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Original Article

Seroprevalence following the First Wave of Pandemic Influenza A (H1N1) in Turkey, 2009

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SUMMARY: In this study, we sought to describe the community seropositivity of pandemic influenza A (H1N1) in order to estimate immunity shortly after the peak of the first pandemic wave in two provinces in Turkey. This cross-sectional study was conducted in the provinces of Diyarbakir and Ankara, after the first wave of H1N1 incidences in 2009. It was designed to evaluate 276 houses in Diyarbakir and 455 houses in Ankara. Everyone living in these houses was included in the study. An antibody titer of $\geq$1:40 was considered as a positive result for all age groups. Antibody titers of $\leq$1:20 were considered as 1 while calculating the log titer and geometric mean. The pandemic H1N1 seropositivity was found to be 24.1% for Ankara and 27.7% for Diyarbakir. In Ankara, seropositivity was statistically associated with the 15–24 age group (odds ratio [OR] = 11.47), pandemic influenza A (H1N1) vaccination (OR = 20.95), and influenza-like illness history (OR = 1.60). In Diyarbakir, H1N1 seropositivity was associated with the 15–24 age group (OR = 8.99) and pandemic influenza A (H1N1) vaccination (OR = 9.94). Because individuals less than 25 years old played an important role in the community transmission of infection and were largely protected against the pandemic influenza A (H1N1) virus, these individuals should be given a high priority for pandemic influenza vaccination in the event of the emergence of another novel pandemic strain.

INTRODUCTION

In April 2009, a new influenza A (H1N1) virus was isolated from humans in Mexico and the United States. After the following widespread infection that occurred worldwide, the World Health Organization (WHO) declared that an influenza pandemic was underway. The 2009 pandemic influenza A (H1N1) virus has a combination of gene segments that have not been identified previously in swine or human populations (1,2).

Under the surveillance system for pandemic influenza, the incidence of infection for various countries was calculated by using the data that focused only on the number of people that received medical care for this illness. However, the people with mild-to-moderate infections were not counted, and this lead to an underestimation of the actual number of cases (3).

Therefore, comprehensive seroprevalence surveys are needed to understand infection rates and population immunity levels before and after the circulation of 2009 pandemic H1N1 virus. Knowledge of the seropositivity rate of the new strain is an important element that can inform decision-makers on public health policy regarding vaccination and other intervention strategies (4,5).

The first imported laboratory-confirmed pandemic influenza A (H1N1) case in Turkey appeared in May 2009. Until the 42nd week of 2009, a limited number of laboratory-confirmed cases have continued to emerge in various provinces. The peak of the epidemic occurred between 47th and 49th weeks of 2009 (6,7).

In Turkey, nasopharyngeal and/or nasal swabs taken from suspected cases were sent to the Refik Saydam National Public Health Agency, National Influenza Center, Ankara, or the Influenza Reference Laboratory at Istanbul University, Faculty of Medicine, Istanbul, or the Regional Public Health Laboratories at the beginning of the pandemic. The number of cases increased sharply in the 43rd week and reached the highest level during the 49th week of the year. However, since the laboratory test was performed on clinical specimens collected from patients requiring hospitalization, and from outbreak cases after the 49th week, the number of cases in this week seems lower than the previous weeks (8).

In this cross-sectional study, we sought to describe the community seropositivity of the 2009 pandemic influenza A (H1N1) in order to estimate immunity shortly after the peak of the first pandemic wave in two provinces in Turkey.

MATERIALS AND METHODS

Study design: The national sentinel surveillance system for influenza covering the 14 provinces (including Ankara and Diyarbakir) has been carried out in Turkey since 2004. In the sentinel surveillance system, the number of influenza-like illness (ILI) cases and the results of laboratory tests performed on clinical specimens ob-

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tained every week from a sample of patients presenting with ILI were registered in each surveillance center. The surveillance is generally carried out between the 20th and 40th weeks each year, but with the declaration of the pandemic in June 2009 (9), the surveillance was continued without break in 2009.

This cross-sectional study was conducted in the provinces of Diyarbakir and Ankara in October, after the first wave of 2009 pandemic H1N1. We analyzed data from the Ankara and Diyarbakir surveys independently. The individuals living in the cities constituted the population of the study. According to the 2008 Address Based Population Registry System of Turkish Statistical Institute (TurkStat), the city populations for Diyarbakir and Ankara were 799,447 and 3,902,956, respectively. The sample size for the community seroprevalence study was calculated using the Epi StatCalc software. Assuming a prevalence of 20% in each age group (0–4, 5–14, 15–24, 25–44, 45–64, and 65 years and older), a sample size of 246 would give a 95% confidence interval (CI) of 15.5% to 25.4%. This was deemed to provide sufficient certainty for the prevalence estimate. This gives a total sample size of 1,476 persons. Since the mean size of a household in the center of Diyarbakir is 6.15 (1,476/6.15) according to the Research of State Planning Institute on Socio-Economic Development Ranking of Counties (2004), we calculated that 240 houses should be accessed. Since it was necessary to choose substitute households, 15% of houses were added in the sample, and it was planned to reach 276 houses in Diyarbakir. In contrast, for the province of Ankara, since household size is 3.73 (1,476/3.73), the number of houses that needed to be accessed was calculated as 396. Since it was necessary to choose substitute houses as well, 15% houses were added in the sample, and the study was thus planned to reach 455 houses in Ankara. The address of the houses was chosen randomly using the Address Based Population Registry System of TurkStat. Everyone living in these houses was included in the study, except for babies younger than 11 months and individuals with any diseases causing primary or secondary immunodeficiency (such as chemotherapy for malignancy, transfer of bone marrow, steroid cortisone use, etc.). Written informed consent was obtained from all participants. The questionnaire, which included demographic data, history of ILI until September, and vaccination history of both seasonal influenza (from 2005 to 2009) and pandemic H1N1, was administered during a face-to-face interview. A 10-ml venous blood sample was collected from all respondents and transported to the Refik Saydam National Public Health Agency as per the appropriate biosafety rules.

Baseline sera: The baseline immunity to 2009 pandemic H1N1 was evaluated in residual samples from sera submitted to the Refik Saydam National Public Health Agency Virology Department for diagnostic testing of arboviruses from Crimean Congo hemorrhagic fever endemic regions in 2008. In total, 360 samples were chosen randomly from the laboratory list: 79 samples from the 0–4 age group, 40 samples from the 5–14 age group, 60 samples from the 15–24 age group, 60 samples from the 25–44 age group, 58 samples from the 45–64 age group, and 60 samples from the 65 years and older group; there was no statistical significance between the age groups by gender.

Antibody detection: All sera were stored at $-20°C$ until they were tested. Hemagglutination inhibition (HAI) assay was performed in the Refik Saydam National Public Health Agency, Virology Laboratory, National Influenza Center. Initially, serum non-specific inhibitors were treated with receptor-destroying enzyme (Denka Seiken, Tokyo, Japan) overnight at 37°C, followed by inactivation at 56°C for 30 min. Then, to detect the titer of the antigen, hemagglutination test was performed; standard antigen was diluted to contain 8 hemagglutinin units/50 μl and back titration was performed. Samples were assayed for antibodies to influenza virus A/California/7/2009 by HAI assay using A/California/7/2009 (H1N1) antigen, which was kindly provided by the National Institute for Biological Standards and Control (NIBSC), Health Protection Agency, and reference antiserum, kindly supplied by WHO for the influenza season 2009–2010. Initially, sera were diluted 1:10, and subsequently, serial twofold dilutions were performed; the starting serum dilution for the assay was 1:20. A 50-μl aliquot of the diluted sera was incubated with an equal volume of the antigen, and then, 50 μl of a 0.75% suspension of Turkey erythrocytes was then added to the mixture. The HAI assay presented the highest reciprocal dilution, which induced complete HAI (10). Seropositivity was defined as an HAI titer 1:40 and above (subject with HAI titer ≥ 40) (11).

Statistical analysis: Data were analyzed after error checks by means of the SPSS 17.0 statistics package software. An antibody titer of $\geq$1:40 was considered positive for all age groups. Variables associated with a seropositive test, with $P \leq 0.20$ as calculated by using univariable statistical tests (chi-square and Fisher's exact test), were then included into a multivariable logistic regression model (1 = seropositivity; 0 = no evidence). Potential confounding factors, such as gender, were forced into the model. A P value <0.05 was considered statistically significant in the multivariable logistic regression model. Antibody titers of $\leq$1:20 were considered to be 1 while calculating the log titer and geometric mean. Kruskal-Wallis tests was used to analyze the survey data for which the Levene's test yielded a P-value <0.05.

RESULTS

The first pandemic influenza A (H1N1) wave in Turkey started in the 42nd week of 2009, peaked between the 47th and 49th weeks of 2009 in various regions of the country, and it declined to baseline again in the 7th week of 2010, according to data confirmed by the Refik Saydam National Public Health Agency. In this study, the serum samples collected from the participants between January and February 2010 were used for analysis, since they were collected after the peak of the epidemic in Diyarbakir and Ankara. The levels of participation in the study were 78.9% (1,164/1,476) and 53.6% (791/1,476) for Ankara and Diyarbakir, respectively.

In Ankara, the percentages of participants who received the seasonal vaccine since 2005 were 3.3% (0–4 age group), 12.8% (5–14 age group), 18.1% (15–24 age group), 15.4% (25–44 age group), 23.7% (45–64 age

group), and 32.7% (65 years or older age group), and the percentages of participants who received the pandemic influenza vaccine were 6.5% (0–4 age group), 7.9% (5–14 age group), 7.8% (15–24 age group), 10.5% (25–44 age group), 9.4% (45–64 age group), and 9.9% (65 years or older age group). The percentages of participants who had a history of ILI until September 2009 were 64.5% (0–4 age group), 52.2% (5–14 age group), 58.1% (15–24 age group), 50.0% (25–44 age group), 38.3% (45–64 age group), and 28.3% (65 years or older age group). In Diyarbakir, the percentages of participants who received the seasonal vaccine since 2005 were 2.2% (0–4 age group), 3.2% (5–14 age group), 0.8% (15–24 age group), 3.8% (25–44 age group), 5.3% (45–64 age group), 13.3% (65 years or older age group); of participants who received the pandemic influenza vaccine were 2.2% (0–4 age group), 1.1% (5–14 age group), 1.7% (15–24 age group), 6.5% (25–44 age group), 3.5% (45–64 age group), and 6.5% (65 years or older age group); and of participants who had ILI history until September 2009 were 51.1% (0–4 age group), 34.4% (5–14 age group), 39.5% (15–24 age group), 36.2% (25–44 age group), 32.4% (45–64 age group), and 29.0% (65 years or older age group).

Pandemic H1N1 seropositivity was found to be 24.1% (281/1,164) for Ankara and 27.7% (219/791) for Diyarbakir. In Ankara, 9.3% of participants stated that they had received H1N1 influenza vaccination, while 3.7% of participants from Diyarbakir stated the same. The prevalence of seropositivity in participants who had

Table 1. Geometric mean titers for pandemic influenza A (H1N1) in Ankara, Diyarbakir, and baseline sera to age groups

Age group	Ankara			Diyarbakir			Baseline			$P^{1)}$
	No. tested	GMT (95% CI)	% with titer ≥1:40	No. tested	GMT (95% CI)	% with titer ≥1:40	No. tested	GMT (95% CI)	% with titer ≥1:40	
0–4	32	10.41 (4.30–25.18)	43.8	53	4.80 (2.82–8.19)	28.3	79	0	0	0.001
5–14	192	10.43 (7.61–14.29)	43.8	200	7.99 (6.01–10.60)	37.5	40	0	0	0.001
15–24	129	12.46 (8.29–18.73)	48.1	121	8.70 (5.91–12.81)	41.3	60	1.11 (0.96–1.27)	0	0.001
25–44	411	2.57 (2.15–3.06)	16.8	270	3.57 (2.85–4.47)	22.2	60	1.14 (0.95–1.38)	3.3	0.001
45–64	288	2.27 (1.95–2.89)	14.6	115	2.34 (1.74–3.17)	13.9	58	1.05 (0.95–1.17)	0	0.001
≥65	112	1.70 (1.31–2.21)	8.9	31	1.65 (1.01–2.68)	9.7	60	1.06 (0.94–1.20)	1.7	0.041

1): *P*-value of differences in geometric mean titers between Ankara, Diyarbakir, and baseline groups, calculated by Kruskal-Wallis test.

Table 2. Univariable analysis results of pandemic influenza A (HIN1) seropositivity

	Ankara				Diyarbakir			
	No. of tested	% with titer ≥1:40	$P^{1)}$	OR (CI)	No. of tested	% with titer ≥1:40	$P^{1)}$	OR (CI)
Age group								
0–4	32	43.8	0.001	7.93 (3.06–20.59)	53	28.3	0.055	3.68 (0.97–13.96)
5–14	192	43.8	0.001	7.93 (3.90–16.13)	200	37.5	0.006	5.60 (1.45–19.06)
15–24	129	48.1	0.001	9.44 (4.52–19.70)	121	41.3	0.003	6.57 (1.89–22.81)
25–44	411	16.8	0.043	2.06 (1.02–4.14)	270	22.2	0.117	2.67 (0.78–9.08)
45–64	288	14.6	0.135	1.74 (0.84–3.64)	115	13.9	0.536	1.51 (0.41–5.55)
≥65	112	8.9		1	31	9.7		1
Gender								
Female	674	23.1	0.352	0.88 (0.67–1.15)	488	27.3	0.730	0.95 (0.69–1.30)
Male	490	25.5		1	303	28.4		1
Household size								
1–3	321	17.8	0.002	1	111	24.3	0.393	1
≥4	843	26.6		1.68 (1.21–2.32)	680	28.2		1.22 (0.77–1.95)
Seasonal vaccine history since 2005								
No	930	22.4	0.018	1	733	28.2	0.286[2)]	1
Yes	213	30.0		1.49 (1.07–2.08)	28	17.9		0.55 (0.21–2.47)
Pandemic influenza vaccine								
No	1,048	19.0	0.001	1	732	26.2	0.001	1
Yes	108	72.2		11.09 (7.08–17.37)	28	71.4		7.03 (3.05–16.23)
ILI history until September 2009								
No	579	19.9	0.001	1	472	25.6	0.039	1.41 (1.02–1.96)
Yes	511	29.7		1.71 (1.29–2.26)	269	32.7		1

1): Chi-square test.
2): Fisher exact test.

received the pandemic H1N1 vaccination was 72.2% and 71.4% in Ankara and Diyarbakir, respectively. In the study, the highest seropositivity was encountered in the 15–24 age group (Ankara, 48.1%; Diyarbakir, 41.3%) ($P = 0.001$). Seropositivity in 25–44 age group was 3.3%, and in the 65 years or older age group, 1.7% of participants from the baseline group had cross-reactive antibodies to the pandemic influenza 2009 virus. The geometric mean titers (GMT) with 95% CI for pandemic influenza A (H1N1) for the different groups is shown in Table 1.

Responses of the participants to the questionnaires were analyzed to identify possible associations with pandemic H1N1 antibody seropositivity; most of the responses showed no association with seropositivity. The statistically significant variables are shown in Table 2. There was no statistically significant correlation between gender of the participants and pandemic HIN1 seropositivity in Ankara. In Diyarbakir, there was no statistically significant correlation between the pandemic H1N1 seropositivity and gender, household size, or having received seasonal vaccine since 2005. In participants from Ankara, pandemic influenza A (HIN1) seropositivity was significantly associated with the following factors: having a household size ≥ 4, having received seasonal vaccine since 2005, having received pandemic influenza vaccine, and having ILI history until September 2009 ($P = 0.002$, $P = 0.018$, $P = 0.001$, and $P = 0.001$, respectively). In participants from Diyarbakir, the association of pandemic influenza A (HIN1) seropositivity with having received pandemic influenza vaccine and ILI history until September 2009 was found to be statistically significant ($P = 0.001$ and $P = 0.039$, respectively). While the odds of seropositivity in individuals with seasonal influenza vaccination history within the last 5 years was found to be 1.49 times higher than the other participants (CI 95% = 1.07–2.08) in Ankara, there was no statistically significant relation between seasonal influenza vaccination and seropositivity in Diyarbakir ($P = 0.286$). Seropositivity was found to be 1.71 times higher in Ankara (CI 95% = 1.29–2.26) and 1.41 times higher in Diyarbakir (CI 95% = 1.02–1.96) for those with a history of ILI from September 2009 until the date of study.

The outputs of the multivariable analysis are shown in Table 3. Pandemic A (H1N1) seropositivity was statistically associated with the 15–24 age group (odds ratio [OR] = 11.47), having pandemic H1N1 vaccination (OR = 20.95), and ILI history (OR = 1.60) in Ankara, as determined by using logistic regression model. In Diyarbakir, being in the 15–24 age group (OR = 8.99) and having pandemic H1N1 vaccination (OR = 9.94) were associated with H1N1 seropositivity.

Seroprevalence in individuals who had no ILI and pandemic H1N1 vaccination history was found to be 14.1% (74/523) in Ankara and 29.9% (112/449) in Diyarbakir.

DISCUSSION

In Turkey, the number of laboratory-confirmed pandemic influenza cases began to increase in the 42nd week of 2009, and reached the peak in the 47th week (7). This cross-sectional study was conducted after the peak

Table 3. Multivariable analysis results of pandemic influenza A (HIN1) seropositivity

	Ankara			Diyarbakir		
	OR	CI 95%	*P*	OR	CI 95%	*P*
Age group						
0–4	9.04	3.03–26.96	0.001	4.89	1.18–20.31	0.029
5–14	9.19	3.91–21.60	0.001	7.61	2.08–27.84	0.002
15–24	11.47	4.79–27.47	0.001	8.99	2.42–33.36	0.001
25–44	1.85	0.81–4.22	0.146	3.11	0.86–11.26	0.084
45–64	1.79	0.76–4.19	0.182	1.78	0.45–7.02	0.408
≥65	1			1		
Gender						
Female	1			1		
Male	1.02	0.73–1.42	0.920	0.91	0.63–1.30	0.589
Household size						
1–3	1					
≥4	1.34	0.88–2.05	0.177	ND		
Seasonal vaccine history since 2005						
No	1					
Yes	0.94	0.60–1.49	0.796	ND		
Pandemic influenza vaccine						
No	1			1		
Yes	20.95	11.94–36.77	0.001	9.94	4.02–24.58	0.001
ILI history until September 2009						
No	1			1		
Yes	1.60	1.15–2.23	0.005	1.23	0.87–1.75	0.240

ND, not done.

of the fall wave and was aimed at improving our understanding of the spread of the pandemic influenza A (H1N1) virus through the population in two provinces where the number of cases declined significantly after the first wave.

Overall, 24.1% and 27.7% of serum samples collected from all age groups were positive for pandemic influenza A (H1N1) virus in Ankara and Diyarbakir, respectively. In Ankara, the percentage of persons found to be positive for H1N1 influenza virus in the 0–24 age group (ranging from 43.8% to 48.1%) was significantly higher than that of the participants aged 25 years and older, with the lowest percentage of positive serum samples among individuals ≥65 years old (8.9%). The same results were observed for the 0–24 age group (28.3–41.3%) in Diyarbakir, with the lowest percentage among individuals ≥65 years old (9.7%). The results of our study were similar to the findings from several other countries that reported the highest rates of infection with pandemic influenza virus in school-age children (5–19 years old) (5,12–14).

The pandemic influenza A (H1N1) virus is affecting children, young adults, and the general population younger than 65 years (13,15,16). According to data from the country, the highest number of the laboratory-confirmed pandemic influenza A (H1N1) cases were reported in the 5–14 (30.5%) age group, followed by the 15–24 (21.9%), 25–44 (20.7%), 1–4 (10.6%), 45–64 (7.5%), and ≥65 (2.0%) age groups (8). In our study, the proportion of samples with titer of ≥1:40 in individuals aged 0–4 and 15–24 was substantially higher than those observed in the above study. These data support the conclusion that the disease is asymptomatic or mainly mild in these age groups and/or household contact is a significant way for transmission. A household size ≥4 was associated with seropositivity (crude OR, 1.68; CI, 1.21–2.32; $P = 0.002$) compared to a small household size (1–3 people) (12,17). However, after applying the multivariable logistic regression model, this effect disappeared (adjusted OR, 1.34; CI, 0.88–2.05; $P = 0.177$) in Ankara.

In our study, seropositivity was 1.49 times more likely ($P = 0.018$) in the people from Ankara who had a seasonal vaccine history since 2005 than in those who had never been vaccinated, and this effect disappeared after adjustment. There was no association between seropositivity and seasonal vaccine history in Diyarbakir. Several studies that have examined the effectiveness of seasonal vaccination against pandemic influenza A (H1N1) virus infection have showed that there was little or no effect of the seasonal vaccines on the pandemic influenza illness (14,18). However, Bandaranayake et all. reported that people who received the seasonal influenza vaccine were 1.8 times more likely to have HI titers ≥1:40 compared with those who had never been vaccinated (12).

Some studies have demonstrated higher levels of antibody titers in prepandemic serum samples from older persons (≥80 years) than from persons in younger age groups, presumably as a result of previous exposure to antigenically related H1N1 influenza viruses circulating since 1918 and a lifetime of exposure to influenza A (13,18–20). In the present study, seropositivity in 25–44 age group was 3.3%, and in the 65 years or older age group, 1.7% of the participants from the baseline group had cross-reactive antibodies to the 2009 pandemic H1N1 virus. The age distribution of laboratory-confirmed pandemic influenza cases (8) is not consistent with the observed lack of seropositivity, particularly in the participants aged ≥65 years. This might be explained by the fact that heterotypic immunity to influenza from antibodies against the neuraminidase or cellular responses to highly-conserved viral epitopes contribute to the apparent protective effect in older individuals (18). Moreover, one of the limitations of this study regarding the representativeness of baseline samples was that these samples were sent to the virology laboratory for serological testing for arboviruses from provinces other than Ankara and Diyarbakir. The other limitation was the relatively small numbers of sera in the baseline samples from individuals who were 65 years or older, and with no information on seasonal influenza vaccination and other important determinants. Moreover, two previous studies have shown that none of the individuals aged >60 years had cross-reactive antibodies to pandemic influenza A (H1N1) virus (21,22).

To our knowledge, this is the first randomized cross-sectional study reported from Turkey that provides information on the population immunity profile of the pandemic H1N1 virus. Such studies are important as they allow for evidence-based decisions on interventions during future influenza threats and vaccination policies. In the present study, a relatively high proportion of people under 25 years may have already developed protective antibodies, given the mild nature of the pandemic. This age group, who played an important role in the community transmission of infection, is largely protected against pandemic influenza A (H1N1) virus, and they should be given a high priority for pandemic influenza vaccination in the event of the emergence of another novel pandemic strain.

Seroprevalence in individuals who had not developed ILI and had a history of pandemic H1N1 vaccination was found to be 14.1% (74/523) in Ankara and 29.9% (112/449) in Diyarbakir. We believe that these figures reflect asymptomatic cases for both cities.

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Conflict of interest None to declare.

Appendix The participants in the study are as follows: Handan Kalaycıoğlu, Dilber Aktaş, and Fatma Bayraktar (Refik Saydam National Public Health Agency); Çiğdem Şimşek, Gönül Çulha, Ahmet Özlü, and Füsun Arıkan (Local Health Authority, Ankara); and Vedat Dorman (Local Health Authority, Diyarbakir).

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