RESEARCH ARTICLE

Serological studies of influenza infection among population in southern region of Kazakhstan during the 2018-2021 epidemic season

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Abstract

Objective: To conduct serological studies of influenza infection rate during an epidemic.

Methods: The retrospective study was conducted at the Research and Production Centre for Microbiology and Virology, Almaty, Kazakhstan, and comprised data, including blood samples, from patients with symptoms of acute respiratory viral infection, bronchitis and pneumonia during 2018-21 from various healthcare institutions in the Almaty region. Serological tests on blood serums were carried out sing haem agglutination inhibition assay and enzyme-linked immunosorbent assay. Data was analysed using Graph Pad Prism 9.

Results: Of the 779 blood samples, 392(50.3%) came from women and 387(49.7%) from men. The overall age range was 0-80 years. Serological analyses using haem agglutination inhibition assay showed the presence of anti-hemagglutinins against pandemic A(H1N1)pdm09 virus in 292(37.5%) samples, influenza A/H3N2 virus in 340(43.6%) and type B virus in 53(6.8%). Antibodies against two subtypes of influenza A virus and type B virus were simultaneously identified in 25(3.2%) cases, whereas against influenza A (H1N1+H3N2) viruses in 69(8.9%). In enzyme-linked immunosorbent assay, antibodies against influenza A/H1N1pdm virus were detected in 108(13.9%) cases, against A/H3N2 virus in 105(13.5%) and type B virus in 65(8.3%). Antibodies simultaneously against two subtypes of influenza A virus were identified in 46(5.9%) of blood serums, and against influenza A and B viruses in 60(7.7%).

Conclusion: Co-circulation of influenza A and B viruses was observed, confirming the role of influenza viruses in the epidemic process.

Keywords: Virus, Influenza, Antigen, Serum, Diagnosis. (JPMA 73: 804; 2023)

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Introduction

Influenza is an acute respiratory viral infection with an aerogenic transmission mechanism. Influenza viruses are constantly circulating among the human population, causing seasonal increases in the disease and periodically acquiring the character of epidemics and even pandemics. Influenza epidemics inflict significant damage to economy and society, like decline in labour productivity, reduction in human resources, loss of human lives, and undue burden on healthcare facilities.

This refers primarily to influenza type A viruses, which cause epidemics every 2-3 years, and pandemics several times a century accompanied by the spread of infection everywhere in the world with the number of cases of around 1-2 billion.[1,2]

In 2019, the World Health Organisation (WHO) launched the Global Influenza Strategy 2019-2030 aimed at strengthening epidemiological surveillance and preparing for future pandemics.[3] In particular, an important role was assigned to the study of the circulation characteristics for influenza viruses and their properties, mechanisms of variability and susceptibility (host factors), minimisation of risk factors for the infection and appearance of severe forms, development of new effective diagnostic systems, vaccines and drugs.

The epidemic process of influenza has undergone considerable changes in recent years. Concurrently with the circulation of known seasons influenza A/H1N1, A/H3N2 and B viruses, a reassortant influenza A/H1N1pdm09 2009 virus has appeared, and the role of respiratory viruses of non-influenza aetiology has increased. To predict the epidemic situation and formulate an epidemiological diagnosis of influenza, a major role in the epidemiological surveillance system is assigned to retrospective analyses.[4-6]

Serological diagnosis is particularly important in the case of an atypical or asymptomatic course of influenza infection. This research method for the surveillance of the influenza virus circulation is used in the diagnosis,

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assessment of the risk group for infection and disease, the degree of their susceptibility as well as the rate of their involvement in the infectious process, which makes it possible to reduce socio-economic damage through the use of effective prophylactic and therapeutic agents. The haemagglutinins inhibition assay (HAI) and enzyme-linked immunosorbent assay (ELISA) are still widely used in the diagnosis of influenza infection to determine specific antibodies in blood serums.[7,8]

Clinical laboratory diagnosis using serological methods can be considered retrospective since the presence of diagnostic increases in antibody titers (at least 4 times) in paired blood serums collected at different stages of the disease or throughout life can serve as confirmation of a previous infection. This is due to the fact that a person throughout his life repeatedly encounters influenza viruses, and anamnestic antibodies against a wide range of strains, with the exception of new antigenic variants, are always detected in the blood serum of each person.[7]

Epidemic rise in the incidence of influenza are recorded annually in various regions of the world, including the Republic of Kazakhstan,[9,10] which is a transit corridor for the passage of epidemic variants of the influenza virus from East to West. This reinforces its geopolitical importance in global influenza surveillance, which is currently conducted in more than 110 WHO national centres.

The current study was planned to conduct serological studies of influenza infection rate during an epidemic.

Material and Methods

The retrospective study was conducted at the Research and Production Centre for Microbiology and Virology, Almaty, Kazakhstan, and comprised data, including blood samples, from patients with symptoms of acute respiratory viral infection, bronchitis and pneumonia during the 2018-21 epidemic from various healthcare institutions in the Almaty region.

The samples were collected after approval from the institutional ethic review committee. The sample was gender-balanced and regardless of age. It was initially divided into children and adults groups, but for a detailed analysis, the sample was divided into 15 age groups: 0-4, 5-9, 10-14, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69 and >70 years.

In order to remove nonspecific inhibitors, three volumes of receptor-destroying enzyme at a working dilution of 1:50 were added to the serums, kept for 18 hours at 37°C; six parts of saline were added to achieve a total dilution of serums of 1:10, and then heated at 56°C for 30 min.

The level of specific antibodies against influenza virus haemagglutinins in blood serums was determined in the HAI and ELISA, according to the WHO recommendations.[11,12] HAI was performed using reference viruses (A/Swine/Iowa/15/30, A/California/04/09 [H1N1] pdm, A/Wisconsin/67/05[H3N2], and B/Victoria/2/ 87) as well as commercial diagnostic ums (A/Michigan/45/ 2015[H1N1]pdm, A/Singapore/INFIMH-16-0019/2016 [H3N2], B/Phuket/3073/13, and B/Colorado/06/2017) (Federal Budgetary Institution Research Institute of Influenza, Ministry of Health and Social Development, St. Petersburg, Russia. ELISA was carried out for influenza viruses of A(H1N1) and A(H3N2) subtypes and type B using relevant kits (Limited Liability Company Enterprise for Production of Diagnostic Preparations, St. Petersburg, Russia).

Data was analysed using Graph Pad Prism 9. Frequencies and percentages were calculated for categorical variables. Chi-square test was used to assess the significance of inter-group differences for antibodies level for HAI and ELISA. $P \leq 0.05$ was considered statistically significant.

Results

Of the 779 blood samples, 392(50.3%) came from women and 387(49.7%) from men. The overall age range was 0-80 years. The most populous age group was >70 years 215(27.6%), while the least populous was that of 10-14 years 9(1.15%) (Table).

Serological analyses using HAI showed the presence of anti-haemagglutinins against influenza A/H1N1pdm 2009 virus in 292(37.5%) samples, influenza A/H3N2 virus in 340(43.6%) and type B virus in 53(6.8%). Antibodies against two subtypes of influenza A virus and type B virus were simultaneously identified in 25(3.2%) cases, whereas against influenza A(H1N1+H3N2) viruses in 69(8.9%) (Figure 1).

In ELISA analysis, antibodies against influenza A/H1N1pdm virus were detected in 13.9% cases, against A/H3N2 virus in 105(13.5%) and type B virus in 65(8.3%). Antibodies simultaneously against two subtypes of influenza A virus were identified in 46(5.9%) of blood serums, and against influenza A and B viruses in 60(7.7%) (Figure 2).

Discussion

Taking into account the simplicity of the serological test and its high diagnostic value, it might be argued that HAI and ELISA can be useful, complementing the standard methods for examining patients with influenza and acute respiratory virus infections (ARVI). The advantage of serological methods is that they are suitable for the determination of virus-specific antibodies in blood serums.

Table-3: Myeloperoxidase (MPO) distribution.

Age group (years)	Sample size n	Numbers of participants that are immune to influenza viruses (%)							
		A/H1N1pdm		A/H3N2		type B		Total	
		HAI	ELISA	HAI	ELISA	HAI	ELISA	HAI	ELISA
0-4	33	11 (33.3)	6 (18.2)	22 (66.7)	9 (27.3)	Nil	Nil	33 (100.0)	15 (45.5)
5-9	15	3 (20.0)	1 (6.7)	3 (20.0)	1 (6.7)	9 (60.0)	13 (86.7)	15 (100.0)	15 (100.0)
10-14	9	7 (77.8)	3 (33.3)	4 (44.4)	2 (22.2)	1 (11.1)	2 (22.2)	12 (133.3)	7 (77.8)
15-19	19	11 (57.9)	5 (26.3)	8 (42.1)	3 (15.8)	Nil	2 (10.5)	19 (100.0)	10 (52.6)
20-24	44	30 (68.2)	15 (34.1)	14 (31.8)	6 (13.6)	12 (27.3)	19 (43.2)	56 (127.3)	40 (90.9)
25-29	36	21 (58.3)	11 (30.6)	15 (41.7)	6 (16.7)	Nil	Nil	36 (100.0)	17 (47.2)
30-34	22	7 (31.8)	3 (13.6)	12 (54.5)	5 (22.7)	5 (22.7)	8 (36.4)	24 (109.1)	16 (72.7)
35-39	29	7 (24.1)	3 (10.3)	15 (51.7)	6 (20.7)	7 (24.1)	11 (37.9)	29 (100.0)	20 (69.0)
40-44	33	16 (48.5)	8 (24.2)	29 (87.9)	12 (36.4)	3 (9.1)	5 (15.2)	48 (145.5)	25 (75.8)
45-49	33	12 (36.4)	6 (18.2)	26 (78.8)	11 (33.3)	2 (6.1)	3 (9.1)	40 (121.2)	20 (60.6)
50-54	58	38 (65.5)	19 (32.8)	42 (72.4)	17 (29.3)	19 (32.8)	30 (51.7)	99 (170.7)	66 (113.8)
55-59	73	25 (34.2)	12 (16.4)	44 (60.3)	17 (23.3)	4 (5.5)	6 (8.2)	73 (100.0)	35 (47.9)
60-64	73	46 (63.0)	23 (31.5)	43 (58.9)	16 (21.9)	3 (4.1)	5 (6.8)	92 (126.0)	44 (60.3)
65-69	87	60 (69.0)	30 (34.5)	46 (52.9)	18 (20.7)	4 (4.6)	6 (6.9)	110 (126.4)	54 (62.1)
>70	215	82 (38.1)	40 (18.6)	96 (44.7)	43 (20.0)	9 (4.2)	15 (7.0)	187 (87.0)	98 (45.6)
Total	779	376 (48.3)	185 (23.7)	419 (53.9)	172 (22.1)	78 (10.01)	125 (16.0)	873 (112.07)	482 (61.9)
***p*-value**		0,0001		0,0001		0,2622		0,0001	

HAI: Hemagglutination inhibition assay, ELISA: Enzyme-linked immunosorbent assay.

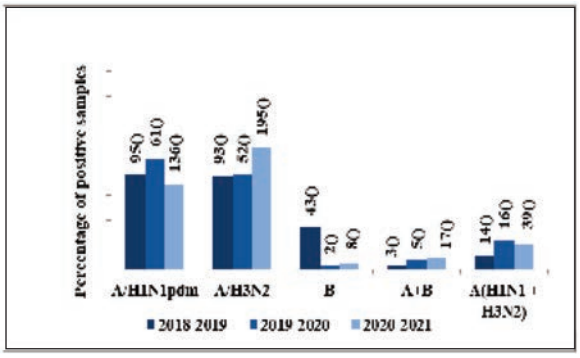

Figure: Detection of antibodies against influenza viruses in blood serums using hemagglutination inhibition assay (HAI).

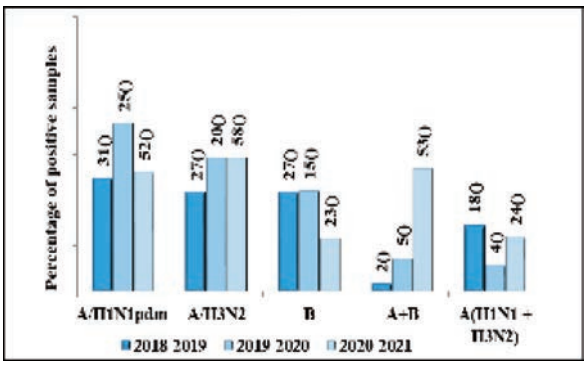

Figure: Detection of antibodies against influenza viruses in blood serums using enzyme-linked immunosorbent assay (ELISA).

The implementation of HAI also does not require much time and the availability of high-tech equipment. The advantages of the HAI include simplicity of implementation, specificity, and the absence of a need for high-tech equipment.

As can be seen from the presented, detection of antibodies for influenza viruses through HAI and ELISA during the epidemic periods of 2018-21 were similar (Figures 1-2).

Susceptibility to influenza, regardless of the strain, can be influenced by various factors, including immune status, pregnancy, gender, concomitant diseases and age.[13]

A high proportion of antibodies against influenza A/H1N1pdm virus was detected in those aged 10-30 years (65.5%). The proportion of positive samples decreased (28%) in those aged 30-40 years, and increased again (50.7%) in participants aged >40 years (Table).

The data resulting from the studies of serums for the presence of antibodies against various strains of influenza virus by age groups was similar for both ELISA and HAI (Table).

Studies of the circulation of influenza viruses among the population in different regions are a necessary element of measures to control the spread of the pathogen. Although classic influenza is a clinically typical disease, laboratory tests are needed at the onset of every influenza epidemic. They must confirm the prognosis for influenza and rule out other illnesses caused by a variety of viral and bacterial agents. Due to the fact, that re assortment leads to the emergence of viruses with new biological and antigenic properties that are capable of wide epidemic spread, surveillance over the infection spread and timely diagnosis of the pathogen are extremely significant trends in combating influenza.[14]

Sero-diagnosis provides determination of the aetiology of influenza by detecting specific antibodies in the blood and is especially important in the atypical or asymptomatic course of influenza infection. The detection of antibodies against influenza A/H1N1pdm, A/H3N2 and B viruses in blood serums collected from patients in the southern region of Kazakhstan through HAI and ELISA is indirect evidence of the role of influenza virus in the occurrence of ARVI, and its interaction with the human body.

Conclusion

Data confirmed the role of influenza A/H1N1pdm, A/H3N2 and B viruses in the epidemic process, and indicated the advisability of carrying out continuous sero-epidemiological monitoring of the circulation of influenza viruses in various regions of Kazakhstan.

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Conflict of Interest: None.

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References

1. WHO. Influenza. [Online] [cited 2021 May 04]. Available from: URL: https://www.who.int/ru/news-room/fact-sheets/detail/influenza-(seasonal).
2. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team; Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; 360: 2605-15.
3. WHO. Global Influenza Strategy 2019-2030. [Online] [cited 2021 May 04]. Available from: URL: WWW.WHO.INT.
4. Lvov DK, Alkhovsky SV, Kolobukhina LV, Burtseva EI. [Etiology of epidemic outbreaks COVID-19 on Wuhan, Hubei province, Chinese People Republic associated with 2019-nCoV (Nidovirales, Coronaviridae, Coronavirinae, Betacoronavirus, Subgenus Sarbecovirus): lessons of SARS-CoV outbreak.]. Vopr Virusol 2020; 65: 6-15.
5. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013; 30: 2725-9.
6. Lvov DK, Burtseva EI, Kolobukhina LV, Fedyakina IT, Bovin NV, Ignatjeva AV, et al. [Peculiarities of the influenza and ARVI viruses circulation during epidemic season 2019-2020 in some regions of Russia]. Vopr Virusol 2021; 65: 335-49.
7. Mukasheva EA, Nikolaeva LI, Makhnovsky PI, Kirillova ES, Kolobukhina LV, Merkulova LN, et al. [Diagnostic capacity of detection of specific antibodies to pandemic influenza A(H1N1)pdm09 virus]. Vopr Virusol 2017; 62: 109-14.
8. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999; 37: 937-43.
9. Klivleyeva NG, Ongarbayeva NS, Baimukhametova AM, Saktaganov NT, Lukmanova GV, Glebova TI, et al. Detection of influenza virus and pathogens of acute respiratory viral infections in population of Kazakhstan during 2018-2019 epidemic season. Russian Journal of Infection and Immunity 2021; 11: 137-47.
10. Glebova TI, Klivleyeva NG, Baimukhametova AM, Saktaganov NT, Lukmanova GV, Ongarbayeva NS, et al. Circulation of influenza viruses in the epidemic season of 2018–2019 among people residing in Northern and Western Kazakhstan. Infekc Bolezni 2021; 19: 70–5.
11. Douwdal WA, Kendal A, Noble GR. Influenza virus. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infection. Washington; 1979.
12. WHO. Manual for the laboratory diagnosis and virological surveillance of influenza. World Health Organization. [Online] 2011 [cited 2018 Sep18]. Available from: URL: https://apps.who.int/iris/bitstream/handle/10665/44518/9789241548090_eng.pdf?sequence=1&isAllowed=y. pdf.
13. Fink AL, Liu Hs, Shaw-Saliba K, Mehoke Th, Evans J, Liu ZY, et al. Regional differences in vaccine uptake and serological responses to vaccine and circulating strains of H1N1 viruses among patients with confirmed influenza. J Clin Virol Plus. 2021; 1: 100034.
14. Allwinn R, Preiser W, Rabenau H, Buxbaum S, Stürmer M, Doerr HW. Laboratory diagnosis of influenza--virology or serology? Med Microbiol Immunol 2002; 191: 157-60.