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Seroprevalence study of antibodies against influenza A(H1N1) 2009 virus after the second pandemic wave in Slovenia

Vesna Šubelj[1], **Katarina Prosenc**[1], **Maja Sočan**[2]

[1]Laboratory of Virology, National Institute of Public Health, Ljubljana, Slovenia
[2]Centre for Communicable Diseases, National Institute of Public Health, Ljubljana, Slovenia

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Seroprävalenz-Studie von Antikörpern gegen das Influenzavirus A/H1N1 (2009) nach der zweiten Pandemiewelle in Slowenien

Zusammenfassung. *Ziel:* Das pandemische Influenzavirus (H1N1) 2009, das aus einer Kombination von porzinen, humanen und aviären Influenzaviren besteht, trat erstmals im April 2009 auf und breitete sich innerhalb der folgenden Monate global aus. Untersucht wurde die serologische Reaktion auf das Influenzavirus A/H1N1 (2009) bei nicht geimpften Personen aus der zentralen Region Slowenien.

Methoden: Nach der zweiten Pandemiewelle wurden 226 Serumproben von gesunden Vorschulkindern, Studenten, medizinischem Personal und Blutspendern, die noch nicht mit Pandemieimpfstoff geimpft worden waren, mittels Mikroneutralisationstests (MN) untersucht. Dabei wurden auch Daten über frühere Impfungen mit saisonalen Impfstoffen und kürzliche Atemwegserkrankungen (ARI) erhoben.

Ergebnisse: Die Seropositivitätsrate gegen das pandemische Influenzavirus war insgesamt hoch: 76.9 % der Teilnehmer hatten Antikörpertiter von ≥20. Titer von ≥40 wurden bei 54 % der geprüften Serumproben festgestellt, wobei die höchste Prävalenz in der Gruppe der 15- bis 17-Jährigen lag. Es wurden keine statistisch signifikanten Unterschiede zwischen den MN-Titern von Personen, die noch nie mit Influenza-Impfstoffen geimpft worden waren und jenen, die mindestens einmal geimpft worden waren. In der Krankengeschichte von Personen mit MN-Titern von ≥20, ≥40 oder ≥80 (mit den jeweiligen *p*-Werten 0,654; 0,755 sowie 1) konnte keine erhöhte Häufigkeit von ARI festgestellt werden.

Schlussfolgerungen: Eine hohe Seroprävalenz gegen das Pandemieinfluenza-Virus wurde in allen Altersgruppen festgestellt, auch wenn das klinische Bild einer akuten Atemwegserkrankung (ARI) nicht vorlag. Frühere Impfungen mit saisonalen Impfstoffen hatten keinerlei Auswirkung auf die serologische Reaktion auf das Influenzavirus A/H1N1 (2009).

Correspondence: Maja Socan, Ph.D., Centre for Communicable Diseases, National Institute of Public Health, Trubarjeva 2, 1000 Ljubljana, Slovenia, E-mail: maja.socan@ivz-rs.si

Summary. *Objective:* The pandemic influenza (H1N1) 2009 virus, which combined genes from swine, human and avian influenza viruses, emerged in April 2009 and spread globally within the next month. The post-pandemic serological response to the influenza A(H1N1) 2009 virus in non-vaccinated individuals from the central part of Slovenia was studied.

Methods: After the second pandemic wave, 226 serum samples from healthy preschool children, students, health care workers and blood donors not previously vaccinated with pandemic vaccine were investigated by use of microneutralization assays (MN). Data on previous vaccinations with seasonal influenza vaccine and recent acute respiratory infections were collected.

Results: The overall rate of seropositivity to the pandemic influenza virus was high: 76.9% of the participants had antibody titres of ≥20. A titre of ≥40 was detected in 54% of the serum samples tested, the highest prevalence being in the 15–17 age group. There was no statistically significant difference in MN titres between individuals who had never been vaccinated with influenza vaccine and those vaccinated at least once. History of ARI was not more frequent in subjects with MN titres ≥20, ≥40 or ≥80 (*p*-value 0.654, *p*-value 0.755 and *p*-value 1, respectively).

Conclusions: High seroprevalence to pandemic influenza has been found in all age groups regardless of the absence of clinical picture compatible with acute respiratory infection. Previous vaccinations with seasonal influenza vaccines had no impact on serological response to the influenza A(H1N1) 2009 virus.

Key words: Influenza, pandemic A(H1N1) 2009 virus, serology, microneutralization assay, antibody titre.

Introduction

A new, triple re-assortment influenza virus emerged in 2009, combining swine, human and avian genes. The influenza A(H1N1) 2009 virus spread rapidly over the globe with the first pandemic wave occurring during late spring and summer. In most European countries, the first wave was relatively mild. In the northern hemisphere, the second wave started in October 2009 and caused an intensive rise in the incidence of influenza-like illness.

Several studies were conducted to gain knowledge of pre-existing and post-pandemic immunity against the pandemic A(H1N1) 2009 strain. Cross-reactive antibodies against the 2009 pandemic H1N1 influenza virus were found in adults [1, 2]. There was no or little cross-reactivity in serum samples taken from children before the pandemic started [1–4]. Individuals born in the first quarter of the 20th century had pre-existing antibodies against the new virus; genetic and structural analyses revealed similarity between the pandemic virus and Spanish influenza virus [4–6]. Young people were disproportional affected in the 2009 pandemic [7–11]. A serological study conducted in the UK demonstrated that around one child in every three was infected in regions with intensive circulation of pandemic strain during the first pandemic wave [12].

The objective of the study was to determine the proportion of individuals with serum antibodies against influenza A(H1N1) 2009 virus in different age groups in the central part of Slovenia after the second pandemic wave.

Methods

Serum samples were collected from healthy children and teenagers during a routine pre-scheduled medical examination before entry to the school: from children aged 5–6 years, from primary school students, aged 10–14-years, and from second grade students attending secondary schools (aged 15–17 years). Sampling was done in five out-patient clinics run by the Ljubljana Community Health Centre. A very short questionnaire was filled by students and by parents/legal guardians of preschool children. Blood samples were taken for basic biochemistry tests as part of the routine health check. Residual serum samples of children and students were sent to the virology laboratory for serological testing. Serum samples were collected in February and March 2010.

Health care workers employed in the Ljubljana Community Health Centre were asked to complete a questionnaire and to give serum samples for serological testing, but it turned out that their willingness to participate was very low, for example, less then 12% of physicians agreed to fulfil the questionnaire and give the blood sample.

Blood donors, aged 18 to 66 years, coming from Ljubljana and its surroundings, were the third group included in the study. Serum samples from the blood donor group were collected from mid-July to the beginning of September 2010.

Data on age, sex, vaccination status (vaccination with pandemic or seasonal vaccine) and acute febrile respiratory infection in the past months were collected. All participants were tested for antibodies against pandemic influenza, but only those not previously vaccinated with pandemic vaccine were included in the final analysis.

The microneutralisation (MN) assay was performed as previously described using MDCK cells (ATCC CCL 34), candidate reference antiserum (NIBSC 08/194) and pandemic H1N1 influenza isolate A/Slovenia/2106/2009 that is A/California/7/2009-like virus [13]. Twofold dilutions of each serum were made, and each was tested at an initial dilution of 1:10 and final dilution of 1:1280. Serum samples were tested separately and in duplicate. If the results showed a difference by a factor of 2, the samples were retested. Inhibition of the citopathogenic effect on MDCK constitutes a positive neutralization reaction and indicates the presence of virus-specific neutralizing antibodies in the serum tested.

Data were summarized as frequencies and percentages. Differences among seropositive subjects regarding history of acute respiratory infection during pandemic season and history of past vaccinations with the seasonal influenza vaccine were assessed by the chi-square test. A p-value of less than 0.05 represented statistical significance.

The research protocol was approved by the National Medical Ethics Committee and been performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Results

A total of 226 serum samples were tested for antibodies against the influenza A(H1N1) 2009 virus by use of MN assay. Serum samples were obtained from 47 preschool chil-

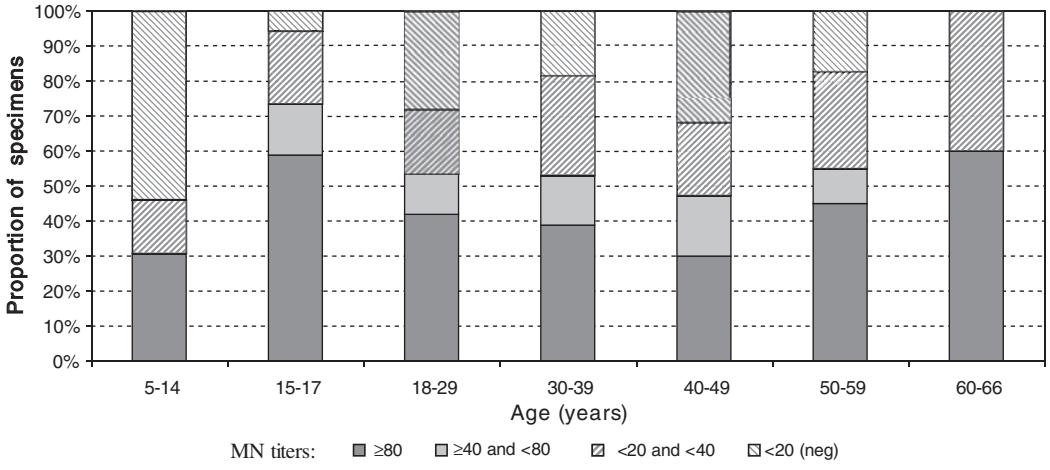

Fig. 1: Proportions of microneutralization (MN) titres against A(H1N1) 2009 in different age groups

Table 1. Microneutralization antibody response to influenza A (H1N1) 2009 virus in children and adults during the post-pandemic period

Antibody titre	Age (years)							
	5–14	15–17	18–29	30–39	40–49	50–59	60–66	Σ
<20 (neg)	7	2	12	9	17	5	0	52
≥20 and <40	2	7	8	14	11	8	2	52
≥40 and <80	0	5	5	7	9	3	0	29
≥80	4	20	18	19	16	13	3	93
Σ	13	34	43	49	53	29	5	226
≥40 and ≥80 (%)	4 (30.8%)	25 (73.5%)	23 (53.5%)	26 (53.1%)	25 (47.2%)	16 (55.2%)	3 (60%)	122 (54%)

dren and students younger than age 18, and from 22 health care workers and 157 blood donors. A MN titre of ≥40 was detected in 54% of all serum samples. The largest proportion of individuals with a MN titre of ≥40 was found among secondary school students (73.5%) (Fig. 1, Table 1).

Information on vaccination with seasonal influenza vaccine in previous years was obtained for 175 participants. Those with uncertain vaccination history were mostly children and young adults. As many as 132 participants had never been vaccinated against seasonal influenza and 38 were vaccinated one to three times. Five participants had been reportedly vaccinated practically every year. A MN titre of ≥40 was found in 60.5% of the participants who had received one to three vaccinations with seasonal influenza vaccine and in 52.2% of the participants not previously vaccinated with a vaccine against influenza. There was no statistically significant difference between the two groups (chi-square test, *p*-value 0.368).

The history of acute febrile illness with respiratory symptoms (ARI) was recorded in 35 participants. One hundred and thirty-one participants denied having ARI during the previous eight months; 60 participants were not sure or did not answer the question. Most of the non-respondents were students. History of ARI was not more frequent in those with MN titres ≥20, ≥40 or ≥80 (chi-square test, *p*-value 0.654, *p*-value 0.755 and *p*-value 1, respectively).

Discussion

In the present study serum samples were obtained from 226 participants not previously vaccinated with the pandemic vaccine. In these samples taken during the post-pandemic period, antibodies against pandemic influenza were measured by use of microneutralization assays. MN is more sensitive compared to hemagglutination inhibition test (HI) and measures a brother range of neutralizing antibodies [1, 12]. This method was chosen to avoid cross-reactivity with A(H1N1) viruses from previous seasons. The overall seropositivity rate was high: 76.9% participants had antibody titre ≥20. Considering the high sensitivity and specificity of MN assay, antibody titre of ≥20 was taken as highly probable marker of infection with A(H1N1) 2009 influenza virus. Individuals with A titre of ≥40 was detected in 54% of the serum samples tested. A comparably high level of protection was found in a Canadian study, which showed that 41% of all persons tested by microneutralization assays had a titre of ≥40. In the above mentioned study the seroprevalence showed *U*-shaped age distribution, with a high proportion of seropositive persons aged 90 or older and those under 19 years of age [11].

The correlate of protection for pandemic influenza is currently unknown and remains to be clarified [12]. Cell-mediated immunity might have a role in shortening virus shedding, but antibodies mediate the immunity to influenza [7]. In general, a titre of ≥40 is considered to be immunoprotective [12]. The highest percentage of participants with titre ≥40 was found among 15–17-year olds. The high proportion of seropositives may have resulted from intensive circulation of pandemic virus among teenagers. Epidemiological and virological surveillance in Slovenia for the 2009/2010 season confirmed that the intensity of pandemic influenza activity was highest among teenagers [14]. As reported by Waalen in his study of the elderly (≥80 years), teenagers and young adults in Norway, seropositivity might be a result of pre-existing antibodies reactive to pandemic influenza, and cannot be explained by cross-reactivity only [9]. In the view of Waalen study, the highest seropositivity in the teenage group in Slovenia might be partly explained also by pre-existing immunity, but the reason of this phenomenon is not clear, because the exposure of this age group to pandemic resembling viruses is not probable. Influenza epidemic in the 2007/2008 season caused by the seasonal influenza A(H1N1) virus may also have increased the prevalence of cross-reactive antibodies [12, 15].

No difference in antibody titres against pandemic influenza was found between individuals previously vaccinated with seasonal vaccine and those never vaccinated. One of the earliest studies indicated that neither contemporary seasonal influenza vaccines nor adjuvanted influenza vaccine can provide a cross-reactive protection to pandemic virus [2].

In the present study, history of ARI was not correlated with seropositivity to pandemic influenza, an observation indicating a high rate of asymptomatic infections during the last pandemic. Similarly, during an outbreak of pandemic influenza in a Finnish garrison half of the seropositive individuals had no history of upper respiratory tract infection with or without fever [15].

In conclusion, high seroprevalence to pandemic influenza has been found in all age groups regardless of the absence of clinical picture compatible with ARI. Previous

vaccinations with seasonal influenza vaccines had no impact on serological response to influenza A(H1N1) virus.

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Conflict of interest

The authors declare that there is no conflict of interest.

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