Journal of Virology Research
ISSN: 0976-8785 & E-ISSN: 0976-8793, Volume 3, Issue 1, 2014, pp.-025-031.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000287

ISOLATION AND IDENTIFICATION OF SEASONAL INFLUENZA VIRUS SUBTYPE (H1N1, H3N2) AND TYPE B FROM BLOOD AND NASAL SWAB OF HUMAN IN ALNAJAF (IRAQ) FROM MARCH 2012 TO APRIL 2013

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Received: December 23, 2013; Accepted: January 25, 2014

Abstract- This study was conducted for the first time in AL-Najaf / Iraq, and the study included the preparation of a local inactivated vaccine (whole and subunit). The number of cases infected with seasonal influenza virus was 647 case out of one thousand suspected case. How were distributed into 11 groups. Seasonal influenza virus was detected by three diagnostic methods (plasma pH, rapid test device and real time PCR).

The present study reflected that the most diagnosed cases infected with seasonal influenza virus during the period extended from 03/26/2012 up to 04/30/2013 represented by were type A (H3N2) of (283). Whereas H1N1 (148) case, H3N2+H1N1, (56) case, H1N1 + H3N2 + B were (30) case. While only 130 case infected with type B.

Keywords- real time PCR, hemagglutinin (HA), neuraminidase (NA)

Citation: Alameedy F.M.M., Alkhafaji Y.A. and Alsaadi A.A. (2014) Isolation and Identification of Seasonal Influenza Virus Subtype (H1N1, H3N2) and Type B from Blood and Nasal Swab of Human in Alnajaf (Iraq) from March 2012 to April 2013. Journal of Virology Research, ISSN: 0976-8785 & E-ISSN: 0976-8793, Volume 3, Issue 1, pp.-025-031.

Introduction

Influenza, commonly called "the flu," is an illness caused by RNA viruses that infect the respiratory tract of many animals, birds, and humans. In most people, the infection results in the person getting fever, cough, headache, and malaise; some people also may develop a sore throat, nausea, vomiting, and diarrhea. The majority of individuals has symptoms for about one to two weeks and then recovers with no problems. However, compared with most other viral respiratory infections, such as the common cold, influenza infection can cause a more severe illness with a mortality rate of about 0.1% [1].

Influenza viruses belong to the Orthomyxoviridae family and are divided into types A, B and C. Influenza types A and B are responsible for epidemics of respiratory illness that are often associated with increased rates of hospitalization and death.

All influenza viruses are negative strand RNA viruses with a segmented genome. As proven in [Table-1], influenza type A and B viruses have 8 genes that code for 10 proteins, including the surface proteins haemagglutinin (HA) and neuraminidase (NA), [2,3]. In the case of influenza type A viruses, further subdivision can be made into different subtypes according to differences in these two surface proteins. To date, 17 HA subtypes and 9 NA subtypes have been identified. However, during the 20th century, the only influenza A subtypes that circulated extensively in humans were A(H1N1); A(H1N2); A(H2N2); and A(H3N2). All known subtypes of influenza type A viruses have been isolated from birds and can affect a range of mammal species. As with humans, the number of influenza A subtypes that have been isolated from other mammalian species is limited. Influenza type B viruses almost exclusively infect humans [4,5].

Table 1- Morphological and genetic features of Influenza viruses

Character	Influenza A	Influenza B	Influenza C
Genetic structure	8 segments	8 segments	7 segments
Viral proteins	11 total	11 total	9 total
Unique viral protein	M2	NB	HEF
Antigenic determinants	HA and NA	HA and NA	HA and NA
Genetic change	Antigenic shift and drift	Antigenic drift	Antigentic drift
Host range	Avians, Humans, Swine, Mammals Marine, Horses	Humans	Humans Swine
Human epidemiology	Pandemics and seasonal epidemics	Seasonal epidemics	No seasonality

Influenza virus particles are highly pleiomorphics, mostly spherical with 80-120 nm in diameter [Fig-2]. Present on its surface two distinct glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA), enchased in the phospholipidic envelope by sequences of hydrophobic amino acids localized close to ends COOH (HA), and NH2 (NA), [6].

The HA and NA molecules range from 10 to 12 nm in length, the mean ratio of HA to NA is (5:1). The HA spikes are rod shaped whereas the NA spikes are mushroom shape with stalk. A third membrane protein, the M2 protein, is present in small quantities in influenza A viruses [7].

The influenza genomes (a genome is the complete gene complement of an organism), for Types A and B Influenza virus consist of eight separate, single - stranded RNA segments containing ten genes. Type C contains only seven RNA segments. These RNA segments are coated by helical or spiral nucleo - proteins creating segments sometimes known as ribonucleo proteins (RNPs). Recall that this combination of genome and protein covering is also known as the nucleocapsid [9].

The nucleocapsid of influenza viruses is surrounded by an envelope. Each of the RNA segments has the code for one or more of the viral proteins. [Table-2] provides the current understanding of the influenza virus genes and their functions. The influenza virus is one of very few viruses to have its genome in separate segments. This segmenting of the genome increases the likelihood that new genetic sequences will develop if two different strains of virus infect a cell at the same time. Gene segments from each of the strains may produce new combinations leading to a new strain of influenza virus. On the positive side, laboratory duplication of the genome segments may lead to new vaccine strains to inoculate people against these viral strains [10,11].

Table 2- Structure of The Influenza virus.

Gens of influenza A and their presumed functions	
1 PB2 gene	Codes for an RNA polymerase involved in cap binding (sealing end of molecule), part of transcriptase, which is an enzyme that converts DNA into types of RNA.
2 PB1 gene	Codes for an RNA polymerase involved in elongation of the molecule; part of transcriptase.
3 PA gene	Codes for an polymerase that may serve as a protease; part of transcriptase.
4 HA gene Code for an RNA polymerase involved	Codes for hem agglutinin; three distinct hem agglutinins are found in human infections (H1,H2,H3), at least nine others have been found in animal flu viruses.
5 NP gene	Codes for the nucleoproteins; types A, B and C have different nucleoproteins, part of transcriptase complex.
6 NA gene	Codes for neuraminidase: involved with release of virus from the host cell, two different neuraminidases have been found in human viruses (N1, N2); at least seven others in others animals, e.g., chickens, pigs, ducks.
7 M1 gene M2 gene	Matrix protein, different sections of the genetic code of the gene are read to produce the two proteins that open channels in the cell membrane and allow charged atoms or molecules (ions) to pass through.
8 NS1 gene NS2 gene	Codes for two different nonstructural proteins whose function is still un known, as above, different sections of the code are used for each.

Material and Methods

Samples Collection

One thousand clinical samples were randomly collected from different areas of AL-Najaf province. Primary health centers and hospitals including (Abdul-Sahib Dakeel, AL-Askary, first and the second AL-Wafaa, AL- Carama, AL-Khawla, AL-Salam, AL-Jawad Imam, AL- Hussani Imam, AL-Judida, AL-Abassia, Kunda, Muslim bin Akeel, AL-Shaheed Mahdi Al- Attar, AL- Ansaar, AL- Adal, AL-Moalemeen, AL-Sadr, AL- Hakeem, Middle of Euphrates).Samples were collected during a period extended from 26 March 2012 upto 31 of April 2013.

Population Groups

Studied samples subject groups were distribution into (11) groups which include (pregnant, diabetic, tumor, renal failure, asthmatic, smokers, chronic heart disease, hepatitis, infant and children, healthy adult, vaccinated). This distribution was made depending on their age and clinical status of both gender.

Detection of Influenza Virus

Three different diagnostic procedures were used for detection of influenza virus including plasma pH, rapid device test, real time PCR.

Plasma pH Test

Plasma pH was performed as a guide for influenza virus diagnosis according to [12]. Three ml of sterile double distilled water was placed in each glass sterile screw caped 10 ml test tubes, after that, 0.2 ml of plasma sample was added, then 3 ml of phosphate solution was mixed to gather thoroughly, following that pH of plasma samples were assayed by Hettich pH meter. The calculated values, were recorded. Then 0.12 mL of aqueous solution of acetyl coline ioded was added for each sample thoroughly mixed then tubes were incubated at 37°C for 30 minutes, and pH values were measured as soon as possible after 30 minutes. The second pH reading for each plasma sample was subscribed from first pH-reading and the resulting values were recored and regared as the final pH value.

Rapid Test Device

The CerTest Influenza A+B Card is a one step colored chromatographic immunoassay for the qualitative detection of influenza type A and type B antigens. It can be used directly with nasal swabs or nasal wash or nasal aspirated specimens. Rapid test device was carried according to restriction manual of manufacturing company (CerTest-Spain), [13].

Real Time PCR Technique

RNA Extraction

Viral RNA was extracted by using Viral Nucleic Acid Extraction Kit (Primer Design Ltd Precision™ Viral RNA/DNA extraction kit) following the manufacturer's instructions directly from chicken egg allantoic fluids, virus-infected cell supernatants, plasma, serum, transport media for nasal swab the concentration and the purity of the extracted total RNA were determined by measuring the absorbance ratio at wavelength 260 nm over 280 nm using a spectrophotometer.

cDNA Synthesis

Conversion of RNA isolated from above step to cDNA by the Power cDNA synthesis kit following the manufacturer's instructions directly.

cDNA Amplification

Amplification was carried out in the Laboratory of Veterinary hospital in Najaf. The viral RNA was extracted from 647 positive clinical samples by rapid test device (nasal swabs, throat swabs, plasma, serum, allantoic fluid, amniotic fluid and propagated in cell culture) of population, using the primer design viral RNA kit (primer design UK) in accordance with the manufacturer's instruction successfully amplified. All of the primers for the HA subtypes, NA subtypes was summarized in [Table-3].

Table 3- Primers of seasonal influenza virus

Type /subtype	Gene	Name	Sequences	Bases	PCR product size
Influenza type B Seasonal	HA	HA(B)-1102Fw HA(B)-1226Rv	ATTGCTGGTTTCTTAGAAGG TTGTTTATRGCTTCTTGMGT	20 20	125 bp
Influenza type B Seasonal	NA	NA(B)-916Fw NA(B)-1069Rv	TACACAGCAAAAAGACCC TCCACKCCCTTTRTCCCC	18 18	254 bp
Seasonal H1N1	HA	H1h-678Fw H1h-840Rv	CACCCCAGAAATAGCCAAAA TCCTGATCCAAAGCCTCTAC	20 20	163 bp
Seasonal H1N1	NA	N1h-1134 Fw N1h-1275 Rv	TGGATGGACAGATACCGACA CTCAACCCAGAAGCAAGGTC	20 20	142 bp
Seasonal H3N2	HA	H3h-177Fw H3h-388Rv	GAGCTGGTTCAGAGTTCCTC GTGACCTAAGGGAGGCATAATC	20 22	211 bp
Seasonal H3N2	NA	N2h-1150 Fw N2h-1344 Rv	GTCCAMACCTAAYTCCAA GCCACAAAACACAACAATAC	18 20	194 bp

Melting Curve Analysis

After completion of 45 cycles PCR amplification, the PCR products were melted by raising the temperature from 53ºC to 95ºC at a rate of 1ºC/min. The Exicycler thermal block software displayed the data collected during melt curve analysis as -dF/dTvs Temperature [Fig-1]. As a result melting temperatures were derived from melting peaks by melting curve analysis of the amplified DNA specimens.

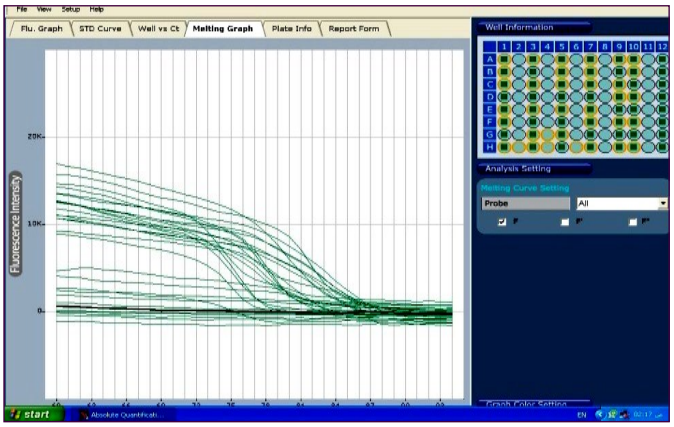

Fig. 1- Melting Temperatures

Results

Influenza Virus Detection

Plasma pH Test

Plasma pH value had been measured in plasma of 1000 influenza virus suspected cases. However 647 case who were detected with rapid test device and real time PCR technique as influenza virus infected cases gave positive results with plasma pH test in which pH values were less than normal (pH 7.15- 7.35) in normal population, in [Table-4].

The study indicated that infected renal failure group gave the lowest mean plasma pH value (6.65) in female and 6.74 in male. Whereas influenza virus infected tumor population group reflecting the highest mean plasma pH value in comparison with other studied infected group. Anyhow the plasma mean pH values extended from 6.65 - 6.77 in female plasma samples, whereas plasma mean pH value in males extended from 6.74 - 6.87.

Rapid Test Device

Of a total one thousand different clinical cases collected, only 647 case were positive influenza virus as detected by rapid test device [Table-5] and [Fig-2].

Table 4- The mean of plasma pH values of studied population groups who were infected with influenza virus in correlation with gender.

Population groups	Case number for each group	pH mean value ± SE	
		Female	Male
Pregnant	65	6.76±0.02	
Diabetic	50	6.72±0.03	6.80±0.02
Tumor	45	6.77±0.02	±0.02 6.87
Renal failure	57	6.65±0.03	6.74±0.02
Asthmatic	30	6.75±0.03	6.78±0.02
Smokers	70	6.68±0.04	6.76±0.03
Chronic heart disease	60	6.73±0.02	6.83±0.02
Hepatitis	15	6.76±0.03	6.81±0.02
Infant & children	80	6.69±0.02	6.86±0.03
healthy adult	135	6.69±0.03	6.84±0.02
vaccinated	40	6.70±0.02	6.78±0.02
Total number	647		

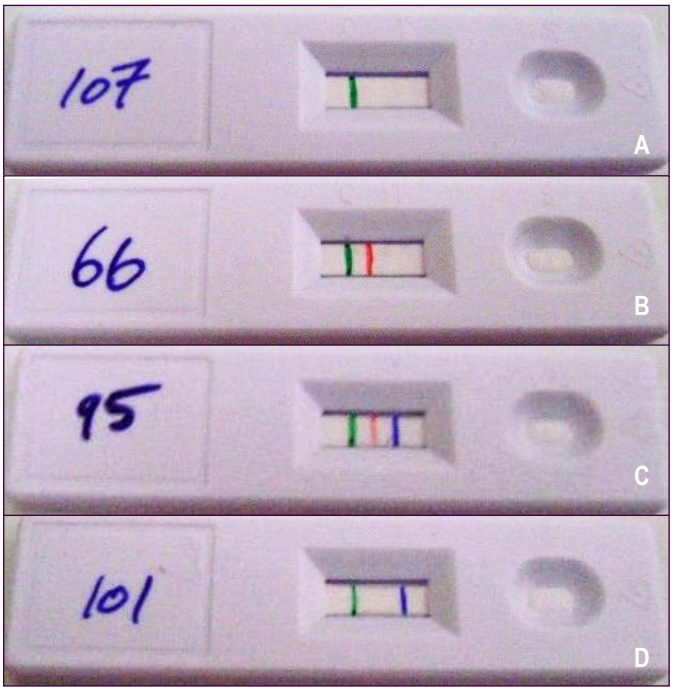

Fig. 2- Rapid test device for detection of seasonal influenza virus.

A: *Negative;* ***B:*** *Positive seasonal influenza virus type A;* ***C:*** *Positive seasonal influenza virus type A + B;* ***D:*** *Positive seasonal influenza virus type B.*

Table 5- Distribution of influenza virus types (A, B and A+B) among different studied population groups.

Type of population groups	Cases of number	%	Type A virus	%	Type B virus	%	Type A+B virus	%
Pregnant	65	10	52	10.6	5	3.8	8	26.7
Diabetic	50	7.7	44	9	2	1.5	4	13.4
Tumor	45	6.9	39	8	6	4.6		
Renal failure	57	8.8	38	7.8	5	3.8	14	46.7
Asthmatic	30	4.6	19	3.9	11	8.4		
Smokers	70	10.8	65	13.3	5	3.8		
Chronic heart disease	60	9.2	44	9	12	9.2	4	13.4
Hepatitis	15	2.3	15	3				
Infant & children	80	12.36	32	6.5	48	36.9		
Healthy adult	135	20.8	123	25.2	12	9.2		
Vaccinated	40	6.1	16	3.2	24	18.4		
Total	647		487		130		30	

The highest number of infected population were healthy adult group (135) representing (20.8%) followed by infant and children group (80) (12.36%), smokers (70) (10.8%), pregnant (65) (10%), whereas hepatitis infected group represented the lowest group of infection (15) (2.3%).

The [Table-5] also appeared that 487 case were infected with type A influenza virus and 130 case of type B, whereas only 30 case were infected with mixed type A+B. However the adult healthy population reflected also the highest percent of type A influenza virus infectivity (25.2%) followed by smoker (13.3%), then pregnant group (10.6%). But diabetic, chronic heart disease infected group and nearly tumor group gave the same percent of infection (9%,9% and 8%) respectively. While hepatitis population group had the lowest percent of type A influenza virus infection (3%).

On the other hand the infant and children group had the highest percent of infectivity with type B influenza virus followed by vaccinated group and adult healthy group then chronic heart disease and asthmatic group (18.4%,9.2%,9.2% and 8.4%) respectively. Pregnant, renal failure and smokers groups gave the same present of type B influenza virus infectivity (3.8%). Yet diabetic group reflected the lowest percent of infecting in the study.

This study also cleared that renal failure group was the highest one infected with mixed type A+B influenza virus infection (46.7%) followed by pregnant group (26.7%) whereas diabetic and chronic heart disease groups gave the same percent of infection with mixed types (13.4%). However tumor, asthmatic, smokers, hepatitis, infant and children, adult healthy and vaccinated groups appeared to be not infected with mixed types A+B influenza virus infection.

Real Time PCR

Of a total 1000 suspected influenza virus infected cases only 647 positive case were detected as influenza virus infected with rapid test device. All the positive cases were undergone diagnosis with real -time - technique. All samples gave positive result with real-time -PCR techniques. They appeared different percent of infection for each group population in correlation with influenza virus type. However adults healthy population group had the highest number of infectivity with H3N2 influenza virus (73) case, which represent (25.7%), but only (8) case of them were infected with mixed H1N1 + H3N2. But no one of this group infected with mixed H3N2 +B type, as well as mixed H1N1+B, yet (12) case of them were infected with type B only (9.23%).On the other hand (41) case of this group reflected infection with H1N1 (27.70%).

Smokers group were represented with (39) case infected by H3N2 (13.78%), eighteen case (12.16%) infected by H1N1, eight case (14.28%) infected by mixed H1N1 + H3N2 and only (5) case (3.84%) were infected by type B influenza virus.

Renal failure group reflected the highest number of infection with mixed H3N2+B (9) out of total (19) case detected (47.36%) and mixed H1N1+B (5) out of total (11) case detected (45.45%).

However the infectivity with type B influenza virus was detected in infant and children group who were represented by (48) case out of (130) total case detected by real time PCR technique represented (36.92%).

Real time PCR technique revealed the same number of infection with influenza virus mixed A type. Pregnant, tumor, smokers and adult healthy groups (8) cases for each represented H1N1+H3N2, (14.28). Whereas renal failure, asthmatic and chronic heart disease group gave (7.14%) infectivity with same mixed subtype H1N1+H3N2. However only (5) case of diabetic subjects were diagnosed as mixed H1N1+H3N2 infected group represented (8.92%). Finally only (2) cases were detected as mixed H1N1+H3N2 influenza virus infected case by real time PCR technique which was represented by (3.57%) in both infant and children group as well as vaccinated group in [Table-6], [Fig-3].

During diagnosis by real time PCR one case positive (epidemic) mixed H1N1+H3N2+B during the that was confirmed by examination at the Central Public Health Laboratory in Baghdad, in addition to the presence of (9) cases of H9N1 in human mixed A and B type influenza virus. Annex (6) describes the difference threshold cycle (C_T) between samples (plasma, nasal and throat swab, fluid of tissue culture fibroblast and chicken egg embryo.

Discussion

Plasma pH Test

Plasma pH test in which pH values were less than normal in all group patients, especially renal failure and tumor patients groups, immunocompromised patients are more susceptible to influenza virus infections because of poor immune response who are not able to combat infection. Examples of immunocompromised people were those with renal failure, tumor, chronic heart disease, hepatitis, asthma and pregnant women, or are undergoing chemotherapy or radiation therapy for cancer. Other conditions, such as certain cancers and genetic disorders, could also cause a person to become immunocompromised. Immunocompromised individuals could sometimes be prone to more serious infections and/or complications than healthy people. These findings are in agreement with Koo [14].

Table 6- Distribution of influenza virus subtypes as detected by real time PCR

population groups	Influenza virus subtypes and its percentage											
	H1N1	%	H3N2	%	H1N1+H3N2	%	H3N2+B	%	H1N1+B	%	Type B	%
pregnant	15	10.13	29	10.24	8	14.28	6	31.57	2	18.18	5	3.84
diabetic	13	8.7	26	9.18	5	8.92	1	5.26	3	27.27	2	1.53
tumor	11	7.43	20	7.06	8	14.28					6	4.61
Renal failure	11	7.43	24	8.48	4	7.14	9	47.36	5	45.45	5	3.84
asthmatic	5	3.37	10	3.53	4	7.14					11	8.46
smokers	18	12.16	39	13.78	8	14.28					5	3.84
chronic heart	13	8.78	27	9.54	4	7.14	3	15.78	1	9.09	12	9.23
hepatitis	4	2.7	8	2.82	3	5.35						
infant & children	11	7.43	19	6.71	2	3.57					48	36.92
healthy adult	41	27.7	73	25.79	8	14.28					12	9.23
Vaccinated	6	4.05	8	2.82	2	3.57					24	18.46
Total	148		283		56		19		11		130	

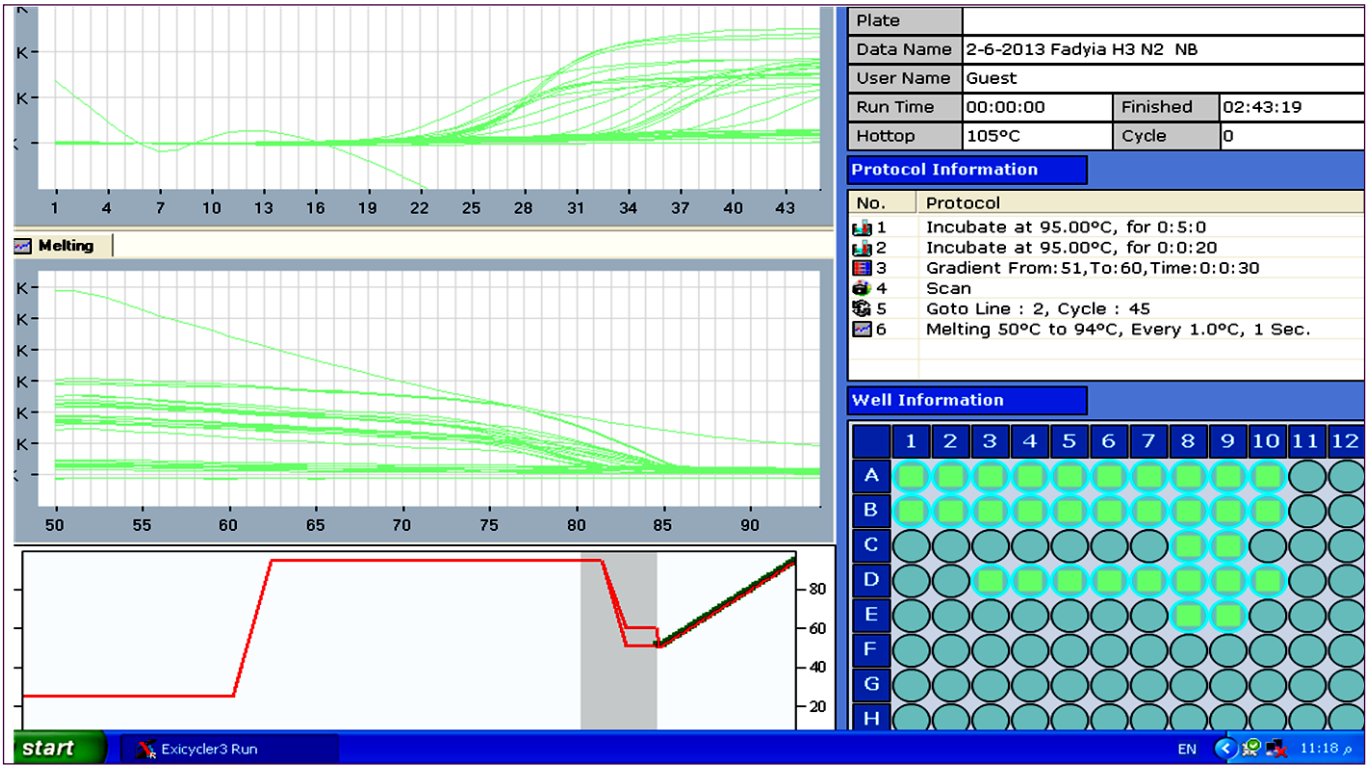

Fig. 3- Detection of seasonal influenza virus subtypes as detected by real time PCR.

Metabolic acidosis is a condition that occurs when the body produces too much acid or when the kidneys are not removing enough acid from the body. If unchecked, metabolic acidosis leads to acidemia, i.e., blood pH is low (less than 7.35) due to increased production of hydrogen ions by the body or the inability of the body to form bicarbonate (HCO3-) in the kidney [15]. Its causes are diverse, and its consequences can be serious, including coma and death. Together with respiratory acidosis, it is one of the two general causes of academia [16]. In most cases, acidosis occurs first for reasons explained below. Free hydrogen ions then diffuse into the blood, lowering the pH [17].

The plasma mean pH values of females are lower than plasma mean pH value of males due to physiological reasons.

Influenza infection lead to lower pH values because, there has been an ongoing effort to develope methods for fusing liposomes with the plasma membrane of living cells. However, although liposomes could be induced to fuse with one another, or with isolated intracellular membranes, liposomes added to cells either adhere or become endocytosed. At the moment, only one method has been indicate to result in controlled and efficient fusion of liposomes with the cell plasma membrane. It makes use of the spike glycoprotein of influenza virus as a fusogen. The spike glycoproteins of a number of enveloped viruses display fusogenic properties at low pH. These viruses normally enter their host cells by endocytosis. In acidic endosome compartment, a fusion reaction is triggered between the viral envelop and the endosomal membrane that delivers the nucleocapsid into the cytoplasm, [18].

Blood pH is regulated to stay within the narrow range of 7.35 to 7.45 in adult, while it is 7.18-7.38 in infants,, making it slightly basic [18]. Blood that has a pH below 7.35 is too acidic, whereas blood pH above 7.45 is too basic. Blood pH is regulated by the partial pressure of oxygen (pO2), partial pressure of carbon dioxide (pCO2), and HCO3- are carefully regulated by a number of homeostatic mechanisms, which exert their influence principally through

the respiratory system and the urinary system in order to control the acid-base balance and respiration. An arterial blood gas test will measure these parameters. Plasma also circulates hormones transmitting their messages to various tissues. The list of normal reference ranges for various blood electrolytes is extensive. Influenza infection was diagnosed through plasma pH for the first time in Iraq 2012-2013.

Rapid Test Device

Rapid influenza diagnostic tests are immunoassays that can identify the presence of influenza A and B viral nucleoprotein antigens in respiratory specimens of influenza by rapid diagnostic test. Potentially the test is of great benefit to the patient and public health. A rapid test is an easy and accurate test performed to diagnose test. A rapid test is performed in the health care practitioner's office. The present results are in agreement with other studies [19,20]. The present study findings are consistent with those of other studies, in the United States of America also indicates the spread of the influenza virus type (A) more than type (B) CDC, (2012). while the studies of the WHO type resulted in blaming A(96%) where as the type B(31%). In Sweden, the percentage of seasonal influenza infection approximately 47.7% of the total 4519 case from different age groups of the type (A) while the type (B) the percentage was 1.6% from 149 case [21]. Seasonal influenza virus infection in AL-Najaf province / Iraq, proved that the type A cause (75.27%), while type B cause (20.09%) while patients with mixed A+B infection comprised (4.63%).

Real Time PCR

These viruses cause the majority of viral respiratory tract infections in healthy adults of seasonal influenza virus type A (H3N2 andH1N1), then type B of infant and children, because the contact with other population also a significant cause of disease in immunocompromised patients. Patients were not reinfected with same viral subtype during period of seasonal influenza virus. However, some were infected with a different viral subtype. The H3N2 viral diagnosis has the highest number than H1N1. A result which is in agreement with [22].

[Table-6], this result is consistent with other studies of (Australasia, South pacific, South east asia, South asia and Africa). The number of infected patients with seasonal influenza virus include H1N1(92), H3N2(150)and B(202) case in Australia out of 2304 examined samples. A study in the South pacific the following results were reported: (H1N1(48), H3N2(69), and B(215) case from a total of 342 samples.

While in South east Asia the following result published (H1N1(5), H3N2(32) and B(23) cases from 721 examined samples. In South Asia a study resulted in the followings: (H1N1(23), H3N2(28)and B (6) case from 102 examined samples while in Africa it was (H1N1 (4), H3N2(12) and B(17) cases from 64 samples during 2012 [22].

The assay was found to be sensitive and specific. Previously, assays using hybridization have been indicate to reduce sensitivity in comparison to a single target PCR. Real-time PCR was found to be more sensitive than cell culture on a range of different respiratory samples, which employed RT-PCR for the detection of viral infections. Conventional respiratory viral cell culture is limited by a lack of speed and therefore has little impact on patient care. Rapid immunological tests partly overcome this problem, but the low sensitivity requires cell culture to be performed on negative specimens.

The real-time PCR generates a diagnostic result within one working day and within a few hours of the rapid antigen detection tests. Elimination of post-PCR processing not only increases the speed but also reduces the risk of cross-contamination.

Real-time PCR applications on respiratory viruses have been described previously. However, new strains of viruses will continue to emerge. Because of the high mutation frequency of RNA viruses and the heterogeneity of the circulating strains, it is theoretically possible that mutations in the primer and probe regions may evolve. The ability to sensitively and rapidly detect both H3 and H1 strains and variants is particularly important as demonstrated by the severity of the most recent 2012/2013 flu season. This season is marked by an early start with a major H3N2 strain that hasn't been seen in circulation for at least five years, making most individuals susceptible to infection. Rapid sub typing is of high clinical value in that during some seasonal influenza virus, determining the subtype at the outset of symptoms can guide early appropriate therapy, helping to decrease morbidity in the population. Additionally, epidemiological surveillance of subtypes is important in determining if the vaccine is sufficient and to evaluate if any antigenic drift has occurred, which would make the vaccine less effective. the assay in the present study with primers detects of (H3, H1 and B) strains, which was determined using database sequence analysisof strains. The result of Central Health Laboratory in Baghdad (CHL-B) gave the following results, the H1N1 percentage was (99.42%) while H3N2 was much less (0.57%). Moreover, the CHL-B did not diagnose any case of type B compared to other subtypes (H1N1and H3N2) because of the different database sequence of primers.

Based on FluNet reporting (as of 10 September 2013), during weeks 34 to 35 (18 August 2013 to 31 August 2013), National Influenza Centres (NICs) and other national influenza laboratories from 69 countries, areas or territories. The result of WHO GISRS laboratories tested more than (21167) specimens. Thousand and five hundred and forty-six (1546) were positive for influenza viruses, of which 1295 (83.8%) were typed as influenza A and 251 (16.2%) as influenza B. Of the sub-typed influenza A viruses, 500 (46.4%) were influenza A(H1N1), 576 (53.5%) were influenza A(H3N2). Of the characterized B viruses, 20 (87%) belong to the B-Yamagata lineage and 3 (13%) to the B-Victoria lineage (WHO, 2013). Variation in the subtype of influenza virus because of human influenza viruses bind glycans containing sialic acid linked α2-6 to the next sugar, and mutations that change the binding specificity might change the host tropism. Viruses isolated in the same season have similar binding specificity profiles but the profiles indicate marked year-to-year variation. None of the 610 glycans on the array (166 sialylatedglycans) bound to all viruses; the closest was Neu5Acα2-6 $(Galβ1\text{-}4GlcNAc)_3$ in either a linear or biantennary form, that bound 42 of the 45 viruses. The earliest human H3N2 viruses preferentially bound short, branched sialylatedglycans while recent viruses bind better to long polylactosamine chains terminating in sialic acid. Viruses isolated in 1996, 2006, 2010 and 2012 bind glycans with α2-3 linked sialic acid. All of these viruses were representative of epidemic strains that spread around the world, so all could infect and transmit between humans with high efficiency. Concluding of the year-to-year variation in receptor binding specificity was a consequence of amino acid sequence changes driven by antigenic drift, and that viruses with quite different binding specificity and avidity are equally fit to infect and transmit in the human population [23]. The temperature gradient feature of the real time PCR system was used to determine the optimal RT temperature. The system was

programmed to perform RT at temperatures ranging from 51-60°C of all primer, but type B has a different optimum ranging from (54-58), followed by a three - step protocol (denaturation, annealing and extension). Threshold cycle (C_T) differenced values of seasonal influenza virus for each H1, H3,N1,N2,NB,HB primer, the natural sample different, whereas plasma and allantoic fluid a best of C_T in comparison with serum, nasal, throat swab and tissue culture.

Whenever value of C_T was lower it mean a high concentration of influenza virus. Selecting Higher RT temperature improves the specificity of the assay but compromises the assay sensitivity, A conclusion agreement with [24].

Conclusions

The following conclusions are extracted from present study: 1-Seasonal influenza virus A (H3N2) is more common than type A (H1N2) and type B. 2-Rapid test device and real time PCR are important in the confirmation for detection of influenza virus. 3-The highest number of infected population are healthy adults compared with other groups.

Conflicts of Interest: None Declared.

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