

Full Length Research Paper

Clinical and laboratory characteristics of *H1N1* infection in Sulaimani Centre of Infectious Disease during the Global Pandemic in 2010

Mohammed O. Mohammed^{*1}, Kosar M. Ali¹, Ahmed Saed², Ali M. Jabari¹, and Aumaid U. Uthman³

¹School of Medicine /Faculty of medical science/University of Sulaimani/Kurdistan- Iraq

²Internist, Director of Medical Emergency Teaching Hospital Sulaimani.

³Department of Microbiology, college of Veterinary medicine/University of Sulaimani/Kurdistan- Iraq

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The aim of this study was to describe the clinical characteristics, risk factors, laboratory investigations and outcome of hospitalised patients with Influenza A (H1N1) during the Global Pandemic in 2010, at the Centre of Infectious Disease in Sulaimani city. This study conducted between 1st January to 1st March 2010. Patients, who presented with clinical feature of flu and had positive Influenza A rapid test, were included in the study. Also some samples proved to have influenza A (H1N1) by real-time reverse transcriptase polymerase-chain-reaction. Molecular biology examination has been performed at Kurdistan Centre for Strategic studies and scientific researches. A total of 157 patients were enrolled in the study, 71 (45.2%) were males and 86(54.8%) were females. Most of them were from city of Sulaimani. 37% were smoker and 8% were pregnant. Cough and fever were the common presenting symptoms (92.8% and 66.2% respectively). History of contact with a person with recent Flu infection was positive in 21% of patients. Chest X-Rays were abnormal in 28.6% of them. The results had been confirmed by PCR test, 5 patients died in hospital and the death rate was 3.2%. Sulaimani City was part of the H1N1 pandemic in 2010; People in big cities are on high risk of getting this disease. More than half of our patients stayed more than 5 days in hospital, and only 7.6% of the patients had required mechanical ventilation; this was very low compare with other studies, which might reflect early initiation of treat and good management plan in our unit. Single mutation have been detected at amino acid sequence of Haemagglutinin gene 267 changing Isoleucine to Valine, which indicate the possibility of antigenic drift.

Keywords: H1N1, Swine flu, virus Sequencing, Sulaimani.

INTRODUCTION

In June 2009, the World Health Organization (WHO) declared the first human influenza pandemic of the 21st century (Malik et al., 2009).

The outbreak began in Mexico in March 2009 and by

the end of July 2009, more than 168 countries reported confirmed cases of pandemic influenza A (H1N1) 2009, and there were more than 162 380 laboratory-confirmed cases and 1154 deaths (Center for disease control and prevention, August 2009- April 2010; World Health Organization (WHO), 2009).

The currently circulating strain of swine origin influenza virus of the H1N1 strain has undergone triple

*Corresponding Author's E-mail: dr_m_omer@yahoo.com

Table 1. Sequences and locations of Primers used in this study (Mahmood et al., 2010).

Primers	sequences 5'- 3'	position and direction in (H) gene
SW-19f	ATGAAGGCCAATACTAGTAGTTC	19- forward
SW-431f	GTTTCATGGCCCAATCATGAC	431-forward
SW- 1031f	TCCCGTCTATTCAATCTAGA	1031-forward
SW- 541r	GATTTGCTGAGCTTTGGGTA	541-reverse
SW-1101r	AACCGTACCATCCATCTACC	1101-reverse
SW-1721r	TCTCATGCTTCTGAAATCCTAAT	1721-reverse

reassortment and contains genes from the avian, swine and human viruses (Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, 2009; Garten et al., 2009; Dawood et al., 2009; Garten et al., 2009).

The symptoms of 2009 H1N1 influenza were similar to the symptoms of regular human seasonal influenza and included fever, cough, sore throat and myalgia. A feature seen more frequently with 2009 H1N1 influenza was gastrointestinal upset with almost a quarter of patients presenting with vomiting and diarrhea (Malik et al., 2009; Dawood et al., 2009; Garten et al., 2009; Morens et al., 2009; Clinical management of human infection with new influenza A (H1N1) virus, 2010).

Swine flu is presumptively diagnosed clinically and a quick test (for example, nasopharyngeal swab sample) is done to see if the patient is infected with influenza A or B virus. Most of these rapid tests are based on PCR technology. Swine flu is definitively diagnosed by identifying the particular antigens associated with the virus type (Kotsimbos et al., 2010).

The Aim of the study was to evaluate the clinical and laboratory manifestations of the patients with signs and symptoms of flu during the Global outbreak of swine flu from a tertiary care referral institute, and to perform viral sequencing at Kurdistan Center for Strategic studies and scientific researches.

Patients and Methods

A hospital based case series study was conducted on patients with signs and symptoms of flu admitted to Sulaimani infectious unit of General teaching hospital, from January 1st to March 1st 2010. Nasal swabs were taken for rapid test (one-step influenza virus A/B H1N1 standard diagnostic, Bioline) from all suspected cases, and positive results were confirmed by RT-PCR test (light cycler 480 Roche) and also by WHO reference laboratory in Baghdad.

A confirmed case of pandemic H1N1 influenza A is defined as an individual with an influenza-like illness with laboratory-confirmed H1N1 influenza A virus detected by RT-PCR. During this period the clinical profile of H1N1 cases were analysed with reference to age distribution, Gender, time distribution, clinical manifestations, risk factors, complications etc. Detailed physical examination and investigations like complete blood count, renal function test, liver function test, ECG, CXR were done for all patients. Data was analysed using statistical SPSS software and using chi square test.

Nasal swabs were taken for molecular biology examination at Kurdistan Centre for Strategic studies and scientific researches. RNA was extracted from the samples using TRIZOL reagent, cDNA was synthesised using olegodT 15 primers, and the products were amplified using three pairs of primers (Table 1) covering 1702 bp Haemagglutinin gene. The PCR products were sequenced repeatedly, partial sequence of the gene Haemagglutinin was loaded in gene Bank accession no: GU595065 (Mahmood et al., 2010).

RESULTS

A total of 157 patients were admitted to Sulaimani emergency infectious unit with signs and symptoms of upper airway infection and positive rapid test for Influenza H1N1.

Most of the patients were below 40 years of age, and the peak incidence was found among the age group 21-30 years (27.3%), as seen in Figure 1.

Table 2 shows that 71 (45%) patients were males, 86 (55%) were females and 8 (9.3%) patients were pregnant. Patients from inside the city of Sulaimani were 102 (65%), while 55 (35%) were from outside the city.

Most of the Patients were residents, only 13(8.3%) were visitors from outside Iraq, and 63% of the patients were married. Most of the patients 74 (47.1%) were employed, 34 (21.6%) were unemployed, 7 (4.5%) were

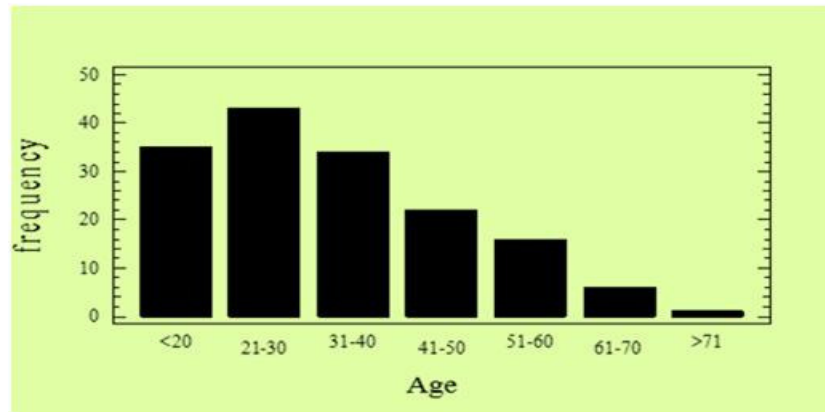


Figure 1. Age distribution of the patients

Table 2. Characteristics of the patients

Characteristic	Value/No (%)
Gender	
Male	71 (45.2%)
Female	86 (54.8%)
Pregnant	8(9.3%)
Non-pregnant	78(90.7%)
Address	
Inside Sulaimani	102 (64.96%)
Outside Sulaimani	55 (35.04%)
Residence	
Resident	144 (91.7%)
Visitor	13 (8.3%)
Marital status	
Married	99 (63%)
Unmarried	58 (37%)
Occupation	
Employed	74 (47.1%)
Unemployed	34 (21.6%)
Health service personnel	7 (4.5%)
Student	42 (26.8%)
Smoking	
Smoker	43 (27.4%)
Non-smoker	114 (72.6%)
Flu contact	
Present	33 (21%)
Absent	124 (79%)
Mode of referral	
Private clinic	33 (21%)
Self-presentation	56 (35.7%)
Acute admission unit	68 (43.3%)

health personnel and 42 (26.8%) were students. The rate of smoking among the patients was 27.4%. Out of those 157 attendants to infection unit, only 33 (21%) patients

had history of contact with other patients with recent flu.

Co-morbidities were found among patients like resp-

Table 3. Clinical characteristics of the patients

	< 40yr n=112 (%)	40-65yr n=40 (%)	> 65yr n=5 (%)	Total n=157 (%)
Co-morbidities				
Asthma	15 (13.4%)	1 (2.5%)	0	16 (10.2%)
COPD	0	9 (22.5%)	3 (60%)	12 (7.6%)
Others	24 (21.4%)	12 (30%)	2 (40%)	38 (24.2%)
None	73 (65.2%)	18 (45%)	0	91 (58%)
Clinical presentation				
Cough	110 (98.2%)	39 (97.5%)	3 (60%)	152 (96.8%)
Sputum	66 (58.9%)	30 (75%)	1 (20%)	97 (61.8%)
Hemoptysis	21 (18.8%)	2 (5%)	1 (20%)	24 (15.3%)
Fever	90 (80.4%)	10 (25%)	4 (80%)	104 (66.2%)
Chills	47 (42%)	0	0	47 (30%)
Dyspnea	59 (52.7%)	27 (67.5%)	5 (100%)	91 (58%)
Headache	54 (48.2%)	7 (17.5%)	2 (40%)	63 (40.1%)
Vomiting	38 (34%)	0	0	38 (24.2%)
Chest pain	39 (34.8%)	0	0	39 (24.8%)
Diarrhea	17 (15%)	3 (7.5%)	1 (20%)	21 (13.3%)
Others	29 (25.9%)	8 (20%)	0	37 (23.5%)
Duration of symptoms				
Less than 1 days	33	0	0	33 (21%)
1-4 days	60	11	0	71 (45.2%)
More than 5 days	19	29	5	53 (33.8%)

Table 4. Vital signs of the patients

	Characteristic	No (%)
Vital signs		
BP	Normal	124 (79%)
	Low	33 (21%)
Pulse	Less than 100 b/min	100 (63.7%)
	More 100 b/min	57 (36.3%)
Respiratory rate	Less 20 /min	111 (70.7%)
	More 20/min	46 (29.3%)
O ₂ saturation	Less 92%	39 (24.8%)
	More 92%	118 (75.2%)
Temperature	< 37.5°C	51 (32.5%)
	> 37.5°C	106 (57.5%)

iratory problems; 16 (10.2%) had asthma and 12 (7.6%) had COPD, and 38 patients had other system co-morbidities like heart failure and renal failure.

The most common presenting symptoms were cough (96.8%) and fever (66.2%). The duration of symptoms, in most of the patients, was between 1-4 days at presentation, Table 3.

Only 33 (21%) patients had low blood pressure and

57 (36.3%) had tachycardia. Respiratory rate was above 20 cycles /minute in 46(29.3%) patients, while oxygen saturation was abnormal in 39 (24.8%) patients. Temperature was high in 106 (67.5%) patients, Table 4.

Only 30 (19%) patients required ventilator support; 18 (11.4 %) in the form of non-invasive, and 12 (7.6%) in the form of invasive (mechanical ventilation).

Figure 2. Deduced amino acid sequence of Haemagglutinin gene of H1N1 virus indicating one single mutation at the position 267aa changing Valine to Isoleucine (underlined) (Mahmood et al., 2010).

"MKAILVLLYTFATANADTLGIGYHANNSTDTVDTVLEKNVTVT
 HSVNLLEDKHNGKLCCKLRGVAPLHLGKCNIAAGWILGNPECESLSTASSWSYIVETSSS
 DNGTCYPGDFIDYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPHAGAKS
 FYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQLYQNADAYVVFV
 GTSRYSKFKFPEAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVIPRYAFAMER
 NAGSGIIISDTPVHDCNTTCQTPKGAINSTLSPFQNIHPITIGKCPKYVKSTKLRLATG
 LRNVPSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKI
 TNKVNVSIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENER
 TLDYHDSNVKNLYEKVRSQKLNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSE
 EAKLNREEIDGVKLESTRIYQILAIYSTVASSLVLVVSGGAISFWMCSNGSLQCRIC"

More than half of the patients stayed more than 5 day in hospital and majority of patients (78%) discharged after recovery, while 5 (3.2%) patients died in hospital.

Amplifying Haemagglutinin gene of influenza A virus using primers specific for H1N1 followed by sequencing showed that 18 patients (8 males and 10 females) were positive. Most of the patients, 17 (94.4%), were from inside the city and only two patients were visitor from outside Iraq. All the patients who died were RT-PCR positive.

Molecular biology examination at Kurdistan Center for Strategic studies and scientific researches has been performed, the nucleotide and the amino acid sequence of Influenza A virus (A/Sulaimani/05/2009(H1N1)) segment 4 hemagglutinin (HA) gene, partial cds, was loaded in GenBank accession no. GU595065.

Total number of 1695 nucleotide was amplified using three sets of primers (Table 1) covering 1695 bp of Haemagglutinin gene. Deduced amino acid sequences of the amplified gene (565 aa) indicate its homology with H1N1 swine flu virus with 99% identity. Analysis of the amino acid sequence indicates, the present of a single mutation at amino acid sequence position 267, changing Valine to Isoleucine (Figure 2). This is the first report of swine flu virus detection in Iraq, with single mutation at 267 aa (Mahmood et al., 2010).

DISCUSSION

A total of 157 cases with suspected Influenza A (H1N1) infection were hospitalised who had positive rapid test for H1N1. Out of these, 18 were confirmed by RT-PCR test.

Seventy one patients (45.2%) were male and 86 (54.8%) patients were female. More than 90% of the

patients were below 65 years of age. These findings are correspondent to a Multicentre Study done in Argentina, which showed that 52% of the cases were females and 48% were males and 92% of the patients were below 65 years of age (Namendys et al., 2010; Kim et al., 2011).

Most of the patients were from the inside city of Sulaimani (65%), and only 8% were visitors from outside Iraq, this might reflect that crowded places like big cities and travelling from and to endemic areas would increase the risk of disease transmission (Jung et al., 2011)

In current study the smoker rate were 27% and 5% of the patients were pregnant, these values are less in comparison with other study results, 37.5 and 8% respectively (Corral et al., 2010).

Nearly 42% of the patients had co-morbidities prior to admission; like (asthma, COPD, heart failure, renal failure, etc.). This rate is less than other studies that showed co-morbidities were higher and ranging from 56-87% in different studies (Mauad et al., 2010; Corral et al., 2010; Kim et al., 2011).

Cough, fever and sputum were common presenting symptoms (96.8%, 66.2%, and 61.8% respectively), which are in concordance with other international studies (Corral et al., 2010; Jung et al., 2011; Patarroyo et al., 2010; Patarroyo et al., 2010).

The median time from onset of illness to hospital admission was 5 days, which is in agreement to a study done in India (5 days) (Chudasama et al., 2012) and 4 days in Argentina (Libster et al., 2010).

Hypotension was recorded in 21% of the patients, pulse rate was above 100 beats /min in 36.3% of patients, while oxygen saturation was abnormal (<92%) in 24.8% of the patients, While other studies recorded higher rate of Hypoxia and tachycardia (Namendys et al., 2010).

All of the patients had CXR and 28.6% had abnormal CXR; (unilateral infiltration, bilateral infiltration, consolidation and rarely pleural effusion), while the CXR finding and lung involvement were more apparent in other studies (Corral et al., 2010; Kharma et al., 2011).

Ventilator support were needed in 30 patients only, 18 patients (11.5%) required non-invasive ventilation and 12 patients (7.6%) required invasive or mechanical ventilation, in other studies ventilatory support applied to 25%, (Corral et al., 2010) and 27% of the patients (Patarroyo et al., 2010; Nikhanj et al., 2010; Abou El Fadl et al., 2012). This might reflect the fact that good earlier treatment decreased the need for mechanical ventilation.

Most of the patients (more than 50%) required more than 5 days hospital stay, 5 patients died in the hospital (3.2%), which is lower than Argentina study 13% (Corral et al., 2010).

Amplification and sequence analysis of Haemagglutinin gene indicate the presence of one single mutation at amino acid position 267 which indicate the possibility of change in virus behaviour. This was for the first time in Iraqi Kurdistan H1N1 swine flu detected and its Haemagglutinin gene analysed, detailed Data are saved in Gene bank (accession no. GU595065) (Mahmood et al., 2010).

Mutations may often be an evolutionary 'dead end' and do not have much significance (Steinhauer, 2010). It is thus important that laboratory surveillance continues to include virus isolation and monitors the circulating viruses antigenically. Concurrent genetic surveillance would facilitate early detection of antigenic sites that are selected for the virus to escape immunological restraint (Mak et al., 2011).

In Conclusion, Sulaimani City was part of the H1N1 pandemic in 2010, this disease affects female more than male. People in Big cities are on high risk of getting this disease. People with co-morbidities at high risk of the disease. Cough and fever were common presenting symptoms in this and other studies. Only 7.6% of the patients' required mechanical ventilation, the death rate was only 3.2%, which is very low in compare with other study. Detection of mutation in Haemagglutinin gene indicates the possibility of antigenic drift in a way that the virus changes its behaviours.

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014 Merit Res. J. Pharm. Pharm. Sci.

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