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Epidemiological assessment of influenza (H1N1- A) in patients hospitalized in Tohid hospital in Sanandaj, Iran during 2013-2014

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ABSTRACT

Background: Influenza is an acute respiratory infection caused by influenza virus which is highly contagious and spread easily. Objectives: The aim of this study was to evaluate the epidemiology of influenza (H1N1) type A in patients admitted to Tohid hospital, Sanandaj, Iran during 2013-2014. Material and Methods: This study was descriptive. Data were collected using a questionnaire which was designed based on study goals. The questionnaires were completed via interview, observation and performing clinical tests. After clinical confirmation of influenza by a specialist, genotype was determined by PCR. Results: Of the total 76 cases, 36 cases (48.6%) were male, mean age was 42.7 ± 16.3 and 13.5% were rural and 86.5 were urban dwellers. 52 patient (70.3%) were hospitalized and 22 (29.7%) were outpatients. Travel history was including, abroad 9.5%, domestic 16.2% and without traveling 75.3%. The average time between referring to the doctor and sampling for H1N1 was 2.7 days. The prevalence of influenza (H1N1 A) was 1.4%. Conclusion: The low incidence of H1N1 influenza type A in this study was probably due to travel to infected areas. Considering virus mutation, the readiness of health services to prevent an epidemic of this disease is essential and recommended.

Keywords: Influenza A virus, Respiratory Tract Infections, Epidemiological Studies

INTRODUCTION

Influenza is an acute respiratory infection caused by influenza virus [1]. In the cold season the virus is in epidemic form and infected 5-15% of the population and also is responsible for 250 to 500 thousand deaths annually.Also in case of pandemic it is causing millions of deaths [2].Influenza viruses are enveloped RNA viruses belonging to Orthomyxoviridae family; they are highly contagious and spread fast and easily. influenza viruses Type A are categorized as 17 H (haemagglutinin) and 10 N (Neuraminidase) subtypes which can give rise to many possible combinations (designated as H1N1, H1N2….H2N1, H2N2….H5N1, H5N2…. and so on) [3].The prevalence of influenza H1N1/A in Portugal and Saudi Arabia has been reported as 4.54and 4.28 percent respectively [4, 5].

The virus has emerged as a result of swine infection to common subtypes of influenza type A and simultaneous amplification and genome displacement which its pathogenic characteristics is more severe than other seasonal subtypes [6,7].According to the World Health Organization in a short time after infection, more than 17,700 cases have lost their lives worldwide [8]. It has been reported that six months after the outbreak of H1N1 in 2009 in Iran from 2662 patients who were positive for H1N1 18.2% have died [9]. According to the WHO in 2009 most disease events occurred in teens and young adults, but most have been reported in hospitalized children. Generally, 1 to 10

percent of the cases would be hospitalized [10].Evaluating 400 cases registered in Iran in 2010 the highest incidence was in the age group 15-24 years and in males [11].

H1N1 virus spraed through coughing or sneezing of infected person to another and also exposure to infected respiratory secretions could lead to infection. This virus has high outbreak during the first 5 days and the first 10days in children [12]. The symptoms of H1N1 influenza include fever, cough, body aches, headache, sore throat, weakness, fatigue, nausea and diarrhea [13].

H1N1 flu is sensitive to Amantadine, Oseltamivir, Rimantadine and Zanamivir, but in 2009 to avoid drug resistance, it was supposed to use only both oseltamivir and zanamivir [14]. Given the importance of this disease and observing new cases, the aim of this study was to evaluate the epidemiology of influenza (H1N1) type A in patients admitted to Tohidhospital,sanandaj, Iran during 2013- 2014.

MATERIALS AND METHODS

This study was descriptive and conducted on all patients suspected to influenza (H1N1) type A, who referred to Tohid hospital in Sanandaj, Iran during 2013-2014. Data were collected using a questionnaire which was designed based on study goals. The questionnaires were completed via interview, observation and performing clinical tests. After visiting or referring patients who were suspected to influenza (H1N1) type A demographic data including age, sex, occupation and place of residence was asked. After clinical confirming of the disease by infectious disease specialist 5ml blood was taken from patients in order to culture. Virus genotype was determined by PCR. In order to evaluate the gene sequences of influenza type A virus matrix the following oligonucleotides were designed using amplified PCR:

(M253R)5‘-AGG GCA TTT TGG ACA AAG/T CGT CTA-3’
M52C (5’-CTT CTA ACC GAG GTC GAA ACG-3’)

The collected samples were stored in a transitional environment. The samples were stored at 4 °C. Transferring environment of the samples include balanced saline solution in addition, the following additives all from ICN Zoetermeer Companies, Netherland: Glycerol 10%, 200 units per ml penicillin, 200 ng per ml streptomycin, Polymyxin B sulfate 100 units per ml, 250 ng per mL gentamicin and 50 units per ml Nystatin.

RNA Isolation was performed using RNA extraction kit from (Roche Molecular Biochemicals) with minor modifications to the manufacturer's instructions. 0.2 ml of the sample was homogenized by vortex then the samples were lysed with 4.0 ml of a lysis buffer. After completing the process of connection to the column, the acquired RNA was washed in 50 nl distilled water without nucleases with a temperature of 80 ° C.

PCR: Reverse transcription polymerase chain reaction (RT-PCR) was used. PCRs were optimized according to enzymes, primer set, and concentration as well as periodic parameters. Samples by one-step RT-PCR at the final volume of 25 nl were reproduced containing;50 micromoles hydrochloride Z (with pH 8.5),50 micromoles of sodium chloride,7 micromoles MgCl2,2 micromoles dithiothreitol,1 micro mol nucleoside triphosphate dioxide at a concentration of 1 micromole, oligonucleotides at a concentration of 0.4 nmol,2.5 units of recombinant RNAsin,10 units of reverse transcriptase avian myeloblastic, 2.5 units of Ampli-Taq DNA polymerase and 5 nl RNA. All used enzymes were made by promegabenelux B.V.

Temperature cycles were set in MJ PTC-200 (Mastercycler gradient, eppendorf) based on the following conditions: 30 min at 42 ° C., once 4 minutes at 95 ° C., 40 cycles at 95 ° C for 1 minute, 45 ° C 1 minute and 72 ° C. for 3 minutes. Any reaction was evaluated by agar gel electrophoresis (10 nl per sample) using staining ethidium bromide [22].

Finally, patients were followed up for the outcome of the disease and they recorded in a questionnaire. After collecting data, they were analyzed using statistical software SPSS, Ver.18, and descriptive statistics including; absolute and relative frequency, mean and standard deviation.

RESULTS

The results of this study showed that out of 76 cases, 36 (48.6%) cases were male and 38(51.4%) cases were female. The mean age was 42.7 ± 16.1 with age ranging from 18 to 84 years .In terms of nationality one case was non-Iranian. 13.5% were from rural and 86.5% were from urban areas. 52 (70.3%) patients were hospitalized and 22

(29.7%) patients were outpatients. In terms of traveling history 9.5% had a history of traveling abroad, 16 % a history of travel within the country and 75.3% had no traveling history.

More than 60% of patients had following four symptoms; shivering, joint pain, headache and cough (Table 1). Only three patients (1.4%) had a history of prophylaxis. The average time between goings to the doctor to sampling for evaluating in terms of influenza H1N1 was 2.7 days. Only one case (1.4%) had influenza H1N1type A. The results also showed that 62.2 % of cases had no the history of previous diseases (table 2).

Table 1. Distribution frequency of symptoms in patients

Symptom	No.	%
Shivering	49	64.5
Joint pain	48	63.2
Headache	46	60.5
Cough	42	55.3
Muscular pain	42	55.3
Rhinorrhea	35	46.1
Sore throat	32	42.1
Shortness of breath	31	40.8
Sneezing	30	39.5
Anorexia	28	36.8
Fever	26	34.2
Chest discomfort	24	31.6
Difficult breathing	24	31.6
Hemoptysis	24	31.6
nausea and vomiting	23	30.3
Eye Redness	15	19.7
Diarrhea	14	18.4
Dizziness	9	11.8

Table 2. The history of previous diseases of the cases

The history of previous diseases	No.	%
Chronic heart disease	11	14.8
Chronic lung disease	9	12.2
Chronic Kidney Disease	5	6.8
Chronic Liver Disease	2	2.7
Chronic Anemia	4	5.7
Seizures	2	2.7
Malnutrition	2	2.7
Diabetes	3	4.1
Without The history of previous diseases	46	62.2

DISCUSSION

Type A influenza virus that regularly its genome is changing is cause of flu outbreaks in human populations. A new type of this virus arises when infecting simultaneously at least by two subtypes occurs. This new type requires new vaccines for immunization and new medicines for treatment [15].Infectivity and mortality of influenza type A compared with seasonal flu is higher. In a study in the US mortality of H1N1 type A was five times more than seasonal flu [16].

In this study 48.6% of participants were male and 51.4% were female, which is in consistent with the study by Babamahmoodi et al in Mazandaran, Iran[17], but In a study by Shariati rad et al. 64% of patients were male and 36% were female [18].In our study the mean age was 42.7 years. In a study by Babamahmoodi et al in Mazandaran the mean age was 39.33 years [17] and in a study by Shariati rad et al. it was 37.8 years for men and 32.6 years for women [18].

In the present study the average time between goings to the doctor to sampling for evaluating in terms of influenza H1N1 was 2.7 days and in a study by by Shariati rad et al. it was 4.2 days [18]. The reason perhaps was that the awareness and sensitivity about disease is high and people were able to seek information on disease and its symptoms, therefore they referred to health centers for treatment quickly.

In our study the prevalence of positive cases of H1N1 type A was 1.4% and there were no deaths. In a study by Babamahmoodi et al in Mazandaran, Iran the prevalence of positive cases of H1N1 was 15.04% and mortality was 2.44% [17]. In a study by Shariati rad et al the prevalence of positive cases of H1N1 was 17.8% [18]. The

prevalence of positive cases of H1N1which has been reported by Al-Tawfigh in Saudi Arabia was 28.4% and mortality was 2.13% [4]. In a study by Malveiro in Portugal positive cases have been reported as 54.4% [19] and in a study by Yang et al which was conducted in Taiwan the positive cases of H1N1 were 8.45 with no mortality [20]. The reason for this difference could be related to the type and severity of flu type which perhaps in our study the virulence was less than other studies.

Hospitalization of patients with suspected influenza H1N1had an important role in reducing mortality, therefore, hospitalization of all patients suspected with symptoms of influenza H1N1 is necessary and they would immediately be admitted and hospitalized.

The synchronous rotation of new type of H1N1 type A virus, type A seasonal virus and Avian influenza virus increases the risk of new genetic recombination, therefore the appropriate antibodies does not exist in individuals and the possibility of rapid progression of the disease is high. People awareness and health system readiness particularly in public places, dormitories, schools, universities and military barracks is necessary [21].

CONCLUSION

It can be concluded that the low prevalence of H1N1-flu in this study considering the high age of incidence was probably due to travel to infected areas. Considering virus mutation the readiness of health services to prevent an epidemic of this disease is essential and recommended.

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