RESEARCH ARTICLE

The fast diagnosis by different methodologies of the influenza virus

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Abstract

This paper presents the causative agent of the epidemic of the influenza in our country during the season 2009-2010. It also shows the effectiveness of the molecular diagnosis for Influenza virus by the means of the real-time PCR method in comparative of classical virological ones. Also in this paper we have presented the antigenic characterization of this virus which caused the pandemic during 2009-2010 years. We have collected and processed with several diagnostic methods like imunoflorescent assay, rapid tests, isolation and molecular method 409 samples. These were collected by the means of a Sentinel Surveillance throughout Albania, (tampon nasal- pharyngeal) from people suspected of influenza in different ages. To isolate the virus of influenza we have used two methods: the method of isolation of influenza in the cell line of MDCK and also the isolation of the viral RNA by the means of the molecular method. The identifications of the isolates were carried out through the reactions of the hem agglutination inhibition and we have used also the method of Immunofluorescence and rapid test for the antigen detection of influenza virus. The results of the virus analyses are given in the relevant figures. The positive isolates were sent to the International Center of Influenza in London to be confirmed and also to have a further genetic analysis through molecular methods. From these tests performed during the season 2009-2010, it came out that our country was affected by one strain of influenza type A, AH1N1 variant A/California/2009/11. This strain circulated in the whole world causing the pandemic of 2009 and was a new variant deriving from the fusion of 4 strains of Influenza a process which occurred in pigs. These variants have affected the majority of the countries in Europe and in the world.

Key words: influenza, isolates, pandemic, virus, analyses, real-time PCR, acute infection, immunofluorescence, cell line etc.

1. Introduction

Influenza, commonly known as "the flu", is an infectious disease of birds and mammals caused by RNA viruses of the family Orthomyxoviridae, the influenza viruses. The most common symptoms are chills, fever, runny nose, sore throat, muscle pains, headache (often severe), coughing, weakness/fatigue and general discomfort. Influenza is an acute infection of the respiratory tract that usually comes in the form of an epidemic.

There are three immunologic types of the virus of influenza: Type A., type B and type C of influenza. The major antigenic differences are often seen inside of influenza type A virus and in a lower scale in type B, while type C seems like it is antigenically stable. Apart from the human subtypes, there are also the subtypes which are hosted in pigs, horses, gooses and hens. Some animal subtypes have similar features to those subtypes spread among people. (1,2,3)

Influenza comes in forms of repeated infections, with a greater incidence during the winter.

It is only the viruses of type A that cause pandemics.

The epidemics start when the virus goes through mutation, changing into a new antigenic type, and in which the antibodies in the population are found in a low level (5,6).

Flu can occasionally lead to pneumonia, either direct viral pneumonia or secondary bacterial pneumonia, even for persons who are usually very healthy. In particular it is a warning sign if a child (or presumably an adult) seems to be getting better and then relapses with a high fever as this relapse may be bacterial pneumonia. Another warning sign is if the person starts to have trouble breathing (3,5).

Typically, influenza is transmitted through the air by coughs or sneezes, creating aerosolscontaining the virus. Influenza can also be transmitted by direct contact with bird droppings or nasal secretions, or through contact with contaminated surfaces. Airborne aerosols have been thought to cause most infections, although which means of transmission is most important is not absolutely clear. Influenza viruses can be inactivated by sunlight,disinfectants and detergents. As the virus can be inactivated by soap, frequent hand washing reduces the risk of infection (2,4).

Influenza spreads around the world in seasonal epidemics, resulting in about three to five million yearly cases of severe illness

2. Materials and methods

There are proceeded out 409 analyses (tampon nasal pharyngeal) throughout Albania, from people during the the first 3 days of the onset of this disease and with the main signs of influenza: temperature 39-40 degrees, headache, dazzling state, sore muscles and wrists, red and tearful eyes, weaker pulse, lack of appetite, vomiting etc.

The nasal pharyngeal secretions taken in tampons, were immediately placed in tubes with 2 ml solution Hanks, with 10% gelatin, 1000 UI/ml penicillin, 250 Gamma/ml streptomycin and bicarbonate sodium, having the right quantity to take the solution working with, in pH 7.6

The materials were sent immediately in ice thermos in IPH virology laboratory and were placed in the freezer on -70 degrees C until the moment of the analyses.

These materials were inoculated in the MDCK cell line, a very sensitive cell line for the isolation of Influenza virus and incubated for 7 days on 35°C and each day we checked to see the appearance of cytopathic effect.

The identification of the isolated viruses was performed through the reaction of hemagglutination inhibition (RHAI), adding to the reaction, apart from the antigenic of the references, our isolates through standard antiserums of the references about the viruses of influenza.(2)

Parallel to this we performed the isolation of the viral RNA by the extraction method with spin columns. Detection of the viral RNA with target primers supported by CDC for Influenza type A, type B.

The identification of the virus types of influenza was also performed through the reaction of direct immune fluorescence and rapid tests with chromatographic principle.

3. Results and discussions

From the 409 examinations carried out through the method of the isolation of the virus in the cell line MDCK, it was made possible in the first passage to notice through the reaction of hem agglutination with cock and guinea pig erythrocytes, the existence of hem agglutination titers of the virus of influenza in 30 of them.

The further passage of these test-tube, made it possible to get a higher level of hem agglutination titers. So the first passage came with the result of hem agglutination titers 1:4 to 1:64; these titers were not enough to identify and find the type of the isolated viruses, so we performed a second passage where we found higher titers which were enough to perform the identification test of these isolates.

The identification of the isolated test-tubes in the cell line was realized through the reaction of hem agglutination inhibition.

We have performed also the Real-Time PCR method for the identification of the RNA viral in these clinical samples and we found that the pandemic virus was present in 30 of them.

With the direct imunoflorescent assay with monoclonal antibodies we found that the type A of Influenza was present in 48 of the whole number of samples that we collected.

We found the perfect match when comparing three methods, the real-time pcr, IFA and rapid test BINAX only in 14 of them.

From 409 tested with the BINAX rapid test we found positive for FluA only 17 of them.

With real-time pcr method we found positive for the pandemic virus and type A of Influenza only 30 of them.

Below we will present these results in table 1. In the table 2 are presented the results for IFA directly from the clinical samples.

Table 1. Display or three different methods for the fast identification of Influenza

Cinical samples tested	BINAX positive	Real-time PCR positive	Direct IFA positive
409	17	30	48

Table 2. The reaction of IFA for the identification of the type directly from the clinical samples.

Clinical samples tested	Serum antiviral (Reaction IF)					
	Flu A	Flu B	Para influenza 1	Para inluenza 2	Para influenza 3	Adenovirus (Group)
409	48	-	-	-	-	-

All the positive isolates went through the reaction of hemaglutination inhibition which is a necessary reaction because by this reaction we can identify not only the type of the influenza viruses, but also the variants of the same type. This method is carried out through adding to the reaction, apart from the known virus antigenic of the reference, the isolated test-tube.

All the positive isolates were tested with RHAI to identify them between types A of Influenza. They were tested against antiserum for A/California/2009/11 (H1N1) virus, A/Panama/2007/99 (H3N2) virus, B/HongKong/330/01 dhe B/Sichuan/379/99.

All the isolates gave enough titers for the A/California/2009/11 virus, which means that all of them were persons affected from the swine influenza virus.

These subtypes, as confirmed by our laboratory and by the International Center of the Influenza in London, were the virus of influenza subtype AH1N1, variant A/California.(1)

Based on the results of the analysis, we conclude that all over our country dominated the the subtype pAH1N1 (all isolated viruses).

Based on the serological and virus examinations this epidemic was moderate local, mainly in family environments; and the group age most affected is the child group, since from the four positive cases one of the cases belongs to the group age of 4 year olds, two cases of the group age of 8 year old and only one case of a 36 year old.

The special thing about the year 2009 is that for the isolation of the virus of influenza, we used parallel to the method of the isolation by the inoculation of the materials (secretions nasal pharyngeal) in the cell line MDCK we introduced for the first time the method of the real-time pcr method which is going to be now a routine method for the fast detection of the influenza virus. The use of these methods increases the possibility for the isolation of the virus of influenza and the help of the clinicians to fill the clinical aspect of the cases. The use of the two procedures to identify the isolated viruses is helpful and useful for the specific characteristics that the virus of influenza has.

The use of the method of Immune fluorescence has the advantage of carrying out the results in a shorter time than the procedures of RHAI, but on the other hand, the reaction of the Immune fluorescence referring to CDC guidelines gives a lot of false-positive results. It gives the results for the types and subtypes but not for the variants and so it cannot be used as a method of confirmation.

The use of the test of RHAI has the disadvantage of carrying out the result for a longer time, but it has a big advantage. That is, it does not only identify the subtype of the virus of influenza, but also the variants of the subtypes of the virus. (1)

Regarding the real-time pcr method it is now the revolution in the diagnostics, it is more costly but, gives results in only 3 hours and this is a way to help clinicians in managing the treatment of different cases. Following the WHO recommendations the real-time pcr method is the method of confirmation.

Many year research about the epidemics of influenza show that our country has been affected by different epidemics caused by three subtypes of the viruses of influenza; AH1N1, AH3N2 and B. But mainly our country has been affected by the two last subtypes, that is subtype AH3N2 and B, which have affected our people more often (5, 7).

Then after this, a question arises: If the defensive level against a virus that has affected people a year ago is high, why do the epidemics of the same subtype come out again not after a longer time than a year?

The fact is that the viruses of influenza, especially those of the subtypes A/H3N2, go through antigenic preventing in the form of slipping (type A and B) antigenic or in the form of antigenic breaking (type A of influenza). These antigenic changes lead into having unprotected people in the country and the spread of this epidemic among people depends on the changing scale of the antigenic features.

4. Conclusions

- In the city of Tirana and in all other districts of our country, it was observed a case of influenza epidemic caused by the type A of the virus of influenza.
- This strain was completely new, never emerged before and the fusion of 4 reassortants gave birth to it. This verified also the onset of a pandemic influenza in the whole regions of the world.
- The virus examinations carried out in our country and confirmed by the WHO-CC of Influenza in Mill Hill London revealed that the cause of the epidemic of influenza in our country was the subtype AH1N1, variant A/California. This strain affected mostly the adolescent and adult ages.
- For isolating the virus of influenza, we used the method of the inoculation in the cell line MDCK.
- For the first time we used the molecular method of the Real-Time PCR, with primers provided from CDC and WHO.

- For the identification of the isolated test-tubes, it was used the method of RHAI, considered the "gold standard" method for important researches of the antigenic differences that occur in the viruses of influenza.
- The number of isolated viruses resulted the same with Real-Time PCR so for that reason we think that this method is the fastest for the diagnosis of the Influenza virus.
- The viruses of influenza, isolated in our country during this period, differed from the viruses of influenza spread in the previous years.

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