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RESEARCH ARTICLE

RELATION BETWEEN OROTRACHEAL INTUBATION, INFLAMMATORY MARKERS, BREATHING AND VOICE IN POST-COVID-19

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Abstract

Introduction: COVID-19, an infectious disease with a wide spectrum of clinical manifestations and intensities in the human body, it can cause respiratory and vocal disorders, with fatigue. Objective: To verify the relation between biological Inflammatory markers D-dimers and C-Reactive Protein, Forced Vital Capacity, Maximum Phonation Time, vocal performance and fatigue, length of hospitalization period and gender of people affected by COVID-19 who were hospitalized, but did not use orotracheal intubation and compare with a group of post-COVID-19 patients with orotracheal intubation. Methods: Data on D-dimers and C-Reactive Protein, spirometry, Maximum Phonation Time, performance and vocal fatigue were collected. The study included 42 adult people affected by COVID-19 who were hospitalized, 22 (52.4%) female and 20 (47.6%) male; 23 (54.8%) critical cases composing the group with orotracheal intubation (average age 48.9 years old) and 19 (45.24%) severe cases in the group without orotracheal intubation (average age 49.9 years old). Results: hospital length of stay was significantly longer for the group with orotracheal intubation; D-dimers were significantly altered in all groups; correlations between maximum phonation times were positive and significant; correlations between maximum phonation times, vocal performance and fatigue were both negative and significant. Conclusion: Patients with orotracheal intubation had longer hospital internment and increased D-dimers and were amazed that, whenever maximum phonation times decreased performance and vocal fatigue increased.

Keywords: Biomarkers. Coronavirus infections. Health evaluation. Phonation. Vital capacity. Voice.

Introduction

COVID-19 (coronavirus disease 2019) is an infectious disease caused by the new coronavirus (SARS-CoV-2), associated with its most serious complication, severe acute respiratory syndrome 2 [1]. It develops in the human body with repercussions on breathing and voice [2,3]. The initial symptoms of COVID-19 may be similar to those of the common flu, and may manifest in the form of pneumonia, severe pneumonia or severe acute respiratory syndrome [4,5,6].

Most infected people have the mild form of the disease, with some symptoms such as malaise, fever, fatigue, cough, mild dyspnoea, anorexia, sore throat, body pain, headache or nasal congestion, and some may also present with diarrhea, nausea, and vomiting. Signs of fatigue, including with voice, may be persistent due to lung and respiratory mucosa impairment [2, 7, 8].

COVID-19 may lead to oxidative damage thus triggering an intense inflammatory response in the human body, being an extremely complex event [9]. There are some biological markers that determine the inflammatory response from the elevation of its serum levels, among them the C-reactive protein (CRP) and the D-Dimer (DD) [3,10,11] as an indication of worsening of the COVID-19 condition.

Such biological markers of inflammation were significantly higher in critically ill patients compared to non-severe ones [12]. Once the inflammatory condition in the body is determined by COVID-19, the person may evolve well, may need non-invasive ventilatory support, or present worsening and necessity of orotracheal intubation (OTI).

Aspects related to sex and COVID-19 vary according to the location in which the studies were carried out, but in general the disease affects more males [3,5,8,13]. In addition, older age, high number of comorbidities such as obesity, diabetes, heart

failure, prominent laboratory abnormalities were associated with critically ill patients and longer hospitalization periods [12,14].

In some post-COVID-19 cases during follow-up, it appears that patients may have clinical complications related to the disease itself, the decompensation of the underlying disease and the instituted treatment [15], such as lung damage that triggers respiratory failure and in an attempt to repair these damages, patients can evolve to pulmonary fibrosis [8]. The reflexes of these alterations can appear in the voice, more specifically in the maximum phonation times (MPT), which are influenced by the Forced vital capacity (FVC) or by the inadequate laryngeal valve effect [2,3], and in the increase of vocal fatigue.

The change in glottic coaptation may be caused by the elevated expression of angiotensin-converting enzyme 2 (ACE-2) that has been found in the vocal fold epithelium in individuals with COVID-19 causing edema [2]. OTI can injure the vocal folds, impairing glottic closure. They can be detected in patients with intubation for periods longer than 24 hours. Lesions occur mainly due to the pressure exerted by tubes larger than 7mm and/or cuff on the laryngotracheal mucosa and/or with the tube angle in relation to the labial commissure different from 90° [16,17].
They are correlated with a greater occurrence of dysphagia and greater degrees of vocal alteration. Clinical signs after extubation are: dysphagia, odynophagia, dysphonia/hoarseness, presence of stridor, pain on palpation in the trachea region and presence of bleeding [16,17,18,19,20].

Despite the use of vaccines and preventive measures, many people still end up being (re)infected and develop changes after COVID-19. Therefore, the importance of verifying the existence of a relation between post-COVID-19 inflammatory, respiratory and vocal variables to reduce the risk of other problems in the general health of the population.

Knowing the relation of biological markers with VC, MPT and between performance and vocal fatigue, assists speech therapy care to be personalized and assertive, reducing hospitalization time, hospital costs, the risk of laryngeal injuries,

with consequences on communication, which may interfere in the quality of life of patients and cause harm to society as a whole [21].

Thus, the working hypothesis of this research was that the people in the sample would have a predominance of OTI, male gender, older age and higher values in the biological markers of inflammation CRP as well as DD and would tend to have a lower VC, since the lung is one of the organs more affected, with lower MPT, higher performance scores and vocal fatigue, along with longer hospitalization.

Based on the above, the objective of this study was to verify the relation between the biological markers of inflammation DD and CRP, VC, MPT, vocal performance and fatigue, furthermore gender of people affected by COVID-19 who were hospitalized, but did not use the OTI to compare with a group of post-COVID-19 patients with OTI.

Materials and Methods

This is a cross-sectional, observational, field research, prospective, comparative and quantitative study, approved by the Human Research Ethics Committee of the institution of origin (n 4.527.287). The data were collected at the post-COVID-19 Rehabilitation Outpatient Clinic of the University Hospital of Santa Maria/RS, from August 2021 to May 2022.

Sampling Process

At the time of hospital discharge, patients returned for reassessment with the Pulmonologist. When the patient attended this return visit, he was referred to the Post-COVID-19 Rehabilitation Outpatient Clinic. If the doctor from another specialty had performed the discharge and had not scheduled a reassessment at the hospital, a nurse carried out an active search in the digital medical records of these patients and, after being contacted, they were invited to participate in this research. At that moment of invitation, all ethical aspects were clarified and, if they were interested in

participating, they should sign the informed consent form and go through the processes described below.

It is also important to add that the time for each assessment varied between 15 and 45 minutes, since most of them depended on the understanding and collaboration of the patient. To complete all assessments, the patient attended the medical clinic on two or three occasions.

The inclusion criteria adopted for patient selection were: male or female genders; ages between 20 and 65 years to exclude vocal changes and presbyphonia [22,23]; having been admitted to the UHSM with a medical diagnosis of COVID-19 with or without OTI. As exclusion criteria were adopted: report or diagnosis of neurological, endocrinological, psychiatric, gastric or laryngeal or vocal changes prior to COVID-19; not passing the audiological screening; declaring oneself to be a smoker or alcoholic and not having complete data in the medical records. For the application of the inclusion and exclusion criteria, the procedures described below were performed with the patients.

Anamnesis

Patients underwent anamnesis to report items such as: sex; age; pre-existing diseases (systemic or neurological); the hospital length of stay; respiratory, auditory and vocal symptoms, as well as smoking and alcoholism. The data were collected at the post-COVID-19 Rehabilitation Outpatient Clinic of the University Hospital of Santa Maria/RS.

Audiological Screening

The patients were submitted to audiological screening, without meatoscopy, performed by airborne pure tone scanning making use of earphones at frequencies of 1000, 2000 and 4000 Hz at 25 dB, with a calibrated audiometer (Interacoustics, AD629) and in an acoustic booth [24]. This evaluation was carried out in the audiology sector of UHSM. The screening was carried out to exclude patients with possible hearing loss that could interfere with the self-monitoring of the voice [25,26].

A total of 79 subjects were evaluated, of which 37 did not pass the adopted criteria. Nineteen male subjects were excluded for: not having CRP (7), not having DD (1), not having CV (1), having discomfort during voice collection (1), failure in the hearing screening (7), did not answer the questionnaires (2). Eighteen female subjects were excluded for: not having the PCR (1), not having the DD (3), not having the PCR or the DD (2), not having the VC (4), having discomfort during vocal collection (2), failed hearing screening (2) or did not answer the questionnaires (4).

Subjects who passed the inclusion and exclusion criteria made up the study sample and were divided into CG (critical group, subjects who required OTI) and SG (severe group, subjects who did not require OTI). So far, the study sample consisted of 42 cases, 22 (52.38%) females and 20 (47.62%) males, and 23 (54.76%) CG (mean age 48.86 years) and 19 (45.24%) SG (mean age 49.89 years). At the time of data collection for our study, no patient had a tracheostomy.

The Sample Power method of Minitab v21.2 software was applied to determine the power of this sample of all 42 cases. For this, we considered the prevalence of OTI in the sample, which was 54.8% (23 cases with OTI). The sample power of this study was 0.7405 (74.05%), a value slightly below the ideal value, which is 0.80 (80%).

After the contraction process, the following procedures were performed with the patients:

Data Collection

For data collection, measurements of the MPT/a/, /s/ and /z/ were performed and the Vocal Performance Questionnaire (VPQ) and Vocal Fatigue Index (VFI) protocols were applied. Data were collected by different evaluators, in a standardized manner, following the protocol of the clinic.

The results of the CRP, DD and spirometry measurements, as well as information on the hospital length of stay (LoS) and use of OTI were consulted in the medical records of each post-COVID-19 patient, as they are part of the Rehabilitation

Clinic post-COVID-19 routine of UHSM. Following the recommendations of the competent bodies [27,28,29,30,31,32,33,34], safety measures were adopted during data collection for this research, especially to keep patient safety [35].

Maximum phonation times

All MPT emissions were collected twice in a place with ambient noise lower than 48 dBC, measured by a digital sound pressure level meter (Icel®, DL 4200) [36,37]. The subjects were instructed to sustainably emit the phonemes /a/, /s/ and /z/ in usual pitch and loudness, in the orthostatic position after deep inspiration, until the end of expiration. The MPT were performed in the audiology sector, as it is an acoustically treated environment at the UHSM.

For further analysis, the highest timed value of each phoneme in seconds (s) was used [38,39]. MPT/a/ values between 14.04 and 26.96 s were considered reference values for adult females and between 16.06 and 26.27 s for adult males [40]. Reference values for both sexes of MPT/s/ and /z/ range from 15 to 25 s [41]. Lower values were suggestive of air escape during phonation (lack of glottic coaptation and/or respiratory flow or control) and higher values were suggestive of glottic hyperfunction [40,41].

Vocal self-assessment protocols

The vocal self-assessment questionnaires were read by the patients and the answers marked by the individuals themselves, leaving the researchers available to clarify possible filling doubts [42,43]. The data were collected at the post-COVID-19 Rehabilitation Outpatient Clinic of the University Hospital of Santa Maria/RS (UHSM).

The VPQ protocol is derived from clinical practice [44], translated and validated into Brazilian Portuguese [45], and includes several aspects of vocal performance, with 12 questions. The patient chose the best option among the five alternatives, with the letter “a” corresponding to one point up to a maximum of five points in the letter “e”. The maximum sum of points is 60 and the minimum score considered normal is

less than 12, any value above 12 indicates a decrease in vocal performance. It is a short, convenient questionnaire and has high internal consistency to measure the degree of vocal deviation [46].

The VFI consists of 17 questions, divided into four vocal factors [47]. Factor 1 has seven items (items 2, 3, 4, 5, 9, 10 and 11) related to “Tiredness and voice impairment”; factor 2 has three items (items 1, 6 and 7) related to "Avoidance of voice use"; factor 3 has four items (items 13, 14, 15 and 16) related to "Physical discomfort"; and factor 4 has three items (items 17, 18 and 19) related to "Improvement of voice symptoms with rest". The protocol was answered by the patient himself, according to the frequency with which he experiences the symptoms: 0 = never, 1 = almost never, 2 = sometimes, 3 = almost always and 4 = always. The threshold values for each factor were: 4.5 for tiredness and voice impairment; 3.5 for avoidance of voice usage; 1.5 for physical discomfort and 8.5 for improvement of vocal symptoms with rest. The threshold value for the total score was 11.5. The score is obtained by the simple sum of the questions. Therefore, the higher the score, the greater the fatigue in the first three factors. Unlike the other domains, the higher the score for the fourth factor, the greater the improvement in symptoms [47,48,49,50,51,52,53,54].

Spirometry

Spirometry is a physiological test that measures the maximum volume of air that an individual can inhale and exhale with maximum effort. The primary signal measured in spirometry is volume or flow as a function of time [55]. The most relevant measure discussed in this document is the FVC, which is the volume released during an expiration performed as vigorously and completely as possible starting from the complete inspiration [55,56]. A digital spirometer was used (MS-IOS, Jaeger).

The subject was seated using a clip for nasal occlusion and the lips should completely occlude the disposable mouthpiece inserted in the turbine. After being well positioned, the subject was instructed to perform both maximum inspiration and expiration maneuvers, according to the recommendations of the Brazilian Society of Pulmonology and Phthisiology [56] until three reproducible maneuvers were recorded

[57]. The VC spirometric data were interpreted making use of the Pereira [56] normative values for the Brazilian Society of Pulmonology and Phthisiology, the Forced vital capacity (FVC) is normally equal to the slow vital capacity (SVC) [58,59,60,61,62]. In individuals without airflow obstruction, FVC and SVC should differ by less than 0.2 l. FVC greater than SVC means, in general, lack of collaboration in the slow maneuver. FVC may be less than SVC in individuals with an obstructive disorder (significant difference above 0.2 l).

Spirometry was performed in the UHSM pneumology sector, but the spirometry results were consulted in the medical records of each post-COVID-19 patient, as they are part of the routine of the post-COVID-19 Rehabilitation Clinic of UHSM.

Biological markers of inflammation

Measurements of CRP and DD were consulted in the medical records of each post-COVID-19 patient, as they are part of the routine during hospitalization in the COVID-19 ward of the UHSM. In this study, for the analysis of the results, we considered that approximately 99% of the healthy population have CRP values below 0.1 mg/dl and, in most cases, the levels do not reach 0.2 mg/dl [63]. In general, mild inflammation and viral infections lead to elevations in the range of 0.1-0.4 mg/dl, while more severe inflammation and bacterial infections show serum concentrations between 0.40-20 mg/dl [64,65]. The material used is the serum, the method is immunoturbidimetry with reference values between 0.2 to 0.9 mg/dl.

The reference value of DD in blood is up to 0.5 mcg/ml. In COVID-19, values above 1 mcg/ml are associated with mortality and hypercoagulability [66,67], which can be predictive of the severity of cases and longer hospitalization periods. The material used is plasma in citrate and the method is immunoturbidimetric with reference values lower than 0.5 mcg/ml.

Statistical methods

The collected data were tabulated and statistically analyzed using the software: SPSS V20, Minitab 16 and Excel Office 2010. The significance level was 0.05 (5%). The Mann-Whitney U test was used to compare the CG across all the sample, segmented by gender. Comparison of the CG for the distribution of relative frequencies of qualitative factors was performed using Fisher's exact test. To analyze the relations between FVC, MPT, VFI and VPQ values, Spearman rank correlation was used. A significance level of p <0.05 was utilized.

Results

Comparisons between the variables age, hospital LoS and time until collection in the SG and CG, and in the entirety sample (All), according to gender are shown in Table 1. The hospital LoS was significantly longer for the CG (Female, Male and All).

TABLE 1 - Comparisons between the groups with and without orotracheal intubation for the variables age, length of hospitalization and time until the collect

			Mean	Median	Standard Deviation	p-value
Age	Female	CG	47.4	48	12.5	0.552
		SG	51	50	8.8	
	Male	CG	50.5	51	8	0.941
		SG	48.7	49	13.8	
	All	CG	48.9	51	10.5	0.781
		SG	49.9	49	11.2	
Hospital LoS (days)	Female	CG	22.4	18	12.5	<0.001*
		SG	9.2	10	3.3	
	Male	CG	29.5	23	15.9	0.002*
		SG	12.3	11	5.2	
	All	CG	25.8	23	14.3	<0.001*
		SG	10.7	10	4.5	
Time until collection (days)	Female	CG	115.9	66	95.2	0.974
		SG	85.7	75.5	57.4	
	Male	CG	172.6	124	152	0.732
		SG	169	182	97.8	

All	CG	143	98	126	0.980
	SG	125.2	87	87.9	

Note: CG (critical group); SG (severe group); LoS, hospital length of stay.

Mann-Whitney U test.

* statistically significant p values; p<0.05

Table 2 shows the comparisons between the CG and SG in the D-dimer and CRP variables according to gender and reference values. There was a statistically significant difference between the CG and SG in the distribution of DD in the totality of the sample.

TABLE 2 - Comparison between the groups with and without orotracheal intubation for the variables D-dimers and C-reactive protein, according to gender and reference values

			CG		SG		Total		p-value
			n	%	n	%	n	%	
DD (mcg/ml)	Female	Deviant	11	91.7	6	60	17	77.3	0.096
		Normal	1	8.3	4	40	5	22.7	
	Male	Deviant	11	100	8	88.9	19	95	0.450
		Normal	0	0	1	11.1	1	5	
	All	Deviant	22	95.7	14	73.7	36	85.7	0.043*
		Normal	1	4.3	5	26.3	6	14.3	
PCR (mg/dl)	Female	Deviant	11	91.7	7	70	18	81.8	0.197
		Normal	1	8.3	3	30	4	18.2	
	Male	Deviant	10	90.9	9	100	19	95	0.550
		Normal	1	9.1	0	0	1	5	
	All	Deviant	21	91.3	16	84.2	37	88.1	0.480
		Normal	2	8.7	3	15.8	5	11.9	

Note: CG (critical group); SG (severe group); DD, D-dimer; PCR, C-reactive protein.

Exact Test of Fisher.

* statistically significant p values; p<0.05

Table 3 shows the comparisons between the CG and SG in the variables VC and MPT/a/, MPT/s/ and MPT/z/, according to gender and reference values.

TABLE 3 - Comparison between the groups with and without orotracheal intubation for the variables forced vital capacity and maximum phonation times of /a/, /s/ and /z/, according to gender and reference values

			CG		SG		Total		p-value
			n	%	n	%	n	%	
FVC	Female	Deviant	2	16.7	3	30	5	22.7	0.301
		Normal	10	83.3	7	70	17	77.3	
	Male	Deviant	3	27.3	2	22.2	5	25	0.383
		Normal	8	72.7	7	77.8	15	75	
	All	Deviant	5	21.7	5	26.3	10	23.8	0.729
		Normal	18	78.3	14	73.7	32	76.2	
MPT/a/	Female	Reduced	11	91.7	8	80	19	86.4	0.351
		Normal	1	8.3	2	20	3	13.6	
	Male	Reduced	8	72.7	6	66.7	14	70	0.684
		Normal	2	18.2	1	11.1	3	15	
		Increased	1	9.1	2	22.2	3	15	
	All	Reduced	19	82.6	14	73.7	33	78.6	0.699
		Normal	3	13	3	15.8	6	14.3	
		Increased	1	4.3	2	10.5	3	7.1	
MPT/s/	Female	Reduced	11	91.7	7	70	18	81.8	0.357
		Normal	1	8.3	2	20	3	13.6	
		Increased	0	0	1	10	1	4.5	
	Male	Reduced	8	72.7	7	77.8	15	75	0.390
		Normal	3	27.3	1	11.1	4	20	
		Increased	0	0	1	11.1	1	5	
	All	Reduced	19	82.6	14	73.7	33	78.6	0.280
		Normal	4	17.4	3	15.8	7	16.7	
		Increased	0	0	2	10.5	2	4.8	
MPT/z/	Female	Reduced	12	100	9	90	21	95.5	0.455
		Normal	0	0	1	10	1	4.5	
	Male	Reduced	7	63.6	6	66.7	13	65	0.156
		Normal	4	36.4	1	11.1	5	25	
		Increased	0	0	2	22.2	2	10	
	All	Reduced	19	82.6	15	78.9	34	81	0.249
		Normal	4	17.4	2	10.5	6	14.3	
		Increased	0	0	2	10.5	2	4.8	

Note: CG (critical group); SG (severe group); FCV, Forced Vital Capacity; MPT, maximum phonation time of /a/, /s/ and /z/.

Exact Test of Fisher.

* statistically significant p values; p<0.05

Table 4 shows the comparisons between the CG and SG in the VFI factors and VPQ according to gender and reference values.

TABLE 4 - Comparison between the groups with and without orotracheal intubation for the factors of the Vocal Fatigue Index and Vocal Performance Questionnaire, according to gender and reference values

			CG		SG		Total		p-value
			n	%	n	%	n	%	
Factor 1 - Tiredness and voice impairment	Female	Deviant	7	58.3	5	50	12	54.5	0.309
		Normal	5	41.7	5	50	10	45.5	
	Male	Deviant	2	18.2	4	44.4	6	30	0.179
		Normal	9	81.8	5	55.6	14	70	
	All	Deviant	9	39.1	9	47.4	18	42.9	0.591
		Normal	14	60.9	10	52.6	24	57.1	
Factor 2 – Avoidance of voice use	Female	Deviant	5	41.7	4	40	9	40.9	0.334
		Normal	7	58.3	6	60	13	59.1	
	Male	Deviant	3	27.3	3	33.3	6	30	0.358
		Normal	8	72.7	6	66.7	14	70	
	All	Deviant	8	34.8	7	36.8	15	35.7	0.890
		Normal	15	65.2	12	63.2	27	64.3	
Factor 3 – Physical discomfort	Female	Deviant	6	50	4	40	10	45.5	0.300
		Normal	6	50	6	60	12	54.5	
	Male	Deviant	3	27.3	3	33.3	6	30	0.358
		Normal	8	72.7	6	66.7	14	70	
	All	Deviant	9	39.1	7	36.8	16	38.1	0.879
		Normal	14	60.9	12	63.2	26	61.9	
Factor 4 – Improvement of voice symptoms	Female	Deviant	8	66.7	8	80	16	72.7	0.299
		Normal	4	33.3	2	20	6	27.3	
	Male	Deviant	9	81.8	7	77.8	16	80	0.409
		Normal	2	18.2	2	22.2	4	20	

with rest	All	Deviant	17	73.9	15	78.9	32	76.2	0.703
		Normal	6	26.1	4	21.1	10	23.8	
VFI Total	Female	Deviant	7	58.3	4	40	11	50	0.236
		Normal	5	41.7	6	60	11	50	
	Male	Deviant	5	45.5	3	33.3	8	40	0.308
		Normal	6	54.5	6	66.7	12	60	
	All	Deviant	12	52.2	7	36.8	19	45.2	0.320
		Normal	11	47.8	12	63.2	23	54.8	
VPQ	Female	Deviant	12	100	10	100	22	100	1.000
		Normal	0	0	0	0	0	0	
	Male	Deviant	11	100	8	88.9	19	95	0.450
		Normal	0	0	1	11.1	1	5	
	All	Deviant	23	100	18	94.7	41	97.6	0.265
		Normal	0	0	1	5.3	1	2.4	

Note: CG (critical group); SG (severe group); VFI, vocal fatigue index; VPQ, vocal performance questionnaire.

Exact Test of Fisher.

* statistically significant p values; p<0.05

Table 5 shows the correlations between VC, MPT, VFI and VPQ. The correlations between MPT, VFI and VPQ were statistically significant. The highest correlation occurred between VPQ and VFI, being positive and strong.

TABLE 5 - Correlations between Forced Vital Capacity, Maximum Phonation Times, Vocal Fatigue Index and the Vocal Performance Questionnaire

		VC	MPT/a/	MPT/s/	MPT/z/	VFI
MPT/a/	Corr (r)	0.018				
	p-valor	0.909				
MPT/s/	Corr (r)	0.047	0.556			
	p-valor	0.768	<0.001*			
MPT/z/	Corr (r)	-0.052	0.699	0.711		
	p-valor	0.746	<0.001*	<0.001*		
VFI	Corr (r)	0.021	-0.520	-0.337	-0.491	
	p-valor	0.895	<0.001*	0.029*	0.001*	
VPQ	Corr (r)	0.019	-0.486	-0.277	-0.423	0.795

p-valor	0.907	0.001*	0.076	0.005*	<0.001*

Note: FVC, Forced Vital Capacity; MPT, Maximum Phonation Time of /a/, /s/ and /z/; VFI, Vocal Fatigue Index; VPQ, Vocal Performance Questionnaire; Corr(r) - correlation.

Spearman Correlation Test

* statistically significant p values; p<0.05

Discussion

In our study, length of stay was significantly longer for the CG (Table 1), which is in line with the literature. Critical illness with the usage of mechanical ventilation can result in the weakness of acquisition and functional changes, increasing the length of hospitalization [68]. At the time of data collection for our study, no patient had a tracheostomy.

Agreeing with our results, the average length of hospitalization in one study was 19 (range: 3-41) days, and longer hospitalization periods were associated with people aged 45 years or older and with severe illness [69], the average hospitalization duration was 9.93 ± 4.45 days with a range of 3 to 37 days and as the severity of COVID-19 increased, the length of hospitalization increased, with the longest being for critically ill patients 25.59 ± 7.30 days and the shortest for mild patients of 8.74 ± 1.80 days [70].

It is possible to observe that the most severe cases of COVID-19 need more time for recovery and more attention in rehabilitation activities [71] due to the clinical manifestations of the infection that exacerbate pre-existing diseases such as: cardiovascular diseases, dyslipidemia, diabetes mellitus, chronic obstructive pulmonary disease, asthma, among others [72]. Even people without comorbidities can have their condition aggravated by innate conditions of the organism that, for some reason, have difficulties in maintaining its balance conditions [73,74]. As a result, they predict poor clinical outcomes and consequently lead to longer periods hospitalized, the necessity for intensive care, duration of mechanical ventilation and need for hospital rehabilitation [75].

Most patients belonged to the CG (Table 1), as this is a study carried out in a hospital environment. Despite this, the average age did not indicate that older adults would be the most likely to present the most severe disease cases, contrary to our working hypothesis based on studies that mention advanced age as a severity factor for COVID-19 [76,77,78]. This demonstrates that individuals of any age can acquire the infection and be prone to critical manifestations. Our results are in line with cohort studies of hospitalized patients with confirmed COVID-19 in which the average ages were 49 years [5]; median 59 years [79]; mean 43.9 years old [72]; and median 47 years old [74].

Although there were no significant differences, our results showed that most patients were female, in disagreement with our working hypothesis and with many studies that reported a higher incidence of males, especially in the critical phase of the disease [76,77,80,81]. However, our observed female predominance agrees with other studies whose main focus was on post-COVID-19 [82,83] (Table 1).

The differences between gender-specific behaviors, genetic and hormonal factors, including sex differences in biological pathways related to the SARS-CoV-2 infection, show sex and gender disparities impacting the incidence and lethality of the disease as well as the adaptation of treatment according to their needs [84,85]. It is also perceived that the female gender is associated with lower chances of clinical outcomes, major adverse events and all-cause mortality [86]. Studies suggest that men are more affected by COVID-19 than females because ACE2 expression is higher in male [87,88].

An epidemiological analysis based on the data provided by OpenDataSUS uncovered that most patients were male and that they tended to have more severe COVID-19 [89]. Male gender was an independent predictor of in-hospital mortality, and the mortality rate among male patients with SARS-CoV-2 was 2.8 times higher when compared to females [90]. Similarly, evidence emerging from China suggests that COVID-19 has a reported fatality rate of 2.8% in Chinese male versus 1.7% in females [84].

Several risk factors are associated with in-hospital mortality from COVID-19, including advanced age, male gender, ICU stay, OTI, comorbidity, SpO2 < 93, respiratory distress, loss of consciousness, headache, anorexia, and cough) [81]. In most studies in different parts of the world, such as in the population of China, Italy, Iran, Holland, Turkey, among other countries) [76,77,81,91,92,93], male gender is a predisposing factor for severe outcomes of COVID-19. 19, regardless of age and comorbidities [94]. A multivariate analysis indicates that females were 27% less likely to have hospital mortality and 24% less likely to be admitted to the ICU [86] .

Just as there is heterogeneity in the acute infectious phase, there are also differences in the complications seen post-COVID-19 [95]. Gender has been found to be an important determinant of the Long-COVID-19 syndrome because it is a significant predictor of persistent symptoms. After an average follow-up time of five months after the acute phase, females were significantly more likely than male to report dyspnea, weakness, chest pain, palpitations, and sleep disturbances, but not myalgia and cough [96]. In another study of subjects who met the inclusion criteria there was a slightly higher proportion (14.9%) of Long-COVID-19 in middle-aged females (50-60 years) compared to the male population (9.5 %) [97].

Consistent with the longitudinal study conducted in Mexico City after hospital discharge, it was found that females were more likely than men to develop long-term symptoms [98]. Added to this, a series of cases in Michigan in which 55.9% were female [82]. Another cohort study was composed of 59% female and 41% male [83].

In our study, there was a significant change in DD within all groups and, despite not being significant, CRP levels were also altered (Table 2), confirming our working hypothesis. The studies are unanimous when they state that COVID-19 is characterized by an increased acute inflammatory response, in which patient serum samples are abundant with mediators such as CRP and DD [12,99,100,101,102,103,104,105] .

It was not pointed out VC significant differences between the groups studied, but most subjects had normal VC, contrary to our working hypothesis (Table 3). The VC did not present a significant correlation with the other factors (Table 5) and was within

the normal range in most subjects (Table 3). This may be due to the time interval between hospitalization and evaluation, in which it is possible a Spontaneous recovery of the body and/or with drug support making use of corticosteroids [106] and prophylaxis for thromboembolism [107,108], that occurred during the period of hospitalization and post-discharge. In most patients who have recovered from severe COVID-19, dyspnea scores and exercise capacity improved over time [109].

In addition, voice impairment related to COVID-19 was mainly associated with decreased respiratory capacity [110]. Vocal disorders caused by COVID-19 were reported by patients, with a decrease in MPT, changes in performance and vocal fatigue. The prevalence of vocal symptoms was considered high from 23.8% to 43.7%, stating that the acute respiratory syndrome caused by COVID-19 that can affect the voice [111]. Other researchers also reported a high rate of dysphonia due to the COVID-19 disease with a prevalence of 26.8% [2]; 43.7% [112]; 22.3% [113]; 79% [114].

In our study, the MPT did not show significant differences between the groups, but most were outside the normative values (Table 3). MPT values were statistically lower than their healthy counterparts [115]. The MPT was significantly lower among survivors than among controls with average time of 15.97 s in the control group, 10.72 s in the pneumonia group and 8.88 s in the severe pneumonia group [116].

The literature suggests that COVID-19 may impact vocal characteristics during the period of infection [115,117]. In auditory-perceptual vocal assessments, patients with COVID-19 had dysphonia and the severity of this dysphonia was significantly different among patients with a different severity of COVID-19, with higher scores on all GRBAS scale items [115,118]. In some other studies just like our, there is a predominance of females, with up to 70.8% of women, and the proportion of women reached 76.6% in the dysphonic group [2]. There was a higher frequency of persistent dysphonia in patients admitted to the ICU (71.4%) compared to patients in the wards (28.6%), but no statistically significant association was found between admission to the ICU and persistent dysphonia [119]. The vocal fatigue of any degree was reported by 43/160 post-COVID-19 patients (26.8%) [112].

As for OTI among hospitalized adults with COVID-19, there were high rates of dysphonia (42%) and dysarthria (23%) and the history of OTI was predictive of voice quality [120]. The dysphonia is one of the common complaints, mainly in patients with OTI [121]. Voice-related complaints are the most common symptom, followed by those related to swallowing and breathing, and many were suggestive of OTI-related injury [122]. Other studies report that 53.7% of the analyzed patients had abnormal GRBAS scores and positive endoscopic findings correlated with self-reported VHI-10 scores [123].

Prolonged OTI and tracheostomy during treatment of severe COVID-19 are major contributors to voice, airway, and swallowing dysfunction, laryngeal problems have also been identified in mild illness [124]. These complications are not limited to patients who needed OTI or tracheostomy, findings similar to ours, in which most patients had vocal disorders that were not restricted to OTI (Table 4). In our study, all CG subjects underwent OIT and, of these, four (17.39%) underwent tracheostomy. At the time of data collection for our study, no patient had a tracheostomy.

When we observed the correlations between MPT, VFI and VPQ, the MPT showed a significant negative correlation with the VFI and VPQ, and VC did not significantly correlate with the other variables (Table 5). No other studies were found that correlated these variables in COVID-19. Despite that, similar studies reported that patients with a previous history of COVID-19 had significantly lower MPT, increased scores in questionnaires of self-reported voice complaints that revealed close correlations with the COVID-19 disease symptom scores, and the overall dysphonia severity score was higher in the study group than in the control group [125].

In disagreement with our data, previous studies concluded that as the MPT increases, the vital capacity also increases, evidencing the interrelation between these variables [126]. Likewise, it was observed that the greater the patient's VC is, the greater his MPT will be [127].

The innovative nature of relating OTI with inflammatory markers and vocal aspects, contribute to scientific evidence in the area and to society. As limitations, we highlight the small number of patients that did not allow stratification by age group, a long time after hospital discharge until the patient's evaluation, not being a multicenter study and, finally, the lack of comparison with a control group. Therefore, multicenter studies are suggested, with a control group, stratified by age correlating these variables plus other pulmonary, vocal and anthropometric measures, such as the body mass index.

Conclusion

Patients with OTI had a longer hospital stay and increased D-dimers and displayed that, whenever MPT decreased, vocal performance and fatigue increased.

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