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Comparative Study of Influenza Virus and Parainfluenza 3 Virus as Causative Agents of Upper Respiratory System in Thi-Qar Province

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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, 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%). 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 HPIV3 based on age group, and represents the highest percentage were 4(2.70%)(57-63)years, 4(2.70%)(64-70)years. H1N1virus infections in the December, January, February and March months was higher than the April, HPIV3 infection in the March and April months were higher than the December, January and February months. The main clinical features caused by H1N1, rhinorrhea ,fever and cough were reported as the highest frequently,15(75.0%), 10(50.0%), 9(45.0%) respectively ,the other signs were dyspnea 7(35.0%), sneezing 5(25.0%). HPIV3, rhinorrhea was reported as the highest frequently 16(76.2%), the other signs were sneezing 5(23.8%), cough and fever 4(19.0%) for each of them,dyspnea 2(9.50%). In the present study, the highest H1N1infection was recorded in Nasiriyah district 6(30.0%), followed by Al-Dawaya 4(20.0%), recorded in both of SuqAlshyuokh and Al-Shatrah 3(15.0%), recorded in both of Al-Garraf and Al-Rifai 2(10.0%). The highest HPIV3 infections were recorded in both Nasiriyah, Al-Rifai, and Al-Shatrah, 7(33.3%) ,5(23.8%), 4(19.0%) respectively, recorded in both of SuqAlshyuokh, Al-Dawaya 2(9.50%), followed by Al-Garraf 1(4.80%).**

***Keywords*: Influenza, parainfluenza, PCR, Thi-Qar, Iraq**

i. Introduction

Respiratory viruses are the most prevalent disease-causing pathogens in humans *(Boncristiani, 2009)* . Influenza viruses, which are negatively stranded,

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segmented, encapsulated RNA viruses with helical ribonucleocapsids, are members of the Orthomyxoviridae family. *(Nayak et al., 2004)* . There are currently three different varieties of influenza viruses: types A, B, and C. Form A is the most common type to infect people worldwide *(Mozhgani et al., 2018*) . Influenza Seasonal epidemics brought on by a virus (IAV) have a large annual impact on human morbidity and mortality rates as well as the global economy *(Eisfeld et al., 2015)***.** The influenza pandemic of 2009 was brought on by the same virus as the seasonal IAV (H1N1) virus that is now circulating *(Aufi et al., 2020)* . Despite varying in length from 2341 to 890 bases, the eight single-stranded negative-sense vRNA segments of IAV (PB2, PB1, PA, HA, NP, NA, M, and NS) share the same basic structure: a main (*Noda, 2021).* Human parainfluenza viruses (HPIV) types 1- 4 are prevalent respiratory infections that cause both upper and lower respiratory tract disorders, with HPIV-3 being more frequently linked to bronchiolitis, bronchitis, and pneumonia. HPIV-3 typically occurs in the spring and summer *(Maykowski et al., 2018)*. The Family Paramyxoviridae includes enclosed, single-stranded negative sense RNA viruses, which are known as parainfluenza viruses (HPIVs). according to genetic and antigenic diversity Typically, pleomorphic enclosed particles between 150 and 300 nm in diameter make up parainfluenza virions *(Yao & Compans, 2000)* . Their genome, which encodes six proteins, is about 15,500 nucleotides long. The surface glycoproteins hemagglutinin-neuraminidase (HN) and fusion (F) protein are two of these and are important antigenic determinants. The majority of HPIV molecular epidemiology studies to date have used the HN gene as their foundation since it exhibits a high level of genetic variability *(Oh et al., 2021)***.**

ii. 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 .

A. Viral nucleic acid extraction

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

B. 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 **.**

C. 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 **.**

D. 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 **.**

iii. The results

According to Influenza Viruses suffered patients as in Table (1) which revealed that 13.3% were caused by H1N1 Virus **,** 14.0% were HPIV3 **.** 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 : Demonstrate of Influenza A Virus (H1N1) positive in patients group according to gender

Gender	NO. of Cases					
	Positive		Negative		Total	
	NO	%	NO	%	NO	%
Male	12	8.00	138	92.0	150	100
Female	8	5.30	142	94.7	150	100
Total	20	13.3	130	86.7	150	100
Pearson Chi-Square	Cal_x^2 : 0.857 *df*: 1 P.value: 0.355 Tab_x^2: 3.840 α: 0.05					

Table 2 : Demonstrate of HPIV 3 Positive in Patients Group According to Gender

Gender	NO. of Cases					
	Positive		Negative		Total	
	NO	%	NO	%	NO	%
Male	11	7.30	139	92.7	150	100
Female	10	6.70	140	93.3	150	100
Total	21	14.0	129	86.0	150	100
Pearson Chi-Square	Cal_x^2 : 0.051 *df*: 1 P.value: 0.821 Tab_x^2: 3.840 α: 0.05					

Table 3 : Age Distribution of Influenza A Virus (H1N1) positive in Patients Groups

Age Groups	NO. of Cases					
	Positive		Negative		Total	
	NO	%	NO	%	NO	%
less than7 year	4	2.70	146	97.3	150	100
7-14 years	2	1.30	148	98.7	150	100
15-21 years	1	0.70	149	99.3	150	100
22-28 years	0	0.00	150	100	150	100
29-35 years	1	0.70	149	99.3	150	100
36-42 years	2	1.30	148	98.7	150	100
43- 49 years	1	0.70	149	99.3	150	100
50-56 years	2	1.30	148	98.7	150	100
57- 63 years	3	2.00	147	98.0	150	100
64-70 years	4	2.70	146	97.3	150	100
Total	20	13.3	130	86.7	150	100
Pearson Chi-Square	Cal_x^2 : 8.108 *df*: 9 P.value: 0.523 Tab_x^2: 16.92 α: 0.05					

Table 4 : Age Distribution of HPIV3 Positive in Patients Groups

Age Groups	NO. of Cases					
	Positive		Negative		Total	
	NO	%	NO	%	NO	%
less than 7 years	3	2.00	147	98.0	150	100
7-14 years	2	1.30	148	97.7	150	100
15-21 years	1	0.70	149	99.3	150	100
22-28 years	0	0.00	150	100	150	100
29-35 years	1	0.70	149	99.3	150	100
36-42 years	1	0.70	149	99.3	150	100
43- 49 years	2	1.30	148	97.7	150	100
50-56 years	2	1.30	148	97.7	150	100
57- 63 years	4	2.70	146	97.3	150	100
64-70 years	4	2.70	146	97.3	150	100
Total	21	14.0	129	86.0	150	100
Pearson Chi-Square	Cal_x^2 : 8.108 *df*: 9 P.value: 0.523 Tab_x^2: 16.92 α: 0.05					

Table 5: Distribution of Influenza A Virus (H1N1) Positive in Patients Groups According to the Months of Study

Months Groups	NO. of Cases					
	Positive		Negative		Total	
	NO	%	NO	%	NO	%
December	6	4.00	144	96.0	150	100
January	4	2.70	146	97.3	150	100
February	4	2.70	146	97.3	150	100
March	4	2.70	146	97.3	150	100
April	2	1.30	148	98.7	150	100
Total	20	13.3	130	86.7	150	100
Pearson Chi-Square	Cal_x^2 : 2.055 *df*: 4 P.value: 0.726 Tab_x^2: 9.490 α: 0.05					

Table 6 : Distribution of HPIV3 Virus Positive in Patients Groups According to the Months of Study

Months Groups	**NO. of Cases**					
	Positive		**Negative**		**Total**	
	NO	**%**	**NO**	**%**	**NO**	**%**
December	**0**	**0.00**	**150**	**100**	**150**	**100**
January	**0**	**0.00**	**150**	**100**	**150**	**100**
February	**2**	**1.30**	**148**	**98.7**	**150**	**100**
March	**10**	**6.70**	**140**	**93.3**	**150**	**100**
April	**9**	**6.00**	**141**	**94.0**	**150**	**100**
Total	**21**	**14.0**	**129**	**86.0**	**150**	**100**
Pearson Chi-Square	**Cal_x^2 : 23.712 *df*: 4** **P.value: 0.001** **Tab_x^2: 9.490** **α: 0.05**					

Real Time quantitative PCR(RT-qPCR)

RT qPCR for H1N1

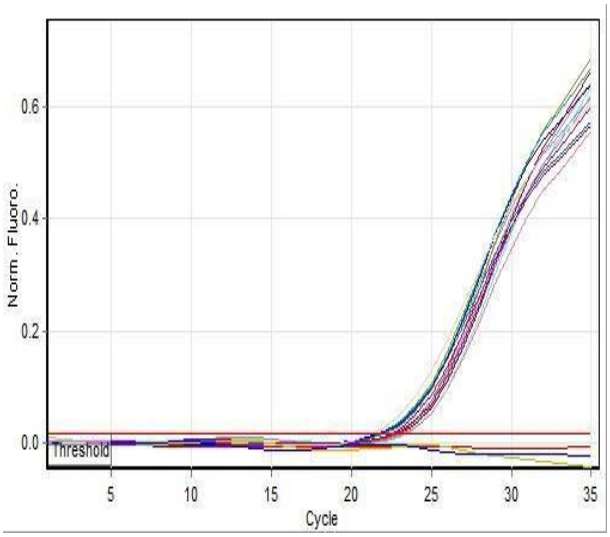

Figure 1 : MATRIX (M) Primer amplification plots by qPCR. Lines included 14 samples. The photograph was taken directly from qPCR machine.

RT qPCR for HPIV3

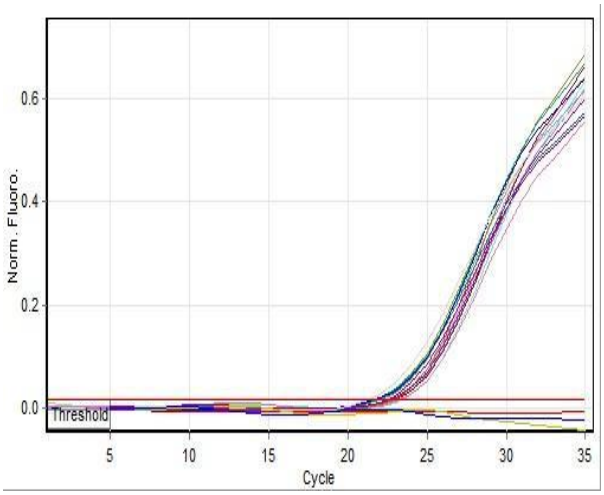

Figure 2: (PI4S1)Primer amplification plots by qPCR. Lines included 14 samples the photograph was taken directly from qPCR machine

iv. Discussion

The current study was aim to determine the infection rate of Influenza A (H1N1) and Parainfluenza HPIV3 in patients with upper respiratory infection, and their association with demographic and some parameters including age ,gender ,and clinical symptoms during the period from December 2021 till April 2022 in Thi-Qar province . In the current study out of 150 sample , 13.3% They were infected with H1N1 virus , 14.0% They were infected with HPIV3, there was 22.0% of total 150 influenza viruses patients considered mixed infections . In this study, we found percentage of males 8.00% a higher than females 5.30% **.** Agree with study In Iraq , Male patients found more vulnerable for endemic Influenza H1N1 infection due to the nature of the Iraqi culture that they are mainly responsible for family finance and support making them more exposed to the infectious agents in large crowded provinces *(Nasser et al., 2015).* Contrasted with a study During the first and second wave of the 2009 H1N1 pandemic, a significant majority of patients hospitalized with severe 2009 H1N1 disease was young adult females (15–49 years of age) (*Klein et al., 2012*) . In this study ,we found percentage 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)* . 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) . which is in accordance with the studies reported from Iran In this study , Where they found most of those infected with the H1N1 virus were than less than 7 years , 64-70 Years . Influenza is contagious in people of all ages and is difficult to be assessed precisely since numerous, if not the majority, of those afflicted, do not require medical attention, and therefore, are not diagnosed (*Aufi et al., 2021)* .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)* . In this study, the peak of the virus was in the month of December ,February, March, the percentage was for each month (2.70%). While in a study conducted in Iraq, Influenza A (H1N1) pdm09 activity start increasing in winter (mainly in January toward the end of February) and disappeared in the rest of the year as in Najaf, Muthanna , Dhi Qar and Basrah *(Mohamed et al., 2016*) . I agree with another study , Influenza peaks were observed during the winter, December to March, which is similar to other northern hemisphere countries *(Elhakim et al., 2020).* We disagree with another study; The reported incidence of influenza A fell at the end of November ; and in December the rates were very low (*Pierangeli et al., 2012).* In this study, the HPIV3 disappeared in the winter while it reached its peak in March (6.70%) and April (6.00%) Also, Peak HPIV-3 activity occurred annually each spring during April–June *(Fry et al., 2006)* . There are generally more cases of hPIV3 in spring and early summer each year *(Gu et al., 2020).* In Korea, HPIV3 was the most prevalent, and outbreaks were observed from late spring to the beginning of summer (April– July) every year *(Park et al., 2018).* All of H1N1 virus positive patients are suffered from Respiratory symptoms. H1N1virus infection has a wide range of clinical manifestations in our study, Rhinorrhea(75.0%) was found to be the predominant symptom followed Fever, (50.0%) ,Cough(45.0%) , Dyspnea(35.0%) , Sneezing(25.0%). I agree with a study conducted in which there was fever, cough, headache, running nose etc. Some cases had difficulty in breathing and required hospitalization *(Mukherjee et al., 2010*) .While , in China , The most common presenting symptoms among the decedents were fever (100%), cough (100%), altered mental status (68%), dyspnea (58%), vomiting (53%), runny nose (42%), coma (26%) and seizures (26%) (*Lu et al., 2018)* . All of HPIV3 positive patients are suffered from Respiratory symptoms. HPIV3 infection has a wide range of clinical manifestations in our

study, Rhinorrhea(76.2%) was found to be the predominant symptom followed , Sneezing(23.8%) Fever, (19.0%) , Cough(19.O%) , Dyspnea(9.50%) . While in some studies the symptoms varied , It was a fever (87.5%) and cough (89.28%) were the most common symptoms. Less common symptoms were earache, vomiting, cyanosis, muscular pain, and wheezing *(Gregianini et al., 2019)*. The study was conducted in Iraq / Thi-Qar province in different cities such as Al-Nasiriyah , Al-Shatrah, Al-Garraf, Al-Rifai, Al-Dawaya, SuqAlshyuokh, The biggest proportion of Influenza A (H1N1) infection were recorded in Al-Nasiriyah . Where the proportions were: Al-Nasiriyah(30.0%), Al-Dawaya(20.0%), Al-Shatrah and SuqAlshyuokh (15.0%) For both of them, It was the lowest in both Al-Garraf and Al-Rifai(10.0%) for both of them. While , The highest prevalence of H1N1 infected people appeared in Diyala province while the least H1N1 prevalence has appeared in Wasit *(Nasser et al., 2015).* Also , In Iran , The influenza A/H1N1 virus caused pandemic flu in 2009 and is still circulating during the winter season in many countries (Mohebbi *et al*., 2019). The study was conducted in Iraq / Thi-Qar province in different cities such as Al-Nasiriyah , Al-Shatrah, Al-Garraf, Al-Rifai, Al-Dawaya, SuqAlshyuokh, The biggest proportion of HPIV3 infection were recorded in Al-Nasiriyah . Where the proportions were: Al-Nasiriyah(33.3%), Al-Rifai(23.8%), Al-Shatrah(19.0%), Al-Dawaya and SuqAlshyuokh (9.50%) For both of them, It was the lowest infection in Al-Garraf(4.80%). In Iran , HPIV-3-positive samples were found in the spring (*Ramezannia et al., 2021)* . In the USA, HPIV-3 causes yearly spring and summer epidemics *(Section et al., 2008)* . Najaf/Iraq HPIV-3 infection occurred in spring and summer *(Khayoon, 2015).*

Conclusions:

According to the previous results the present study concludes the following:

1. Most of the influenza and parainfluenza viruses infections were mixed.
2. The frequency of male patients was higher than females .
3. Age specific frequency in patients caused by IAV (H1N1) and HPIV3 is high in aged (less than 7 years), (57- 63) and (64-70) year .
4. Most of the H1N1 virus infections were in December, January and February months while HPIV3 were in March and April.

v. ACKNOWLEDGMENT

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vi. ETHICAL CONSIDERATION

The ethical permission was obtained from all hospitals and participants in this work (patients and healthy) to conduct the research.

vii. CONFLICT OF INTEREST

The authors declare no conflicts of interest

viii. References

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