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Hemagglutinin antigenic sites changes of pandemic influenza viruses A(H1N1)PDM09 isolated in 2009-2017 in Ukraine

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Background. The influenza A(H1N1)pdm2009 (H1N1/2009) virus, that emerged during March and early April 2009, spread rapidly among humans to develop into the first human influenza pandemic over 40 years. Hemagglutinin is known to be a major target region of neutralizing antibodies, which inhibit binding with sialic acid receptors effectively. The virus evades these antibodies primarily by accumulating amino-acid substitutions in the HA's antigenic sites. It is known that the H1 molecule has five antigenic sites: Sa, Sb, Ca1, Ca2, and Cb. The antigenic sites Sa and Sb, which contain the largest number of amino acid residues, are key in neutralizing epitopes that are adjacent to the receptor-binding pocket. The aim of our work was to analyze variability of [the] influenza viruses A(H1N1)pdm09 amino acid substitutions in antigenic sites of hemagglutinin. **Methods.** Nasal-throat swabs taken from influenza-affected patients from different regions of Ukraine, collected during 2009-2017, were used in the study. The samples were analyzed using real-time polymerase chain reaction (RT-PCR). Influenza viruses were isolated in MDCK and MDCK-SIAT cell culture. Sequencing of influenza viruses genes, isolated in our laboratory, was performed in the World Influenza Center in London using the technology of RNA-SEQ, which allows sequencing coding and noncoding mRNA. [The] Nucleotide sequences were translated into [the] amino acid sequences using MEGA 6 software. **Results.** Ukrainian isolates between 2009-2017 years clustered in the influenza genetic groups 2, 6, 7, and 8. Genetic changes were observed in each of the antigenic sites: Sa – S162T, K163Q, K163I; Sb – S185T, A186T, S190G, S190R; Ca1 – S203T, R205K, E235V, E235D, S236P; Ca2 – P137H, H138R, A141T, D222G, D222N; Cb – A73S, S74R, S74N. The greatest number was detected on the sites Ca1 and Ca2. The smallest number of amino acid substitutions was detected in the antigenic site Cb. In spite of detected mutations in antigenic sites, Ukrainian isolates retained the similarity to the vaccine strain A/California/07/09 during 2009-2017. **Conclusions.** Information about the changes in antigenic sites is very important for prediction of the next dominant strains. It is well-documented that antigenic changes of HA occasionally result in the acquisition of carbohydrate side chains on the HA molecule. Since the carbohydrate side chains in the vicinity of antigenic sites mask the neutralizing epitopes on the HA surface, the amino acid substitutions associated with the acquisition of carbohydrate chains are believed to efficiently generate antigenic variants.

Oxidative stress caused by ozone as factor for changing index of sphericity of red blood cells at hypothermic storage

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Background. Autotransfusions of ozonated blood or infusions of gaseous ozone into blood vessels and body cavities are effective in curing some pathological states, but the mechanism of ozone interaction with blood components is still unknown (Gornicki A., 2013). That is why, research of the ozone influence on the structural and functional parameters of blood components and particular erythrocytes, is an important aspect in modern science. It is known that warmed ovine erythrocytes in addition to their possible use in veterinary medicine are widely used in various studies such as immunological researches (Sparrow R.L. *et al.* 2015). Therefore, finding new and improving existing methods of storage is a perspective direction in cryobiology. For example, it has previously been shown that using the inducers of oxidative stress (particular, ozone) before freezing, increased efficiency of cryopreservation of red blood cells (Zinchenko V.D. *et al.*, 2013). It is known that parameters of mechanical resistance are widely used to assess red blood cell state under conditions of stress factors (Shpakova N.M. *et al.*, 2015). Therefore, the **aim** of this work was to study the effect of ozone treatment on erythrocyte sphericity index and mechanical stability at hypothermic storage. **Methods.** The washed human and ovine erythrocytes were ozonated (the dose of ozone was 120 μg/ml) and resuspended in Olsver solution (in 1:1) or 5 % mannitol solution on saline solution. Control and ozonized erythrocytes were stored in hypothermic conditions for 2 months. To study the distribution density of the eretrocytes sphericity index, we prepeared an experimental curve of osmotic fragility of erythrocytes. For this purpose, with the use of the low-angle scattering, the part of the preserved cells in the hypotonic NaCl solutions with concentrations of 0.9, 0.8, 0.7, 0.6, 0.5, 0.4 and 0.3 % was determined. The curves of distribution of erythrocytes according to the spherical index were built using experimental curves of osmotic fragility based on the physical and mathematical model of hypotonic hemolysis in the solution of the non-penetrating substance. **Results.** Hypothermic storage of control and ozonated ovine erythrocytes for 2 months results in a shift of the density curve of its distribution by the sphericity index to the range of higher spherical indexes, and in the medium with mannitol, this shift is more distinct. After hypothermic storage of human red blood cells, the proportion of cells with low sphericity index increases sharply in the medium with mannitol as well as in the Olsver medium. **Conclusions.** The density of distribution of erythrocytes by the index of sphericity after hypothermic storage depends on the preservation medium.