminal branches in order to observe their ultrastructural differences. However, we could not find any ultrastructural differences in between these branches. Although the injuries of the terminal branches of facial nerve are very common and they may lead to physical and psychological disorders, there is no study in the literature performing the quantitative analysis of them. Additionally, these numerical data will have an importance during the nerve repair process of terminal branches of facial nerve in various injuries.

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