Türkiye'de 2007 – 2008 influenza sezonunda oseltamivir dirençli influenza A (H1N1) virüslerinin H274Y mutasyonu ile saptanması

Detection of oseltamivir-resistant influenza A (H1N1) viruses with H274Y mutation during the 2007 - 2008 influenza season in Turkey

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ÖZET

Amaç: Kuzey yarım kürede 2007-2008 influenza sezonunun başlangıcından itibaren dahaönce tanımlanmış olan influenza A (H1N1) virüsünün oseltamivir direncinin arttığısaptanmıştır. Bu direncin göstergesi, nöraminidaz (NA) geninin 274. aminoasidinin (H274Y) histidinden tirozine dönüşümü ile ortaya konmaktadır. Avrupa'da influenza A (H1N1) suşları arasında oseltamivire direnç oranı %25 olarak saptanmıştır. Bu oran ülkeler arasında farklılık göstermekle birlikte Norveç'te en yüksek (%67), İspanya'da en düşük (%2) olarak tespit edilmiştir. Bu çalışmada 2007-2008 influenza sezonunda Türkiye'deki influenza A H1N1 izolatlarında oseltamivir direncini tespit etmek amaçlanmıştır.

Yöntem: Refik Saydam Hıfzıssıhha Merkezi Başkanlığı (RSHMB) Ulusal İnfluenza Merkezi'nde 2007 Kasım - 2008 Mayıs ayları arasında real-time RT-PCR ile test edilerek influenza A (H1N1) pozitif olarak bulunan toplam 73 örnek arasından rastlantısal olarak 20 örnek seçilmiştir. Oseltamivir direncini tespit etmek için influenza A H1N1 suşlarının NA gen bölgesi 274. aminoasitini hedefleyen RT-PCR işlemi, histidinden tirozine dönüşümü göstermek

ABSTRACT

Objective: In the beginning of the 2007-2008 influenza season in Northern Hemisphere, the frequency of influenza A (H1N1) viruses bearing a previously defined oseltamivir resistance increased dramatically. This is conferring to the amino acid exchange from histidine to tyrosine at position 274 (H274Y) in neuraminidase (NA) gene. The overall frequency of oseltamivir resistance in influenza A (H1N1) strains in Europe was reported as being 25%. Although it varied between countries, it was shown that the highest percentage was in Norway (67%), and the lowest was in Spain (2%). In this study, it was aimed to evaluate the oseltamivir resistance in influenza A H1N1 isolates from Turkey during the 2007-2008 influenza season.

Method: During November 2007 – May 2008, 20 samples were randomly selected between 73 influenza A (H1N1) positive samples detected with real-time RT- PCR in National Influenza Center (NIC) of Refik Saydam National Public Health Agency (RSNPHA). To detect such resistant viruses in Turkey, an RT-PCR assay was performed targeting amino acid position at 274 in NA gene of H1N1 influenza strain to investigate the presence or absence of histidine to tyrosine mutation.

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Geliş Tarihi / Received : 25.04.2011
Kabul Tarihi / Accepted : 15.02.2012

DOI ID : 10.5505/TurkHijyen.2012.23600

Çarhan A, Albayrak N, Altaş AB, Uyar Y, Özkaya E. Türkiye'de 2007 – 2008 influenza sezonunda oseltamivir dirençli influenza A (H1N1) virüslerinin H274Y mutasyonu ile saptanması. Turk Hij Den Biyol Derg, 2012; 69(1): 1-6.

için uygulanmıştır. İnfluenza virüslerinin NA geni sekanslanmış ve daha önceden tanımlanmış gen dizileri ile karşılaştırılarak direnç belirlenmiştir.

Bulgular: Çalışmamızda değerlendirilen toplam 20 influenza A (H1N1) izolatının iki (%10)'sinde oseltamivir direncinin göstergesi olan 274. (N1 numaralandırmasında 275) aminoasitte histidinden (H) tirozine (Y) değişim saptanmıştır. NA segmentinin kısmi dizi analizi sonuçları National Center for Biotechnology Information (NCBI) GenBankasında GQ369800, GQ369799, GQ369798 giriş numaraları ile sunulmuştur.

Sonuç: NA dizi analizi ile ülkemiz orta ve doğu kısımlarından gelen influenza A (H1N1) suşlarının H274Y oseltamivir direncinin değerlendirilmesiyle Türkiye'de 2008 yılında nöraminidaz inhibitörlerine direncin görülmeye başlandığını belirlenmiştir.

Anahtar Sözcükler: İnfluenza A (H1N1), oseltamivir direnci, H274Y mutasyonu, Türkiye

The NA genes of influenza, viruses were sequenced. The presence of resistance was inferred by comparison with published sequences and known resistant mutations.

Results: In our study, the histidine (H) to tyrosine (Y) substitution at position 274 (275 in N1 numbering) of the NA gene was evaluated. The change indicating resistance to oseltamivir was observed in two isolates of the total 20 influenza A (H1N1) strains (10%). The partial sequence analysis results of NA segments were submitted to the National Center for Biotechnology Information (NCBI) GenBank with accession numbers GQ369800, GQ369799, GQ369798.

Conclusion: Resistance to neuraminidase inhibitors was determined by NA gene sequencing in the isolates of A/H1N1 strains taken from central and eastern parts of Turkey by monitoring the presence of H274Y oseltamivir-resistant in 2008.

Key Words: Influenza A (H1N1), oseltamivir resistance, H274Y mutation, Turkey

INTRODUCTION

The prevalence of oseltamivir-resistant influenza viruses A (H1N1) dramatically increased worldwide at the beginning of 2007-2008 influenza season in the northern hemisphere. Recent reports indicated that by early 2009 most influenza A (H1N1) strains were resistant to oseltamivir (a neuraminidase inhibitor). These were associated with a specific "histidine-to-tyrosine mutation" at position 274 (H274Y; H275Y in N1 numbering system) in the neurominidase (NA) protein that confers high-level resistance to oseltamivir (1-5). An increased number of influenza A viruses (H1N1) with resistance to oseltamivir was first reported by World Health Organization (WHO, Norway) in January 2008 and high levels of oseltamivir resistance were determined among influenza A (H1N1) viruses in European countries and USA (4,6-8). WHO Global Influenza Surveillance Network (GISN) showed that 16% of community isolates (0%–67% by country) of influenza A viruses (H1N1) were oseltamivir resistant in the 2007 – 2008 season. The overall frequency of oseltamivir resistance in influenza A (H1N1) strains from Europe was approximately 25%. The frequency of seasonal influenza A (H1N1) viruses resistance to oseltamivir varied between countries. The highest percentage was observed in Norway (67%), followed by France (47%), while increased levels of resistance to oseltamivir were detected worldwide (2-6,8).

The aim of this study was to determine the resistance to oseltamivir (neuraminidase inhibitor) in some influenza A H1 isolates obtained during the 2007-2008 influenza season in central and eastern parts of Turkey. In order to detect such histidine to tyrosine mutation in amino acid position 274 in NA gene, RT-PCR assay was performed.

MATERIAL and METHODS

To detect the dominant circulating influenza virus types and the efficiency of seasonal vaccine, Turkish Ministry of Health established in 2005 the National İnfluenza Surveillance Program (NISP). Influenza surveillance is conducted as sentinel surveillance system by two centers within 14 provinces, Refik Saydam National Public Health Agency (RSNPHA) National Influenza Center (NIC) and Istanbul University Faculty of Medicine Virology Laboratory which are the members of international information networks. The RSNPHA NIC is responsible for nine provinces in the central and eastern parts of Turkey for Sentinel Influenza Surveillance Programme (Figure 1). The clinical specimens were collected from nine sentinel collaborators as part of routine national virologic influenza surveillance during November 2007 - May 2008 influenza season. From all parts of the country, influenza activity was measured monthly to determine the predominant strain circulating in Turkey.

In this study, total of 20 influenza viruses, confirmed as influenza A (H1) by real-time reverse transcription-PCR (RT-PCR), were selected from 73 influenza A (H1) positive specimens of 2007-2008 influenza season. The specimens that contain high titer of the viruses were selected in order to work sequencing. The NA genes of influenza viruses were sequenced and resistance was evaluated by comparison sequence of obtained GenBank and known resistant mutations.

Total RNA was extracted using QIAamp viral RNA mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. The samples were analyzed for influenza A and B as described in U.S. Centers of Disease Control (CDC)'s protocol, and positive result for influenza A virus were subtyped for H1 with subtype-specific primers provided from CDC (9). The neuraminidase inhibitor oseltamivir's resistance was tested by detecting the H274Y mutation using sequence analysis, through partial-length cycle sequencing of the coding region for the viral NA. A set of primers was designed to determine a residue of the neuraminidase protein at the amino acid position 274 and PCR was performed as previously described (10). The sequence results were subjected to BLAST analysis by using the National Center for Biotechnology Information (NCBI) GenBank.

RESULTS

In 2007-2008 influenza season, Refik Saydam National Public Health Agency National Influenza Center collected specimens from nine provinces.

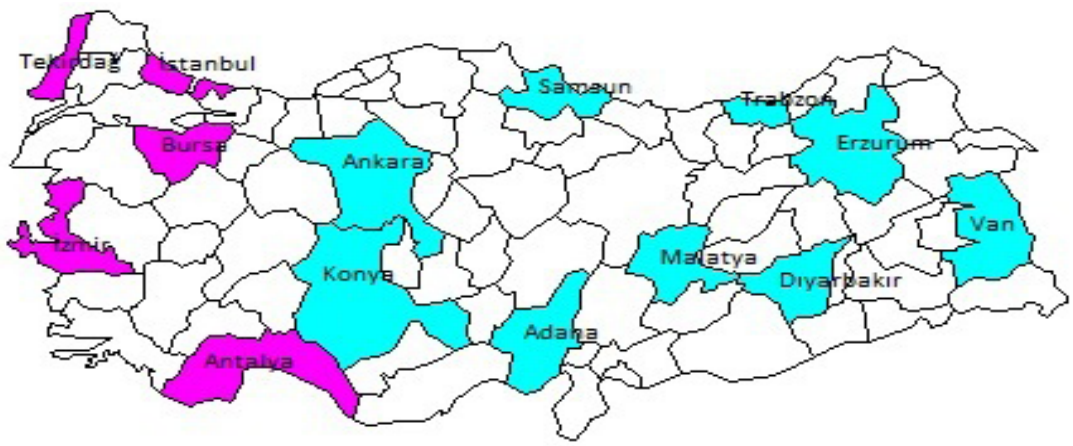

Figure 1. Sentinel Centers for Influenza Surveillance Programme *
* Purple coded provinces send samples to the labortories in İstanbul, while green coded provinces send samples to the laboratory in Ankara.

All of the influenza A positive samples were tested for inluenza A (H1N1) viruses by real-time RT-PCR. In this study, we were able to amplify sequences for 20 seasonal influenza A (H1N1) viruses. Total of 20 influenza A (H1N1) isolates were selected from 73 influenza A (H1N1) positive specimens of 2007-2008 influenza season. The NA genes of influenza viruses were sequenced and resistance was inferred by comparison with published sequences and known resistance mutations. The neuraminidase genes of all 20 seasonal influenza A (H1N1) viruses were successfully sequenced, and two of them (10%) had histidine (H) to tyrosine (Y) substitution at position 274 (275 in N1 numbering) of the NA gene which indicates resistance to oseltamivir. The partial sequence analysis results of NA segments were submitted to National Center for Biotechnology Information (NCBI) GenBank with accession numbers GQ369800, GQ369799, GQ369798. According to the topological phylogenetic analysis, phylogenetic relationship of isolates from different parts of Turkey is shown in Table 1.

DISCUSSION

In the beginning of 2007–2008 influenza season, the emergence of resistance to oseltamivir among influenza A (H1N1) viruses was reported especially in Europe and the prevalence of oseltamivir-resistant influenza A (H1N1) viruses dramatically increased worldwide. By the early 2009 influenza season,

Table 1. Phylogenetic relationship of influenza A (H1N1) isolates from different provinces in central and eastern parts of Turkey in 2007-2008 influenza season

Sequence product no	Isolate date / Isolate no	Isolate location	274 amino acid changes
4	2007/1370	Konya	Histidine to tyrosine substitution at amino acid position 274
5	2007/1558	Van	No change
6	2008/1	Ankara	
7	2008/47	Trabzon	
8	2008/117	Van	
9	2008/136	Trabzon	
10	2008/141	Trabzon	
11	2008/149	Diyarbakir	
12	2007/1530	Diyarbakir	
13	2008/11	Adana	
14	2008/30	Erzurum	
16	2008/38	Ankara	
17	2008/43	Trabzon	
18	2008/68	Malatya	
19	2008/77	Van	
20	2008/112	Malatya	
29	2008/155	Hakkari	
30	2008/156	Hakkari	
31	2008/223	Trabzon	
33	2008/261	Adana	Serine to alanine substitution at amino acid position 265 Histidine to tyrosine substitution at amino acid position 274

recent reports showed that most influenza A (H1N1) virus strains were resistant to oseltamivir (1-8, 11).

According to our previous study, total of 1157 clinical specimens were collected from nine provinces in 2007-2008 influenza season, and all of these samples were tested for influenza viruses by real-time RT-PCR. In this influenza season, 276 clinical specimens were found positive for influenza viruses. The predominant virus strain was evaluated as influenza A (55.7 %), and the dominant subtype was detected as H3 (61.2%). The 2007-2008 influenza season in Turkey was characterized by moderate clinical activity, and a dominance of influenza A (H3) (12).

The specimens were selected according to the CT level of the real-time PCR results. According to our knowledge, the resistance results were not affected by the specimen selection criteria, that we were used in this study.

In this study, we determined the resistance to neuraminidase inhibitors in influenza A H1 isolates obtained during 2007-2008 influenza season in central and eastern parts of Turkey. Results of our analysis of 2007-2008 influenza season isolates revealed that 10% of the tested isolates were resistant to oseltamivir. During November 2007-April 2008, oseltamivir resistance was reported in 6 out of 30 (20%) H1N1 isolates from western part of Turkey by Istanbul University Virology Laboratory (13). Up to June 2008, 52 countries worldwide reported similar results. The influenza A H1N1 viruses carried a specific neuraminidase mutation (H274Y) that confers high-level resistance to oseltamivir, despite of low antiviral drug usage (4, 5, 11).

In this report, we determined the presence of H274Y oseltamivir-resistant influenza A (H1N1) strains in central and eastern parts of Turkey. The results indicate that resistance to neuraminidase inhibitors (NAIs) has begun to be detected in A/H1N1 isolates in Turkey in 2007-2008 influenza season.

ACKNOWLEDGEMENTS

Authors would like to thank to Turkish Ministry of Health, Primary Health Care Directarote and Health Directorates of Provinces and physicians and also thank to the technical staff of RSNPHA Virology Reference and Research Laboratory for their kind support.

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