TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

INVESTIGATION OF INFLUENZA ACTIVITY DURING 2019-2020 WINTER SEASON IN NORTHERN CYPRUS

SAHEM MOWAFAQ A. ABUJAMOUS

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY DEPARTMENT

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According to the relevant articles of the Near East University Postgraduate study - Education and Examination Regulations, the members of the thesis committee and the decision of the Board of Directors of the Institute have approved this thesis.

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DECLARATION

I hereby declare that the work in this thesis entitled "Investigation of Influenza Activity during 2019-2020 Winter Season in Northern Cyprus" is the product of my own research efforts undertaken under the supervision of Dr**.** AYŞE ARIKAN SARIOGLU. No part of this thesis was previously presented for another degree or diploma in any university elsewhere, and all information in this document has been obtained and presented in accordance with academic ethical conduct and rules. All materials and results that are not original to this work have been duly acknowledged, fully cited and referenced.

SAHEM MOWAFAQ A. ABUJAMOUS

Signature

Date:

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SAHEM MOWAFAQ A. ABUJAMOUS

ABSTRACT

The current study is mainly focused on the investigation of Influenza Activity During the 2019-2020 Winter Season in Northern Cyprus. It was carried out on 844 patients with flu-like symptoms all age group patients. It is a cross-sectional study conducted from December 2019-March 2020 in Department of Microbiology, Near East University Hospital, Near East University, Faculty of Medicine, North Nicosia, Northern Cyprus. The study subjects included patients with suspected influenza symptoms of any age attending Near East University Hospital. The specimens were collected during 1 to 7 days onset of illness. The samples were collected from the period the winter season of 2019 and the beginning of 2020 spring and processed by rapid influenza diagnostic test method (ABON Biopharm Co., Ltd, Hangzhou, China). Out of total 844 clinical samples of patients with Influenza like illness (ILI), we encountered 401 (48%) male patients and 443 (52%) female patients. It was found that 11.5% (97/844) of Influenza A positive samples and 88.5% (747/844) were negative. However, 16.2% (137/844) of Influenza B positive samples and 83.8%(707/844) were negative. In additional, 49.5% (48/97) of Influenza A positive samples were male and 50.5% (49/97) were female, but 43.1% (59/137) of Influenza B positive samples were males and 56.9% (78/137) were females. In terms of gender, 47.3% (353/747) of Influenza A negative samples were male and 52.7% (394/747) were female, but 48.4% (342/707) of Influenza B negative samples were males and 51.6% (365/707) were females. The results of the study revealed that the highest positivity of Influenza virus was observed in the months of January, with a total of 980 analyzes. Of which, 65 (13.3%) were positive for influenza A and 83 (16.9%) were positive for influenza B, followed by February and December. Based on the results, it is concluded that Influenza virus was distributed throughout the 2019–2020 study period. Highest positivity of Influenza virus was found during the winter and the lowest prevalence in the spring season. In addition, it is concluded that the distribution of seasonal influenza types was not associated with gender during the period. However, the rate of both influenza type A and B have a statistically significant relationship with months and age. This initial study may also help surveillance and vaccine studies in the future.

ÖZET

Bu çalışmaesasolarakKuzeyKıbrıs'ta 2019-2020 KışSezonunda Grip Aktivitesininaraştırılmasınaodaklanmıştır. Tümyaşgrubuhastalarda grip benzerisemptomlarıolan 844 hasta üzerindegerçekleştirildi. YakınDoğuÜniversitesiHastanesi, YakınDoğuÜniversitesiTıpFakültesi, KuzeyLefkoşa, KuzeyKıbrısMikrobiyolojiAnabilimDalı'ndaAralık 2019-Mart 2020 tarihleri arasındayürütülenkesitselbirçalışmadır. ÇalışmakonularıYakınDoğuÜniversitesiHastanesinebaşvuran her yaştanşüpheli influenza semptomlarıolanhastalarıiçeriyordu. Örnekler, hastalığınbaşlangıcısırasında 1 ila 7 güntoplandı. Örnekler, 2019 kışsezonuve 2020 baharınınbaşlangıcıdönemindentoplanmışvehızlı grip teşhistestiyöntemi (ABON Biopharm Co., Ltd, Hangzhou, Çin) ileişlenmiştir. İnfluenza benzerihastalığı (GBH) olanhastalarıntoplam 844 klinikörneğinden 401 (% 48) erkekve 443 (% 52) kadın hasta ilekarşılaştık. İnfluenza A pozitiförneklerinin% 11,5'inin (97/844) ve% 88,5'inin (747/844) negatifolduğutespitedildi. Bununlabirlikte, İnfluenza B pozitiförneklerinin% 16,2'si (137/844) ve% 83,8'i (707/844) negatiftir. Ekolarak, İnfluenza A pozitiförneklerinin% 49,5'i (48/97) erkekve% 50,5'i (49/97) kadındır, ancak İnfluenza B pozitiförneklerinin% 43,1'i (59/137) erkekve% 56,9'u (78/137))kadındı. Cinsiyetaçısından, İnfluenza A negatiförneklerinin% 47,3'ü (353/747) erkekve% 52,7'si (394/747) kadındır, ancak İnfluenza B negatiförneklerinin% 48,4'ü (342/707) erkekve% 51,6'sı (365 / 707) kadındı. Çalışmanınsonuçları, influenza virüsününenyüksekpozitifliğinintoplam 980 analizleOcakaylarındagörüldüğünüortayakoydu. Bunlardan 65'i (% 13,3) influenza A içinpozitif, 83'ü (% 16,9) influenza B içinpozitiftirveonuŞubatveAralıkizlemektedir. Sonuçlaragöre, İnfluenza virüsünün 2019-2020 çalışmadönemiboyuncadağıldığısonucunavarıldı. Influenza virüsününenyüksekpozitifliğikışaylarındaveendüşükprevalansilkbaharmevsimindebul undu. Ayrıcamevsimsel influenza türlerinindağılımınındönemboyuncacinsiyetileilişkilendirilmediğisonucunavarılmıştır . Bununlabirlikte, hem influenza tip A hem de B'ninoranı, ay

veyaşileistatistikselolarakanlamlıbirilişkiyesahiptir. Bu ilk çalışma, gelecektesürveyansveaşıçalışmalarına da yardımcıolabilir.

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LIST OF ABBREVIATIONS

CDC: Centers for Disease Control and Prevention.

COPD: Chronic Obstructive Pulmonary Disease.

DFA: Direct Fluorescent Antibody.

EIA: Optical Immunoassay.

EIA: Enzyme Immunoassay.

H1N1: Hemagglutinin Type 1 and Neuraminidase Type 1.

HA: Haemagglutinin Activity.

HEF: Hemagglutinin Esterase Fusion.

HI: Hemagglutination Inhibition.

HRSV: Human Respiratory Syncytial Virus.

ILI: Influenza-like Illness

NA: Neuraminidase Activity.

NAT: Nucleic Acid Testing.

NEP: Nuclear Export Protein.

PCR: Polymerase Chain Reaction

POC: Point-of-care.

RNA: Ribonucleic Acid.

RNP: Ribonucleoprotein.

RSV: Respiratory Syncytial Virus.

VTM: Viral Transport Medium.

WBC: White Blood Cell.

WHO: World Health Organization

INTRODUCTION

1.1 General Information

Influenza is commonly known as flu. It is an infectious viral infection that infects the upper and lower respiratory tracts in the human body. Itis caused by various subtypes of influenza viruses (Schaffner *et al.*, 2018).

Influenza virus belong to *Orthomyxoviridae* family includes major viral pathogens, `Influenza virus being an important member of this group. Enveloped viruses belong to this group. The genome of this type of viruses is made up of negative sense single stranded segmented RNA. In terms of classification, the viruses of influenza are categorized into types A, B, C, and D. As proven scientifically, these types can generally inflict the same symptoms. However, they are antigenically unrelated. To explain this further, the infection caused by any one of these type does not necessarily guarantee immunity against the others. It has been identified that the viruses of type A are mostly responsible for spread of the great influenza epidemics in the world. IN contrast, the viruses of B type are not extensive but limited to localized outbreaks of smaller geographical spread. With regards to the viruses of type C, it has been noted that these types are only responsible for mild respiratory illness in humans. In addition, the type D of influenza viruses have not been reported as being infectious to humans. These viruses have only been identified in pigs and cattle (Hayden and Palese, 2009).

More specifically, the type A of influenza viruses is further divided into certain subtypes. This further classification is according to the type of surface proteins contained (Haemagglutinin (HA) and Neuraminidase (NA)). Evidently, influenza A (H1N1), Influenza A (H3N2) and Influenza A (H1N1) pdm09 viruses are found in humans. With regard to Influenza B viruses, they are not categorized into subtypes. They are rather grouped into lineages and strains. As of today, the Influenza B virus possesses mainly two co-circulating lineages. These lineages are commonly referred to as B/Yamagata and B/Victoria. In the study of these viruses, it is revealed that the surface glycoprotein antigens (HA and NA) can have at least two types of antigenic

variations. These two types are shift (major) and drift (minor). As it has been demonstrated scientifically, viruses of influenza Type A go through the two types of antigenic variation. On the contrary, influenza type B viruses have only type of antigenic variation, which drift only. It is asserted that antigenic drift happens all the time. Most of the studies of influenza indicated that antigenic shift of variation does not occur frequently but occasionally (Sambala et al., 2019).

Seasonal influenza is very common, and its major cause is mainly related caused to numerous subtypes of influenza viruses. It is commonly understood that the influenza viruses are usually prevalent in winter season. Nevertheless, these types of viruses are very much common all year round in the tropical regions (Acharya, Acharya, Phuyal, & Subramanya, 2020).The influenza virus causes damage to health. It principally attacks the upper and lower respiratory system of humans causing severe consequences on the normal functioning of the body. The symptoms resulting from the infection of these viruses are either mild or severe(Grohskopf et al., 2019).Generally, the well-known symptoms are in the form of high fever, runny nose, sore and throat. In addition, seasonal influenza may cause muscle and joint pain. More commonly, headache and coughing are symptoms of influenza. These symptoms can be felt after two days from infection and they largely last for less than a week. Nevertheless, coughing is different. It may last for some more time, approximately more than fourteen days(WHO, 2019).Influenza infection may also produce others symptoms in children. If infected by this seasonal disease, children might undergo diarrhea and vomiting, which are not prevalent in grownups. More specifically, such types of diarrhea and vomiting that may affect children are found to occur more in the form of gastroenteritis. This disease, gastroenteritis, is an unrelated. Many wrongly refer to as “stomach flu” or the "24-hour flu"(Engelkirk, 2020). Influenza infection can also cause severe complications. These complications can be in the form of viral pneumonia and sometimes secondary bacterial infection. In addition, sinus infections are considered as complications of influenza. More importantly, influenza damages and worsens previous deteriorating health conditions. It can complicate the health condition of patients suffering from asthma or heart failure(Boyd, 2006).

Worldwide, between 290,000 and 650,000 deaths happen every year, and they mainly affect to reducing the quality of life of individuals due to secondary infection (WHO, 2019). It is reported that people with the age range over 65 are susceptible to infection, and death among this age group is estimated at 90% (Kovács, 2014; WHO, 2019). Moreover, children are considered a vulnerable group to this type of infection. Globally, the annual attack rates among children reached20% to 30%. These rates of children are responsible for in home flu transmission (WHO, 2019). Consequently, the economic burdens during the influenza illness play a major role in transmitting the infection. Hospitalization can become difficult for them financially and they find no ways to go for medical visits (Fragaszy et al., 2018).

The seasonal epidemic is caused by the viruses of Influenza A, Influenza B and Human Respiratory Syncytial Virus (HRSV). These viruses inflict most of the people, causing viral respiratory infections. They in particular affect new-born below 96 weeks of age and those aged persons(Papillard Marechal et al., 2011). They are also responsible for morbidity and mortality in young kids, aged peoples and immunocompromised individuals. The most familiar symptoms of these viral infections are fever, cough, sore throat, and myalgia to more severe complication like bronchitis, pneumonia and death(Boivin, 2004).

Only three types of Influenza viruses, as indicated in many research studies, that have the ability to circulate in the human populations (Influenza virus A, B and C), Influenza A and B viruses are clinically important(Zambon; MC Stockton, JD Clewley, JP Fleming, & DM, 2001). Respiratory Syncytial Virus (RSV) can be subtyped into Type A and Type B, these types are commonly circulating in the human population and can cause respiratory complications. Influenza viruses cause respiratory illness in millions resulting in 500,000 deaths occurs each year (Nicholson). Annually, about 20% of children and 5% of adults all over the world get infected by influenza (Turner et al., 2003). Similarly, RSV also 40% - 90% of bronchiolitis cases were observed in less than 5 years age group children and 50% of pneumonia cases were observed in less than 2 years age group children (Paramore, Ciesla, & Liu, 2004). Many research studies confirm that respiratory virus infections remain a challenge for public health. This can be attributed to their prevalence all over the world and the ability to spread very quickly

in societies. The tremendous morbidity that influenza viruses possess and the mortality rate that result from infection continue pose threats to human lives. Despite all the efforts, new respiratory viruses will continue to occur because of their genomic nature (Kesson, 2007).

1.2 Virus types

In virus classification, influenza viruses can be categorized as negative sense RNA viruses. They are closely related to human parainfluenza viruses. Further classification of these viruses places them in the *paramyxovirus* family. This family of viruses is largely responsible for causing respiratory infections in children such as croup. Moreover, the same viruses are also known to cause influenza-like illness in adults. (Grohskopf et al., 2019; Hall, 2001).

1.2.1 Influenza Virus A

This type of virus mainly exists in wild aquatic birds. These birds are considered as the main natural hosts for this influenza A type of viruses. Nevertheless, viruses often spread and inhabit other species. When this happens, viruses spread and ultimately cause outbreaks in domestic poultry. They sometimes spread among humans leading to epidemics. Influenza A virus are classified and divided into several serotypes. This is done with regard to the compliance with the antibody response that these type of viruses get (Hall, 2001).

The serotypes of influenza viruses which are confirmed to exist and infect in the human body are outlined below:

- H1N1, caused Spanish flu, (in 1918).
- H2N2, caused Asian Flu, (in 1957).
- H3N2, caused Hong Kong Flu, (in 1968)
- H5N1, caused Bird Flu (in 2004)
- Swine Flu (in 2009)
- H6N1, only infected one person.
- H7N7, which has extraordinary zoonotic potential
- H1N2, endemic in humans, also in pigs and birds

- H9N2, H7N2, H7N3, H10N7 and H7N9, which were classified in 2018 as having the greatest epidemiological potential among the Type A subspecies.

1.2.2 Influenza Virus B

This virus belongs to one species. It is mainly associated with humans, and it is considered less common, compared to the other types. The type B of influenza virus has the ability to make mutilations rated 2–3 times slower than type A. Hence, it is regarded as less genetically diverse. It possesses only one influenza B serotype. Since it does not have high antigenic diversity, this type of virus is easily overcome. People can acquire immunity against this type of virus from a very young age. Its low antigenic changes prevent it from occurring. In addition, many studies concluded that this type of virus has limited host range. It inhibits cross species antigenic shift, and thus limiting its occurrence (Gatica-Wilcox, 2014; Nobusawa & Sato, 2006; Osterhaus, Rimmelzwaan, Martina, Bestebroer, & Fouchier, 2000).

1.2.3 Influenza Virus C

Influenza C virus affects humans, dogs, and pigs. It very often causes severe complications. Moreover, it has the ability to spread as a local epidemic. Nevertheless, this type of viruses is not very common. Besides, only mild symptoms are report about the infection of this virus(Matsuzaki et al., 2006; Taubenberger & Morens, 2008).

1.2.4 Influenza Virus D

It is the fourth type of influenza viruses. It was identified in 2016 and it was first isolated in 2011. This type of virus belongs to one species that only inhabits in pigs and cattle. However, it has been reported that Virus D can affect humans(Su, Fu, Li, Kerlin, & Veit, 2017).

1.3 Pathology of Influenza Virus Infections

The pathology of influenza viruses can be described as an antigenic type of viruses. They are genetically part of the family *Orthomyxoviridae*. This family of viruses contains a negative sense and they possess a segmented RNA genome (single-stranded).

1.3.1 Pandemic

Influenza viruses are able to cause global outbreak. Nevertheless, this can mainly happen when they possess a new strain of influenza A virus, which exclusively contain the hemagglutinin protein. What characterizes this type is that it can easily be transmitted among people.

1.3.2 Pneumonia

Influenza viruses are largely responsible for causing inflammation in the lower respiratory tract. This inflammation results from the infection of many microorganisms (bacteria and viruses), among them the influenza viruses(Hayden and Palese, 2009).

1.3.3 Seasonal (Epidemic and Interpandemic)

In most of the cases, influenza viruses are seasonal. They affect millions of people in winter. The annual occurrence of influenza A or influenza B virus in winter season is very common(Schaffner *et al.*, 2018).

1.3.4 Highly Pathogenic Avian Influenza (HPAI)

Influenza viruses can mutate and get transformed. One of the types of avian influenza virus mutate at the site of hemagglutinin division producing a fatal virus that infects poultry(Swayne and Suarez, 2000).

1.4 Virus Transmission

Influenza is a very common disease. It is prevalent during winter. Generally, influenza viruses spread during the colder months of the year. In principle, the Influenza viruses can be transmitted in three main different ways:

1- Through a direct transmission from one person to the other. This happens usually through the respiratory tract by inhaling the infected droplets that come out from coughing and sneezing

2- Through hand to eye, nose, mouth transmission.

3- Through surfaces contaminated or direct personal contact(Hall, 2007; Weber & Stilianakis, 2008).

The order of these three steps is not according to importance. The virus can spread in either ways, and there is no clear-cult method of transmission. What is evident is that all these three steps can contribute to the fast spread of the virus. Since they can be transmitted through air, one small droplets of cough (0.5 to 5 μm in diameter) is enough to cause an infection of influenza. When the virus particles enter into the human body, they start attacking the ciliated epithelial cells that line the upper respiratory tract. They selectively destroy them causing damage to the bronchial tubes and trachea. It has been confirmed that the incubation period of the influenza infection can extend from one to two days. The symptoms begin to appear after this period causing sudden and distinct chills, and muscle aches (Brankston, Gitterman, Hirji, Lemieux, & Gardam, 2007; Cole & Cook, 1998; Weber & Stilianakis, 2008).

1.5 Influenza Diagnosis

The flu can be difficult to diagnose, because it's more pronounced symptoms (cough, fever, and aches) have much in common with other illnesses. Clinicians have several different methods of identifying and determining whether a patient is infected by the influenza virus or not. Today healthcare providers have developed certain diagnostic tests which give results within less than half an hour. To test the patient for the presence of influenza, the lab technician takes samples from the patient's nose or throat. Nevertheless, such types of test are not 100% reliable. They sometimes bring out misleading results, either false positive or negative. Hence, they cannot be used to determine the specific strain of influenza that a patient may carry (Deslandes, & Frost, 2003).

Nevertheless, there are other reliable methods that can be used. These methods are found to be more accurate. However, using these methods requires healthcare providers to dispatch certain samples of a patient's throat cells, saliva, or other biological materials

to be examined in a specialized lab. These methods, as it has been proven, often cultivate the virus itself, or sometimes they can be used to cultivate the RNA inside it, allowing for a more accurate assessment of the virus's properties and origin. Therefore, the results of these methods take much time. The delay in diagnosis may affect the effectiveness of the treatment. However, these testing methods are still applied particularly during epidemics. Given all these testing methods, there are some more common tests that are used for detecting the flu. These tests are viral culture as well as rapid antigen testing. The rapid molecular assays are also widely used in which reverse transcription polymerase chain reaction is employed (PCR) (Formica, Treanor, & Walsh, 2003).

It is a common event among respiratory viral pathogens to cause similar clinical conditions for which differential diagnosis of the pathogens is essential. Currently used methods for diagnosis of respiratory viruses are rapid antigen detection assessments, virus culture, immune fluorescence and real time PCR (Falsey, Formica, Treanor, & Walsh, 2003; Ruest, Michaud, Deslandes, & Frost, 2003).

Molecular diagnostics such as RT-PCR, Real time PCR are currently used for detecting most of respiratory viruses because of their better sensitivity. These methods can also be employed to identify more than one pathogen in a single reaction (Multiplex PCR). The aim of all this is to reduce time and cost, which make the assay cumbersome, error prone, expensive and increases chances of cross contamination. Commercially available multiplex PCR assays are more costly and need dedicated instruments and kits (Hindiyeh, Hillyard, & Carroll, 2001).

During the COVID-19 pandemic outbreak, numerous studies have shown that it can be possible to detect and diagnose both influenza and COVID-19. Hence, a patient might be infected by the two types of viruses. Recently, on the first of this year, the COVID-19 appeared, which has flu-like symptoms, such as fever and body aches, but the symptoms of influenza usually do not include shortness of breath (Khani, Tabarraei, & Moradi, 2018).

1.6 Treatment

Usually, all we need to do to get over the flue is to take enough rest. In addition, it is advisable to take plenty of fluids to treat the flu. However, when there are serious complications, the prescription of some antiviral medications is a must. Doctors usually prefer prescribing oseltamivir (Tamiflu), zanamivir (Relenza), peramivir (Rapivab) or baloxavir (Xofluza). In many clinical studies, these drugs have been proven effective in shortening the period of influenza effectiveness, and they also help in preventing serious complications from occurring(Couch, 2000).

Oseltamivir is an oral medication usually prescribed for treating the flu. It is inhaled and used only by those patients suffering from certain chronic respiratory problems in the form of asthma and lung disease. There are some side effects associated with using antiviral medications. These side effects range from nausea and vomiting. Nonetheless, they can be reduced especially when the patient takes the prescribed medicine with food. Surprisingly, most of the circulating flu strains have developed certain resistance to some medications. For example, amantadine and rimantadine (Flumadine) are revisited by the flue, and they no longer have an effect. Given this reason, these medications are not recommended any more (Couch, 2000)

.

Because of the increasing threats that are repeatedly posed by seasonal influenza annually, the World Health Organization (WHO) highly recommends the use of non-pharmaceutical and pharmaceutical interventions (R. Lehnert, M. Pletz, A. Reuss, & T. Schaberg, 2016),(WHO, 2018). The suggested procedures and medications are deemed effective in preventing and mitigating the effects of influenza infection. The recommended non-pharmaceutical interventions are represented by basic hand hygiene, coughs covering, and the use of personal protective equipment. These protective equipment are masks, gloves, and gowns, and they are now very common in the fight against COVID-19. These are the first-line methods that are supposed to containing and prevent the infection. These procedures can be effective in delaying the spread of the virus, and reducing transmission. Besides, pharmaceutical interventions are still widely used to prevent the flue. These medications are in the form of trivalent, quadrivalent

vaccines or therapeutic, including the use of antiviral medications, such as oseltamivir, zanamivir. More importantly, it has been reported that the effective use of antivirals against influenza requires treatment within the first 48 hours of infection. Once the symptoms appear, it is essential to start treatment so that to prevent the virus can be successful(R. Lehnert, M. Pletz, A. Reuss, & T. Schaberg, 2016).Vaccination, according to many scientific studies, is still considered a perfect and proven strategy to limit the spread and curtail the transmission of influenza. However, to yield the best outcomes, the vaccines that are given should be administered every year. The vaccination process should be done before start of the flu season. WHO recommends that viruses be included in the formulation of the influenza vaccine formulation (WHO, 2018).

While vaccination remains the primary strategy for protecting a population from influenza, it apparently seems that a global shortage of supplies and medication can deprive people from getting hospitalized at the right time. In addition, the unequal access to influenza vaccines remains an obstacle for many nations. Although capacity to produce influenza vaccine has multiplied, only few of these vaccines have been distributed despite their introduction(Sambala et al., 2019).

1.7Influenza and Immune response

Upon detection of influenza virus infection, human immune system aims to defend against and clear the viral infection by innate and adaptive immune responses(Kreijtz, Fouchier and Rimmelzwaan, 2011).

The initial phase of the influenza virus infection activates the Innate immune response as a first line of defense against the infection. The innate immune response consisting of physical barriers (mucus and collections), alveolar macrophages, natural killer cells, dendritic cells, group of cytokines, interferon's (IFNs), and IFN-stimulated gene. The innate immunity plays a critical role in initiation the adaptive immunity response. The cellular adaptive immunity is mediated by lymphocyte cells (B cells and T cells). T cells are mainly known as $CD4^+$ T and $CD8^+$ T cells are characterized with antigen-specific memory cells, capturing and neutralizing the pathogen. The activation of $CD8^+$ T cells leads to migration to the infection site where they detect the infected cells and eliminate them via lyric activity and inhibit the virus progeny production.

Whereas, the humeral adaptive immunity response produces antibodies against different influenza antigens. $CD4^+$ T cells contribute to B cell maturation and proliferation to plasma cells for enhances the antibody production. So, the inherited factors and age-related changes are associated with the host immune responses. A decline number of lymphocytes in the elderly lead to more susceptibility to infection and consequently, responsiveness of vaccine is compromised, especially in elderly individuals(Kreijtz, Fouchier and Rimmelzwaan, 2011; Bahadoran *et al.*, 2016; Chen *et al.*, 2018).

1.8 Influenza and COVID-19

Based on the clinical features and mechanism of spread, it seems that both Influenza and corona viruses in terms of spread mechanism (COVID-19) are similar in many ways. These two viruses are described as contagious, and they ultimately cause respiratory illnesses. However, they are seemingly caused by different viruses. Recently designated as a pandemic, COVID-19 is caused by a novel virus, namely coronavirus 2 (SARS-CoV-2). On the other hand, Influenza is similarly an infectious respiratory disease. Its main cause belongs to types A and B of influenza viruses (Kaur, Posimreddy, et al., 2020).

In terms of origin, Coronavirus disease (COVID-19) was first identified in Wuhan city of Chinese in a Seafood Market. Its spread is pervasive, and it has killed millions of people till date. The whole world crimpled in the attempt to face this pandemic. It has posed a dangerous global health threat that recurrently results in many consequences. The COVID-19 symptoms are manifested in a spectrum of clinical effects. The commonly reported symptoms are fever and cough. In addition, the symptoms extend to myalgia and dyspnea. The less frequent symptoms that have been recorded include vomiting, diarrhoea and nausea. More prominently, COVID-19 is associated with severe respiratory symptoms. Nevertheless, there can be many other problems resulting from the infection of this virus. These include multiple organ dysfunctions. Coagulopathy has also been regarded as a prominent feature of COVID-19.It is indicated that severe coagulation dysfunction may because due to poor prognosis

(Kaur, Posimreddy, et al., 2020; Kaur, Qaqa, et al., 2020). In addition to all this, COVID-19 has been reported to cause severe neurological and cardiovascular complications. Till date, there has been no declared effective for the treatment of COVID-19 (Kaur, Qaqa, et al., 2020).

Zhiqi Yang and et al. (2020) studied the features of COVID-19 and they reported that there are some prominently different features that differentiate COVID-19 from pneumonia caused by influenza. In the early stages, it was reported that the crazy-paving pattern is regarded as the most powerful imaging feature. In later stages, the study found out that WBC count resulted in the highest diagnostic efficacy in clinical manifestations(Z. Yang et al., 2020).

Due to the paucity of published data on the widespread of influenza infection in Northern Cyprus [23], the current study, we aimed to investigate the influenza activity during Winter Season (2019-2020).

REVIEW OF LITERATURE

2.1 The Influenza Viruses

Influenza viruses pose a definite challenge to mankind and numerous animals.

The disease caused is responsible for high mortality rate and causes enormous economic loss among domesticated animals each year. Epidemics appear in the human population at frequent intervals. The Influenza virus continues to be a constant threat to the human population and animals as it was evident from the 2009 pandemic and the strain circulation thereafter at frequencies(Kawaoka & Neumann, 2012).

2.1.1 Classification

Influenza viruses, in terms of classification, are grouped under the family Orthomyxoviridae. This family is further divided up into the following five genera- Influenza virus A, Influenza virus B, Influenza virus C, Thogotovirus and Isa virus(Kawaoka & Neumann, 2012). Influenza A and B viruses infects human beings and cause annual epidemics. Influenza A viruses are further divided into sub-types based on the surface proteins (HA and NA). During the last century, H1N1, H3N2, H2N2 and H1N2of Influenza A subtypes are reported to have been circulated in humans. There are no Influenza B virus subtypes recognized (Osterhaus et al., 2000).

As indicated by many researchers, the influenza A, B, and C viruses, account to three genera out of the five genera that constitute the family *Orthomyxoviridae*. These genera are known well by their segmented type of negative-strand RNA genomes. In terms of sequencing, all these viruses reflect that they have a common genetic ancestry. However, research confirms that these viruses are genetically diverged. Re-assortment, for instance, and the viral RNA segments exchange that occurs between the different types of viruses clearly indicate that these procedures can occur within each genotype. Nevertheless, these procedures cannot happen across types. More importantly, a further classification of Influenza A viruses brings us several subtypes based on their surface of glycoproteins, the hemagglutinin (HA), and the neuraminidase (NA). Regardless of all classifications, influenza viruses are named

in a standardized way. This nomenclature divides viruses according to their type, species, location, and also isolate number and year. With regard to influenza A viruses, they are categorized as HA and NA sub-types. Despite the large number of genetically distinct subtypes, such as – 16 for HA and 9 for NA, it has been observed that influenza A viruses are grouped under three HA (H1, H2, and H3) and two NA (N1 and N2) subtypes. Only these viruses have been reported to cause human epidemics (Shaw & Palese, 2013).

2.1.2 Virus Structure and Organization

There are around eight segmented RNA molecules within the Influenza A genome, which are packed into ribonucleoprotein (RNP) complexes (Palese, 1977). As demonstrated by many studies, in the RNPs, it has been noted that the nucleoproteins (NP) are in association with the RNA molecules. These molecules are in possession of an RNA dependent RNA polymerase complex. This complex is composed of 3 sub-units. These sub-units are namely, (1) polymerase basic protein 1 (PB1), (2)polymerase basic protein 2 (PB2) and (3) polymerase acidic protein (PA)(Lamb, Etkind, & Choppin, 1978).

The major protein constituent of the RNP happens to be the NP, nucleoprotein which forms a core. The RNA is wrapped by RNP to form a helical structure around it. The matrix protein 1 (M1) surrounds the RNP, which is again enclosed by an envelope (Figure 2.1). The precise interaction of M with the RNPs and viral envelope has not been ascertained. The major antigenic determinants that contribute to the classification of the virus are HA and NA. The variations like antigenic drift and antigenic shift are responsible for the occurrence of outbreaks, epidemic and pandemics. The NEP or NS2 is present in the virion which interacts with the M1 protein (Lamb, Etkind, & Choppin, 1978).

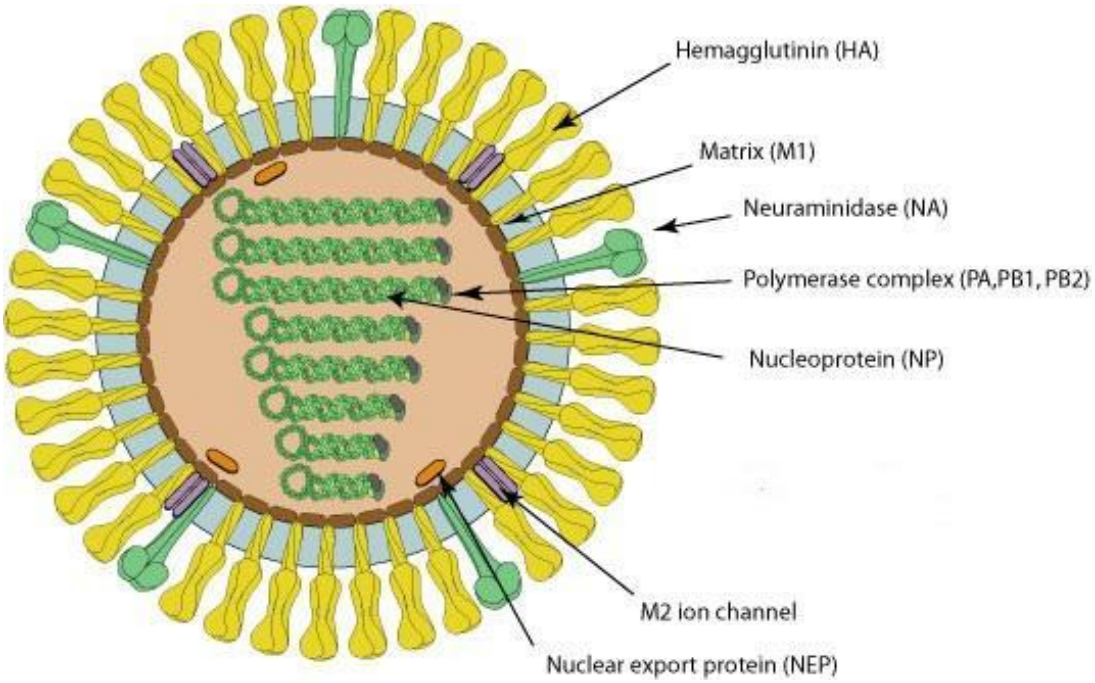


Figure 2.1: Influenza A virus (SURESH V, 2017)

By electron microscopy, it is not easy make a difference between the viruses of Influenza A and those of B viruses. In terms of shape, influenzaisspherical or filamentous. It can have spherical forms, and these forms are typically ordered in 100 nm in diameter. As it is shown, the filamentous forms of the influenza virus are often in more than 300 nm in length. The virion that constitutes influenza A is composed of glycoprotein spikes that are HA and NA. It is of a ratio that is approximately four to one(Shaw & Palese, 2013). In the structure, there appears a smaller number of matrix (M2) in the form of ion channels. These types of channels traverse the lipid envelope (Zebedee & Lamb, 1988).

As indicated in the schematic view, there are three integral membrane proteins (HA, NA, and M2) that are seen overlaying with an envelope forming a matrix of M1 protein. This matrix covers the virion core. In the Internal side of the M1 matrix, we find a nuclear export protein (NEP). We also find the ribonucleoprotein (RNP) complex. This RNP is seen composed of the viral RNA segments. These segments are fully coated with nucleoprotein (NP), in addition to the heterotrimeric RNA-dependent RNA polymerase. In a similar manner, the influenza B virion is found to be of the same organization. It possesses four different envelope proteins. They are namely HA, NA, NB and BM2. Structured differently, influenza C virions are unique in their form.

They are completely different from the A and B viruses. The notable difference is in their reaction to the infected cell surfaces. These virions can form long cordlike structures showing more than 500 μm. Nonetheless, in terms of composition, it is confirmed that influenza C virions are the same. They are made up of a glycoprotein-studded lipid envelope. In their structure, we find overlying protein matrix and the RNP complex. The influenza C viruses, however, possess only one surface glycoprotein, the hemagglutinin-esterase-fusion (HEF) protein, corresponding functionally to the HA and NA of influenza A and B viruses. It also has one minor envelope protein, CM2 (Shaw & Palese, 2013).

2.1.3 Genome Structure

The genomes of influenza A and B viruses are made up of eight negative-sense, single-stranded RNA segments. Contrarily, the influenza C virus harboursonlya specific seven-segment genome as shown in table 1. The segments of the types A and B of influenza are arranged in a decreasing order. In the same manner, the segments of the type C of influenza virus carry numbers in decreasing length. In the viruses of the influenza type A and B, there are segments that start from 1 to 5. They only encode one protein for each segment: the PB2, PA, HA and NP proteins. All influenza viruses, as indicated above, have the ability to encode the polymerase subunit PB1 on segment 2. However, in some of the strains of influenza typically the A virus, the segment contained can also make codes for the accessory protein PB1-F2(Chen et al., 2001). Mainly, it has been reported that the PB1-F2 has identifiable analogue either for B or C types of influenza viruses. On the other hand, it is realized that the segment number 6 of the influenza A virus has the ability to encode only the NA protein. This limited ability is also contrasted with that of the influenza B viruses. This type of the viruses can encode both of the NA protein(Hatta & Kawaoka, 2003). Moreover, studies have shown that the segment number 7 that is part of both the viruses of influenza A and B types can make the necessary codes for the M1 matrix protein. Generally, the genome of the influenza A is also expressed differently from the segment number 7.It is separated by the RNA splicing process(Horvath, Williams, & Lamb, 1990). Influenza B virus encodes its BM2 membrane protein in a +2 alternate reading frame (Briedis, Lamb, & Choppin, 1982; Horvath et al., 1990). Finally, it is found that both the viruses of the influenza A and B have a single RNA segment. They also possess segment 8,

which they utilize to express the interferon-antagonist NS1 protein (Dauber, Heins, & Wolff, 2004; Kochs, García-Sastre, & Martínez-Sobrido, 2007). and, by mRNA splicing, the NEP/NS2 (Briedis & Lamb, 1982; Lamb, Choppin, Chanock, & Lai, 1980). In terms of genomic structure, it is noted that the viruses of influenza Care in possession of pattern that is similar to that of influenza A and B viruses. Nevertheless, the HEF protein, which is an important component of influenza C, is positioned to replace the HA and NA proteins. This process thus leaves the genome of the influenza virus type C with one segment less, compared to those in influenza A or B viruses.

Segmenting	Length of Segment in nucleotide	Encoded Protein(s)	Protein length in amino acids	Protein function
1	2341	PB2	759	Polymerase subunit; mRNA cap recognition
2	2341	PB1	757	Polymerase subunit; RNA elongation, endonuclease activity
		PB1-F2	87	Pro-apoptotic activity
3	2233	PA	716	Polymerase subunit;protease activity
4	1778	HA	550	Surface glycoprotein; major antigen, receptor binding and fusion activities
5	1565	NP	498	RNA binding protein nuclear import regulation
6	1413	NA	454	Surface glycoprotein; sialidase activity, virus release
7	1027	M1	252	Matrix protein; vRNP interaction, RNA nuclear export regulation, viral budding
		M2	97	Ion channel; virus uncoating and assembly
8	890	NS1	230	Interferon antagonist protein; regulation of host gene expression
		NEP/NS2	121	Nuclear export of RNA

Table2.1: The genomic segments and the encoded proteins of influenza A (Puerto Rico, no date).

The source of the data in the table above is from Puerto Rico/8/1934 (H1N1). It is reported that the ends that form each of the vRNA segment composes a helical

hairpin. This helical hairpin is surrounded by the heterotrimeric RNA polymerase complex. In general, the rest of the segmenting endsaretotallycoveredbyarginine-rich NP. As it is indicated in the table, it seems that the net positive charge of arginine-rich NP is in binding position with the negatively charged phosphate backbone of the vRNA(Baudin, Bach, Cusack, & Ruigrok, 1994; Murti, Webster, & Jones, 1988). Clearly, the table shows that the segments and each of their vRNAhave non-coding regions. In most of the cases, these regions are composed of several varying lengths, ranging from three to five ends. Despite all this, the extreme ends of all the segments can clearly be found in all the types of influenza virus. They are partially considered as complementary terminals in pairs, and they mostly serve as promotion for replicating the vRNA replication. They are also in a position to induce transcription process that function as the viral polymerase complex (Baudin, Bach, Cusack, & Ruigrok, 1994).

The most important task of a segmented genome is to enable antigenic shift. In this process, the virus A type of influenza and its constituting strain usually attempt to possess the HA segment. It may also possibly try to possess the NA segment from another type of influenza virus, even if it is of a different sub-type. This is often known as segment re-assortment. It happens in cells that are infected by viruses that are able to inhabit both human and animals. The result is usually encoding completely novel antigenic proteins. Unfortunately, human populations have no pre-existing immunity to such novel antigenic proteins. The Pandemic that can result from influenza is created when the antigenic shift of the influenza is able to produce a virus that the immunity of the human body cannot resist. This can occur due to the naivety of the immune system. It is argued that the Antigenic shift was the reason behind the Spanish flu that spread during 1918–1919. The lethality of that virus was unprecedented and it killed millions of people. By clinically examining the characteristics of the virus of 1918(Spanish flu), it was revealed that its genes and the uniqueness of their constellation were very much behind the virulence that the virus had. In addition, it was reported that other factors such as the HA, NA, and also acquiring PB1 genes were all responsible and contributed much to the pathogenicity that distinguished the Spanish flu(Pappas et al., 2008; Tumpey et al., 2005). It seemed that the international

spread and outbreak of that pandemic (Spanish flu) was largely ascribed to its ability to acquiring antigenic novel surface proteins. Most of the people in the world at that time were immunologically naïve to that type of virus (Ahmed, Oldstone, & Palese, 2007).

2.1.4 Cycle of the Influenza Replication

2.1.4.1 Attachment of the Virus

The different types of Influenza viruses have the ability to recognize N-acetylneuraminic sialic acid on the surface of the cell that hosts it. These types of acids are composed of nine-carbon acidic monosaccharides and they are to be found positioned at the terminals of many glycoconjugates. Given its ability to attach itself, The carbon-2is part of the terminal sialic acid. It therefore attaches itself to tightly to the walls of the carbon-3 or carbon-6 of galactose. They subsequently form α-2,3- or α2,6-linkages. The linkages, namely those of α-2,6, are predominantly found in the tracheal epithelial cells of the human body. More specifically, the linkages of α-2,3 are largely found in the gut epithelium of ducks. It is known that human respiratory epithelium possesses also sialic acids. These acids are themselves in possession of certain terminalα-2,3-linkages.However, these linkages are less in number compared to those linkages of α-2,6 (Couceiro, Paulson, & Baum, 1993; Matrosovich, Matrosovich, Gray, Roberts, & Klenk, 2004); consequently, with the presence of the sialic acids, it is possible that humans can catch infection caused by the several types of avian influenza viruses. Nevertheless, such type of infection is considered of less effective when compared with that of human strains(Beare & Webster, 1991; Tian et al., 1985).

In terms of differential expression, the sialic acids are very important since it demonstrates the low infectivity in the respiratory tract of the mammalian. It can also assist in measuring the extent of pathogenicity that some avian strains possess. It is realized that the proteins of α-2,3-linked sialylated are very much common in humans. They cause problems in the lower respiratory tract (bronchioles and alveoli). Technically, the upper respiratory tract is most susceptible to airborne virus particles. The lungs, however, are less accessible, and this fact explains the reasons why it is rare

to find the infections caused by avian virus in the human body. Despite all this, the strains of the avian can make serious infections to the lungs of humans, and when that happens, it immediately leads to severe complications.

In terms of structure, the molecule of HA is characterized by a trimer, consisting of two regions of distinct structures. These regions are made up of a stem, which ideally comprises alpha-helices. They are in the structure of a triple-stranded coiled-coil. There is also a head of globular shape and beta-sheet that is antiparallel.(Wilson, Skehel, & Wiley, 1981). The head of this type of structure comprises the sialic acid and the site that serves as a receptor(Webster, Laver, Air, Palese, & Kingsbury, 1983).In the replication process that the virus undergoes, serine proteases cleaves the HA protein into HA1 and HA2. This type of modifying seems very significant in terms of the virus's ability to infect. Moreover, the portion that constitutes the HA2 plays a very important role in mediating the fusion process of the envelope of the virus. The HA1 portion of the virus is also seen to contain the binding of the receptor. It also controls the antigenic sites of the virus(reviewed in (Steinhauer, 1999). Most of the studies in these regards have confirmed that the antibodies that are against HA have a role in neutralizing the virus infectivity. Based on this, the strains of the virus cultivate amino acid more frequently. Hence, they modify to a certain extent the structure of the antigenic sites. On the other side, the HA molecule configuration, that is stem-head in shape, is positioned firmly within numerous strains and many different sub-types. As indicated below, these observed changes form a process that is often termed as 'antigenic drift'. After all of these processes, some antigenic sites undergo multiple mutations. The outcomes usually a virus type that is of less affectivity virus (David Tuller . 2009;Shaw & Palese, 2013).

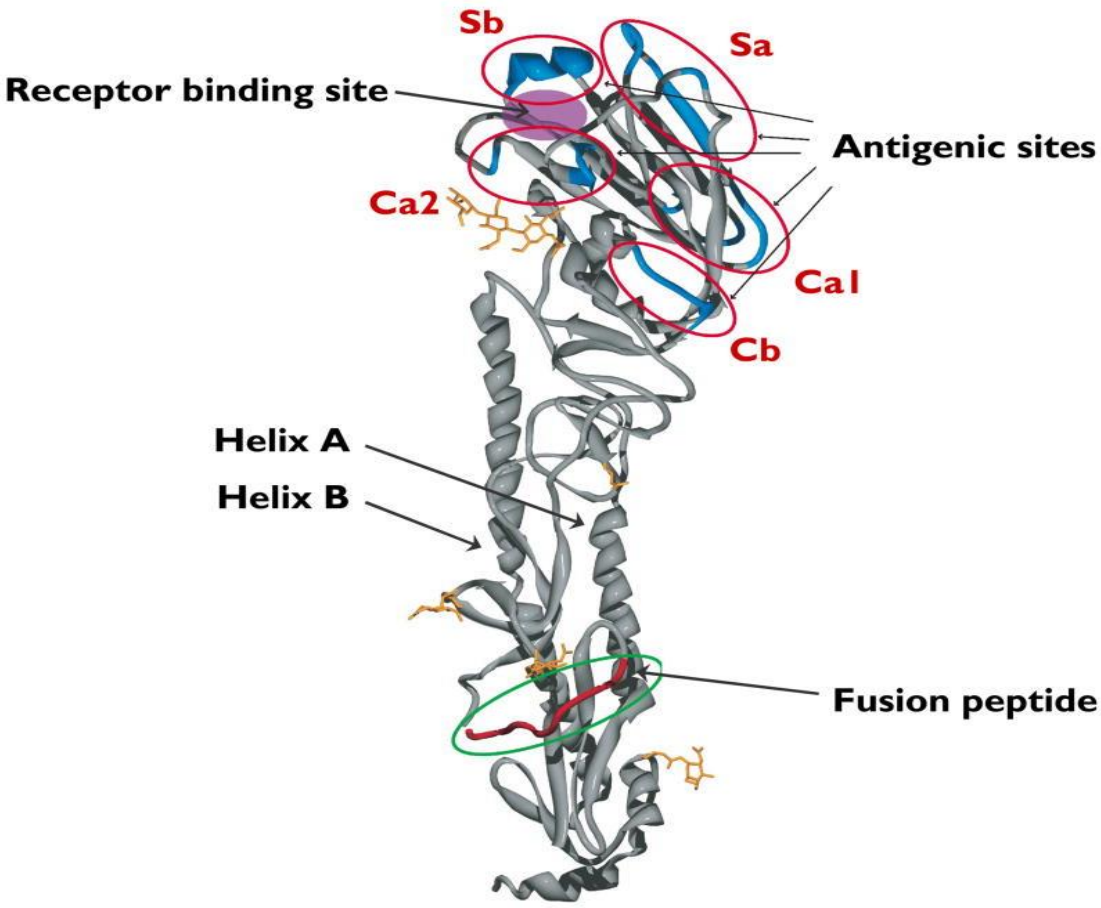


Figure 2.2: Ribbon diagram featuring 1918 influenza A virus(Shaw & Palese, 2013)

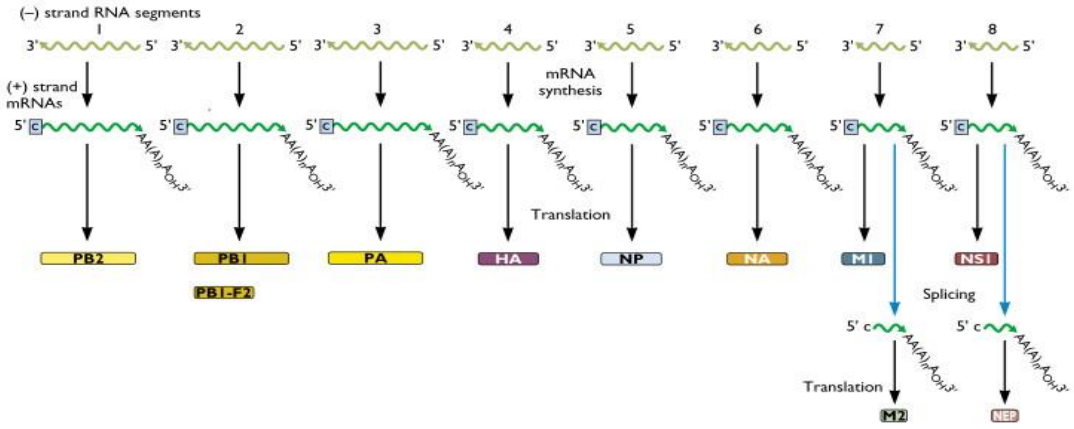


Figure 2.3: Influenza Virus RNA Genome (David Tuller . 2009)

There are eight segments that constitute the general structure of viral RNA within the

virion of the influenza A. As it is shown above, the molecules of this type of virus carry all the information that is required in the process of making new particles of influenza virus. Apparently, the 8 RNAs of the virion are displayed schematically. They appear as olive-green lines as it can be seen at the top of the illustration. The 8 numbers of RNAs are characterized as a chain of nucleotides; all appear in four different chains of A, C, G, U. With respect to the structure of the influenza virus, it is confirmed that the eight RNAs which exist in the structure of this virus constitute more than 14,000 nucleotides in total length. The genetic code of this virus is made up of the nucleotides(Han *et al.*, 2018; Xue *et al.*, 2018).

The viral aspects of these RNAs are deemed significant and we must consider them for certain reasons. Firstly, it can be seen that the several ends that are part of the RNAs have labels of 3′ and 5′. Hence, the nucleic acids of these possess a certain type of polarity. One end of the chain, as it can be recognized, is totally different from the other only chemically. This type of polarity can be noticed clearly as it is reflected in the labels either by 5′ or 3′. The second point that must be considered is the fact that a nucleic acid is when it is copied or duplicated; there emerges a new strand of the complementary polarity. Based on all this, the influenza viral RNAs are classified as (-). They can also be regarded as negative strand RNAs. This is because these strands are regarded as the opposite in terms of polarity of the RNA. They are translated repeatedly in order to facilitate making the protein. The molecules of the RNA are in essence templates merely for the process of synthesis required for proteins. They are explained as molecules that have (+), or simply positive polarity. When they enter the cell, there happen certain changes. The (-) strand of the influenza and its viral RNAs are instantly copied into the complementary type of (+) strands. This process is accomplished in order to facilitate creating templates for proteins synthesis. The enzyme RNA polymerase serves as an agent in copying the viral RNAs. It is also transferred directly to the cell along with the virus(Fodor and Velthuis, 2020).

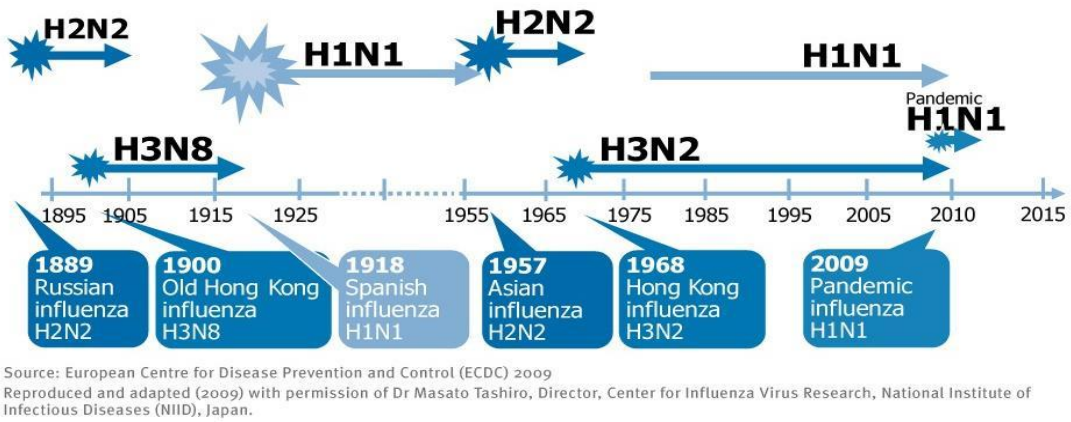
The scheme shown above presents the olive-green lines which are the (-) strand RNAs. These strands are found to be components of the influenza virion. When entering the cell, the virion along with the8 RNAs, as manifested in the scheme above, are copied into (+) strand mRNAs. More importantly, the mRNAs functions primarily as templates for the creation and the synthesis of proteins. The illustration above shows

the specific viral proteins that can be made by the different types of viral mRNA. As it is displaced above, the RNA segment has four codes that are all for the viral HA protein. The RNA segment can copy 6 codes for the viral NA protein. Moreover, it is noted that some RNA segments have the ability to encode for more than one protein. In their structure, the influenza type A and B viruses possess 8 RNA segments. The other influenza virus, namely C, possesses 7 RNA segments(Xue *et al.*, 2018).

The types of the viruses of Influenza are generally considered as (-) strand RNA viruses. This is due to the polarity that is an apparent characteristic of the RNA. It is in all processes carried in the virion. The other viruses of RNA – such as poliovirus – are classified as (+) strand types of RNA viruses. As explained above, the genomic structure of RNA type of viruses can be carried and transmitted into protein when they enter the cell (Robb, Smith, Vreede, & Fodor, 2009).

2.2 Epidemics and Pandemics

Type A virus undergoes antigenic shift and hence cause pandemics which can occur at intervals of minimum of ten to forty years. After the pandemic subsides, epidemics can occur due to frequent antigenic drifts. Past (since 1885) human Influenza pandemics are shown in Figure 2.4. These are known as (i) the Spanish flu (1918), (ii) Asian flu (1957), (iii) the Hong Kong flu (1968), (v) Russian flu (Re-emergence of H1N1)(1977), (vi) Bird influenza H5N1, and H9N2 outbreak that happened in Hong Kong (1997), Swine-OriginH1N1 Pandemic (2009) (Day, 1998; P. Wright, Neumann, & Kawaoka, 2007; P. F. Wright & Webster, 2001).



Source: European Centre for Disease Prevention and Control (ECDC) 2009
Reproduced and adapted (2009) with permission of Dr Masato Tashiro, Director, Center for Influenza Virus Research, National Institute of Infectious Diseases (NIID), Japan.

Figure 2.4: Recorded Human Pandemic Influenzas since 1885(Johansen, Nicoll, Ciancio, & Kramarz, 2009)

2.3 Influenza Virus Transmission

Influenza virus can take three main ways for transmission to humans: (Bandaly, LE CANN, & ANDRES, 2006). The first process of transmission for the virus is usually direct by contacting the infected individuals (Carrat et al., 2008). The second possible way of transmission is either direct by contacting the contaminated objects in their different forms. The third way of transmission according to Mubareka et al., 2009)is by inhaling the virus-laden aerosols. However, the contribution that each of the processes described above to the overall spread of influenza is still not understandable. The CDC recommendations that are made in regard to controlling the transmission of the viruses of influenza in various healthcare environments necessitate taking certain measures that are supposed to minimize the spread of aerosol and fomite mechanisms (Carrat et al., 2008).

Transmission of influenza virus from animals and birds to humans occurs repeatedly. This results from the direct contact with the infected birds and animals. The risk and the severity of the transmission can be very high especially during slaughtering and preparing the animal for consumption. Nevertheless, if we eat meat that is properly cooked, it is confirmed that result in no health or infection risks. Avian influenza, in addition, can be transmitted to humans via direct exposure to the surfaces and water that have been infected through the droppings of birds(Zhang *et al.*, 2020)

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More importantly, the viruses of influenza can rapidly be transferred from one person to another. This transmission spreads through aerosols that are made particularly by an infected individual coughing or sneezing. When the person inhales the aerosol, he gets infected. Nevertheless, this infection occurs only within the individuals with less immunity(Zhang *et al.*, 2020).

In most of the cases, the transmission depends on the aerosols. If the aerosols contain virus particles, transmission of the virus can take place. For this process to be successful, activities such as speaking, singing, and normal breathing are ideally processing that

produce aerosols. Coughing and sneezing are also processing of transmission but they lead to a more forceful expulsion of the aerosol particles. All of these processes contribute to the transmission of influenza virus but at varying degrees and sizes. The largest droplets usually fall immediately onto the ground. In this case, they don't transmit the infection effectively. They transmit the virus only to those in close contact. Those droplets that have ability to travel for a distance due to their small size are more prone to cause more infection. Size here plays a very important role in transmitting the virus infection. These droplets are usually of 1-4 microns in diameter, and they are termed as 'droplet nuclei'. Given their small size, they have the ability to stay in the air for a very long period of time. Though they may not only have the ability of travelling long distances, but they also possess the ability of reaching even to the lower respiratory tract easily (Fouchier, 2015).

In addition, the nasal secretions can be responsible for transmission of the virus. When they contain virus particles, they can directly transmit the virus either through a third party (contaminated object) or to the individual. It is common that the person frequently touches their nose or conjunctiva, this process ensures putting the virus on the hands. Subsequently, any type of contact, either intimate or non-intimate, in the form of shaking hands will definitely transmit the virus. This process continues from one individual to the author, resulting in the spread of the virus and infection of many people. More importantly, contaminated hands also transfer the virus. By touching other objects, the virus gets easily transferred from one medium to another. In one of the studies conducted on the transmission of the influenza, it was found out that 23-59% of objects that are available in homes and day care facilities contain influenza viral RNA. Other studies have also demonstrated that the infectious influenza virus lingers on paper currency. It remains of papers currencies for many weeks, and through this period, the virus gets transmitted to a large number of people (Carrat et al., 2008).

Despite all the many ways of transmission, Influenza spread can be contained. It can be curtailed to a great extent primarily by following protective measure. It is proved that covering one's nose and mouth when the person coughs or sneezes reduces the spread and contamination. In addition, the frequent washing of hands is proven effective in

reducing virus transmission when often done with soap and water or alcohol-based hand cleaners. Face masks, however, are not recommended by CDC. They do not reduce the viral spread (CDC, 2013).

2.4 Diagnosis of Influenza Virus Infections

2.4.1 laboratory Diagnosis of Influenza Virus

Diagnosing the different viruses of influenza in a laboratory has become very essential. For the effective prevention of the virus, certain measured must be taken into consideration. The outbreak of many viruses such as H5N1, gave the laboratory a significant role to play in the diagnosis. The functions that the lab can perform include isolation and the sub-typing of the virus which help in the disease surveillance and the development of vaccines as well. There are also other viruses that infect the respiratory system. They can also produce similar symptoms and they frequently co-circulate during the epidemics caused by influenza. Because of this, it seems that the establishment a particular diagnosis of influenza mainly based on the clinical symptoms cannot be adequate, and it can very often be problematic (Pachucki, 2005; Peltola, Reunanen, Ziegler, Silvennoinen, & Heikkinen, 2005).

Nevertheless, laboratory detection and testing of influenza has not been usually successful. Historically, it has remained questionable due to the type of management that is employed for the patients infected with influenza. It is considered limited in terms of test sensitivity. More importantly, there is a considerable lack of effective antiviral therapies. To tackle these issues, there is a need for more development with regard to accurate testing mechanism. More investment should be done in the detection of influenza. New technologies will definitely enable the medical laboratories to provide reliable definitive diagnosis. More development in this regard means clinicians would be able to initiate antiviral therapy. In addition, improving the diagnostic aspect will reduce the anti-bacterial use of injections. It will lead to a more infection-controlled measures, which contribute to decreasing the duration of hospitalization. In general, any improvement in the diagnostic process will have a significant impact on the reduction ancillary testing. It will also reduce health care

costs (Barenfanger, Drake, Leon, Mueller, & Troutt, 2000; Bonner, Monroe, Talley, Klasner, & Kimberlin, 2003; Woo, Chiu, Seto, & Peiris, 1997).

2.4.2 Current Diagnostic Testing Options

There are several techniques that are employed for detecting influenza infection. These can be done either by detecting the virus or how the immune system of the patient responds to the virus. In addition, the approaches of diagnosis that are well-know and recognized worldwide are mostly related to isolating the virus or detecting the viral antigen. These can only be done by immune-specific assays. Immunofluorescence microscopy is one of the procedures in this regard. Moreover, point-of-care (POC) testing (e.g., EIA or optical immunoassay) is very common. Lastly, detecting the viral nucleic acid can also be performed based on the amplification techniques (i.e., nucleic acid testing [NAT]). It is affirmed that detecting the antibody is mostly performed by applying virus neutralization (virus NT) technique. The process also involves hemagglutination inhibition (HI) tests that are carried out with an aim to detect seroconversion to a specific virus strain. These can be used to check the immune status mostly after vaccination (Cox, Wakelin, Gillespie, & Despommier, 2007; Julkunen, Pyhälä, & Hovi, 1985; Newton, Treanor, & Menegus, 2000)

Despite the development, the diagnosis of the influenza remains limited. Sometimes the current employed techniques may produce misleading results. To avoid any of the false results; the reports have to be understood only in when considering thepatient'sclinical history. It is possible that false-negative test outcomes may happen. This can be ascribed to the lack of adequate quantities of the viral analyte. Moreover, the inappropriate collection and handling of the samples can affect the results. Sometimes these misappropriate procedures along with the presence of viral inhibitors need to be considered since they will have effect on the outcomes of tests (Cox, Wakelin, Gillespie, & Despommier, 2007).

2.5 Factors Influencing the Selection of Tests

There are many factors that govern selecting an appropriate test. In this regard, several factors may come into play. The size as well as the capacity of the lab, for

instance, can be a determining of the whole process of selection. It can have a critical impact on the selection of tests. Generally, small labs are found in physician offices or sometimes they can be part of small hospitals. One disadvantage is that they are mostly suffering from many restrictions. In such a situation, the use and choice of selecting the rapid POC tests may not be frequent. Being small, it means minimal use of required infrastructure. In addition, this type of labs can be operated and managed by staffs that possess no specialist knowledge of virology. In this type of setting, cost is made up for by having few infrastructure facilities. Nevertheless, the seeming simplicity of Clinical Laboratory Improvement Amendment–waived testing should not be taken for granted. Technicians and healthcare personnel should be extra careful about maintaining good laboratory practices. They should maintain adequate training. More importantly, there must be an access to quality control testing, and all the procedure has to supervised (Ebell, 2005).

For large hospitals and reference laboratories, there are many options and alternatives. Such types of facilities are usually staffed with trained technologists, and thus they may have access to the use of POC tests, and DFA. These have higher sensitivity and they are also affordable in terms of price per test. There is a possibility to do Isolation in cell culture in these settings. This is mostly done after a negative DFA result to confirm the test for quality assurance purposes. More importantly, this procedure can be taken to amplify the virus for additional sub-typing or resistance testing. The HI test, for instance, can be done to detect and monitor seroconversion, and in certain cases, it can be employed to sub-type viral isolates. More recently, it has been noticed that the well-established and large laboratory are increasingly using nucleic acid–based technologies with an aim to diagnose influenza and infection with other respiratory pathogens. However, it is found out that the implementation of such technique is complex, and it requires more expensive infrastructure. Moreover, it demands well-trained technologists and sufficient space that can minimize amplicon contamination(Baz *et al.*, 2012)

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2.6 Influenza Testing

In times of pandemics, laboratory testing is highly required. It is therefore essential in the pre-pandemic stages, and it can also be compounded in the early pandemic stages. However, there is a need for appropriately certified BSL-3 laboratory facilities. Rapid diagnosis during pandemics requires well-qualified lab technicians and adequate capacity. Instruments such as NAT, in addition to high-volume automated nucleic acid extraction, should have their say in the testing process, and they are on demand to save the time for the lab staff. Most importantly, the RT-PCR assay is used basically at the initial treatment process for the detection of all types of influenza viruses. This type of assay can also be reflexed to a more specific RT-PCR assay with HA-specific primers for the identification of a pandemic strain. As recommended by many studies, NAT is a recommended approach. However, it can adversely be impacted by problems of supply due the increasing demands due to virus outbreaks. It can however be used for primers and probes(Vos *et al.*, 2019)

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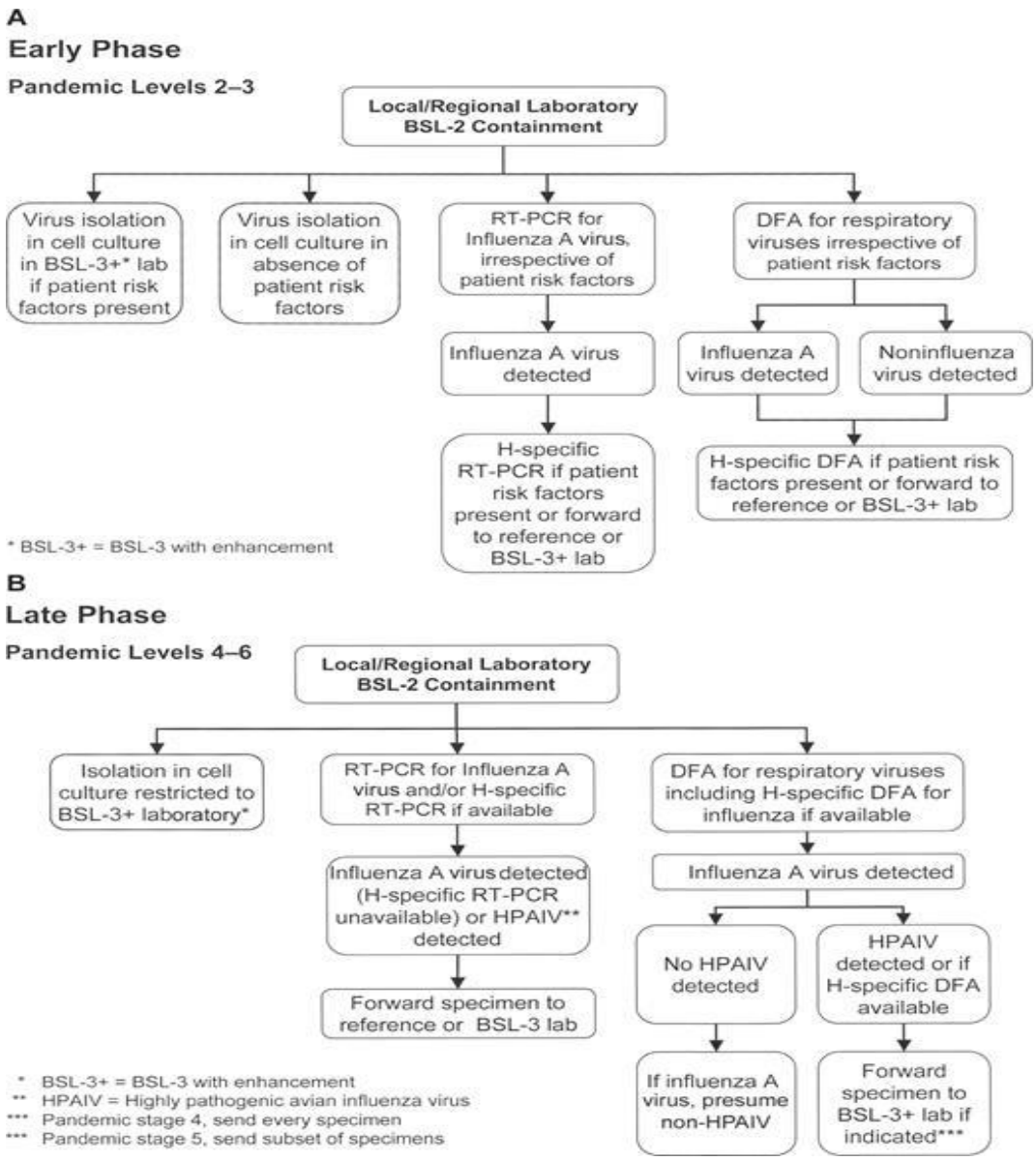

Figure 2.5: DFA and RT-PCR(Petric, Comanor, & Petti, 2006).

2.7 Treatment

Testing is the very important step in treatment. The doctor usually conducts a physical exam of the patient. The aim of this physical exam is to search for signs and symptoms of influenza. After this step, the doctor may ask the patient to do a test for detecting influenza viruses. Nevertheless, during the prevalence times of influenza, the doctor may just diagnose the patient with requesting him to do a test. Based on the sign

and symptoms, the doctor builds his evaluations of the patient's case(Omoto *et al.*, 2018).

In certain cases, the doctor may request doing a test to detect influenza. Polymerase chain reaction (PCR) testing is more popular by several healthcare institutions. This type of testing is more sensitive than other tests. It can identify the influenza strain. During the COVID-19 pandemic, several studies and lab results demonstrated that testing for both and diagnosing both influenza and COVID-19 can be done altogether. A test for these two viruses can simultaneously be done at the same time (Simonson, 2019).

In most of the cases, there is no specific treatment for the people infected by the influenza and are relatively healthy. People of such category do not require special drugs or a specific type of treatments. However, when a person is infected by influenza, the common simple treatment methods include taking enough rest, drinking more fluids, and eating a light diet. In addition, infected people have to remain at their homes, and they are advised to take acetaminophen for the reduction off ever and also for relieving muscle aches (R. Lehnert, M. Pletz, A. Reuss, & T. J. D. Ä. I. Schaberg, 2016; Simonson, 2019).

In severe circumstances and the infected person is seriously ill, certain medication might be prescribed, such as antiviral drugs. The common antiviral drugs for severe cases of influenza are categorically as oseltamivir phosphate, zanamivir, peramivir, and baloxavir (Kohno *et al.*, 2010).

2.7.1 Oseltamivir phosphate

This medication is commonly used for treating the cases of influenza. It is widely-approved and receives good rating. It can be administered for patients from two weeks of age and older. Clinical experiments demonstrated that this type of medicine can function well in that patient who sought treatment immediately after two days from catching the flu. This product has a generic version, yet it considered more expensive than the brand name. The recorded side effects of this medication are simple and range from nausea, vomiting. In addition, the side effects can include rare nosebleeds, light occurrences of headaches, and sometimes tiredness(Shin *et al.*, 2017).

2.7.2 Zanamivir

This medical product received wide approval for the treatment of influenza. It is prescribed for patients seven-years-old and older. Moreover, it can be administered for patients who are five-year-old and older. Taking this kind of medicine is usually through inhalation. Therefore, it is best prescribed for patients with respiratory illnesses, such as COPD or asthma. Its side effects are mild. They largely cause little headaches and nausea. In some cases, the side effects can range from diarrhea to nose irritation. Vomiting is not common side effect for this medicine(Freund *et al.*, 1999).

2.7.3 Peramivir

This drug received approval for treatment in many countries. It is now used to treat influenza patients who are of two years old and older. This medication can be applied through an injection to the vein (intravenously). The side effects of this product are limited. A commonly registered side effect is only diarrhea (Kohno *et al.*, 2010).

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2.7.4 Baloxavir

This medication in the form of tablet is also approved for treating the flu in people over twelve years old. It can be used for both healthy people and those developing influenza-related complications of high risks. The common side effects that have been recorded include diarrhea and bronchitis. Moreover, nausea is very rare, while headaches have little presence.

Generally speaking, all the side effects that are mentioned above for all the common influenza medications that are used to treat influenza are only the most common ones as recorded by many medical institutions. There might be other side effects, other than what is mentioned above. This is common to all types of medication; the patient might be allergic to one medicine but not to another. It is hence advisable to consult a doctor and discuss the possible side effects according to the case of the patient. Amantadine and rimantadine have also been accepted as approved medicines that can be used for treating patients suffering from influenza. However, it has been reported that the influenza viruses have the ability to resist these medicines(Omoto *et al.*, 2018).

2.7.5 Complications

Being infected with influenza, it means that the patient is also more susceptible to bacterial infections. Infections from bacteria, as have been confirmed, are more likely whenever the individual has influenza. Till today, healthcare providers mostly use antibiotic drugs for the treatment of influenza. In most of the cases, the common secondary infections include that are currently in use are bacterial pneumonia, ear infections and sinus infections (Rothberg and Haessler, 2010).

2.8 Prevention

As known in many countries, vaccination annually is regarded as one of the primary strategies that can be used for the prevention of the Influenza virus infection. Since the composition of the Influenza viruses change every year, this makes the vaccine that was used in the previous year's totally ineffective. To prevent the virus, several vaccines have been developed. There are today many influenza vaccines. The common vaccines types that are today available are whole virus vaccines, Split virus vaccines, Subunit virus vaccines, or live attenuated vaccines. WHO Recommended Northern hemisphere Influenza virus vaccine candidates (2011-2018) showed in Table 2.2

Year	Vaccine Components
2011-2012	A/California/7/2009 (H1N1)-like virus; A/Perth/16/2009 (H3N2)-like virus; B/Brisbane/60/2008-like virus.
2012-2013	A/California/7/2009 (H1N1)pdm09-like virus; A/Victoria/361/2011 (H3N2)-like virus; B/Wisconsin/1/2010-like virus
2013-2014	A/California/7/2009 (H1N1)pdm09-like virus; A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011b; B/Massachusetts/2/2012-like virus.
2014-2015	A/California/7/2009 (H1N1)pdm09-like virus; A/Texas/50/2012 (H3N2)-like virus; B/Massachusetts/2/2012-like virus.
2015-2016	A/California/7/2009 (H1N1)pdm09-like virus; A/Switzerland/9715293/2013 (H3N2)-like virus; B/Phuket/3073/2013-like virus
2016-	A/California/7/2009 (H1N1)pdm09-like virus;
2017	A/Hong Kong/4801/2014 (H3N2)-like virus; B/Brisbane/60/2008-like virus.
2017-2018	A/Michigan/45/2015 (H1N1)pdm09-like virus; A/Hong Kong/4801/2014 (H3N2)-like virus; B/Brisbane/60/2008-like virus.

Table 2.2: Northern hemisphere Influenza virus Vaccine candidates Recommended by WHO (2011-2018)

3 AIM AND OBJECTIVE

3.1 Aim

The current study is mainly focused on the investigation of Influenza Activity During the 2019-2020 Winter Season in Northern Cyprus.

3.2 Objective

- To investigate the annual rate of seasonal influenza A and B viruses in circulation during 2019 winter through 2020 spring in Northern Cyprus.
- To identify the highest prevalence type of Influenza between A and B within the study period.
- To evaluate the effect of age and gender on the rate of Influenza A and B viruses
- To determine the peak period for the seasonal spread of both influenza A and B viruses.

4 MATERIALS AND METHODS

4.1 Study population

The present study was carried out on 844 patients with flu-like symptoms all age group patients.

4.2 Study design

The study used in this research was a cross-sectional.

4.3 Study duration

From December 2019-March 2020

4.4 Place of the study

Department of Microbiology, Near East University Hospital, Near East University, Faculty of Medicine, North Nicosia, Northern Cyprus.

4.5 Subject recruitment procedure

Study subjects included patients with suspected influenza symptoms of any age attending Near East University Hospital.

4.6 Ethical issues

Ethical approval of the study was taken from Near East Ethical Committee with the permission number NEU/2020. Since our study was retrospective, informed consent form was not used.

4.7 Sample and data collection

The specimens have been collected within 1 to 7 days onset of illness.

Laboratory testing of samples from suspected cases of influenza was conducted properly. Throat swabs and / or nasal swabs were collected from the patients who agreed to participate in this study. The patients were admitted to departments of Pediatrics, Cardiology, Chest Diseases, Emergency Service, Intensive Care, Infectious

Diseases Departments, and the main Microbiology Laboratory of the Near East University Hospital, Near East University.

After collecting the swabs, immediately placed at 4°C in viral transport medium (VTM) and transported to the laboratory.

A detailed lab request form was constructed and document the symptoms, date of illness, clinical features, laboratory examination and demographic data for each patient. For ensuring the ethical considerations, the author obtained a written informed consent from the patients or their parents/guardians before collecting the sample. Patient Proforma and Consent forms were enclosed.

Characteristics including age, gender, clinics and rapid test results are documented and evaluated. Influenza positivity was assessed between different age groups in the ranges 0-4, 5-14, 15-44 and over 45 in all individuals during the time period.

4.8 Sample Processing and Storage

The samples were processed as per WHO standard protocol(Organization, 2011). The samples were processed in Biosafety Cabinet. The collection vials with swab were agitated vigorously on vortex mixer.

The prevalence of seasonal influenza specifically type A & B were determined by using rapid influenza diagnostic test (ABON Bio pharm Co., Ltd, Hangzhou, China) which provides a specific, low-cost and rapid test, as the result appears within 10 minutes. A total of 1,688 test kits were used during analysis. The positive result for Influenza type A and Influenza type B virus was assessed by using the strip of the kit which is coated with antibody against nucleoproteins (NP) in different positions.

The validity of laboratory test is dependent on proper sample collection and handling which if done inappropriately can lead to incorrect diagnostic results.

For laboratory diagnosis of influenza, there are certain recommended procedures for collecting and storing the human clinical samples. These recommendations are outlined in Guidelines for Collection, Storage and transportation of Human Clinical samples for Laboratory Diagnosis of Influenza.

4.9 Biosafety measures

4.9.1 Basic Biosafety Requirements

- Personal protective equipment (PPE)
  - Gloves (latex)
  - Laboratory coats or gowns (front closed full- length apron)
  - Head cover
  - Protective eyewear
  - Face protection (triple layered mask, N95 mask when handling novel sample)
  - Shoe cover
- Puncture resistant autoclavable yellow coloured and red coloured biosafety bag
- Biosafety symbols
- Biosafety spill kit
- Chemical disinfectants: sodium hypochlorite, 70% ethanol, quaternary ammonium compounds, detergents, iodophors, phenolic compounds

4.9.2 Biosafety practices

The standard protocol for pre-analytic biosafety measures, analytic biosafety measures, and Post analytic biosafety measures were all followed.

5 RESULTS

5.1 Patient characteristics

The present study is a cross-sectional study. The study population was 844 participants suspected of influenza infection. Of all the patients, 48% (401/844) were males and 52% (443/844) were females (Figure 5.1). All the participants hospitalized at the Near East University Hospital, Near East University during the period from December 2019-March 2020.

The swab samples were collected from different departments including Pediatrics (356, 42.2%), emergency services (344, 40.8%), main laboratory (91,10.8%), internal medicine (19, 2.3%), infectious disease (13,1.5%), chest disease and allergy (8,0.9%), ear-nose-throat (8,0.9%), cardiology (3,0.4%) and dialysis (2, 0.2%) clinics were screened for influenza A and B in this study (Figure 5.2) and (Table 5.1).

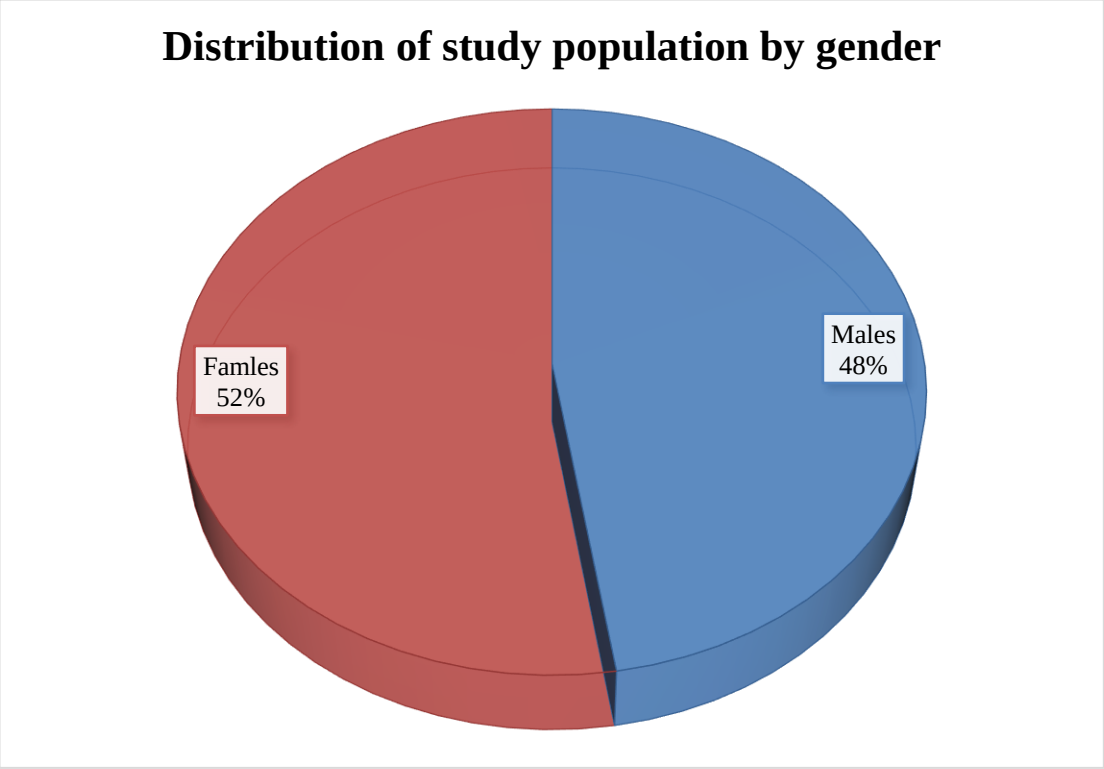

Figure 5.1: Distribution of population by gender

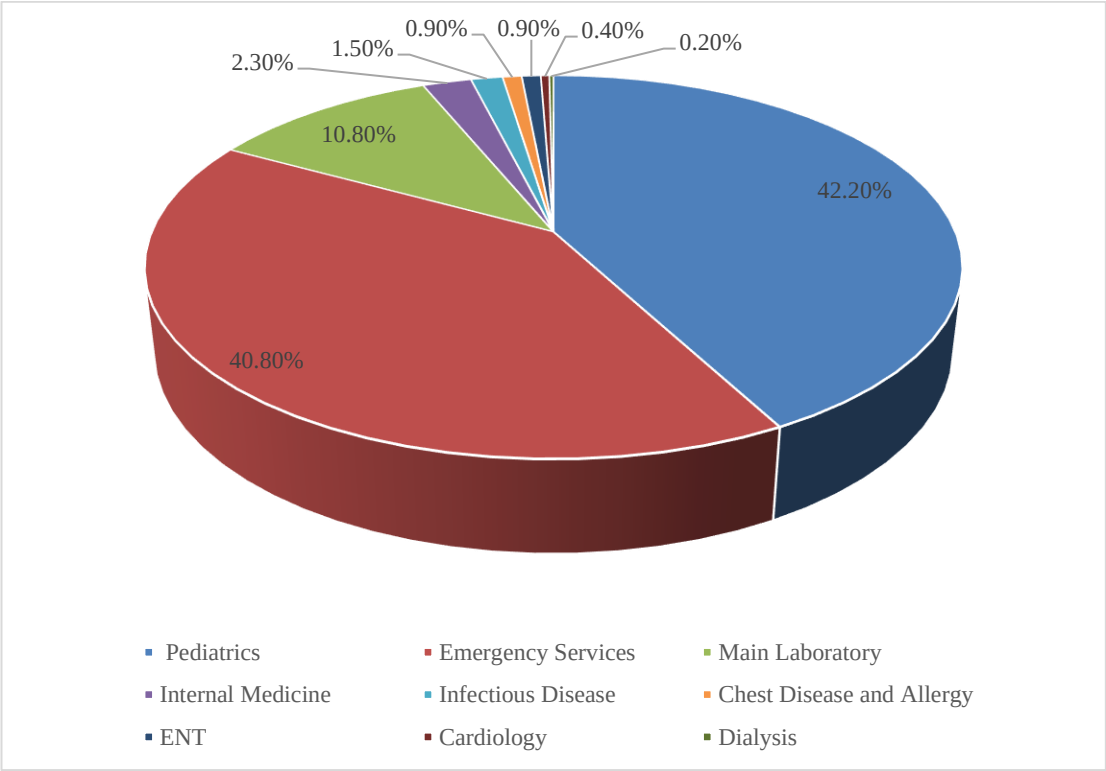

Figure 5.2: Distribution of the study samples

The figure above clearly presents the distribution of the study participants. The distribution shows the different clinics from which patient samples were collected. This distribution is according to clinics.

Table5.1: Distribution of the study population according to hospital departments

Characteristics	**Patient group**
Patients, n	844
Gender, M/F, n (%)	401(47.5%)/ 443 (52.5%)
Clinics	Patients, n (%)
Emergency service	344 (40.8%)
Paediatrics	356 (42.2%)
Internal medicine	19 (2.3%)
Dialysis	2 (0.2%)
Infectious disease	13 (1.5%)
Chest disease and allergy	8 (0.9%)
Cardiology	3 (0.4%)

Ear nose and throat	8 (0.9%)
Laboratory	91 (10.8%)

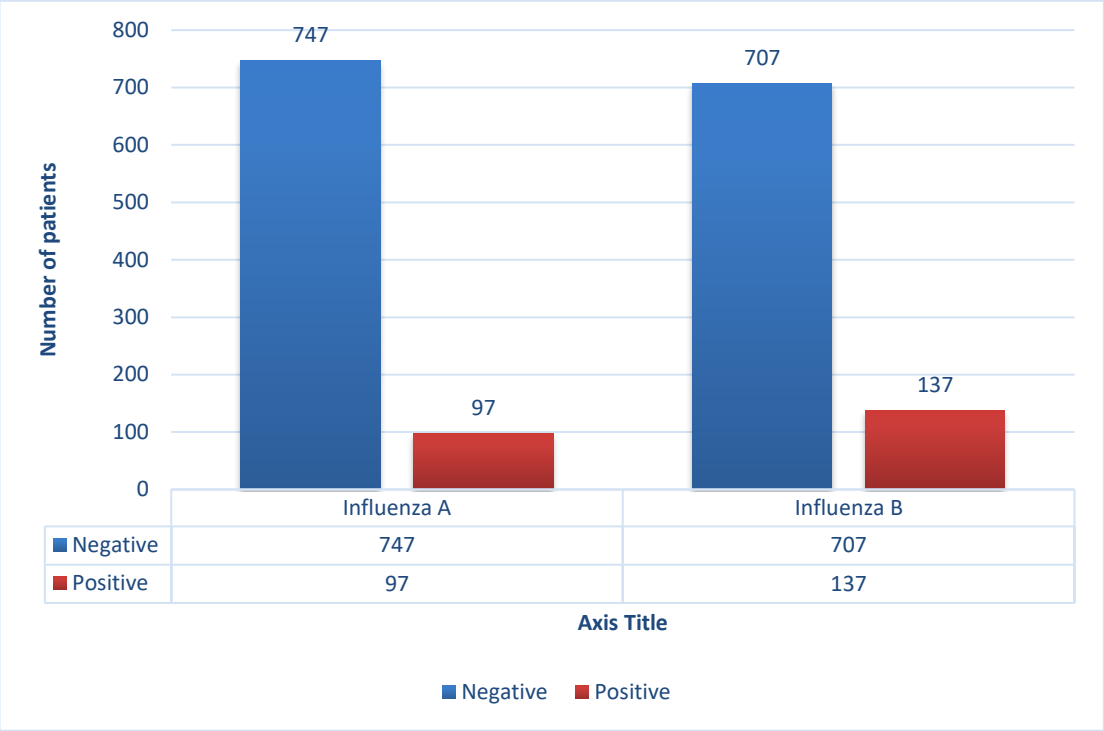

Figure 5.3: Influenza A and influenza B positivity

Table5.2: Influenza A and influenza B positivity among genders

	Influenza A			**Influenza B**		
	Negative	**Positive**	**P**	**Negative**	**Positive**	P
Males	353 (%88)	48 (%12)	0.67	342 (%85.3)	59 (%14.7)	0.25
Females	394 (%88.9)	49 (%11.1)		365 (%82.4)	78 (%17.6)	
Total	747 (%88.5)	97 (%11.5)		707 (%83.8)	137(%16.2)	

The table 5.2 clearly presents the different numbers of males and females distributed by positive and negative for influenza A and B. Of these, 443 (52.5%) were females and 401 (47.5%) were males. Overall individuals screened for influenza viruses, 234 (27.7%) were determined as positive for influenza A or B. Of the infected patients, 97 (11.5%) were positive for influenza A, 137 (16.2%) were positive for influenza B. Among influenza A positive patients, 48 (49.5%) were males and 49

(50.5%) were females. Moreover, 59 (43.1%) males and 78 (56.9%) females were determined as influenza B positive. The difference between either influenza type A or influenza B positivity and genders are not statistically significant (P = 0.67, P = 0.25) respectively.

Table 5.3: The rate of influenza A and influenza B positivity among different age groups

Influenza Types	0-4 yes	5-14 yes	15-44 yes	>45 yes	p
INFLUENZA A					
Negative	307 (86%)	187 (87.4%)	172 (91.5%)	81 (95.3%)	0.047*
Positive	50 (14%)	27 (12.6%)	16 (8.5%)	4(4.7%)	
INFLUENZA B					
Negative	313(87.7%)	162 (75.7%)	153 (81.4%)	79 (92.9%)	0.000*
Positive	44 (12.3%)	52 (24.3%)	35 (18.6%)	6 (7.1%)	

As shown in table5.3, among the patients with influenza suspected, the majority of positive influenza A cases were 14% (n= 50) in the age group of 0 to 4 years while the minimum, 4.7% (n=4) was in the age group above 45 years. However, the negative influenza in same groups were (86% (n = 307), 95.3 % (n = 81)) respectively.

But, the majority of positive influenza B cases were 24.3% (n = 52) in the age group of 5 to 14 years while the minimum, 7.1% (n = 6) was in the age group above 45 years. However, the negative influenza in same groups were (75.7% (n = 162), 92.9 % (n = 79)) respectively. The different between all groups in influenza A was significant with P- value = (0.047), but strong significant with influenza B. P- value < 0.0001).

Table5.4: The distribution rate of influenza A and B positivity by months

Influenza Types	December 2019 n (%)	January 2020 n (%)	February 2020 n (%)	March 2020 n (%)	p
INFLUENZA A					
Negative	29 (82.9%)	425 (86.7%)	223 (91.0%)	70 (94.6%)	0.088
Positive	6 (17.1%)	65 (13.3%)	22 (9.0%)	4 (5.4%)	
INFLUENZA B					
Negative	32 (91.4)	407 (83.1)	198 (80.8)	70 (94.6)	0.034*
Positive	3 (8.6)	83 (16.9)	47 (19.2)	4 (5.4)	

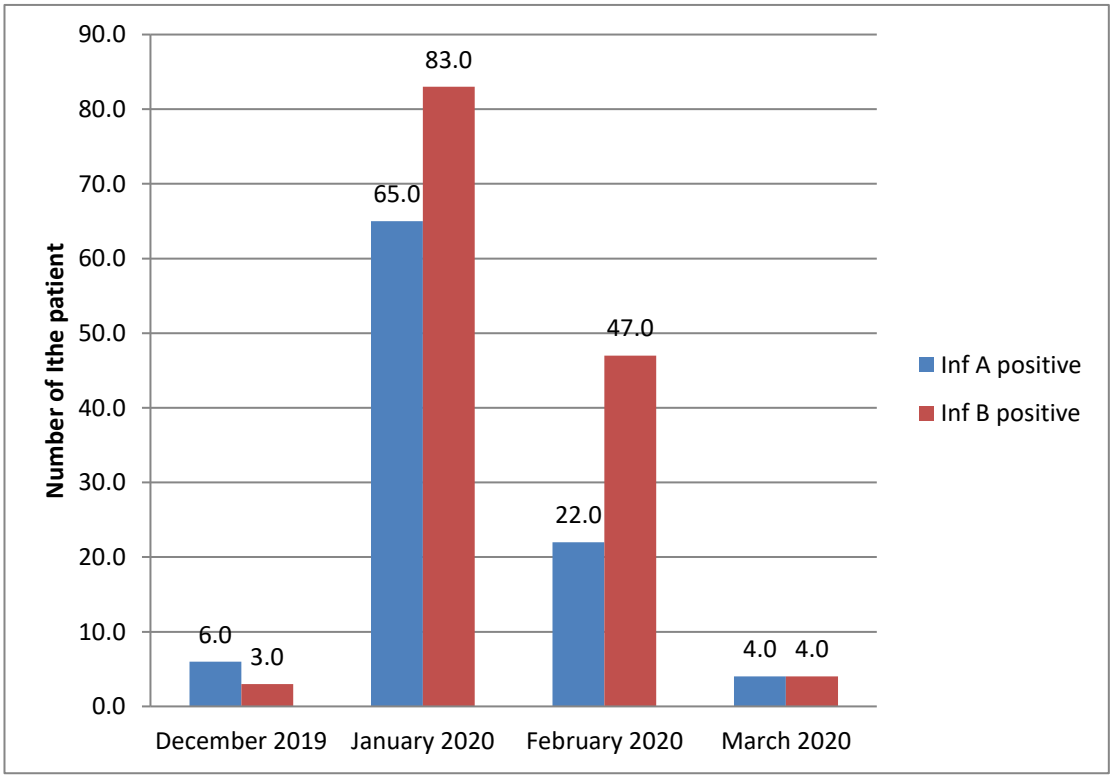

Figure 5.4: The distribution rate of influenza A and B positivity by months.

The Table No. 5.4, and bar chart No 5.3, shows the distribution rate of influenza A and B positive versus months. During December 2019, a total of 70 influenza and B tests were performed. The results of the testing revealed that 6 (17.1%) and 3 (8.6%) of the participants of the study were tested positive for influenza A and B respectively.

The peak month of the influenza activity was determined in January with a total of 980 analyses. Of which, 65 (13.3%) were positive for influenza A and 83 (16.9%) were positive for influenza B. In February 2020, a total of 490 tests were enrolled and 9% and 19.2% of the tests were positive respectively for influenza A and B. During March 2020, 148 test kits were involved of which, 5.4% of them were positive for both influenza A and influenza B. After February 2020, the number of tests carried out in March decreased (70%). There was no statistically significant relationship between the rate of influenza A and months (P = 0.088).

However, the relationship between the prevalence of influenza B and months were statistically significant (P =0.034) and statistically, influenza B infection was most common in February 2020.

6 DISCUSSION

Influenza is an acute respiratory disease which usually has mild manifestations and it is caused by influenza type A or type B viruses. However, due to the inherent nature of the virus to mutate, and the ability to infect across species combined with its route of transmission (respiratory), makes Influenza Virus one of the most obnoxious agents to manage. As has been witnessed in the past, influenza has caused many a pandemic with heavy mortality and morbidity. The Spanish flu of 1918 being a classical case, wherein approximately 50 million people were killed in a single flu season (Gupte, Gupte, & Kumar, 2012).

The incidence of influenza virus ranges from 5% to 20% each year (Tokars, Olsen, & Reed, 2018). This current study presents the estimation rate of seasonal influenza type A and/or B in Northern Cyprus for the 2019 – 2020 influenza season. During this period, as seen in figure 5.3, overall individuals screened for influenza viruses, 234 (27.7%) were determined as positive for influenza A or B. The positivity rate for influenza B infections was higher (16.2%) comparing to influenza A infections (11.5%) (See figure 5.3). Similar to a study in France, the number of influenza B cases (29.7%) was higher than the number of influenza A cases and influenza B outbreak peaked after influenza A outbreak(Mosnier et al., 2017). In contrast, another study by Tamerius at all. from the United States of America shows that the number influenza A infection with 136,793 patients was higher than influenza B with 86,498 patients(Tamerius, Uejio, & Koss, 2019).

Risk factors including months, age and gender play significant role in the distribution of influenza illness and its treatment which may also have important public health implications (Morgan & Klein, 2019; Nguyen & Noymer, 2013; Wong, Luscombe, & Hawke, 2019). We correlated the rate of influenza-positivity versus months between December 2019 and March 2020. Our findings show that influenza A or B positivity was detected at the highest rate in December 2019. However, the highest number of tests was performed in January with a total of 980 analyses. Unusually, most of the positive patients were infected with influenza B in our study. Influenza type A viruses are more common than influenza type B viruses due to

antigenic shift of proteins present on the virus surface or a combination with viruses circulating in animals (Paulonis, 2019). In studies related to seasonal flu research, influenza type A is found more frequently than type B. In a recent study by Yang et al. in 2019-2020 in China and other neighboring Asian countries such as Korea and Japan, the positivity rate for influenza type A (77.1%) was higher than type B (22.9%).Also, in another study that shows the epidemiology of seasonal influenza in Iraq by Aufi et al., the incidence rate of influenza type A and B respectively were 16.7 and 4.7 per 100000 people-years (Aufi, Fadhil, Ali, Alhamdani, & Owaid, 2020).After the 2009 pandemic in the WHO European Region, the results of a retrospective study by Mook et al., that focused on alternating patterns of seasonal influenza activity from 2010 to 2018, influenza type A was the predominant circulating influenza viruses during five seasons (2010-2011, 2011-2012, 2013-2014, 2014-2015, and 2016-2017) (Mook et al., 2020).

Furthermore, in February 2020, half of the tests compared to January were performed due to decrease in the number of the patients and the positivity rate was determines to be higher for Influenza B infection (19.2%). During March 2020, 148 test kits were involved of which, 5.4% of them were positive for both influenza A and influenza B. After February 2020, the number of tests started to drop by 70% until March, but there was no statistically significant relationship between influenza A prevalence and months ($P = 0.088$). In contrast, the relationship between the prevalence of influenza B and months was statistically significant ($P = 0.034$). During the period between December 2019 to March 2020, the influenza B positivity was more common (59%) than influenza A (41%). Referring to the influenza type B incidence, some studies showed that the majority of seasonal influenza during the season of 2017-2018 was for influenza type A, while another study in 2017–2018 by Basileet al., indicated that influenza type B positivity was higher (63%) in comparison to influenza type A(Basile et al., 2019; Mook et al., 2020). In addition, the results of a study by Karolyi et al., for the hospitalized patients after testing by routine PCR (polymerase chain reaction), 75.8% of influenza positive cases were for influenza type B. A study conducted by Yang et al.in China and other neighboring Asian countries during 2019-2020 seasons showed that influenza type B was the predominant in

Mongolia and Russia (80.7% and 77.2 respectively)(Karolyi et al., 2019; J. Yang et al., 2020).

The distribution of influenza virus types also varies by age and sex and this has important public health implications (Wong et al., 2019; J. Yang et al., 2020).Therefore, we compared to age and sex differences among patients infected with influenza virus type A or B in the current study. Our findings suggest that gender is not associated in the rate of influenza infections in Northern Cyprus for 2019-2020 seasons (See Table 5.2). However, our findings suggest that the seasonal influenza positivity is associated with the patient's age and it is an important factor in getting infected with the virus. The overall age distribution of influenza type A cases was the highest among young children ages 0 - 4 (14.0%) with ($P = 0.047$) and the lowest was detected among the elderly (4.7%). Moreover, influenza type B was most frequently determined in the 5-14 ages with ($p < 0.0001$). Similar to the results of a study by Mosnier et al., influenza type B was very common especially in school-age children. For influenza species, it shows that the number of cases decreases with age and indicated a statistically significant relationship in an inverse relationship(Mosnier et al., 2017).

7. CONCLUSION AND SUMMARY

The study was carried out to evaluate the prevalent the circulating strains of influenza virus in Northern Cyprus. This study was based on the 844 clinical (throat and nasal swab) samples collected from patients having ILI symptoms from Near East university hospital.

The samples were collected from the period the winter season of 2019 and the beginning of 2020 spring and processed by rapid influenza diagnostic test method (ABON Bio pharm Co., Ltd, Hangzhou, China). Following conclusions were made from the study:

Out of total 844 clinical samples of patients with Influenza like illness (ILI), we encountered 401 (48%) male patients and 443 (52%) female patients.

We found that 11.5% (97/844) of Influenza A positive samples and 88.5% (747/844) were negative. However, 16.2% (137/844) of Influenza B positive samples and 83.8% (707/844) were negative.

In additional, 49.5% (48/97) of Influenza A positive samples were male and 50.5% (49/97) were female, but 43.1% (59/137) of Influenza B positive samples were males and 56.9% (78/137) were females.

47.3% (353/747) of Influenza A negative samples were male and 52.7% (394/747) were female, but 48.4% (342/707) of Influenza B negative samples were males and 51.6% (365/707) were females.

The study was carried out during December 2019 –March 2020, with an aim to identify the prevalence of the influenza virus in Northern Cyprus region and it was observed that:

December 2019 –March 2020, out of 844 samples collected and processed, 27.7 %(234/844) positivity of Influenza virus was observed. Out of which 11.5% (97/844) positive were influenza type A virus, 16.2% (137/844) were influenza type B virus.

The highest positivity of Influenza virus was observed in the months of January, with a total of 980 analyzes. Of which, 65 (13.3%) were positive for influenza A and 83 (16.9%) were positive for influenza B, followed by February and December.

From this study, we conclude that Influenza virus was distributed throughout the 2019–2020 study period. Highest positivity of Influenza virus was found during the winter and the lowest prevalence in the spring season.

Our study sets out that the distribution of seasonal influenza types was not associated with gender during the period. However, the rate of both influenza type A and B have a statistically significant relationship with months and age. This initial study may also help surveillance and vaccine studies in the future.

Study limitations

1. The sample size and the technique we used point the limitations of our study.

2. We could only test samples of individuals who attended to NEU Hospital by using rapid antigen detection test kits.

3. Although these tests provide rapid test results, they have limited analytical sensitivity to detect influenza viruses in respiratory specimens and they do not provide information on subtypes of specific virus strains. Therefore, a larger sample set will be needed to estimate the accurate rate of seasonal influenza viruses of whole population in Northern Cyprus by using molecular tests.

4. The data was only collected from patients were admitted to NEAR EAST HOSPITAL where shows that out study do not reflect the data in whole Cyprus.

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Appendix A

YAKIN DOĞU ÜNİVERSİTESİ BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

ToplantıTarihi : 28.05.2020

Toplantı No **:**2020/79

ProjeNo **:**1069

YakınDoğuÜniversitesiTıpFakültesiöğretimüyelerindenYrd. Doç. Dr. Ayşe Sarıoğlu'nunsorumluaraştırmacısıolduğu, YDU/2020/79-1069 projenumaralıve**"Investigation of Influenza Activity during 2019-2020 Winter Season in Northern Cyprus"** başlıklıprojeönerisikurulumuzca online toplantıdadeğerlendirilmişolup, etikolarakuygunbulunmuştur.

Prof. Dr. Rüştü Onur

YakınDoğuÜniversitesi

BilimselAraştırmalarEtikKuruluBaşkanı

Appendix B

Seasonal Influenza Activity

ORIGINALITY REPORT

9%	7%	7%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet Source	2%
2	www.frontiersin.org Internet Source	1%
3	academic.oup.com Internet Source	1%
4	en.wikipedia.org Internet Source	1%