

# Early Epidemiological analysis of CoVID-19: First report from South of Iran

**Ali Akbari**

Shiraz University of Medical Sciences

**Amir Emami** (✉ [emami.microbia@gmail.com](mailto:emami.microbia@gmail.com))

Shiraz University of Medical Sciences

**Fatemeh Javanmardi**

Shiraz University of Medical Sciences

**Neda Pirbonyeh**

Shiraz University of Medical Sciences

**Nima Fadakar**

Shiraz University of Medical Sciences

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## Research Article

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# Abstract

**Background:** Despite the whole world's effort for controlling, an ongoing global outbreak of lower respiratory tract disease, caused by new corona virus; led to a major public health issue. Current study aims to evaluate the characteristics of infected case in Fars, Iran

**Methods:** According to the referral of suspicious patients during one month, 2538 samples were evaluated and extracted for Viral RNA nucleic acids by using the Invitrogen ChargeSwitch® Total RNA Cell Kit (Invitrogen Co.). Data were recorded based on the standardized data collection forms shared by WHO and the International Severe Acute Respiratory and Emerging Infection Consortium.

**Results:** By 19 March 2020, 440 admitted patients had been identified with CoVID-19 confirmation. More than 50% of cases were men. Mean age were estimated  $48.15 \pm 18.07$ . Of all the patients, 30 (6.81%) had a history of travel to Qom city, which was the first infected zone in Iran, also 92(20.90%) had close contact with contaminated or suspected individuals. The mortality rate was estimated 2.95% (13 cases). Cardiovascular disease (25, 5.68%), diabetes (33, 7.5%), and hypertension (35, 7.95%) were the most common co-existing disorders. Patients with underlying disease are more in danger of death [OR: 11.64, (95%CI 3.14 – 43.18),  $p= 0.0002$ ].

**Conclusion:** Human to human transmission of SARS-CoV-II causes Fars province in southwest of Iran become infected and 440 cases identified during one month. The SARS-Cov-2 is more likely to affect male sex and individuals with old age and underlying disorders.

## Introduction

The emergence of a novel coronavirus in December 2019 in Wuhan city in china; show the threat of cross-species transmission events which make an outbreak in human society. The quick spreading of this contagious virus causes a global concern of worldwide public health issues which world health organisation (WHO) declared CoVID-19 a pandemic in Mar 11, 2020 [1, 2]. At the time when the pandemic was announced, total of 118,000 people were infected and more than 4,000 were murdered in 114 countries. Due to genetic similarity of 2019 novel coronavirus with sever acute respiratory syndrome coronavirus in 2003 (SARS); this new virus called SARS-CoV-II and the related disease defined as "CoVID-19"[3, 4]. This new Coronavirus is the third widespread outbreak relating to this virus family in the 21 century. According to the first reports; there was a strong association between direct exposure to the Huanan seafood wholesale Market and animal to human transmission. Nevertheless; it soon became clear that subsequent transmissions were person to person[5, 6]. Various evaluation about mechanisms of transmission, showed that SARS-CoV-II may spread through droplets due to sneeze and cough, which is similar to another respiratory viral pathogens (i.e. Flu and Rhinovirus).[7]

Due to different reports about SARS-CoV-II incubation time; estimating the infected people and contaminated community became complicated. Based on the first reports; the incubation time could be within 3 to 7 days and the duration time from infection to symptoms develop may estimate 12.5 days

(95%CI: 9.2–18)[8]. Hence the infected individuals are unaware of disease in the early phase and have low level of vigilance. So, this makes the global spreading of this unlimited border virus. Given what happened and according to the high contagious ability of SARS-CoV-II it has been conducted that travel restrictions are not a solution to the prevention CoVID-19[9, 10]

Outbreaks of SARS-CoV-II and rapidly evolving situation especially outside of China shocked the health ministers in other countries. Although the health policy makers; scramble to delay the spreading of the virus, but CoVID-19 is affecting 187 Countries and territories around the world with more than 300,000 confirmed cases until March 22, 2020.

According to epidemiological information about the CoVID-19 in the world, it was thought that IRAN would soon become infected with the disease. Due to this and the existing warnings, most of the provinces in IRAN, consequently Fars province (4th biggest province in the south), established standard diagnostic methods, control and treatment fields based on experiences of involved areas. According to this readiness, the first suspected cases were evaluated, diagnosed and were subjected under specific and isolated treatments. Based on the first available documentary information, the first positive case for CoVID-19 was reported at 18 February 2020 in Fars province. Although Fars was in a better situation of CoVID-19 epidemiology compare with most northern provinces, and despite the initial necessary preparations, unfortunately, the incidence of SARS-CoV-II related disease increased dramatically within a short period of one month. Considering the importance of early investigation of epidemic diseases and their application in the management of infection control, this study was conducted to report comprehensive descriptive data in Fars as the first report of the CoVID-19 in southern Iran.

## Methods

### Sample collection

Since the first detected of clinical suspected CoVID-19 in southern Iran (18 February 2020), total of 2538 samples were collected from referral patients with suspected criteria in favor of corona. Due to the protocols the patients with acute onset of respiratory symptoms (respiratory frequency  $\geq 30$ /minute), shortness of breath which accompanied by fever, blood oxygen saturation  $\leq 93\%$  and PaO<sub>2</sub>/FiO<sub>2</sub> ratio  $<300$  who have been hospitalized and were sampled. According to the CDC recommends samples were collected at the first time of symptoms onset. For this purpose, samples were collected from upper respiratory both nasopharyngeal AND oropharyngeal (NP/OP swabs) and inserted in the same tube contain viral transport media. For analysis, collected samples were send to the referral medical microbiology laboratory in 2-8°C containers (Affiliated with Shiraz University of Medical Sciences).

### RNA isolation

All submitted samples to the referral laboratory were extracted for Viral RNA nucleic acids by using the Invitrogen ChargeSwitch® Total RNA Cell Kit (Invitrogen Co.). The procedure was performed according to the manufacturer's protocol. extracted RNAs were stored at -70°C until the following tests. According to

the importance of the results accuracy before testing, the RNA extraction procedure was qualified and validated for recovery and purity.

### **Detection by Real Time-PCR**

Given that, several assays have been introduced interim guidance on laboratory detection of SARS-CoV-II, we setup berlin protocol based on detection of E and RdRP target genes. [11]

As the first line of screening assay, the Real Time-PCR assay was done to amplify the highly conserved envelope related gene (E gene). For confirmation of the test results, E positive samples were tested for RNA-dependent RNA polymerase gene (RdRp gene). All assays used the same conditions. The specification and sequences of the used primers and probs have been shown in table 1. In brief, a 25- $\mu$ l reaction containing 5 $\mu$ l of extracted RNA, 12.5 $\mu$ l of 2X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (0.4mM of each deoxyribonucleotide triphosphates (dNTP) and 3.2mM magnesium sulfate), 1 $\mu$ l of reverse transcriptase/Taq mixture, 0.4 $\mu$ l of magnesium sulfate solution (50mM), (Invitrogen Co.), and 1 $\mu$ g of nonacetylated bovine serum albumin (Roche). All oligonucleotides were ordered from 1<sup>st</sup> step company (Malaysia), according to the berlin protocol (Jan 17th, 2020) approved by World Health Organisation [12]. Thermal cycling was performed at 55°C for 10 min for reverse transcription, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s, 58°C for 30 s. The assays were performed on The LightCycler<sup>®</sup> 96 instrument as a *real-time* PCR system. Positive control for the assay was provided by pastor institute (Iran). If the CT value exceeded 36 cycles, intended sample was considered negative.

### **Statistical analysis:**

Continuous measurements were presented with mean, median, and interquartile range (IQR). Categorical variables were described as frequency rates and percentage (%). Independent sample t-test was used for age comparison. The statistical analysis was carried out by using SPSS software version 22. Statistically, a significant difference was considered when  $p < 0.05$ .

## **Results**

In the recent study, from 2538 tested cases, 440 (17.34%) were confirmed with SARS-CoV-II in Fars. All of the positive patients were on average 48.15 years old with standard deviation 18.07. The median age was 46.50 (IQR: 35–61). According to sex distribution, it was revealed that 248 (54.4%) were male (mean age  $48.50 \pm 18.67$ ) and 192 (43.6%) were female (mean age  $47.78 \pm 17.63$ ). Of all the patients, 30 (6.81%) had a history of travel to Qom city (the first CoVID-19 epidemic zone in IRAN), and 92 (20.90%) had close contact with contaminated or suspected individuals. Other infected patients with SARS-CoV-II had a different occupation as follows: 18 (4.09%) were medical staff, 196 (44.54%) were self-employed, 156 (35.45%) were house wife and 56 (12.72%) were employee, 8 (1.81%) were students and 6 (1.36%) were in pre-school age.

Of all the patients some of them were especial cases: two pregnant women, one 4-month baby, and two men were devotee with history of pulmonary problem. In the confirmed population moreover of 5 cases were in a family and 10 persons were a couple.

## Clinical features

Of all the 440 patients, 17 cases (3.86%) had a history of chronic disease. Cardiovascular diseases (25, 5.68%), diabetes (33, 7.5%), and hypertension (35, 7.95%) were the most common co-existing disorders. According to clinical outcomes; 80 (18.18%) of patients were recovered and discharged; and 186 (42.27%) remained in the hospital and need more care. During the study period, 13 patients were died because of CoVID-19, where the mortality rate was estimated 2.95%. The characteristics of dead patients were as follow: 8 males and 5 females, so that 4 with diabetic mellitus, 2 with hypertension, 2 with cardiovascular disease, one asthma, and one liver disease. The mean age was estimated  $64.53 \pm 21.98$  for these victims. According to results, it was revealed that patients with underlying diseases deteriorated more rapidly in compare with patients with no comorbidities [OR: 11.64, (95%CI 3.14–43.18),  $p = 0.0002$ ]

Of all patients, 11 cases were received intensive care and hospitalized in ICU. A significant difference was seen in age of patients with ICU and without ICU admission ( $P < 0.05$ ). Cases who required intensive care were significantly older and also were more likely to have underlying comorbidities.

Table 1  
 Characteristics of 440 patients admitted with CoViD-19 in Fars, Iran

Age		Underlying disorders	
(Mean ± SD)	48.15 ± 18.07	Asthma	8 (1.81%)
Range	2.5–95	Cardiovascular disease	25(5.68%)
≤ 18	13 (2.95%)	Liver disease	2 (0.45%)
19–40	148 (33.63%)	Renal disease	9 (2.04%)
41–60	169 (38.40%)	Pulmonary	6 (1.36%)
≥ 61	110 (25%)	Diabetic mellitus	33 (7.5%)
Sex		Malignancy	4 (0.90%)
Male	248 (56.4%)	Hepatitis B	1 (0.22%)
Female	192 (43.6%)	Chronic disease	17 (3.86%)
Occupation		Clinical Outcome	
Self – employed	197 (44.77%)	Remained in Hospital	186 (42.27%)
Employee	56 (12.72%)	Discharge	80 (18.18%)
Medical staff	18 (4.09%)	Died	13 (2.95%)
House-wife	156 (35.45%)	ICU admission	11 (2.5%)
Students	7 (1.59%)		
Pre-school	6 (1.36%)		
Exposure History			
Travel to Qom	30 (6.81%)		
Contact with infected cases	92 (20.90)		

## Discussion

Chinese people started the New Year 2020 with severe pneumonia symptoms which the reason was not clear. In the following of the virus epidemic, the agent was known as new Coronavirus which has a contagious power in rapid person to person transmission. In recent month, after knowing more about the virus, SARS-CoV-2 has been named to the disease cause. The disease caused by this virus is now known worldwide as Covid-19. Contagion power of the virus in asymptomatic phase in infected cases and travelling may be the main reasons for this rapid distribution [13]. Given that Iran is also a major tourist country in the middle east, then it was not exception of this global outbreak, particularly near Nowruz

ceremony (Persian New Year Celebration). According to an interesting report which was conducted based on Data-Driven Coding and statistical methods, a total of  $14450 \pm 6244$  individuals will be infected in Iran and spread to 31 provinces. Also the infection, peaks during 3 weeks and the epidemic is expected to end in June 2020[14]. Another prediction computes the basic reproduction number ( $R_0$ ) of CoVID-19 in Iran, by the mean estimation 3.6; which means; 3.6 susceptible individuals would be infected by confirmed positive cases. There is uncertainty about many of the factors that go into estimating  $R_0$ , such as in estimating the number of cases. Based on these current estimates, projections of the future number of cases of coronavirus are fraught with high levels of uncertainty and will likely be somewhat inaccurate. The most important reason for ambiguities of these predictions is unknown impact of future impacts of current disease control measures, such as travel restrictions, social and self-quarantine efforts will influence the virus's continued spread especially in holidays of the new year in IRAN.

As the virus moves into new regions and country, it encounters communication with different health settings that affect their vulnerability to disease (i.e different social structures). The current analysis revealed that the majority of patients were male, which was similar to other studies in China. Our estimation for male prevalence was 56.4% while the percentage was less than Chaolin and Chen's reports (73% and 68%)[15, 16]. Possible explanation may be related to sex hormones and protection of female due to X chromosome. Another likelihood reason may be due to smoking history. According to primary literature, there is an association between male sex and smoking status in CoVID-19[17]. In another hand, it was revealed that cigarette smoking in Chinese male is more than Iranian male[18]. So, this sex predisposition may be related to much higher smoking rate in men in China, than in Iran.

Another point about current results which is seen in the over world was the high prevalence of CoVID-19 in vulnerable people; it means elderly and individuals with underlying disorders are more susceptible to the SARS-CoV-2. Along with other studies; hypertension, diabetes, and cardiovascular diseases are other important risk factors for CoVID-19 and there is a strong association between mortality rate and having co-existing comorbidities [19–22].

According to that the medical staff represent the front line in the fight against the SARS-CoV-2, their protection is as crucial as ever. Based on this, the notable point in our results was low contamination rate in our medical staff which was 4%, while it was reported 20% in Italy[23]. According to the evaluation of various reports in the countries involved in the disease, it was found that their health staff are either contaminated and many of them have positive tests for CoVID-19[24]. Although the results of different studies on personnel contamination are varying together, the important point is that the medical staff are infected during the human-to-human transmission chain, which is a great threat for other persons especially their patients and their families due to its long incubation time. Another hypothesis regarding clinical staff contamination is that they may be infected by contaminated surfaces that constantly contact with. Due to this highly recommended that clinical staff clean their workspaces and personal items such as stethoscopes, mobile phones, dictation devices, nametags, and other items with hospital-provided disinfectants or alcohol-based disinfectants[25]. Another threat for health staff is their physical

and mental exhaustion. In addition to infection risk, missing patients and colleagues make their situation more difficult. [23, 26].

Data analysis of recent study showed approximately 3% mortality rate in total of 440 infected patients, while this rate in China and Italia were reported 11% and 7.2% respectively[15, 27].

Our results showed that all of the nine patients who died during the study time, had underlying diseases. It seems old age and co-existing health disorders increase the mortality rate. Similar results in other studies showed that weaker immune function of these patients justify these occurrences and the low level of immunity, causes secondary infections, which makes the anti-infective treatment more difficult [15, 28].

Information about patients who receive intensive care in this study, showed resemble pattern in the aspect of comorbidity, fatality, and age in comparison with other studies [15, 16]

Up to date, no specific treatment has been found for SARS-CoV-2 except meticulous supportive care. Currently the best approach is prevention and applying infection control strategies as follow: Immunocompromised individuals should avoid to be in the public gathering, everyone should wash their hands frequently during the day, keep the social distance and evade close contact with animals and raw products. Considering that there is no proper treatment and vaccine for CoVID-19 yet, we should believe that follow the sanitary instructions are the best and least costly way to control and prevent CoVID-19 [29–31].

This study has several strength points: To our knowledge it is the first report from Fars province, southern of Iran. Moreover, from 2538 number of suspected patients during the study time, only 440 cases were confirmed that expresses good infection control strategies have performed despite rapid distributing in compare with other countries with better equipment.

Another notable point in the recent study was that, despite hostile sanctions against IRAN and lack of access to many international facilities, we were able to significantly control the spread of the disease and the number of patients in the first few days of exposure. Despite the limited availability of many medical, pharmaceutical, and laboratory facilities but proper management of resources and extensive health culture in Iran could help in preventing the beyond spread of the disease in the country especially in the southern region[32]. This is while other countries such as European territories and the United States shows a similar pattern of infection spread and even worse in somehow. Another case that has led to successful infection control in Iran, especially in the general population was, a lot of personal protective production line have developed in every city in Iran. Charities, private sector groups and independent manufactures cause that our country be in a good position in compare with developed countries which are not in sanctions and access to all facilities. In fact, Iran, like their counterparts globally, can more or less afford to protect themselves. Nevertheless, limitations should not be ignored in this study. Detailed patient's information especially about clinical characteristics was un available at the time of analysis.

# Conclusion

In summary, since the outbreak of CoVID-19 in Iran is occurred and cover the whole country approximately, vulnerable individuals at the extreme of ages and those that are immunocompromised are more in the risk of infection. Further, health professionals should be aware of the precautions necessary to avoid the contraction and spread of the disease.

# Abbreviations

WHO

World Health Organization

CoVID-19

Coronavirus disease

ICU

Intensive care unit

SARS-CoV

Severe acute respiratory syndrome coronavirus

# Declarations

## Ethical Approval and Consent to participate

This study was approved by the Ethical Committee of Shiraz University of Medical Sciences (Code: IR.SUMS.REC.1398.1379)

## Consent for publication

All authors read and approved the final manuscript and agree for publishing the article in this journal.

### Consent for publication

All authors are agree for publication in this journal.

## Availability of data and materials

Data will be available for reviewer only, not for publication.

## Competing interests

All authors declare that, they have no conflict of interest.

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## Authors' contributions

A.E and A.K designed the study and writing the primary draft. F. J did the statistical analysis and write the results section. N.F and N.P revised and edited the paper

## Author's information

**Affiliation:** Shiraz university of Medical Science

**Corresponding author:** Amir Emami

## ORCID

**Ali Akbari** : 0000-0002-2970-2052

**Amir Emami**: [0000-0002-4510-1820](https://orcid.org/0000-0002-4510-1820)

**Fatemeh Javanmardi**: 0000-0001-8841-0861

**Neda Pirbonyeh**: 0000-0001-5700-3913

**Nima Fadakar**: 0000-0002-4083-5577

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