

## Original Article

# Epidemiological and Laboratory Investigation of Influenza in Hospitalized Patients at Yazd with Emphasis on Improving Influenza Registry Deficiencies

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#### ABSTRACT

#### Article history

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#### **Keywords**

Characteristics H1N1 Influenza Influenza B Iran **Introduction:** Influenza is an acute respiratory illness caused by three types of viruses: A, B, and C. Types A and B are responsible for the majority of human infections. This study represents the first epidemiological and laboratory investigation of influenza in Yazd Province in recent years.

**Materials and Methods:** This study was conducted on patients at Shahid Sadoughi Hospital, with a clinical diagnosis of influenza. Polymerase chain reaction (PCR) tests for influenza B and H1N1 were performed on all patients.

**Result:** Among these patients, PCR results were positive for 417 patients (73.8%), of which 64 were positive for type B and 353 for subtype H1N1 (62.5%). The remaining patients had other influenza-like illnesses. The clinical outcome for 18 H1N1 patients was death. The most common underlying condition was hypertension, present in 63 patients (11.2%). The most common clinical symptoms of H1N1 were cough, fever, and shortness of breath in 63.5%, 62.3%, and 51.6%, respectively. Platelet counts were significantly lower in both H1N1 and influenza B patients compared to PCR-negative patients (p = 0.002). Neutrophil and lymphocyte percentage differences were most prominent in H1N1 cases, with H1N1 showing the highest neutrophils and lowest lymphocytes (p = 0.001).

**Conclusion:** Prominent clinical symptoms and laboratory findings, such as decreased platelet counts and altered neutrophil and lymphocyte ratios, were key in supporting the diagnosis of influenza, especially H1N1. While most patients recovered, the higher mortality among H1N1 cases highlights the importance of early diagnosis, prompt treatment, and seasonal preparedness.



### Introduction

Influenza, or the flu, is an acute respiratory infection caused by RNA viruses from the Orthomyxoviridae family, primarily affecting the lungs of both humans and animals [1-3]. The disease is characterized by the sudden onset of fever, headache, muscle pain, weakness, and lethargy [4], and it can occur sporadically or lead to widespread outbreaks, epidemics, or even pandemics [2, 3]. There are three main types of influenza viruses: A, B, and C [5]. Type A infects both humans and animals and is responsible for most influenza pandemics, while type B is restricted to humans and occasionally causes seasonal epidemics [6-9]. Type C is less common and typically associated with mild illness. Among the subtypes of influenza A, H1N1 is particularly notable. It caused the 2009 global pandemic and is believed to have been the agent behind the 1918 "Spanish flu" pandemic, which infected over 500 million people and caused an estimated 50 to 100 million deaths—equivalent to 3% to 5% of the global population [10, 11]. Although the H1N1 strain originated in swine, human-to-human transmission occurs via respiratory droplets or contact with contaminated surfaces, rather than direct contact with pigs [12, 13].

H1N1 infection can vary in severity, ranging from mild, self-limiting illness to severe respiratory complications such as pneumonia and acute respiratory distress syndrome (ARDS). While mild cases may not require hospitalization, severe cases demand timely intervention, including antiviral therapy and supportive care [14]. Given the serious complications of influenza in certain high-risk groups, it is crucial to identify which

populations are most vulnerable and need preventive measures. Additionally, with the emergence of the Coronavirus disease (COVID-19) and the overlap in symptoms between COVID-19 and influenza [15], it has become necessary to collect epidemiological, clinical and paraclinical information specific to our region. In addition, this study represents the first epidemiological and laboratory investigation of influenza in Yazd Province in recent years. The primary objective of this study was to analyze the epidemiological patterns and laboratory findings of hospitalized influenza patients, to support the establishment of an influenza registry.

#### **Materials and Methods**

#### Sample selection

This descriptive cross-sectional study was conducted on patients with a clinical diagnosis of influenza at Shahid Sadoughi Hospital in Yazd from March 2023 to March 2024. Participants were selected using a convenience sampling method based on their availability.

**Inclusion criteria:** Patients over 18 years of age with polymerase chain reaction (PCR) confirmed influenza and clinical manifestations, as evaluated by an infectious disease or internal medicine specialist at the hospital.

**Exclusion criteria:** Patients with incomplete medical records or those discharged at their own request.

#### **Procedure**

Information collected included age, gender, underlying diseases, pregnancy status, and a history of immunosuppressive drug use (e.g.,

corticosteroids). Clinical symptoms recorded were fever, cough, shortness of breath, weakness, lethargy, headache, diarrhea, sore throat, anorexia, and myalgia. Additionally, data on relative recovery and laboratory findings including complete blood count (CBC), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were extracted from medical records.

#### Statistical analysis

Data were entered into SPSS, version 26, and analyzed using the Chi-Square test and ANOVA test. P < 0.05 was assumed significant.

#### Results

The minimum and maximum lengths of hospitalization were 1 and 45 days, respectively.

The most frequent durations of hospitalization were 4 days, 5 days, and 3 days, with frequencies of 114, 113, and 82 patients, respectively. Additionally, 3% of patients required hospitalization for more than 2 weeks. The frequency of PCR results is shown in Table 1. Figure 1 shows the distribution of PCR responses among 565 hospitalized patients. As shown in Table 1 and Figure 1, a positive PCR result was found in 417 patients (73.8%) [the total of positive (type B) and positive (H1N1)]. Figure 2 shows the frequency of patients in the inpatient ward [infectious ward (normal), intensive care unit (ICU), emergency room]. Table 2 illustrates the distribution of patients among the negative PCR, Influenza B, and H1N1 influenza groups according demographic characteristics, including age range, gender, inpatient department, status of pregnancy, and month of hospitalization.

Table 1. The frequency of PCR results

PCR response	Number (%)
Negative	148 (26.2)
Positive (type B)	64 (11.3)
Positive (H1N1)	353 (62.5)
Total	565 (100)

PCR= Polymerase chain reaction

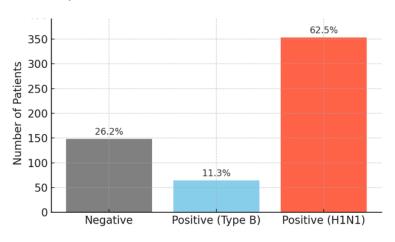


Fig. 1. Distribution of polymerase chain reaction responses among 565 hospitalized patients

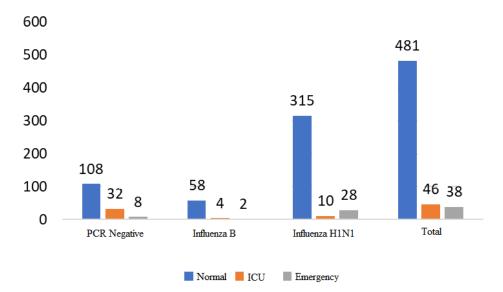


Fig. 2. The frequency of patients in terms of inpatient wards

**Table 2.** The distribution of patients in different groups in terms of demographic variables

Variables	Negative PCR N (%)	Influenza B virus N (%)	H1N1 influenza N (%)	Total N (%)	P-value
Gender Men Women	69 (46.6) 79 (53.4)	33 (51.6) 31 (48.4)	171 (48.4) 182 (51.6)	273 (48.3) 292 (51.7)	0.82
Age range < 30 30-39 40-49 50-59 60-69 70-79 ≥ 80	18 (12.2.) 36 (24.3) 23 (15.5) 17 (11.5) 18 (12.2) 21 (14.2) 15 (10.1)	7 (10.9) 12 (18.8) 27 (42.2) 7 (10.9) 2 (3.1) 3 (4.7) 6 (9.4)	31 (8.8) 57 (16.1) 48 (13.6) 58 (16.4) 67 (19) 57 (16.1) 35 (9.9)	56 (9.9) 105 (18.6) 98 (17.3) 82 (14.5) 87 (15.4) 81 (14.3) 56 (9.9)	<0.001
Inpatient department Ward Intensive care unit Emergency	108 (73) 32 (21.6) 8 (5.4)	58 (90.6) 4 (6.3) 2 (3.1)	315 (89.2) 10 (2.8) 28 (7.9)	481 (85.1) 46 (8.1) 38 (6.7)	<0.001
Status of pregnancy No Yes	139 (93.9) 9 (6.1)	62 (96.9) 2 (3.1)	341 (96.6) 12 (3.4)	542 (95.9) 23 (4.1)	0.35
Month of Hospitalization January February March April May June July August September October November December	27 (18.2) 3 (20) 7 (4.7) 3 (2) 1 (0.7) 3 (2) 1 (0.7) 0 (0) 4 (2.7) 22 (14.9) 51 (34.5) 26 (17.6)	16 (25) 13 (20.3) 7 (10.9) 7 (10.9) 3 (4.7) 4 (6.3) 3 (4.7) 0 (0) 0 (0) 1 (1.6) 2 (3.1) 8 (12.5)	23 (6.5) 2 (0.6) 0 (0) 1 (0.3) 0 (0) 0 (0) 5 (1.4) 0 (0) 15 (4.2) 77 (21.8) 162 (45.9) 68 (19.3)	66 (11.7) 18 (3.2) 14 (2.5) 11 (1.9) 4 (0.7) 7 (1.2) 9 (1.6 0 (0) 19 (3.4) 100 (17.7) 215 (38.1) 102 (18.1)	<0.001

As shown in Table 2, there was a significant difference among the groups in terms of age range. In the overall patient population, the 30-39 age group had the highest rate of hospitalization due to illness, followed by the 40-49 age group. Although there were fewer patients aged over 80 and under 30 compared to other age groups, the difference was not statistically significant. Contrary to the general trend, the highest incidence of influenza B occurred in the 40-49 age group (42.2%), which showed a significant difference compared to other age groups. The most common age range for H1N1 infection was 60-69 years (p < 0.05).

Among the hospitalized patients, 481 were admitted to the infectious disease or internal medicine departments, while 46 patients were admitted to the ICU due to deterioration in their condition and the need for more intensive care. Additionally, 38 patients were admitted to the emergency department and later discharged. The results showed that out of the 46 patients admitted to the ICU with a diagnosis of influenza, only 10 had H1N1 influenza, 4 had influenza B, and the remaining 32 patients (69%) had influenzalike illness. The data indicated no significant difference between influenza B and H1N1 influenza in terms of overall hospitalization rates (p>0.05). However, patients diagnosed with influenza were significantly more likely to be hospitalized in the general wards (p < 0.05). The highest prevalence of the disease occurred in November. Across the autumn months, the highest rate of hospitalizations due

to influenza was observed. With the onset of winter, the number of cases decreased, reaching its lowest level in August, when no cases of hospitalized influenza were reported. The incidence of negative PCR and H1N1 cases started increasing in September, peaked in November, and then declined. In contrast, influenza B started with a low prevalence in October and reached its peak in January (25%) and February (20%) before declining. H1N1 influenza had the lowest prevalence in the spring, with only one case out of 22 hospitalized patients diagnosed with H1N1; 14 patients had influenza B, and 7 had other illnesses. These findings suggest that in cases of clinical suspicion of influenza during the spring, influenza B should be the primary consideration, followed by other viral diseases, with H1N1 being less likely. Conversely, in autumn, H1N1 should be the top diagnostic consideration, while influenza B is less likely to occur (p < 0.05).

Table 3 presents the comparison of patient frequencies across different groups based on clinical variables. Fever and cough were the most common symptoms in patients with influenza, with prevalence of 58.9% and 57.7%, respectively. Shortness of breath and myalgia were also common, affecting 44.4% and 23.7% of patients. Coryza was present in only 10% of patients. In some cases, digestive symptoms were observed. There was a significant difference among the groups in terms of fever, cough, and shortness of breath, nausea, and vomiting (p < 0.05). A total of 65 patients had underlying conditions based on

Centers for Disease Control and Prevention criteria. The most common condition was hypertension, present in 63 patients, and this showed a significant association with influenza (p < 0.05). Chronic obstructive

pulmonary disease was the second most common underlying condition, affecting 46 hospitalized patients. The frequency of clinical outcomes of patients is shown in Table 4.

Table 3. The comparison of patient frequencies across different groups based on clinical variables

Variables	Negative PCR N (%)	Influenza B virus N (%)	H1N1 influenza N (%)	Total N (%)	P-value
Use of immunosuppressive drugs and corticosteroids No Yes	148 (100) 0 (0)	62 (96.9) 2 (3.1)	353 (100) 0 (0)	563 (99.6) 23 (4.1)	0.6
Underlying disease Hypertension Chronic obstructive Pulmonary disease Malignancy Coronary artery disease Asthma Chronic kidney disease Cerebrovascular accident	11 (7.4)	3 (4.7)	49 (13.9)	63 (11.2)	0.024
	6 (4.1)	6 (9.4)	34 (9.6)	46 (8.1)	0.106
	10 (6.8)	0 (0)	15 (4.2)	25 (4.4)	0.087
	4 (2.7)	0 (0)	21 (5.9)	25 (4.4)	0.051
	3 (20)	0 (0)	15 (4.2)	18 (3.2)	0.132
	3 (20)	2 (3.1)	12 (3.4)	17 (30)	0.713
	4 (2.7)	1 (1.6)	5 (1.4)	10 (1.8)	0.603
Clinical signs Fever Cough Shortness of breath Myalgia Anorexia Headache Sore-throat Diarrhea Abdominal pain Nausea and vomiting Infectious coryza Weakness and lethargy Chest pain	70 (47.3)	43 (67.2)	220 (62.3)	333 (58.9)	0.003
	68 (45.9)	34 (53.1)	224 (63.5)	326 (57.7)	0.001
	52 (35.1)	17 (26.6)	182 (51.6)	251 (44.4)	< 0.001
	35 (23.6)	12 (18.8)	87 (24.6)	134 (23.7)	0.549
	5 (3.4)	5 (7.8)	24 (6.8)	34 (6)	0.277
	16 (10.8)	8 (12.5)	42 (11.9)	66 (11.7)	0.920
	5 (3.4)	6 (9.4)	13 (3.7)	24 (4.2)	0.096
	11 (7.4)	4 (6.3)	31 (8.8)	46 (8.1)	0.741
	5 (3.4)	1 (1.6)	13 (3.7)	19 (3.4)	0.678
	14 (9.5)	12 (18.8)	65 (18.4)	91 (16.1)	0.038
	13 (8.8)	12 (18.8)	32 (9.1)	57 (10.1)	0.05
	20(13.5)	12 (18.8)	67 (19)	99 (17.5)	0.328
	3 (20)	1 (1.6)	13 (3.7)	17 (3)	0.473

Table 4. The frequency of clinical outcomes of patients

Diagnosis of influenza	Clinical outcome	N (%)
	Recovery	536 (94.9)
Based on clinical symptoms	Transfer to another center	3 (0.5)
	Death	26 (4.6)
	Recovery	396 (94.9)
Based on positive PCR	Transfer to another center	1 (0.2)
	Death	20 (4.7)

In Clinical symptoms: of the 565 patients, 536 recovered and were discharged, 3 were transferred to other centers, and 26 died. Among the deceased, 18 had H1N1 influenza, 2 had influenza B, and 5 had other illnesses. Among the H1N1 influenza deaths, 10 were men and 8 were women. No significant difference was observed in clinical outcomes between the three groups or between genders (p> 0.05). Out of 565 patients, 417 had a positive PCR result, and 396 of these patients

recovered and were discharged. One patient was transferred to another center, and 20 died. Among the deaths, 18 were due to H1N1 influenza, and two were due to influenza B. Again, no significant difference in clinical outcomes was found between the three groups or between genders in patients with positive influenza PCR results (p> 0.05). Table 5 shows the mean and standard deviation of laboratory findings.

**Table 5.** The mean and standard deviation of laboratory findings

Laboratory findings		Number	Mean ± SD
White blood cell	Negative PCR	131	9764 ± 595
	Influenza B virus	62	6800± 5749
	H1N1 influenza	349	8134 ± 7924
Lymphocyte (%)	Negative PCR	120	23.16±16.76
	Influenza B virus	56	28.27±18.27
	H1N1 influenza	320	20.24±14.24
Neutrophil (%)	Negative PCR	120	71.26±17.90
	Influenza B virus	56	66.26± 18.60
	H1N1 influenza	320	73.80± 15.59
Platelet	Negative PCR	131	267358±122270
	Influenza B virus	62	206919±110130
	H1N1 influenza	349	226449±115291
Hemoglobin	Negative PCR	131	12.40±2.1
	Influenza B virus	62	11.94±2.04
	H1N1 influenza	346	12.46±2.28
Erythrocyte sedimentation rate	Negative PCR	100	54.34±30.65
	Influenza B virus	56	46.13±30.50
	H1N1 influenza	268	44.23± 29.39
Aspartate aminotransferase	Negative PCR	91	46.04±109.73
	Influenza B virus	48	47.33±47.43
	H1N1 influenza	271	44.58±118.34
Alanine aminotransferase	Negative PCR	91	39.84±85.79
	Influenza B virus	48	39.00±50.09
	H1N1 influenza	270	37.27±91.20
Lactate dehydrogenase	Negative PCR	26	599.08± 564.46
	Influenza B virus	21	537.62± 324.21
	H1N1 influenza	82	549.83± 320.94
Oxygen saturation upon arrival	Negative PCR	26	91.67±3.15
	Influenza B virus	21	92.02±92.02
	H1N1 influenza	82	91.55±3.44
Oxygen saturation upon discharge	Negative PCR	148	95.66±2.82
	Influenza B virus	64	95.49±3.68
	H1N1 influenza	353	95.13±3.26

PCR= Plymerase chain reaction

There was no significant difference in white blood cell (WBC) count between influenza A and negative cases. However, statistical analysis showed that in influenza B cases, the WBC count was significantly lower than in negative cases (p= 0.02). In terms of neutrophil and lymphocyte percentages, the highest percentage of neutrophils was found in influenza A cases (73.80%), while the lowest was in influenza B cases (66.26%). The opposite pattern was observed for lymphocytes. This difference was significant (p= 0.001). However, no significant difference was found between negative and positive cases. Platelet counts in both influenza A and B patients were significantly lower than in patients without influenza (p = 0.002). For the other laboratory parameters, no significant differences were observed between the groups.

#### **Discussion**

Our study shows that out of a total of 565 patients suspected of having influenza, PCR results were positive for 417 patients (73.8%), of which 64 (11.3%) tested positive for the B strain and 353 (62.5%) for the H1N1 strain, with PCR-negative cases accounting for 26.2%. The remaining patients had other illnesses. This result indicates that H1N1 was the most frequent strain detected.

In the study by Ayora-Talavera et al., 53% of the patients were infected with H1N1 [16]. In contrast, a study by Pandita et al. in India found that only 30% of patients were infected with H1N1 [17]. These differences may be due to varying diagnostic criteria, different

prevalence of disease strains, or differing methods and tools used for PCR testing.

Most hospitalized patients were in the age group of 30-39 years. However, for influenza B, the most common age group was 40-49 years (42.2%), which showed a significant difference compared to other age groups. A similar finding was observed in the study by Kim et al., indicating that the average age of influenza B infection was higher [18].

Among the patients hospitalized for influenza B or H1N1, most were admitted to the general ward (about 90%), while PCR-negative patients had a significantly higher rate of ICU admissions (21.6%). These results suggest that when a patient requires hospitalization in the intensive care unit, the likelihood of a disease other than influenza increases.

The most common clinical symptoms of H1N1 were fever, cough, and shortness of breath, which were present in 62.3%, 63.5%, and 51.6% of patients, respectively. Myalgia (24.6%), weakness and lethargy (19%), and nausea and vomiting (18.4%) also ranked next in terms of symptom prevalence. Sore throat was noted in 13 patients (3.7%), and symptoms of coryza were present in 9.1% of patients, making these among the less common symptoms.

In the study by Ling et al., fever (91%) and cough (88%) were the most common symptoms of the disease [19]. However, this study also reported a high prevalence of sore throat (66%) and rhinorrhea (56%) among patients. In the study by Mehta et al., fever (97.7%), cough (86.4%), and shortness of

breath (45.45%) were also the most common symptoms, similar to our findings. However, sore throat was reported as one of the common symptoms of influenza as well [20]. Many other studies have also reported similar symptoms [21-24].

Khandaker et al. assessed the clinical symptoms in patients with H1N1 influenza and reported that cough (84.9%) and fever (84.7%) were the most common symptoms in patients with H1N1 influenza [25]. The study by Kaji et al. also stated that there was no difference in the presence of fever between H1N1 and influenza B [26]. However, this study reported that influenza B cases exhibited more severe gastrointestinal symptoms, whereas our study found no difference between the two types of influenza. In contrast, the research by Kim and colleagues showed that cough, sputum, rhinorrhea, vomiting, diarrhea, and headache were more prevalent in influenza B infections compared to H1N1 infections [18].

In the current study, the lowest level of lymphocytes was observed in patients with H1N1 influenza. Oh et al. compared hematological factors in patients influenza A and B and revealed that the proportion of neutrophils was higher in influenza A than in influenza B infections, although the values were within normal limits for both influenza types [27]. This indicates that neutrophil count alone may not be a definitive marker for distinguishing between influenza types.

Wang et al. studied 150 children with H1N1 who tested positive for the rapid influenza diagnostic test, 152 children with negative

H1N1 tests, and 75 children with influenzalike illness but not H1N1 [28]. They found that combining a low lymphocyte count with a low CRP level in the early stages of illness could help screen for H1N1 in children with falsenegative rapid influenza diagnostic test results, aiding in differential diagnosis.

Wang et al. also examined hematologic markers in H1N1 cases and noted a decrease in lymphocytes in some instances. Additionally, the counts of T lymphocyte subgroups were significantly reduced in the acute phase, dropping to very low levels after a few days before returning to normal during recovery [29]. The researchers concluded that T lymphocyte subgroups could be used as markers to track the progression of H1N1, offering valuable insights for early diagnosis, disease monitoring, and prognosis evaluation. These findings provide important hematologic criteria for improving the accuracy of H1N1 diagnosis and treatment monitoring. Regarding the months of illness onset, significant differences were observed among the groups. The cases of PCR-negative and H1N1 peaked in November, reaching their peak in January (25%) and February (20%) before declining. Influenza H1N1 had the lowest prevalence in the spring months; among 22 hospitalized patients, only one had H1N1, while 14 had influenza B and 7 had other diseases. The study by Kim et al. also reported a higher prevalence of influenza B in the spring [18]. These results suggest that if there is a clinical suspicion of influenza in the spring season, influenza B should be prioritized in the differential diagnosis, with other viral diseases occurring before H1N1. Conversely, in the fall, H1N1 is the primary diagnosis, while influenza B has the lowest likelihood of occurrence. Similar results have been reported in studies conducted in Mexico, Singapore, and Italy [16, 30, 19]. In the study by Kang et al., the highest prevalence of influenza was also reported in November [31]. In contrast, studies in India reported that the highest prevalence of the disease occurred in January, February, and March. This statistical difference may be due to climatic and geographical variations among countries [17, 32, 331.

In our study, 18 PCR-confirmed H1N1 patients died, resulting in a mortality rate of 5.1%, while the remaining patients were discharged after recovery. Among the 18 fatalities, 8 were women and 10 were men. No statistically significant differences in clinical outcomes were observed based on PCR status or gender. In a study conducted by Afzali et al. in Kashan, involving 86 hospitalized patients, the reported mortality rate was 8.1%, with two female and seven male deaths [34]. In comparison, a hospital-based study in India reported a mortality rate of 11%, with 236 confirmed cases and 26 deaths [35]. Lower mortality rates were observed in other countries, including Guatemala (2.7%; 6 deaths among 198 confirmed cases) [36], southeastern Brazil (1.8%) [37], and the United States (7%) [38].

Although the mortality rate observed in our study is lower than that in India and the United States, it remains higher than rates reported in Guatemala and Brazil. This highlights the clinical severity of H1N1 infection in hospitalized patients in our region. Several potential contributing factors may explain this mortality rate, such as delayed healthcare-seeking behavior, underlying comorbidities, or limited access to critical care services.

Nonetheless, the findings underscore the need for improved early detection, timely antiviral therapy, and enhanced preparedness to manage severe H1N1 cases more effectively at the local level.

#### Conclusion

Prominent clinical symptoms and laboratory findings, such as decreased platelet counts and altered neutrophil and lymphocyte ratios, were key in supporting the diagnosis of influenza, especially H1N1. While most patients recovered, the higher mortality among H1N1 cases highlights the importance of early diagnosis, prompt treatment, and seasonal preparedness. In summary, this study can serve as a basis for physicians and public health to understand the clinicalpersonnel epidemiological characteristics of H1N1 influenza cases for analysis, treatment, and the development of preventive strategies in the near future. The fall months are associated with a significant increase in cases, often accompanied by clinical symptoms such as fever, shortness of breath, and cough. In our study, influenza B and H1N1 strains were examined using PCR tests, while other strains, such as H3N2, can also spread during disease outbreaks. Additionally, with the emergence of the COVID-19 virus, which can present symptoms similar to influenza in many cases,

it is recommended that similar studies be conducted, including COVID testing and other influenza strains. Given the potential for recurrent influenza epidemics in the future, further evaluation of various characteristics depending on the type of virus and preparation for them is essential.

#### **Ethical Considerations**

This study was approved by the Ethical Committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.MEDICINE.REC.1402.259).

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None.

#### **Conflict of Interest**

The author declared no conflict of interest.

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### **Authors' Contribution**

F.H, AS.AB, J.A, designed, collected, and edited the manuscript, SA.MA, M.Sh, and Z.A. wrote the draft, edited it, and interpreted the statistical analysis.

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