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TANAFFOS

Spectrum and Burden of Influenza Infection: An Approach to Identify Predictors of Morbidity and Mortality Rate from the Patients of the Northwest of Iran

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Background: The objective of this research is to analyze influenza-induced complications, symptoms, and the interaction of morbidity and mortality rates in hospitalized influenza cases based on age-sex dispersion, influenza virus subtype, prescribed medications, and underlying conditions.

Materials and Methods: We performed this retrospective study using a dataset of 10,517 hospitalized individuals, including 3,101 laboratory-confirmed influenza cases from patients of all ages who had attended hospitals in the Northwest of Iran due to respiratory complications.

Results: The most prevalent strain which circulated annually was influenza A/H3N2. In contrast to previous studies, our findings suggested that influenza A/H1N1 has the highest mortality rate and the most severe complications. Regardless of virus type/subtype, the most susceptible age group for influenza was 0-9 years old in both males and females. Meanwhile the high-risk age group among males was 50-59 years old and among females were over 80 age group (mortality rate ≈ 20%). Chronic obstructive pulmonary disease (COPD) (32%) and cardiovascular disease (CVD) (30%) were the most prevalent active underlying diseases among the patients who died, with the latter being more prevalent in males over the age of 70. Patients with a history of chemotherapy had the highest mortality rate. Patients who were prescribed a combination of antibiotics and antivirals had better outcomes with lowest mortality rate.

Conclusion: Our findings demonstrated that annual influenza seasons are often marked by changes in influenza types and subtypes, with variations in the severity. Development of a standardized set of arrays that best correspond with infections, can be useful in guiding diagnostic and therapeutic decisions.

Key words: Influenza; Clinical manifestations; Epidemiology; Vaccination

INTRODUCTION

Influenza is a multifactorial infectious disease with a large burden of illness. Infectious disease mechanisms have a broad range of intriguing and unexplained phenomena; nevertheless, there is a discrepancy about how different studies incorporate immunology, epidemiology, virology, and mathematics to present a picture of influenza seasonality. Local seasonal influenza outbreaks are often associated with increased incidence of two or more influenza types or subtypes (1). In general, the compound influenza activity is accompanied by the co-circulation and interaction of seasonal H3N2, seasonal H1N1, and influenza B. However, the cause behind the asymmetry of seasonality is still undisclosed. Influenza circulation

activity may be influenced by viral evolution, host susceptibility, environmental, and biological factors (2).

Regarding the World Health Organization (WHO), influenza triggers 3-5 million cases of severe illness and 250-500 thousand deaths worldwide per year (3). Influenza outbreaks occur in the southern hemisphere from May to October and in the northern hemisphere from October to May, implying an alternative influenza transmission in each of these hemispheres (3). Sex disparities among different individuals, which are based on the variations in sex hormones and chromosomes, are one of the physiological factors linked to influenza morbidity, severity, and mortality. In general, testosterone has an immunosuppressive effect on the immune system, while estrogen has an immune-enhancing effect. According to the evidence, females have a much greater immune response than males, which may contribute to meliorating females during infectious diseases. The X-linked microRNAs have also been found to contribute to sex disparities in immune responses, culminating in far higher responses in females. Though, because the outcome of infectious diseases is reliant on multifactorial circumstances and sex hormone levels may not remain constant throughout life, a definitive perspective about comparing females' and males' status following an infectious disease is difficult to ascertain (4, 5).

Other factors contributing to influenza infection outcomes include age-dependent phenomena, which have a complex relationship with susceptibility and severity due to significant physiological changes in the body, and immunity acquired from previous exposures, which often comes as a result of aging. It suggests the fact that the high-risk age groups can shift over periods. So, through aging, older individuals become well cross-protected which younger individuals and children lack, making them more susceptible to developing the severe disease (6). It's reflected in the fact that these younger individuals may have an overactive immune response to the infection, which can be coupled with multi-organ damage and a worsening of their condition (7). Furthermore, in recent years, virologic features of the influenza virus, such as virus types and subtypes, have emerged as important factors associated with the severity of influenza infection in patients (8). Although there is limited evidence to indicate whether the diversity of influenza virus types, subtypes, and lineages impacts clinical manifestations, illness severity, or case fatality ratio (9). Currently, the utilization of antibiotics in the case of secondary infection and antivirals or a combination of them is the most conventional therapeutic approach for influenza. The M2 ion channel blockers and the neuraminidase inhibitors (NAIs) are two classes of influenza antivirals that have already received widespread licensure around the world. Considering the multilateral risk factors of influenza is a key step in preventing and managing influenza (10). Given the above reflection, this paper attempts to provide epidemiological analyses to evaluate the correlation between age, sex, underlying diseases, influenza-induced complications, and mortality in influenza-positive hospitalized cases from the Northwest of Iran.

MATERIALS AND METHODS

We performed this retrospective study by focusing on a wide spectrum of influenza disease characteristics and risk factors. 10,517 individuals were recruited of all ages and were hospitalized in the northwest of Iran from February 2012 to February 2021. The sampling strategy was the same in each of the 9 consecutive years.

The covariates in the dataset included the type of diagnosing test, location, date of onset of the symptoms, date of death or hospital discharge, and date of testing, influenza virus type and subtype, age of the group at onset (in 10-year bands), sex (male, female), underlying diseases (diabetes mellitus, severe obesity, chronic neurological disorder/epilepsy, chronic renal disease, chronic obstructive pulmonary disease, cardiovascular disease, malignancy, chronic liver disease) prescribed medication for influenza (antivirals and antibiotics) vaccination status, and influenza induced symptoms and complications.

Laboratory confirmation was performed following the protocol described by the WHO for the detection and characterizations of influenza viruses.

The samples were tested for influenza infection by reverse transcription-polymerase chain reaction (RT-PCR). Positive notifications make up approximately 29.5% of all notifications, which was extracted accordingly: the number of laboratory-confirmed cases for any influenza virus type or subtype, A/H1N1, A/H3N2, B (with unknown lineage), the age-standardized notification rates', underlying diseases, and prescribed medications for influenza correlation with morbidity and mortality rate for each type and subtype of influenza, and the dominance of influenza type and subtype during each year. We assessed the prevalence of influenza (A, A/H1N1, A/H3N2, B) and hospitalization rate for overall males and females, as well as individually for each sex and age group, to study them statistically. Also, our data addresses the displaying of periodic annual functions which describe the peak of activity and intensity of the seasonality during each year from 2012 to 2021. Descriptive statistics were calculated for all variables used in the study by IBM SPSS v24 and considered $P < 0.05$ to demonstrate the statistical significance.

RESULTS

Demographics

A series of preliminary studies were conducted to assess the association between demographic variables (age, sex, underlying diseases, medication history, and vaccination history) and the main variables of interest (symptoms, morbidity, and mortality rate). This research contained data from 10,517 samples, comprising 5,181 (49.26%) males and 5,336 (50.74%) females, with 1,628 (52.50%) females and 1,473 (47.50%) males who tested positive for influenza (Table1). Our study included 1,588 (51.21%) cases of influenza A/H3N2, 870 (28.06%) cases of influenza A/H1N1, and 643 (20.73%) cases of influenza B. The cases ranged in age from 0 to 98 years old. The population of each sex was divided into nine groups based on age. The age range of 0-9 years old had the highest number of cases. Although there were minor differences, this tendency was maintained over 9 years and different months. Moreover, we perused the underlying diseases of the influenza-positive cases and found out that the most common underlying diseases were chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and epilepsy respectively. 165 (5.32%) cases in our study were vaccinated. The vaccination rate was about 4.7% in the younger generation (under 18 years old) and 7.9% in cases with underlying chronic disease.

Table 1. Annual influenza-associated morbidity by type and subtype of the virus

	Specimens	No. of positives	Influenza A		Influenza B
			H1N1	H3N2	
2012	690	27	22	5	0
2013	1211	381	339	37	5
2014	746	226	9	186	31
2015	2358	892	347	206	339
2016	1141	402	26	341	35
2017	759	143	71	25	47
2018	633	124	53	34	37
2019	2395	833	3	747	83
2020	575	73	0	7	66
2021	9	0	0	0	0

Analyses by sex and age group

Our analysis demonstrated that sex disparities have a significant impact on influenza outcomes, and since the influence of sex disparities may be age-dependent, the importance of reproductive status in influenza outcomes should not be overlooked. Our findings revealed that the highest rate of influenza-positive cases among males and females, regardless of virus subtype, was in the 0-9-year-old population, especially in the 0-4-year-old age group, which was about 2 folds higher than the 5-10-year-old age group, and the lowest was in the 10-19-year-old population. According to our findings, influenza mortality rates and severity were higher prior to the onset of puberty (0-9 years old) than during adulthood (10-50 years old). We discovered a statistically significant link between mortality rate and sex; males had a higher overall mortality rate than females. We perused mortality rates in each age group to

assess underlying factors that contributed to males having a higher mortality rate than females. Except for the 20-29-year-old age group and cases over 70 years old, males had a more severe influenza infection than females in all age groups (Figure1). Our findings revealed that the male mortality rate was highest in the 50-59-year-old age group, whereas the female mortality rate was highest in the over 80-year-old age group (both about 20%). The current finding suggests that females over the age of 70 and those aged 20 to 29 had a higher death rate than males. However, males had a higher mortality rate among those aged 10 to 60. Since the sex-age-related distribution of some underlying illnesses can increase influenza mortality, we investigated how the underlying diseases were distributed among different age groups of males and females. The most common underlying lethal disease among influenza-positive patients in our study were COPD and CVD. They were more prevalent in males, particularly in cohorts aged over 50. We compared symptoms presented by each sex to evaluate age-related clinical and laboratory variations, but no significant differences were noted.

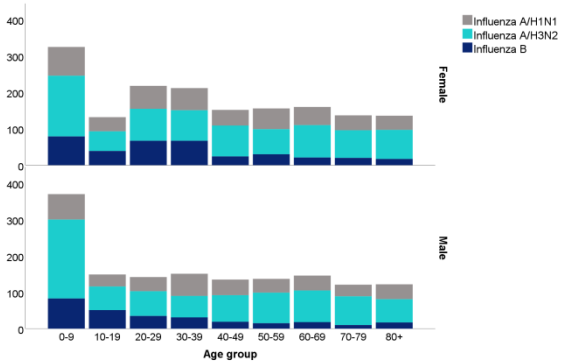

Figure 1. Sex differences between influenza-positive cases by age group, number of influenza-infected patients regarding each age group in each sex is presented by subtype of the influenza virus

Clinical characteristics and severity

We evaluated the mortality rate and symptomatology of each influenza virus type and subtype (Figure 2). According to our analysis, influenza is characterized by sudden onset of fever, cough, myalgia, headache, malaise, pharyngitis, and nasal congestion. Additionally, gastrointestinal manifestations such as nausea, vomiting, and diarrhea are prevalent. Fever higher than 38 degrees was the most common symptom amongst all influenza types and subtypes (87.7%), and cough was the second most common symptom amongst all influenza types and subtypes (76.5%). In addition, conjunctivitis (6.2%) and convulsion (5.9%) were the least frequent influenza symptoms. According to our findings, the majority of cases manifesting each symptom was higher in influenza-positive cases compared to influenza-negative cases. Our analyses revealed that among the patients who died, the most prevalent symptom prior to death was a fever higher than 38 degrees (84%), and the second most prevalent symptom was hemoptysis (34%). To estimate the severity of each subtype and type of influenza, we analyzed the mortality rate for each type and subtype of influenza virus. The mortality rate of patients with H1N1 infection was approximately 9.6%, while influenza B and H3N2 mortality rates were about 4.6 and 5.5%, respectively. These results suggest that influenza-positive cases of H1N1 had more severe outcomes than other cohorts.

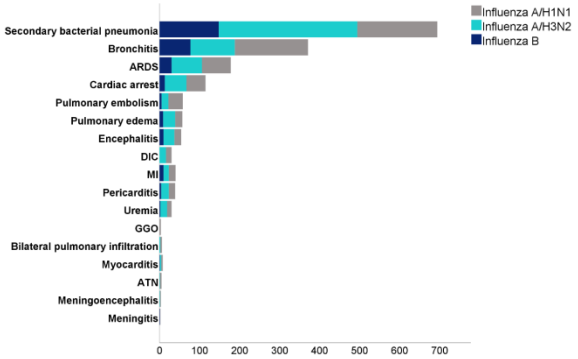

Figure 2. Influenza-induced complications. This figure illustrates the number of influenza-infected cases manifesting each complication by each type and subtype of the influenza virus

Influenza infection can induce extrapulmonary complications, which are less common. Further analyses showed that among the influenza-positive patients, secondary bacterial pneumonia was the most prevalent complication (22.4%). Other common pulmonary complications in our study include: bronchitis (12%), acute respiratory distress syndrome (ARDS) (5.7%), cardiac arrest (3.7%), pulmonary embolism (1.9%), and pulmonary edema (1.8%). Moreover, extrapulmonary complications of

influenza included: encephalitis (1.7%), myocardial infarction (MI) (1.3%), pericarditis (1.3%), disseminated intravascular coagulation (DIC) (1%), Uremia (1%), myocarditis (0.3%), meningoencephalitis (0.1%), acute tubular necrosis (ATN) (0.06%), and meningitis (0.06%) (Figure 3). According to table 2, our analysis revealed that patients infected with influenza H1N1 had a greater risk of influenza-induced complications compared to patients infected with influenza H3N2 or B.

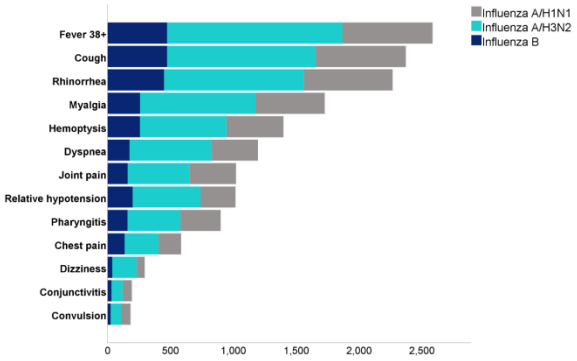

Figure 3. Influenza symptomatology. This figure illustrates the number of influenza-infected patients manifesting each symptom by each type and subtype of the influenza virus.

Table 2. Influenza-associated mortality for various underlying disease and medication history

Comorbidity	No. of cases	Mortality (%)
Diabetes mellitus	253	45(18)
Severe obesity	42	10(24)
Chronic neurological disorder/epilepsy	133	31(23)
Chronic renal disease	110	20(18)
Chronic obstructive pulmonary disease	360	115(32)
Cardiovascular disease	357	107(30)
Malignancy	94	27(29)
Chronic liver disease	18	4(22)
Medication history		
Prolonged immunosuppressants	79	18(23)
Prolonged aspirin	260	48(18)
Chemotherapy	63	18(29)

Medication therapy

We analyzed the medication administered for influenza-positive cases. Antivirals were given to 2,646 (42.9 percent) influenza-positive individuals, with oseltamivir accounting for nearly 99% of them. 0.66% of the patients had received only antibiotics, whereas about 57% had received a combination of antivirals and antibiotics. The mortality rates of patients who received only antibiotics, only antivirals, or a combination of the two were 100, 9.5, and 8.3%, respectively, indicating that the combination of antivirals and antibiotics had the greatest effectiveness in the treatment of influenza-positive patients.

Chronic underlying diseases and medication history

Our analyses demonstrated that 467 (15.06%) patients who tested positive for influenza had underlying diseases. We appraised the mortality rate of influenza-positive cases regarding each comorbidity and concluded that patients with COPD had the highest mortality rate (32%), while patients with CVD had the second-highest mortality rate (30%). Besides, the impact of medication history on the outcome of influenza-positive patients with underlying diseases was evaluated. 4.5% of the influenza-positive cases had a history of long-term immunosuppressant administration, 21.36% had a history of long-term aspirin administration, and 5.1% had a history of chemotherapy. By assessing the mortality rate per patient group, we discovered that the death rate for patients with a history of chemotherapy was the highest (28.5%) (Table 3).

Table 3. Influenza-induced complications by type and subtype of the influenza virus

Complication	Influenza A/H1N1	Influenza A/H3N2	Influenza B
ARDS	76(8.3%)	72(4.3%)	30(4.8%)
ATN	4(0.2%)	1(0.1%)	-
Bilateral pulmonary infiltration	4(0.5%)	2(0.2%)	-
Bronchitis	183(21%)	111(6.9%)	77(12%)
Cardiac arrest	48(5.5%)	54(3.4%)	13(2%)
DIC	14(1.6%)	16(1%)	-
Encephalitis	17(1.9%)	27(1.7%)	10(1.5%)
GGO	3(0.3%)	-	-
Meningitis	1(0.1%)	-	1(0.2%)
Meningoencephalitis	2(0.2%)	1(0.1%)	-
MI	17(2%)	13(0.8%)	10(1.5%)
Myocarditis	4(2%)	3(0.8%)	1(1.6%)
Pericarditis	16(1.8%)	19(1.1%)	4(0.6%)
Pulmonary edema	30(2.1%)	18(1.9%)	9(1.4%)
Pulmonary embolism	36(2. %)	17(1.9%)	5(1.1%)
Secondary bacterial pneumonia	200(23%)	346(23%)	148(21.8%)
Uremia	11(1.3%)	17 (1%)	2(0.3%)

Prophylactic vaccination

Our data analysis revealed 165 of the cases in our study had been vaccinated. The vaccinated cohorts comprised 80 (48.48%) females and 85 (51.52%) males. We compared the effectiveness of influenza vaccination among males and females through evaluating the positivity rate of influenza among vaccinated males and females. We found that the positivity rate of influenza among vaccinated males was about 1.6-fold higher than that of females (32.6% vs 21.7%). Further analysis of influenza-induced complications of vaccinated influenza-positive cases revealed that only 2 (1.1%) cases of the influenza-positive vaccinated cases developed secondary bacterial pneumonia, 4 (2.2%) cases developed bronchitis, and the rest did not manifest any complication. Moreover, our analyses of influenza symptomatology showed that fever higher than 38 degrees was the most common symptom which was manifested by 30 (18.8%) cases and cough was the second most common symptom which was manifested by 25 (15.1%) cases. We evaluated influenza vaccination outcomes and discovered that 45.5% of cases were tested positive for influenza. Further analysis indicated that 66.6% of vaccinated patients with a history of immunosuppressant were tested positive for influenza, while this rate was 40% among patients with a history of chemotherapy. Another determinant factor associated with vaccination efficacy was age. 78.9% of vaccinated influenza-positive patients were over the age of 50. According to our findings, 47 (23.9%) cases tested positive for H3N2, while 41 (87.2%) cases were infected with H3N2. About 11.7% of the vaccinated cases who were tested positive for influenza died.

Seasonality

To peruse peaks of influenza activity over time, we analyzed influenza peaks throughout 9 years. Our analyses presented that the influenza peak was between November and March of each year ($P < 0.05$). Positive influenza cases accumulated from November to March of the nine consecutive years accounted for 96% of all positive cases. On February 21, 2012, the first confirmed case of influenza H3N2 was reported, and the first case of influenza H1N1 was reported on December 1, 2012. The highest peak of influenza B and H1N1 was observed in January 2020. The influenza H3N2 incidence peaked in January 2017. According to our findings, there was no influenza H1N1 virus circulation from January 2020 to January 2021 and no influenza B virus circulation from January 2012 to January 2013. However, there was a circulation of influenza H3N2 annually; thus, cases infected by influenza A/H3N2 were detected more frequently than influenza B and influenza A/H1N1 with annual peaks. The subtypes detected in each season differed unpredictably. Nevertheless, our data analysis revealed that annual peaks of H3N2 were higher than influenza B and H1N1 in 2014, 2017, 2019, and 2020. Our analyses also indicated that the number of influenza-positive cases during 2020 had a significant reduction compared to former years; the number of influenza-positive cases during 2020 was approximately 20% of the average annual positive cases of former years (Figure 4).

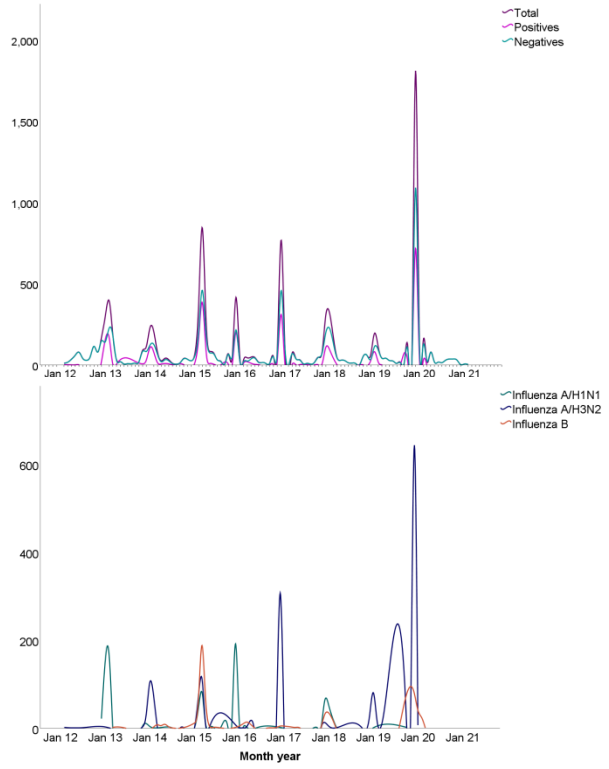

Figure 4. Number of cases tested for influenza from 2012 to 2021. This figure illustrates the number of monthly cases that were tested in a total sample, the positive ones and negative ones regarding the type and subtype of the influenza virus.

DISCUSSION

Health systems in Iran, as well as a number of other countries, have begun to pay greater attention to the detection of different types and subtypes of influenza, as well as the restriction of transmission (11). According to the evidence, the disease emerges year-round in tropical and subtropical countries like Iran, though seasonal peaks of increased activity are noticeable for each type and subtype of influenza (12). Likewise, our findings suggest that approximately 95.6% of influenza-positive cases were detected between November and March for 9 consecutive years. Variations of solar ultraviolet (UV) radiation trigger these seasonal phenomena and may play a key role in the seasonal incidence of influenza outbreaks. In this respect, according to a study by Hosseini et al., more than 52% of influenza cases occur in the winter and 31% in the autumn. According to the findings, local influenza outbreaks occur in Iran at 80 weeks of the year, with more than half of them occurring during the winter. Several studies have shown that within interpandemic periods, influenza-induced complications and mortality are statistically higher among patients with chronic underlying diseases, particularly among the elderly (13).

Furthermore, as compared to the prior 5 years, our findings revealed that the least number of influenza-positive cases were detected in 2020. That could be attributable to the consequences of the COVID-19 pandemic and nonpharmaceutical interventions (NPIs) like lockdowns and border closures on mitigating or even eliminating severe influenza outbreaks (14, 15). In this respect, regarding a report by Solomon et al., as there is a special emphasis on seasonal influenza vaccination during the Coronavirus Disease 2019 (COVID-19) pandemic, there might be justification for a drop in influenza-positive cases within 2020 (16). Analyzing the role of underlying diseases in the severity of the disease showed that patients with COPD had the highest mortality rate. According to the study by Mallia and Johnston on hospitalized influenza-infected patients, during the influenza epidemic, chronic pulmonary disease was the most common underlying disease, implying that pulmonary disease including COPD is considered the most important risk factor for an adverse outcome of influenza infection (17). CVDs are the second most common underlying disease and a major risk factor for mortality in influenza-positive hospitalized patients. The prevalence of CVD rises during the cold seasons, which coincide with influenza outbreaks. Low temperatures, along with influenza infection, tend to worsen CVD symptoms, increasing hospitalization and mortality rates. In line with this, Nguyen et al. observed that CVD mortality and influenza incidence peaked at about the same times during the study period (18).

Medication history in influenza patients may be considered a risk factor for influenza patients. According to our statistics, approximately 28.5% of influenza-infected patients with a history of chemotherapy died as a consequence of influenza infection. This suggests that cancer patients or who have recovered from cancer would have more severe outcomes. Chemotherapy can exacerbate infectious diseases like influenza by impairing the function of peripheral T cells and suppressing T-cell proliferation through activation of the lymphocyte-specific protein tyrosine kinase (LCK) (19). Furthermore, according to our findings, about 22.7% of the influenza-positive patients who had a history of using immunosuppressants had died. This population is considered a high-risk group for infectious diseases, like influenza. Several studies have also shown that immunocompromised patients are more vulnerable to infectious disease and are more likely to develop impaired vaccination responses, decreased cytokine response, and immunoglobulin levels (19-21).

According to our findings, 9.8% of the influenza-positive cases with a history of long-term aspirin use died, indicating that long-term aspirin use is a contributing factor for severe outcomes in patients with influenza infection. A study by Eyers et al. suggested that the use of antipyretics for human influenza infection may lead to the development of secondary bacterial pneumonia (22). In this way, our analyses revealed that approximately 29% of the influenza-positive patients with a history of long-term

aspirin use developed secondary bacterial pneumonia. Besides that, according to Bancos et al., aspirin may interfere with the development of immune responses following vaccination and can lead to immune tolerance in dendritic cells (23). Likewise, animal studies have demonstrated the role of aspirin in increasing mortality following influenza virus infection (22, 24)

Other than underlying diseases and medication history, sex and age are two important factors influencing mortality and disease severity. Our findings revealed that influenza-positive males aged 30 to 70 died at a greater rate than females. We also noticed that males aged 50 to 59 had the highest mortality rate. Meanwhile, among males, the prevalence of CVD and COPD was highest in this age group, which is well associated with the highest mortality rate in this age group. The higher mortality rate of males in their pre-puberty age among males aged younger than 50 years old may indicate a positive effect of testosterone during infections (25). Regarding the fact that serum testosterone levels begin to decline with age and are associated with an increased mortality rate. It is hypothesized that lower serum testosterone levels in males can lead to more severe outcomes. Vom Steeg et al. supported this hypothesis by showing that testosterone levels in young male mice decline during the acute phase of infection. However, testosterone levels return to normal during the recovery phase of infection, while testosterone levels in old mice remained low during the infectious disease (26). Overall, it has been demonstrated that the outcome of influenza A virus infection in male mice and testosterone levels is correlated with improved outcomes following infection (27-29). Tuko et al. showed that there is a negative correlation between testosterone level and interleukin 1β (IL-1β) level, which is an inflammatory factor (25) that highlights the positive effect of testosterone in preventing severe disease. Altogether, testosterone plays an essential positive role in young males as opposed to old males. Furthermore, studies have shown that in pre-puberty ages, boys have shorter or equal airway length compared to girls, and after puberty, as airway length is normalized for body height, boys have significantly greater airway length than girls. This may explain why the younger boys are more likely to be hospitalized and have a higher mortality rate (30).

Our findings indicated the mortality rate of females rises sharply after the age of 40, which corresponds to the estimated age of estrogen reduction. Since sex hormone levels begin to increase one pattern and females around the age of 10, peak in the early 20s and then begin to fall around the age of 45, Thus, in this way, we speculated that there may be a link between sex hormone patterns and female mortality rate. In line with this hypothesis, a study by Taneja proposed that estrogen plays a positive role in the immune system. According to this study, estrogen has an immune-enhancing effect on the immune system (4). Estrogen has been shown to regulate immune response by altering the negative selection of high affinity auto-reactive cells, modulating B cell activity, and enhancing T helper 2 (Th2) response (4). Another justification for females' tendency to have a lower mortality rate than males is a disparity in immune response after vaccination. By analyzing the vaccination rate of the patients included in our study, we discovered that 32.6% of the vaccinated males tested positive for influenza, while approximately 21.7% of the vaccinated females tested positive, indicating that women appear to develop greater immunity after influenza vaccination than males. Furthermore, the effect of age on vaccine effectiveness cannot be overlooked. Potluri et al. performed a study to assess the effect of age-sex on the humoral immune response following vaccination, and it was proposed that young females had the highest IL-6 level following vaccination, which may be an indication of the fact that young females acquire the highest level of vaccine-induced humoral immunity(31). Even though no research has been done on the connection between reproductive status and vaccine-induced immunity to date. During the 2009 H1N1 pandemic, serum antibody levels were compared among different sexes and age groups, and it was revealed that the association between age and vaccine-induced immune response was

only found in females. In addition, the amount of serum estradiol in females was shown to be linked to seroconversion of neutralizing antibodies Voigt et al. investigated the effect of sexual dimorphism on influenza vaccination-induced acquired immunity and achieved some noteworthy findings. The proportion of influenza A-induced memory B cells, the level of the cluster of differentiation (CD) 4^+ cells, the capacity of $CD4^+$ cells to express estrogen receptors, and their function in the immune response against influenza were all reported to be greater in vaccinated females than in males (32). Regarding the strong evidence, males have higher levels of natural killer (NK) cells, which could make it challenging for older males to develop a protective vaccine-induced immune response due to higher activity of pro-inflammatory NK cells-related genes and a larger fraction of NK cells in males' peripheral blood mononuclear cells (PBMCs). Also, regardless of age, females tend to show greater antibody responses than males, higher basal immunoglobulin levels, and higher B cell numbers. Even though further studies are required to determine sex differential response to influenza vaccination. Additionally, since females' humoral immune responses differ from males before puberty, it is concluded that sex chromosomes are determinants of immune response and therefore disease outcome (33). The X chromosome comprises a range of immune-related genes as well as regulatory microRNAs. Many X-linked genes are implicated in innate and adaptive immune responses, which are much more active in female immune cells than in males (34). These findings suggest that sex disparities should be taken into account when designing new antiviral medications and vaccination protocols for influenza in order to promote more efficient approaches.

Our findings showed that children aged 0 to 10, or those born after the 2009 H1N1 pandemic, were more likely to be infected with H1N1 than other age groups. Xu's analysis yielded a similar pattern of results (35). It has been well established that individuals can maintain a lifelong bias in immune memory, even after prolonged exposure to or vaccination against heterologous influenza subtypes (36). Birth year, rather than age, influences subtype-specific variations in seasonal influenza attack risk. According to the evidence, the 1968 H3N2 outbreak did not lead to an increase in mortality in the elderly who had previously been exposed to the virus as children or young adults to an H3N2 virus that circulated in the late 1800s (35, 37). Our studies confirm that the roles of virus type and subtype in morbidity and mortality should not be overlooked. As shown by our analysis, the mortality rate for patients infected with influenza B was about 4.7%, while the rates for patients infected with influenza H1N1 and H3N2 were 9.7 and 5.5%, respectively, implying that the severity of influenza H1N1 was the highest. Given the fact that some studies have identified no significant variation in clinical presentations of different influenza viruses, implying that virus type or subtype does not appear to be a major determinant of severity, especially whenever the patient's age, sex, and pre-existing health conditions are taken into consideration (8).

Our data analysis revealed that secondary bacterial pneumonia was the most common pulmonary complication in influenza-positive cases. In accordance with our results, other studies on hospitalized patients with respiratory viral infection found that higher mortality risk was mostly attributed to secondary pneumonia rather than primary viral infection (28% vs 15%) (38, 39). Several extrapulmonary complications such as cardiovascular and neurologic can also be observed in addition to pulmonary complications, notwithstanding their rareness (40). Cardiovascular complications are the second leading cause of death during the influenza season. Acute myocarditis is a well-known influenza complication that impacts 0.4% to 13% of hospitalized patients (41). Influenza myocarditis may manifest with symptoms ranging from fever, myalgia, palpitations, shortness of breath, and chest pain to hemodynamic instability and collapse. Another cardiovascular complication that manifested in 4% of influenza A-infected patients was myocardial infarction (42). Even though few studies have assessed the association between infection and hemorrhagic stroke. Our

data as well as a large prospective study didn't show such an association between influenza and hemorrhagic stroke. Several mechanisms underlie influenza-induced cardiovascular diseases. Influenza and other respiratory infections can lead to atherosclerosis through nonspecific immune stimulation or plaque rupture. Moreover, endothelial dysfunction, hypercoagulability, or increased viscosity can occur following the fever (41, 43, 44). Although neurologic complications of influenza were not common in our study, all the patients with neurologic complications like meningitis, meningoencephalitis, and encephalitis in our study died soon after (45). It is noteworthy to state that about 96% of these groups were pediatric cases, in other words, about 6.6% of the pediatrics had neurologic dysfunctions. Khandaker et al. substantiated our findings by demonstrating that influenza is associated with various neurologic complications, mostly in pediatric populations, such as Reye syndrome, Guillain-Barré syndrome (GBS), transverse myelitis, seizures, and encephalopathy, which can be fatal (46).

We noticed that among the vaccinated influenza-positive patients, only 2 cases (1.1%) of bacterial pneumonia and 4 cases (2.2%) of bronchitis were found. Our analysis also revealed that patients with a history of vaccination have fewer influenza-induced complications. In line with our findings, research by Deiss et al. showed that after vaccination, there was a substantial reduction in the severity of symptoms among individuals with breakthrough influenza A/H3N2 infection (47). Analyses of the prescribed medication for influenza patients revealed that using a combination of antivirals and antibiotics had the highest efficacy rather than using only antivirals, with oseltamivir accounting for almost 99% of the prescribed antivirals (48, 49). Oseltamivir and zanamivir are highly recommended and well prescribed for the patients in our study due to their high effectiveness in the treatment of influenza-infected patients (7). Even though oseltamivir may have neuropsychiatric side effects, its administration in Japan is restricted in teenagers aged 10 to 19 (50). We perused the complications of influenza-positive young patients who had used oseltamivir and realized that 5 cases of influenza-positive patients aged 0 to 18 with no underlying diseases have died due to epilepsy, meningitis, and meningoencephalitis, all of which are considered oseltamivir side effects. Furthermore, because of the risks associated with zanamivir, it is not approved for children under the age of 7 (10, 51).

CONCLUSION

In summary, this population-based study investigated influenza clinical complications and outcomes regarding several factors. The findings demonstrated that annual influenza seasons are often marked by changes in influenza types and subtypes, with variations in the severity. Moreover, our analysis revealed a relationship between the date of birth and morbidity rate, which may refer to the "antigenic sin" phenomenon, however, we found that sex hormone levels may also have a determinant role in the age-dependent pattern of influenza severity. The fact that female notifications continue to rise after menopause suggests that there is a link between age and sex when it comes to influenza virus susceptibility. Besides, we showed the relationship between underlying diseases and clinical characteristics of the disease. Taking these factors into account, as well as insights into strain-specific pathogenicity, can be useful to guide messaging about influenza vaccination, treatment recommendations, and influenza prevention.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethics committee approval

The present study was approved by the National ethic committee with registration number IR.TBZMED.VCR.REC.1395.401.

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