

Morphometric analysis of pulpal myelinated nerve fibers in human teeth with chronic periodontitis and root sensitivity

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Key words: myelinated nerve fibers; dental pulp; chronic periodontitis; root sensitivity.

Summary. Background. The reasons why root sensitivity occurs in some periodontally diseased teeth are still unknown. It is possible that root sensitivity may be related to changes of intradental myelinated nerve fibers, which are responsible for dentine sensitivity.

Objective. The aim of this study was to define the pattern of myelinated nerve fiber changes in the pulps of teeth with and without root sensitivity in the presence of chronic periodontitis.

Materials and methods. A total of 33 cross-sectioned human dental pulp specimens were collected from noncarious, intact, permanent teeth sensitive to electric and thermal (cold) stimulus (10 hypersensitive teeth with chronic periodontitis (HTPP group), 15 nonsensitive teeth with chronic periodontitis (NTPP group), and 8 nonsensitive teeth with healthy periodontium (control group)). The morphometric parameters were estimated using light microscopy and image-analyzing computer program Image-Pro Plus.

Results. The means of myelinated nerve fiber density, fiber and axon diameter, area, perimeter, length, width, g ratio, index of circularity, and myelin sheath thickness in NTPP group significantly differed from HTPP group and the control group teeth ($p < 0.001$). The great reduction in the density of myelinated nerve fibers in NTPP group was accompanied by unequal decrease in the number of very large-diameter myelinated nerve fibers. The mean values of morphometric parameters of all myelinated nerve fibers in HTPP group were almost the same as those in the control teeth, and no significant difference was observed.

Conclusion. The findings of the present study suggest that the reason for enhanced root sensitivity has likely nothing to do with changes of the innervation of myelinated nerves in the dental pulp. While, decreased sensitivity of periodontally diseased teeth may be related to the degeneration of myelinated nerve fibers in the pulp.

Introduction

Root sensitivity (RS) is a common problem in patients with chronic periodontitis. According to the data of a few epidemiological studies, the prevalence of this painful condition is high and range between 72.5% and 98% (1, 2). It is known that intradental myelinated A-fibers are responsible for the sensitivity of dentine and can actively respond to injury, resulting in profound morphological changes (3). Unfortunately, the physiopathological mechanism of root sensitivity in periodontally diseased teeth is not fully understood. In accordance with the findings that bacteria can penetrate root cementum and radicular dentin tubules (4), it has been postulated that an extent of chronic inflammatory process in periodontium may play a role in the development of RS. The histological investigations of the human dental pulp in patients with chronic

periodontitis are rather scarce and, in general, include the morphological changes of odontoblasts, fibroblasts, and nerve fibers, developed due to chronic periodontitis (5). However, these studies were not designed to determine the correlations between the neural changes and RS. Although the changes in the regional sensitivity of the affected teeth might depend on the morphological state as well as on the density of the innervation in the dentine and pulp (6), the morphometric parameters of the nerve fibers in the human dental pulp with chronic periodontal lesions have not received proper attention.

Since the correlations between root sensitivity and morphometric changes of the nerve fibers in the human dental pulp have not been described, the aim of the present study was to define the pattern of myelinated nerve fiber (MNF) changes in the pulps

of teeth with and without root sensitivity in the presence of chronic periodontitis.

Materials and methods

A total of 33 noncarious, intact teeth from volunteers ($n=29$) aged from 30 to 50 years in general good health and with chronic periodontitis or with healthy periodontium were observed during this study in the Department of Dental and Oral Diseases at Kaunas University of Medicine, Lithuania. Patients who had received any periodontal and desensitizing treatment during the previous 3 months as well as the ones who had been using desensitizing toothpastes within the past 6 weeks or receiving regular medication were not included in this study.

Case records were screened, and full oral examination was performed. The research protocol and informed consent form were approved by the Bioethical Committee of Kaunas University of Medicine. All patients received detailed particulars of the principle and purpose of the study and signed appropriate informed consent forms.

To be included into this study, teeth were tested for pulpal vitality. The method used included electric stimulation and cold (ice) test. Teeth with nonvital dental pulp were excluded.

Each tooth with chronic periodontitis included in this study had at least one pocket of 5 mm or more in combination with the attachment loss of 2 mm or more and was stimulated by applying one-second airflow (45 psi, 20°C) from the air syringe of the dental unit to estimate root sensitivity. The syringe was held perpendicularly, 2–3 mm from the root surface. The adjacent teeth were protected by the dentist's fingers. After stimulation, the patients were asked to grade the sensitivity using a visual analogue scale (VAS). According to VAS scores, periodontally diseased teeth were divided into two groups: hypersensitive teeth with chronic periodontitis (HTPP) (VAS scores ≥ 20 mm) and nonsensitive teeth with chronic periodontitis (NTPP) (VAS scores < 20 mm).

Teeth were extracted under local 2% lidocaine anesthesia for orthodontic, prosthetic reasons or because of tooth mobility. The control group was formed from intact third molars and for orthodontic reasons or because of mechanic injuries of the jawbones extracted teeth with healthy periodontium without root sensitivity signs.

Tissue removal and processing. After extraction, the tooth was sectioned along its longitudinal axis using high-speed diamond burs with an abundant water spray cooling. The pulp was taken out with a preparation

needle, and transverse sections were obtained from the coronal third of the root pulp using scissors. The tissue samples were immersed in a fixative containing 2.5% glutaraldehyde in 0.1 M potassium cacodylate buffer (pH 7.4) solution for at least 4 h at room temperature or overnight at 4°C. Afterwards, the samples were postfixed in 1% osmium tetroxide solution in 0.1 M potassium cacodylate buffer (pH 7.4) for 2 h, dehydrated with serial transfers in increasing concentrations of ethanol and embedded in a mixture of Epon 812 and Araldite. Semithin (1 μ m) sections were stained with methylene blue according to R. L. Ridgway (1986) and examined under a light microscope.

Morphometric analysis. All morphometric parameters were counted by light microscopy using image-analyzing computer program Image-Pro Plus Version 4.5. The cross-sectional profile of each pulp using a magnification of $\times 5$ and representative fields under a $\times 100$ oil-immersion objective were photographed with a digital camera Nikon COOLPIX 4500 and a microscope Zeiss Axioimat. A stage micrometer was used to calibrate the measurements.

The number of the nerve fiber bundles was counted; total cross-sectional area was measured using a magnification of $\times 5$, and the density (expressed as N/mm^2) was calculated in the full circumference pulps.

The total number of MNFs in the whole pulp section and in the discrete MNF bundles was counted in the photographs taken at $\times 1000$ magnification, and then the density of MNFs was determined. Only MNFs the contour of which were completely within a photograph and in good transverse section was counted. MNFs with invaginated or evaginated myelin and with free myelin loops were excluded from analysis.

For morphometric analysis at least 25% of the MNFs in each pulp were taken by a systematic random sampling. The outline of each axon and its myelin sheath was drawn with a digitizing pen. Then the maximum, mean, and minimum diameter, area, perimeter, length, and width of axons and fibers were determined using the same equipment.

Myelin sheath thickness was calculated as the difference between the fiber diameter and the axon diameter, divided by two (7). The g ratio was calculated as the quotient obtained by dividing the axon diameter by the fiber diameter (8). The index of circularity (IC) was calculated as the ratio between the measured axonal area and the area of a circle with the same circumference (8).

Statistical analysis. Data of the study were processed using SPSS for Windows (Statistical Package for Social Science) version 12 software. The data were

analyzed by descriptive statistics with frequency distribution. The Student's criterion was used for comparison of means. Multiple comparisons were analyzed by applying analysis of variance using the Bonferroni post hoc test. The usage of these criteria is possible when sample variables are distributed normally. The Kolmogorov-Smirnov goodness-of-fit test was performed in order to assess the normality of distribution. Some elements of the data appeared not to be normally distributed, so a nonparametric analysis of variance was applied to these data sets. Nonparametric data were analyzed using the Kruskal-Wallis test. Statistical significance of the difference between the means was accepted at the level of $p < 0.05$, very significant at the level of $p < 0.01$ and strongly significant at the level of $p < 0.001$.

Results

A total of 33 histological sections of the dental pulps (10 in HTPP, 15 in NTPP, and 8 in control group) were selected for morphometric analysis. The mean age (mean \pm SD) of patients whose teeth were attributed to control group was 39.13 ± 0.34 , to HTPP group – 39.56 ± 0.29 , to NTPP group – 40.04 ± 0.27 years.

The area of the pulps ranged between 0.48 mm^2 and 0.69 mm^2 (mean \pm SD, 0.58 ± 0.13 in HTPP, 0.56 ± 0.66 in NTPP, and 0.59 ± 0.11 in control group); the difference among the groups was not statistically significant.

The lowest mean number of MNFs per pulp was found in NTPP group (101.73 ± 27.57) compared with the HTPP (497.80 ± 102.87) and control (475.25 ± 61.53) groups (Fig. 1). In the NTPP group, the number of nerve fiber bundles per pulp was also smaller (3.93 ± 1.03) and statistically differed from HTPP (9.30 ± 3.09) and control (8.00 ± 2.20) groups ($p < 0.001$). A statistically significant difference between HTPP and control groups was not found.

The morphometric analysis of cross-sectioned dental pulps also revealed a higher MNF and nerve fiber

bundle density per 1 mm^2 of pulp in HTPP and control groups (Table 1).

The detailed morphometric analysis of total 4977 myelinated fibers (1892 in HTPP, 1434 in NTPP, and 1651 in control group) revealed some differences among the groups.

The MNF size varied widely, ranging from 2.78 to $120.5 \text{ }\mu\text{m}^2$ in HTPP group; from 2.63 to $119.96 \text{ }\mu\text{m}^2$, in control; and from 0.69 to $65.66 \text{ }\mu\text{m}^2$, in NTPP group. The parameter values distributed unequally. Very small MNF area ($\leq 1.0 \text{ }\mu\text{m}^2$) was seen only in NTPP group, and very large area ($\geq 100 \text{ }\mu\text{m}^2$) of MNF was found only in HTPP and control groups. The largest mean area of MNF was in control group – $19.42 \pm 15.60 \text{ }\mu\text{m}^2$ – and did not differ significantly from that in HTPP group – $18.53 \pm 14.92 \text{ }\mu\text{m}^2$. The smallest mean area of MNF – $10.04 \pm 6.49 \text{ }\mu\text{m}^2$ – was found in NTPP group and it differed statistically significantly from HTPP and control groups ($p < 0.001$). The distribution of axon areas in different study groups corresponded roughly to that of MNF (Fig. 2).

Histograms of axon and fiber diameters in all groups are shown in Fig. 3 and 4. Both axon diameter and fiber diameter appeared to be uniformly distributed. The mean fiber diameter ranged from $3.42 \text{ }\mu\text{m}$ to $4.73 \text{ }\mu\text{m}$, and there were significant differences only between NTPP and other two groups ($p < 0.001$). The axon diameter distribution corresponded roughly to that of fibers, similarly skewed to the right.

The percentage of large-diameter fibers ($> 3.5 \text{ }\mu\text{m}$) was higher in HTPP and control group (Fig. 4) and differed from NTPP group where the small-diameter MNFs ($< 3.5 \text{ }\mu\text{m}$) dominated.

The mean values of perimeter, length, and width of the MNFs as well as of the axons were significantly ($p < 0.001$) smaller in NTPP group than in the other groups (Table 2). No significant differences in all three parameters between HTPP and control group were found.

The results concerning g ratios in the pulps show

Table 1. Comparative morphometry of MNF and MNFB density in HTPP, NTPP, and control groups

Groups	MNF density (per mm^2)	MNFB density (per mm^2)
Hypersensitive teeth with periodontal pathology (HTPP)	878.22 ± 117.06	16.00 ± 2.64
Nonsensitive teeth with periodontal pathology (NTPP)	$182.35 \pm 49.65^*$	$7.06 \pm 2.01^*$
Control	831.23 ± 191.45	13.89 ± 4.46

* A statistically significant difference as compared to hypersensitive teeth with periodontal pathology. Values are expressed as mean \pm SD.

MNF – myelinated nerve fiber; MNFB – myelinated nerve fiber bundle.

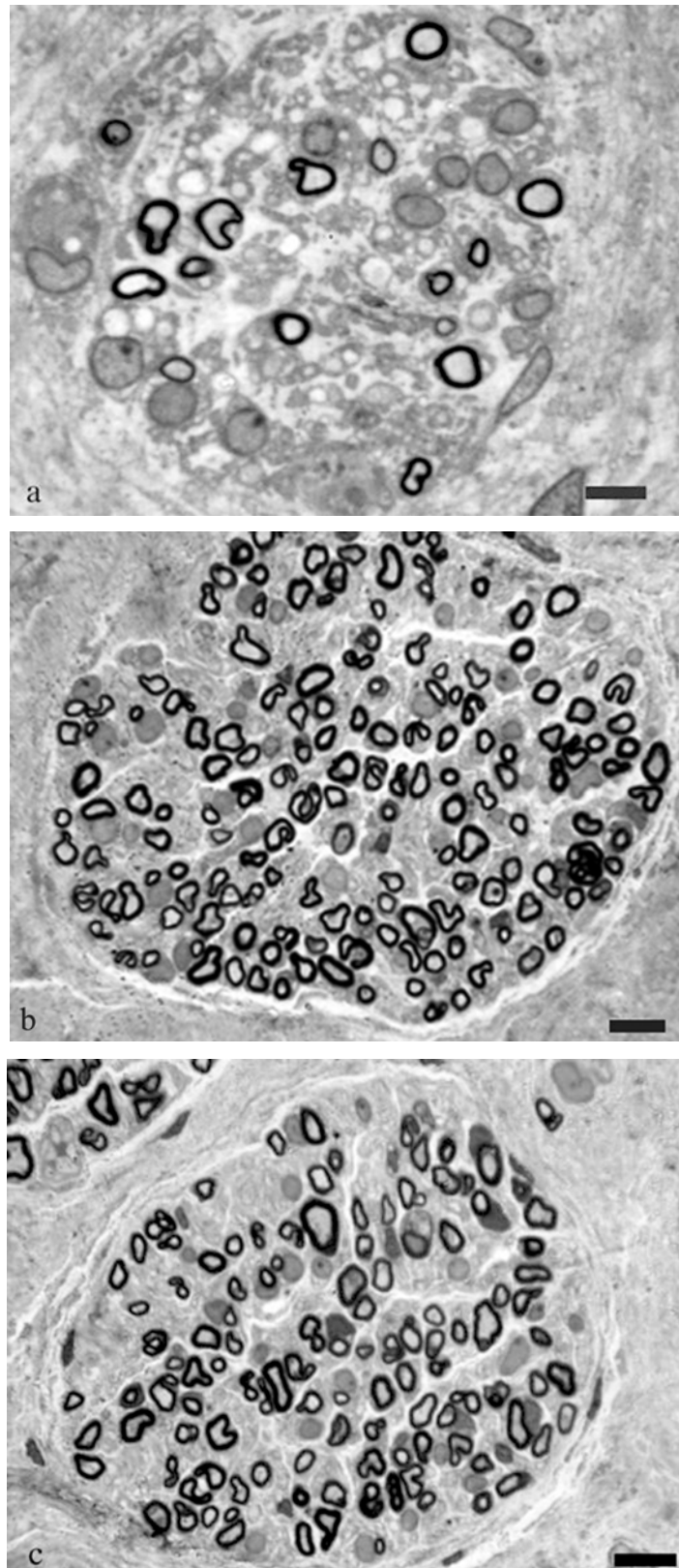


Fig. 1. Light micrographs showing different patterns of density of MNFs within MNF bundles of human dental pulp

There is evident loss of MNFs in NTPP group (a) compared to HTPP group (b), which is similar to control group (c). Methylene blue; bars – 10 μ m.

MNF – myelinated nerve fiber; HTPP – hypersensitive teeth with periodontal pathology;
NTPP – nonsensitive teeth with periodontal pathology.

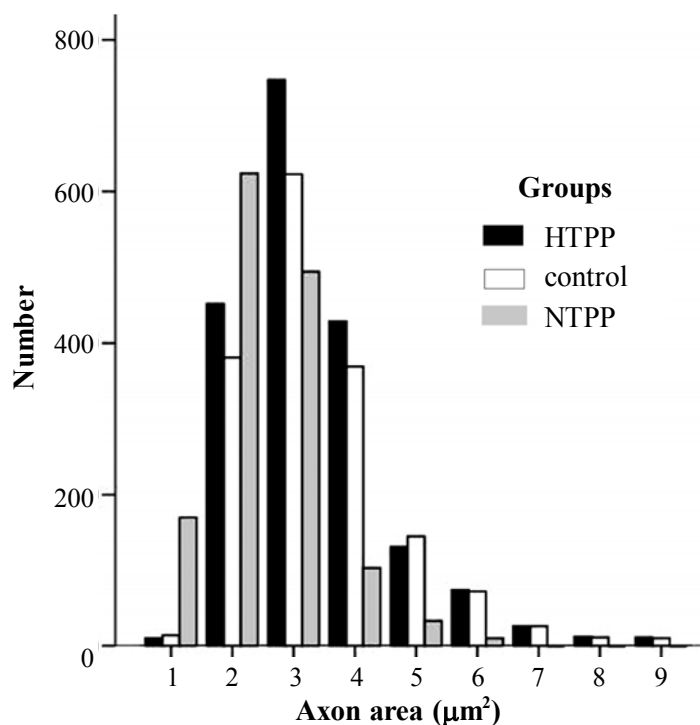


Fig. 2. Histogram of MNF axon areas in HTPP, NTPP, and control groups
 MNF – myelinated nerve fiber; HTPP – hypersensitive teeth with periodontal pathology;
 NTPP – nonsensitive teeth with periodontal pathology.

Table 2. Comparative morphometry of MNF and axon perimeter, length, and width in HTPP, NTPP, and control groups

Groups	Perimeter (μm)		Length (μm)		Width (μm)	
	MNF	Axon	MNF	Axon	MNF	Axon
Hypersensitive teeth with periodontal pathology (HTPP)	17.17±6.46	11.45±5.39	5.96±2.15	4.07±1.73	4.00±1.60	2.33±1.21
Nonsensitive teeth with periodontal pathology (NTPP)	12.50±4.34*	7.87±3.77*	4.38±1.52*	2.87±1.30*	2.95±1.05*	1.60±0.81*
Control	17.45±6.54	11.57±5.44	6.04±2.20	4.11±1.79	4.11±1.66	2.40±1.25

* A statistically significant difference, as compared to hypersensitive teeth with periodontal pathology. Values are expressed as mean±SD.
 MNF – myelinated nerve fiber.

that mean g ratio varied from 0.57 to 0.61. The lowest mean g ratio (0.57±0.10) was in NTPP group, and it statistically differed from HTPP (0.61±0.10) and control groups (0.61±0.10) ($p<0.001$).

The statistical analysis of myelin sheath thickness, like other parameters in the groups, showed the same tendency. This parameter ranged from 0.87 to 0.90 (mean 0.89±0.35) in HTPP; from 0.89 to 0.92 (mean 0.90±0.35), in control; and from 0.71 to 0.73 (mean

0.72±0.24) in NTPP groups. The mean value in NTPP group was significantly smaller than in HTPP ($p<0.001$) and that in control group ($p<0.001$).

Table 3 shows the mean IC for the axonal profiles (range 0.61–0.65) and fiber profiles (range 0.73–0.76).

Discussion

Certain previous studies have presented the histomorphometric analysis of the majority of peripheral

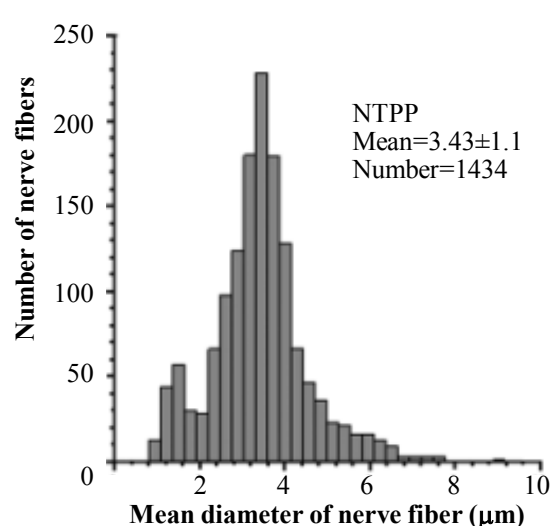
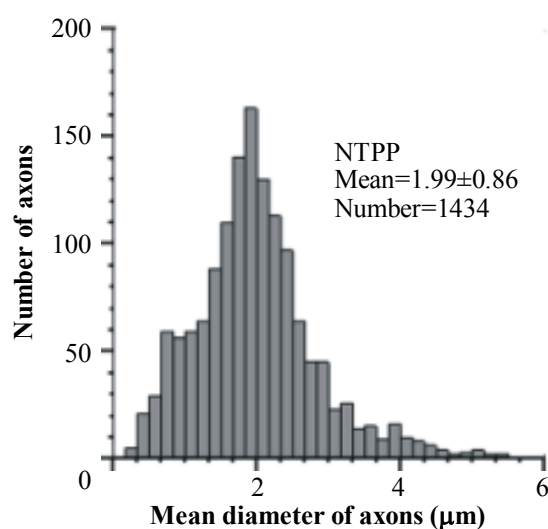
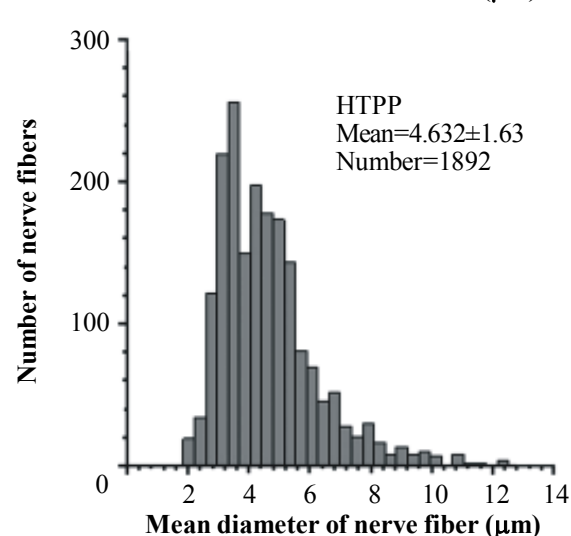
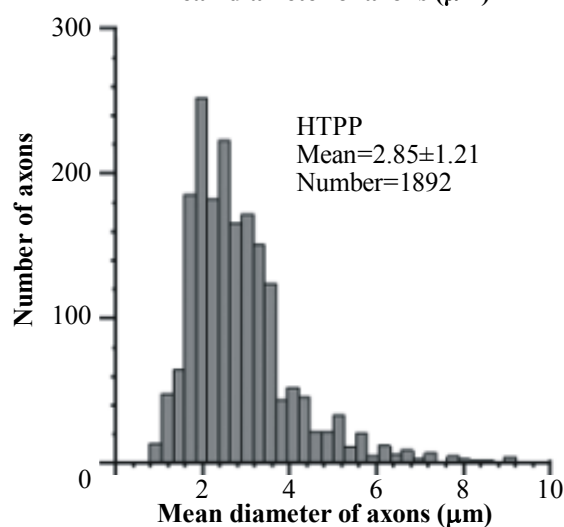
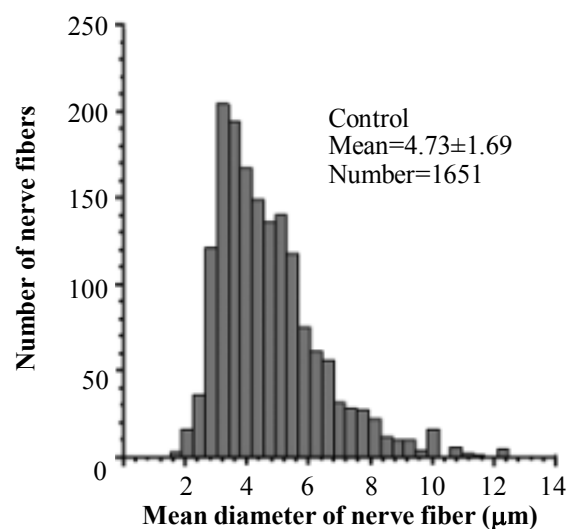
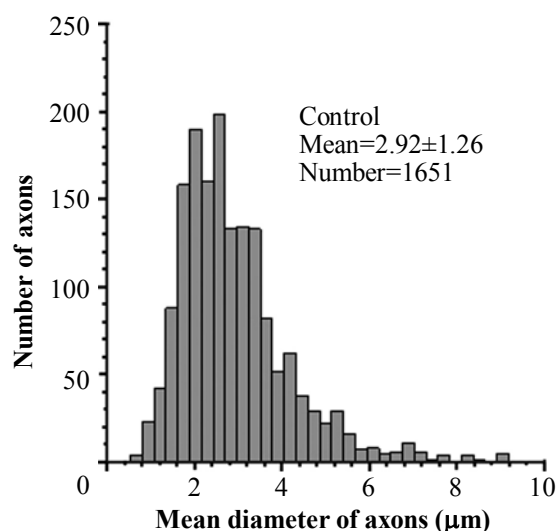


Fig. 3. Histograms of MNF axon diameter in the studied groups

MNF – myelinated nerve fiber; HTPP – hypersensitive teeth with periodontal pathology; NTPP – nonsensitive teeth with periodontal pathology.

Fig. 4. Histograms of MNF diameter for the groups

MNF – myelinated nerve fiber; HTPP – hypersensitive teeth with periodontal pathology; NTPP – nonsensitive teeth with periodontal pathology.

Table 3. Mean index of circularity of axonal and myelinated nerve fiber in HTPP, NTPP, and control groups

Groups	HTPP	NTPP	Control
Axonal IC	0.61±0.22	0.65±0.23*	0.63±0.22
Fiber IC	0.73±0.15	0.76±1.33*	0.74±0.15

* A statistically significant difference as compared to hypersensitive teeth with periodontal pathology. Values are expressed mean±SD.

HTPP – hypersensitive teeth with periodontal pathology; NTPP – nonsensitive teeth with periodontal pathology; IC – index of circularity.

nerves; however, only some of them have dealt with human dental pulp (9–11). Most of these studies used the apical part of the radicular pulp to identify the quantity of nerve fibers entering a tooth. W. Graf and G. Björlin (9), having examined 12 teeth of different groups using light microscopy, were the first to determine that a tooth is reached by 536 to 600 MNFs. The numbers of MNFs (359 to 361) in incisors and canines, presented by the later D. Johnsen's and S. Johns' study (10), were insignificantly smaller. The axons which penetrate through the apical foramen and lateral root canals when proceeding in the coronal direction branch insignificantly, then some of them end at blood vessels or enter dentinal tubules. Most of the nerve fibers reach the coronal part of dental pulp where they branch and form a plexus. This study has dealt with the upper part of the dental root pulp which could have caused a slightly higher number of MNFs in the dental pulps of the control group as well as of HTPP group than in the case of the studies mentioned above. Whereas, the mean number of MNFs as well as the mean number of their bundles found in the pulp cross-sections of the NTPP group was several times smaller.

The absolute numbers of MNFs and their bundles were found to be directly dependent on the study area. That is why the density of nerve fibers gives more exact characteristics of the innervation of the dental pulp tissues and its changes. The mean area of the dental pulps in different groups investigated in this study was found not significantly different, whereas the smaller mean densities of MNFs and MNF bundles as well as their absolute numbers were determined in the dental pulps of the NTPP group. This could account for weaker nerve response to thermal stimulus and also support the opinion of a number of investigators claiming that dynamic changes in the intra-dental neural density may have functional significance in terms of sensitivity (6). On the other hand, the difference in the mean density of MNFs when comparing the control and HTPP groups was found to be insignificant. The latter finding indicates that the ground for

the RS in HTPP group is other than the changes in the innervation of the dental pulp tissues.

The evaluation of each of the parameters has contributed to disclosing a certain tendency in differences among the groups. The distribution of the values of all MNF parameters in HTPP group was almost the same as that in the control group, and no significant difference was observed. However, the MNF parameter results of the NTPP group showed a significant difference in comparison with both, the control and the HTPP groups.

The smallest mean area of MNF could have been equally resulted by the disappeared large-area ($\geq 70 \mu\text{m}^2$) MNFs in NTPP dental pulps and very small area ($\leq 1 \mu\text{m}^2$) MNFs found there.

This could also be the reason for the findings that the mean length, width, and perimeter were smaller in the above-mentioned group too.

The MNF diameters found in this study coincided with the already published data that the tooth is innervated by A δ (2–5 μm in diameter) and A β (5–12 μm in diameter) MNFs (11, 12). According to the study by P. N. R. Nair *et al.* (12), 93% of the myelinated nerve fibers that enter human premolars are A δ fibers, and the remaining 7% are A β fibers. Our study has found the similar pattern only in dental pulps of NTPP group where A δ fibers made up 92.1% and A β – 7.9%. Moreover, on the contrary, the composition of MNF in dental pulps of control and HTPP groups was different, showing smaller number of A δ , as well as greater number of A β fibers. It is not known what might have caused a reduction in the number of A β fibers in the NTPP group. However, a similar loss of selective large-diameter MNFs was found in the dental pulps of the aged mammals (13, 14). This leads to a supposition that early disappearing large-diameter MNFs might be more vulnerable than those of a smaller diameter. On the other hand, it seems that even a significant loss of large-diameter MNFs could not have influenced the reduction of root sensitivity in the NTPP group, because the majority of the remaining MNFs

was formed from A δ fibers, directly related to the appearance of sharp and acute pain characteristic of RS.

The results of this study demonstrated that alongside with the absolute reduction of the number of MNFs in the teeth of NTPP group, the mean area of axons, as well as length, width, and diameter and myelin sheath thickness in this group were also smaller than in the other groups.

All these findings are characteristic of slowly progressing axonopathy with relative hypomyelination (15) which causes disorder of the function of nerve fibers. Similar MNF changes were found in the peripheral nerves in cases of diabetes mellitus (15) and some of the disorders of nervous system (16). In this study, the difference in these parameters between the control and HTPP groups was not found.

The g ratio is an important indicator of myelinated fiber function. The optimal action potential conduction in MNF occurs when g ratio values are close to 0.6 (17). The present study showed that the g ratio values in the human pulp ranged from 0.57 to 0.61 and were slightly greater in the control and HTPP groups. It can be explained by the findings showing that g normally increased with increasing fiber size. The previous reports on g ratio values for MNF of human dental pulp were not available. However, similar g ratio values were found in human sural (18) and intracardiac (19) nerves where this index varied extremely close to 0.7.

Index of circularity, where a result of 1 is equivalent to a circle and 0 represents a straight line (20), has been described in a few studies of the mammalian

peripheral nerves with a range of 0.55 to 0.95 (20–22). In this study, the fiber IC and the axon IC were slightly greater in the NTPP group. These findings can be explained by reasoning that MNFs with invaginated or evaginated myelin and with free myelin loops were excluded from analysis.

Extensive changes in the nociceptor structure could have a significant effect on the sensory function of the pulpal nociceptors (23). However, some authors have found that the correlation between the actual pulpal morphological changes and the clinical pain symptoms is poor (24). The results of the present study showed that RS variability in teeth with chronic periodontitis might be related to dental MNF degeneration rather than to exaggerate MNF response. The reasons for sharp alterations of MNFs are still unknown. According to the study by K. Langeland *et al.* (25), the pulp succumbed only when periodontal lesions involved the apical foramen, otherwise only minor changes occurred in the pulp. Therefore, it seems that degenerative changes of MNFs in intact periodontally diseased teeth with vital pulp may occur due to open dentinal tubules and lateral root canals. This suggests that occlusion of these canals may prevent teeth hypersensitivity as well as pulp tissue from alteration.

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Žmogaus danties pulpos mielininių nervo skaidulų morfometrinė analizė sergant lėtiniu periodontitu ir esant padidėjusiam dantų šaknų jautrumui

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Raktažodžiai: mielininės nervo skaidulos, danties pulpa, periodontitas, dantų šaknų jautrumas.

Santrauka. Priežastys, dėl ko padidėjęs dantų šaknų jautrumas randasi tik kai kuriuose lėtinio periodontito pažeistuose dantyse, iki šiol nežinomos. Tikėtina, kad padidėjęs dantų šaknų jautrumas galėtų būti susijęs su danties mielininių nervo skaidulų, atsakingų už danties jautrumą, pokyčiais.

Tyrimo tikslas. Apibūdinti sergančiųjų lėtiniu periodontitu mielininių nervo skaidulų pokyčius dantų pulpose esant padidėjusiam dantų šaknų jautrumui ir normaliam.

Medžiaga ir metodai. Ištirti 33 išoriškai nepažeistų, ėduonies nepažeistų bei į juntamąjį dirgiklį reaguojančių dantų pulpų skerspjūviai (10 dantų esant lėtiniam periodontitui ir padidėjusiam dantų šaknų jautrumui (HTPP grupė), 15 dantų, diagnozavus lėtinį periodontitą, bet neradus padidėjusio dantų šaknų jautrumo (NTPP grupė) ir 8 dantys be minėtos patologijos (kontrolinė grupė)). Morfometriniai rodikliai apskaičiuoti naudojant šviesinį mikroskopą ir vaizdus analizuojančią kompiuterinę programą „Image-Pro Plus“.

Rezultatai. Mielininių nervo skaidulų tankumo, skaidulos ir aksonų diametro, ploto, perimetro, ilgio, pločio, dydžio g, apvalumo indekso ir mielino dangalo pločio vidurkiai NTPP grupėje statistiškai reikšmingai ($p < 0,001$) skyrėsi nuo HTPP ir kontrolinės grupės dantų. Mielininių nervo skaidulų tankumas šioje grupėje žymiai sumažėjo dėl netolygaus labai didelio diametro skaidulų sumažėjimo. Visi morfometriniai mielininių nervo skaidulų rodikliai HTPP ir kontrolinėje grupėse buvo panašūs ir statistiškai reikšmingai nesiskyrė.

Išvados. Tyrimo duomenimis, dantų šaknų jautrumo sustiprėjimas nesusijęs su danties pulpos mielininių nervo skaidulų pokyčiais. O dantų šaknų jautrumo susilpnėjimas, sergant lėtiniu periodontitu, gali būti susijęs su danties pulpos mielininių nervo skaidulų degeneracija.

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