Biotech Health Sci. 2015 May; 2(2): e28414.

Published online 2015 May 23.

Research Article

Study of the Epidemiological Features and Clinical Manifestations of the Preceding Epidemic of Influenza A (H1N1) as a Guide for Dealing With the 2015 Outbreak in the Qazvin Province, Iran

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Received: March 5, 2015; **Accepted:** April 14, 2015

Background: In 2009, a pandemic associated with a new type of influenza A virus (H1N1) affected many countries worldwide. After five years of silence, in 2015 we encountered another outbreak of H1N1 influenza A.

Objectives: The present study aimed to study the epidemiological and clinical features of this disease in the cold and dry climate of Qazvin province, Iran in the last epidemic, during 2009.

Patients and Methods: This was a cross-sectional study in which the demographic characteristics and clinical manifestations of confirmed cases of influenza A virus (H1N1) in the province of Qazvin were investigated. The definite diagnosis of cases was performed using real time Polymerase Chain Reaction (PCR) on oropharyngeal washing specimens from adults and throat swabs from children and severely ill patients.

Results: During the time course between July to December 2009, 76 confirmed cases of influenza A (H1N1) were discovered in the province of Qazvin. The mean age of patients was 25.67 ± 16.9 years. The most affected people were students and housewives. Coughing was found to be the most common clinical symptom (96.1%) followed by fever (92.1%), myalgia (48.5%), and diarrhea and vomiting (34.2%). In laboratory confirmed patients, 62 were hospitalized and two cases deceased. Regarding the total population of the Qazvin province (1,100,000), the rate of hospitalization was calculated at 5.42 per 100,000 individuals, with a mortality rate of 0.175 per 100,000 individuals (3.2% of hospitalized cases).

Conclusions: Concerning the higher prevalence of disease in younger age groups, and more severe disease in high-risk groups, including overweight patients and pregnant women, the authors recommend special attention to clinical symptoms such as diarrhea and vomiting, cough, myalgia and fever in patients with cold symptoms. Also, for severely ill patients, the allocation of adequate intensive care units should be of prime importance.

Keywords: Influenza A Virus, H1N1 Subtype; Comorbidity; Epidemiology

1. Background

In 2009 the world experienced the emergence of a pandemic associated with a new type of influenza with a genetic arrangement that was never seen in viruses isolated from humans or animal sources (1, 2). By the 20th of September 2009, cases of disease were reported from 191 countries worldwide (3). This virus contains gene segments from human, swine, and avian influenza viruses (4). Although some authorities believe in exaggeration of the extent of danger of the 2009 pandemic flu, later studies did not show evidence of "media over-hyping" in the swine flu pandemic (5). Due to the extremely widespread transmission of the disease in societies, even with extensive sampling, it was impossible to estimate the real number of infected people (6). Therefore, from the July 2009 sampling of all suspected cases was discontinued and samples were taken only from those with severe disease. The first confirmed case of the disease in the province of Qazvin was discovered in July 2009 followed by another 75 laboratory-proven cases within the whole province afterwards. In subsequent years cases of influenza A (H1N1) were scant, yet after five years another outbreak emerged in 2015. The province of Qazvin with a population of 1 143 178 (according to the latest national census) is located on the southern margin of the Alborz mountain ranges within a latitude between 35° 24' and 36° 48' N with a cold and dry climate.

2. Objectives

The present study aimed to evaluate the epidemiological and clinical aspects of this disease during the last epidemic within the cold and dry climate of the Qazvin province.

3. Patients and Methods

This was a cross-sectional study performed in the province of Qazvin during the time course between October and February 2009. Samples were taken from all patients with severe clinical symptoms of influenza who had visited the health centers within the entire province of Qazvin. Specimens were obtained through oropharyngeal gargling of Dulbecco's Modified Eagle's Medium (DMEM) in adult patients, and throat swab in children and severely ill patients. Samples, while in viral transport medium, were transferred to the molecular diagnostic department of the reference laboratory of the province. The samples were kept at -70°C until laboratory analysis. Viral RNA extraction was performed using commercial kits (High Pure Viral RNA, Qiagen and Roche Co, USA). Laboratory diagnosis of the causative agent was performed by Real Time-Polymerase Chain Reaction (RT-PCR) using two step PCR kits (Qiagen and Invitrogen Co, USA) and application of the World Health Organization (WHO) defined sets of primers and probes specific for universal influenza A, swine influenza A and swine H1N1 (sequences are shown in Table 1). Later, using the RT-PCR technique and AB1 7500 instrument, the target sequences were amplified and the Cycle Threshold (CT) value was automatically recorded and the results were qualitatively reported for each set of reactions. Those specimens that gave positive CTs with each set of primers and probes for universal influenza A, swine influenza A, and swine H1N1, were considered as positive. Demographic characteristics and clinical signs of all patients were recorded. For those complaining of dyspnea, pulse oximetry was performed, and arterial oxygen saturation of less than 90%, during the course of the disease, was considered as hypoxemia. Also, the height and weight of patients were measured while the Body Mass Index (BMI) was calculated only for those over 18 years of age, except pregnant women, for whom the BMI was not determined. Descriptive data were presented as frequencies (percentages) for discrete variables and as means (± Standard Deviation) or medians for continuous variables. Univariate analysis of data was performed using the chi-square and Fisher's exact test. A p-value of less than 0.05 was considered statistically significant. Epi Info ™ 3.5.1 software was used for data analyses.

4. Results

From July to December 2009, a total of 518 patients were examined, from whom 76 confirmed cases of pandemic influenza A (H1N1) were discovered (Figure 1). Medical care was provided for patients through ten hospitals within the five cities of the province. During the epidemic, school closures took place in different cities of the province, during the second half of November 2009. The age of patients ranged from two months to 72 years with a median of 23 years and a mean age of 25.67 ± 16.9 years. Of all patients, 30 were allocated to the under 18-year-old group and 46 to the over 18-year-old group. During this epidemic most cases (51 patients) of disease were reported from the city of Qazvin and the least (one patient) from Abyek (Figure 2). The distribution of demographic characteristics, underlying conditions and the clinical symptoms of disease in under 18-year-old and over 18-year-old age groups are shown in Table 2. In the course of disease, 30 patients had hypoxemia (arterial oxygen saturation under 90%). The univariate analysis indicated that the presence of chronic pulmonary diseases (including asthma) and Congestive heart failure (CHF) or valvular heart disease was associated with hypoxemia in the

Table 1. Sequence of Primers and Probes Used for RT-PCR in the Present Study

Primers and Probes	Sequence (5'>3')	Working Concentration, μM
InfA		
Forward	GAC CRA TCC TGT CAC CTC TGA C	40
Reverse	AGG GCA TTY TGG ACA AAK CGT CTA	40
Probe	TGC AGT CCT CGC TCA CTG GGC ACG	10
SW InfA		
Forward	GCA CGG TCA GCA CTT ATY CTR AG	40
Reverse	GTG RGC TGG GTT TTC ATT TGG TC	40
Probe	CYA CTG CAA GCC CA"T" ACA CAC AAG CAG GCA	10
SW H1		
Forward	GTG CTA TAA ACA CCA GCC TYC CA	40
Reverse	CGG GAT ATT CCT TAA TCC TGT RGC	40
Probe	CA GAA TAT ACA "T"CC RGT CAC AAT TGG ARA A	10

course of disease. Also, in our study, BMI was significantly higher in the hypoxemic patients (Table 3). Multiple logistic regression analysis with the mentioned variables, showed a significant association between BMI (OR: 1.21, CI: 1.06-1.38, P = 0.004) and chronic pulmonary diseases (OR: 4.8, CI: 1.01-23.26, P = 0.048), and hypoxemia in the course of Swine-Origin Influenza A (H1N1). Amongst patients with confirmed disease, 62 were hospitalized in whom oseltamivir was administered as antiviral therapy. The reported complications were negligible and limited to nausea and vomiting in two patients. In hospitalized patients, 12 were admitted to intensive care units and 11 needed assisted ventilation. Also, of the 62 hospitalized patients, 60 (86%) were discharged. Two patients (3.2%) died (a 37-year-old man with chronic pulmonary disease and a 39-year-old woman without any known co-morbidity). Considering the total number population within the province, the rate of hospitalization was calculated at 5.42 per 100 000 individuals and that of mortality rate at 0.175 per 100 000 individuals (3.2% of hospitalized cases).

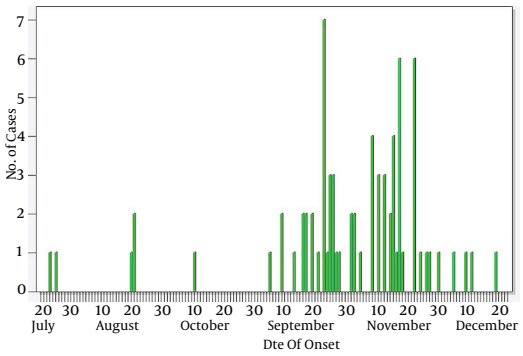

Figure 1. Seasonal Distribution (Epidemiologic Curve) of Confirmed Cases of Human Infection With Swine-Origin Influenza A (H1N1) Virus in the Qazvin Province (July-December 2009)

Figure 2. Regional Distribution of Seventy-Six Confirmed Cases of Human Infection With Swine-Origin Influenza A (H1N1) Virus in the Qazvin Province (July-December 2009)

The figures in brackets indicate the number of cases per 100,000 individuals, based on the total population of each city according to the latest national census in 2007 (incidence in the Qazvin province: 6.648 per 100,000 individuals).

Table 2. Demographic Characteristics, Underlying Conditions and Clinical Manifestations of the Study Population (n = 76) [a, b]

Characteristics	Age Group		P Value
	≤ 18 years	> 18 years	
Occupation			
Worker	0/30 (0.0)	5/45 (11.1)	0.0708
Teacher	0/30 (0.0)	2/45 (4.4)	0.3567
Housekeeper	1/30 (3.3)	18/45 (40.0)	0.0003
Health Care Worker	0/30 (0.0)	2/45 (4.4)	0.3567
Office Employer	0/30 (0.0)	3/45 (6.7)	0.2101
Military	0/30 (0.0)	5/45 (11.1)	0.708
Shop Keeper	0/30 (0.0)	6/45 (13.3)	0.0404
Driver	0/30 (0.0)	2/45 (4.4)	0.03567
Unemployed	29/30 (96.7)	2/45 (4.4)	0.0000
Gender			
Female	10/30 (33.3)	21/46 (45.7)	0.2854
Male	20/30 (66.7)	25/46 (54.3)	0.2854
Comorbidities			
Asthma or chronic pulmonary disease	1/28 (3.6)	11/46 (23.9)	0.0188
CHF or valvular heart disease	3/28 (10.7)	3/46 (6.5)	0.4092
Neurologic and neuromuscular disorders	3/28 (10.7)	1/46 (2.2)	0.1488
DM	0/28 (0.0)	9/46 (19.6)	0.0099
Hypertension	0/30 (0.0)	4/46 (8.7)	0.1272
Pregnancy	0/30 (0.0)	3/46 (6.5)	0.2159
Clinical Manifestations			
Cough	27/30 (90.0)	46/46 (100.0)	0.0577
Fever	28/30 (93.3)	42/46 (91.3)	0.5556
Myalgia	2/20 (10.0)	30/46 (65.2)	0.0000
Diarrhea	9/30 (30.0)	17/46 (36.9)	0.5321

[a] Abbreviations: DM, Diabetes mellitus; CHF, congestive heart failure.
[b] Data are presented as No/total No. (%).

Table 3. Demographic Characteristics and Underlying Medical conditions of Patients According to Hypoxemia [a,b]

Variables	Hypoxemic	Non Hypoxemic	P Value
Continuous Variables			
Age	23.3 ± 16.2	29.3 ± 17.6	0.135
BMI	22.2 ± 27.3	27.2 ± 21.3	0.000
Dichotomous variables			
Gender, Female	13/30 (43.3)	18/46 (39.1)	0.715
Asthma or chronic pulmonary disease	14/30 (46.7)	3/46 (6.5)	0.000
CHF or valvular heart disease	8/30 (26.7)	3/46 (6.5)	0.018
Neurologic and neuromuscular disorders	2/30 (6.7)	2/46 (4.3)	0.517
DM	5/30 (16.7)	4/46 (8.7)	0.243
Hypertension	3/30 (10.0)	1/46 (2.2)	0.166
Pregnancy	1/33 (3.3)	2/46 (4.3)	0.657

[a] Abbreviation: DM, Diabetes mellitus; CHF, congestive heart failure.
[b] Data are presented as No/total No. (%) or Mean ± Standard Deviation (SD).

5. Discussion

When the first epidemic Influenza A virus (H1N1) was reported from Mexico in the beginning of 2009, there were a few known health authorities who could have predicted the emergence of a pandemic. Following numerous cases of H1N1-associated influenza A reported from other countries in the beginning of summer 2009, the WHO, due to widespread human-to-human transmission of the disease at least in two countries, announced the maximum level of pandemic alert (phase 6 pandemic). Gradually, more cases of disease were reported from other countries including Asian and Middle East countries. The rate of confirmed case detection in the Middle East was variable; in our study it was 6.65 per 100,000 individuals. Ahmed et al. reported 18.5 per 100.000 individuals in Abu Dhabi, Emirates (7). In Iran, Afrasiabian et al. reported 10.5 per 100.000 individuals in the Kurdistan province (157 cases in 1 494 000 individuals) (8). In this study most cases were reported from northern regions of the province with cold climates. The possible reason for fewer incidence of disease within the southern regions could be due to a warmer climate, as well as lack of adequate resources in case-finding and laboratory facilities in these regions. In our study, the rate of hospitalization in pandemic influenza A (H1N1) was 5.42 per 100,000 individuals, which was less than reports from many developed countries (9, 10). It is important to note that the rate of hospitalized cases is more related to the sensitivity of the health care system and also the availability of sufficient hospital beds rather than the incidence rate and severity of disease in the community. In contrast to the former strains of influenza, patients with pandemic influenza A (H1N1) were mostly from younger age groups. As reported in some published studies, at the beginning of the epidemic within developed countries, the incidence rate of disease among the age group below 18 years was calculated at up to 60% (1). In the Middle East, Ahmed et al. reported the highest incidence of disease in the 15 - 19 year-old age group amongst 344 confirmed cases in Abu Dhabi, Emirates (7). In the present study, 60.5% of cases were under 25 years old. As shown in Figure 1, the epidemiological curve (seasonal distribution) is indicative of a person-to-person transmission. In an experimental study using laboratory animals, the release of respiratory droplets was confirmed as the principal route of transmission (11). In a human study carried out in south Asia, wet cough was shown to act as an important risk factor for transmission of H1N1-2009 virus from index cases to household contacts (OR: 1.56, CI: 1.22-1.99) (12). In a consultation meeting held in the Eastern Mediterranean region, large particle droplets were introduced as the main route of transmission of the virus (13). Contact with fomites contaminated with respiratory or digestive secretions of patients was also considered as a route of infection (14). Social distancing such as school closures has had a dramatic effect on transmission of pandemic influenza (15). It is possible that school closures in the time interval between 15 and 30 November in different cities of the province had an effect on the decline of the incidence of new cases of influenza, as illustrated in Figure 1. The symptoms of pandemic influenza A (H1N1) were to a great extent similar to those of seasonal flu that occurred during the recent years (16). However, in the studies reviewing the pandemic, the occurrence of gastrointestinal manifestations (diarrhea and vomiting) was emphasized (17). As shown in Table 4, except for the study of Tang et al. (18) incidence of diarrhea was relatively high in the 2009 influenza pandemic. Although most cases with 2009 pandemic influenza were mild and self-limiting, yet, about 0.5% of all cases required assisted ventilation (1). In most previous studies, the majority of patients were children less than five years old, people with chronic cardiopulmonary diseases, and pregnant women. In our study, despite higher co-morbidity rate among hypoxemic patients, the significant difference was only found in those with pulmonary co-morbidity. However, the presence of underlying diseases, in general, was lower (35.5% of hospitalized cases) than that of many other similar studies. In a report by Webb et al. from Australia and New Zealand on hospitalized patients at Intensive Care Units (ICUs) alone, no underlying disease was found in 229 (27.9%) cases (19). Also, in another study by Louie from the USA, there was no underlying disease in 347 patients (32%) out of a total of 1088 individuals (20). In the Eastern Mediterranean region, 75% of admitted patients had underlying medical conditions such as immunosuppression, heart or lung disease (including asthma), diabetes and obesity (13). The lowest number of underlying diseases was reported from Mexico, where the absence of underlying diseases was proved in 10 cases (55.5%) from a total of 18 confirmed hospitalized cases with pandemic influenza (21). In a study on the pediatric age group, asthma and neurologic impairment were the most prevalent underlying risk factors (22). Regarding the role of obesity and being overweight, there are established evidences concerning the place of high BMI in severity of disease (19, 23, 24). In our study, BMI was shown to be an independent risk factor of Hypoxemia in the course of the 2009 pandemic influenza A (H1N1). Considering the susceptibility of pandemic influenza A (H1N1) virus to oseltamivir, as indicated by studies during the past epidemics (1, 2), and the recommendation by the Infectious Diseases Society of America concerning the administration of antiviral drugs for all cases of severe influenza (25), all hospitalized cases in this study were placed under oseltamivir therapy. In this study, similar to some other studies involving hospitalized patients with pandemic influenza A (H1N1) (26), the side effects were mild and scant. The rate of admission to ICUs was reported to be diverse in studies carried out in different countries. In a study by Bishop in Australia, the rate of admission to ICUs was calculated to be around 3.5/100 000 individuals (9). Likewise, in another study by Webb

Table 4. Comparison of Clinical Symptoms and Outcome Measures of the Present Study and Studies From Other Countries [a]

Clinical Symptoms	Present Study	Study Population	Other Articles	References
Fever	70/76 (92.1)			
		Confirmed cases in the United States	371/394 (94.0)	(1)
		Confirmed inpatient cases in China	287/426 (67.4)	(26)
		Confirmed inpatient cases in California (USA)	972/1088 (89.0)	(20)
		Confirmed inpatient cases in Mexico City	18/18 (100.0)	(21)
		Confirmed cases and close contacts in Singapore	434/547 (79.3)	(18)
Cough	73/76 (96.1)			
		Confirmed cases in the United States	365/397(92.0)	(1)
		Confirmed inpatient cases in China	296/426 (69.5)	(26)
		Confirmed inpatient cases in California (USA)	939/1088 (86.0)	(20)
		Confirmed inpatient cases in Mexico City	18/18(100.0)	(21)
		Confirmed cases and close contacts in Singapore	482/547 (88.1)	(18)
Diarrhea	26/76 (34.2)			
		Confirmed cases in the United States	82/323 (25.0)	(1)
		Confirmed inpatient cases in China	12/426 (2.8)	(26)
		Confirmed inpatient cases in California (USA)	215/1088 (20.0)	(20)
		Confirmed inpatient cases in Mexico City	4/18 (22.0)	(21)
		Confirmed cases and close contacts in Singapore	4/547 (0.7)	(18)
Myalgia or arthralgia	32/66 (48.5)			
		Confirmed inpatient cases in China	43/426 (10.1)	(26)
		Confirmed inpatient cases in California (USA)	359/1088 (33.0)	(20)
		Confirmed inpatient cases in Mexico City	8/18 (44.0)	(21)
		Confirmed cases and close contacts in Singapore	111/547 (20.3)	(18)
Outcome Measures				
Mechanical ventilation in hospitalized patients	11/62 (17.7)	Confirmed cases in the United States	4/22 (18.0)	(1)
		Confirmed inpatient cases in the United States	42/260 (16.1)	(3)
		Confirmed inpatient cases in Mexico City	12/18 (66.0)	(21)
		Confirmed inpatient cases in California (USA)	227/915 (25.0)	(20)
		Confirmed pediatric inpatient cases Toronto (Canada	7/58 (12.0)	(22)
ICU admission of hospitalized patients	12/62 (19.4)	Confirmed cases in the United States	8/22 (36.0)	(1)
		Confirmed inpatient cases in California (USA)	340/1088 (31.0)	(20)
		Confirmed pediatric inpatient cases in Toronto (Canada)	12/58(21.0)	(22)
Mechanical ventilation in ICU	11/12 (91.7)	Confirmed cases in the United States	4/8 (50.0)	(1)
		Confirmed ICU cases in Australia and New Zealand	456/706 (64.6)	(19)
		Confirmed inpatient cases in California (USA)	193/297 (65.0)	(20)
		Confirmed inpatient cases in the United States	42/67 (62.7)	(3)
		Confirmed pediatric inpatient cases in Toronto (Canada)	7/12(58.0)	(22)
Mortality in hospitalized patients	2/62 (3.2)	Confirmed cases in the United States	2/36 (5.0)	(1)
		Confirmed inpatient cases in the United States	19/272 (7.0)	(3)
		Confirmed inpatient cases in Mexico City	7/18 (38.8)	(21)
		Confirmed inpatient cases in California (USA)	118/1088 (11.0)	(20)
		Confirmed inpatient cases in China	0/426 (0.0)	(26)
		Confirmed pediatric inpatient cases Toronto (Canada)	0/58 (0.0)	(22)
		Confirmed cases in Menoufia, Egypt	5/125 (4.0)	(27)
Mortality in confirmed cases	2/76 (2.6)	Confirmed cases in the United States	2/642 (0.31)	(1)
		Confirmed cases in Colombia	7/183 (3.8)	(29)
		Confirmed cases in Menoufia, Egypt	5/400 (1.25)	(27)

[a] Data are presented as No/total No. (%).

et al. covering both Australia and New Zealand, the admission rate was reported to be 2.86/100,000 individuals (19). This rate in our study was calculated at 1.497/100,000 individuals. In Table 4, the rate of assisted ventilation, admission to ICU and mortality is compared with the findings of studies reported from different countries. It is important to note that due to the shortage of ICU beds within the province, the number of cases admitted to ICUs was probably less than the real number of cases with the indications of the need for admission to these units in our study. As seen in the Table 4, while the rate of assisted ventilation in hospitalized cases in our study was lower than most similar studies, this rate among the patients admitted to ICUs in the present study was clearly higher than that reported from other countries emphasizing the severity of cases who were admitted to ICUs. According to several studies, published in the beginning of the 2009 pandemic, mortality rate among the hospitalized patients was reported to be high, as demonstrated in a report by Perez-Padilla et al. from Mexico in which 38% of hospitalized cases with confirmed disease at a tertiary center died (21). However, in studies performed later, mortality rates of 4% (27) and 11% (20) in hospitalized patients were reported. Even in some studies such as the study by Cao et al. (26) on 426 confirmed cases (China), Ahmed et al. (7) on 356 confirmed cases (Abu Dhabi Emirates) and Afrasiabian et al. (8) on 157 confirmed cases (Kurdistan, Iran), no case of mortality was reported (7, 26). The mortality rate among the hospitalized patients in the present study was 3.2% and this low mortality rate was likely to be due to the later occurrence of the epidemics, readiness and mobilization of the health care system, and also early administration of oseltamivir. Nevertheless, the possible inadequacy of laboratory coverage and missing cases could not be ruled out. The mortality rate of confirmed cases of our study was calculated as 2.63%, which was higher than that found by other similar studies, such as 1.73% in a study from Mexico (21) and 0.31% in a study from the USA (1). Likewise, the possible reason for this difference is probably due to allocation of sampling only for severely ill people in recent studies. In the Eastern Mediterranean region, the mortality rate in confirmed cases was calculated as 0.174 per 100,000 individuals (1019 deaths in 583,000,000 individuals in 22 countries) (13). In the present study, the mortality rate was 0.175 per 100,000 individuals, which is comparable to figures reported for the entire region. Regarding the prevalence of disease in the younger age group and housewives, considering the clinical symptoms such as diarrhea, vomiting, cough, myalgia and fever amongst these two groups is of prime importance. Moreover, the observation that the severe form of novel influenza was more obvious among patients with higher BMI, a trend not seen in seasonal influenza (28), is of interest and necessitates greater attention for the management of this disease in overweight patients. Meanwhile, allocation of sufficient financial resources to ICUs in developing countries for severely ill patients is of crucial priority.

5.1. Limitations and Recommendations

Regarding the inclusion of only laboratory confirmed cases of pandemic influenza A (H1N1) in the present study, and also the possibility of failure in including all cases of disease in the study due to lack of laboratory test or proper sampling, the results of the current study could not be decisively attributed to all cases of H1N1 influenza. It is proposed to undertake studies in order to determine the reasons for the emergence of the new outbreak of H1N1 influenza A in 2015.

Acknowledgements

We would like to express our gratitude for all the assistance provided throughout the study by the health care personnel of Qazvin University of Medical Sciences.

Authors' Contributions

Study concept and design: Behzad Bijani, Reza Qasemi Barqi and Mohammad Reza Sarokhani. Acquisition of data: Shiva Leghaie, Ebrahim Amini, Mohammad Reza Sarokhani and Behzad Bijani. Analysis and interpretation of data: Behzad Bijani and Mohammad Reza Sarokhani Statistical analysis: Behzad Bijani. Drafting of the manuscript: Ali Asghar Pahlevan, Behzad Bijani, Reza Qasemi-Barqi and Mohammad-Reza Sarokhani.

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