

Full Length Research Paper

Clinical and laboratory characteristics of *H1N1* infection in Sulaimani during the global pandemic in 2010

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Abstract

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The aim of this study was to describe the clinical characteristics, risk factors, laboratory investigations and outcome of patients with Influenza A (*H1N1*) during the Global Pandemic in 2010, and to perform viral sequencing to see if same global strain of *H1N1* is responsible to the epidemic 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, and proved to have influenza A (*H1N1*) by real-time reverse transcriptase polymerase-chain-reaction(PCR-test) were included in the study. Molecular biology examination of *H1N1* 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. 5 patients died in hospital and the death rate was 3.2%. Single mutation have been detected at amino acid sequence of Haemagglutinin gene 267 changing Valine to Isoleucine. Sulaimani City was part of the *H1N1* pandemic in 2010; only 7.6% of the patients had required mechanical ventilation; this was very low compared with other studies, which might reflect early initiation of treatment and good management plan in our Unit. Single mutation have been detected which indicate the possibility of antigenic drift.

Keywords: *H1N1*, Swine flu, PCR test, virus sequencing.

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 (Martin and Jon, 2009; World Health Organization WHO, 2010).

The currently circulating strain of swine origin influenza virus of the *H1N1* strain has undergone triple reassortment and contains genes from the avian, swine and human viruses (Dawood et al., 2009; Garten et al., 2009; Gupta et al., 2013; Vijaykrishna et al., 2010).

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	TTCATGCTTCTGAAATCCTAAT	1721-reverse

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; Gupta et al., 2013; Vijaykrishna et al., 2010; Morens et al., 2009; Hajjar et al., 2009).

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, and to perform viral sequencing to see if same strain of *H1N1* is responsible to the epidemic in Sulaimani or not?

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. A form designed to register demographic data like age, Gender, address, clinical manifestations, risk factors, complications. Detailed physical examination and

basic investigations, ECG, Chest X ray (CXR) were done for all patients.

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 Oleg odT 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).

Data was analysed using statistical SPSS software, P-value less than 0.05 regarded as statistically significant.

RESULTS

Most of the patients were below 40 years of age, and the peak incidence 43 patients (27.3%), was found among the age group 21-30 years, Figure 1.

Table 2 shows the demographic and epidemiologic data of the participants;

Co-morbidities were found among patients like respiratory 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.

The vital signs of the patients are shown in Table 4.

Forty five (28.6%) patients had abnormal CXR; (unilateral infiltration, bilateral infiltration, consolidation and pleural effusion).

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).

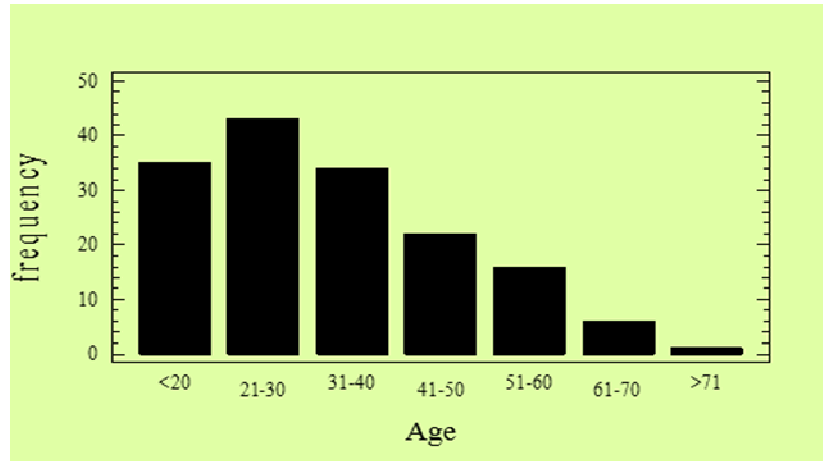


Figure 1. Age distribution of the patients

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%)

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%)

Table 3. Continue

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%)

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.

The nucleotide and the amino acid sequence of Influenza A virus segment 4 hemagglutinin (HA) genes, 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 it's 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

In this study, Seventy one (45.2%) were males and 86(54.8%) were females. More than 90% of the patients were below 65years 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 Chest X ray 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 et al., 2009). 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 that *H1N1* swine flu 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).

CONCLUSION

People with co-morbidities at high risk of the disease. Only 7.6% of patients required mechanical ventilation. The death rate was only 3.2%. 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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