

RESEARCH PAPER

Epidemiological and clinical characteristics of the COVID-19 epidemic and associated factors for mortality in Golestan province, Iran: a retrospective cohort study

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Keywords

COVID-19 • Acute Respiratory Distress Syndrome • Death • Iran

Summary

Background. We aimed to further clarify the epidemiological and clinical characteristics of 2019-nCoV pneumonia and risk factors associated for mortality.

Methods. In this study, we included inpatient with acute respiratory distress syndrome at Golestan Hospitals who had been discharged or had died in 2020. Epidemiological, and clinical data were extracted from electronic medical records and compared between recovered and died cases. We used multiple logistic regression methods to explore the risk factors associated with in-hospital death.

Results. Overall 2,835 acute respiratory distress syndrome patients were included in this study, and 874 (30.83%) were positive for 2019-nCoV. Five hundred and sixty-three patients (19.86%) died, 1,687 patients (59.51%) were recovered. Of the total deaths, only 288 (10.15%) were attributed to COVID-19. The most common symp-

oms at onset of illness were respiratory distress [1,795 (63.32%)], fever [1,601 (56.47%)], dry cough [1,595 (56.26%)], sore throat [445 (15.70%)], and myalgia [342 (12.06%)]. One thousand and twelve (35.7%) had 1 or more coexisting medical conditions. In multiple logistic regression analysis, risk factors associated with the death included older age [OR (Odds Ratio) = 1.03; 95% CI: 1.02-1.04], blood oxygen level ($SPO_2 < 93\%$) (OR = 2.44; 95% CI: 1.79-3.31), comorbidities (OR = 2.15; 95% CI: 1.62-2.84), respiratory distress (OR = 1.74; 95% CI: 1.28-2.37), and headache (OR = 0.44 95% CI; 0.21-0.92).

Conclusions. The 2019-nCoV infection caused collections of severe respiratory illness and was associated to a high ratio of hospitalization in ICU and high mortality. Older age and comorbidities were associated with more risk of death among patients with 2019-nCoV.

Introduction

In December 2019, a number of cases of pneumonia by unknown origin were identified in Wuhan, the capital city of Hubei province, China [1]. Clinical evidence showed that these cases were very similar to viral pneumonia [2-4]. Most patients reported to have worked or lived near the seafood market, as well as had contacts with live wild animals. In the early stages of the pneumonia, severe acute symptoms of respiratory infection occurred, with some patients rapidly developing acute respiratory distress syndrome (ARDS), acute respiratory failure, and other serious complications. On January 7th, a new coronavirus was detected by the Chinese Centers for Disease Control and Prevention, which was subsequently named by the

World Health Organization as the new Coronavirus 2019 (nCoV-2019) [5].

Emergency of 2019-nCoV has attracted global attention, and WHO has declared the COVID-19 a public health emergency of international concern (PHEIC) [6, 7]. Since the outbreak of Acute Respiratory Syndrome (SARS) in Guangdong, China, in 2003, WHO has announced five PHEIC cases: H1N1 (2009), polio (2014), Ebola in West Africa (2014), Zika (2016) and Ebola in the Democratic Republic of the Congo (2019) [8]. Announcing a PHEIC is an immediate call at the highest level for the international community to launch a global concerted effort to stop the spread of the disease, which requires serious public health activities, high-level political commitment and a sufficient budget [6]. Common symptoms of this infection include respiratory

symptoms, fever, cough, and respiratory distress. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death [9].

According to the latest World Health Organization report, since the announcement of the first case of COVID-19, 4,248,389 cases and 294,046 deaths due to the disease have been reported in more than 180 countries. So that more than 80% of deaths in the United States and Europe, as well as 37% and 55.5% of deaths, were reported in the United States and Europe, respectively. In Iran, 112,725 cases and 6,783 deaths have been reported [10]. Due to the fact that the disease has spread to all countries of the world, including Iran, this study was conducted to determine epidemiological, clinical characteristics and risk factors for mortality of inpatients with COVID-19 in Golestan province, Iran.

Methods

This is a retrospective cohort study, conducted on 2,835 patients acute respiratory distress syndrome aged < 1 to 99 years and hospitalized at Golestan University of Medical Sciences Hospitals in Golestan provinces, Iran. The study was approved by the Research Ethics Commission of Golestan University of Medical Sciences (IR.GOUMS.REC.1398.385). The patients with acute respiratory distress syndrome admitted to Hospitals of Golestan university of Medical Sciences in 2020, were enrolled. We obtained the medical records and compiled data for hospitalized patients with laboratory-confirmed COVID-19. A confirmed case of COVID-19 was defined as a positive result on RT-PCR assay of nasal and pharyngeal swab specimens and suspected cases of 2019-nCoV or Acute Respiratory Distress Syndrome was defined the negative test of PCR and hospitalized. We obtained epidemiological, demographic, clinical, laboratory, management and outcome data from patients' medical records. The primary end point was admission to an intensive care unit (ICU), or the use of mechanical ventilation. Secondary end point involved patients' death.

Descriptive analyses of the variables were expressed as median [interquartile range (IQR)], or number (%) or simple ranges, as appropriate. No imputation was made for missing data. Means for continuous variables were compared using independent group t tests when the data were normally distributed; otherwise, the Mann-Whitney test was used. Proportions for categorical variables were compared using the χ^2 test, although the Fisher exact test was used when the data were limited. All epidemiological, demographic and clinical exposures were tested for association with end point in univariate analysis and those with $p < 0.20$ were then entered into a logistic regression model to examine their independent effect. All statistical analyses were performed using STATA 12.0 software (Stata Corporation, College Station, TX, USA).

Results

In overall, 2,835 hospitalized patients with suspected 2019-nCoV nasal and pharyngeal swab specimens were prepared and send to the laboratory for PCR. Of these patients, 874 (30.83%) were positive for 2019-nCoV, 1,046 (36.90%) were negative and 915 (32.28%) were not available for PCR result (Tab. I).

The demographic and clinical characteristics of the patients are shown in Table I. The median age was 54 years (IQR: 41-67; range: 0-99 years), and 1,635 (57.67%) were males. The median durations from first symptoms to hospital admission and death were 2 days (IQR: 0-3), and 7 days (IQR: 4-13), respectively. Among them, there were 32 patients with exposure history of COVID-19 (Tab. I). Of these patients, 17.92% were ventilated, 14.50% had SpO₂ less than 93%, and 11.99% were admitted to the ICU.

Of the 2,835 patients, 1,012 (35.7%) had one or more coexisting medical conditions. Cardiovascular disease [486 (17.14%)], diabetes [423 (14.92%)], chronic lung disease [177 (6.24%)] and hypertension [77 (2.72%)] were the most common coexisting conditions (Tab. I).

The most common symptoms at onset of illness were respiratory distress [1,795 (63.32%)], fever [1,601 (56.47%)], dry cough [1,595 (56.26%)], sore throat [445 (15.70%)], and myalgia [342 (12.06%)]. Less common symptoms were headache, fatigue, abdominal pain, chest pain, diarrhea, nausea and vomiting (Tab. I). A secondary end-point (death) event occurred in 563 patients (19.86%), that 1,687 patients (59.51%) were recovered, and 585 (20.63%) under treatment. However, of the total deaths, only 288 deaths were confirmed by the Golestan University of Medical Sciences Death Committee for COVID-19 (Tab. I). Thus, the mortality rate was 10% for COVID-19.

Table II demonstrates that when compared with death and recovered cases, the death cases were older ($p < 0.001$) and most of them were males (54.53%; $p = 0.03$). More death cases presented with initial symptoms of respiratory distress compared with those recovered (77.08 vs 59.63%; $p < 0.001$) and with lower oxygen saturation (SpO₂ < 93%) (33.68 vs 12.27%; $p < 0.001$). Compared with recovered cases, death cases had a higher proportion of comorbidities, including cardiovascular disease (27.78 vs 13.22%), diabetes (23.61 vs 12.98%; $p < 0.001$), chronic lung disease (11.81 vs 4.68%; $p < 0.001$), chronic kidney disease (7.64 vs 2.37%; $p < 0.001$) and cancer (3.47 vs 0.95%; $p = 0.001$).

Multiple logistic regression models showed that several factors were associated with death in confirmed cases of 2019-nCoV, which included age of patents (OR = 1.03, 95% CI: 1.02-1.04), oxygen saturation (SpO₂ < 93%) (OR = 2.44, 95% CI: 1.79-3.31), comorbidities (OR = 2.15, 95% CI: 1.62-2.84), respiratory distress (OR = 1.74, 95% CI: 1.28-2.37) and headache. Although most of the variables mentioned were associated with higher likelihood of death, but headache was negatively associated with death (OR = 0.31; 95% CI, 0.13-0.70) (Tab. III).

Tab. I. Baseline characteristics of patients suspected and infected with 2019-nCoV.

Variables	Total (n = 2,835)	Confirmed cases (n = 874)	Suspected cases (n = 1,046)	Not available of PCR result (n = 915)	P-value
Age, median (IQR), y	54 (41-67)	56 (43-68)	52 (39-65)	55 (42-67)	F = 11.60 < 0.01*
Sex, n (%)					
Female	1200 (42.33)	366 (41.88)	453 (43.31)	381 (41.64)	$\chi^2(1) = 0.39$ 0.71 **
Male	1635 (57.67)	508 (58.12%)	593 (56.69)	534 (58.36)	
Onset of symptom to, median (IQR)					
Hospital admission	2 (0-3)	2 (0-4)	1 (0-4)	2 (1-3)	F = 0.23 0.63*
Death	7 (4-13)	8 (4-13)	8 (3-14.5)	6 (3-8)	F = 0.03 0.86*
Discharge	8 (5-12)	8 (5-12)	8 (5-12)	6 (4-9)	F = 1.15 0.78*
History of exposure with COVID-19, n (%)					
Yes	32 (1.13)	11 (1.26)	21 (2.01)	0 (0.0)	$\chi^2(1) = 1.63$ 0.2**
No	2803 (98.87)	863 (98.74)	1025 (97.99)	915 (100)	
Ventilation, n (%)					
Yes	508 (17.92)	227 (25.97)	179 (17.11)	102 (11.15)	$\chi^2(1) = 0.12$ 0.72**, ***
No	289 (10.19)	105 (12.01)	88 (8.41)	96 (10.49)	
Admitted to ICU, n (%)					
Yes	340 (11.99)	105 (12.01)	127 (12.14)	108 (11.80)	$\chi^2(1) = 0.45$ 0.49**, ***
No	1153 (40.67)	377 (43.14)	412 (39.39)	364 (39.78)	
SPO₂, n (%)					
93% >	411 (14.50)	168 (19.22)	150 (14.34)	93 (10.16)	$\chi^2(1) = 8.21$ 0.001**, ***
93% <	408 (14.39)	119 (13.62)	179 (17.11)	110 (12.02)	
Comorbidities, n (%)					
Cardiovascular disease, n (%)	1012 (35.70)	356 (40.73)	335 (32.03)	321 (35.08)	$\chi^2(1) = 15.66$ <0.001**
Diabetes, n (%)	486 (17.14)	168 (19.22)	160 (15.30)	158 (17.27)	$\chi^2(1) = 5.18$ 0.02**
Chronic lung disease, n (%)	423 (14.92)	155 (17.73)	131 (12.52)	137 (14.97)	$\chi^2(1) = 10.19$ 0.01**
Hypertension, n (%)	177 (6.24)	65 (7.44)	71 (6.79)	41 (4.48)	$\chi^2(1) = 0.30$ 0.58**
Hypertension, n (%)	77 (2.72)	22 (2.52)	25 (2.39)	30 (3.28)	$\chi^2(1) = 0.03$ 0.85**
Chronic kidney disease, n (%)	106 (3.74)	46 (5.26)	39 (3.73)	21 (2.30)	$\chi^2(1) = 2.65$ 0.10**
Chronic liver disease, n (%)	21 (0.74)	6 (0.69)	12 (1.15)	3 (0.33)	$\chi^2(1) = 1.08$ 0.29**
Cancer, n (%)	44 (1.55)	13 (1.49)	20 (1.91)	11 (1.20)	$\chi^2(1) = 0.50$ 0.47**
Immune deficiency disease, n (%)	34 (1.20)	5 (0.57)	15 (1.43)	14 (1.53)	$\chi^2(1) = 3.43$ 0.06**
Signs and symptoms					
Respiratory distress, n (%)	1795 (63.32)	565 (64.65)	692 (66.16)	538 (58.80)	$\chi^2(1) = 0.48$ 0.48**
Fever, n (%)	1601 (56.47)	495 (56.64)	599 (57.27)	507 (55.41)	$\chi^2(1) = 0.07$ 0.78**
Dry cough, n (%)	1595 (56.26)	478 (54.69)	575 (54.97)	542 (59.23)	$\chi^2(1) = 0.01$ 0.90**
Sore throat, n (%)	445 (15.70)	160 (18.31)	176 (16.83)	109 (11.91)	$\chi^2(1) = 0.72$ 0.39**
Myalgia, n (%)	342 (12.06)	89 (10.18)	155 (14.82)	98 (10.71)	$\chi^2(1) = 9.22$ 0.002**
Headache, n (%)	157 (5.54)	43 (4.92)	36 (3.93)	78 (7.46)	$\chi^2(1) = 5.19$ 0.02**
Confusion, n (%)	140 (4.94)	39 (4.46)	62 (5.93)	39 (4.26)	$\chi^2(1) = 2.05$ 0.15**



Tab. I. Baseline characteristics of patients suspected and infected with 2019-nCoV.

Variables	Total (n = 2,835)	Confirmed cases (n = 874)	Suspected cases (n = 1,046)	Not available of PCR result (n = 915)	P-value
Nausea and Vomiting, n (%)	136 (4.80)	45 (5.15)	59 (5.64)	32 (3.50)	$\chi^2(1) = 0.23$ 0.63**
Chest pain, n (%)	112 (3.95)	34 (3.89)	48 (4.59)	30 (3.28)	$\chi^2(1) = 0.56$ 0.45**
Temperature C, median (IQR)	39 (39-39)	39 (38.5-39)	39 (39-39)	39 (39-39)	F = 0.42 0.07*
Secondary end point, n (%)					
Death	563 (19.86)	288 (32.95)	190 (18.16)	85 (9.29)	$\chi^2(1) = 60.13$ < 0.01**
Recovered	1687 (59.51)	414 (47.37)	648 (61.95)	625 (68.31)	
Hospitalization	585 (20.63)	172 (19.68)	208 (19.89)	205 (22.40)	

* This test compared the Positive PCR group vs Negative PCR group, One-way analysis of variance (ANOVA); ** Chi-squared test (χ^2); *** Some of the status variables are missing.

Tab. II. Epidemiological and clinical characteristics among death and recovered cases.

Variables	Death (n = 288)	Recovered (n = 1687)	Total (n = 1975)	P-value
Age, median (IQR), y	65 (54-76)	50 (39-62)	53 (41-65)	T = 12.01 <0.01*
Sex, n (%)				
Female	119 (41.32)	681 (40.37)	800 (40.51)	$\chi^2(1) = 0.09$ 0.76**
Male	169 (58.68)	1006 (59.63)	1175 (59.49)	
Onset of symptom to, median (IQR), day				
Hospital admission	1 (0-4)	2 (1-4)	2 (0-4)	T = 0.98 0.32*
Discharge	4 (2-10)	7 (4-11)	7 (4-11)	T = 0.77 0.44*
History of exposure with COVID-19, n (%)				
Yes	5 (1.74)	20 (1.19)	25 (1.27)	$\chi^2(1) = 0.59$ 0.44**
No	283 (98.26)	1667 (98.81)	1950 (98.73)	
Ventilation, n (%)				
Yes	96 (76.19)	235 (57.18)	331 (61.64)	$\chi^2(1) = 14.74$ <0.01**
No	30 (23.81)	176 (42.82)	206 (38.36)	
Admitted to ICU, n (%)				
Yes	36 (22.22)	197 (22.70)	233 (22.62)	$\chi^2(1) = 0.01$ 0.89**
No	126 (77.78)	671 (77.30)	797 (77.38)	
SPO₂, n (%)				
93%>	191 (66.32)	1480 (87.73)	1671 (84.61)	$\chi^2(1) = 86.59$ < 0.01**
93%<	97 (33.68)	207 (12.27)	304 (15.39)	
Comorbidities, n (%)				
Cardiovascular disease, n (%)	80 (27.78)	223 (13.22)	303 (15.34)	$\chi^2(1) = 40.14$ < 0.01**
Diabetes, n (%)	68 (23.61)	219 (12.98)	287 (14.53)	$\chi^2(1) = 22.37$ < 0.01**
Chronic lung disease, n (%)	34 (11.81)	79 (4.68)	113 (5.72)	$\chi^2(1) = 23.13$ < 0.01**
Hypertension, n (%)	13 (4.51)	39 (2.31)	52 (2.63)	$\chi^2(1) = 4.65$ 0.03**
Chronic kidney disease, n (%)	22 (7.64)	40 (2.37)	62 (3.14)	$\chi^2(1) = 22.45$ < 0.01**
Chronic liver disease, n (%)	1 (0.35)	14 (0.83)	15 (0.76)	$\chi^2(1) = 0.76$ 0.38**
Cancer, n (%)	10 (3.47)	16(0.95)	26 (1.32)	$\chi^2(1) = 12.06$ < 0.01**





Tab. II. Epidemiological and clinical characteristics among death and recovered cases.

Variables	Death (n = 288)	Recovered (n = 1687)	Total (n = 1975)	P-value
Immune deficiency disease, n (%)	2 (0.69)	14 (0.83)	16 (0.81)	$\chi^2(1) = 0.05$ 0.81**
Signs and symptoms				
Respiratory distress, n (%)	222 (77.08)	1006 (59.63)	1228 (62.18)	$\chi^2(1) = 31.85$ < 0.01**
Fever, n (%)	162 (56.25)	1037 (61.47)	1199 (60.71)	$\chi^2(1) = 2.81$ 0.09**
Dry cough, n (%)	161 (55.90)	1041 (61.71)	1202 (60.86)	$\chi^2(1) = 3.47$ 0.06**
Sore throat, n (%)	49 (17.01)	284 (16.83)	333 (16.86)	$\chi^2(1) = 0.005$ 0.94**
Myalgia, n (%)	25 (8.68)	241 (14.29)	266 (13.47)	$\chi^2(1) = 6.63$ 0.01**
Headache, n (%)	9 (3.13)	120 (7.11)	129 (6.53)	$\chi^2(1) = 6.40$ 0.01**
Confusion, n (%)	11 (3.82)	80 (4.74)	91 (4.61)	$\chi^2(1) = 0.47$ 0.49**
Nausea and vomiting, n (%)	11 (3.82)	94 (5.57)	105 (5.32)	$\chi^2(1) = 1.50$ 0.22**
Chest pain, n (%)	17 (5.90)	57 (3.38)	74 (3.75)	$\chi^2(1) = 4.34$ 0.03**
Temperature C, median (IQR)	39 (39-39)	39 (39-39)	39 (39-39)	T = -0.80 0.42*

* Two-Sample t-Test; ** Chi-squared test (χ^2).

Tab. III. Multiple logistic regression analysis showing factors independently associated with death caused by 2019-nCoV.

Variables	AOR (CI 95%)	P-value
Age	1.03 (1.02-1.04)	< 0.001
SPO ₂ < 93%	2.44 (1.79-3.31)	< 0.001
Comorbidities	2.15 (1.62-2.84)	< 0.001
Respiratory distress	1.74 (1.28-2.37)	< 0.001
Myalgia	0.77 (0.48-1.22)	0.27
Headache	0.44 (0.21-0.92)	0.03

AOR: Adjusted Odds Ratio.

Discussion

During this study, the clinical status of patients admitted to hospitals of Golestan province including the common symptoms of COVID-19 patients and their companions, as well as the clinical outcomes and characteristics of patients were evaluated. In addition, this study reviewed the influencing factors of fatality as a retrospective cohort study. As of April 20th, 2020, 2,835 cases of suspect COVID-19 were hospitalized, of which about 31% were reported to be a definite case of COVID-19. About 12% of the patients had to be admitted to the ICU, and about 20% of the patients expired. Among them, only 51% tested positive for COVID-19. Therefore, fatality rate for COVID-19 about 10%. Moreover, about 60% of the patients were discharged from the hospitals with complete recovery.

Coronavirus is among the basic pathogens of respiratory diseases. SARS-Corona and MERS-Corona virus are the two most robust pathogens that have caused an epidemic

in recent years. These two pathogens cause severe respiratory symptoms in patients. These viruses caused the involvement of a huge part of human society in the world [11]. However, the new coronavirus has caused a totally different condition all over the world.

The experience of previous viruses had shown that the mortality rate for the SARS virus was more than 10% and for the MERS was more than 35% [12-14]. However, this value is different for the new coronavirus, in a way that the mortality rate has shown to be 1.5% in the present study. Nevertheless, it should be noted that future death records should be reported in inpatients.

The most common clinical symptoms of the patients of the trial include shortness of breath, fever and dry cough. Unlike the present study, the most common one in Wuhan city of China was fever (in 98% of patients) [1]. A reason for this difference in the clinical signs between the patients in Iran and China is the way of providing services based on the type of patient. In Wuhan, all patients with mild symptoms were kept in hospital or tested for the virus; however, between Iranian patients, only those with severe respiratory symptoms were admitted or PCR-tested. This difference in methods led to the fact that most of the patients included in the present study had severe symptoms of coronavirus including severe shortness of breath. However, in general, the basic presented symptoms in patients were similar to the previous coronavirus diseases, including SARS and MERS [15, 16].

During our study, it was shown that the total reported cases of coronavirus in males were higher than in females. The same rate of infection incidence was also seen in MERS and SARS. The lower susceptibility of

females to the disease may be partly due to the female hormones that give them immunity [17]. In addition, about half of the patients of coronavirus in the present study had at least one underlying disease, which was mainly cardiovascular and diabetes disease. This finding was similar to that of MERS [18]. Basically, our findings indicate that the disease regularly invades males of elder ages with underlying diseases and suppression of the immune system.

Moreover, the risk factors of deaths due to the coronavirus were investigated in our study. It was shown that the age, SpO₂ of less than 93%, having underlying diseases and the onsets of symptoms with respiratory distress are among the main risk factors or predictors of fatalities due to coronavirus. The findings of the present study are consistent with the results of the study of Wu et al. [19]. These findings are also consistent with the results of the study of Wu et al. [19] and Zhou et al. [20]. Nonetheless, these findings can be explained by the fact that the PCR test does not have a high sensitivity in detecting coronavirus in COVID-19 patients, so that a number of patients with CT scan results showing evidence of severe pulmonary involvement and had negative PCR results. On the other hand, during the early and middle periods of the epidemic, there were multiple sampling errors for PCR because of the fear of the healthcare staff from being infected by the virus, as well as not having the proper skills. As a result, these errors led to misclassification of patients. Therefore, it should be noted that dead patients with a negative PCR result were necessarily not free of the Coronavirus infection. This is the reason for the similar death risk factors for both groups. Interestingly, the onset of symptoms with a headache in cases where their PCR test was positive showed an inverse relationship with fatality and reduced chance of death up to 70%. However, it should be noted that obtaining a history for the headache is a subjective concept that may not be recorded accurately in the epidemic situation. Therefore, this finding should be cautiously interpreted and used.

There were a number of limitations in our study that may have affected the results. First, there was a number of missing data in some variables that may have reduced the study's power to assess the effect of that variable on death. This shows that there is a need for a more robust database of patients in Iran [21], and unfortunately, there is a severe weakness. Second, in this study, we were not able to use laboratory data. This was because of the fact that the laboratories data were not accurate enough. Third, we did not have access to the CT scan reports, because the patients' database only included the CT images, not the reports, which were used in the clinical setting. We were also not able to re-examine the whole CT images because of the current emergency situation.

Conclusions

However, according to the conducted search, this is the first retrospective cohort report in Golestan province.

The 2019-nCoV infection caused collections of severe respiratory illness and was associated high ratio of hospitalization in ICU and high mortality. This study generally identified two important factors for fatality including old age and underlying diseases. On that account, we can try to decrease the fatality by planning on care for these groups. Among the underlying diseases, diabetes, as well as cardiac, lung, and kidney diseases play an important role in the fatality of Coronavirus patients.

Abbreviations

ARDS: Acute Respiratory Distress Syndrome; nCoV-2019: New Coronavirus 2019; PHEIC: Public Health Emergency of International Concern; SARS: Sever Acute Respiratory Syndrome; RT-PCR: Reverse-Transcriptase-Polymerase-Chain-Reaction; ICU: Intensive Care Unit; CT: Computerized Tomography.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Research Ethics Commission of Golestan University of Medical Sciences (IR.GOUMS.REC.1398.385).

Acknowledgements

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors would like to thank Specialized Hospital of Golestan University of Medical Sciences for making the data for this research undertaking available. The authors are also grateful to data collectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

MRH, GR and AR originated the research idea and analyzed the data. HS, AT, MG, AJ and MM designed the study protocol. AT and AT confirmed the samples. AF, SA, and NJ managed the data collection. AR, MRH and GR drafted the manuscript. All authors confirmed that they meet ICMJE criteria for authorship.

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Received on December 9, 2020. Accepted on March 9, 2021.

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How to cite this article: Honarvar MR, Roshandel G, Shirzad-Aski H, Tabarraei A, Tahamtan A, Ghelichi-Ghojogh M, Fazel A, Arefnia S, Jafari N, Mansoury M, Jafari A, Rajabi A. Epidemiological and clinical characteristics of the COVID-19 epidemic and associated factors for mortality in Golestan province, Iran: a retrospective cohort study. *J Prev Med Hyg* 2021;62:E584-E304. <https://doi.org/10.15167/2421-4248/jpmh2021.62.2.1910>

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