High incidence of severe influenza among individuals aged over 50 years

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Running title: Epidemiology of pandemic influenza

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Abstract

Age-specific epidemiological data of asymptomatic, symptomatic and severe infection are essential for public health policies in combating influenza. In this study, we incorporated data of microbiologically-confirmed infection and seroprevalence to comprehensively describe the epidemiology of pandemic H1N1 2009 influenza. Seroprevalence was determined from 1795 random serum samples collected in January 2007 (before the first wave) and March 2010 (after the second wave) in our hospital. Data on microbiologically-confirmed infection and severe cases were obtained from Centre for Health Protection in Hong Kong. Severe cases were most common in the 51-60 year age group. Microbiologically-confirmed incidence rate was highest in children aged ≤10 years, and dropped sharply in the adult population ($\rho$=-1.0, $p<0.01$), but the incidence rate for severe disease was highest in the 51-60-year age group. For the 51-60-year age group, the seroprevalence was similar to the younger age groups, but the proportion of severe case relative to seroprevalence was significantly higher than that of age groups 11-50. As judged from the percentage of specimens positive for other respiratory viruses compared with that of pandemic H1N1 virus, the impact of symptomatic disease due to pandemic H1N1 was higher than other respiratory viruses in people aged ≤50 years. In conclusion, the 51-60-year age group, which had the highest incidence and incidence rate of severe disease but currently not considered by the World Health Organization to be a risk factor, should be prioritized for influenza vaccination in areas where universal influenza vaccination is not practiced.
Introduction

One of the major criticisms on the handling the pandemic H1N1 2009 influenza by the World Health Organization is the apparent overestimation of its disease severity. Data of age-specific incidence rate from the pandemic influenza can provide the scientific basis for formulating public health policies and insights on the age-related susceptibility to and severity of influenza. Currently, there are discrepancies in the age cutoff in the recommendations for influenza vaccination especially in adults. While the World Health Organization considers individuals aged $\geq 65$ years to be at a higher risk of severe influenza and includes them as one of the target groups for influenza vaccination and antiviral treatment or prophylaxis (50), the United States Centers for Disease Control and Prevention has extended influenza vaccination to all persons aged $\geq 6$ months, and in the event of vaccine shortage, to those aged $\geq 50$ years (18).

To accurately assess the incidence rate and severity, the choice of the denominator is crucial (36). If the total number of microbiologically-confirmed cases is used as the denominator, this would overestimate the severe case incidence rate because infection without microbiological confirmation would be excluded. On the other hand, influenza-like illness (ILI) is often used as a surrogate marker for total number of cases, but this may be inaccurate especially if the surveillance period extends over a long period of time when many ILIs may be due to other respiratory pathogens. Other epidemiological studies have used seroprevalence as a measure of the infected population, and these studies have reported high prevalence in young adults and more severe disease in the pediatric and the geriatric populations (27, 30), which may be explained by the immature
immune system and immunosenescence respectively (37, 40). However, most of these studies did not incorporate age-specific data of asymptomatic, symptomatic and severe infection in their analysis.

In this study, we sought to integrate clinical and laboratory data to evaluate the relative impact of the pandemic H1N1 2009 influenza on different age groups of the population. The change in seroprevalence provides an estimate of the overall burden of infection (49), whereas the incidence rate of microbiologically-confirmed infections represents an estimate of symptomatic cases, as samples were mostly collected from symptomatic patients. The proportion of microbiologically-confirmed cases or severe cases to seropositive population was used to estimate the burden of symptomatic or severe disease in those infected. To assess for the relative impact of pandemic H1N1 2009 influenza virus when compared to other respiratory viruses, we have analyzed the positivity rate of all respiratory specimens tested for respiratory viruses.
Methods

Samples for the determination of antibody titre

This study was approved by the institutional review board of the Hospital Authority of Hong Kong. Antibody titre was determined from archived serum samples randomly selected at the clinical biochemistry division of Queen Mary Hospital in January 2007 before the first wave of the pandemic at the summer of 2009, and in March 2010 after the peak of the second wave. This laboratory provides service to both inpatients and outpatients with an estimated catchment population of 0.53 million people, or 8% of the Hong Kong population which should be reasonably representative of our total population of 7 million. Redundant serum samples were excluded by using their unique identity card number for Hong Kong residents. All samples were coded and remained anonymous during the analysis.

Serological assay

Antibody titre against the pandemic H1N1 2009 influenza virus was determined by hemagglutination inhibition (HI) assay as previously described (7). Briefly, non-specific inhibitors in the serum were removed with receptor-destroying enzyme (RDE II, SEIKEN). The treated serum samples were 2-fold serially diluted with phosphate buffered saline, starting from 1:10. Diluted serum samples were then mixed with 4 haemagglutinin units of pandemic H1N1 2009 influenza virus (A/HK415742/2009) and incubated at room temperature for 1 hour. 0.5% of turkey red blood cells were then added and incubated at room temperature for 30 minutes before examination for haemagglutination. An HI titre of $\geq 40$ was considered to be positive.
Microbiologically-confirmed influenza cases

Microbiologically-confirmed pandemic H1N1 2009 influenza infections confirmed by the Virology Division, Public Health Laboratory Services Branch of the Centre for Health Protection (CHP) in Hong Kong from May 1, 2009 to February 28, 2010 were included in our analysis. This laboratory processed clinical specimens requested for respiratory virus detection from all government hospitals or out-patient clinics spread all over Hong Kong. Respiratory specimens included nasopharyngeal aspirate, nasopharyngeal swab, throat swab, tracheal aspirate and bronchoalveolar lavage. The case definition for microbiologically-confirmed cases was a positive reverse transcription-polymerase chain reaction (RT-PCR) test for pandemic H1N1 2009 influenza virus H1 gene or a positive viral culture in respiratory specimens as previously reported (32). The decision for microbiological testing was made by the attending clinician. All severe cases during this period were reported to CHP. The case definition for severe pandemic H1N1 2009 influenza requiring notification to CHP include admission to the intensive care unit, critical condition requiring assisted ventilation or a change of severity from critical to fatal, and microbiological confirmation of pandemic H1N1 2009 influenza virus. The total number of laboratory requests for testing influenza virus or “other respiratory viruses” is the number of respiratory tract specimens which have been sent to the Virology Division, Public Health Laboratory Services Branch of the CHP in Hong Kong, and requested for virus detection. “Other respiratory viruses” include seasonal influenza A(H1N1), influenza A(H3N2), influenza B, adenovirus, parainfluenza virus, respiratory syncytial virus and rhinovirus.
Definitions

The seroprevalence(%) is obtained by the number of serum samples with HI titre of ≥40 over the total number of non-redundant sera tested. The infected population in each age group was estimated by the percentage change in seroprevalence between 2010 and 2007 multiplied by the population number (34). The incidence rate of symptomatic disease is the number of microbiologically-confirmed cases over the population of the age group, whereas the incidence rate of severe disease is the number of notified severe disease over the population of the age group. The burden of symptomatic or severe disease is estimated by the ratio of their incidence over the infected population.

Statistical analysis

The relationship between age and incidence rate was assessed by Spearman correlation. The Chi Square test for trends was used to assess the proportion of positive specimens for pandemic H1N1 2009 influenza virus and other respiratory viruses in different age groups. The risk ratio of influenza between the 51-60-year age group and the younger age groups were calculated. The statistical analysis was performed using SPSS software, version 18.0 for Windows (SPSS), R statistical environment version 11.1 or VasserStats (http://faculty.vassar.edu/lowry/VassarStats.html).
Results

The seroprevalence was estimated by HI testing of 1795 serum samples of which 795 and 1000 samples were collected in 2007 and 2010 respectively (Table 1). In 2007, the seropositive rate was 8.8%. Pre-existing cross-reactive antibodies against pandemic H1N1 2009 influenza virus were mainly found in patients aged ≥71 years, and more prevalent in the older age groups (Figure 1). Cross-reactive antibodies were also found in 2.1% of individuals from age 21 to 50. No children ≤10 years old or adults between 51 to 70 years old were seropositive in 2007. In 2010, the overall seropositive rate was 22.9%. Difference in HI titre between baseline and 2010 were not statistically significant for age groups 71 years or above. Therefore we could not use seroprevalence to accurately predict the overall incidence rate of infection in the population ≥71 years old.

Between May 1, 2009 and February 28, 2010, a total of 27,116 microbiologically-confirmed cases of pandemic H1N1 2009 influenza infection were found. Of the 255 severe cases reported, 157 were male and 98 were female, with a median age of 51 years. The 51-60-year age group has the highest number of severe cases, accounting for 29.4% of the total number of severe cases (Figure 2). The incidence rate of microbiologically-confirmed pandemic H1N1 2009 influenza was highest in the ≤10-year age group, and dropped sharply with increasing age ($\rho=-1.0, p<0.01$), while the incidence rate of severe cases showed an apparent bimodal distribution, with higher incidence rate in the age group ≤10 and those older than 50 years old (Figure 3). The highest incidence rate of severe disease also occurred in the 51-60-year age group with significantly higher risk than other younger age groups (Table 2).
The proportion of microbiologically-confirmed incidence or severe disease relative to infected population was used to estimate the burden of symptomatic or severe disease in the infected population of different age groups (Figure 4). While the burden of symptomatic disease was highest in the ≤10-year age group, severe disease most frequently occurred in the older population, which started to rise in the 51-60-year age group (Figure 4 and Table 2).

We also assessed the relative impact of pandemic H1N1 2009 influenza infection with other respiratory viral infection in different age groups by analyzing the positivity rate in respiratory tract specimens. The pandemic H1N1 2009 influenza virus was more frequently detected than other respiratory viruses in age groups ≤60, but other respiratory viruses predominated in age groups >60 years (Figure 5). The percentage of specimens positive for pandemic H1N1 2009 influenza virus decreased with increasing age (chi square test for trend $\chi^2 = 13380.73$, $p<0.001$). Though a significant trend was still observed for other respiratory viruses, its magnitude was much less than that for pandemic H1N1 2009 influenza virus (chi square test for trend $\chi^2=366.969$, $p<0.001$). It should be noted that the percentage of specimens positive for other respiratory viruses is not significantly different ($p>0.05$) between age groups 51-60 and 41-50, which suggested that there is no sampling bias in terms of specimen collection in these age groups.
Discussion

In this study, we have systematically analyzed and compared the age-specific incidence rate of the pandemic H1N1 2009 influenza using data generated by serial serological data and microbiologically-confirmed infections encompassing all age groups. The pandemic H1N1 2009 influenza virus more frequently affected children and adolescents than older adults, evidenced by the greater difference between pre-pandemic and post-pandemic seroprevalence, the higher age-specific incidence rate of microbiologically-confirmed infection, and the higher positivity rate in respiratory tract specimens when compared to other respiratory viruses. The 51-60-year age group accounts for the highest number of severe cases, and this age group also has the highest incidence rate of severe cases. By analyzing the positivity rate in respiratory tract specimens, we have also shown that the pandemic H1N1 2009 influenza virus was the predominant respiratory virus affecting the population aged 50 or below.

The main advantage of using the difference in pre-pandemic and post-pandemic seroprevalence to estimate the incidence rate is that even asymptomatic cases are captured. Numerous seroprevalence studies of pandemic H1N1 2009 influenza have been published (1-4, 8, 9, 11, 15, 16, 19, 20, 29, 31-35, 39, 41, 43, 44, 46, 51-54), but unlike our study, many of them did not incorporate the incidence rate of microbiologically-confirmed cases nor severe cases. Other studies which have compared seroprevalence with microbiologically-confirmed data either did not include all age groups (51), nor performed the age-specific analysis (33).
The World Health Organization included adults aged 65 years or older as a risk factor for severe disease and included them as one of the target groups for influenza vaccination and antiviral treatment or prophylaxis (50). This is supported by our finding that the burden of severe disease is highest in the 61-70-year age group, and consistent with other studies which have shown that those aged \( \geq 60 \) or 65 has higher case-fatality ratio (10, 14, 17). However, we have also demonstrated that 51-60-year age group has the highest incidence rate of severe disease among all age groups and higher burden of severe disease when compared to younger age groups. Our result is comparable to the worldwide estimates using laboratory-confirmed cases as the denominator in the calculation for the rate of severe cases, in which the 50-64-year age group had the highest relative risk of admission to intensive care unit for most countries (47). Likewise, a study from California showed that the highest incidence rate of death occurred in those aged 50-59 years (30). However both studies did not estimate the burden of severe disease in all infected individuals by monitoring the change in seroprevalence before and after the first wave. Rather they used the total population as the denominator for the analysis. Findings of these two studies and our study are consistent with the recommendation from the United States Centers for Disease Control and Prevention in that individuals aged \( \geq 50 \) years should have priority for vaccination in the event of vaccine shortage (18). Our finding was unlikely to be caused by specimen collection bias as the positivity rate for other respiratory virus was not different from those of age group 41 to 50. The high incidence rate of severe disease in this age group therefore justifies lowering the age cutoff for high risk group from 65 to 51 years old.
The burden of symptomatic disease was lowest in the age groups 21-60, suggesting that there were many patients with mild disease who did not require medical attention and therefore not tested for respiratory viruses in this age group. The result is consistent with the findings in another large prospective study, in which most patients in this age group were only mildly symptomatic (6). The burden of severe disease was also lower in the age groups 21-60 when compared to older age groups. Despite such a low burden of severe disease, the absolute number of severe cases in these age groups were actually higher than in those above 60 years old, similar to previous studies (13, 25, 28, 38, 45, 48), because a high proportion of the population in these age groups were infected due to the lack of pre-existing cross-reactive antibodies against the pandemic H1N1 virus. We also speculate that this particular age group has developed a solid cell-mediated immunity due to cytotoxic T lymphocyte response or non-neutralizing antibody dependent cellular cytotoxicity against the highly conserved viral antigens on the viral matrix or nucleoprotein (12, 26), which is important in the recovery from the illness. Such relative protection induced by repeated exposure before and after the age of 20 decayed with age-associated immunosenescence or impaired by major underlying disease in those over 50 years of age.

The overall post-pandemic seroprevalence of 22% in our study is in the lower range of the estimates from previous seroprevalence studies, which have shown an estimated infection rate of 5-60% (5). The wide range of estimates may be accounted by the difference in the ethnic group of the studied population, the serological assay (HI vs viral neutralization assays) and the timing of specimen collection (after first or second wave).
In our study, we have also noted a bimodal distribution of pre-pandemic seroprevalence, with cross-reactive antibodies (HI titre ≥40) found in a small number of individuals aged 21-50 years, and more in those aged over 70 years. This finding is consistent with other studies in which the seropositive rate was slightly higher in those born in the 1970s and 1980s (2, 19, 22, 32). However, this observation is not seen in other studies (24, 33). The reason for their low level of cross-reactive antibodies is not known.

There are several limitations in this study. Factors associated with lower rates of seroconversion have been described, including older age, female, pregnant individuals or milder disease (7, 9, 32, 42). Notably, 10% of convalescent plasma donors for influenza had an HI titre <40 (21). These factors contribute to a lower seroprevalence rate, which leads to an underestimation of the incidence of infection. On the contrary, vaccination leads to seroconversion, and therefore seroprevalence may overestimate the incidence of infection where vaccine coverage is high. However, the pandemic H1N1 vaccination uptake rate was very low in Hong Kong after one widely publicized report of suspected influenza-related complication of Guillain Barre Syndrome in a medical doctor. Up to 31 March, 2010, only 188,622 individuals in the Hong Kong influenza vaccination program (2.7% of the total Hong Kong population) have received the monovalent pandemic H1N1 vaccine, while an additional 61,107 doses of vaccine have been distributed to private practitioners for individuals who are excluded from the at risk target group in the vaccination program. However, the exact number of vaccinees in the private sector outside the government vaccination program is not known as this data is not reported to the CHP. But even if this batch of vaccine were all administered to patients, the
percentage of vaccinated population will only be 3.6%. Therefore, the effect of
vaccination is negligible. Furthermore, the number of available pre-pandemic serum
collection in the ≤10-year age group was low, we may not be able to detect individuals who
were seropositive. However, since other studies for this age group showed very low
prevalence of pre-existing antibody of <5% (34), the interpretation of our results should
be similar for this age group. Finally, the difference in the rate of severe infection may be
confounded by factors such as underlying diseases. In our study, we have found a higher
incidence of severe infection in the 51-60-year age group. In Hong Kong, 22.5% of the
population in the age group 50-59 has chronic diseases, compared to 2.8-10.7% in those
aged below 50 years (23). We were not able to access the clinical data of each patient as
the serum samples were coded anonymously. Further studies incorporating underlying
diseases will allow an assessment of the independent effect of age on the severity in this
age group.

While the adolescents and younger adults were most commonly affected by the pandemic
H1N1 2009 influenza virus, the clinical consequence is most alarming in older adults. In
contrast to the usual age cutoff of 65 years old, our results clearly demonstrated that
individuals aged older than 50 years are more prone to severe infection than the younger
population. In view of our findings, health authorities should consider lowering the at risk
groups from age 65 to age 51 years.

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Figure legend.

Figure 1. Proportion of seropositive individuals in years 2007 and 2010. Y-axis represents the percentage of seropositive individuals detected by hemagglutination inhibition antibody titer of more than or equal to 40.

Figure 2. Incidence of severe cases of pandemic H1N1 2009 infection. Y-axis represents the absolute number of severe cases as defined by admission to the intensive care unit, critical condition requiring assisted ventilation or a change of severity from critical to fatal.

Figure 3. Microbiologically-confirmed incidence rate of pandemic H1N1 2009 influenza (per 100,000 population). The Y-axis on the left represents the incidence rate for all cases with positive virological test for pandemic H1N1 2009, while the Y-axis on the right is the incidence rate of all severe cases.

Figure 4. Burden of symptomatic or severe disease. Population infected is estimated by seroprevalence. The Y-axis on the left represents the burden for all cases, while the Y-axis on the right is for severe cases.
Figure 5 shows the proportion of clinical specimens positive for pandemic H1N1 2009 influenza virus and other respiratory viruses. The Y-axis represents the percentage of clinical specimens tested positive for influenza and other respiratory viruses.
Table 1. Number of serum samples collected in each age group

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>2007</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>11-20</td>
<td>74</td>
<td>100</td>
</tr>
<tr>
<td>21-30</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td>31-40</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td>41-50</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td>51-60</td>
<td>72</td>
<td>100</td>
</tr>
<tr>
<td>61-70</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td>71-80</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>81-90</td>
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<td>100</td>
</tr>
<tr>
<td>≥91</td>
<td>121</td>
<td>100</td>
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</tbody>
</table>
Table 2. The risk ratio of severe disease between 51-60-year age group and the younger age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Risk ratio (incidence rate)</th>
<th>95% confidence interval</th>
<th>Risk ratio (burden)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 or below</td>
<td>1.164</td>
<td>1.041-1.301</td>
<td>1.326</td>
<td>1.186-1.483</td>
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<td>11-20</td>
<td>1.496</td>
<td>1.361-1.645</td>
<td>1.933</td>
<td>1.758-2.126</td>
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<td>21-30</td>
<td>1.608</td>
<td>1.458-1.772</td>
<td>1.838</td>
<td>1.667-2.025</td>
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<tr>
<td>31-40</td>
<td>1.610</td>
<td>1.446-1.794</td>
<td>1.464</td>
<td>1.314-1.631</td>
</tr>
<tr>
<td>41-50</td>
<td>1.500</td>
<td>1.317-1.710</td>
<td>1.741</td>
<td>1.528-1.983</td>
</tr>
</tbody>
</table>
Figure 1.
Proportion of seropositive individuals in years 2007 and 2010

![Bar chart showing the proportion of seropositive individuals by age group in years 2007 and 2010, with the difference between the two years also depicted.](http://cvi.asm.org/)
Figure 2:
Incidence of severe cases of pandemic H1N1 2009 infection
Figure 3.
Microbiologically-confirmed incidence rate of pandemic H1N1 2009 influenza (per 100,000 population)
Figure 4.
Burden of symptomatic or severe disease. Population infected is estimated by seroprevalence.
Figure 5.
Proportion of clinical specimens positive for pandemic H1N1 2009 influenza virus and other respiratory viruses

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total no. of request</th>
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<tbody>
<tr>
<td>10 or below</td>
<td>37888</td>
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<td>11-20</td>
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<td>91 or above</td>
<td>4877</td>
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