Srp Arh Celok Lek. 2016 Nov-Dec;144(11-12):626-632
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1612626R
UDC: 616.921.5-06

Risk factors for severe influenza A virus infections in post-2009 pandemic period

Jelena Radovanov[1], Ivana Hrnjaković-Cvjetković[1,2], Nataša Nikolić[1,2], Aleksandra Jovanović-Galović[1], Gordana Kovačević[1], Aleksandra Patić[1,2], Uroš Milošević[2,3], Vesna Milošević[1,2]

[1]Institute of Public Health of Vojvodina, Center of Virology, Novi Sad, Serbia;
[2]University of Novi Sad, Medical Faculty, Novi Sad, Serbia;
[3]Clinical Centre of Vojvodina, Novi Sad, Serbia

SUMMARY

Introduction Literature data concerning risk factors for severe influenza in post-2009 pandemic period, from low- and middle-income Central and Eastern European countries are very limited.

Objective The aim of this study was to investigate the risk factors for severe A(H1N1)pdm09 and A(H3N2) influenza during the post-2009 pandemic period.

Methods During four consecutive seasons of 2010/2011–2013/2014, nasopharyngeal or nasal and pharyngeal swab samples from 153 patients with mild and 147 patients with severe influenza were tested using real-time reverse transcription polymerase chain reaction (real-time RT PCR) assays.

Results The study indicated three statistically significant risk factors of influenza severity, including presence of chronic underlying illness/condition [odds ratio (OR) of 15.2, 95% confidence interval (CI) of 1.8–125.4, p = 0.001), age ≥15 years (OR 9.2, 95% CI 3.5–24.1, p < 0.001), and delay in medical care of more than two days after the symptoms onset (OR 3.2, 95% CI 1.6–6.4, p = 0.001).

Conclusion Obtained results confirmed that patients with chronic underlying illness/condition and older than 15 years had the highest risk for serious complications from influenza and highlighted the importance of start of antiviral therapy within the first two days of illness in order to reduce the risk for the most severe outcomes of influenza, such as acute respiratory distress syndrome and lethal outcome.

Keywords: influenza A; acute respiratory infections; real-time RT PCR

INTRODUCTION

Influenza is usually mild-to-moderate, self-limited, acute upper respiratory tract disease, but each year severe complications develop in about three to five million people worldwide [1]. During the pandemic of 2009, it became clear that the existing systems of influenza surveillance provide very limited data on severity of an influenza season. To overcome this problem, WHO recommended strengthening the monitoring of influenza viruses and underlying risk conditions that are specifically associated with severe clinical presentations of influenza [2]. According to the WHO case definition, influenza-like illness (ILI) is an acute respiratory illness with the onset within the previous 10 days, body temperature of ≥38°C and cough, while severe acute respiratory illness (SARI) is a type of ILI with pronounced difficulties in breathing, demanding hospitalization [3]. SARI may be presented as primary viral or secondary bacterial pneumonia and exacerbation of chronic diseases. In some cases SARI may progresses to the most severe form of acute lung injury named acute respiratory distress syndrome (ARDS).

Manifestations and clinical outcome of influenza virus infection is a result of complex interplay between viral and host factors. Development of severe influenza virus infections depends on viral type- and strain-specific virulence determinants, immunological and physiological characteristics of host, and may also be influenced by health-related behavior and medical treatment of patients [4, 5, 6]. Advanced age, chronic medical conditions including chronic respiratory and cardiovascular diseases, diabetes mellitus, chronic liver or renal diseases and immunodeficiencies, as well as pregnancy, are well known factors for complications of influenza [1]. During the pandemic influenza outbreak in 2009, obesity was also associated with severe A(H1N1)pdm09 infections [7]. Most of the literature data concerning risk factors for severe outcome of influenza are focused on the influenza A(H1N1)pdm09 infections during the 2009 pandemic outbreak in developed, high-income countries, while data for other influenza subtypes and from low- and middle-income Central and Eastern European countries are scarce [8, 9].

OBJECTIVE

The aim of this study was to investigate the risk factors for severe A(H1N1)pdm09 and A(H3N2) influenza during the post-2009 pandemic period in Vojvodina province, Serbia.

METHODS

Patients and real-time RT PCR testing

Centre for Virology at the Institute of Public Health of Vojvodina conducts continuous viro-

Correspondence to:
Jelena RADOVANOV
Institute of Public Health of Vojvodina
Center of Virology
121 Futoška St.
21000 Novi Sad
Serbia
virusologija@izjzv.org.rs

logical surveillance of influenza in the Vojvodina region in collaboration with primary healthcare institutions, hospitals and institutes of public health in the Vojvodina region, Serbia. During four consecutive seasons, from 2010/2011 to 2013/2014, nasal and throat or nasopharyngeal swab samples were taken from 887 patients when the WHO definitions for ILI or SARI [3] were met. Patients were asked a set of questions about their age, height, weight, vaccine status, and pre-existing health-conditions including malignancies, diabetes, chronic cardiovascular, respiratory, liver, and kidney diseases, immunosuppressive conditions, and pregnancy. The time from symptom onset to the first medical contact and antiviral treatment was also recorded. The body mass index (BMI) was calculated for each patient, except for pregnant women, using self-reported weight and height and the following formula: weight in kilograms divided by height in meters squared. Among adults aged >19 years obesity was defined as BMI ≥30. For patients aged up to 19 years, obesity was determined using appropriate WHO BMI-for-age charts (http://www.who.int/dietphysicalactivity/childhood/en/) and criteria that BMI value of more than 2 SD above the mean is equivalent to BMI >30 at 19 years.

Samples were transported to the Centre for Virology and tested by real-time reverse transcription polymerase chain reaction (real-time RT PCR) assays. Extraction of viral RNA was done by QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Real-time RT PCR testing was performed using influenza A type-specific and A(H1N1)pdm09 and A(H3N2) subtype-specific primer and probe sets provided by Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) and AgPath-ID™ One-Step RT-PCR Reagents (Applied Biosystems, Foster City, CA, USA). Real-time RT PCR assays were performed on the Applied Biosystems 7500 thermocycler according to the protocol developed by the CDC (enclosed with the reagents). A total of 300 patients with real-time RT PCR-confirmed influenza A infections were included in this study.

Statistical analyses

Descriptive statistics was performed for all study variables. For continuous variables, the data were reported as mean [with 95% confidence interval (CI)] and median (with range), and for categorical variables as percentage. For comparison of different variables between patients with ILI and patients with SARI/ARDS, Student's t-test and Fisher's exact test were used where appropriate. Variables with p-values <0.05 were entered into logistic regression models to identify factors associated with influenza severity. Severe influenza was assigned as a dependent variable in logistic regression analysis, while independent variables were the following: age group, influenza A subtype, the average time from symptoms onset to the first medical contact, the presence of any chronic underlying illness/condition (CUI/C), the presence of more than 1 CUI/C, and the presence of individual CUI/C. Age of the patient and time from symptoms onset to hospitalization were classified as two categories (<15 or ≥15 years, and ≤2 or >2 days, respectively). Odds ratios (OR) and 95% CI were calculated for each variable. Identified risk factors with a p-value <0.05 in the univariate analysis were included in a multivariate logistic regression model to assess independent association with severity. A factor was defined significant for $p < 0.05$ in the multivariate analysis. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The age distribution, distribution of influenza A virus subtypes and underlying illnesses/conditions by clinical manifestations of influenza are summarized in Table 1. In the group of 300 influenza A positive patients, there was approximately equal number of those who experienced severe disease (49%, 153/300), and those with mild forms of illness (51%, 147/300). A highly significant difference ($p < 0.001$) between the mean and median age of those who had uncomplicated influenza (mean age of 20.7 years, 95% CI 17.6–23.8, median age of 13 years, range of 1–81 years) and of those with severe illness (mean age of 47.8 years, 95% CI 44.7–50.9, median age of 51 years, range of 2–81) was found. Children aged 5–14 years dominated among ILI cases (47.1%, 72/153), while most of the SARI/ARDS cases were 30–64 (58.5%, 86/147) years of age.

Among 300 positive cases, A(H1N1)pdm09 and A(H3N2) subtypes were almost equally represented (48%, 144/300 and 52%, 156/300, respectively). Significantly more SARI/ARDS cases were caused by influenza A(H1N1)pdm09 (66%, 97/147, $p < 0.001$), while significantly more ILI cases were associated with A(H3N2) (69.3%, 106/153, $p < 0.001$) subtype.

The presence of one or more CUI/C was recorded in 40.7% (122/300) of patients positive for influenza A. Chronic cardiovascular disease was the most common underlying medical condition in positive patients (14.7%, 44/300). CUI/C were significantly more present in patients with complications (73.5%, 108/147) compared to those with mild form of disease (9.1%, 14/153, $p < 0.001$). All detected comorbidities were statistically more common in patients with SARI/ARDS than in patients with ILI.

Differences in age distribution, and mean and median age, between patients with severe A(H1N1)pdm09 and A(H3N2) influenza, were not significant, with the exception of children aged 5–14 years, which were recorded only among A(H3N2) SARI/ARDS patients (10%, 5/50, $p = 0.004$) (Table 2). Persons 30–64 years old predominated in the group of influenza A(H1N1)pdm09 with SARI/ARDS (57.7%, 56/97), as well as in the A(H3N2) SARI/ARDS group of patients (60%, 30/50). CUI/C were more prevalent in the group of influenza A(H1N1)pdm09 SARI/ARDS patients (80.4%, 78/97) then in A(H3N2) SARI/ARDS patients (60%, 30/50, $p = 0.0105$). In regard to the individual CUI/C, the only significant difference was observed in the case of malignant diseases which were detected

Table 1. Age distribution, distribution of influenza A virus subtypes, and underlying illnesses/conditions among patients with mild and severe influenza

Characteristic		Positive n = 300	ILI (%) n = 153 (51)	SARI/ARDS (%) n = 147 (49)	p
Age (years)	0–4	18 (6)	16 (10.5)	2 (1.4)	0.001
	5–14	77 (25.7)	72 (47.1)	5 (3.4)	<0.001
	15–29	47 (15.7)	20 (13.1)	27 (18.4)	>0.05
	30–64	127 (42.3)	41 (26.8)	86 (58.5)	<0.001
	≥65	31 (10.3)	4 (2.6)	27 (18.4)	<0.001
	Mean (95% CI)	34 (31.3–36.7)	20.7 (17.6–23.8)	47.8 (44.7–50.9)	<0.001
	Median (range)	33 (1–84)	13 (1–81)	51 (2–84)	<0.001
Influenza A virus subtypes	H1N1pdm09	144 (48)	47 (30.7)	97 (66)	<0.001
	H3N2	156 (52)	106 (69.3)	50 (34)	<0.001
CUI/C*	With CUI/C	122 (40.7)	14 (9.1)	108 (73.5)	<0.001
	One CUI/C	87 (29)	13 (8.5)	74 (50.3)	<0.001
	More than one CUI/C	35 (11.7)	1 (0.6)	34 (23.2)	<0.001
	Cardiovascular disease	44 (14.7)	11 (7.2)	33 (22.4)	0.0003
	Respiratory disease	20 (6.7)	2 (1.3)	18 (12.2)	0.001
	Diabetes mellitus	13 (4.3)	-	13 (8.8)	<0.001
	Malignancy	22 (7.3)	-	22 (14.9)	<0.001
	Immunodeficiency	35 (11.7)	1 (0.6)	34 (23.1)	<0.001
	Liver/renal disease	23 (7.7)	-	23 (15.6)	<0.001
	Obesity**	8 (2.7)	-	8 (5.4)	0.003
	Pregnancy	9 (3)	1 (0.6)	8 (5.4)	0.0177

ILI – influenza-like illness; SARI – severe acute respiratory illness; ARDS – acute respiratory distress syndrome; CI – confidence interval; CUI/C – chronic underlying illness/condition

* Patients with multiple chronic underlying illnesses or conditions were counted for each
** Body mass index equal to or greater than 30

Table 2. Age distribution and distribution of underlying illnesses/conditions among patients with severe A(H1N1)pdm09 and A(H3N2) influenza

Characteristic		Severe A(H1N1) pdm09 influenza n = 97	Severe A(H3N2) influenza n = 50	p
Age (years)	0–4	2 (2.1)	-	>0.05
	5–14	-	5 (10)	0.004
	15–29	22 (22.7)	5 (10)	>0.05
	30–64	56 (57.7)	30 (60)	>0.05
	≥65	17 (17.5)	10 (20)	>0.05
	Mean (95% CI)	47.06 (43.28–50.85)	49.14 (43.44–54.84)	>0.05
	Median (range)	51 (2–83)	51.5 (6–84)	>0.05
CUI/C*	With CUI/C	78 (80.4)	30 (60)	0.0105
	One CUI/C	52 (53.6)	22 (44)	>0.05
	More than one CUI/C	26 (26.8)	8 (16)	>0.05
	Cardiovascular disease	23 (23.7)	10 (20)	>0.05
	Respiratory disease	11 (11.3)	7 (14)	>0.05
	Diabetes mellitus	10 (10.3)	3 (6)	>0.05
	Malignancy	19 (19.6)	3 (6)	0.0298
	Immunodeficiency	23 (23.7)	11 (22)	>0.05
	Liver/renal disease	17 (17.5)	6 (12)	>0.05
	Obesity**	5 (5.1)	3 (6)	>0.05
	Pregnancy	4 (4.1)	4 (8)	>0.05

CI – confidence interval; CUI/C – chronic underlying illness/condition

* Patients with multiple risk factors were counted for each
** Body mass index equal to or greater than 30

in 19.6% (19/97) of severely ill patients with A(H1N1) pdm09 infections, but in only 6% (3/50, p = 0.0298) of patients with severe A(H3N2) influenza.

Differences in health seeking behavior and antiviral treatment of patients with different clinical manifestation of influenza are presented in Table 3. The mean time from the onset of influenza symptoms to the first medical contact was 2.3 days (95% CI 2.1–2.5 days) with a median of two days (range 1–6) for ILI patients, and 3.3 days (95% CI 2.9–3.6) with a median of three days (range 1–10 days) for SARI/ARDS patients, which was significantly different (p < 0.001). Patients with ARDS had the longest mean delay before consultation (3.5, 95% CI 2.8–4.2). Data on antiviral treatment were available for 91 patients with ILI and 113 patients with SARI/ARDS. Antivirals were administrated to 41.6% (47/113) of SARI/ARDS patients, but

Table 3. Health care behavior and antiviral treatment of patients with different clinical manifestation of influenza

Characteristic		ILI	SARI/ ARDS	SARI**	ARDS	Fatal cases***	p*
Health care behavior	Total number of patients	153	147	126	21	9	
	Mean time from the symptoms onset to the first medical contact in days (95% CI)	2.27 (2.1–2.5)	3.31 (2.9–3.6)	3.27 (2.9–3.6)	3.52 (2.8–4.2)	2.89 (1.8–3.9)	<0.001
	Median time from the symptoms onset to the first medical contact in days (range)	2 (1–6)	3 (1–10)	2.5 (1–10)	3 (1–6)	3 (1–5)	<0.001
Antiviral therapy	Total number of patients	91	113	99	14	7	
	Number of patients who received the antiviral treatment (%)	6 (6.6)	47 (41.6)	40 (40.4)	7 (50)	5 (71.4)	<0.001
	Number of patients who received the antiviral treatment within 48h from symptoms onset (%)	4 (66.7)	31 (65.9)	25 (62.5)	1 (14.3)	1 (20)	>0.5
	Number of patients who received the antiviral treatment >48h from symptoms onset (%)	2 (33.3)	16 (34.1)	15 (37.5)	6 (85.7)	4 (71.4)	

ILI – influenza-like illness; SARI – severe acute respiratory illness; ARDS – acute respiratory distress syndrome; CI – confidence interval

* Significance of difference between ILI and SARI/ARDS cases

** SARI cases without ARDS

*** SARI and ARDS cases with fatal outcome

Table 4. Results of the logistic regression analysis with severe influenza as depended variable

Risk factor		Univariate regression analysis			Multivariate regression analysis		
		OR	95% CI	p	OR	95% CI	p
Age	≥15	27.1	11.8–61.7	<0.001	9.2	3.5–24.1	<0.001
Influenza A subtype	A(H1N1)pdm09	4.4	2.7–7.1	<0.001	1.7	0.8–3.3	0.146
Health care behavior	More than two days from the symptom onset to the first medical contact	2.6	1.6–4.2	<0.001	3.2	1.6–6.4	0.001
CUI/C	With CUI/C	27.5	14.2–53.2	<0.001	15.2	1.8–125.4	0.011
	More than 1 CUI/C	5.9	0.7–47.5	0.091	-	-	-
	Cardiovascular disease	3.9	1.9–8.1	<0.001	0.7	0.3–2.8	0.107
	Respiratory disease	10.5	2.4–46.3	0.002	0.9	0.4–3.6	0.382
	Diabetes mellitus	6.5	1.4–29.7	0.016	0.5	0.2–4.5	0.575
	Malignancy	28.4	3.8–113.4	0.001	1.6	0.1–24.3	0.741
	Immunodeficiency	45.7	6.2–339.1	<0.001	4.7	0.3–70.4	0.262
	Liver/renal disease	31.5	4.2–135.9	0.001	2.0	0.2–24.0	0.583
	Obesity*	3.4	0.7–17.1	0.138	-	-	-
	Pregnancy	8.7	1.1–70.8	0.042	0,7	0.1–13.5	0.815

OR – odds ratio; CI – confidence interval; CUI/C – chronic underlying illness/condition

* Body mass index equal to or greater than 30

only 6.6% (6/91, p>0.001) of ILI patients received antiviral therapy. Among cases with serious complications of influenza, more patients with SARI received antivirals within two days from the symptoms onset (62.5%, 25/40), comparing to the patients with ARDS (14.3%, 1/7, p = 0.0347), and the patients who died (20%, 1/5). Out of 300 patients, only five patients (1.7%) – four with ILI and one with SARI – had a history of vaccination against influenza.

Three percent (9/300) of all positive cases and 6.1% (9/147) of all severe cases had fatal outcome. All patients who died had A(H1N1)pdm09 influenza, and most of them were 30–64 years old (66.7%, 6/9) and had the CUI/C (88.9%, 8/9 (data not shown in the table). The mean time from the onset of symptoms to hospital admission was 2.9 days (95% CI 1.8–3.9) with a median of three days (range of 1–5 days). The mean time from symptoms onset to death was 8.9 days (5.5–12.3) with a median of 9 days (range of 1–15 days) (data not shown in the table).

The univariate logistic regression analysis revealed that age ≥15 years (OR 27.1, 95% CI 11.8–61.7), being infected with influenza A(H1N1)pdm09 (OR 4.4, 95% CI 2.7–7.1), having CUI/C (OR 27.5, 95% CI 14.2–53.2), and more than two days from the symptoms onset to the first medical contact (OR 2.6, 95% CI 1.6–4.2) were significantly (p < 0.001) associated with severe diseases (Table 4). All individual CUI/C, except obesity, were associated with complicated forms of influenza. Among them, immunocompromised patients had the highest risk of having SARI/ARDS (OR 45.7, 95% CI 6.2–339.1, p < 0.001). In the multivariate analysis, three variables remained statistically significant prediction factors of influenza severity, including CUI/C (OR 15.2, 95% CI 1.8–125.4, p = 0.001), age of ≥15 years (OR 9.2, 95% CI 3.5–24.1, p < 0.001), and delay in medical care more than two days after the symptoms onset (OR 3.2, 95% CI 1.6–6.4, p = 0.001).

DISCUSSION

The results of our study demonstrated that almost 60% of ILI cases were children aged <15 years, and more than 90% of these children had an uncomplicated form of influenza. The lack of preexisting immunity together with factors facilitating the virus transmission (shedding the greater

quantities of virus for longer periods of time than adults; prolonged stay in crowded indoor environments such are schools and daycare facilities), make children more susceptible to influenza virus infections. However, their efficient innate immune mechanisms and adaptive T cell immune response, most likely, protect them from developing severe disease [10]. On the other hand, children younger than five years of age are at higher risk from influenza complications due to the immature immune system [8]. Still, in the present study, the children aged <5 years were significantly more present among ILI (10.5%) than among SARI/ARDS (1.4%) cases, and almost 90% of the children of that age had a mild form of illness.

It is well known that despite having no greater risk of infection comparing to younger adults, older adults suffer the highest rates of hospitalization and mortality due to influenza [11]. In this study, almost 60% of SARI/ARDS and almost 70% of fatal cases were patients 30–64 years old. About two thirds of patients from that age group had SARI/ARDS. Moreover, patients ≥65 years old accounted for only about 10% of the whole sample, but almost 90% of them had complicated forms of illness. Similarly to our findings, Zhang et al. [12] discovered that severe influenza virus infections occurred most frequently in the oldest population (>60 years old), but the absolute number of adults aged 20–60 with severe illness was higher than in those >60 years old. It can be assumed that adults are partially protected against influenza due to cross-reactive immunity generated during previous exposures to influenza A viruses. However, such relative protection is probably impaired by underlying diseases which are more common in adults than in children. Chronic diseases themselves and low immunity caused by them can induce progression of influenza to more serious forms, and influenza might in turn aggravate patients' chronic illness. In this investigation, CUI/C were present in almost 75% of severely ill patients and in less than 10% of patients with mild form of the disease. Moreover, eight out of nine fatal cases had one or more CUI/C, which was in accordance with the McCullers's and Hyden's [13] suggestion that seasonal influenza rarely kills without a secondary cause.

Of all patients with severe influenza enrolled into our study, 5.4% were pregnant, 5.4% were obese, and obesity was recorded in 25% of patients who died. The specific changes in physiology (increased heart rate, stroke volume, oxygen consumption, and decreased lung capacity) and alterations in cell-mediated immunity in pregnancy increase the risk for severe influenza [7]. Obesity is associated with various comorbidities that are risk factors for influenza complications, such as diabetes, chronic cardiovascular or pulmonary diseases [14], and it is also an independent risk factor for severe influenza probably due the decreased pulmonary function and immunodysregulation involving adipokines [15]. Van Kerkhove et al. [11] reported that, according to pooled data from 19 countries and regions, during the pandemic of 2009, pregnant women were about seven times more likely to be hospitalized comparing to non-pregnant women, and that the proportion of obese patients increased with disease severity and represented a median of 6%, 11.3%, and 12% of all hospitalized, ICU-admitted, and fatal cases, respectively.

This investigation revealed that serious complications developed in 26.5% of previously healthy individuals without CUI/C. That finding may be explained by the immunosenescence in the elderly (a decline in functionality of the innate and adaptive immune system) [16], or by heritable predisposition to severe outcome of influenza independent of viral strain, preexisting immunity and the age, leading to pathological immune response (the so-called cytokine storm) [17].

The present study demonstrated that patients with ILI had significantly shorter delay before medical consultation than patients with SARI/ARDS (2.3 vs. 3.3 days). In relation to our results, Van Cauteren et al. [5] reported slightly shorter (1.9 days) mean time from the symptoms onset to the first medical consultation for ILI patients, while Kusznierz et al. [14] recorded 4.3 days for non-fatal hospitalized patients. Among patients who died, the disease progression was rapid, with a median time of three days from the symptoms onset to hospital admission, and a median time of nine days to death. During the pandemic of 2009, Santa-Olalla Peralta et al. [18] recorded that fatal cases had the median time of three days from the onset of the illness to the hospitalization, and a median time of 13 days to death.

The time from the onset of symptoms to initiation of antiviral treatment is the key factor in reducing the severity of influenza infections [6, 7]. In this study, among severely ill cases, antiviral therapy was administrated within two days from the onset of symptoms significantly more often to patients with SARI (62.5%) than to patients with ARDS (14.3%) and, although nonsignificantly, to patients who died (20%). These results suggest that prompt administration of antivirals could reduce the risk for most severe complications of influenza, ARDS and the fatal outcome. Although flu vaccine effectiveness can vary depending on characteristics of the person being vaccinated (such as their age and health) and antigenic similarity between the vaccine and circulating influenza viruses, the vaccination can significantly reduce the risk of getting ill from flu and the risk of more serious outcomes [19]. The proportion of vaccinated persons in this study was only 1.7%, which is consistent with overall low flu vaccine coverage in our country and in some other Eastern European countries [9].

Both influenza subtypes were approximately equally represented among positive patients, but their distribution in groups with different clinical manifestations of influenza varied significantly. Two thirds of SARI/ARDS cases were caused by influenza A(H1N1)pdm09 subtype, while almost 70% of ILI cases were associated with A(H3N2). There are few published comparisons of manifestation and outcomes of A(H1N1)pdm09 and A(H3N2) influenza. During the pandemic of 2009, it was recorded that infections caused by the new A(H1N1)pdm09 virus appear to be indistinguishable in severity and symptoms from seasonal A(H1N1) or A(H3N2) influenza [4]. Most patients experienced uncomplicated illness, while severe disease developed in only a small subset of patients. The majority of the A(H1N1)pdm09 viruses contain none of the known

doi: 10.2298/SARH1612626R

genetic hallmarks of highly pathogenic influenza viruses and there is no evidence for the significant antigenic changes [4, 15]. Only minor proportion of currently circulating A(H1N1)pdm09 strains (<1.8%) contain D222G mutation in hemagglutinin gene, which allows deep access to the lungs [20]. However, recent studies have reported changes in the epidemiology of the A(H1N1)pdm09 infection which are reminiscent of outbreaks in historical post-pandemic periods. Namely, the age-specific shift of A(H1N1)pdm09 infections towards adults, consistent with a development of A(H1N1)pdm09-specific immunity in younger populations that had the highest infection rates during the pandemic of 2009, has been observed [21]. The synergism between A(H1N1)pdm09 viruses and chronic underlying conditions mostly prevalent in older adults resulted in increased severity of influenza A(H1N1) pdm09 infections, which has been detected in our study and which is in line with the results of other researchers [21]. In contrast, high levels of immunity against A(H1N1) pdm09 viruses naturally acquired during the pandemic of 2009 made children more susceptible to infections with A(H3N2) viruses in subsequent years.

Our conclusions were also confirmed by the results of logistic regression analysis. Three independent risk factors for severe clinical disease were identified. Patients with CUI/C had about 15 times, and patients aged ≥15 years had about nine times higher the risk from severe influenza. The odds of severe disease in patients who asked for medical help more than two days after the symptoms onset (OR 3.2) was lower, but still significant.

This study had a number of limitations. The patients enrolled into this study represented only a small fraction of the total number of influenza cases in the population of Vojvodina, due to limitations of the passive surveillance system. Passive sentinel influenza surveillance system is simple and cost-effective, but it detects only symptomatic individuals who seek medical care and obtain laboratory tests. Moreover, selection of patients, data collection, and submission of samples to virological testing is performed voluntarily by physicians and might be influenced by their willingness and capacity to perform them. It should also be noted that BMI was calculated using self-reported height and weight, which are subject to substantial measurement error. Self-reported BMI tend to have lower values than true BMI based on physical measurement of height and weight, resulting in underestimates of the prevalence of obesity in study population [22]. The relatively small number of children younger than five years and patients with certain CUI/C identified through surveillance, reduced the statistical power of our results and probably affected their precision. Thus, our findings need to be interpreted with some caution because of the selection bias and small number of cases in certain groups.

CONCLUSION

In conclusion, our study confirms that during the first four consecutive seasons after the 2009 pandemic, individuals with CUI/C, older than 15 years, and who delayed medical consultation for more than two days after the illness onset, had significantly higher risk for serious complications from influenza. Furthermore, they highlight the importance of an early start of antiviral therapy, in order to reduce the risk for the most severe outcomes of influenza such as ARDS and the fatal outcome. Results from this study may help in targeting vulnerable populations and improvement of patient management. This is especially relevant in the light of an overall low vaccination coverage and very limited use of antivirals in our country.

REFERENCES

1. WHO. Influenza (Seasonal). [cited 2015 june 15] Available from: http://www.who.int/mediacentre/factsheets/fs211/en/
2. WHO. WHO Regional Office for Europe guidance for sentinel influenza surveillance in humans. [cited 2015 june 12] Available from: http://www.euro.who.int/data/assets/pdf_file/0020/90443/E92738.pdf
3. WHO. Global epidemiological surveillance standards for influenza. Geneva: WHO Press; 2013. [cited 2015 june 20] Available from: http://www.who.int/influenza/resources/documents/influenza_surveillance_manual/en/
4. Tscherne DM, Garcia-Sastre A. Virulence determinants of pandemic influenza viruses. J Clin Invest. 2011; 121(1):6–13. [DOI: 10.1172/JCI44947] [PMID: 21206092]
5. Van Cauteren D, Vaux S, de Valk H, Strat YL, Vaillant V, Levy-Bruhl D. Burden of influenza, healthcare seeking behaviour and hygiene measures during the A(H1N1)2009 pandemic in France: a population based study. BMC Public Health. 2012; 12:947. [DOI: 10.1186/1471-2458-12-947] [PMID: 23127166]
6. Manabe T, Higuera Iglesias AL, Vasquez Manriquez ME, Martinez Valadez EL, Ramos LA, Izumi S, et al. Socioeconomic factors influencing hospitalized patients with pneumonia due to influenza A(H1N1)pdm09 in Mexico. PLoS One. 2012; 7(7):e40529. [DOI: 10.1371/journal.pone.0040529] [PMID: 22808184]
7. Abdelaty NM. Risk factors and prognostic criteria in 230 patients with influenza A (H1N1) infection. Egypt J Chest Dis Tuberc. 2013; 62(1):1–8. [DOI: 10.1016/j.ejcdt.2013.02.006]
8. Mertz D, Kim TH, Johnstone J, Lam PP, Science M, Kuster SP, et al. Populations at risk for severe or complicated influenza illness: systematic review and meta-analysis. BMJ. 2013; 347:f5061. [DOI: 10.1136/bmj.f5061] [PMID: 23974637]
9. Meerhoff TJ, Simaku A, Ulqinaku D, Torosyari L, Gribkova N, Shimanovich V, et al. Surveillance for severe acute respiratory infections (SARI) in hospitals in the WHO European region – an exploratory analysis of risk factors for a severe outcome in influenza-positive SARI cases. BMC Infect Dis. 2015; 15:1. [DOI: 10.1186/s12879-014-0722-x] [PMID: 25567701]
10. Tran CH, Sugimoto JD, Pulliam JRC, Ryan KA, Myers PD, Castleman JB, et al. School-located influenza vaccination reduces community risk for influenza and influenza-like illness emergency care visits. PLoS ONE. 2014; 9(12):e114479. [DOI: 10.1371/journal.pone.0114479] [PMID: 25489850]
11. Van Kerkhove MD, Vandemaele KAH, Shinde V, Jaramillo-Gutierrez G, Koukounari A, Donnelly CA, et al. Risk Factors for Severe Outcomes following 2009 Influenza A (H1N1) Infection: A Global Pooled Analysis. PLoS Med. 2011; 8(7):e1001053. [DOI: 10.1371/journal.pmed.1001053] [PMID: 21750667]
12. Zhang AJX, To KKW, Tse H, Chan KH, Guo KY, Li C, et al. High incidence of severe influenza among individuals over 50 years of age. Clin Vaccine Immunol. 2011; 18(11):1918–24. [DOI: 10.1128/CVI.05357-11] [PMID: 21900532]
13. McCullers JA, Hayden FG. Fatal influenza B infections: Time to reexamine influenza research priorities. J Infect Dis. 2012; 205:870–2. [DOI: 10.1093/infdis/jir865] [PMID: 22291194]

14. Kusznierz G, Uboldi A, Sosa G, Torales S, Colombo J, Moyano C, et al. Clinical features of the hospitalized patients with 2009 pandemic influenza A (H1N1) in Santa Fe, Argentina. Influenza Other Respir Viruses. 2012; 7(3):410–17. [DOI: 10.1111/j.1750-2659.2012.00405.x] [PMID: 22817648]
15. Cheng VCC, To KKW, Tse H, Hung IFN, Yuen KY. Two years after pandemic influenza A/2009/H1N1: What have we learned? Clin Microbiol Rev. 2012; 25(2):223. [DOI: 10.1128/CMR.05012-11] [PMID: 22491771]
16. Mathews JD, McBride ES, McVernon J, Pallaghy PK, McCaw JM. Prior immunity helps to explain wave-like behaviour of pandemic influenza in 1918-9. BMC Infectious Diseases. 2010; 10:128. [DOI: 10.1186/1471-2334-10-128] [PMID: 20497585]
17. Albright FS, Orlando P, Pavia AT, Jackson GG, Albright C. Evidence for a heritable predisposition to death due to influenza. J Infect Dis. 2008; 197(1):18–24. [DOI: 10.1086/524064] [PMID: 18171280]
18. Santa-Olalla Peralta P, Cortes-García M, Vicente-Herrero M, Castrillo-Villamandos C, Arias-Bohigas P, Pachon-del Amo I, et al. Risk factors for disease severity among hospitalised patients with 2009 pandemic influenza A(H1N1) in Spain, April-December 2009. Euro Surveill. 2010; 15(38). [PMID: 20929651]
19. CDC. Preventing seasonal flu with vaccination. [cited 2015 july 15] Available from: http://www.cdc.gov/flu/protect/vaccine/ index.htm
20. Resende PC, Motta FC, Oliveira M de LA, Gregianini TS, Fernandes SB, Cury ALF, et al. Polymorphisms at residue 222 of the hemagglutinin of pandemic influenza A(H1N1)pdm09: Association of quasi-species to morbidity and mortality in different risk categories. PLoS ONE. 2014; 9(3):e92789. [DOI: 10.1371/journal.pone.0092789] [PMID: 24667815]
21. Davila J, Chowell G, Borja-Aburto VH, Viboud C, Grajales Muñiz C, Miller M. Substantial morbidity and mortality associated with pandemic A/H1N1 influenza in Mexico, winter 2013-2014: Gradual age shift and severity. PLOS Curr. 2014; 26;6. [DOI: .1371/currents.outbreaks.a855a92f19db1d90ca955 f5e908d6631] [PMID: 24744975]
22. Stommel M, Schoenborn CA. Accuracy and usefulness of BMI measures based on self-reported weight and height: findings from the NHANES & NHIS 2001-2006. BMC Public Health. 2009; 9:421. [DOI: 10.1186/1471-2458-9-421] [PMID: 19922675]

Фактори ризика за настанак тешких инфлуенца А вирусних инфекција после пандемије 2009. године

Јелена Радованов[1], Ивана Хрњаковић-Цвјетковић[1,2], Наташа Николић[1,2], Александра Јовановић-Галовић[1], Гордана Ковачевић[1], Александра Патић[1,2], Урош Милошевић[2,3], Весна Милошевић[1,2]

[1]Институт за јавно здравље Војводине, Центар за вирусологију, Нови Сад, Србија;
[2]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[3]Клинички центар Војводине, Нови Сад, Србија

КРАТАК САДРЖАЈ

Увод У литератури постоје веома оскудни подаци о факторима ризика за настанак тешких облика инфлуенце после пандемије 2009. године у земљама Централне и Источне Европе.

Циљ рада Циљ овог рада био је да се испитају фактори ризика за настанак тешких A(*H*1*N*1)*pdm*09 и A(*H*3*N*2) инфлуенца вирусних инфекција после пандемије 2009. године.

Методе рада Узорци назофарингеалних брисева или брисева носа и грла 153 пацијента са благим и 147 пацијената са тешким обликом инфлуенце тестирани су *real-time RT PCR* тестовима на присуство инфлуенца A(*H*1*N*1)*pdm09* и A(*H*3*N*2) вируса.

Резултати Истраживање је указало на четири статистички значајна фактора ризика за настанак тешких случајева инфлуенце, укључујући присуство хроничне болести/стања (*OR* 15,2; 95% *CI* 1.8–125,4; $p = 0,001$), узраст ≥15 година (*OR* 9,2; 95% *CI* 3,5–24,1; $p < 0,0001$) и одлагање медицинске консултације више од два дана након почетка болести (*OR* 3,2; 95% *CI* 1,6–6,4; $p = 0,001$).

Закључак На основу резултата овог истраживања може се закључити да су пацијенти са хроничним обољењима/стањима и старији од 15 година били под највећим ризиком од озбиљних компликација инфлуенце, као и да је примена антивирусне терапије у прва два дана болести значајна за смањење ризика од најтежих компликација.

Кључне речи: инфлуенца А; акутна респираторна инфекција; *real-time PCR*

Примљен • Received: 16/10/2015 **Ревизија • Revision:** 17/08/2016 **Прихваћен • Accepted:** 04/10/2016