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ABSTRACT

Introduction: Rotaviruses are one of the most leading causes of severe gastroenteritis in children less than five years of age worldwide. This study describes prevalence of rotavirus A (RVA) genotypes in Uzbekistan during the period October-December in 2014 and 2015-2016 after introduction of rotavirus vaccination.

Methods: In total, 17546 stool specimens testing for the presence of rotavirus antigen by EIA was performed by using the Prospect Rotavirus Kit (Oxoid Ltd.UK). In total 318 EIA positive samples were randomly selected and genotyped by using one-step conventional reverse transcription polymerase chain reaction (RT-PCR). RT-PCR was performed using a Qiagen One-Step RT-PCR kit (Qiagen, Inc., Valencia, CA) and Rotavirus Genotyping Oligonucleotide Primers (CDC, Atlanta).

Results: The results showed a change in the circulating genotypes towards the prevalence of the genotype G2P[4] and a decrease in the prevalence of the genotype G1P[8].

Conclusion: The prevalence of the genotype G2P[4] is not necessarily due to vaccine escape, but can also occur in the course of the natural fluctuation of RVA genotypes, both geographically and temporally and this tendency requires further monitoring.

Keywords: Rotaviruses; Antigen; Vaccine; Enzyme immunoassay; RVA genotype

INTRODUCTION

Acute diarrheal diseases of childhood remain one of the important public health problems due to the high incidence rate, the development of severe forms, complications and high mortality. To date, the etiological structure of acute diarrheal diseases has changed significantly. Despite the fact that the etiological structure of acute diarrhea differs in different countries of the world, but still the dominant are diarrhea caused by viruses [1,2]. Rotaviruses are the most common cause of severe diarrhea in children less than 5 years of age around the world and globally 215 000 deaths associated with this infection have occurred in 2013 [3]. The genome of rotavirus group A (RVA) consists of 11 double-stranded RNA (dsRNA) segments that contains six structural (VP1, VP2, VP3, VP4, VP6, VP7) and six nonstructural (NSP1-NSP6) proteins [4]. Viral classification rotaviruses are based on the serological characteristics and sequence diversity of the VP7 and VP4 proteins determining G and P genotypes, respectively [4,5].

To date, much information has been accumulated on the genetic diversity of rotaviruses. At the present time 36 G genotypes and 51 P genotypes [6] have been identified, and the most widespread combinations of genotypes of RVA are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8] [7-9]. There are geographic differences in the prevalence of different rotavirus genotypes and the frequency of occurrence of these genotypes, which may change with time. Change in the landscape of genotypes can be observed after the introduction of vaccination. In the Republic of Uzbekistan since June 2014, vaccination against rotavirus disease (Rotarix GlaxoSmithKline Inc., Rixensart, Belgium) has been included into the national immunization calendar. To assess the effectiveness of vaccination, it is necessary to know the genetic spectrum of rotaviruses common in a given area. The choice of the pathogen

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detection methods plays an important role. In some studies RT-PCR assays targeting gene segments of the RVA (VP4, VP6, VP7) have shown a higher sensitivity in the rate of RVA detection in comparison with EIA [1,10-12].

The aim of this study was to investigate the landscape of circulating RVA genotypes in Uzbekistan during October-December in 2014 and 2015-2016 after introduction of rotavirus vaccination.

MATERIALS AND METHODS

Epidemiological surveillance and stool specimen collection

In 2014, sentinel epidemiological surveillance of acute cases of diarrhea in two hospitals of the Republic of Uzbekistan was organized on the basis of the WHO guidelines [13] for assessing the effectiveness of vaccination and preventing severe cases of rotavirus infection (city infectious diseases hospital No.4 in Tashkent city and the regional infectious diseases hospital in the Bukhara city). Epidemiological surveillance included children aged from 0 to 59 months who had a liquid stool not less than 3 times in the last 24 hours but not longer than 7 days. In sentinel surveillance included stool samples from every third child incoming to hospital with diarrhea. The stool samples were collected and held in a special container during the first 24 hours from the moment the patient entered the hospital and were stored at 2°C -8°C not more than 2 weeks before delivery to the laboratory. All samples were sent to the Reference Laboratory of the Research Institute of Virology and stored at minus 20°C. On October-December 2014 and 2015-2016, 17,546 stool samples were collected from children 5 years old hospitalized with acute gastroenteritis.

Enzyme immunoassay (EIA)

Stool samples testing for the presence of rotavirus antigen by EIA was performed by using the Prosyp Rotavirus Kit (Oxoid Ltd., UK) according to the manufacturer's instructions.

Viral RNA extraction and RVA genotyping

Viral RNA was extracted from 10% stool suspensions prepared in phosphate buffered saline (PBS) using QIAamp Viral RNA Mini kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions. One-step conventional reverse transcription polymerase chain reaction (RT-PCR) was performed using a Qiagen One-Step RT-PCR kit (Qiagen, Valencia, CA) and Rotavirus Genotyping Oligonucleotide Primers (CDC, Atlanta). For the VP7 genotyping (G1, G2, G3, G4, G9, G12) the forward primer 9con1-L and the reverse primers 9T-1, 9T-2, 9T-3P, 9T-4, 9T-9B, G12S were used and for VP4 genotyping (P[4], P[6], P[8], P[9], P[10]) the forward primer con3 and the reverse primers 1T-1D, 2T-1, 3T-1, 4T-1, 5T-1 as described previously [14,15]. After denaturing RNA (5 min at 95°C), the RT-PCR reaction was carried out in the Applied Biosystems Thermal Cycler "Veriti". The reaction conditions were: 30 min at 50°C for the RT step; 15 minutes at 95°C; 30 PCR cycles (30 seconds at 94°C, 30 seconds at 50°C and 45 seconds at 72°C); 7 minutes at 72°C; 4°C storage. Detection of amplification products was performed by gel electrophoresis in 3% of SeaKem ME Agarose (Lonza Inc, Rockland, ME) which was stained with GelRed (Biotium Inc) using Track it 100 bp DNA Ladder (Thermo Fisher Scientific) in 1X TAE buffer at 110 Volts for 90 min. VP7 (G) and VP4 (P) genotyping was carried out according the size of amplicons as described previously.

RESULTS

In total, 17,546 stool specimens were collected after introduction rotavirus vaccination on (October-December) 2014 and 2015-2016 from children<5 years of age, who were hospitalized with acute gastroenteritis in two hospitals in Uzbekistan (city infectious diseases hospital No.4 in Tashkent city and the regional infectious diseases hospital in Bukhara city). Antigen of RVA was detected by EIA in 2,547/17,546 (14.5%) samples. The results of EIA during the study period were as follows: (October-December) 2014 – 680/2466 (27.6%), 2015–899/7806 (11.5%), 2016 – 968/7274 (13.3%). Analysis of the results of our study by EIA showed a decrease in the rate of rotavirus infection [16]. In total 318 EIA positive samples were randomly selected and genotyped: 61 samples from (October-December) 2014, 185 samples from 2015 and 72 samples from 2016. The analysis of the obtained results shows that 313 (98.4%) samples were typeable for both, VP7 (G) and VP4 (P). G types were determined for 317 (99.7%), and 1 (0.3%) sample was untypeable. P types were determined for 314(98.7%), and 4 (1.3%) were untypeable. Mixed genotypes i.e. more than one genotype founded in an individual sample were determined for 4 (1.3%).

In 2014-2016, genotype G2P[4] was the most common genotype and detected in 141 (44.3%) samples. Other the most common genotypes in 2014-2016 were identified in the following order: G9P[8] – 69 (21.7%), G4P[8] – 29 (9.1%), G1P[8] – 27 (8.4%), whereas, common genotypes G3P[8] was only detected in 2014. In 2014-2016, uncommon genotype G2P[6] was detected in 5 (1.6%) samples, genotype G9P[4] accounted for 34 (10.5%) samples in 2014-2015, and prevalence other genotypes are presented in the Table 1.

Table 1: Prevalence RVA genotypes after the introduction of rotavirus vaccination

<table>
<thead>
<tr>
<th>Genotype</th>
<th>2014 (n=61)</th>
<th>2015 (n=185)</th>
<th>2016 (n=72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1P[8]</td>
<td>2 3.3</td>
<td>20 10.8</td>
<td>5 6.9</td>
</tr>
<tr>
<td>G2P[4]</td>
<td>26 42.6</td>
<td>72 38.9</td>
<td>43 59.7</td>
</tr>
<tr>
<td>G2P[6]</td>
<td>1 1.6</td>
<td>1 0.5</td>
<td>3 4.2</td>
</tr>
<tr>
<td>G2P[8]</td>
<td>0 0</td>
<td>2 1.1</td>
<td>0 0</td>
</tr>
</tbody>
</table>
According to the WHO, by the end of 2016, rotavirus vaccines have been introduced in 90 countries, and global coverage is estimated at 25%, and the efficacy and safety of these vaccines have been proven in many clinical trials [17-22].

In 2005-2009, in order to assess the burden of rotavirus infection in three countries of Central Asia (Uzbekistan, Kazakhstan, Kyrgyzstan) was conducted a prospective hospital-based surveillance for rotavirus diarrhea. The results of these studies presented an epidemiological picture of rotavirus infection in Central Asia and showed a substantial rotavirus burden which can be prevented by vaccination [23]. The results of this large prospective study also formed the basis for the decision to introduce vaccination against rotavirus infection in the Republic of Uzbekistan since June 2014, and since January 2014 was organized sentinel surveillance of rotavirus infection. In Uzbekistan, rotavirus vaccination coverage reached 95%-99% [24].

Conducting molecular genetic studies allows us to determine the circulating genotypes characteristic of a particular region. The existing geographical differences in the prevalence of different rotavirus genotypes and the frequency of occurrence of these genotypes can vary from year to year. Results of studies of circulating genotypes in Uzbekistan conducted prior to the introduction of rotavirus vaccination in the period 2005 show the prevalence of the genotype G1P[8] – 52.0%, as well as the prevalence of genotypes G2P[4]:19.0% [16]. In neighboring Central Asian Republics i.e. in Kyrgyzstan and Kazakhstan in 2007-2009, the predominant genotypes were G1P[8], G2[4], and G3P[8]. In Kyrgyzstan, the dominant genotype was G1P[8]:51.0-64.7% of cases, and in Kazakhstan G2P[4] prevailed [25]. These research results show similarity in the prevalence of circulating genotypes in the period of time before vaccination in neighboring Republics. Similarly, predominated genotype G1P[8] in 2007-2012 were reported in African Region (AFR), Western Pacific Region (WPR), South East Asia Region (SEAR), Eastern Mediterranean Region (EMR), European Region (EUR), the only exception was American Region (AMR) where genotype G2P[4] was relatively more commonly prevalence [8].

In 2014, G2P[4] was the predominant genotype-42.6%, and the occurrence of the genotype G1P[8] was 3.3%. But, in 2015, predominated two genotypes G2P[4]:38.9% and G9P[8]:32.9%, and the prevalence of G1P[8] was 10.8%. The predominant two genotypes G2P[4] and G9P[8] were also observed in post-vaccination period in Scotland where a monovalent vaccine was used [26]. Whereas in Yemen where the monovalent vaccine was also used, after introduction vaccination the predominant genotypes were G1P[8] and G9P[8] [27]. Transient increase in the frequency of genotypes G9P[8] and G12P[8] were reported in some high vaccine coverage areas within the USA [8]. In 2016, G2P[4] was also the predominant genotype – 59.7%, and genotype G1P[8] was found in 6.9% of cases. An increase in the prevalence of G2P[4] was observed every year, whereas the circulation of the genotypes G4P[8], G9P[4], G9P[8] varied. After the introduction of monovalent rotavirus vaccine, such changes in the proportion of genotypes, i.e. the decrease in the prevalence of the genotype G1P[8] and the prevalence toward the genotype G2P[4] was also observed in Saudi Arabia [28]. Genotype G2P[4] was predominant in Belgium, Austria, Colombia after introduction rotavirus vaccination [29-31]. An increase of G2P[4] RVA isolates was also observed in Brazil after introduction of Universal Mass Vaccination (UMV) using the Rotarix vaccine [32]. However, the vaccine was efficacious in reducing the absolute number of natural RV infections/disease [33]. In Brazil before vaccine introduction and in neighboring countries of S America where UMY with RV vaccine has not been introduced, increases of G2P[4] RVA isolates were observed [34].

Thus, the relatively higher frequency of G2P[4] RVA isolates is not necessarily due to vaccine escape, but can also occur in the course of the natural fluctuation of RVA genotypes, both geographically and temporally. The prevalence of G2P[4] strains reported some countries after introduction Rotarix vaccine is unusual, and this issue requires further monitoring [8]. In order to find out whether there are differences between the strains of genotype G1P[8] that circulate in the post-vaccination period, further in-depth molecular genetic studies are needed.

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<tr>
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<tbody>
<tr>
<td>G4P[8]</td>
<td>12</td>
<td>19.7</td>
<td>4</td>
</tr>
<tr>
<td>G9P[4]</td>
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<td>16.4</td>
<td>24</td>
</tr>
<tr>
<td>G9P[8]</td>
<td>6</td>
<td>9.8</td>
<td>61</td>
</tr>
<tr>
<td>Other†</td>
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<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Mixed‡</td>
<td>2</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td>Untypable §</td>
<td>1</td>
<td>1.6</td>
<td>1</td>
</tr>
</tbody>
</table>

† Other: genotypes which identified in isolated cases; ‡ Mixed: more than one genotype found in one individual sample; § Untypable: one of genotypes (G or P) untypable.

**DISCUSSION**

Our results obtained after introduction of vaccination show a change in circulating RVA genotypes towards the prevalence of the genotype G2P[4] and a decrease in the prevalence of G1P[8] (Figure 1).
CONCLUSION

Thus, analysis of the genotypes of rotaviruses will allow identifying regional genetic characteristics of the pathogen, monitoring the vaccination strategy as well as forecasting the epidemic situation and carrying out anti-epidemic measures.

ACKNOWLEDGMENTS

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REFERENCES


