Sudan University of Science and Technology

College of Graduate Studies

Prevalence of *Influenza A and B* Virus among Suspected COVID-19 patients by RT-PCR In Khartoum state

مدى انتشار فيروس الانفلونزا (أ) و (ب) بين مرضى الكوفيد -19 المشتبه بهم بواسطة جهاز تفاعل البلمرة المتسلسل في الوقت الحقيقي في ولاية الخرطوم

A Dissertation Submitted in Partial Fulfillment for the Requirementsof M.Sc. Degree in Medical Laboratory Science (Microbiology)

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الآيـــــــة

قال تعالى :

(وَلَوْلَا فَضْلُ ٱللَّهِ عَلَيْكَ وَرَحْمَتُهُۥ لَهَمَّت طَّآئِفَةٌ مِّنْهُمْ أن يُضِلُّوكَ وَمَا يُضِلُّونَ إِلَّآ أَنفُسَهُمْ ۖ وَمَا يَضُرُّونَكَ مِن شَىْءٍ ۚ وَأَنزَلَ ٱللَّهُ عَلَيْكَ ٱلْكِتَٰبَ وَٱلْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ ۚ وَكَانَ فَضْلُ ٱللَّهِ عَلَيْكَ عَظِيمًا (113) .

سورة النساء الاية (113)

صدق الله العظيم

DEDICATION

I dedicate this work

I would be very grateful for anybody helped me .I would like to

dedicate it to my Mother and Father who offers me the

inspiration of success and keenness throughoutmy life . . .

To my sisters Zeinab and Esraa . . .

Who have always helped me and believed that I could do it . . .

To my lovely family…… My Uncles Abd Elnasir Abd El Kader , Tarig Abd El Kader , Misnad Awad. . .

Who always encourage me with passion and endless support. I am so Lucky to have Uncles Love me so much and stand beside me the way you have!

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ABSTRACT

This study was performed during the outbreak period of coronavirus disease (COVID-19), in order to detect the prevalence of *Influenza A and B* viruses among suspected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This study was conducted in the period 25 May to 8 August 2022, all Nasopharyngeal samples collected from patients with suspected COVID- 19 received in the virology lab in Jabra Hospital for Emergency and injuries Khartoum, were tested for SARS-CoV-2 by GeneXpert System , then Real Time –PCR technique was used to detect the *Influenza A and B* virus.

A total of 100 Nasopharyngeal swabs were collected from suspected COVID-19 patients participated in this study, the result showed that out of 54 (54%) COVID positive patients, there were 6 (6%) have *Influenza A*, 2 (2%) have *Influenza B*, 1 (1%) have *Influenza A& B* and among 46 (46%) COVID-19 negative patients there were 6 (6%) have *Influenza A*, 1 (1%) have *Influenza B* and 1 (1%) have *Influenza A&B*.

In conclusion, the percentage of *Influenza A* 12 (12%) was higher prevalence among patients with COVID-19 than *Influenza B* 3(3%) and also showed infection with both A and B 2(2%).study highlights the importance of screening for co- infecting viruses in COVID-19 patients, given the high prevalence of *Influenza* viruses.

المستخلص

أجريت هذه الدراسة خلال فترة تفشي مرض فيروس كورونا (COVID-19) ، من أجل الكشف عن انتشار فيروسات الأنفلونزا A و B بين فيروس كورونا 2 (SARS-CoV-2) المشتبه به. أجريت هذه الدراسة في الفترة من 25 مايو إلى 8 أغسطس 2022 ، وتم اختبار جميع عينات البلعوم الأنفي التي تم جمعها من المرضى المشتبه في إصابتهم بـ COVID-19 في مختبر علم الفيروسات في مستشفى جبرا للطوارئ والإصابات بالخرطوم ، وتم اختبار SARS-CoV-2 ، تم استخدام تقنية Real Time –PCR للكشف عن فيروس الأنفلونزا A و B.

تم جمع ما مجموعه 100 مسحة من البلعوم الأنفي من مرضى COVID-19 المشتبه بهم الذين شاركوا في هذه الدراسة ، وأظهرت النتيجة أنه من بين 54 (54 %) من المرضى المصابين بفيروس COVID ، كان هناك 6 (6 %) مصابون بالإنفلونزا A، (2 %)2 لديهم ا نفلونزا B، 1 (1%) مصابون بالإنفلونزا A & B ، ومن بين 46 (46%) من المرضى السلبيين لـ COVID-19 كان هناك6 (6%) مصابون بالإنفلونزا A، 1 (1%) مصابون بالإنفلونزا B و 1 (1%) لديهم الأنفلونزا A & B.

في الختام ، كانت النسبة المئوية للإنفلونزا A 12 (12%) أعلى انتشارًا بين المرضى المصابين بـ COVID-19 من أنفلونزا B (3%) 3 وأظهرت أيضًا الإصابة بكل من A و B 2(2%). تسلط الدراسة الضوء على أهمية فحص الإصابة بالفيروسات في مرضى COVID-19 ، نظرًا لارتفاع معدل انتشار فيروسات الإنفلونزا.

Table of contents

List of tables

List of figures

Chapter 1

Introduction

CHAPTER ONE

1.1 Introduction

In December 2019, an outbreak of virus pneumonia caused by a novel coronavirus with high similarity to *severe acute respiratory syndrome coronavirus* (*SARS-CoV*) emerged and was subsequently named as *SARS-CoV-2* by the World Health Organization (WHO) (Zhou *et al*.,2020).

In 2020, the ongoing pandemic of Coronavirus disease 2019 (COVID-19) has posed a great challenge to health care systems globally and has therefore been recognized as a crucial public health emergency of international concern (Cheng *et al*.,2021).

To date (August 4, 2020), WHO has declared more than 197 million confirmed cases and 4,219,861 deaths worldwide (Who *et al*.,2021). As *SARS-CoV-2* continues to spread globally, it will overlap with *influenza* virus infection in every coming flu season (Belongia and Osterholm.,2020).

Influenza virus and *SARS-CoV-2* infection share similar respiratory illness symptoms, including fever, couch, dyspnea, sore throat, and fatigue, whereas they lack distinctive symptoms to differentiate COVID-19 from influenza infection. Additionally, both viruses can lead to life-threatening outcomes, involving acute respiratory distress syndrome (ARDS), septic shock, and multiple organ failure, especially in older adults and those with chronic diseases andco-infections (Gold *et al*.,2020;Wu *et al*.,2020) .

Effects of co-infection on *Influenza* pneumonia patients have already been well-focused (Alfaraj.,2017). However, it is still not completely understood whether simultaneously infected with SARS-CoV2 and *Influenza* virus contributes to increased disease severity, in terms of mortality, incidence of shock, being admitted to an intensive care unit (ICU) or requiring ventilator support. Additionally, knowledge of pathogenic interactions

between SARS-CoV-2 and *Influenza* virus is also limited up to now. Therefore, it is crucial to determine the epidemiological impacts of such interaction at present so as to inform treatment and control strategies to contain co infection with SARS-CoV-2 and *Influenza* virus. Since the beginning of COVID-19 pandemic, co-infection with *influenza* virus in COVID-19 patients has been widely detected and a number of case reports were subsequently published (Xiang *et al*.,2021 ; Singh *et al*.,2020) . The aim of this study to detect co infection of *Influenza A and B* among suspected COVID-19 patients to improve the management of these co infection patients.

2.1 Rationale

SARS-CoV-2 continues to spread globally, The meta-analysis of prevalence studies revealed that the frequency of influenza virus co-infection among patients with COVID-19 in Asia and American. There were no reports of co-infection with *Influenza virus A/B* in patients with COVID-19 from Africa or Oceania at the time of this study **(**Dadashi *et al*.,2021). Also no reports were introduced in Sudan So due to similarity of symptoms between COVID-19 and Influenza virus infections and presence of high prevalence of co infection in previous studies (Belongia and Osterholm) ,this study was conducted to detect the prevalence of co-infection with *Influenza virus A/B* in patients with suspected COVID-19 which reflects the necessity of effective diagnosis through which treatment decisions are made.

1.3 Objectives

1.3.1 General objectives

To detect the prevalence of *Influenza A and B* Viruses among suspected COVID-19 by RT-PCR in Khartoum state.

1.3.2 Specific objectives

1- To detect prevalence of *Influenza A and B* viruses in nasopharyngeal swabs among suspected COVID-19 patients by RT- PCR.

2- To evaluate the co-infections of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with *Influenza A and B* viruses.

3- To associate between the COVID-19 positive and *influenza A and B* infections

4- To associate between the COVID-19 negative and *influenza A and B* infections.

Chapter 11

Literature Review

CHAPTER TWO

2. Literature review

2.1 Historical background

Coronaviruses are a group of related RNA viruses that cause diseases in mammals and birds. In humans and birds, they cause respiratory tract infections that can range from mild to lethal. Mild illnesses in humans include some cases of the common cold (which is also caused by other viruses, predominantly rhinoviruses), while more lethal varieties can cause SARS, MERS and COVID-19, which is causing the ongoing pandemic. In cows and pigs they cause diarrhea, while in mice they cause hepatitis and encephalomyelitis. (Fan *et al*., 2019).

Coronaviruses constitute the subfamily *Orthocoronavirinae*, in the family

Coronaviridae, order *Nidovirales* and realm *Riboviria* (Fan *et al*., 2019). They are enveloped viruses with a positive-sense single- stranded RNA genome and a nucleocapsid of helical symmetry(Cherry *et al*.,2017). The genome size of coronaviruses ranges from approximately 26 to 32 kilobases, one of the largest among RNA viruses (Woo *et al*.,2010). They have characteristic club-shaped spikes that project from their surface, which in electron micrographs create an image reminiscent of the stellar corona, from which their name derives (Almeida *et al*.,1968).

The name "coronavirus" is derived from Latin *corona*, meaning "crown" or "wreath", itself a borrowing from Greek κορώνη *korṓ nē*, "garland, wreath", The name was coined by June Almeida and David Tyrrell who first observed and studied human coronaviruses (Tyrrell and Bynoe .,1965). The word was first used in print in 1968 by an informal group of virologists in the journal *Nature* to designate the new family of viruses (Almeida *et al*.,1968). The name refers to the characteristic appearance

of virions (the infective form of the virus) by electron microscopy, which have a fringe of large, bulbous surface projections creating an image reminiscent of the solar corona or halo (Tyrrell and Fielder.,2002). This morphology is created by the viral spike peplomers, which are proteins on the surface of the virus (Sturman and Holmes *et al*.,1983).

The scientific name *Coronavirus* was accepted as a genus name by the International Committee for the Nomenclature of Viruses (later renamed International Committee on Taxonomy of Viruses) in 1971 (Lalchhandama .,2020). As the number of new species increased, the genus was split into four genera, namely *Alphacoronavirus*, *Betacoronavirus*,

Deltacoronavirus, and *Gammacoronavirus* in 2009 (Carsten.,2010). The common name coronavirus is used to refer to any member of the subfamily *Orthocoronavirinae* (Fan *et al* .,2019). As of 2020, 45 species are officially recognized.

2.1.1 Structure

Coronaviruses are large, roughly spherical particles with unique surface projections (Goldsmith *et al*.,2004). Their size is highly variable with average diameters of 80 to 120 nm. Extreme sizes are known from 50 to 200 nm in diameter (Masters .,2006). The total molecular mass is on average 40,000 kDa. They are enclosed in an envelope embedded with a number of protein molecules (Lalchhandama .,2020). The lipid bilayer envelope, membrane proteins, and nucleocapsid protect the virus when it is outside the host cell (Neuman *et al*.,2011).

The viral envelope is made up of a lipid bilayer in which the membrane (M), envelope (E) and spike (S) structural proteins are anchored (Lai and Cavanagh.,1997). The molar ratio of E:S:M in the lipid bilayer is approximately 1:20:300 (Godet *et al*.,1992). The E and M protein are the structural proteins that combined with the lipid bilayer to shape the viral envelope and maintain its size (Fehr and Perlman .,2015). S proteins are needed for interaction with the host cells. But human coronavirus NL63 is peculiar in that its M protein has the binding site for the host cell, and not its S protein (Naskalska *et al*.,2019). The diameter of the envelope is 85 nm. The envelope of the virus in electron micrographs appears as a distinct pair of electron-dense shells (shells that are relatively opaque to the electron beam used to scan the virus particle)(Fehr and Perlman.,2015; Neuman *et al*.,2006).

2.1.2 Genome

Coronaviruses contain a positive-sense, single-stranded RNA genome. The genome size for coronaviruses ranges from 26.4 to 31.7 kilobases (Woo *et al*.,2010). The genome size is one of the largest among RNA viruses. The genome has a 5′ methylated cap and a 3′ polyadenylated tail (Fehr and Perlman.,2015).

The genome organization for a coronavirus is 5′-leader-UTR-replicase (ORF1ab)-spike (S)-envelope (E)-membrane (M)-nucleocapsid (N)-3′UTR-poly (A) tail. The open reading frames 1a and 1b, which occupy the first two-thirds of the genome, encode the replicase polyprotein (pp1ab). The replicase polyprotein self cleaves to form 16 nonstructural proteins (nsp1–nsp16) (Fehr and Perlman.,2015).

The later reading frames encode the four major structural proteins: spike, envelope, membrane, and nucleocapsid (Snijder *et al*.,2003). Interspersed between these reading frames are the reading frames for the accessory proteins. The number of accessory proteins and their function is unique depending on the specific coronavirus (Fehr and Perlman.,2015).

2.1.3 Classification

Coronaviruses form the subfamily *Orthocoronavirinae,*(Fan *et al*.,2019) which is one of two sub-families in the family

Coronaviridae, order *Nidovirales,* and realm *Riboviria (Groot et al.,2011).*

They are divided into the four genera: *Alphacoronavirus*,

Betacoronavirus, *Gammacoronavirus* and *Deltacoronavirus*.

Alphacoronaviruses and betacoronaviruses infect mammals, while gammacoronaviruses and deltacoronaviruses primarily infect birds (Wertheim *et al*.,2013).

- Genus: ***Alphacoronavirus***;(Decaro .,2011).
  - Species: *Alphacoronavirus 1* (TGEV, Feline coronavirus, Canine coronavirus), *Human coronavirus 229E, Human coronavirus NL63, Miniopterus bat coronavirus 1, Miniopterus bat coronavirus*

HKU8, *Porcine epidemic diarrhea virus*, *Rhinolophus bat coronavirus HKU2*, *Scotophilus bat coronavirus 512*

- Genus ***Betacoronavirus***;(Decaro.,2011).
  - Species: *Betacoronavirus 1* (*Bovine Coronavirus*, *Human coronavirus OC43*), *Hedgehog coronavirus 1*, *Human coronavirus HKU1*, *Middle East respiratory syndrome-related coronavirus*, *Murine coronavirus*, *Pipistrellus bat coronavirus HKU5*, *Rousettus bat coronavirus HKU9*, *Severe acute respiratory syndrome–related coronavirus* (*SARS-CoV*, *SARS-CoV-2*), *Tylonycteris bat coronavirus HKU4*
- Genus ***Gammacoronavirus***; (Decaro.,2011).
  - Species: *Avian coronavirus*, *Beluga whale coronavirus SW1*
- Genus ***Deltacoronavirus***; (Decaro.,2011).
  - Species: *Bulbul coronavirus HKU11*, *Porcine coronavirus HKU15*

2.1.4 Replication cycle

Infection begins when the viral spike protein attaches to its complementary host cell receptor. After attachment, a protease of the host cell cleaves and activates the receptor-attached spike protein. Depending on the host cell protease available, cleavage and activation allows the virus to enter the host cell by endocytosis or direct fusion of the viral envelope with the host membrane (Simmons *et al*.,2013).

On entry into the host cell, the virus particle is uncoated, and its genome enters the cell cytoplasm. The coronavirus RNA genome has a 5′ methylated cap and a 3′ polyadenylated tail, which allows it to act like a messenger RNA and be directly translated by the host cell's ribosomes. The host ribosomes translate the initial overlapping open reading frames ORF1a and ORF1b of the virus genome into two large overlapping polyproteins, pp1a and pp1ab (Fehr and Perlman.,2015).

The larger polyprotein pp1ab is a result of a -1 ribosomal frame shift caused by a slippery sequence (UUUAAAC) and a downstream RNA pseudoknot at the end of open reading frame ORF1a (Masters.,2006). The ribosomal frameshift allows for the continuous translation of ORF1a followed by ORF1b (Fehr and Perlman.,2015).

The polyproteins have their own proteases, PLpro (nsp3) and 3CLpro (nsp5), which cleave the polyproteins at different specific sites. The cleavage of polyprotein pp1ab yields 16 nonstructural proteins (nsp1 to nsp16). Product proteins include various replication proteins such as RNA-dependent RNA polymerase (nsp12), RNA helicase (nsp13), and exoribonuclease (nsp14) (Fehr and Perlman.,2015).

A number of the nonstructural proteins coalesce to form a multi-protein replicase-transcriptase complex (RTC). The main replicase-transcriptase protein is the RNA-dependent RNA polymerase (RdRp). It is directly involved in the replication and transcription of RNA from an RNA strand. The other nonstructural proteins in the complex assist in the replication and transcription process. The exoribonuclease nonstructural protein, for instance, provides extra fidelity to replication by providing a proofreading function which the RNA-dependent RNA polymerase lacks (Sexton *et al*.,2016).

Replication – One of the main functions of the complex is to replicate the viral genome. RdRp directly mediates the synthesis of negative-sense genomic RNA from the positive-sense genomic RNA. This is followed by the replication of positive-sense genomic RNA from the negative-sense genomic RNA (Fehr and Perlman.,2015).

Transcription – The other important function of the complex is to transcribe the viral genome. RdRp directly mediates the synthesis of negative-sense subgenomic RNA molecules from the positive-sense

genomic RNA. This process is followed by the transcription of these negative-sense subgenomic RNA molecules to their corresponding positive-sense mRNAs (Fehr and Perlman.,2015). The subgenomic mRNAs form a "nested set" which have a common 5'-head and partially duplicate 3'-end (Payne.,2017).

Recombination – The replicase-transcriptase complex is also capable of genetic recombination when at least two viral genomes are present in the same infected cell (Payne.,2017). RNA recombination appears to be a major driving force in determining genetic variability within a coronavirus species, the capability of a coronavirus species to jump from one host to another and, infrequently, in determining the emergence of novel coronaviruses(Su *et al*.,2017) The exact mechanism of recombination in coronaviruses is unclear, but likely involves template switching during genome replication (Su *et al*.,2017)

The replicated positive-sense genomic RNA becomes the genome of the progeny viruses. The mRNAs are gene transcripts of the last third of the virus genome after the initial overlapping reading frame. These mRNAs are translated by the host's ribosomes into the structural proteins and many accessory proteins (Fehr and Perlman.,2015). RNA translation occurs inside the endoplasmic reticulum. The viral structural proteins S, E, and M move along the secretory pathway into the Golgi intermediate compartment. There, the M proteins direct most protein-protein interactions required for the assembly of viruses following its binding to the nucleocapsid. Progeny viruses are then released from the host cell by exocytosis through secretory vesicles. Once released the viruses can infect other host cells (Fehr and Perlman.,2015).

2.1.5 Transmission

Infected carriers are able to shed viruses into the environment. The interaction of the coronavirus spike protein with its complementary cell receptor is central in determining the tissue tropism, infectivity, and species range of the released virus (Masters.,2006;Cui *et al*.,2019). Coronaviruses mainly target epithelial cells (Groot *et al*.,2011) .They are transmitted from one host to another host, depending on the coronavirus species, by either an aerosol, fomite, or fecal-oral route (Decaro.,2011).

Human coronaviruses infect the epithelial cells of the respiratory tract, while animal coronaviruses generally infect the epithelial cells of the digestive tract (Groot *et al*.,2011) SARS coronavirus, for example,infects the human epithelial cells of the lungs via an aerosol route by binding to the angiotensin-converting enzyme 2 (ACE2) receptor. Transmissible gastroenteritis coronavirus (TGEV) infects the pig epithelial cells of the digestive tract via a fecal-oral route (Decaro.,2011).by binding to the Alanine aminopeptidase (APN) receptor ,(Fehr and Perlman.,2015).

2.1.6 Infection in humans

Coronaviruses vary significantly in risk factor. Some can kill more than 30% of those infected, such as MERS-CoV, and some are relatively harmless, such as the common cold (Fehr and Perlman.,2015). Coronaviruses can cause colds with major symptoms, such as fever, and a sore throat from swollen adenoids (Lau *et al*.,2015). Coronaviruses can cause pneumonia (either direct viral pneumonia or secondary bacterial pneumonia) and bronchitis (either direct viral bronchitis or secondary bacterial bronchitis)(Forgie and Marrie,2009). The human coronavirus discovered in 2003, SARS-CoV, which causes severe acute respiratory syndrome (SARS), has a unique pathogenesis because it causes both upper and lower respiratory tract infections)(Forgie and Marrie,2009).

Six species of human coronaviruses are known, with one species subdivided

into two different strains, making seven strains of human coronaviruses together.

Four human coronaviruses produce symptoms that are generally mild, even though it is contended they might have been more aggressive in the past: (King and Anthony.,2020)

Human coronavirus OC43 (HCoV-OC43), β-CoV
Human coronavirus HKU1 (HCoV-HKU1), β-CoV
Human coronavirus 229E (HCoV-229E), α-CoV
Human coronavirus NL63 (HCoV-NL63), α-CoV–

Three human coronaviruses produce potentially severe symptoms:

1. Severe acute respiratory syndrome coronavirus (SARS-CoV), β-CoV (identified in 2003)
2. Middle East respiratory syndrome-related coronavirus (MERS-CoV), β-CoV (identified in 2012)
3. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), β-CoV (identified in 2019)

These cause the diseases commonly called SARS, MERS, and COVID-19 respectively.

2.1.7 Prevention and treatment

A number of vaccines using different methods have been developed against human coronavirus SARS-CoV-2. Antiviral targets against human coronaviruses have also been identified such as viral proteases, polymerases, and entry proteins. Drugs are in development which target these proteins and the different steps of viral replication (Dong *et al*.,2020).

Vaccines are available for animal coronaviruses IBV, TGEV, and Canine CoV, although their effectiveness is limited. In the case of outbreaks of highly contagious animal coronaviruses, such as PEDV, measures such as

destruction of entire herds of pigs may be used to prevent transmission to other herds. (Fehr and Perlman.,2015)

2.2 History of Influenza virus

Influenza, commonly known as "the flu", is an infectious disease caused by *influenza viruses*. Symptoms range from mild to severe and often include fever, runny nose, sore throat, muscle pain, headache, coughing, and fatigue. These symptoms begin from one to four days after exposure to the virus (typically two days) and last for about 2–8 days. Diarrhea and vomiting can occur, particularly in children. Influenza may progress to pneumonia, which can be caused by the virus or by a subsequent bacterial infection. Other complications of infection include acute respiratory distress syndrome, meningitis, encephalitis, and worsening of pre-existing health problems such as asthma and cardiovascular disease.(Call *et al*,. 2005)

Transmission through aerosols and intermediate objects and surfaces contaminated by the virus also occur.(Fabian *et al*.,2008)

In a typical year, 5–15% of the population contracts influenza. There are 3–5 million severe cases annually, with up to 650,000 respiratory-related deaths globally each year. Deaths most commonly occur in high-risk groups, including young children, the elderly, and people with chronic health conditions. In temperate regions of the world, the number of influenza cases peaks during winter, whereas in the tropics influenza can occur year-round. Since the late 1800s, large outbreaks of novel influenza strains that spread globally, called pandemics, have occurred every 10–50 years. Five flu pandemics have occurred since 1900: the Spanish flu in 1918–1920, which was the most severe flu pandemic, the Asian flu in 1957, the Hong Kong flu in 1968, the Russian flu in 1977, and the swine flu pandemic in 2009.(Fabian *et al*.,2008)

2.2.1Signs and symptoms

The time between exposure to the virus and development of symptoms, called the incubation period, is 1–4 days, most commonly 1–2 days. Many infections, however, are asymptomatic (Dharmapalan., 2020) . The onset of symptoms is sudden, and initial symptoms are predominately non-specific, including fever, chills, headaches, muscle pain or aching, a feeling of discomfort, loss of appetite, lack of energy/fatigue, and confusion. These symptoms are usually accompanied by respiratory symptoms such as a dry cough, sore or dry throat, hoarse voice, and a stuffy or runny nose. Coughing is the most common symptom (Krammer *et al*.,2018). Gastrointestinal symptoms may also occur, including nausea, vomiting, diarrhea (Ghebrehewet and Macpherson.,2016), and gastroenteritis (Sederdahl and Williams.,2020), especially in children. The standard influenza symptoms typically last for 2–8 days (Peteranderl *et al* 2016). A 2021 study suggests influenza can cause long lasting symptoms in a similar way to long COVID .

Symptomatic infections are usually mild and limited to the upper respiratory tract, but progression to pneumonia is relatively common. Pneumonia may be caused by the primary viral infection or by a secondary bacterial infection. Primary pneumonia is characterized by rapid progression of fever, cough, labored breathing, and low oxygen levels that cause bluish skin. It is especially common among those who have an underlying cardiovascular disease such as rheumatic heart disease. Secondary pneumonia typically has a period of improvement in symptoms for 1–3 weeks (Kalil and Thomas.,2019) followed by recurrent fever, sputum production, and fluid buildup in the lungs (Krammer et al.,2018), but can also occur just a few days after influenza symptoms appear (Kalil and Thomas.,2019) . About a third of primary pneumonia

cases are followed by secondary pneumonia, which is most frequently caused by the bacteria *Streptococcus pneumoniae* and *Staphylococcus aureus* (Dharmapalan.,2020; Krammer *et al*.,2018).

2.2.2 Types of virus

Influenza virus nomenclature (for a Fujian flu virus)

Influenza viruses comprise four species. Each of the four species is the sole member of its own genus, and the four influenza genera comprise four of the seven genera in the family *Orthomyxoviridae*. They are:(Krammer *et al*.,2018) *Influenza A virus* (IAV), genus *Alphainfluenzavirus*

- *Influenza B virus* (IBV), genus *Betainfluenzavirus*
- *Influenza C virus* (ICV), genus *Gammainfluenzavirus*
- *Influenza D virus* (IDV), genus *Deltainfluenzavirus*

IAV is responsible for most cases of severe illness as well as seasonal epidemics and occasional pandemics. It infects people of all ages but tends to disproportionately cause severe illness in the elderly, the very young, and those who have chronic health issues. Birds are the primary reservoir of IAV, especially aquatic birds such as ducks, geese, shorebirds, and gulls,(Li *et al*.,2019 ;Joseph *et al*.,2017), but the virus also circulates among mammals, including pigs, horses, and marine mammals. IAV is classified into subtypes based on the viral proteins haemagglutinin (H) and neuraminidase (N) (Asha and Kumar.,2019). As of 2019, 18 H subtypes and 11 N subtypes have been identified. Most potential combinations have been reported in birds, but H17-18 and N10-11 have only been found in bats. Only H subtypes H1-3 and N subtypes N1-2 are known to have circulated in humans (Asha and Kumar.,2019), the current IAVsubtypes in circulation being H1N1 and H3N2 (Sautto *et*

al.,2018). IAVs can be classified more specifically to also include natural host species, geographical origin, year of isolation, and strain number, such as H1N1/A/duck/Alberta/35/76 (Krammer *et al*.,2018 ; Ghebrehewet *et al*.,2016).

IBV mainly infects humans but has been identified in seals, horses, dogs, and pigs (Asha and Kumar.,2019). IBV does not have subtypes like IAV but has two antigenically distinct lineages, termed the B/Victoria/2/1987-like and B/Yamagata/16/1988-like lineages (Krammer *et al* .,2018), or simply (B/)Victoria(-like) and (B/)Yamagata(-like)(Asha and Kumar.,2019 ; Sautto *et al*.,2018). Both lineages are in circulation in humans (Krammer *et al* .,2018), disproportionately affecting children(Ghebrehewet *et al*.,2016). IBVs contribute to seasonal epidemics alongside IAVs but have never been associated with a pandemic (Asha and Kumar.,2019).

ICV, like IBV, is primarily found in humans, though it also has been detected in pigs, feral dogs, dromedary camels, cattle, and dogs (Sederdahi and Williams.,2020 ; Asha and Kumar.,2019). ICV infection primarily affects children and is usually asymptomatic (Krammer *et al*.,2018 ; Ghebrehewet *et al*.,2016) or has mild cold-like symptoms, though more severe symptoms such as gastroenteritis and pneumonia can occur (Sederdahl and Williams.,2020). Unlike IAV and IBV, ICV has not been a major focus of research pertaining to antiviral drugs, vaccines, and other measures against influenza (Asha and Kumar.,2019). ICV is subclassified into six genetic/antigenic lineages.(Sederdahl and Williams.,2020 ;Su *et al*.,2017)

IDV has been isolated from pigs and cattle, the latter being the natural reservoir. Infection has also been observed in humans, horses, dromedary camels, and small ruminants such as goats and sheep (Asha and

Kumar.,2019 ; Su *et al*.,2017). IDV is distantly related to ICV. While cattle workers have occasionally tested positive to prior IDV infection, it is not known to cause disease in humans (Krammer *et al*.,2018 ;Ghebrehewet *et al*.,2016 ;Sederdahi and Williams.,2020). ICV and IDV experience a slower rate of antigenic evolution than IAV and IBV. Because of this antigenic stability, relatively few novel lineages emerge (Su *et al*.,2017).

2.2.3 Genome and structure

Influenza viruses have a negative-sense, single-stranded RNA genome that is segmented. The negative sense of the genome means it can be used as a template to synthesize messenger RNA (mRNA) (Dharmapalan.,2020).

IAV and IBV have eight genome segments that encode 10 major proteins. ICV and IDV have seven genome segments that encode nine major proteins (Sederdahal and Williams.,2020). Three segments encode three subunits of an RNA-dependent RNA polymerase (RdRp) complex: PB1, a transcriptase, PB2, which recognizes 5' caps, and PA (P3 for ICV and IDV), an endonuclease (McCauley *et al*.,2011). The matrix protein (M1) and membrane protein (M2) share a segment, as do the non-structural protein (NS1) and the nuclear export protein (NEP) (Krammer *et al*.,2018). For IAV and IBV, hemagglutinin (HA) and neuraminidase (NA) are encoded on one segment each, whereas ICV and IDV encode a hemagglutinin-esterase fusion (HEF) protein on one segment that merges the functions of HA and NA. The final genome segment encodes the viral nucleoprotein (NP) (McCauley *et al*.,2011). Influenza viruses also encode various accessory proteins, such as PB1-F2 and PA-X, that are expressed through alternative open reading frames (Krammer *et al*.,2018 ;Shim *et al*.,2017) and which are important in host defense suppression, virulence, and pathogenicity (Hao *et al*.,2020).

The virus particle, called a virion, is pleomorphic and varies between being filamentous, bacilliform, or spherical in shape. Clinical isolates tend to be pleomorphic, whereas strains adapted to laboratory growth typically produce spherical virions. Filamentous virions are about 250 nanometers (nm) by 80 nm, bacilliform 120–250 by 95 nm, and spherical 120 nm in diameter (Dadonaite *et al*.,2016). The virion consists of each segment of the genome bound to nucleoproteins in separate ribonucleoprotein (RNP) complexes for each segment, all of which are surrounded by a lipid bilayer membrane called the viral envelope. There is a copy of the RdRp, all subunits included, bound to each RNP. The envelope is reinforced structurally by matrix proteins on the interior that enclose the RNPs (Allen and Ross.,2018), and the envelope contains HA and NA (or HEF(Su *et al*.,2017)) proteins extending outward from the exterior surface of the envelope. HA and HEF (Su *et al*.,2017) proteins have a distinct "head" and "stalk" structure. M2 proteins form proton ion channels through the viral envelope that are required for viral entry and exit. IBVs contain a surface protein named NB that is anchored in the envelope, but its function is unknown (Krammer *et al*.,2018).

2.2.4 Life cycle

The viral life cycle begins by binding to a target cell. Binding is mediated by the viral HA proteins on the surface of the envelope, which bind to cells that contain sialic acid receptors on the surface of the cell membrane. (Krammer *et al*.,2018 ; Li *et al*.,2019 ; Allen and Ross.,2018) For N1 subtypes with the "G147R" mutation and N2 subtypes, the NA protein can initiate entry. Prior to binding, NA proteins promote access to target cells by degrading mucous, which helps to remove extracellular decoy receptors that would impede access to target cells (Allen and Ross.,2018). After binding, the virus is internalized into the cell by an endosome that contains

the virion inside it. The endosome is acidified by cellular vATPase (Shim *et al*.,2017),to have lower pH, which triggers a conformational change in HA that allows fusion of the viral envelope with the endosomal membrane (Hao *et al*.,2020). At the same time, hydrogen ions diffuse into the virion through M2 ion channels, disrupting internal protein-protein interactions to release RNPs into the host cell's cytosol. The M1 protein shell surrounding RNPs is degraded, fully uncoating RNPs in the cytosol (Shim *et al*.,2017 ;Allen and Ross.,2018).

RNPs are then imported into the nucleus with the help of viral localization signals. There, the viral RNA polymerase transcribes mRNA using the genomic negative-sense strand as a template. The polymerase snatches 5' caps for viral mRNA from cellular RNA to prime mRNA synthesis and the 3'-end of mRNA is polyadenylated at the end of transcription (McCauley *et al*.,2011). Once viral mRNA is transcribed, it is exported out of the nucleus and translated by host ribosomes in a cap-dependent manner to synthesize viral proteins(Shim *et al*.,2017). RdRp also synthesizes complementary positive-sense strands of the viral genome in a complementary RNP complex which are then used as templates by viral polymerases to synthesize copies of the negative-sense genome (Krammer *et al*.,2018 ;Allen and Ross.,2018). During these processes, RdRps of avian influenza viruses (AIVs) function optimally at a higher temperature than mammalian influenza viruses (Peteranderl *et al*.,2016).

Newly synthesized viral polymerase subunits and NP proteins are imported to the nucleus to further increase the rate of viral replication and form RNPs (McCauley *et al*.,2011). HA, NA, and M2 proteins are trafficked with the aid of M1 and NEP proteins (Hao *et al*.,2020) to the cell membrane through the Golgi apparatus (McCauley *et al*.,2011). and inserted into the cell's membrane. Viral non-structural proteins including

NS1, PB1-F2, and PA-X regulate host cellular processes to disable antiviral responses (Krammer *et al*.,2018 ; Allen and Ross.,2018 ; Hao *et al*.,2020). PB1-F2 aso interacts with PB1 to keep polymerases in the nucleus longer (Joseph *et al*.,2017). M1 and NEP proteins localize to the nucleus during the later stages of infection, bind to viral RNPs and mediate their export to the cytoplasm where they migrate to the cell membrane with the aid of recycled endosomes and are bundled into the segments of the genome (Krammer *et al*.,2018 ; Allen and Ross.,2018).

Progenic viruses leave the cell by budding from the cell membrane, which is initiated by the accumulation of M1 proteins at the cytoplasmic side of the membrane. The viral genome is incorporated inside a viral envelope derived from portions of the cell membrane that have HA, NA, and M2 proteins. At the end of budding, HA proteins remain attached to cellular sialic acid until they are cleaved by the sialidase activity of NA proteins. The virion is then released from the cell (Krammer *et al*.,2018 ; Allen and Ross.,2018). The sialidase activity of NA also cleaves any sialic acid residues from the viral surface, which helps prevent newly assembled viruses from aggregating near the cell surface and improving infectivity. Similar to other aspects of influenza replication, optimal NA activity is temperature- and pH-dependent (Peteranderl *et al*.,2016). Ultimately, presence of large quantities of viral RNA in the cell triggers apoptosis, i.e. programmed cell death, which is initiated by cellular factors to restrict viral replication (Shim *et al*.,2017).

Two key processes that influenza viruses evolve through are antigenic drift and antigenic shift. Antigenic drift is when an influenza virus's antigens change due to the gradual accumulation of mutations in the antigen's (HA or NA) gene (Li *et al*.,2019). This can occur in response to evolutionary pressure exerted by the host immune response. Antigenic

drift is especially common for the HA protein, in which just a few amino acid changes in the head region can constitute antigenic drift (Allen and Ross.,2018 ;Su *et al*.,2017) .The result is the production of novel strains that can evade pre-existing antibody-mediated immunity (Krammer *et al*.,2018 ; Ghebrehewet *et al*.,2016). Antigenic drift occurs in all influenza species but is slower in B than A and slowest in C and D (Su *et al*.,2017). Antigenic drift is a major cause of seasonal influenza (Saunders Hastings and Krewski.,2016), and requires that flu vaccines be updated annually. HA is the main component of inactivated vaccines, so surveillance monitors antigenic drift of this antigen among circulating strains. Antigenic evolution of influenza viruses of humans appears to be faster than influenza viruses in swine and equines. In wild birds, within-subtype antigenic variation appears to be limited but has been observed in poultry immunity (Krammer *et al*.,2018 ; Ghebrehewet *et al*.,2016).

Antigenic shift is a sudden, drastic change in an influenza virus's antigen, usually HA. During antigenic shift, antigenically different strains that infect the same cell can reassort genome segments with each other, producing hybrid progeny. Since all influenza viruses have segmented genomes, all are capable of reassortment (Sederdahl and Williams.,2020 ; Su *et al*.,2017). Antigenic shift, however, only occurs among influenza viruses of the same genus (McCauley *et al*.,2011) and most commonly occurs among IAVs. In particular, reassortment is very common in AIVs, creating a large diversity of influenza viruses in birds, but is uncommon in human, equine, and canine lineages (Lycett *et al*.,2019). Pigs, bats, and quails have receptors for both mammalian and avian IAVs, so they are potential "mixing vessels" for reassortment (Asha and Kumar.,2019). If an animal strain reassorts with a human strain (Sautto *et al*.,2018), then a novel strain can emerge that is capable of human-to-human transmission.

This has caused pandemics, but only a limited number have occurred, so it is difficult to predict when the next will happen (Krammer *et al*.,2018 ; Ghebrehewet *et al*.,2016) .

2.2.5 Pathophysiology

In humans, influenza viruses first cause infection by infecting epithelial cells in the respiratory tract. Illness during infection is primarily the result of lung inflammation and compromise caused by epithelial cell infection and death, combined with inflammation caused by the immune system's response to infection. Non-respiratory organs can become involved, but the mechanisms by which influenza is involved in these cases are unknown. Severe respiratory illness can be caused by multiple, non-exclusive mechanisms, including obstruction of the airways, loss of alveolar structure, loss of lung epithelial integrity due to epithelial cell infection and death, and degradation of the extracellular matrix that maintains lung structure. In particular, alveolar cell infection appears to drive severe symptoms since this results in impaired gas exchange and enables viruses to infect endothelial cells, which produce large quantities of pro-inflammatory cytokines (Kalil and Thomas.,2019).

Pneumonia caused by influenza viruses is characterized by high levels of viral replication in the lower respiratory tract, accompanied by a strong pro-inflammatory response called a cytokine storm (Krammer *et al*.,2018). Infection with H5N1 or H7N9 especially produces high levels of pro-inflammatory cytokines (Li *et al*.,2019). In bacterial infections, early depletion of macrophages during influenza creates a favorable environment in the lungs for bacterial growth since these white blood cells are important in responding to bacterial infection. Host mechanisms to encourage tissue repair may inadvertently allow bacterial infection. Infection also induces production of systemic glucocorticoids that can reduce inflammation to

preserve tissue integrity but allow increased bacterial growth (Kalil and Thomas.,2019).

The pathophysiology of influenza is significantly influenced by which receptors influenza viruses bind to during entry into cells. Mammalian influenza viruses preferentially bind to sialic acids connected to the rest of the oligosaccharide by an α-2,6 link, most commonly found in various respiratory cells (Krammer *et al*.,2018 ; Li *et al*.,2019 ; Allen and Ross.,2018), such as respiratory and retinal epithelial cells (Shim *et al*.,2017). AIVs prefer sialic acids with an α-2,3 linkage, which are most common in birds in gastrointestinal epithelial cells and in humans in the lower respiratory tract (Shao *et al*.,2017). Furthermore, cleavage of the HA protein into HA_1, the binding subunit, and HA_2, the fusion subunit, is performed by different proteases, affecting which cells can be infected. For mammalian influenza viruses and low pathogenic AIVs, cleavage is extracellular, which limits infection to cells that have the appropriate proteases, whereas for highly pathogenic AIVs, cleavage is intracellular and performed by ubiquitous proteases, which allows for infection of a greater variety of cells, thereby contributing to more severe disease (Krammer *et al*.,2018; Lycett *et al*.,2019; Steinhauer.,1999).

2.2.6 Diagnosis

Diagnosis based on symptoms is fairly accurate in otherwise healthy people during seasonal epidemics and should be suspected in cases of pneumonia, acute respiratory distress syndrome (ARDS), sepsis, or if encephalitis, myocarditis, or breaking down of muscle tissue occur (Kalil and Thomas.,2019). Because influenza is similar to other viral respiratory tract illnesses, laboratory diagnosis is necessary for confirmation. Common ways of collecting samples for testing include nasal and throat swabs (Krammer *et al*.,2018). Samples may be taken from the lower respiratory

tract if infection has cleared the upper but not lower respiratory tract. Influenza testing is recommended for anyone hospitalized with symptoms resembling influenza during flu season or who is connected to an influenza case. For severe cases, earlier diagnosis improves patient outcome (Chow *et al* .,2019). Diagnostic methods that can identify influenza include viral cultures, antibody- and antigen-detecting tests, and nucleic acid-based tests (Vemula *et al*.,2016).

Viruses can be grown in a culture of mammalian cells or embryonated eggs for 3–10 days to monitor cytopathic effect. Final confirmation can then be done via antibody staining, hemadsorption using red blood cells, or immunofluorescence microscopy. Shell vial cultures, which can identify infection via immunostaining before a cytopathic effect appears, are more sensitive than traditional cultures with results in 1–3 days (Krammer *et al*.,2018; Chow *et al*.,2019; Vemula *et al*.,2016). Cultures can be used to characterize novel viruses, observe sensitivity to antiviral drugs, and

Monitor antigenic drift, but they are relatively slow and require specialized skills and equipment (Krammer *et al*.,2018).

Serological assays can be used to detect an antibody response to influenza after natural infection or vaccination. Common serological assays include hemagglutination inhibition assays that detect HA-specific antibodies, virus neutralization assays that check whether antibodies have neutralized the virus, and enzyme-linked immunoabsorbant assays. These methods tend to be relatively inexpensive and fast but are less reliable than nucleic-acid based tests(Krammer *et al*.,2018; Vemula *et al*.,2016).

Direct fluorescent or immunofluorescent antibody (DFA/IFA) tests involve staining respiratory epithelial cells in samples with fluorescently-labeled influenza-specific antibodies, followed by examination under a fluorescent microscope. They can differentiate between IAV and IBV but can't subtype

IAV (Vemula *et al*.,2016). Rapid influenza diagnostic tests (RIDTs) are a simple way of obtaining assay results, are low cost, and produce results quickly, at less than 30 minutes, so they are commonly used, but they can't distinguish between IAV and IBV or between IAV subtypes and are not as sensitive as nucleic-acid based tests (Krammer *et al*.,2018; Vemula *et al*.,2016).

Nucleic acid-based tests (NATs) amplify and detect viral nucleic acid. Most of these tests take a few hours (Vemula *et al*.,2016), but rapid molecular assays are as fast as RIDTs (Chow *et al*.,2019) Among NATs, reverse transcription polymerase chain reaction (RT-PCR) is the most traditional and considered the gold standard for diagnosing influenza (Vemula *et al*.,2016). because it is fast and can subtype IAV, but it is relatively expensive and more prone to false-positives than cultures (Krammer *et al*.,2018), Other NATs that have been used include loop-

Mediated isothermal amplification-based assays, simple amplification-based assays, and nucleic acid sequence-based amplification. Nucleic acid sequencing methods can identify infection by obtaining the nucleic acid sequence of viral samples to identify the virus and antiviral drug resistance. The traditional method is Sanger sequencing, but it has been largely replaced by next-generation methods that have greater sequencing speed and throughput (Vemula *et al*.,2016).

2.2.7 Treatment

Treatment of influenza in cases of mild or moderate illness is supportive and includes anti-fever medications such as acetaminophen and ibuprofen adequate fluid intake to avoid dehydration, and resting at home (Ghebrehewet *et al*.,2016). Cough drops and throat sprays may be beneficial for sore throat. It is recommended to avoid alcohol and tobacco use while sick with the flu. Aspirin is not recommended to treat influenza

in children due to an elevated risk of developing Reye syndrome (Banday *et al*.,2021). Corticosteroids likewise are not recommended except when treating septic shock or an underlying medical condition, such as chronic obstructive pulmonary disease or asthma exacerbation, since they are associated with increased mortality (Chow *et al*.,2019) If a secondary bacterial infection occurs, then treatment with antibiotics may be necessary (Ghebrehewet *et al*.,2016).

Antiviral drugs are primarily used to treat severely ill patients, especially those with compromised immune systems. Antivirals are most effective when started in the first 48 hours after symptoms appear. Later administration may still be beneficial for those who have underlying immune defects, those with more severe symptoms, or those who have a higher risk of developing complications if these individuals are still shedding the virus. Antiviral treatment is also recommended if a person is hospitalized with suspected influenza instead of waiting for test results to return and if symptoms are worsening (Krammer *et al*.,2018; Chow *et al*.,2019). Most antiviral drugs against influenza fall into two categories: neuraminidase (NA) inhibitors and M2 inhibitors (Peteranderl *et al*.,2016).Baloxavir marboxil is a notable exception, which targets the endonuclease activity of the viral RNA polymerase and can be used as an alternative to NA and M2 inhibitors for IAV and IBV (Dharmapalan.,2020; Li *et al*.,2019; Lampejo.,2020).

NA inhibitors target the enzymatic activity of NA receptors, mimicking the binding of sialic acid in the active site of NA on IAV and IBV virions (Krammer *et al*.,2018) so that viral release from infected cells and the rate of viral replication are impaired (Ghebbrehewet *et al*.,2016). NA inhibitors include oseltamivir, which is consumed orally in a prodrug form and converted to its active form in the liver, and zanamivir, which is a powder that is inhaled nasally. Oseltamivir and zanamivir are effective for

prophylaxis and post-exposure prophylaxis, and research overall indicates that NA inhibitors are effective at reducing rates of complications, hospitalization, and mortalityand the duration of illness (Peteranderl *et al*.,2016; Chow *et al*.,2019; Lampejo.,2020). Additionally, the earlier NA inhibitors are provided, the better the outcome (Lampejo.,2020), though late administration can still be beneficial in severe cases (Krammer *et al*.,2018; Chow *et al*.,2019). Other NA inhibitors include laninamivir (Krammer *et al*.,2018) and peramivir, the latter of which can be used as an alternative to oseltamivir for people who cannot tolerate or absorb it(Chow *et al*.,2019).

The adamantanes amantadine and rimantadine are orally administered drugs that block the influenza virus's M2 ion channel (Krammer *et al*.,2018), preventing viral uncoating (Lampejo.,2020), These drugs are only functional against IAV (Chow *et al*.,2019).but are no longer recommended for use because of widespread resistance to them among IAVs (Lampejo.,2020). Adamantane resistance first emerged in H3N2 in 2003, becoming worldwide by 2008. Oseltamivir resistance is no longer widespread because the 2009 pandemic H1N1 strain (H1N1 pdm09), which is resistant to adamantanes, seemingly replaced resistant strains in circulation. Since the 2009 pandemic, oseltamivir resistance has mainly been observed in patients undergoing therapy (Krammer *et al*.,2018),

especially the immunocompromised and young children (Lampejo.,2020).

Oseltamivir resistance is usually reported in H1N1, but has been reported in H3N2 and IBVs less commonly (Krammer *et al*.,2018). Because of this, oseltamivir is recommended as the first drug of choice for immunocompetent people, whereas for the immunocompromised, oseltamivir is recommended against H3N2 and IBV and zanamivir against H1N1 pdm09. Zanamivir resistance is observed less frequently, and resistance to peramivir and baloxavir marboxil is possible(Lampejo.,2020).

2.2.8 Prevention

Vaccination

Annual vaccination is the primary and most effective way to prevent influenza and influenza-associated complications, especially for high-risk groups (Krammer *et al* 2018; Dharmapalan.,2020; Chow *et al*.,2019).

Vaccines against the flu are trivalent or quadrivalent, providing protection against an H1N1 strain, an H3N2 strain, and one or two IBV strains corresponding to the two IBV lineages (Dharmapalan.,2020; Sautto *et al*.,2018). Two types of vaccines are in use: inactivated vaccines that contain "killed" (i.e. inactivated) viruses and live attenuated influenza vaccines (LAIVs) that contain weakened viruses (Krammer *et al* 2018). There are three types of inactivated vaccines: whole virus, split virus, in which the virus is disrupted by a detergent, and subunit, which only contains the viral antigens HA and NA (Tregoning *et al*.,2018). Most flu vaccines are inactivated and administered via intramuscular injection. LAIVs are sprayed into the nasal cavity(Krammer *et al* 2018).

Vaccination recommendations vary by country. Some recommend vaccination for all people above a certain age, such as 6 months (Chow *et al*.,2019), whereas other countries recommendation is limited for high at risk groups, such as pregnant women, young children (excluding newborns), the elderly, people with chronic medical conditions, health care workers(Krammer *et al* 2018) , people who come into contact with high-risk people, and people who transmit the virus easily (Ghebrehewet *et al*.,2016). Young infants cannot receive flu vaccines for safety reasons, but they can inherit passive immunity from their mother if inactivated vaccines are administered to the mother during pregnancy (Principi and Esposito.,2018). Influenza vaccination also helps to reduce the probability of reassortment (Peteranderl *et al*.,2016).

In general, influenza vaccines are only effective if there is an antigenic match between vaccine strains and circulating strains (Sautto *et al*.,2018; Dharmapalan.,2020). Additionally, most commercially available flu vaccines are manufactured by propagation of influenza viruses in embryonated chicken eggs, taking 6–8 months (Sautto *et al*.,2018). Flu seasons are different in the northern and southern hemisphere, so the WHO meets twice a year, one for each hemisphere, to discuss which strains should be included in flu vaccines based on observation from HA inhibition assays (Dharmapalan.,2020; Allen and Ross.,2018). Other manufacturing methods include an MDCK cell culture-based inactivated vaccine and a recombinant subunit vaccine manufactured from baculovirus overexpression in insect cells (Sautto *et al*.,2018;*Barr et al*.,2018).

Antiviral chemoprophylaxis

Influenza can be prevented or reduced in severity by post-exposure prophylaxis with the antiviral drugs oseltamivir, which can be taken orally by those at least three months old, and zanamivir, which can be inhaled by those above seven years of age. Chemoprophylaxis is most useful for individuals at high-risk of developing complications and those who cannot receive the flu vaccine due to contraindications or lack of effectiveness (Dharmapalan .,2020) . Post-exposure chemoprophylaxis is only recommended if oseltamivir is taken within 48 hours of contact with a confirmed or suspected influenza case and zanamivir within 36 hours (Dharmapalan .,2020;Ghebrehewet *et al*.,2016). It is recommended that it be offered to people who have yet to receive a vaccine for the current flu season, who have been vaccinated less than two week since contact, if there is a significant mismatch between vaccine and circulating strains, or during an outbreak in a closed setting regardless of vaccination history

(Ghebrehewet *et al*.,2016).

2.2.9 Infection control

Hand hygiene is important in reducing the spread of influenza. This includes frequent hand washing with soap and water, using alcohol-based hand sanitizers, and not touching one's eyes, nose, and mouth with one's hands. Covering one's nose and mouth when coughing or sneezing is important (Jefferson *et al*.,2020). Other methods to limit influenza transmission include staying home when sick (Krammer *et al* 2018), avoiding contact with others until one day after symptoms end (Ghebrehewet *et al*.,2016), and disinfecting surfaces likely to be contaminated by the virus, such as doorknobs(Krammer *et al* 2018). Health education through media and posters is often used to remind people of the aforementioned etiquette and hygiene (Dharmapalan .,2020).

There is uncertainty about the use of masks since research thus far has not shown a significant reduction in seasonal influenza with mask usage. Likewise, the effectiveness of screening at points of entry into countries is not well researched (Jefferson *et al*.,2020). Social distancing measures such as school closures, avoiding contact with infected people via isolation or quarantine, and limiting mass gatherings may reduce transmission ((Krammer *et al* 2018.,Jefferson *et al*.,2020).but these measures are often expensive, unpopular, and difficult to implement. Consequently, the commonly recommended methods of infection control are respiratory etiquette, hand hygiene, and mask wearing, which are inexpensive and easy to perform. Pharmaceutical measures are effective but may not be available in the early stages of an outbreak (Saunders-Hastings *et al*.,2017).

In health care settings, infected individuals may be cohorted or assigned to individual rooms. Protective clothing such as masks, gloves, and gowns is recommended when coming into contact with infected individuals if there

is a risk of exposure to infected bodily fluids. Keeping patients in negative pressure rooms and avoiding aerosol-producing activities may help (Dharmapalan .,2020), but special air handling and ventilation systems are not considered necessary to prevent the spread of influenza in the air (Lampejo .,2020). In residential homes, new admissions may need to be closed until the spread of influenza is controlled. When discharging patients to care homes, it is important to take care if there is a known influenza outbreak (Ghebrehewet *et al*.,2016).

Since influenza viruses circulate in animals such as birds and pigs, prevention of transmission from these animals is important. Water treatment, indoor raising of animals, quarantining sick animals, vaccination, and biosecurity are the primary measures used. Placing poultry houses and piggeries on high ground away from high-density farms, backyard farms, live poultry markets, and bodies of water helps to minimize contact with wild birds (Krammer *et al* 2018). Closure of live poultry markets appears to the most effective measure (Li and Linster *et al*.,2019)and has shown to be effective at controlling the spread of H5N1, H7N9, and H9N2 (Li and Linster *et al*.,2019). Other biosecurity measures include cleaning and disinfecting facilities and vehicles, banning visits to poultry farms, not bringing birds intended for slaughter back to farms changing clothes, disinfecting foot baths, and treating food and water(Krammer *et al* 2018).

If live poultry markets are not closed, then "clean days" when unsold poultry is removed and facilities are disinfected and "no carry-over" policies to eliminate infectious material before new poultry arrive can be used to reduce the spread of influenza viruses. If a novel influenza viruses has breached the aforementioned biosecurity measures, then rapid detection to stamp it out via quarantining, decontamination, and culling may be necessary to prevent the virus from becoming endemic (Krammer

et al.,2018). Vaccines exist for avian H5, H7, and H9 subtypes that are used in some countries (Li and Linster *et al*.,2019). In China, for example, vaccination of domestic birds against H7N9 successfully limited its spread, indicating that vaccination may be an effective strategy (Lycett *et al*.,2019),if used in combination with other measures to limit transmission (Krammer *et al*.,2018). In pigs and horses, management of influenza is dependent on vaccination with biosecurity (Krammer *et al*.,2018).

Chapter 3

Materials and methods

CHAPTER THREE

3. Materials and methods

3.1 Study design

The study was Analytical Cross sectional study.

3.2 Study period

This work carried out in the period between 25 May to 8 August 2022

3.3 Study area

This study was carried out in Jabra Hospital for Emergency and an injury is central hospital in Khartoum state and it is the Top Emergency and Trauma Hospitals and it is the first COVID-19 isolation Center.

3.4 Study population

Patients suspected to COVID-19 attended to Jabra hospital with different age and gender.

3.5 Inclusion criteria

Patients suspected to *COVID-19*.

3.6 Exclusion criteria

Patients who no symptoms of *COVID-19.*

3.7 Sample size

A total of 100 subjects of suspected *COVID*-19 patients.

3.8 Data collection

The data and samples are collected from Jabra Hospital for Emergency and injuries Khartoum .

3.9 Ethical consideration

An approval for the work was taken from Research Ethical Committee in Sudan University of Science and Technology, the hospital were informed for the purpose of the study and its objectives, the hospital was taken already the approval from patients.

3.10 Specimen collection and preservation

Nasopharyngeal swabs were collected following specimen collection guidelines and standard recommended procedures.(Who.,2020)

3.11 Laboratory work

Nasopharyngeal swabs were agitated vigorously Centrifuged at 1000g/min for 5 min .The supernatant was Discarded and leave about 100 µl of solution for RNA extraction.

3.12 Principle of RT-PCR

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 c DNA. *Influenza virus A and 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 and 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.

3.13 procedure

3.13.1RNA Extraction

RNA Extraction according to manufacturer guidelines (QIAamp Viral RNA Mini Handbook 07/2020) .

All reagents were brought to room temperature approximately 30 minutes before used .560 µl were Pipetted from prepared Buffer AVL containing carrier RNA into a 1.5 ml microcentrifuge tube, 140 µl of sample is added to the Buffer AVL–carrier RNA in the microcentrifuge tube. Mixed by pulse-vortexing for 15 s, incubated at room temperature for 10 min. Briefly centrifuged the tube to remove drops from the inside of the lid, then 560 µl ethanol (96–100%) was added to the sample, and mixed by pulse-vortexing for 15 s. After mixing, briefly centrifuged the tube to remove drops from inside the lid, carefully 630 µl of the solution was applied to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Closing the cap, and centrifuged at 6000 x g (8000 rpm) for 1 min. The QIAamp Mini column was added into a clean 2 ml collection tube, and discarded the tube containing the filtrate. Carefully the QIAamp Mini column was opened, and repeat the previous step. If the sample volume was greater than 140 µl, repeat this step until all of the lysate has been loaded onto the spin column. Carefully the QIAamp Mini column was opened, and 500 µl Buffer AW1 was added, centrifuged at 6000 x g (8000 rpm) for 1 min. Placed the QIAamp Mini column in a clean 2 ml collection tube (provided), and discarded the tube containing the filtrate.Carefully the QIAamp Mini column was opened, and add 500 µl Buffer AW2 and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. Placed the QIAamp Mini column in a new 2 ml collection tube (not provided), and discarded the old collection tube with the filtrate. Centrifuged at full speed

for 1 min, the QIAamp Mini column was placed in a clean 1.5 ml microcentrifuge tube (not provided),discarded the old collection tube containing the filtrate and carefully the QIAamp Mini column was opened and added 60 µl Buffer AVE equilibrated to room temperature, closeed the cap, and incubated at room temperature for 1 min. Centrifuged at 6000 x g (8000 rpm) for 1 min. A single elution with 60 µl Buffer AVE is sufficient to elute at least 90% of the viral RNA from the QIAamp Mini column. Performing a double elution using 2 x 40 µl Buffer AVE will increase yield by up to 10%. Elution with volumes of less than 30 µl will lead to reduced yields and will not increase the final concentration of RNA in the eluate. Viral RNA is stable for up to 1 year when stored at −30 to −15°C or at –90 to −65°C.

3.13.2 Reverse Transcription

Reaction mix was prepared: for 12 reactions,by adding 5,0 µl RT-G-mix-1 into the tube containing RTmix and vortexed for at least 5-10 seconds, centrifuged briefly. This mixed is stable for 1 month at -20°C. 6 µl M-MLV was added into the tube with Reagent Mix, mixed by pipetting, vortexed for 3 sec, centrifuged for 5-7 sec (must be used immediately after the preparation). 10 µl of Reaction was Mixed into each sample tube.10 µl RNA samples was added to the appropriate tube. The tubes were placed into thermalcycler and incubated at 37 oC for 30 minutes, diluted 1: 2 each obtained cDNA sample with TE-buffer (by adding 20 µl TE-buffer to each tube). cDNA specimens could be stored at -20oC for a week or at -70oC during a year.

3.13.3 Real Time amplification:

Required quantity of tubes or PCR plate are prepared .15 µl of Reaction Mix was added into each tube, then 10 µl of cDNA sample was added to appropriate tube with Reaction Mix, and Prepared for each panel 2 controls: 10 µl of DNA-buffer was added to the tube labeled Amplification Negative Control and 10 µl of Pos cDNA Infl. A / Infl. B / IC was added tothe tube labeled Cpos;

3.14 Calculation and interpretation of the result

Analysis of results were performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three channels: − The signal of the IC cDNA amplification product was detected in the channel for the FAM fluorophore. − The signal of the Influenza virus B cDNA amplification product was detected in the channel for the JOE fluorophore. − The signal of the Influenza virus A cDNA amplification product was detected in the channel for the ROX fluorophore. The sample was considered to be positive for Influenza Virus A if in the channel Rox (Orange) the value of Ct is different from zero and is less than the boundary value specified in the table below.The sample is considered to be positive for Influenza Virus B if in the channel Joe (Yellow) the value of Ct is different from zero and is less than the boundary value specified in the table below. The sample is considered to be negative for Influenza A/B if Ct value is not determined (absent) in ROX and JOE channels, whereas the Ct value is determined in FAM channel and is less than the value specified in the table below :

Table (3.1) : Interpretation of the result of the real-time PCR instrument

Sample	**Stage for control**	**Ct channel FAM(green)**	**Ct channel Joe(yellow)**	**Ct channel Rox(orange)**	**Result**
NCE	RNA isolation	<28	Neg	Neg	Valid result
NCA	PCR	Neg	Neg	Neg	Valid result
Pos cDNA Infl.A/ Infl.B / IC	PCR	<25	<25	<25	Valid result
Clinical samples	RNA isolation	<28	<33	<33	Valid result

Key :-

NCA :Negative control extraction Neg : negative
NCE : negative control amplification POS : positive
IC : Internal control c DNA: complementary DNA

3.15 Data analysis

Collected data were analyzed using the statistical package of social science (SPSS) program version 25. Chi-square statistical analysis were used to determine P. value significance range. Data represented by mean ± 1 frequency, P-Value less than 0.05 were regarded as significant.

Chapter 1v

Results, Discussion and conclusion

CHAPTER FOUR
4.1 Results

Out of 100 patients suspected COVID-19 patients (56 Female and 44 Male), were randomly selected to participate in this study. Nasopharyngeal swab samples from the patients were subjected to Polymerase Chain Reaction (PCR) for the detection of COVID-19. The result showed that: 54(54%) were positive for COVID-19, while 46(46%) were negative.

Patients' frequency distribution according to *Influenza* infections

The results demonstrated that showed that vast majority of patients (83%) showed negative result for *Influenza virus*. The rest (17%) with positive *influenza* virus; (2%) were *influenza both A, B.*, (12%) with *Influenza A* and (3%) with *Influenza B*.

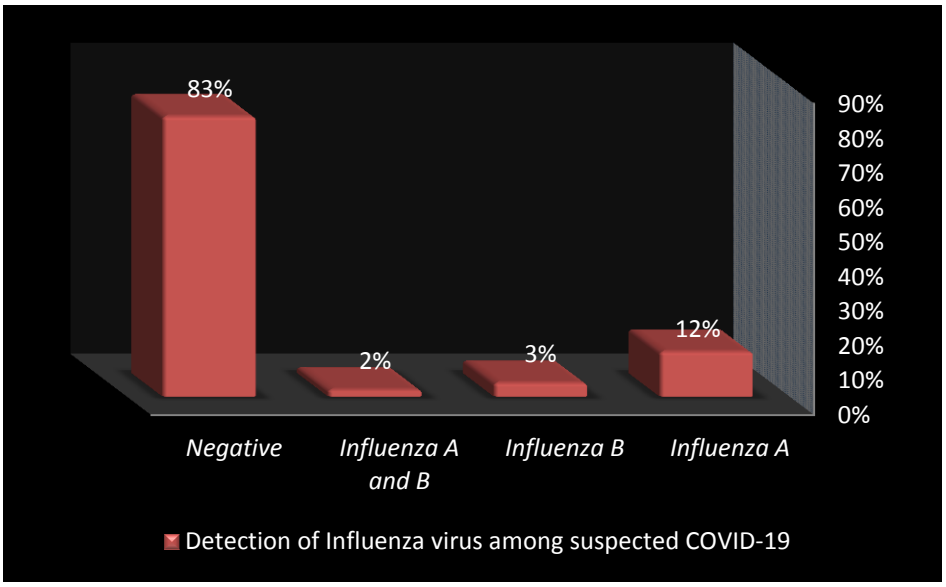

Figure (4.1): Patients' frequency distribution according to *Influenza* infections

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Table (4.1): The relationship between patients' COVID test and being *Influenza* positive

COVID		adene				Total	Chi-square	p-value
		type 1	Type2	both	negative			
	-ve	6(6%)	1(1%)	1(1%)	38(38%)	46(46%)	0.286	0.963
	+ve	6(6%)	2(2%)	1(1%)	45(45%)	54(54%)		
Total		**12(12%)**	**3 (3%)**	**2 (2%)**	**83(83%)**	**100(100%)**		

The above table showed that out of 54(54%) COVID positive patients, therewere 6 (6%) *have Influenza A,* 2(2%) have *Influenza B,* and 1 (1%) have *Influenza A and B.* Out of 46(46%) COVID negative patients, there were 6(6%) have *Influenza A,* 1 (1%) have *Influenza B* and 1 (1%) have *Influenza A and B.* The test showed no significant relationship between patient's COVID positive and being positive with Influenza, (Chi- squre = 0.286, **p**=0.963, in significance).

4.2 Discussion

The COVID-19 pandemic has reached most countries throughout the world, making the global situation serious. Due to the insufficient diagnostic sensitivity of the tests used to detect SARS-CoV-2 and the similarity between COVID-19 and influenza, early diagnosis of SARS-CoV-2 and influenza virus co-infection may be more problematic (Wu *et al*., 2020). A total of 100 suspected COVID-19 patients participated in this study, the result showed that out of 54(54%) COVID positive patients, there were 6(6%) have *Influenza A,* 2(2%) have *Influenza B,* and 1(1%) have *Influenza A and B* ,this result disagreed with (Dadashi *et al*., 2021) the rate of co- infection in Influenza type distribution was (A = 22 , B = 6 , both = 1) in the prevalence of *influenza A and B* this may be due to variation in sample size and geographical area. And agreed with co infection with both .these results also showed that *Influenza A* was higher prevelance among patients with COVID-19 than *Influenza B.* The result agreed with previous study (Dadashi *et al*., 2021) ,Also agreed with previous study (Simin *et al*.,2020) stated that most co infections were co-infected with *influenza virus A.*

Among 46(46%) COVID-19 negative patients there were 6(6%) have *Influenza A*, 1(1%) have *influenza B* ,and 1(1%) have *Influenza A&B* . This result agreed with (Sophie *et al*.,2020) showed (*influenza* A=34, B=22, both=1) in prevalence of COVID -19 negative and infection with both viruses , In the other hand disagreed with the rate of prevalence of *influenza A* and *influenza B*. which disagree with our study, this may be due to variation in sample size and geographical area.

Based on the results of this study suggested the no evidence association between patients COVID-19 postive and being positive with Influenza.

4.3 Conclusion

Based on the results of this study showed high prevalence of *Influenza A* co infection than *Influenza B* , and also showed infection with both viruses influenza A and B 2(2%) at the same time . This study highlights the importance of screening for co- infecting viruses in COVID-19 patients, or suspected COVID-19 patients due to the high prevalence of *Influenza* viruses

4.4 Recommendation

1. Further studies needed to be done on large scale in order to cover more cases to obtain more accurate and better results.
2. Implementation of preventive measures such as social distancing and mask wearing will decrease the infections.
3. Vaccination is strongly recommended to reduce the hospitalization and associated mortality.
4. Testing all COVID-19 patients for other viruses and /or bacteria.

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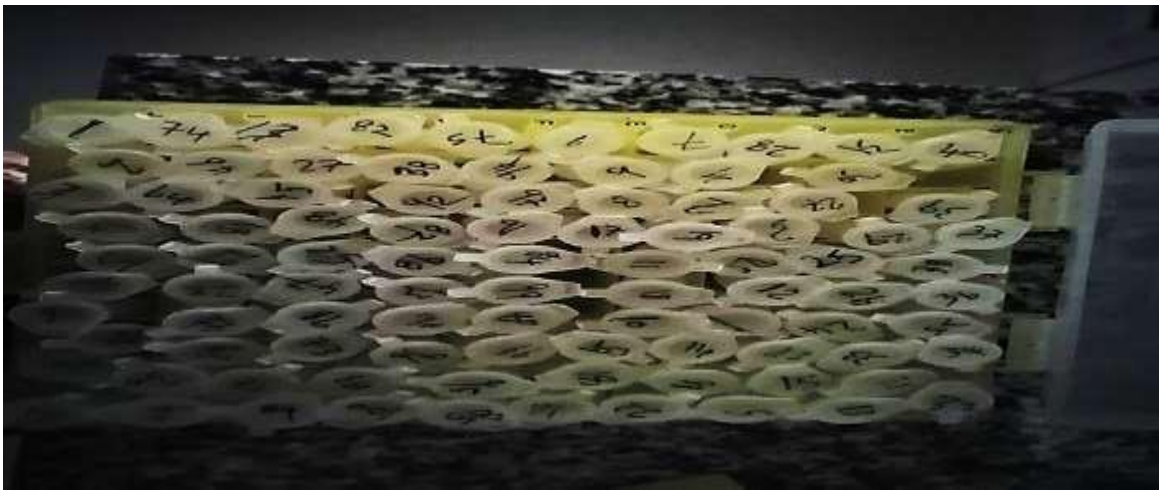
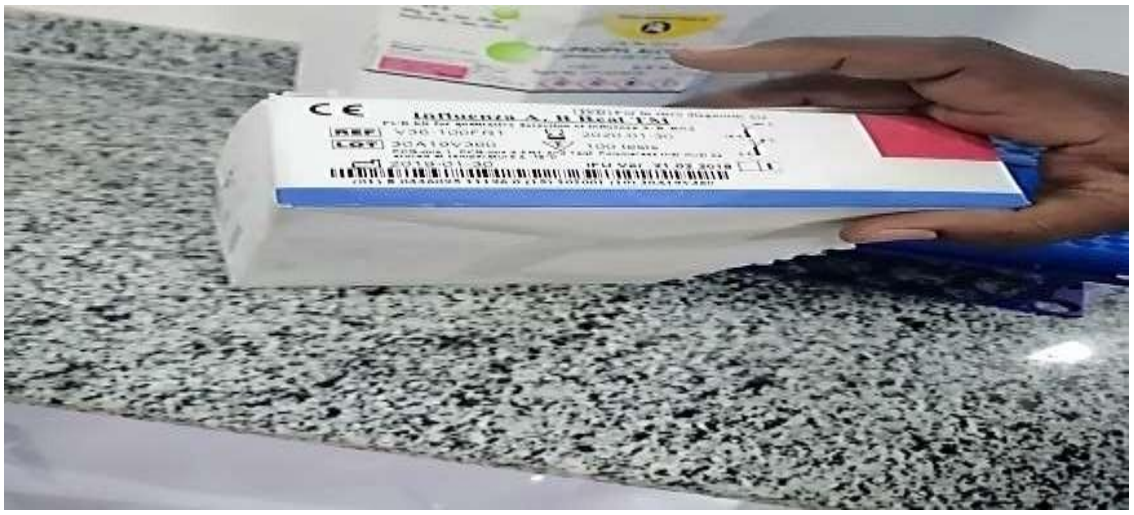
Appendices

APPENDICES



Appendix 1 Collected samples

Appendix 2 .RNA extraction kits, pipettes used in extraction and vortex mixer in Biosafety cabinets class 2



Appendix 3 Real Time PCR kits

Appendix 4.Real Time PCR machine connect with laptop