

Brief Communication Comunicações Breves

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Aged voice: does advancing age generate different impacts?

Voz do idoso: o avanço da idade gera diferentes impactos?

ABSTRACT

Purpose: To verify if there are differences in the vocal aspects of older people from three different age groups with presbyphonia diagnosis. **Methods**: Sixty older adults joined this study. They were both female and male, with an age range from 60 to 90 years old (average: 72.3) and with presbyphonia diagnosis established after otolaryngology evaluation. From their voice recordings, it was possible to make the acoustic and auditory-perceptual analysis. The data collected was compared through statistical tests considering the division of the participants into the following groups: 60-70 years old, 71-80 years old, and 81-90 years old. **Results**: Even though the older people from all of the three groups have presented deviation in multiple vocal aspects such as instability and vocal noise in low frequencies, those with more than 80 years old have presented a higher deviation of the general grade of dysphonia, roughness, breathiness, and pitch. In this group, it was also observed higher deviations in jitter, shimmer, vocal breaks, and the GNE measure on the edge of normality. All the differences were statistically significant. The majority of the older participants from that group presented even a deviation in the phonatory deviation diagram and frequency break. **Conclusion**: Various acoustic and auditory-perceptual aspects had a higher deviation of the vocal aging impacts and also in the development of actions to minimize vocal declination.

RESUMO

Objetivo: Verificar se existem diferenças em aspectos vocais entre idosos com presbifonia, divididos em três faixas etárias. **Método**: Participaram 60 idosos de ambos os sexos com idades entre 60 e 90 anos (média=72,3), com diagnóstico de presbifonia estabelecido em avaliação otorrinolaringológica. A partir da gravação das vozes foi realizada análise perceptivo-auditiva e acústica e os dados foram comparados por meio dos testes estatísticos com os participantes divididos nos grupos etários 60-70 anos, 71-80 anos e 81-90 anos. **Resultados**: Ainda que os idosos dos três grupos tenham apresentado alterações em aspectos vocais variados, como instabilidade e ruído em frequências graves, aqueles com mais de 80 anos apresentaram maior grau de disfonia, rugosidade, soprosidade e pitch. Também foram observados neste grupo maiores desvios em jitter, shimmer e irregularidade e a medida GNE no limite da normalidade. Todas as diferenças foram estatisticamente significativas. A maioria dos idosos dessa faixa etária apresentou ainda alteração no diagrama de desvio fonatório e quebra de frequência. **Conclusão**: Diversos aspectos perceptivo-auditivos e acústicos se apresentaram mais desviados nos idosos mais velhos, o que reforça a necessidade de serem consideradas essas especificidades tanto na avaliação dos efeitos do envelhecimento na voz quanto no desenvolvimento de ações para minimizar o declínio vocal.

Study conducted at Universidade de São Paulo - USP - São Paulo (SP), Brasil.

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INTRODUCTION

With advancing age, there is a gradual deterioration of laryngeal structures and all physiology responsible for phonation^(1,2), with an impact on perceptual-auditory and acoustic vocal aspects⁽³⁾.

The muscular decline of the aging process causes patients with presbyphonia to present vocal fold arching, with the formation of a glottal cleft during phonation^(1,4). The arytenoid cartilages are more prominent than in adults and young people, and asymmetry or reduction in the amplitude of the mucous wave is observed, in addition to the predominance of the open phase in the vibratory cycle of the vocal folds^(4,5). In addition, microscopic changes in the superficial layer of the lamina propria may include thickening or edema, atrophy of elastic fibers, and a decrease in the number of fibroblasts. Histologically, there is a decrease in the total number of cells, changes in protein synthesis, and a reduction in the production of extracellular matrix⁽⁵⁾.

Aging also has an impact on breathing and resonance mechanisms, and the comorbidities and use of medications frequently observed in the older adults interfere with vocal production as a whole⁽⁶⁾. The aged voice may have reduced harmonics, alteration of the fundamental frequency, increased jitter and shimmer, restricted vocal extension, pneumophonoarticulatory incoordination, increased breathiness, roughness and tremor, difficulties in modulating intensity, in addition to decreased vocal resistance and maximum times phonatory⁽⁷⁾.

With advancing age, metabolism becomes slower and endocrine functioning is reduced, especially after menopause, in the case of women. The glandular tissues are progressively atrophied and this can lead to a reduction in vocal extension and changes in fundamental frequency - decrease in women and elevation in men. This means that the voices of both genders can be more easily confused in old age than in youth or adult life⁽⁸⁾. The complaints reported by the older people may be weak and breathy⁽⁹⁾, decreased vocal resistance and hoarseness⁽¹⁾, in addition to difficulty in projecting the voice⁽⁹⁾, being heard in the presence of noise⁽¹⁾, and having to repeat many times what they say⁽²⁾. These factors have a direct impact on their communication and sociability, although to varying degrees⁽⁵⁾. Losses in communication affect the psychological well-being and autonomy of the older population^(2,6).

It is estimated that the prevalence of voice disorders in older people varies between 4.8% and 39.3%^(1,2,9). Kost and Sataloff⁽⁵⁾ describe more significant changes resulting from aging in the larynx of men. It should be noted that the aging process has changed with the advancement of science, technology, and life habits, which has an impact on increasing longevity. In just ten years, the number of older people grew by 40.3% in Brazil and there was a decrease in hospital admissions for this group, which may be the result of the expansion of primary health care services and improved quality⁽¹⁰⁾. In this scenario, it is essential to consider that the older population are increasingly heterogeneous, and it is not enough to develop health strategies considering them only as a single group of individuals over 60 years old. Even health conditions can be very different among elderly people of different ages⁽²⁾. Aside from that, the professional use of the voice is something that lasts for many of them; in these cases, in addition to signs of presbilarynx and pictures of varying degrees of presbyphonia, other vocal impairments, such as signs and symptoms related to phonotrauma, may be associated, for example.

The increasing number of people in this age group who need more effective communication justifies the development of studies that delve deeper into the topic. With a view to the complexity of communication in the older population, knowledge about the characteristics of presbyphonia in different age groups from the age of 60 will guide the development of more specific actions to minimize vocal decline and its effects on their quality of life. This study aimed to verify possible differences in perceptualauditory and acoustic vocal aspects in older people diagnosed with presbyphonia divided into three age groups.

METHODS

This is a retrospective observational cross-sectional study, approved by the Ethics Committee for Analysis of Projects and Research of the Hospital das Clínicas, School of Medicine, Universidade de Sao Paulo (CAAE 57164516.3.0000.0068). In the database of the institution's voice outpatient clinic, all individuals over 60 years of age were selected, with otorhinolaryngological diagnosis of presbyphonia; and who had performed voice recording.

The exclusion factors were the presence of comorbidities of a neurological, auditory, laryngeal, or respiratory nature, in addition to a previous history of voice, speech, or language alterations. All participants signed an Informed Consent Form at the time of data collection.

Information was collected from 60 older people, 36 (60%) women and 24 (40%) men, aged between 60 and 90 years old (average: 72.3 years old). For data analysis, participants were divided into three groups, according to the age group:

- G1: ages between 60 and 70 years old; 27 participants, mean of 66.3 years old [minimum 65.19; maximum 67.44; standard deviation 3.11]; 16 women and 11 men;
- G2: ages between 71 and 80 years old; 25 participants, average: 74.8 years old [minimum 73.84; maximum 75.96; standard deviation 2.76]; 15 women and 10 men);
- G3: ages between 81 and 90 years old; 8 participants, mean 84.2 years old [82.13, 86.63; standard deviation 3.5]; 5 women and 3 men.

The voice recording of patients takes place in the outpatient care routine, in an acoustically treated room, using a desktop computer (HP, USA), Audacity software (Audacity team, USA), Edirol UA-101 interface (Roland, United Kingdom), and AKG microphone model 520 (AKG, Germany). In the evaluation guide, the Brazilian adaptation of the Consensus Auditory-Perceptual Evaluation of Voice (CAPE-V)⁽¹¹⁾ is used. As proposed in CAPE-V⁽¹²⁾, the degree of deviation is indicated on a 100 mm analog-visual scale from listening to the recordings of all vocal tasks - emitting vowels, reading sentences, and spontaneous speech. Based on these guidelines⁽¹²⁾, a speech-

language pathologist specialized in the voice analyzed the general degree of dysphonia, roughness, breathiness, tension, pitch and loudness. This professional had over ten years of experience in using this instrument and in the analysis of voices from older people and who presented high intra-judge reliability (intraclass correlation coefficient of 0.975).

The acoustic vocal analysis was performed using the Voxmetria 2.6 software (CTS Informática, Brazil). The emission of the sustained vowel ϵ / for about five seconds, produced in the usual frequency and intensity, was edited to use the more stable medial third. In the vocal quality analysis module, we extracted the measures of fundamental frequency (minimum, average, maximum, and variability), jitter, shimmer, glottal to noise excitation (GNE ratio), vocal break, and noise. The means were considered and, for data interpretation, the classification as normal/altered according to parameters of normality provided by the software: PPQ jitter between 0.0 and 0.6%, EPQ shimmer between 0.0 and 6.5%, proportion GNE between 0.5 and 1.0, the vocal break between 0.0 and 4.75 and noise between 0.0 and 2.5.

The same vowel was used to position vocal production in the quadrants of the Phonatory Deviation Diagram (PDD), with quadrant 1 being considered in the software as the region of normality. For the spectrographic analysis, the vowel $/\epsilon/$ was used again and the presence/absence of the following aspects was considered: instability, sub-harmonic, noise at high frequencies, noise at low frequencies, frequency drop, and sound drop. The series of harmonics was classified as adequate or altered.

To extract the values related to the vocal intensity (minimum, average, and maximum), we used the Voice Analysis module of the same software, in which the use of some part of speech is required. The phrase "Érica drank pear and blackberry juice" was randomly selected, which contains all the vowels of Brazilian Portuguese and is the first one read by individuals when recording vocal tasks. The descriptive analysis considered data related to age and gender and measures of the fundamental frequency.

To define the inferential statistical analysis, the Kolmogorov-Smirnov normality test was initially applied. For comparison between the three groups in the numerical variables, either the Kruskall-Wallis non-parametric test or the ANOVA One-Way parametric test was applied, using multiple Tukey comparisons to analyze the significant differences two by two, when necessary. Fisher's exact test was applied to compare categorical variables. The level of significance adopted was 5%.

RESULTS

When initially considering all the older participants, regardless of age groups, the following averages stood out: general degree of dysphonia of 52.5 and roughness 48.8, jitter 0.75%, average intensity 59.9 dB, series of altered harmonics (100.0%), noise in low frequencies (96.7%), instability (82.8%), sub-harmonics (58.3%) and noise in high frequencies (56.7%).

In the comparison between the groups, the older adults in G3 presented greater breathiness and pitch deviation (p=0.025, p=0.018) than G1; higher shimmer values (p=0.017) than G2; and greater vocal break than G1 and G2 (p=0.042, p=0.011) (Table 1). In G3, most of the participants had altered PDD and also a drop in frequency, which was not observed in G1 and G2 (Table 2).

Table 1. Comparison between the elderly of the three age groups concerning the numerical variables resulting from the auditory-perceptual and acoustic analyzes

Appente applyzed			Groups		Divelue	Comparisons	
Aspects a	naryzeu	G1 (N=27)	G2 (N=25)	G2 (N=25) G3 (N=8)		Multiples 2x2	
General degree	Average	46.7	50.1	50.1 60.6			
	Median	44.0	53.0	58.5	0.179		
	SD	19.2	17.4	18.5			
Roughness	Average	42.7	44.4	59.3			
	Median	40.0	42.0	58.5	0.110		
	SD	19.9	19.9	16.6			
Breathiness	Average	33.6	36.2	56.5		(60-70)x(81-90) (p)=0.025*	
	Median	32.0	32.0	52.5	0.029*	(71-80)x(81-90) (p)=0.054	
	SD	21.4	20.9	21.0			
Tension	Average	21.7	18.6	27.0			
	Median	18.0	8.0	35.5	0.625		
	SD	23.3	22.2	23.4			
Pitch	Average	13.0	22.8	33.4		(60-70)x(81-90) (p)=0.018*	
	Median	0.0	25.0	34.0	0.015*		
	SD	15.9	19.9	17.8			
Loudness	Average	17.2	21.1	32.0			
	Median	0.0	22.0	37.0	0.280		
	SD	27.1	23.2	29.9			
Jitter	Average	0.72	0.53	1.01			
	Median	0.31	0.17	0.89	0.482		
	SD	1.26	0.66	0.76			

*statistically significant; One-way ANOVA or Kruskal-Wallis test

Caption: SD = standard deviation; GNE = Glottal-to-Noise Excitation Ratio

Table	1.	Continued

Aspects analyzed			Groups			Comparisons Multiples 2x2		
		G1 (N=27)	G2 (N=25)	G3 (N=8)	F-value			
Shimmer	Average	5.01	4.25	8.49		(60-70)x(81-90) (p)=0.058		
	Median	3.95	3.47	7.62	0.010*	(71-80)x(81-90) (p)=0.017*		
	SD	4.48	2.45	4.02				
Vocal break	Average	3.76	3.52	4.85		(60-70)x(81-90) (p)=0.042*		
	Median	3.63	3.43	4.71	0.015*	(71-80)x(81-90) (p)=0.011*		
	SD	1.23	0.99	0.83				
GNE measure	Average	0.71	0.72	0.58				
	Median	0.78	0.80	0.63	0.201			
	SD	0.23	0.20	0.22				
Noise	Average	1.42	1.41	1.99				
	Median	1.15	1.08	1.77	0.201			
	SD	0.94	0.85	0.91				
Average intensity	Average	60.5	59.4	59.8				
	Median	58.8	57.5	59.2	0.777			
	SD	6.2	5.4	6.1				
Maximum	Average	77.9	77.5	78.0				
intensity	Median	78.1	76.0	77.9	0.972			
	SD	7.2	5.4	4.8				
Minimum	Average	33.0	27.6	31.9				
intensity	Median	34.4	24.0	30.0	0.069			
	SD	9.1	8.9	10.1				

*statistically significant; One-way ANOVA or Kruskal-Wallis test Caption: SD = standard deviation; GNE = Glottal-to-Noise Excitation Ratio

Table 2. Comparison between the elderly of the three age groups concerning the categorical variables

	_	Groups					Total (NI-60)			
Aspects analyzed		G1 (N=27)		G2 (N=25)		G3 (N=8)		10tal (N=00)		P-value
		Ν	%	Ν	%	Ν	%	Ν	%	
Gender	Female	16	59.3	15	60.0	5	62.5	36	60.0	> 0.999
	Male	11	40.7	10	40.0	3	37.5	24	40.0	
PDD	altered	11	40.7	7	28.0	5	62.5	23	38.3	0.235
	normal	16	59.3	18	72.0	3	37.5	37	61.7	
Instability	absent	6	22.2	3	12.0	1	12.5	10	16.7	0.710
	present	21	77.8	22	88.0	7	87.5	50	83.3	
Sub-harmonic	absent	11	40.7	10	40.0	4	50.0	25	41.7	0.876
	present	16	59.3	15	60.0	4	50.0	35	58.3	
high freq noise	absent	12	44.4	10	40.0	4	50.0	26	43.3	0.880
	present	15	55.6	15	60.0	4	50.0	34	56.7	
low freq noise	absent	1	3.7	1	4.0	0	.0	2	3.3	> 0.999
	present	26	96.3	24	96.0	8	100.0	58	96.7	
harmonic series	altered	27	100.0	25	100.0	8	100.0	60	100.0	NC
frequency drop	absent	17	63.0	15	60.0	3	37.5	35	58.3	0.482
	present	10	37.0	10	40.0	5	62.5	25	41.7	
sound drop	absent	23	85.2	21	84.0	7	87.5	51	85.0	> 0.999
	present	4	14.8	4	16.0	1	12.5	9	15.0	

Fisher's exact test Caption: PDD = Phonological deviation diagram; NC = not calculable

DISCUSSION

This study aimed to analyze differences in the voice of older people of varying ages, of both genders, diagnosed with presbyphonia and without comorbidities that could impact vocal production. The older adults in the three groups presented perceptual-auditory and acoustic vocal alterations described in the literature, resulting from physiological changes, and which are interrelated, such as roughness, breathiness, high jitter, altered harmonic series, instability, noise in low and high frequencies, presence of sub-harmonics and reduced vocal intensity^(1,4,5,7,9,13). A study with older adults choristers and non-choristers found auditory-perceptual alterations to a lesser extent⁽¹⁴⁾.

In the comparison between the groups, we could verify a worse degree of breathiness and a more altered pitch in the age group above 80 years old in the older participants aged 60 to 70 years old, indicating a greater impact of physiological changes^(1,3-5,7) in the older ones. Older people with presbyphonia, of different ages, differed from older people without presbyphonia in all aspects proposed in CAPE-V, except pitch⁽⁶⁾. Considering the normality parameters of the software used, the G3 average jitter was the most altered, with G1 and G2 staying at the normal limit, but it is worth noting that the program does not explain whether this parameter applies to the older people or only adults.

We observed some other changes commonly described in aspects such as jitter, shimmer, and vocal break^(1,7) only in the group of older people over 80 years old. This may indicate that the impact of the reduction in the amplitude of the mucous wave, of the vocal break vocal, fold vibration, and vocal fold thickening and arching observed in the older adults^(1,4,5,7,9) is higher in this range. Older people with presbyphonia may have altered shimmer than older people without presbyphonia, regardless of age⁽⁶⁾. This greater impact of physiological changes in the older people can also justify the GNE proportion at the limit of normality and change in PDD and drop in frequency in most of the older participants in this group. The impossibility of complete adduction of the vocal folds during phonation can interfere with the stability and frequency control⁽¹⁵⁾. In general, we could verify deterioration of some aspects only in the older adults aged over 80 years old, as well as some alterations with greater severity.

The limitation of this study was the comparison between genders, which may have impacted the comparison between the groups in the pitch aspect. Due to the anatomophysiological differences between men and women, such as the smaller amplitude of the mucus wave motion of the vocal folds in older men than women⁽¹³⁾, it is intended, with the continuity of this study, to analyze all aspects separately by gender. In addition, factors related to the professional use of the voice and consumption of medications that can negatively impact the voice must be investigated⁽¹⁾.

This research differs from others by the participation only of older people diagnosed with presbyphonia and the absence of comorbidities that could impact phonation. This choice, on the one hand, hindered the composition of the sample, on the other hand, it avoided bias due to the presence of other diseases that could lead to greater vocal degradation. A study that compared older people with and without presbyphonia found a relationship between the presence of the disorder and a lower level of physical activity, higher stress levels, vocal hyperfunction, and lower quality of life⁽⁶⁾.

A speech-language pathologist can positively impact the vocal performance and social communication of the older adults, as well as improving their quality of life⁽¹⁾. For this reason, it is important to identify these individuals at a higher risk of vocal problems due to age⁽⁶⁾. The longitudinal monitoring of older people with and without presbyphonia submitted or not to speech-language pathologist intervention would enable to understand the benefits for these older people, especially from the age of 80.

In a society in which life expectancy increases, providing better communicative conditions for these older people is extremely important. Thus, the findings proved to be relevant and can be considered when planning specific actions for this population according to the age group to delay vocal decline.

CONCLUSION

Several perceptual-auditory and acoustic aspects were more deviated in older participants, which reinforces the need to consider these specificities both in the evaluation of the effects of aging on the voice and in the development of actions to minimize vocal decline.

REFERENCES

- Mezzedimi C, Di Francesco M, Livi W, Spinosi MC, De Felice C. Objective evaluation of presbyphonia: spectroacoustic study on 142 patients with Praat. J Voice. 2017;31(2):257.e25-32. http://dx.doi.org/10.1016/j. jvoice.2016.05.022. PMid:27427181.
- Wong HYK, Ma EPM. Self-Perceived voice problems in a nontreatment seeking older population in Hong Kong. J Voice. 2021;35(4):597-603. http://dx.doi.org/10.1016/j.jvoice.2019.12.012. PMid:31911022.
- Gregory ND, Chandran S, Lurie D, Sataloff RT. Voice Disorders in the elderly. J Voice. 2012;26(2):254-8. http://dx.doi.org/10.1016/j.jvoice.2010.10.024. PMid:21530163.
- Kendall K. Presbyphonia: a review. Curr Opin Otolaryngol Head Neck Surg. 2007;15(3):137-40. http://dx.doi.org/10.1097/MOO.0b013e328166794f. PMid:17483679.
- Kost KM, Sataloff RT. Voice disorders in the elderly. Clin Geriatr Med. 2018;34(2):191-203. http://dx.doi.org/10.1016/j.cger.2018.01.010. PMid:29661332.
- Angadi V, McMullen C, Andreatta R, Dietrich M, Uhl T, Stemple J. Biobehavioral measures of Presbylaryngeus. J Voice. 2020;34(3):415-25. http://dx.doi.org/10.1016/j.jvoice.2018.11.005. PMid:30503609.
- Martins RH, Gonçalvez TM, Pessin AB, Branco A. Aging voice: presbyphonia. Aging Clin Exp Res. 2014;26(1):1-5. http://dx.doi.org/10.1007/s40520-013-0143-5. PMid:24068559.
- Menezes LN, Vicente LCC. Envelhecimento vocal em idosos instucionalizados. Rev CEFAC. 2007;9(1):90-8. http://dx.doi.org/10.1590/ S1516-18462007000100012.
- Gois ACB, Pernambuco LA, Lima KC. Factors associated with voice disorders among the elderly: a systematic review. Braz J Otorhinolaryngol. 2018;84(4):506-13. http://dx.doi.org/10.1016/j.bjorl.2017.11.002. PMid:29331352.
- Miranda GMD, Mendes ACG, Silva ALA. Population aging in Brazil: current and future social challenges and consequences. Rev Bras Geriatr Gerontol. 2016;19(3):507-19. http://dx.doi.org/10.1590/1809-98232016019.150140.

- Behlau M, Rocha B, Englert M, Madazio G. Validation of the Brazilian Portuguese CAPE-V Instrument-Br CAPE-V for Auditory-Perceptual Analysis. J Voice. 2020. In press. http://dx.doi.org/10.1016/j.jvoice.2020.07.007. PMid:32811691.
- 12. ASHA: American Speech-Language-Hearing Association. Consensus Auditory-Perceptual Evaluation of Voice (CAPE-V). ASHA Special Interest Group 3, Voice and Voice Disorders [Internet]. Washington: ASHA; 2009 [citado em 2020 Abr 24]. Disponível em: https://www.asha. org/uploadedFiles/ASHA/SIG/03/CAPE-V-Procedures-and-Form.pdf
- Sulter AM, Schutte HK, Miller DG. Standardized laryngeal videostroboscopic rating: differences between untrained and trained male and female subjects, and effects of varying sound intensity, fundamental frequency and age. J Voice. 1996;10(2):175-89. http://dx.doi.org/10.1016/S0892-1997(96)80045-2. PMid:8734393.
- Aquino FS, Silva MA, Teles LC, Ferreira LP. Características da voz falada de idosas com prática de canto coral. CoDAS. 2016;28(4):446-53. http:// dx.doi.org/10.1590/2317-1782/20162015109. PMid:27652927.
- Meirelles RC, Bak R, Cruz FC. Presbifonia. Revista do Hospital Universitário Pedro Ernesto. 2012;11:77-82.

Author contributions

ABPG was responsible for organizing and analyzing data and writing the article; MSZ participated in the data analysis and discussion of the results and was responsible for the correction and finalization of the article; KN was responsible for the design and coordination of the study, for the analysis and discussion of the data and revision of the manuscript.