

ANALYZING THE AGE RELATED CHANGES IN THE HUMAN COCHLEAR NERVE: A QUALITATIVE MICROSCOPIC STUDY

Dhinesh Kumar T ^{*1}, Tara Sankar Roy ², Tony George Jacob ³, Charanjeet Kaur ⁴, Dinesh Kumar V ⁵.

^{*1} Assistant Professor, Department of Anatomy, Academic block, Dhanalakshmi Srinivasan Medical College & Hospital, Siruvachur, Perambalur District, Tamil Nadu, India.

² Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.

³ Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.

⁴ Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.

⁵ Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India.

ABSTRACT

Background: Cochlear nerve serves as one of the nodal point for enabling the passage of sound in both directions. The knowledge of age related morphological changes in human cochlear nerve is important to understand its role in the manifestation of sensori-neural presbycusis.

Materials and Methods: The study was conducted in 21 human cochlear nerve samples, collected in 3 different age groups (0-30 years, 31-50 years, 51 years and above). Resin embedding of cochlear nerves was done. Semi-thin (1 µm) cross sections of the nerves were cut by glass knife on Reichert Ultra-microtome. Under light microscope, toluidine blue stained nerve sections were studied for shape, organization of connective tissue and number of fascicles.

Results: Cochlear nerve was comma-shaped across all the age groups studied. Majority of the nerve sections had a blunt round head and sharp tail. Few sections had blunt tail also. Nerve fascicles were well defined in all the 21 samples studied. The approximate number of fascicles across the various age groups varied from 60 to 85 per nerve. Numerous Schwann cells and numerous small sized blood vessels were found in the endoneurium of older age group compared to younger and middle aged groups.

Conclusion: However, we didn't observe major qualitative changes across different age groups, but the present study provides novel baseline morphological data on the human cochlear nerve.

KEY WORDS: Cochlear Nerve, Morphology, Shape, Number of fascicles, Connective tissue organization.

Address for Correspondence: Dr Dhinesh Kumar, Assistant Professor, Department of Anatomy, Academic block, Dhanalakshmi Srinivasan Medical College&Hospital, Siruvachur, Perambalur District, Tamil Nadu -621113, **E-mail:** drdhineshkumar@gmail.com

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INTRODUCTION

It is pertinent to understand the age related changes in the auditory system because of its profound effect it can have in the social and professional life of an individual. With the

rapidly increasing elderly population in any part of the world, one of the major disabilities is presbycusis i.e. age related hearing loss. A general survey conducted among United Kingdom household in 1998 found that 53% of

men and 41% of women over the age of 75 reported some difficulty with hearing [1]. Owing to this, it is imperative to understand the changes in the central auditory system related to aging. Amidst the patients suffering particularly from sensori-neural presbycusis, those who have low speech discrimination scores are purportedly said to have loss of hair cells and neurons of the cochlea. Scientific reports in the past postulated that most of the degeneration of cochlear nerve fibres occur secondary to the damage incurred to the sensory hair cells [2] or as a resultant of genetic aetiology [3]. Similarly with the increasing age, a reduction is expected in the diameter of the cochlear nerve (CN) could be observed and this can be corroborated with the neuronal losses [4]. On the other hand, documenting the natural course of CN degeneration is salient and this would help to differentiate the normal age related process from the pathological or occupational related hyper-degeneration.

Among the various components of the intricate chain of auditory pathway, CN serves as one of the nodal point for enabling the passage of sound in both directions. In one of the older studies conducted by Schuknecht et al. [5], even after sectioning more than half of the cochlear nerve fibres, the cats didn't had any significant changes in their audiograms. But later it was found that the effect of lesion varies according to the site and the loss of neuronal fibres would typically manifest with patients having difficulty in hearing when they are in complex noisy environments [6]. In the due course, Schuknecht [7] presented four old age cases with extensive age related degeneration in the cochlea and losses of about 50 % of the hair cells of the macula sacculi. Later studies [7-9] demonstrated the variability of audiogram pictures depending upon the site of cochlear lesion. When there is a predominant loss in the sensory component of the cochlea, it would manifest as abruptly sloping high frequency loss above the speaking range and predominant neuronal component loss would result in progressive loss of speech discrimination in the presence of stable pure-tone thresholds.

Understanding the varied manifestations depending upon the site and type of degeneration

is important in deciding the treatment modality. Furthermore, the diversity in the processing of sound signal is supposed to be due to the intensity driven activation of the two sub-populations of auditory fibres [10]. As these fibres form the auditory input to the cochlear nuclei, they can be distinguished on the basis of their threshold activity [11]. Animal studies which aimed at documenting the age related morphological changes in the inner ear could not portray the entire magnitude of the process largely because of controlled laboratory settings in which the experiments are performed. In addition, the age related process in the humans are compounded by oxidative stress from exposure to noise, underlying single nucleotide polymorphisms which leads to malfunction of supporting cells, micro-vascular changes in the supplying vessels [12].

Hoeffding et al., [13] studied the age related morphological changes in the (CN) in rats ranging from young adulthood to advanced age. Their light microscopic study showed that nerves of older animals had similar general appearance to those collected from young adults. They also observed a progressive increase in the cross-sectional area of the nerve. On the other hand, Felder et al., [14] analysed myelinated nerve fibres and hair cells in cochleae of eight patients with high-tone hearing loss and found that 30-40% reduction in nerve fibres in cochleae

MATERIALS AND METHODS

Collection of specimens: After obtaining due clearance from the Human Ethics Committee (Ref. Noynour thesis), All India Institute of Medical Sciences, New Delhi, specimens were procured from the post-mortem cadavers. A total of 21 CN samples were collected from young, adult and aged cadavers at the post-mortem interval of 6-12 hours. All samples were collected during the winter months (November to February) and within 6-12 hours of the individual's death.

The samples were then divided into three groups - Group A had cases from zero to 30 years of age; group B, 31-50 years and group C, 51 years and above.

The cases that had history of ear diseases, hearing loss or head trauma were excluded from this study.

Table 1: Details of the cochlear nerve samples collected for the study.

S. No	Side of sample	Age	Sex	Cause of death
1	Right	68	M	Natural death
2	Right	45	M	Acute Renal Failure
3	Right	55	M	Heart disease
4	Right	50	M	Heart disease
5	Left	48	M	Septicaemia
6	Right	60	M	Acute Drug Overdose
7	Right	36	M	Electrocution
8	Right	55	M	Hanging
9	Right	60	M	Natural death
10	Right	5	M	Drowning
11	Right	42	M	Acute Drug Overdose
12	Right	60	M	Cirrhosis
13	Left	22	F	Hanging
14	Right	29	F	Hanging
15	Right	61	M	Natural death
16	Right	35	M	Septicaemia
17	Right	26	F	Electrocution
18	Right	35	F	Acute Drug Overdose
19	Right	22	M	Hanging
20	Right	17	M	Acute Drug Overdose
21	Right	20	M	Hanging

Dissection methodology: After craniotomy, petrous temporal bones were collected and immersed in chilled 4% buffered paraformaldehyde (0.1 M phosphate buffer, pH 7.4) for fixation. The fixative was changed periodically after every 12 hours. Thereafter, the temporal bones were fixed in a bone holder and the internal acoustic meatus (IAM) were de-roofed with a diamond drill in order to expose the cochlear nerves within the meatus. The part of the CN close to its origin from the cochlea was excised and collected for the present study. The nerves were then transferred immediately into modified Karnovsky's fixative (4% paraformaldehyde and 1% glutaraldehyde) for 24 hours.

Tissue processing for resin embedding: Tissues were fixed in freshly prepared Karnovsky's fixative for 24 hours at 4°C and washed in 0.1 M phosphate buffer thrice over 15 minutes. Following this, the specimens were post fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 to 2 hours. After washing them once again, they were subjected to differential gradients of acetone over 30 minutes in each gradient, till they reach the highest concentration to achieve adequate dehydration. After that, they were cleared in toluene twice.

Infiltration: The processed specimens were infiltrated in following sequence:

[Embedding medium: Araldite CY212- 10 ml; Dodecyl succinic anhydride (DDSA) - 10 ml; 2,4,6 tri (dimethylamino methyl) phenol 30 (DMP-30) - 0.4 ml; Plasticizer (dibutyl phthalate) - 1.0 ml]

- Embedding medium: Toluene: 1:3 - Overnight
- Embedding medium: Toluene: 2:2 - 1 hour
- Embedding medium: Toluene: 3:1 - 1 hour (under vacuum)
- Pure Embedding medium - 1 hour
- Pure Embedding medium - 1 hour at 50°C

The embedded tissues were placed in the flat moulds in such a manner that the longitudinal axis of the nerve was perpendicular to the cutting surface. Embedded blocks were kept at 50°C to achieve polymerization for 12-14 hours then the temperature was raised to 60°C and kept for 48-72 hours (the end-point was the hardening of the resin). Semi-thin (1 µm) cross-sections of the nerves were cut by glass knife on Reichert Ultra-microtome (Germany).

Staining of sections for light microscopy:

The semi-thin (1 µm) sections were floated in water and placed on a clean glass slide. The slide was dried at 80°C and stained using Toluidine blue (1% solution in 1% borax) Sections were washed, dried and mounted using resin. The shrinkage factor was not calculated in the present study because shrinkage is reportedly least with resin embedding [15, 16].

RESULTS

Observations during dissection: The CN were well identified at the level of the internal acoustic meatus in all the dissected cadavers. It was observed as compact bundle of fibres coming out from the basal turn of the cochlea. It was accompanied by facial and vestibular nerve in the internal acoustic meatus. The CN and inferior vestibular nerve occupied a lower plane compared to facial and the superior vestibular nerve.

Qualitative observations: All the samples were qualitatively observed under light microscope (make of microscope Nikon or Olympus). In the cross sections of the toluidine blue stained samples, CN was like comma-shaped across all the age groups studied (Fig.1). Majority of the

comma-shaped cross sections of the nerve had a blunt round head and sharp tail (Fig. 1b and 1c). Few nerve cross sections had blunt tail also (Fig. 1a). The topographic arrangement of the fibres of the CN mimicked the spiral of the cochlea. The fibres originating from the basal turn of the cochlea were located in the peripheral part of the CN whereas, fibres from the apical and middle turn of the cochlea were arranged in the central and middle part of the CN respectively [17].

Fig. 1: Cross section of cochlear nerves showing well defined axon fascicles in all the age groups. $\frac{1}{2}A\frac{1}{4}$ represents the tail portion of the CN. Note the blunt tail in 'a' and sharp tail in 'b' and 'c'. a=22 years; b=42 years; c=58 years. Scale bar =500 μ m.

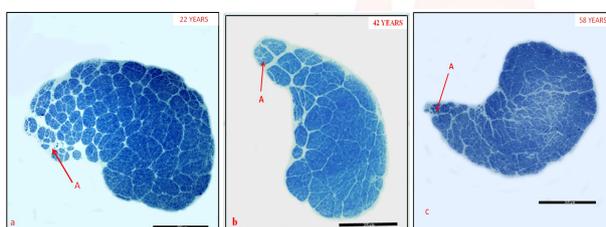


Fig.2: Photomicrograph of CN (a) Showing large myelinated axons (LMA) and small myelinated axons (SMA) in the peripheral zone close to epineurium (EPI) in 55 year-old individual. Scale bar=30 μ m. (b) Showing endoneurial (ENDO) and perineurial (PERI) connective tissue surrounding individual axons and nerve fascicles, respectively in 26 year-old individual. Scale bar=20 μ m.

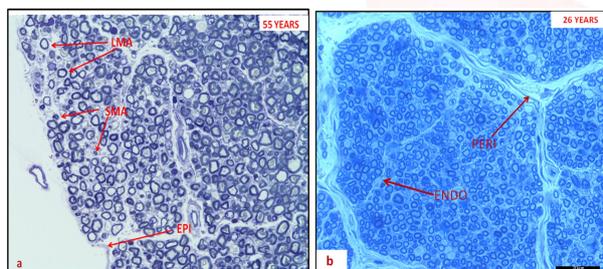
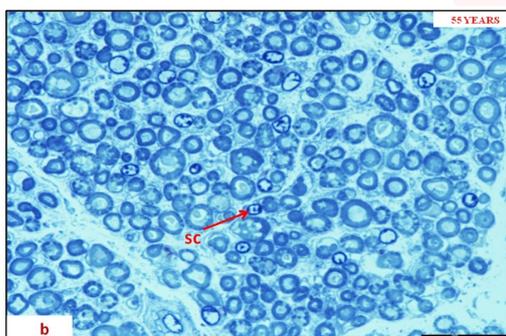


Fig. 3: Showing numerous Schwann cells (SC) in 55 year-old individual. Scale bar=30 μ m.



Organization of connective tissue: On the outer surface, the CN was covered by dense irregular thin layer of connective tissue – the epineurium. The epineurium which sheathed the nerve fascicles contained few blood vessels. The

perineurium was made of thin sleeve of connective tissue, whereas the endoneurium was a delicate layer of connective tissue enclosing the individual axons with minute blood vessels. In the cross-sections, the nerve fascicles were well defined in all the 21 samples studied across all the age groups (Fig.1). The approximate number of fascicles varied from 60 to 85 per nerve.

Organization of myelinated fibres in the fascicles: We could identify myelinated fibres in majority of the toluidine blue stained sections. In light microscopy, the axons appeared as round empty spaces with darkly stained rim of myelin surrounding the axons. Some specimens showed autolytic changes in axons as well as myelin sheath, which were discovered only after examining the resin blocks under the microscope.

Connective tissue organization: The epineurium surrounding the CN was thin (Fig.2a). We could not find significant amount of adipose tissue in the epineurium in any of the samples studied. Perineurium (Fig.2b) and perineurial cells were distinctly visible in all the sections. These perineurial cells appeared as flattened with euchromatic oval shaped nuclei. Endoneurium (Fig.2b) was darkly stained compared to lightly stained perineurium. Numerous Schwann cells (Fig.3) were found in the endoneurium of older age group compared to younger and middle aged groups. Smaller blood vessels (Fig.2b) were abundant in endoneurium of older age group compared to younger and middle aged groups. The blood vessels from the epineurium traversing through the perineurium carried a distinct sleeve of connective tissue in the course of reaching the endoneurium.

DISCUSSION

It can be said that manifestations of the age related hearing loss in terms of decline in the auditory perceptual abilities serve as the unique window for observing the neurobiological changes taking place in the nervous system [18]. Various studies [19-21] have proposed putative mechanisms of hearing loss and usually it is aging along with environmental factors such as excessive noise, predisposition to genetic determinants and lifestyle factors such as cigarette smoking and micro-vascular diseases

The loss of spiral ganglion neurons (SGNs) and auditory nerve fibres has been reported in aged rats [13,23] and many strains of aged mice [12,22]. However, the literature based on human specimens are sparse owing to following reasons: a) difficulty in accessing the CN by surgical biopsy b) typically long post-mortem fixation interval for human temporal bones; and c) very limited access to well-fixed nerve samples during surgical procedures [24]. Radiological studies (25, 26) have shown that age-related hearing loss presents with neuronal loss, which could cause reduction in the CN cross sectional area. The main aim of the present study is to compare the qualitative morphological features of CN across various age groups.

The connective tissue organization of the CN is of great clinical importance as it provides the primary structural framework and biomechanical protection and acts as a vehicle for carrying blood vessels to the nerve. We found that CN in all age groups had well defined axonal arrangement in the fascicles and the epineurium was thin and weak.

The thickness of the CN fibres is certainly important as conduction time plays a very important role in synchronisation of activities and in periodicity pitch sensation. It is reasonable to assume that alterations in fibre diameter which would reflect the underlying neuronal losses are followed by changes in conduction velocity. There were no changes observed in the axonal distribution in all ages studied.

Morphology of Cochlear nerve:

Shape	Comma
Epineurium	Thin and weak
Number of fascicles	60 to 85

The myelination pattern differs between the components of auditory system itself because of the transition between peripheral nervous system and central nervous system. A histological study done on the rat CN showed that myelin along the axon segment between the SGNs cell body and the transitional zone is comprised of peripheral myelin proteins while myelin between the transitional zone and brainstem is exclusively of the central type (27). In the present study, small thin myelinated fibres dispersed among the thick fibres were

found in most of the specimens of different age groups.

Since we have excluded the cases with ear injury and related pathology, the possibilities of acquired sensori-neural hearing loss are ruled out. Direct injuries in form of vascular, trauma or infections would present with visible degenerations in the morphology [28] and occult mechanisms such as ototoxic drugs, acoustic over-exposure and conditions producing localised anoxia would result in minor degenerative manifestations. The signs of degeneration range from development of oedema around the nerve fibres to the visible tears in the myelin sheath. In the final stages, the fibres tend to collapse and manifest as reduction in cross sectional area [29].

In the present study, apart from the increase in Schwann cells and increase in the number of small sized blood vessels in the endoneurium of older age group, we could not make out significant qualitative morphological changes. Similarly, no changes could be observed between the specimens gathered from male and female cadavers. It can be presumed that gross morphological changes cannot be observed as a part of age related hearing loss and with a minimal sample size it becomes more unlikely. To add, the magnitude of neuronal loss should be very high to get manifested in the qualitative morphology aspect and gradual / minimal neuronal losses cannot be made out easily. Furthermore, apart from CN the aging changes can also be in other components such as stria, organ of Corti, spiral ganglion and cochlear nucleus which weren't included as study parameters. These are the possible reasons due to which we could not detect gross morphological variations in the cochlear nerve belonging to different age groups.

CONCLUSION

Being one of the important senses of the body, hearing ability undergoes diminution with age and its impact is profound considering the social repercussions. We aimed at studying the gross morphological changes of the CN in different age groups. Even though, the study based on qualitative morphological data of CN is not suggestive of major changes across

different age groups, the normative data obtained out of it gives us pertinent understanding about the morphology. Further studies based on quantitative analysis using stereology, immunohistochemistry and electron microscopy would widen our knowledge regarding the different types of presbycusis and the optimal management protocol for them in longer run.

Conflicts of Interests: None

REFERENCES

- [1]. Bridgwood A. General household survey: living in Britain—results from the 1998 general household survey. London: The Stationery Office Books, 2000.
- [2]. Bohne BA, Harding GW. Degeneration in the cochlea after noise damage: primary versus secondary events. *Am J Otol* 2000;21:505-9.
- [3]. Bahmad F Jr, Merchant SN, Nadol JB Jr, Tranebjaerg L. Otopathology in Mohr-Tranebjaerg syndrome. *Laryngoscope* 2007;117:1202-8.
- [4]. Schuknecht HF, Gacek MR. Cochlear pathology in presbycusis. *Ann Otol Rhinol Laryngol* 1993; 102:1-16.
- [5]. Schuknecht HF, Woellner RC. An experimental and clinical study of deafness from 19 lesions of the cochlear nerve. *The Journal of laryngology and otology* 1955; 69, 75-97.
- [6]. Frisina DR, Frisina RD. Speech recognition in noise and presbycusis: relations to 11 possible neural mechanisms. *Hear Res* 1997;106:95-104.
- [7]. Schuknecht HF. Further observations on the pathology of presbycusis. *Arch Otolaryngol* 1964;80:369.
- [8]. Belal A, Glorig A. The ageing ear a clinico pathological classification. *J Laryngol Otol* 1997; 10: 1131-35.
- [9]. Hawkins JE. Comparative otopathology: aging, noise, and ototoxic drugs. *Adv Otorhinolaryngol* 1973; 20: 125-141.
- [10]. Viemeister NF. Auditory intensity discrimination at high frequencies in the presence of noise. *Science*. 1983;221:1206-1208.
- [11]. Taberner AM, Liberman MC. Response properties of single auditory nerve fibres in the mouse. *J. Neurophysiol.* 2005;93:557-569.
- [12]. Ohlemiller KK. Age-related hearing loss: the status of Schuknecht's typology. *Curr. Opin. Otolaryngol. Head Neck Surg.* 2004; 12:439-443.
- [13]. Hoeffding V, Feldman ML. Changes with age in the morphology of the cochlear nerve in rats: Light Microscopy. *J Comp Neurol.* 1988; 276: 537-546.
- [14]. Felder E, Schrott-Fischer A. Quantitative evaluation of myelinated nerve fibres and hair cells in cochleae of humans with age-related high-tone hearing loss. *Hear Res.* 1995; 91: 19-32.
- [15]. Schüz A, Palm G. Density of neurons and synapses in the cerebral cortex of the mouse. *J. Comp Neurol.* 1989; 286: 442-455.
- [16]. Mathieu O, Cruz-Orive LM, Hoppeler H, Weibel ER. Measuring error and sampling variation in stereology. Comparison of the efficiency of various methods for planar image analysis. *J Microsc.* 1981; 121: 75-88.
- [17]. Spoendlin H. Differentiation of cochlear afferent neurons. *Acta Otolaryngol.* 1981; 91: 451-456.
- [18]. Frisina RD, Ding B, Zhu X, Walton JP. Age-related hearing loss: prevention of threshold declines, cell loss and apoptosis in spiral ganglion neurons. *Ageing (Albany NY).* 2016; 8(9):2081-99.
- [19]. Yamasoba T, Lin FR, Someya S, Kashio A, Sakamoto T, Kondo K. Current concepts in age-related hearing loss: epidemiology and mechanistic pathways. *Hear Res.* 2013;303:30-8.
- [20]. Christensen K, Frederiksen H, Hoffman HJ. Genetic and environmental influences on self-reported reduced hearing in the old and oldest old. *J Am Geriatr Soc* 2001; 49:1512-17.
- [21]. Lin FR, Thorpe R, Gordon-Salant S, Ferrucci L. Hearing loss prevalence and risk factors among older adults in the United States. *J Gerontol A Biol Sci Med Sci.* 2011;66:582-590.
- [22]. Sha SH, Kanicki A, Dootz G, et al. Age-related auditory pathology in the CBA/J mouse. *Hear Res.* 2008;243(1-2):87-94.
- [23]. Keithley EM, Feldman ML. Spiral ganglion cell counts in an age-graded series of rat cochleas. *J Comp Neurol.* 1979;188:429-442.
- [24]. Xing Y, Samuvel DJ, Stevens SM, Dubno JR, Schulte BA, Lang H. Age-related changes of myelin basic protein in mouse and human auditory nerve. *PLoS One.* 2012;7(4):e34500.
- [25]. Felder E, Kanonier G, Scholtz A et al. Quantitative evaluation of cochlear neurons and computer-aided three-dimensional reconstruction of spiral ganglion cells in humans with peripheral loss of nerve fibres. *Hear Res* 1997; 105:183-190
- [26]. Spoendlin H, Schrott A. Analysis of the human auditory nerve. *Hear Res* 1989; 43:25-38
- [27]. Toesca A. Central and peripheral myelin in the rat cochlear and vestibular nerves. *Neuroscience letters.* 1996;221:21-24.
- [28]. Spoendlin H. Retrograde degeneration of the cochlear nerve. *Acta Otolaryngol* 1975; 79:266-75.
- [29]. Hoeffding V, Feldman ML. Degeneration in the cochlear nerve of the rat following cochlear lesions. *Brain Res* 1988; 24:104-115.

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