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Chest CT scan findings six months after COVID-19 pneumonia: A prospective cross-sectional study

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ABSTRACT

The severity of pulmonary complications in survivors of coronavirus disease 2019 (COVID-19) pneumonia is not well understood, but there are concerns about potential long-term effects. In a prospective cross-sectional study, 43 consecutive patients with confirmed COVID-19 infection and respiratory symptoms were eligible. They were followed-up in Razi Hospital in Rasht, Iran from February 20, 2020, to September 22, 2021. These patients underwent chest CT scans six months after disease onset. The average age of the patients included in this research was 51.72±14.25. Out of the 43 examined patients, 25 patients (58.1%) had no residual radiological manifestations of COVID-19 pneumonia (complete recovery), 11 patients (25.6%) had remaining ground glass opacities (GGO), and 7 patients (16.3%) had residual lung disease (Sub-pleural line). The statistical test did not show a significant relationship between age groups, gender, underlying diseases, duration of hospitalization, the extent and type of involvement, number of involved lobes, degree of involvement, average preliminary O2 saturation, and the O2 therapy method with the remaining pulmonary radiologic appearances caused by COVID-19 pneumonia. The study's findings suggest that half of the patients develop chest CT-scan finding, with the most common being GGO followed by subpleural lines. We believe that if patients are followed up for more than six months, it is possible to gradually decrease the residual pulmonary manifestations in their CT scan findings.

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1. Introduction

Coronavirus disease 2019 (COVID-19) known as a respiratory illness caused by the novel coronavirus SARS-CoV-2. It was first identified in Wuhan, China in late 2019 and has since spread globally, leading to a pandemic. The symptoms of COVID-19 can range from mild to severe and include fever, cough, shortness of breath, fatigue, and loss of taste or smell. The virus is primarily spread through respiratory droplets when an infected person coughs, sneezes, or talks. Preventative measures such as wearing masks, practicing social distancing, and frequent handwashing are recommended to reduce the spread of the virus. Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infection has affected more than 100 million of people worldwide, and is responsible for at least 880,000 deaths [1]. Chest CT scans have played a crucial role in the diagnosis and management of COVID-19 pneumonia. These imaging studies provide valuable information on the extent and severity of lung involvement, allowing healthcare professionals to assess the disease progression and guide through applying the appropriate treatment strategies. While the acute manifestations of COVID-19 pneumonia have been extensively studied, there is growing interest in understanding the long-term effects and the residual radiological findings in survivors of the disease [2].

Several studies have reported various pulmonary complications and radiological findings in patients with COVID-19 pneumonia, both during the acute phase and in the early recovery period. However, there is limited research investigating the chest CT scan findings several months after the initial infection. Understanding these late-stage radiological manifestations is crucial for determining the potential long-term consequences and providing appropriate follow-up care for COVID-19 survivors [1-4].

In a retrospective study conducted in China, 79.3% of 145 patients with confirmed COVID-19 infection showed bilateral pneumonia, 18.6% showed unilateral pneumonia, 61.4% showed ground-glass opacity, and only 2.1% showed no abnormal chest CT result [5]. Radiologic and post mortem studies of patients show that lung injury in severe COVID-19 infection is not a classic diffuse alveolar damage on patients with acute respiratory distress syndrome (ARDS). It is rather an acute fibrinous organizing pneumonia (AFOP) characterized by an extensive intra-alveolar fibrin deposition called fibrin « balls», rather than hyaline membranes. Many patients' secondary consolidation of lesions resembles organizing pneumonia. Extensive injury of alveolar epithelial cells and endothelial cells, with secondary fibroproliferation is a signature of pulmonary COVID-19 infection indicate a potentiality for chronic vascular and alveolar remodeling leading to lung fibrosis and/or pulmonary hypertension [6, 7].

The purpose of this study is to evaluate the chest CT scan findings six months after COVID-19 pneumonia. This research aims to identify any remaining pulmonary radiological appearances and assess their prevalence and characteristics by examining a cohort of patients who had previously tested positive for COVID-19 and had also experienced respiratory symptoms. Furthermore, the study aims to analyze the potential factors associated with these residual findings, such as age, gender, underlying diseases, and the duration of hospitalization.

The findings from this research will contribute to our understanding of the long-term pulmonary complications of COVID-19 while providing insights on the potential sequelae that survivors may face. This knowledge is crucial for developing appropriate monitoring and management strategies to ensure the optimal care of individuals who have recovered from COVID-19 pneumonia. Additionally, it may help healthcare professionals identify patients who require additional interventions or specialized follow-up to address any ongoing respiratory issues.

Overall, investigating the chest CT scan findings six months after COVID-19 pneumonia will provide valuable information on the persistence and characteristics of radiological manifestations in survivors. This knowledge can contribute to the development of evidence-based guidelines for the long-term management and care of individuals who have experienced COVID-19 pneumonia, ultimately improving their overall health outcomes and quality of life.

2. Materials and Methods

This prospective cross-sectional study was conducted at the Razi hospital located in Rasht, Guilan, Iran. This study was approved by the Ethics Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1399.136). At beginning of this study, the informed consent was filled by all participants.

In the current study, 43 consecutive patients with confirmed COVID-19 infection (positive RT-PCR on nasopharyngeal swab) and respiratory symptoms were followed-up in Razi hospital, Rasht, Iran from 20 February 2020 to 22 September 2021. They were referred for chest CT-scan six months after the disease onset were eligible. Patients were excluded if they had not performed initial or six-month chest-CT. Also, patients who were re-infected with COVID-19 within 6 months of follow-up were excluded from the study.

The variables of this study were age groups, gender, underline disease, hospitalization, severity of lung involvement, primary O2 saturation, duration of Oxygen therapy, intubation, the duration the epidemic period of COVID-19 Delta variant.

Non-idiopathic interstitial pulmonary fibrosis describes a group of diseases causing fibrosis to the lung parenchyma due to a known cause which can occur in a wide range of clinical settings by multiple reasons. Radiographic manifestations of pulmonary or interstitial fibrosis include honeycombing, traction bronchiectasis, lung ar-

chitectural distortion, reticulation and interlobular septal thickening. Ground glass opacity (GGO) is a radiological term that describes an area of increased opacity in a hazy lung through which vessels and bronchial structures may still be seen [8].

2.1. Statistical analysis

In this study, the collected data were coded and entered into SPSS software (IBM SPSS Statistics for Windows, version 20.0, New York: IBM Corp). To describe quantitative variables, the mean and standard deviation and qualitative variables were also described using numbers and percentage. To determine the normality of the variables, the Kolmogorov test was used; in case of normality, parametric tests were used; otherwise, according to the research variables, chi-square test and Pearson's and Spearman's correlation coefficients were used to determine the frequency and the relationship. Regression test was also used to determine factors related to clinical findings.

3. Results

3.1. The descriptive characteristics of the patients

Table 1 demonstrates the description of quantitative variables, and Table 2 shows the description of qualitative variables of the patients under study. Out of 43 patients examined whose mean age was 51.72±14.25 years, with 23 individuals (53.5%) being female and 20 individuals (46.5%) being male (Table 1).

At the beginning of the study, results showed that out of 43 patients examined, 12 patients (27.9%) had a severity of involvement greater than 75% in the initial CT scan, and 12 patients (27.9%) had a severity of involvement between 26% to 50% in the initial CT scan (Table 2). The results of the current study showed that out of 43 patients examined, 33 patients (76.7%) had involvements of all 5 lung lobes, and only 5 patients (11.6%) had involvements of 2 lung lobes.

Moreover, after six months of fallow-up, the results of the study demonstrated that out of 43 patients examined, 25 patients (58.1%) had no residual radiological manifestations of COVID-19 pneumonia (complete recovery), 11 patients (25.6%) had remaining GGOs, and 7 patients (16.3%) had residual lung disease (Sub-pleural line) (Table 2).

Figure 1 shows that there were bilateral residual ground glass opacities seen in both the peripheral and central regions of the lungs. The arrow indicates the presence of a specific ground glass opacity. GGOs are radiological findings on computed tomography (CT) scans that indicate areas of increased lung density, but with preserved bronchial and vascular markings.

GGOs are often seen in various pulmonary conditions, including infections, inflammation, and interstitial lung diseases (Figure 1). There are bilateral subpleural lines and the arrow points to a particular subpleural line. Subpleural line (also known as **pleural lines**) refers to thin curvilinear opacities, 1-3 mm in thickness, lying less than 1 cm from and parallel to the pleural surface lines which are seen typically in the lower lung zones. The causes of a subpleural line, which is observed in the dependent area of the lung in a patient lying in a supine position (disappears when prone images are taken), can include dependent atelectasis of a normal lung, pulmonary edema, and fibrosis (other signs are typically present). While this sign is commonly associated with asbestosis, it is not specific to this condition. (Figure 2).

The results of this study showed that there was no significant association between gender and respiratory complications due to COVID-19 pneumonia in the study patients, as determined by the chi-square test (p=0.365) (Table 3). Additionally, there was no significant association between underlying comorbidities and respiratory complications due to COVID-19 pneumonia in the study patients, as determined by the chi-square test (p=0.112). Furthermore, the present study demonstrated that there was no significant statistical relationship between the length of hospital stay and pulmonary complications due to COVID-19 pneumonia in the patients, as determined by the chi-square test (p=0.313) (Table 3).

This study showed no statistically significant relationship between the severity of lung involvement in CT scan (p=0.424), the location of lung involvement (p=0.562), the number of lung lobes involved (p=0.919), the type of lung involvement (p=0.035), the degree of lung involvement (p=0.452), initial O2 saturation (p=0.987), O_2 therapy method (p=0.419), type of treatment (p=0.873), and the duration of COVID-19 Delta variant (p=0.771) with respiratory complications due to COVID-19 pneumonia in the study patients (Table 3).

Table 1: Description of quantitative variables.

Variables	No	Mean	SD	Median
Age (years)	43	51.72	14.25	54 (40 - 62)
Length of hospital stay (days)	43	8.5	6.04	7 (5- 10)
Initial O_2 saturation	43	90.4	69.58	90 (89 - 94)
Secondary O_2 saturation	43	97.1	0.3	97 (96 - 98)

Table 2: Distribution of qualitative variables.

Variables		Frequency	Percentage
Age group	20-40 years	12	27.9
	41-60 years	21	48.8
	Over 60 years	10	23.3
Sex	Men	20	46.5
	Female	23	53.5
t disease	Hypertension (HTN)	12	27.9
	Diabetes Mellitus (DM)	16	37.2
	Hyperlipidemia (HLP)	6	13.9
	Ischemic Heart Disease (IHD)	6	13.9
	Heart Failure (HF)	2	4.6
	Fatty Liver	3	6.9
	Rheumatology	2	4.6
	Liver Transplant	1	2.3
	Underlying Lung Disease	2	4.6
	Renal Failure (RF)	1	2.3
	Tuberous Sclerosis	1	2.3
	No underlying disease	20	46.5
Initial O2 saturation	93>	31	72.1
	≤93	12	27.9
O2 therapy method	BIPAP*	1	2.3
	O2 with mask	42	97.7
Severity of involvement on initial CT	No involvement	0	0
	Less than 25%	11	25.6
	26% to 50%	8	18.6
	51% to 75%	12	27.9
	More than 75%	12	27.9
Position of conflict	Unilateral	3	7.0
	Bilateral	40	93.0
Number of lobes involved	Two	5	11.6
	Three	3	7.0
	Four	2	4.7
	Five	33	76.7
Type of conflict	Ground Glass Opacities (GGO)	27	62.8
	GGO & consolidation	16	37.2
Degree of conflict	Moderate	14	32.6
	Severe	29	67.4
Duration the epidemic period of COVID-19 variant	Non-delta	25	58.1
	Delta	18	41.9
Treatment	Antiviral	11	34.4
	Antiviral and corticosteroid	18	56.2
	Corticosteroid	3	9.4
Pulmonary complications	No residual (complete recovery)	25	58.1
	Remaining GGO	11	25.6
	Residual lung disease (Sub-plural line)	7	16.3

Table 3: Frequency distribution of respiratory complications caused by COVID-19 pneumonia

Variables		Pulmonary complications		P-value
		(Yes) %	(No) %	
Age groups	20 to 40 years	33.3 (4)	66.7 (8)	0.159
	41 to 60 years	33.3 (7)	66.7 (14)	
	Over 60 years	70.0 (7)	30.0 (3)	
	Total	41.9 (18)	58.1 (25)	
Sex	Male	50 (10)	50 (10)	0.365
	Female	34.8 (8)	65.2 (15)	
	Total	41.9 (18)	58.1 (25)	
Underline diseases	Present	52.0 (13)	48.0 (12)	0.112
	Absent	27.8 (5)	72.2 (13)	
	Total	41.9 (18)	58.1 (25)	
Length of hospital stay (days)	Less than 7 days	50.0 (10)	50.0 (10)	0.313
	7 or more days	34.8 (8)	65.2 (15)	
	Total	41.9 (18)	58.1 (25)	
Severity of lung involvement in CT-Scan	Less than 25%	45.5 (5)	54.5 (6)	0.679
	26 to 50%	37.5 (3)	62.5 (5)	
	51 to 75%	25.0 (3)	75.0 (9)	
	Over 75%	58.3 (7)	41.7 (5)	
	Total	41.9 (18)	58.1 (25)	
Involved position of the lung	Unilateral	66.7 (2)	33.3 (1)	0.562
	Bilateral	40.0 (16)	60.0 (24)	
	Total	41.9 (18)	58.1 (25)	
The number of lung lobes involved	Two	40.0 (2)	60.0 (3)	0.919
	Three	66.7 (2)	33.3 (1)	
	Four	50.0 (1)	50.0 (1)	
	Five	39.4 (13)	60.6 (20)	
	Total	41.9 (18)	58.1 (25)	
Type of lung involvement	GGO & consolidation	62.5 (10)	37.5 (6)	0.035
	GGO	29.6 (8)	70.4 (19)	
	Total	41.9 (18)	58.1 (25)	
Degree of lung involvement	Moderate	50.0 (7)	50.0 (7)	0.452
	Severe	37.9 (11)	62.1 (18)	
	Total	41.9 (18)	58.1 (25)	
Initial O_2 saturation	93>	41.9 (13)	58.1 (18)	0.987
	≤93	41.7 (5)	58.3 (7)	
	Total	41.9 (18)	58.1 (25)	
O_2 therapy method	BIPAP	100 (1)	0.0 (0)	0.419
	O_2 with mask	40.5 (17)	59.5 (25)	
	Total	41.9 (18)	58.1 (25)	
Duration the epidemic period of COVID-19 variant	Delta	44.4 (8)	55.6 (10)	0.771
	Non-delta	40.0 (10)	60.0 (15)	
	Total	41.9 (18)	58.1 (25)	

*Bilevel Positive Airway Pressure

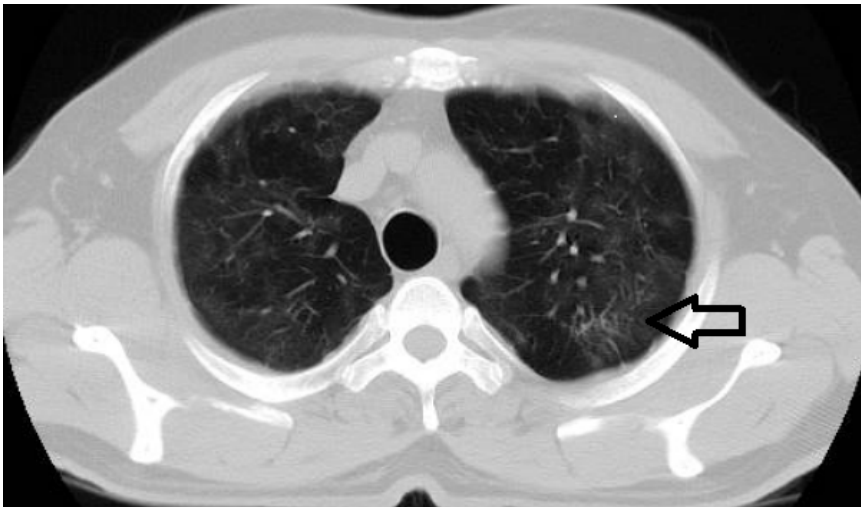

Figure 1: Bilateral residual peripheral and central ground glass opacities. The arrow shows ground glass opacity.

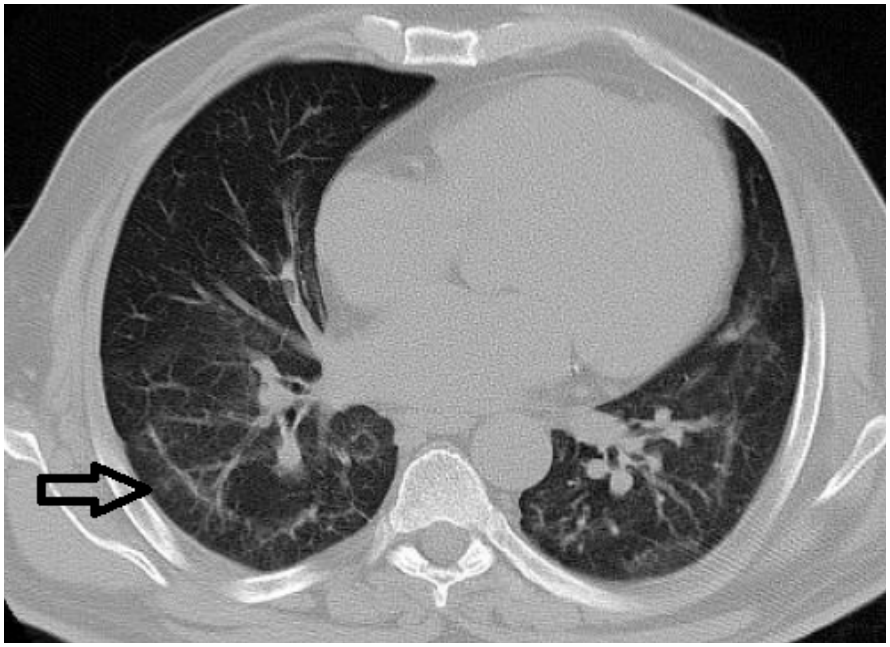

Figure 2: Bilateral subpleural lines.

4. Discussion

After six months of hospital discharge from COVID-19 pneumonia, chest CT scan findings can vary among individuals. Some common findings may include residual lung abnormalities that in some cases, CT scans may show residual lung abnormalities such as ground-glass opacities, consolidation, or fibrotic changes. These findings can indicate persistent lung damage or scarring due to previous infections. Also, CT scans may show resolution of lung abnormalities while some patients may show complete resolution of lung abnormalities seen on previous CT scans. This indicates that the lungs have healed and returned to their normal state. In other cases, CT scans may show persistent lung involvement. This could indicate ongoing inflammation or complications related to COVID-19 pneumonia; whereas, in rare cases, new lung abnormalities may be observed on the CT scan after six months [9], yet they could be unrelated to the previous COVID-19 infection and may require further evaluation. Vural et al. found that pulmonary fibrotic-like changes in patients recovering from COVID-19 pneumonia by analyzing follow-up chest CT scans. A total of 84 patients with moderate-to-severe pneumonia were included, with 35% showing fibrotic-like changes on follow-up CT scans. Factors such as longer hospital stay and higher CT scores at diagnosis were identified as predictors for these sequelae. The findings suggest that over one-third of COVID-19 pneumonia survivors may develop fibrotic changes in lung tissue, particularly in cases of severe pneumonia and extended hospitalization [10]. The results of the current study provide valuable insights into the ra-

diological manifestations and recovery status of patients who have experienced COVID-19 pneumonia. We found that a significant majority of patients had achieved complete recovery with no residual radiological manifestations. This is an encouraging finding and suggests that a substantial proportion of patients can fully recover from COVID-19 pneumonia without any long-term lung complications. However, it is noteworthy that a considerable number of patients, still exhibited GGO on radiological imaging. GGO is a common finding in COVID-19 pneumonia and is associated with lung inflammation and consolidation. Although these residual GGOs may not indicate active infection, they suggest that some degrees of lung involvement persist even after the resolution of clinical symptoms. It is important to closely monitor these patients to assess the long-term implications of these residual GGOs and to ensure appropriate management and follow-up take place.

Additionally, the patients in the study had residual lung disease in the form of subpleural lines. Subpleural lines are linear opacities seen adjacent to the pleural surface and can be indicative of fibrotic changes or scarring. The presence of residual lung disease highlights the potential for long-term complications and underscores the importance of comprehensive follow-up care for COVID-19 survivors.

Overall, the study highlights the heterogeneity of radiological manifestations and recovery outcomes in COVID-19 pneumonia. While a majority of patients experienced complete recovery, a significant proportion still had residual GGOs or lung disease. These findings emphasize the need for continued research to better understand the long-term effects of COVID-19 on the respiratory system and to develop targeted interventions and follow-up protocols for patients with persistent radiological abnormalities. Therefore, it appears that the most common long-term complication in the patients studied in the present study was GGO. It should be noted that during the acute phase of the disease and in the initial few days, GGO is visible as the predominant finding on plain radiography and CT scans. These changes are more commonly observed in the lower regions of the lungs and tend to involve the peripheral areas near the subpleural regions.

In the second week of the disease, GGO patterns predominantly progress towards consolidation. Then, as the recovery process begins, the extent of consolidation gradually decreases, and ultimately, in many patients, the parenchymal lung changes are completely resolved [11]. In a cohort study of 103 COVID-19 recovered patients, parenchymal lung changes were evaluated on CT scans after 3 months of follow-up. In 25% of patients, residual radiological changes, mainly in the form of GGO areas were observed. In 20% of cases, fibrotic parenchymal bands were seen [12]. In another study, 12 patients with severe pulmonary involvement requiring admission to the intensive care unit were evaluated. The average time of radiological findings assessment in these patients was approximately 56 days from the onset of symptoms. Despite relative improvement in acute lesions such as GGO, and consolidation, parenchymal lung changes, mainly in the form of fibrotic findings, were still observable on the CT scans of the patients [13]. Shi H et al., examined 81 patients who had confirmed COVID-19 infection using PCR testing. The results of this study showed that lung damage during the first to third week leads to the appearance of GGO on CT scans, which are subsequently accompanied by consolidation [14]. In the study conducted by Yang et al., the most common and suspicious pattern observed on CT scans for COVID-19 was the involvement of GGO or mixed opacities in the subpleural regions of the lungs [15]. Xu et al., found that after recovery, fibrotic changes can be observed in the subpleural regions of the lungs [16]. However, the results of the CT scan evaluation of patients after 6 months showed that none of the patients in this study exhibited typical manifestations of fibrosis. This could be due to the fact that none of the patients in this study had experienced mechanical ventilation. Pulmonary fibrosis can occur as an idiopathic and genetically related process, or can be caused by age-related factors. However, chronic inflammatory processes in the context of respiratory infections can also contribute to the development of fibrosis. Additionally, lung fibrosis can be a recognized complication following ARDS. In such cases, abnormal secondary immune mechanisms and cytokine storms play a role in the initiation and progression of lung fibrosis [17]. In another study conducted by Yun et al., it was found that patients with more severe initial disease have a slower radiological improvement and a higher likelihood of developing pulmonary fibrosis compared to those with milder manifestations. These findings can occur early in recovered patients from the acute phase of COVID-19. On the other hand, the persistence of pulmonary fibrosis findings in long-term follow-ups indicates a slow recovery trend and, in some cases, the likelihood of their persistence [18].

The frequency of residual radiological manifestations of COVID-19 pneumonia in patients with unilateral lung involvement was found to be two out of three patients, while in patients with bilateral lung involvement, it was 18 out of 40 patients. These findings are consistent with our study. In the study conducted by Shi et al., the pattern of involvement in COVID-19 patients was predominantly bilateral and peripheral [14]. In the study by Yang W et al., the most common and suspicious pattern on CT scan for COVID-19 included bilateral involvement in the lower lung regions [15]. In current study, we cannot find any significant correlation between the severity of lung involvement and the residual radiological manifestations of COVID-19 pneumonia.

Based on the scoring system for lung involvement in CT scans, no involvement in the lungs is assigned a score of zero. The scores for each lobe are then summed up. Scores up to 8 indicate moderate involvement, while

scores exceeding 8 indicate severe involvement [19].

Zheng et al. reported a mean age of 45 years in their study of 161 COVID-19 patients, which is consistent with the findings of the present study [20]. Rodriguez-Morales et al., through a review of 18 different studies, showed that the average age of COVID-19 patients is 51.97 years, which is also in line with the present study [21]. Additionally, Wu et al. reported a summary of clinical data from 72,000 confirmed COVID-19 patients, where the majority of patients were between 30 and 79 years old [22]. In the study by Yang et al., the average age of deceased COVID-19 patients was 69.8 years, which is consistent with the present study [23]. The data indicate that older age is a risk factor for contracting this disease.

In the studies conducted by Li et al. and Du et al. on deceased patients, a higher rate of infection was reported in females [24, 25]. Similarly, according to the results of the study by Talebi et al. the majority of COVID-19 patients and deceased patients were females [26]. However, in the study by Samasami et al. in Tehran in 2020, 62.5% of trauma patients who had no signs of infectious diseases and were suspected of having COVID-19 were males [27]. Hashemian et al., found that the boys may be more prone to developing severe cases of COVID-19, particularly in school-age children. They also consider that the manifestations of the disease in children may be milder compared to adults. Additionally, the presence of underlying diseases seems to be associated with more severe cases [28]. Additionally, in the study by Rodriguez-Morales et al., the rate of infection was higher in males [21]. The difference in results among different studies may be due to variations in sample sizes. However, there was no significant correlation between gender and residual radiological manifestations of COVID-19 pneumonia in the patients studied.

According to the results of our study, the majority of patients had an underlying disease. Consistent with our findings, in the study by Talebi et al. a higher proportion of deceased patients had at least one underlying disease, with common conditions such as high blood pressure, heart disease, and diabetes [26]. Additionally, regarding the significance of underlying diseases in COVID-19 patients, the study by Li et al. showed that all deceased patients had underlying diseases [24]. However, it should be noted that older individuals are more susceptible to mortality, and the prevalence of underlying diseases such as high blood pressure, diabetes, and chronic kidney disease is higher among the elderly, which has increased the risks of death among the study samples [29].

To understanding long-term effects of COVID-19 pneumonia, Anna K. Luger et al., characterized the patterns and rates of improvement of chest CT abnormalities one year after COVID-19 pneumonia. A total of 142 participants were enrolled in the study, with 91 undergoing a one-year follow-up CT examination. The results showed that 54% of participants exhibited CT abnormalities, with different manifestations observed. Factors such as age over 60 years, critical COVID-19 severity, and male sex were associated with persistent CT abnormalities at the one-year follow-up. The study found that 49% of participants experienced complete resolution of CT abnormalities during the study period, while some participants showed no further improvement after six months. In conclusion, long-term CT abnormalities were prevalent one year after COVID-19 pneumonia [30].

According to the results of this study, there was no significant correlation between the severity of involvement, degree of involvement, number of lobes involved, duration of hospitalization, initial average O_2 saturation, and the type of O_2 therapy method with residual radiological manifestations of COVID-19 pneumonia in the studied patients. In other words, the findings of this study indicate that the clinical conditions of patients in the acute stage of the disease will not have much relevance to the occurrence of chest CT-scan findings in the future. In fact, residual radiological manifestations of chronic lung disease in patients with high risk at the time of initial infection cannot predetermine the stage of the disease for the physicians.

Various and numerous complications have been reported following COVID-19 infection; however, respiratory involvement remains as the most important factor in mortality. Although the disease generally improves after various clinical phases, some patients experience short-term or long-term complications. The results of the current study indicate that approximately half of the patients develop chest CT-scan finding, with the most common being GGO followed by subpleural lines. We believe that if patients are followed up for more than six months, it is possible that the residual pulmonary manifestations in their CT scan findings would gradually decrease.

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Authors' contributions

Conceptualization: EH, AP. Data curation: EH, AP, SFA, ST. Investigation: EH, AP, SFA, SNSS, LM. Methodology: EH, AP, AAF, TYK, HAK. Original draft preparation: EH, AP. Critical revision and editing: EH, AP, AAF. All authors read and approved the final version of article.

Conflict of interests

The authors have no conflicts of interests.

Ethical declarations

This study protocol was approved by the Ethics Com-

mittee of Guilan University of Medical Sciences (IR.GUMS.REC.1399.136). The informed consent was filled by all participants.

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