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SHORT COMMUNICATION

Low Level of Cross-Reactive Antibodies to Pandemic Influenza (H1N1) 2009 Virus in Humans in Pre-Pandemic Period in Maharashtra, India

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Abstract In India, the first outbreak of pandemic influenza (H1N1) 2009 (H1N1pdm) was reported from Panchgani, Maharashtra, in June 2009. Studies from several countries have revealed different levels of pre-existing immunity to H1N1pdm 2009 in various age groups. This study was undertaken using age-stratified pre-pandemic human sera to understand baseline cross-reactivity of antibodies against H1N1pdm. Using cut off antibody titers 20 and 40, overall cross-reactivity was 2.1 and 0.9% respectively by microneutralization assay; 1.2% and 0.7% by haemagglutination inhibition assay, respectively. Results showed higher baseline antibodies and cross-reactive antibodies in the 0–19 age group whereas the elderly age group (≥60) showed no cross-reactivity to H1N1pdm. The higher baseline and cross-reactive antibodies in 0–19 years age group could be because of higher positivity to seasonal H1N1 in that age group. Overall, low level of cross-reactive antibodies to H1N1pdm virus were found in humans in pre-pandemic period in Maharashtra, India.

Keywords Pandemic influenza (H1N1) 2009 · Hemagglutination inhibition assay · Microneutralization assay · Cross-reactive antibodies

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The first case of pandemic influenza (H1N1) 2009 (H1N1pdm) in India was reported from Hyderabad on 16th May 2009 while the first outbreak was reported from Panchgani, Maharashtra in June 2009. Antibodies to H1N1pdm were found in 52% subjects in the schools and 9% in the residents of Panchagani [4]. Considerable morbidity and mortality due to H1N1pdm has been reported from Pune, India [6]. Seroepidemiological studies conducted in Pune during August-December 2009 revealed 6–25% seropositivity in different risk groups and general population indicating widespread infections in all sections of the community [9]. There are no reports of seroprevalence of H1N1pdm from other parts of India. We undertook this study in Pune and other five districts of Maharashtra to understand the level of cross-reactive antibodies against H1N1pdm. Studies from several countries have revealed different levels of pre-existing immunity to H1N1pdm 2009 in various age groups. Our findings on the baseline and cross-reactive antibodies to H1N1pdm in age-stratified pre-pandemic serum samples in Maharashtra are presented in this report.

A total of 560 pre-pandemic archived human serum samples were tested, which were collected during the years 2005–2008 and stored at −20°C. These samples were from the age groups 0–19, 20–39, 40–59 and ≥60 years (Fig. 1) and were from six districts of Maharashtra state namely Pune, Satara, Mumbai, Raigad, Nandurbar and Beed. As there is no baseline data from India, sample size was determined based on the cross-reactivity reported by the other studies globally. Sample size was determined by considering 5% prevalence of cross-reactive antibodies and 5% precision with 95% confidence interval. H1N1pdm Indian virus isolate A/India/Jln-NIV 9436/2009 (GenBank accession numbers—HM204573; HM241701-07) [7] and seasonal influenza A(H1N1) virus similar to A/New

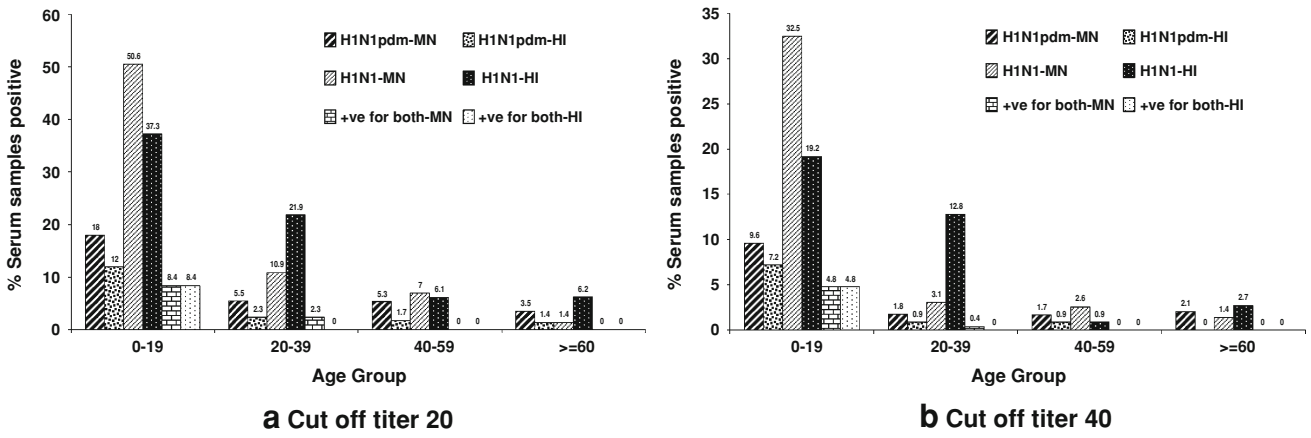

Fig. 1 Cross-reactivity to H1N1pdm in pre-pandemic sera tested by microneutralization (MN) and haemagglutination inhibition (HI) at cut off antibody titers 20 and 40

Caledonia/20/99 isolated at the National Institute of Virology (NIV), were used in the study.

All experiments were conducted in biosafety level 2 (BSL-2) laboratory with BSL-3 practices (www.cdc.gov/h1n1flu/guidelines_labworkers.htm Accessed on 4/27/2009). Microneutralization (MN) assays were performed using Madin-Darby canine kidney cells obtained from the Centers for Disease Control, Atlanta, USA. The cells were used for a maximum of 25 passages and maintained in Dulbecco's modified Eagle's medium (Gibco/BRL) containing 10% fetal bovine serum (Hyclone Laboratories Inc), 2 mM L-glutamine and the antibiotics penicillin and streptomycin. The assays were performed as per Rowe et al., 1999 [8]. Serum samples were heat-inactivated at 56°C for 30 min before using in the assay. Hemagglutination inhibition (HI) assays were performed for the detection of antibodies using 0.5% turkey red blood cells (RBCs). The initial dilution of the serum was 1:10 [12]. The NIV-SF 9436 and seasonal influenza A(H1N1) viruses were grown in 10-day-old SPF embryonated chicken eggs, inactivated using beta-propiolactone, were used as antigens in HI assay. Serum samples were treated with receptor destroying enzyme (Denka Seiken, Japan) for the removal of non-specific inhibitors, and turkey RBCs to remove non-specific agglutinins before using in the HI assay. Cut off antibody titers of 20 and 40 were used in HI and MN assays to calculate positivity [5]. Serum samples, positive for both H1N1pdm and seasonal H1N1 were considered as samples having cross-reactive antibodies.

Cross-reactivity data with both 20 and 40 cut off titers are shown in Fig. 1. Using cut off titers 20 and 40, overall cross-reactivity was 2.1 and 0.9% respectively by MN assay, and 1.2 and 0.7% respectively by HI assay. The 0–19 age group showed the highest baseline and cross-reactive antibodies. The probable reason could be the higher seropositivity observed for seasonal H1N1 virus in these children. These children may have experienced the seasonal H1N1 influenza illness in the recent past. Gurav et al., also reported significantly higher infection rates in school-aged children in Panchgani, Maharashtra [4]. Similarly, Tandale et al., have reported higher seropositivity to H1N1pdm in 15–19 years age group in schools (42.2%) and in general population (20.3%) in Pune, Maharashtra [9].

The elderly age group (≥60) showed no cross-reactivity to H1N1pdm by both MN and HI assays (Fig. 1). Seropositivity to H1N1pdm was lower in elderly population during the serosurveys in early pandemic period indicating low infection rates in Pune [9]. The incidence, severity and mortality of pandemic H1N1 were also lower in elderly than in other age groups in Pune, Maharashtra [6]. China, Singapore and New Zealand have reported lower positivity in adults and elderly [9]. Higher percentages of antibodies to H1N1pdm in pre-pandemic sera have been reported in the adult and elderly populations from Germany, Finland, USA and UK [11]. It indicates that the pre-existing immunity and cross-reactivity levels vary in populations and age groups [10]. The likely hypotheses being forwarded include the differential exposures [3], the role of cell-mediated immunity [1] and immune epitopes or genetic differences [13].

Chen H et al. suggested that vaccination against seasonal influenza might generate partial protection against the new H1N1pdm virus. The negligible uptake of seasonal influenza vaccination in India may have resulted in lower cross-reactivity levels [2, 10]. The lower seropositivity to H1N1pdm and seasonal H1N1 in this study could be the other reason for minimal cross-reactive antibodies to H1N1pdm. The higher baseline and cross-reactive antibodies in 0–19 years age group could be because of higher seropositivity to seasonal H1N1 in that age group, as suggested by Miller et.al [5]. We observed higher percent

positivity in adults (20–39 years) and elderly by HI assay as compared to MN assay with seasonal influenza. Such lesser percent positivity in virus neutralization than HI assay with both H1N1pdm and seasonal influenza has been reported [2]. The limitation of this study is that these results could not be generalized to the Indian population. In conclusion, minimal baseline and cross-reactive antibodies to H1N1pdm by both MN and HI assays were observed in pre-pandemic sera in Maharashtra, India.

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