Original Article

Epidemiological and Molecular Study of Influenza and Parainfluenza Viruses of Respiratory System Infections in Thi-Qar Province, Iraq

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Abstract

The current study is targeted for detection of the some important respiratory pathogen viruses which responsible for respiratory tract infections, Influenza A virus (IAV) and human Parainfluenza viruses (HPIVs) are considered of the causes of respiratory diseases . The current study included the examination of 150 nasal swab samples from the age of one year to the age of 70 years. The collected specimens were kept in viral transport media (VTM). RNA were extracted .The results revealed from this study are summarized in the follows : out of 150 patients suffering from respiratory tract infection only 20(13.3%) were encountered with the H1N1 virus ,23(15.3%) were HPIV1 virus , 11(7.30%) were HPIV2 ,21(14.0%) were HPIV3 .Co-infection of number of viruses appeared in 33(22.0%) patient. The sample are categorized into sex groups were H1N1viruses male 12(8.00%) higher than female 8(5.30%). HPIV1 male 13(8.70%) higher than female 10(6.70%),HPIV2 female 6(4.00%) higher male 5(3.30%), HPIV3 male 11(7.30%) higher female 10(6.70%).The distribution of H1N1virus based on age groups ,and it represents the highest percentage were 4(2.70%) less than 7 years, 3(2.00%) (57-63)years and 4(2.70%)(64-70)years .The distribution of HPIV1,2,3 based on age group , and represents the highest percentage were HPIV1 , 5(3.30%) less than 7 years,4(2.70%)(57-63)years and 5(3.30%)(64-70) years ,HPIV2, 3(2.00%)(less than 7 years) ,HPIV3 ,4(2.70%)(57-63)years ,4(2.70%)(64-70)years. H1N1virus infections in the December, January, February and March months were higher than the April , HPIV1 infections in the December, January and February months was higher than the April and March ,HPIV2 infections in the December, and January months were higher than the February, March and April ,HPIV3 infection in the March and April months were higher than the December, January and February months.

Keywords: Influenza –parainfluenza –PCR –Thi-Qar, Iraq.

INTRODUCTION

The most common disease-causing agents in humans are respiratory viruses, which have a major impact on morbidity and mortality globally, particularly in children. Acute respiratory infections (ARIs) are thought to be a contributing factor in about one-fifth of all juvenile mortality globally) (Boncristiani, 2009) . The three kinds of the influenza virus A, B, and C are regarded as belonging to different genera , The illness is referred to as "flu" in humans, and type A is the most common, leading to influenza disease ; The subtypes of influenza A virus (IAV) are determined by the surface antigens hemagglutinin and neuraminidase (Aufi et al., 2021) .Consequently, viral glycoproteins are crucial to the influenza virus's pathogenicity and pathogenesis (Javanian et al., 2021). All influenza viruses are negative strand RNA viruses with segmented genomes (Alameedy et al., 2014) . Since the global pandemic of 1918 caused by a subtype H1N1 influenza A virus, five genome segments have maintained an unbroken evolutionary history within humans—those encoding the nucleocapsid protein (NP), the matrix proteins (M1 and M2) and the nonstructural proteins (NS1 and NS2), and two encoding polymerase proteins (PB2 and PA) (Rambaut et al., 2008) . Human parainfluenza viruses (HPIVs) which cause respiratory tract infections, are enveloped, negative strand RNA viruses with non-segmented genomes from the Paramyxoviridae family, HPIV1 and HPIV3 are two species that are within the Respirovirus genus, whereas HPIV2 and HPIV4 are under the Rubulavirus genus (Yea et al., 2009). The genomes of all three HPIVs are approximately 15,500 nucleotides in length and encode six common proteins in the invariant order N-P-M-F-HN-L (Schmidt et al., 2011) .These viruses are pleomorphic (Marcink et al., 2020). From filamentous to spherical (Badham & Rossman, 2016).

Material and method

A total of 150 nasopharyngeal swabs were collected from patients were suffering from respiratory tract illness ,the database of them were registered in this study which involved ,date ,age ,gender, address and the major clinical symptoms of patients .

Viral nucleic acid extraction

Viral RNA was extracted from throat swab samples by using EasyPure® Viral DNA/RNA extraction kit (china)

Estimation of extracted RNA

The extracted RNA were estimated by using Nanodrop spectrophotometer that used to measurement the RNA concentration and purity at absorbance 260/280 nm at ratio 1.8 as pure RNA .

Real-Time quantitative PCR (RT-qPCR)

The method is quick and preferable to use when quantifying the amount of amplified product during each PCR cycle .

Statistical Analysis

The statistical analysis produced all samples of study descriptive statistics analyzed by chi-square (p-value 0.01) was considered to be significant .

The Results

According to Influenza Viruses suffered patients as in Table (1) which revealed that 13.3% were caused by H1N1 Virus while diagnostic as 15.3% HPIV1, 7.30% were HPIV2 , 14.0% were HPIV3 on the other hand, there were 22.0% of total 150 influenza viruses patients considered mixed infections , This result showed there was statistical significant differences ($P<0.01$).

Table (1): Distribution of Influenza Viruses Type Detected in Nasal Swab Specimens .

Type of Virus	NO. of Cases					
	Positive		Negative		Total	
	NO	%	NO	%	NO	%
Influenza A (H1N1)	20	13.3	130	86.7	150	100
HPIV 1	23	15.3	127	84.7	150	100
HPIV 2	11	7.30	139	92.7	150	100
HPIV 3	21	14.0	129	86.0	150	100
Mixed Infection	33	22.0	117	78.0	150	100
Pearson Chi-Square	Cal_x^2 : 13.370 *df*: 4 P. value: 0.01 Tab_x^2: 9.490 α: 0.05					

Table (2): Demonstrate of influenza A (H1N1) and parainfluenza (1,2,3) positive in patients group according to gender .

variable	Influenza A (H1N1)	HPIV 1	HPIV 2	HPIV 3
Sex groups	No .of +ve cases	No .of +ve cases	No .of +ve cases	No .of +ve cases
Male	12 (8.0%)	13 (8.70%)	5 (3.30%)	11 (7.30%)
Female	8 (5.30%)	10 (6.70%)	6 (4.0%)	10 (6.70%)
Total	20 (13.3%)	23 (15.3%)	11 (7.30%)	21 (14.0%)

Table (3): Age distribution of influenza A (H1N1) and parainfluenza (1,2,3) positive in patients groups

Variable	Influenza A(H1N1)	HPIV 1	HPIV 2	HPIV 3
Age groups (years)	No .of +ve cases	No .of +ve cases	No .of +ve cases	No .of +ve cases
Less than 7 years	4 (2.70%)	5 (3.30%)	3 (2.0%)	3 (2.0%)
7-14	2 (1.30%)	2 (1.30%)	1 (0.70%)	2 (1.30%)
15-21	1 (0.70%)	1 (0.70%)	0 (0.0%)	1 (0.70%)
22-28	0 (0.0%)	1(0.70%)	1 (0.70%)	0 (0.0%)
29-35	1 (0.70%)	0 (0.0%)	1 (0.70%)	1 (0.70%)
36-42	2 (1.30%)	1 (0.70%)	1 (0.70%)	1 (0.70%)
43-49	1 (0.70%)	1 (0.70%)	1 (0.70%)	2 (1.30%)
50-56	2 (1.30%)	3 (2.00%)	1 (0.70%)	2 (1.30%)
57-63	3 (2.0%)	4 (2.70%)	1 (0.70%)	4 (2.70%)
64-70	4 (2.70%)	5 (3.30%)	1 (0.70%)	4 (2.70%)
Total	20 (13.3%)	23 (15.3%)	11 (7.30%)	21 (14.0%)

Table (4): Distribution of influenza A (H1N1) and parainfluenza viruses (1,2,3) positive in patients groups according to the months of study .

Variable	Influenza A (H1N1)	HPIV 1	HPIV 2	HPIV 3
Month	No .of +ve cases	No .of +ve cases	No .of +ve cases	No .of +ve cases
December	6 (4.0%)	8 (5.30%)	5 (3.30%)	0 (0.0%)
January	4 (2.70%)	7 (4.70%)	4 (2.70%)	0 (0.0%)
February	4 (2.70%)	6 (4.0%)	1 (0.70%)	2 (1.30%)
March	4 (2.70%)	2 (1.30%)	1 (0.70%)	10 (6.70%)
April	2 (1.30%)	0 (0.0%)	0 (0.0%)	9 (6.0%)
Total	20 (13.3%)	23 (15.3%)	11 (7.30%)	21 (14.0%)

Real Time qPCR

RT qPCR for H1N1

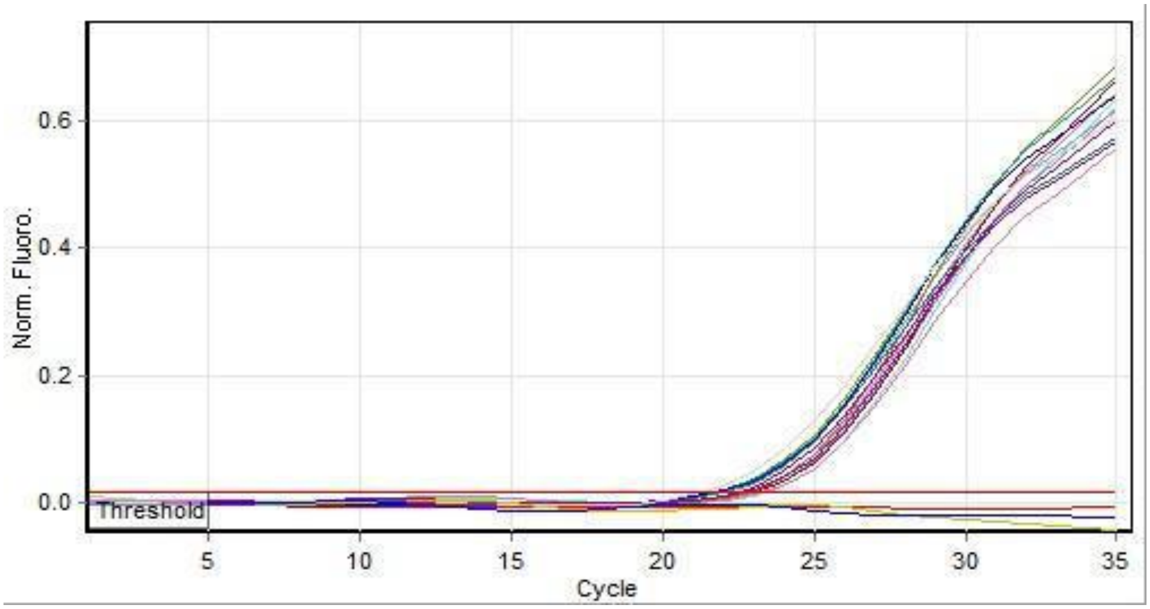

Figure (1): MATRIX (M) amplification plots by qPCR. Lines included 14 samples. The photograph was taken directly from qPCR machine.

RT qPCR for HPIV1

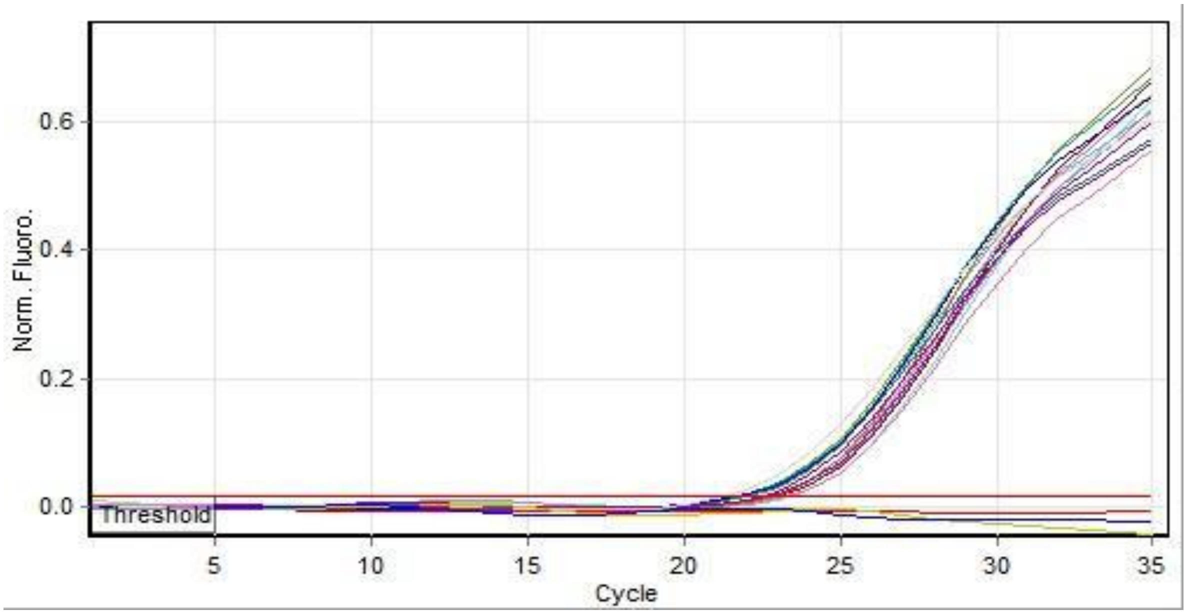

Figure (2): (PI4P1) amplification plots by qPCR. Lines included 14 samples. The photograph was taken directly from qPCR machine

RT qPCR for HPIV2

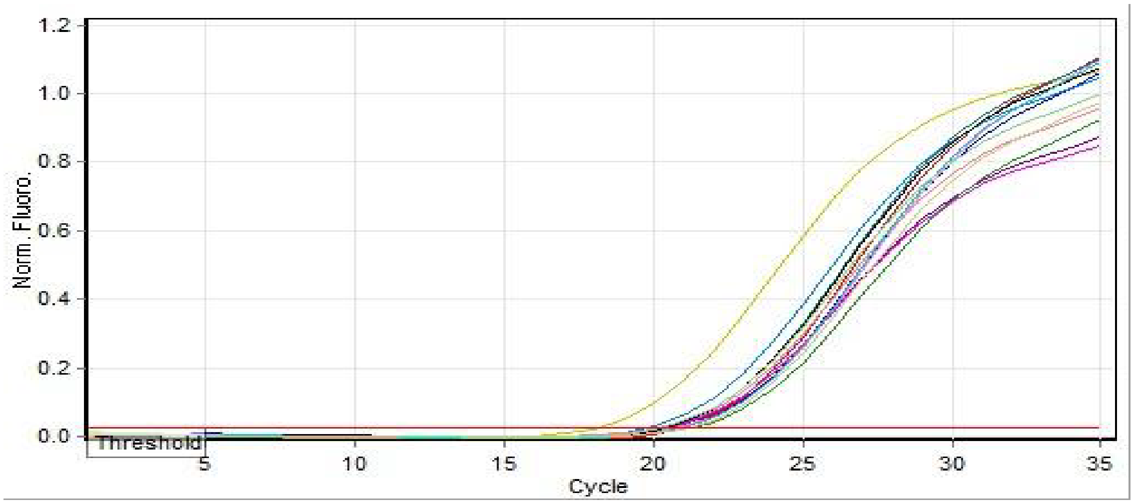

Figure (3): (PI4S1) amplification plots by qPCR. Lines included 14 samples. The photograph was taken directly from qPCR machine .

RT qPCR for HPIV3

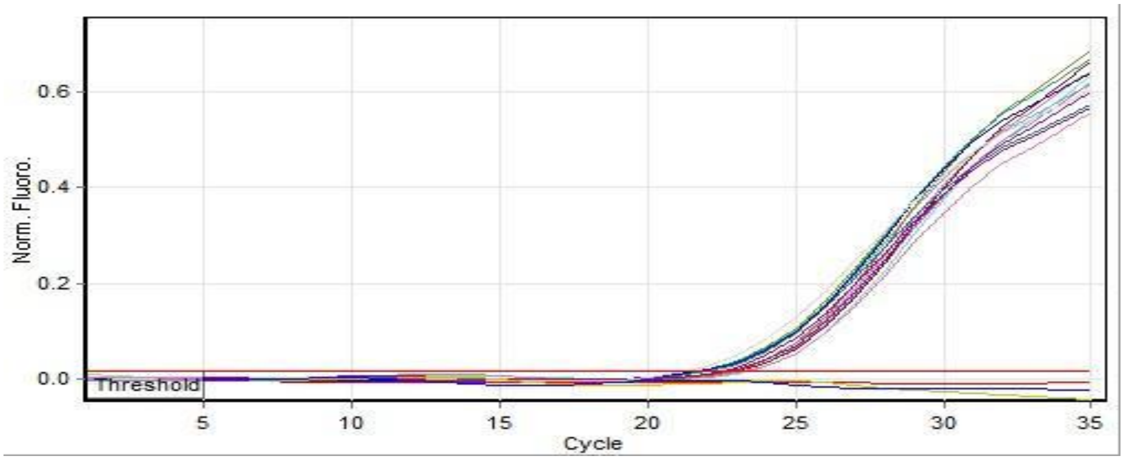

Figure (4): (PI4S1) amplification plots by qPCR. Lines included 14 samples The photograph was taken directly from qPCR machine

Discussion

The current study was aim to determine the infection rate of Influenza A (H1N1) and Parainfluenza (HPIV1,HPIV2,HPIV3)in patients with upper respiratory infection, and their association with demographic and some parameters including age ,gender . Influenza is a contagious respiratory disease and is preventable. Influenza can cause severe complications and death in healthy individuals of all ages (Javanian et al., 2021) . Its distribution varies in different countries and seasons in the current study out of 150 sample , 13.3% are positive with H1N1 virus ,15.3% are positive with HPIV1 ,7.30% are positive with HPIV2 ,14.0% are positive with HPIV3 , there was 22.0% of total 150 influenza viruses patients considered mixed infections . This result is comparable with several studies conducted in different area such as the conducted in study in Georgia in 2004 showed the prevalence rates of 3% and 7% of influenza and parainfluenza infections, respectively (Mirzaei, 2019) . According to a study carried out in Iran (Zahedan City), the prevalence of influenza virus was 81.7%, while no patients infected by parainfluenza viruses were detected (Mirzaei, 2019) . Similar to another study In Iran , In this case-control study, samples were obtained from 50 influenza A (H1N1) from upon evaluation of the 3672 confirmed cases of influenza A (H1N1) (Keshavarz et al., 2019). The mechanisms that determine the differences between genders are complex and can include : hormonal, immunological, behavioral, and genetic factors ; It has been revealed that females generate higher adaptive and innate)immune responses, compared to males ;also, The uneven susceptibility of females and males to infectious diseases has been attributed to mating competition and diet as behavioral and environmental factors (Aufi et al., 2021) .In this study, we found percentage of males 8.00% a higher than females 5.30% .Agree with study conducted in Iraq , the positive H1N1 male percentage (59.99%) was higher than female (39.99%) in different age groups (Mohamed et al., 2016) . In this study ,we found percentage HPIV1 of males 8.70% a higher than females 6.70% . Immunity to HPIVs is incomplete, and infections occur throughout life . HPIVs were isolated with higher frequency from males than females (Liu et al., 2013) . In the study ,we found percentage HPIV2 of females 4.00% a higher than male 3.30% .Don't agree with Some studies show a higher proportion of young male infected by HPIV than young female, and such an incidence of infection becomes similar to the sex as age increases (Gregianini et al., 2019) . In another study, the ratio of males to females infected with the HPIV2 was 3:5 (Rafeek et al., 2021). In this study ,we found percentageHPIV3 of males 7.30% a higher than females 6.70% .In another study, the ratio of males to females infected with the HPIV3 was 19:15 (Rafeek et al., 2021) . Also in another study, the proportion of males(50.0%) is higher than that of females (49.0%) (Fathima et al., 2016). The results of present study revealed there are significant differences between the age groups and the percentage of H1N1virus positive patients with highest value was (2.70%) at both age group of (less than 7 years) and (64-70 years), and the lowest one was (0%) at age group of (22-28 Years) . reported a study at the U.S. Centers for Disease Control and Prevention published in May 2009 found that children had no preexisting immunity to the new strain but that adults, particularly those older than 60, had some degree of immunity(this explain that only one case above 65years) (AL-Ameri et al., 2016) . While in another study , H1N1 infection in female patients have recorder highest incidence in age group 19-40 years (16.07%) followed by (12.54%) in the >40 years age groups (Mohamed et al., 2016) . The results of present study revealed there are significant differences between the age groups and the percentage of HPIV1 positive patients with highest value was (3.30%) at both age group of (less than 7 years) and (64-70 Years), after that (2.70) was age (57- 63 Years) . the lowest one was (0%) at age group of (29-35 Years) . HPIV1 infection was more common in children younger than 4 years old with the higher frequencies in age groups of 1–2 years (1.63%), 2–3 years (1.54%), and 6–12 months (1.52%) (Wang et al., 2015) . The results of present study revealed there are significant differences between the age groups and the percentage of HPIV2 positive patients with highest value was (2.00%) of (Less Than 7 Years) . the lowest one was (0%) at age group of (15-21 Years). While in another study ; Depending on the age group, the prevalence of HPIV types showed significant differences. As patients aged, HPIV2 became increasingly prevalent but the prevalence of hPIV3 decreased (Gu et al., 2020) . The results of present study revealed there are significant differences between the age groups and the percentage of HPIV3 positive patients with highest value was (2.70%) at both age group of (57- 63 Years) and (64-70 Years). the lowest one was (0%) at age group of (22-28 Years) . I agree with who demonstrated that HPIV-3 seropositivity increase with age (Sale et al., 2010) . While in another study HPIV-3 was mainly detected in paediatric patients under 3 years old, while HPIV-1 and HPIV-2 were isolated from a broader age distribution than HPIV-3 (Liu et al., 2013). In this study, the peak of the virus was in the month of December ,February, March, the percentage was for each month (2.70%). In Iranithat prevalence of influenza virus in Iran during thee2015-2016eason peakedi in November and December to similar proportions of circulating seasonal influenza virusesi in most European countries (Mohebbi et al., 2019) . In this study, the HPIV1 peaked in December (5.30%), January(4.70%) and February(4.00%), while it disappeared in the spring months .Seasonal patterns of HPIV infections are distinct among the subtypes and geographic regions. In the USA, HPIV-2 can cause yearly outbreaksoorrbiennial epidemics in co-circulation with HPIV-1 during the fall seasons, whileeHPIV-3 causes yearly spring and summer epidemics (Section et al., 2008). In this study, the HPIV2 peaked in December (3.30%) and January(2.70%), while decreasing in the spring . Agree with study In temperate climates, HPIV-1 and -2 infections occureannually during late fall and early winter (Ren et al., 2011). While in Germany, HPIV-2 detections were observed toobe highest between October and January during the 2016/17 and 2018/19 seasons (Oh et al., 2021).While In Beijing, 2014- 2016: HPIV- 2 in September to December 2014(Pan et al., 2017). In this study, the HPIV3 disappeared in the winter while it reached its peak in March (6.70%) and April (6.00%) .Agree with study , In the USA , HPIV-3 causes yearly spring and summer epidemics (Section et al., 2008). Studies in tropical countriessare scarce, although one investigationrreported seasonal peaks of HPIV3 at the end of winter and in spring in Rio de Janeiro, while other studies carried out in Northeastern and Southern Brazil both showed peaks during October,ccoinciding with spring (Pecchini et al., 2015) . Also, Peak HPIV-3 activity occurred annually eachdspring during April–June (Fry et al., 2006) .

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