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REPORT

Detection of Influenza virus variants circulating in Cuba in 2011-2013 and its impact on influenza prevention

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ABSTRACT

Influenza is a seasonal acute infectious disease of the upper respiratory tract, which main etiological agents are the influenza A and B viruses. In Cuba, acute respiratory infections generate in average 6 million medical visits yearly, and influenza together with pneumonia is the fourth cause of death among the general population and the first among infectious diseases. Annual vaccination is the main measure of prevention and control, its effectiveness relying on the antigenic homology of vaccine strains and those circulating. However, it is demanding to molecularly characterize the circulating strains to detect any divergence from vaccine strains. Hence, the aim of this work was to genetically characterize influenza viruses circulating in Cuba in the period 2011-2013 and define its impact on the effectiveness of vaccination. Up to 124 clinical samples were selected (nasopharyngeal exudates, bronchial washes and lung biopsies), positive to viruses Influenza A and B, at the beginning, in the middle and at the end of each epidemic period. Viral isolates were characterized by PCR and nucleotide sequencing of the HA1 subunit of the hemagglutinin gene. The phylogenetic analysis of Influenza viruses subtype A and B identified new viral variants with previously unreported antigenic variations in the country, corresponding to subtype A (H1N1pdm09), A (H3N2) and B/Yamagata lineage viruses. This last was different from the respective component of the vaccine strain administered. The results provided evidence suggesting a change in the vaccination strategy for Cuba. This work received the Annual Award of the Cuban Academy of Sciences for the year 2017.

Keywords: Influenza, seasonal viral strains, vaccine, immunization campaigns, hemagglutinin gene

RESUMEN

Detección de variantes genéticas de los virus influenza circulantes en cuba en el periodo 2011-2013 y su impacto en la prevención de la influenza. La influenza es una enfermedad estacional, infecciosa y aguda del tracto respiratorio superior, cuyos agentes etiológicos principales son los virus de la influenza A y B. En Cuba, las enfermedades respiratorias agudas generan, como promedio anual, seis millones de consultas médicas, y la influenza junto a la neumonía son la cuarta causa de muerte y la primera de tipo infeccioso. La vacunación anual es la principal medida de prevención y control, mientras que su eficacia depende de la homología antigénica de las cepas vacunales con las circulantes. Sin embargo, esto requiere evaluar cualquier divergencia de las cepas circulantes respecto a las vacunales. En este trabajo se caracterizó genéticamente a los virus de influenza circulantes en Cuba en el periodo 2011-2013 y se definió su impacto en la efectividad de la vacunación. Se seleccionaron 124 muestras positivas a virus de la influenza A y B (exudados nasofaríngeos, lavados bronquiales, biopsias pulmonares), al inicio, a mediados y al final del periodo epidemiológico. Se caracterizó a los aislamientos virales mediante PCR y secuenciación nucleotídica de la subunidad HA1 en el gen de la hemaglutinina. Mediante el análisis filogenético se identificó a nuevas variantes virales con modificaciones antigénicas no reportadas en Cuba, pertenecientes a los subtipos A (H1N1pdm09), A (H3N2) y B/Yamagata. Este último fue diferente al componente de virus de la influenza subtipo B incluido en la vacuna administrada. Los resultados permitieron proponer un cambio en la estrategia de vacunación en Cuba. Este trabajo recibió el Premio Anual de la Academia de Ciencias de Cuba para el año 2017.

Palabras clave: Influenza, cepas virales estacionales, vacuna, campañas de inmunización, gen de la hemaglutinina

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Introduction

Influenza is considered the most contagious of the acute respiratory infections, with Influenza viruses as the most common etiologic agents [1]. These viruses are characterized by their great antigenic and genetic variability, properties that allow its continuous circulation in the human population and making its behavior unpredictable. The variability of influenza viruses is due to antigenic changes on the surface proteins of the virus (hemagglutinin (H) and neuraminidase (N) proteins). When new variants devoid of pre-existent natural immunity either from previous infections or vaccination are introduced in the population, they can cause major epidemics or pandemics caused due to host susceptibility to the new established variant [1, 2].

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The World Health Organization (WHO) created 65 years ago the Global Health Influenza Surveillance [3]. It has contributed to the knowledge and understanding of the epidemiology of these viruses and annually offers the updated formulations of the vaccine against the seasonal circulating strains of the virus. Since 2010, a trivalent formulation has been produced containing representative strains of influenza A (H1pdm09) and A (H3N2) viruses, a strain of one of the known lineages for influenza B viruses (B/Victoria or B/Yamagata) and a tetravalent components composed of representative strains of the two subtypes of influenza A and the two influenza B virus lineages [4, 5].

In Cuba, influenza associated with pneumonia has remained between the fourth and fifth place among the main causes of general mortality since 1984 [6]. In the year 2000 the Cuban Ministry of Public Health approved and put into effect the National Comprehensive Program for the Prevention and Control of ARIs, aimed to reduce the mortality and morbidity associated to ARIs in the Cuban population [7]. This Program prioritizes annual anti-influenza vaccination using the trivalent vaccine for the Northern Hemisphere in risk groups established since 2000. The Cuban National Center of Influenza, located at the Pedro Kouri Institute of Tropical Medicine (IPK) is the facility recognized by WHO as Reference Laboratory responsible for laboratory surveillance of influenza viruses in the country.

In this setting, the molecular characterization of circulating Influenza virus strains is essential to select the seasonal vaccine components effective for yearly vaccination campaigns, among other applications. The advances in molecular biology have revolutionized the biological sciences and are applied to the studies of influenza viruses. They are used to elucidate the causes of frequent and often lethal epidemics due to influenza virus. The analysis of the changes in the nucleotide sequence in the genes of the Influenza viruses by nucleic acid sequencing is helpful to determine the extension and the nature of genomic variation. Worldwide, these studies are of extraordinary importance when determining the components of an anti-influenza vaccine and to monitor the emergence of genetic variants. At the same time, they help the health authorities for the selection and annual acquisition of the formulation suitable for said vaccine [4, 7]. Considering this background, the present research was aimed to genetically characterize Influenza viruses circulating in Cuba in the period 2011-2013 and further define its impact on vaccination efficacy. This work received the Annual Award of the Cuban Academy of Sciences for the year 2017.

Results

Genetic characterization of circulating Influenza virus subtype A strains in Cuba, 2011-2013

Up to 35 sequences of influenza A (H1N1) pdm09 virus were obtained. The analysis phylogenetic showed circulating strains corresponding to subtype A/H1N1pdm09, groups 3, 6A, 6B and 7. Mutations were detected in antigenic sites and in the receptor binding sites of the HA1 domain of the hemagglutinin gene. Genetic variants carried the mutations S174P, S179N, K180Q and S220T, detected for the first time in Cuba, and S202T and R222K. All the sequences analyzed, corresponding to the subtype A/H1N1pdm09 were highly homologous with the vaccine strain A/California/07/2009, which is one of the component of the vaccine applied in both hemispheres in the seasons 2011-2012 and 2012-2013, bearing an amino acid homology of 97.6 % [8].

High quality sequences were obtained for all the 47 positive influenza A (H3N2) samples, with mutations detected for the strains that circulated in the 2011-2013 period. They corresponded to vaccine strain A/Perth/16/2009 (96-99.1 % protein sequence homology), one of the components of the vaccine for the Southern Hemisphere, and with the genetic group A/Victoria/361/2011 (groups 6 and 3; 97.4 and 97.3 % protein sequence homology, respectively).

Five mutations were identified in the sequences of antigenic sites of Cuban strains circulating in the period 2011-2012, when compared to the vaccine strain: D53N and E280A (epitope C), Y94H (epitope E), I230V (epitope D) and S199A (outside the antigenic site). Notably, mutations occurring outside the antigenic sites can indirectly influence on the antigenic capacity of the H protein, as a mechanism for the evolution of new viral variants. The Cuban viral isolated influenza A (H3N2) circulating in the period 2012-2013, corresponded to group 3, specifically to subgroups 3C.2 and 3C.3 [9].

The sequences included in group 3 were found as carrying mutations N145S (epitope A) and V223I (epitope D). Specifically, subgroup 3C was defined by mutations T48I (epitope E), A198S (epitope B) and S45N, which involved the acquisition of a new N-glycosylation site, which is one of the most common forms of protein modification during the early stages of protein synthesis. This host cell process is used by viruses to modify the proteins present on their surface, thereby influencing the stability of viral glycoproteins, their antigenicity and function during the host cell entry, and ultimately on the survival and transmissibility of the virus.

In general, when Cuban sequences were compared with those of vaccine strains and reference strains, all the sequences presented four mutations in three relevant antigenic sites, which were associated to viral strains circulating in the Southern Hemisphere.

Genetic characterization of circulating Influenza virus subtype B strains in Cuba, 2011-2013

In the case of influenza B virus, 42 sequences were analyzed. All the sequences of the circulating strains studied in the period 2011-2012 were related to the vaccine strain B/Brisbane/60/2008, corresponding to the lineage B/Victoria and showing with a 99 % similarity. Particularly, a H137Q mutation was identified in the loop 120, a structure crucial for viral antigenicity, due to the strong selective pressure normally found in this site of the protein.

At the end of 2013, there was identified a re-emergence of the B/Yamagata lineage, which did not circulate since seasons prior to 2005. In fact, it replaced the lineage B/Victoria from circulation and an epidemiological warning was issued by health authorities at the Cuban National Center of Influenza

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due to the possible increase in morbidity rates. The Cuban sequences of Influenza subtype B viruses belonging to the B/Yamagata lineage that circulated in 2013 genetically diverged from vaccine strain B/Wisconsin/01/2010, this last recommended by WHO authorities for the Northern hemisphere for the analyzed period [10]. That vaccine strain was the one applied in our country. The circulating Cuban sequences also showed three mutations in antigenic sites of the H protein, related to receptor binding and the ability to induce neutralizing antibodies.

In summary, new genetic variants of Influenza virus subtype A (H1N1pdm09) virus were identified, circulating in Cuba from 2011 to 2013. The Influenza A (H3N2) viruses characterized were different from the vaccine strain used, highly homologous in protein sequence the viruses circulating in the Southern Hemisphere what impacted in the efficacy of the vaccine. Furthermore, the phylogenetic characterization of Influenza B virus strains demonstrated the re-emergence of the B/Yamagata lineage, which was absent in the vaccine applied in Cuba, and it was associated with increased morbidity and acute myositis in pediatric patients. These results provided scientific evidence supporting the recommendation made to the National Comprehensive Program for the Prevention and Control of ARIs on a change in the vaccination strategy, in order to adopt the vaccine used for the Southern hemisphere. Moreover, these results of molecular characterization were determinant for the monitoring strategies on the evolution of Influenza viruses, providing useful information to the WHO Global Surveillance Program for the selection of the adequate vaccine strains, and to timely detect the emergence of new or previous genetic variants.

Relevance of the study

New genetic variants of influenza A virus circulating in the country during the period 2011-2013 were characterized. They can potentially cause variations in the clinical and epidemiological manifestations derived from the incidence of these viruses, with impact on the population and the healthcare system. Therefore, our findings aided on the identification of potential threats and also on the early implementation of actions to reduce morbidity and mortality attributable to those circulating variants.

From the phylogenetic analyzes of the influenza A and B viruses obtained, a lower genetic similarity of circulating viruses with vaccine components was shown, something that affects the effectivity of the seasonal vaccination campaigns. This knowledge allowed to program healthcare actions to minimize its consequences, including changes in the national immunization strategies for prevention and control of the disease.

Overall, these results emphasize on the need for a continued action, to sustain a surveillance system based on molecular monitoring techniques, for the timely detection of emerging influenza viruses. This also provides information to the WHO Global Influenza Surveillance Program for the appropriate annual selection of the component strains of vaccines for both hemispheres.

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