Disease and Diagnosis

→ **Original Article**

High Prevalence of Influenza A (H1N1 and H3N2) Infection in South Iran

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Abstract

Background: Influenza A is divided into a number of subtypes, the most common of which are circulating H3N2 and H1N1. Influenza A and B viruses, which normally circulate in humans (human influenza viruses), are responsible for seasonal influenza pandemics each year. This study evaluated the prevalence of influenza in Hormozgan province and determined the type and subtypes of influenza circulating in the community.

Materials and Methods: The present study was conducted in a single center on 1237 patients with acute respiratory syndrome and flu-like symptoms in Hormozgan province, Iran from August 2022 to December 2022. Eventually, patients' data such as the age, gender, and hospitalization rate of patients with influenza underwent investigation.

Results: Out of a total of 1237 samples of patients, 349 (28.2%) cases were diagnosed with positive influenza and 888 (71.80%) cases were negative. Of 349 cases of positive influenza, 3 and 346 cases were diagnosed with influenza B and A, respectively. The results represented that out of a total of 349 positive cases of influenza, 216 (61.90%) and 133 (38.10%) people were hospitalized and visited as outpatients, respectively.

Conclusion: From August 2022 to December 2022, Hormozgan province in the south of Iran witnessed an influenza peak with an increase in the prevalence of H1N1 and H3N2 subtypes. The dominant subtype of this peak was H3N2 influenza, but the prevalence of the H1N1 subtype also demonstrated a significant increase. Finally, the cases of hospitalization of patients with positive influenza represented an increase.

Keywords: Influenza, H1N1 subtype, H3N2 subtype, Hormozgan province, Outbreak

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Introduction

Influenza is an infectious disease caused by viruses enveloping RNA of the Orthomyxoviridae family. There are three types of influenza virus called A, B, and C. A and B types are found in humans and animals, as well as humans and mammals, respectively, and type C is only found in humans. Influenza A and B viruses that normally circulate in humans (human influenza viruses) are responsible for seasonal influenza pandemics each year (1, 2). Influenza A is divided into a number of subtypes, the most common of which are circulating H3N2 and H1N1 (also known as H1N1pdm09). Influenza B is classified by lineage; the circulating lineages are Yamagata (B/Y) and Victoria (B/V) (3). This disease causes an acute respiratory tract infection that manifests itself with sudden headaches, muscle pain, fever, severe weakness, and lethargy. Normally, the duration of the disease is 3-4 days. Influenza causes a short-term but severe respiratory infection in all people, especially adults. It is a threat to the elderly, children, and people with cardiorespiratory diseases and may lead to death if not controlled and monitored. Swine influenza is a respiratory viral disease of pigs that originates from the influenza A virus. Every year, cases of human infection with this disease are reported, especially through contact with pigs. This disease is spread in humans through contact with an infected person, and its symptoms are usually similar to those of human influenza (2). The global influenza epidemic in 1918 and 1919 was also caused by the spread of this type of influenza. A combination of swine, human, and bird influenza viruses is a new type of influenza that was discovered in Mexico and the United States in 2009. Prior to the swine influenza outbreak in Mexico in 2009, cases of widespread livestock and human infection with the disease were reported in the United States and the Philippines in 1976 and 2007, respectively (4-6). In

previous influenza epidemics, the global spread of the virus and the creation of an epidemic took more than 6 months, but in the pandemic H1N1 (A) in the United States of America, it took only 6 weeks for the virus to spread globally (5, 7, 8). The seasonal influenza viruses are identified throughout the year, and influenza viruses typically circulating during the fall and winter are known as influenza season. The exact timing and duration of influenza vary, but influenza activity often begins to increase in October. Influenza activity often peaks between December and February, although significant activity can continue into late May (9, 10). Since the beginning of the COVID-19 epidemic, the time and duration of influenza activity have been a less predictable set. There are many reports about the epidemiology, clinical consequences, and molecular diversity of the virus in Iran and around the world. Considering that many conflicting results have been reported in studies related to influenza in Iran, it seems that factors such as population, gender, occupation, and initial conditions should play a role in this regard. To date, the prevalence of influenza viruses, including H3N2, H1N1, and B, has not been well evaluated in Iran (10, 11). The seasonal intensity of influenza 2021-2022 was low and with two waves of influenza A activity. Influenza activity continued from October 2021 to mid-June 2022, and H3N2 subtype viruses predominated throughout the season. Before the emergence of severe acute respiratory syndrome coronavirus 2, influenza activity in the United States was normal. It began to increase in autumn and reached its peak in February. During 2021-2022, influenza activity began to increase with two distinct waves, with A (H3N2) viruses for the entire season. This report summarizes influenza activity. The new influenza viruses are the subtypes of the influenza A virus that is different from the seasonal human influenza that is currently circulating. Of H1 and H3 viruses, four new influenza A viruses (H1N2), including A (H3N2), A (H1), and A (H5N1), have been identified in humans during the 2021-2022 influenza season (7, 9, 12). According to the studies, one of the three types of H1, H2, and H3 is the cause of the recent influenza epidemic (7). The hemagglutinin (HA) and neuraminidase (NA) nucleotide sequences of H1N1 influenza that were available from Iran and its neighbouring countries (i.e., Pakistan, Afghanistan, Turkmenistan, Armenia, Azerbaijan, Turkey, and Iraq) in 2020 were analyzed in this study. According to the results of this study, the age of the appearance of the H1N1 influenza virus serotype in Iran was higher than in the neighbouring countries (1). Influenza A's propensity for extreme changes in its surface glycoproteins (i.e., HA and NA) has caused deadly pandemics. The most severe pandemic on record was the swine-like H1N1 outbreak during 1918-1919, which killed more than 20 million people and lasted until 1957. The new influenza is a viral disease caused by influenza (A) H1N1 that appeared as an emerging disease in the Americas in early 2018 and then in other continents of the world; although its global spread is fast, its severity is moderate (4, 5). This infection occurs once every two to three years as an epidemic and once every 10-30 years as a pandemic. The aforementioned epidemics occur in temperate regions, in the cold months of the year, namely, from late autumn to early spring (4). The aim of the current study was to evaluate the prevalence of influenza and determine the subtypes of influenza circulating in the community, as well as the hospitalization rate of patients with influenza. Finally, it was sought to determine the prevalence in different age groups in Hormozgan province in the south of Iran.

Materials and Methods
Data Collection
The population of this cross-sectional descriptive study included patients with acute respiratory syndrome and flu-like symptoms who had referred to Hormozgan Medical Centers from August 2022 to December 2022. In this evaluation, samples were taken from 1237 patients with these symptoms. The samples were evaluated in terms of influenza type, influenza subtype, age, and gender of the patients. Suspected influenza patients were sampled from the nasopharynx with a special swab. Patient information was extracted from the checklist prepared in Hormozgan comprehensive laboratory.

RNA Extraction and Real-time Polymerase Chain Reaction (RT-PCR)
Two samples of nasopharyngeal and oropharyngeal throat swabs were taken from patients and tested for influenza type A and B and H1N1 and H3N2 subtypes for each patient. Viral RNA was extracted from 140 μL of each clinical sample using a high-purity viral nucleic acid kit (RNJia Virus Kit, Yazd, Iran) according to the manufacturer's instructions. RT-PCR assay was separately performed for 1237 clinical samples, including influenza A (H1N1 and H3N2 subtypes) and B.

Statistical Analysis
The obtained data were statistically analyzed by one-way ANOVA test using SPSS (Version 20.0, IBM, Armonk, NY, USA) and GraphPad Prism (Version 5.0 GraphPad Software, Inc., San Diego, CA) software, and P values less than 0.05 were considered statistically significant.

Results
General Characteristics of COVID-19 Patients
In the present study, which was conducted from August 2022 to December 2022, out of a total of 1237 samples of patients with the symptoms of acute respiratory syndrome that were sent to the central health laboratory from different areas of Hormozgan province, 505 (38.43%) and

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807 (61.41%) of cases were males and females, respectively. There was no statistically significant difference between patients in terms of gender (*P* > 0.05). The average age of patients was 44.08 ± 28.7 years. Out of all these tests, 349 cases (28.20%) were positive for influenza, while 888 cases (71.80%) were negative (Figure 1a). The results of the analysis showed that there is a significant relationship between the age of the patient and infection with the influenza virus (*P* < 0.001), and with increasing age, the probability of getting influenza is higher. The current study also examined the cases of influenza that led to hospitalization. The results demonstrated that out of a total of 349 positive cases of influenza, 216 people (61.90%) were hospitalized and 133 people (38.10%) were visited as outpatients (Figure 1b). Out of 349 cases of positive influenza, 3 and 346 cases were diagnosed as influenza B and A, respectively (Figure 2a). Investigations to determine the subtypes of influenza A represented that 82 (23.70%), 240 (69.4%), and 24 (6.9%) cases were H1N1, H3N2, and untyped, respectively (Figure 2b). Most cases of influenza were in the age group of 14-40 years. At the same time, the number of influenza A H3N2 subtype cases in the age group of 60-80 years showed the highest number of cases after the age group of 14-40 years (Table 1). Examining the statistics of influenza patients in relation to gender indicated that there was no significant difference between male (58.74%) and female (41.26%) cases.

Discussion

Influenza is an acute, self-limited respiratory illness caused by influenza A and B viruses. During recent centuries, influenza has caused outbreaks of respiratory diseases in the fall and winter every 1-3 years (13). The emergence of a new strain of swine influenza, the H1N1 virus, in 2009 has raised serious concerns among health experts due to the increased risk of pandemic disease and mortality (14). Our findings revealed that approximately 28.20% of patients with acute respiratory symptoms from August 2022 to December 2022 in Hormozgan province were infected with influenza A (subtype H1N1 and H3N2) and B. Several studies in recent years have shown that the prevalence of influenza has significantly decreased since the beginning of the COVID-19 pandemic (15-18). These studies concluded that it was associated with the onset and increase of social restrictions to deal with COVID-19. In addition, in our previous study regarding the spread of influenza and COVID-19 in Hormozgan province, it was found that with the start of the COVID-19 pandemic, the cases of infection decreased a lot (11). In the present study, a total of 1237 samples of patients with the symptoms of the acute respiratory syndrome were sent to the central health laboratory from different areas of Hormozgan province. Out of these, 888 cases were negative and 349 cases were diagnosed with influenza, including 144
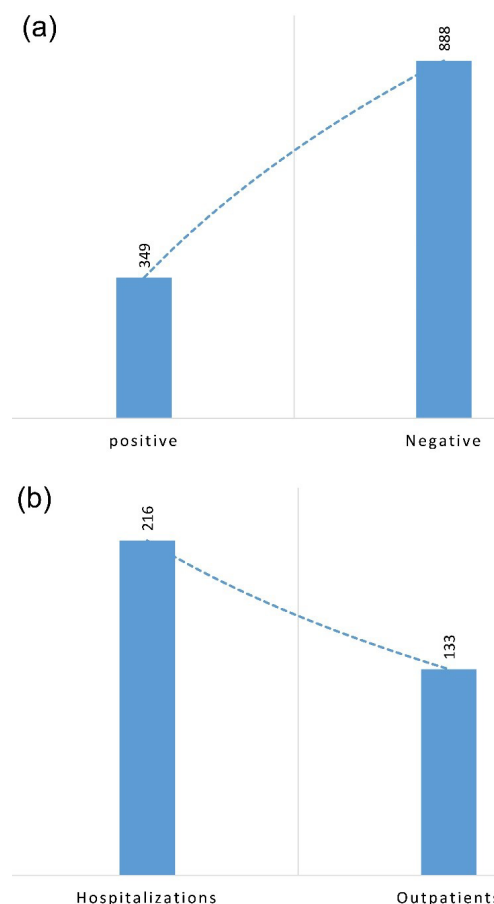

Figure 1. (a) The Plot of the Prevalence of Influenza From August 2022 to December 2022 in Hormozgan province and (b) Statistics of Hospitalizations Among Patients With Influenza

female (41.26%) and 205 male (58.74%) patients. The statistical analysis of Influenza patients indicated that no significant difference was observed between the two genders (*P* > 0.05), which is in agreement with the finding of our previous study (11). The results demonstrated that out of a total of 349 positive cases of influenza, 216 people (61.90%) were hospitalized and 133 people (38.10%) were visited as outpatients. Considering that 61.9% of patients with influenza were hospitalized, it can be concluded that the symptoms of the disease were severe in these people. Based on the analysis of the results, this year's influenza peak has been associated with an increase in cases and the severity of the disease. The evaluation of the prevalence of influenza A subtypes in Hormozgan province showed that 23.70% and 69.40% were H1N1 and H3N2 subtypes, respectively. In a 2022 survey, among 19 127 reported seasonal influenza A virus cases, only 25 cases (0.1%) were identified as H1N1, and 19 102 cases (99.9%) were influenza A (H3N2) (9). The comparison of our study with this report represents that the prevalence of the H1N1 subtype was significantly high in Hormozgan province (9). Our results indicated that most positive cases of influenza were related to the age group of 14-40 years.

Table 1. The Prevalence of Influenza Viruses in Various Age Ranges and genders

Virus	Type	Subtype	Age Range (Number)					Gender (Number)		Total
			0-14	15-40	41-60	61-80	81-100	Female	Male	
Influenza	A	H1N1	6	34	25	13	4	34	48	82 (23.49%)
Influenza	A	H3N2	20	118	38	52	9	100	140	240 (68.76%)
Influenza	A	Untyped	1	13	6	2	2	7	17	24 (6.78%)
Influenza	B		0	3	0	0	0	3	0	3 (0.85%)
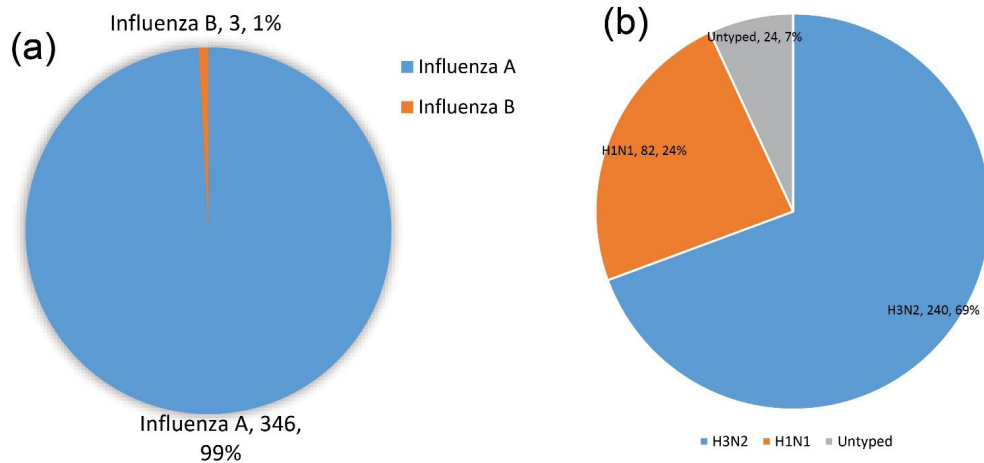
Figure 2. (a) Prevalence of Influenza Type A and B From August 2022 to December 2022 in Hormozgan province and (b) Prevalence of Subtypes H1N1, H3N2, and Untyped Influenza A

Among patients with H3N2 influenza, the age group of 60-80 years demonstrated the most cases of influenza after the age group of 14-40 years. A few studies have focused on the relationship between age groups and cases of influenza. Our finding is in agreement with that of the study of Merced-Morales et al. In their study, most cases were related to the age group of 5-64 years (9). In a study, Suzuki et al investigated the interaction between A/H1N1, A/H3N2, and B influenza viruses in Japan from 2010 to 2019. Their analysis revealed that influenza B peaked later in the season than influenza A, while influenza A/H1N1 and A/H3N2 peaked almost simultaneously (19). Our results also showed the simultaneous prevalence of A/H1N1 and A/H3N2 viruses. By examining the relative incidence and severity of disease at the individual level of seasonal influenza A H3N2 compared to the 2009 H1N1 pandemic, Kwok et al concluded that the clinical incidence of pH1N1 was more severe in the elderly. Although this virus has been circulating in humans since 1968, sH3N2 is significantly more virulent per infection than the pH1N1 strain (20). The interpretation of all influenza outbreak studies suggests that social restrictions can be implemented to reduce the incidence of influenza pandemics, particularly in populations at risk of severe disease or those with underlying diseases. Especially this year, due to the widespread vaccination of the country's population against COVID-19, we witnessed severe peaks

of influenza in Iran and the world. However, influenza vaccination is still the best way to prevent the disease for everyone over 6 months of age. As the first reports of the 2022-2023 influenza season begin with an early increase in influenza outbreaks in most countries, the Centre for Disease Control and Prevention recommends that everyone 6 months and older should be vaccinated and states that vaccination should continue as long as influenza viruses are circulating (21). Using a modelling study, Ali et al predicted the global infection burden of future influenza seasons after the reduction of public and social health measures during the COVID-19 pandemic. They concluded that full implementation of influenza vaccination programs is the best preventive measure to reduce the impact of influenza virus infections in the community (22). Our research has some limitations. This study did not examine the possibility of vaccine efficacy and any cross-protection between influenza and COVID-19 infections in influenza virus infections, which could affect our prediction of the burden of future influenza seasons. Finally, we were unable to investigate influenza data related to individuals with a specific underlying disease and its impact on disease severity.

Conclusion

Hormozgan province witnessed an influenza peak with an increase in the prevalence of H1N1 and H3N2 subtypes

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from August 2022 to December 2022. The dominant subtype of this peak was H3N2, but the prevalence of the H1N1 subtype also showed a significant increase. With the increase in the hospitalizations of patients with influenza, the severity of this disease increased as well. The way to deal with the spread of this infection is to follow health protocols and vaccination.

Authors' Contribution
Conceptualization: Khadijeh Ahmadi, Hamed Gouklani.
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Funding acquisition: Hamed Gouklani.
Investigation: Khadijeh Ahmadi, Zahra Gharibi, Razieh Gorgi, Hamed Gouklani.
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Supervision: Khadijeh Ahmadi, Hamed Gouklani.
Validation: Khadijeh Ahmadi, Hamed Gouklani.
Visualization: Khadijeh Ahmadi, Hamed Gouklani.
Writing–original draft: Khadijeh Ahmadi, Zahra Gharibi, Razieh Gorgi, Hamed Gouklani.
Writing–review & editing: Khadijeh Ahmadi, Hamed Gouklani.

Competing Interests
The authors declare that they have no competing interests.

Data Availability Statement
Not applicable.

Ethical Approval
The current study was approved by the Ethics Committee of the Hormozgan University of Medical Sciences, Bandar Abbas, Iran [IR.HUMS.REC.1398.470]. There was no need for informed consent because this study was retrospective without posing any potential risk to patients.

Informed Consent
Not applicable.

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