AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

The Cause of SARI in Related to COVID and Influenza: A Comparative study

Ahmed Majid Yousif[1], Anfal Mohammed Khudhair[2], Iman M. Aufi[3]

Central Public Health Laboratory, Ministry of Health Department of Public Health, Baghdad, Iraq[1]
Department of Microbiology, College of Medicine, Al-Iraqia University, Baghdad, Iraq[2]
Central Public Health Laboratory, Ministry of Health Department of Public Health, Baghdad, Iraq[3]

Keywords:
SARI, COVID-19 and influenza.

ABSTRACT

The current study aims to determine the main cause of severe respiratory infections (SARI) for Iraqi patients hospitalized in Baghdad hospitals. A total of (249) people who had diagnosis case of severe acute respiratory infection (SARI) and a high temperature. Nasophrangial swab samples were collected from each participant by specific swabs from Sigma Virocult Company in the UK with viral transport medium according to Bradford and Slavin (1940), and stored frozen at -70°C until used for RNA extraction and polymerase chain reaction to detect human influenza and SARS-CoV-2 among others. All samples were tested in the Central Public Health Laboratory (CPHL) at National Influenza Center (NIC), during the period of study. For total SARI patients in current study, When Out of 249 investigated records of patients, 13 (5.2%; 95% CI: 2.4 – 8.0) found to had a positve influnza A (H3N2) results. Wherase, 65 (26.1%; 95% CI: 20.9 – 31.7) found to had a positve positve PCR result. On the other hand, zero perecent out of 249 investigated templates identifed to have a influnza B positive results. Through the current study, we conclude that most cases of severe respiratory infection in Iraq are cause by COVID-19.

1. Introduction

The second-leading cause of years of life lost worldwide across all ages is respiratory infections, which are also the main cause of death in children under the age of five [1]. The Severe Acute Respiratory Infection (SARI) case definition was developed by the World Health Organization (WHO) in 2011 in an effort to standardize global surveillance of influenza-related hospitalizations, enabling national health authorities to interpret their data from a global perspective [2].

The clinical presentation of flu is frequently difficult to distinguish from that of other respiratory viruses, making it particularly challenging to monitor [3]. In addition to influenza viruses, non-influenza respiratory viruses with zoonotic potential constitute a substantial new hazard to countries where large populations of people and animals mix [2]. For instance, coronaviruses are susceptible to interspecies transmission [3],

which has led to widespread human respiratory illness outbreaks including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and most recently, coronavirus. 2019 illness (COVID-19) [4]. It should be noted that the severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and current coronavirus disease (COVID-19) pandemics were all brought on by the zoonotic spread of highly pathogenic coronaviruses, specifically SARS CoV, MERS CoV, and SARS CoV 2, respectively. These epidemics occurred in 2002 to 2003 and 2012, respectively, and are now being blamed on zoonotic transmission [6], [7]. The illnesses brought on by these different viruses range from self-resolving upper respiratory tract infections like the common cold, sore throat, or laryngo-tracheobronchitis to potentially fatal lower respiratory tract infections like bronchiolitis, pneumonia, or acute respiratory distress syndrome (ARDS), as well as sporadically disseminated illnesses.

One of the following three mechanisms can result in the involvement of the lower respiratory tract: (i) direct infection of lung cells without sustained virological replication in the upper respiratory tract (as with some influenza viruses that prefer the sialic acids of the deeper airways), (ii) contiguous spread from upper respiratory tract viral infections (e.g., coronaviruses), and (iii) hematogenous spread [9]. The severity of each infection and the potential progression from the upper to the lower respiratory tract depend on the intrinsic virulence of the causing virus, on potential coinfections, and on patient-related factors like age, underlying illnesses (such as concurrent respiratory and cardiovascular comorbidities), or degree of immunodeficiency [10]. Coronavirus infection is more contagious in 2019 than influenza but less contagious than measles. It also spreads in clusters, where infections can be linked to a reference case or specific geographic area [9]. A significant influence is exerted by "super-spreading episodes," in which a single person infects a large number of people [10], [11]. A person who is infected can transmit the virus to others up to two days before their own symptoms show up. Even if no symptoms develop, a person can still be contagious for up to two weeks in severe instances, even if no symptoms do [12]. The third highly pathogenic human coronavirus disease to date is COVID-19. Although less deadly than SARS and MERS, the fast spread of this extremely contagious illness has posed the greatest threat to global health during this century. It's likely that the SARS-CoV-2 pandemic will be around and coexist with people for a very long time [15].

The current study aim to clarify the co-infection of influenza and SARS-CoV2 in SARI patients.

2. Methodology

A cross-sectional study was carried out after receiving ethical approval from the College of Medicine-University of Al-Iraqia. From October 2021 to March 2022, the study population included 249 people who Diagnosed cases of severe acute respiratory infection (SARI) and a with severe clinical symptoms. Each patient's information was entered into a specially constructed questionnaire format, which included their name, gender, age, residence, asthma history, nasal discharge, kind of respiratory infection, Nasophrangial swab samples were collected from each participant by specific swabs from Sigma Virocult Company in the UK with viral transport medium according to Bradford and Slavin (1940), and stored frozen at -70°C until used for RNA extraction and polymerase chain reaction to detect human influenza and (SARS-CoV-2 some variants by NGS) . All samples were tested in the Central Public Health Laboratory (CPHL) at National Influenza Center (NIC), during the period of study.

2.1 RNA extraction

Viral RNA Mini Kit simplifies the isolation of viral RNA by fast spin-column.

2.2 Preparation of reagents

ISSN: 0005-2523
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2.2.1 Addition of carrier RNA to buffer AVL (Viral Lysis Buffer)

About 310 µl Buffer (elusion buffer AVE) was added to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. The carrier RNA was dissolved thoroughly, and divided into conveniently sized aliquots, and stored at –20 °C and freezing –thawing of carrier RNA aliquots was avoided. For larger numbers of samples, volumes can be calculated using the following sample calculation:

n x 0.56 ml = y ml

y ml x 10 µl/ml = z µl where:

n = number of samples to be processed simultaneously

y = calculated volume of Buffer AVL

z = volume of carrier RNA–Buffer AVE to be added to Buffer AVL.

2.3 Molecular detection of Influenza A, B virus

2.3.1 Qualitative reverse transcription real-time-PCR (qRT-

Influenza Virus A B Real-TM Test is based on three major processes: isolation of virus RNA from specimens, reverse transcription of the RNA, Real Time amplification of the cDNA. *Influenza virus* A&B detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers and detection via fluorescent dyes. These dyes are linked with probes of oligonucleotides which bind specifically to the amplified product. The real-time PCR monitoring of fluorescence intensities allows the accumulating product detection without reopening of reaction tubes after the PCR run. Influenza Virus A B Real-TM PCR kit is a qualitative test which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the process of each individual sample extraction and serves also to identify possible reaction inhibition.

2.3.2 Molecular detection of Influenza A H3N2, H1N1 virus

Influenza A H1N1 & H3N2 Real-TM is Real-Time amplification test for typing of Influenza virus A (identification to subtypes H1N1 and H3N2) RNA in Influenza virus cultures and in clinical material (nasal and oropharyngeal swabs; sputum, bronchial lavage, autopsy material).

Influenza A H1N1 & H3N2 Real-TM Test is based on four major processes: isolation of *Influenza A virus* RNA from specimens, reverse transcription of the RNA and Real Time amplification of the cDNA of *Influenza A virus*. Influenza A H1N1 & H3N2 Real-TM PCR kit is a qualitative test which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the process of each individual sample extraction and serves also to identify possible reaction inhibition.

AccuPower® Molecular detection of SARS-CoV-2 Multiplex RT-PCR Kit
explain how reaction mixtures prepared.

Reaction mixture preparation

Components/N samples	Volume
Master Mix	5µl
Enzyme Mix	5 µl

Total volume	10 µl

When IPC was not used as an extraction control, So 1 µl of IPC was added in the reaction mixture preparation.

2.4 Statistical analysis

Data were entered, checked and analyzed using computer software programs of Statistical Package of Social Science (SPSS) version 26 and STATISTICA version 12. Descriptive statistics of frequency distribution tables of number and percentage were used for qualitative data, whereas, mean, standard deviation and range were used for quantitative data.

Inferential statistics of Unpaired students T-test, Chi-square test and Likelihood Ratio test (alternative to Chi-square test) were used to identify the significant differences between study comparable groups regarding different quantitative and categorial parameters respectively. A P-value of < 0.05 was used as the criterion for determining statistical significance throughout study's tests.

3. Results

The patients' age was approximately normally distributed and ranged from 1 to 99 years with a mean of 52.81 ± 22.872 years old, mostly at age group of 70-79 years old (18.5%). The majority of study sample were female gender (55.4%) with female to male ratio was 1.2:1. However, among female patients, only 7.2% were found to have a positive history of current pregnancy. Almost most of study sample found to have positive history of co-morbidity like hypertension, diabetes mellitus, heart failure and etc. (96.8%) (Table 1).

Table 1: Baseline socio-demographic characteristics of the study sample (n=249)

Characteristics	Description	Number (%)
Age (years)	Mean ± SD	52.81 ± 22.872
	Range (min-max)	98 (1-99)
Age groups	< 10	12 (4.8)
	10-19	9 (3.6)
	20-29	25 (10)
	30-39	24 (9.6)
	40-49	30 (12)
	50-59	36 (14.5)
	60-69	38 (15.3)
	70-79	46 (18.5)
	80-89	19 (7.6)
	> 89	10 (4)
Gender	Female	138 (55.4)
	Male	111 (44.6)

ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

Pregnancy (n=138) [a]	Yes	10 (7.2)
	No	128 (92.8)
History of co-morbidity	Yes	241 (96.8)
	No	8 (3.2)

[a]: Total number among only female patients.

Furthermore, in respect to clinical characteristics of the study's patient, the majority of them have clinical manifestation of combination symptoms of cough and fever as compared to those with single either fever or cough symptoms (90.8% vs. 8.4% vs. 0.8%) respectively. One third of study's sample found to have a positive history of previous clinical manifestation in the last two weeks from current infection (36.5%). Few proportions of study's sample found to have a positive history of animal (birds) contact (1.6%). Similarly, a low proportion of study's sample identified to have positive history of covid-19 vaccination (0.8%), who received proportionally an equal single and full dose among them (50% each). (Table 2)

Table 2: Baseline clinical characteristics of the study sample (n=249)

Characteristics	Description	Number (%)
Clinical manifestation	Cough	2 (0.8)
	Fever	21 (8.4)
	Combination of cough and fever	226 (90.8)
History of similar manifestation two weeks ago	Yes	91 (36.5)
	No	158 (63.5)
History of birds' contact	Yes	4 (1.6)
	No	245 (98.4)
Vaccination	Yes	2 (0.8)
	No	247 (99.2)
Vaccination dosage (n=2) [a]	Single dose	1 (50)
	Full dose (2 doses)	1 (50)

[a]: Total number among only patients with positive history of covid-19 vaccination (Yes).

Out of 249 investigated records of patients, 13 (5.2%; 95% CI: 2.4 – 8.0) found to had a positve influnza A (H3N2) results (Figure 1). Wherase, 65 (26.1%; 95% CI: 20.9 – 31.7) found to had a positve positve PCR result (Figure 2). On the other hand, zero perecent out of 249 investigated templates identifed to have a influnza B positive results.

Figure 1 Prevalence of positive Flu A (H3N2) result among study's sample (n=249)

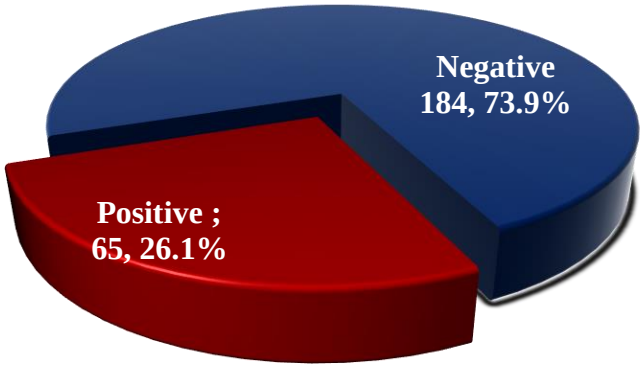

Figure 2 Prevalence of positive covid-19 PCR result among study's sample (n=249)

When compared between COVID-19 PCR and influenza-A template results among study's sample, significant differences were identified, as positive PCR results were entirely prevalent among patients with negative influenza-A template results as compared to none postive PCR results among patients with positive influenza-A results (100% vs, zero %) respectively. On the other hand, positive influenza-A results was overall prevalent among patients with negative PCR results (100% vs. zero %) respectively (x^2: 4.845, df: 1, *P* = 0.028) (Table 1).

Table 1 Distribution of patients by their Covid-19 PCR and influenza-A template results (n= 249)

Patients' covid-19 PCR template results	Influenza-A template results						Total
	Positive (13)			Negative (236)			
	n	%	% [a]	n	%	% [a]	
Positive	-	-	-	65	100	27.5	184
Negative	13	7.1	100	171	92.9	72.5	65

x^2: 4.845, df: 1, *P* = 0.028, [a]: Percentage by total number of influenza-A results (Column).

ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

4. Discussion

The current study designed to confirm the cause of SARI if just from SARS-CoV-2 or influenza viruses subtypes. A total of (249) SARI Iraqi patients were enrolled in the present study. For every patients mRNA was extract and molecular diagnosis multiplex rt PCR to check all strain of SARS-CoV-2.

According to questioner, all data such as age, gender, chronic diseases, symptoms, duration of disease, vaccinations status for last 6 months and other information's were recorded.

In the present study, the patients' age was approximately normally distributed in wide range, mostly at age group of 70-79 years old. The majority of sample were female gender. In the previous study, similar susceptibility to SARS-CoV-2 between males and females was observed in large sample patients who survived the disease. While other study collected from a public data set and in a case series of hospitalized patients found that male gander more effected than female [16]. Although the deceased patients were significantly older than the patients who survived COVID-19, ages were comparable between males and females in both the deceased and the patients who survived [17]. Therefore, gender is a risk factor for higher severity and mortality in patients with COVID-19, independent of age and susceptibility. This gender factor, as well as higher incidences in men for most of the diseases, could correlate with a general demographic fact of a shorter life expectancy in men compared to women in China and in the world in general [18].

In present study, most cases of SARI were caused by COVID-19 when compared to inflaunza. Several studies have reported co-infection of respiratory pathogens along with SARS-CoV-2 [19], [20]. There are also multiple reports of COVID-19 and influenza co-infections globally [21], [22]. A systematic review and meta-analysis of 26 studies through September 2020 reported the pooled prevalence of COVID-19 and influenza co-infection as 0.8% [23]. Another meta-analysis of 118 co-infection studies through February 2021 reported a pooled prevalence of 19% co-infections and 24% superinfections in COVID-19 cases. A total of 92% of the studies were conducted in hospital settings, and influenza A, B, and Respiratory Syncytial Viruses (RSV) were seen to be the most common co-infecting pathogens [24]. Co-infections of SARS-CoV-2 with influenza virus have been highlighted as a cause of concern due to demonstrated worsening of the clinical picture in such cases. A hospital-based study in Saudi Arabia noted increased mortality and ICU admissionsin influenza A/COVID-19 co-infections [18]. Triple co-infections with both influenza A and B and SARS-CoV-2 have also been reported [23].

In view of the emerging global recommendations to launch comprehensive surveillance programs for other respiratory pathogens alongwith SARS-CoV-2 [22] and availability of only limited, sporadic reports of co-infections from India [20], concerted efforts were made by the Indian Council of Medical Research to establish pan-India surveillance for SARS-CoV-2 and influenza viruses.

Aufi and her colleges reported that 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. The uneven susceptibility of females and males to infectious diseases has been attributed to mating competition and diet as behavioral and environmental factors [25].

In conclusion, through the current study concluded that most cases of severe respiratory infection in Iraq are cause by COVID-19.

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