Journal of Clinical Virology 61 (2014) 248–254

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Clinical epidemiological and molecular aspects of rubella outbreak with high number of neurological cases, Tunisia 2011–2012

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ARTICLE INFO

Article history:
Received 26 November 2013
Received in revised form 30 June 2014
Accepted 6 July 2014

Keywords:
Rubella
Outbreak
Mild rash
Encephalitis
CRS
Genotypes

ABSTRACT

Background: A large and unusually prolonged rubella outbreak occurred in Tunisia from April 2011 to July 2012 and was characterized by a high number of neurological cases.
Objectives: To describe the outbreak and to perform virus genotyping of isolated virus strains.
Study design: From January 2011 to December 2012, 5000 sera for serological diagnosis of acute rubella and 31 cerebrospinal fluid from patients with neurological symptoms were tested for the presence of rubella immunoglobulins G and M. Real-time PCR was performed on 49 throat swabs, 21 cerebrospinal fluid and 27 serum samples. Positive samples were assessed for virus genotyping by sequencing and the obtained sequences were compared to those previously isolated in the country.
Results: Acute rubella was confirmed in 280 patients including 15 neonates, 217 children and adults with mild rash and 48 patients with severe rubella (mainly encephalitis, $n = 39$). Most of acquired rubella cases (60.7%) were aged over 12 years with a male predominance observed in the age group 12–25 years (79%). Females belonged essentially to the unvaccinated age groups under 12 and over 25 years. Among the 23 samples tested positive by real-time PCR, six could be genotyped and clustered with either the 1E genotype, previously detected in Tunisia, or the 2B genotype which has never been isolated in Tunisia before.
Conclusions: Gender and age distributions of the patients reflect the impact of the selective rubella vaccination program adopted in Tunisia since 2005. Genotype 1E continues to circulate and genotype 2B was probably recently introduced in Tunisia.

1. Background

Rubella is an acute usually mild viral disease traditionally affecting susceptible children and young adults worldwide [1]. The rubella virus (RV) is the only member of the *Rubivirus* genus, within the Togaviridae family. It is an enveloped positive sense single-stranded RNA virus. Although RV is a single serotype, sequence analysis revealed that distinct genotypes of wild-type RV exist. An update of the nomenclature describes the upgrading of 3 provisional genotypes (1h, 1i, and 1j) established in 2007 [2] to give 12 recognized genotypes which are divided into two major phylogenetic groups: Clade 1 containing 9 genotypes (1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, and 1J) and Clade 2 including 3 genotypes (2A, 2B, and 2C); this update retains only one provisional genotype (1a) [3].

Clinically, rubella infection is characterized by a nonconfluent maculopapular rash and lymphadenopathy. However, 20–50% of infections are subclinical. Exceptionally, rubella can lead to serious complications like encephalitis and hemorrhagic manifestations. The risk of rubella infection is mainly due to the teratogenic potential of the RV which can lead to serious abnormalities in the fetus (congenital rubella syndrome, CRS) when it affects pregnant women during the first trimester of pregnancy [4].

The primary goal of rubella vaccination is to prevent congenital rubella infection, including CRS. There are two general approaches

Abbreviations: RV, Rubella virus; CRS, Congenital rubella syndrome; CSF, Cerebrospinal fluid; PCR, Polymerase chain reaction; WHO, World Health Organization.

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http://dx.doi.org/10.1016/j.jcv.2014.07.003
1386-6532/

to the use of rubella vaccine. One approach focuses exclusively on reducing CRS by immunizing adolescent girls or women of childbearing age, or both groups. The other approach is more comprehensive, focusing on interrupting transmission of rubella virus, thereby eliminating rubella as well as CRS [1]. Tunisia adopted the first strategy. The program which began in March 2005 consisted in a selective immunization of 12-year-old schoolgirls and seronegative post-partum women. In order to reduce the number of susceptibles among the female population, a 1-time campaign targeted at girls aged from 13 to 18 years (catch-up campaign) was conducted in 2005.

During the first semester of 2011, the national surveillance system represented by the national network for monitoring measles/rubella suspected cases in Tunisia (supervised by the Ministry of Health) reported 1173 cases of febrile rash which represent more than three times the number of reported cases during the same period in the previous years [5]. The alert was given by the health authorities and the laboratory investigations confirmed rubella acute infection in 66% of cases while measles was diagnosed in only 6 patients [5]. This rubella outbreak was unusually extended and lasted up to December 2012. Its beginning coincided with the massive influx of refugees of different nationalities from Libya, after the beginning of the Libyan revolution in February 2011. The epidemic was also characterized by a high number of severe forms essentially consisting in encephalitis cases.

2. Objectives

To describe the rubella outbreak and to perform genotyping and molecular characterization of RV that circulated during this period.

3. Study design

3.1. Serological analysis

From January 2011 to December 2012, 5000 sera were received for serological diagnosis of acute rubella from clinically suspected rubella cases (congenital or acquired rubella) or for prenatal screening from pregnant women. The diagnosis of acute rubella was based on the detection of specific IgM (ETI Rubek-M reverse PLUS kit, DiaSorin, Italy) and IgG (VIDAS® RUB IgG II kit, bioMérieux®, France). Acute rubella was confirmed once IgM associated or not with IgG, was detected in serum. For litigious IgM serologic result (when the ratio of the optical density obtained for the test serum to the optical density obtained for a standard (cutoff) serum is low) especially in asymptomatic pregnant women, rubella IgG avidity was measured for dating infection (Dade Behring Enzygnost, Germany). Results were interpreted according to the manufacturer's instructions.

Anti-rubella IgG and IgM were also assessed in 31 cerebrospinal fluid (CSF) samples collected in serum-rubella IgM-positive patients with neurological complications, in order to search for intrathecal secretion of anti-rubella antibodies.

3.2. Virus detection and sequencing

3.2.1. Studied samples

Virus detection was performed on a total of 97 samples, collected in 87 serologically confirmed rubella cases (with positive IgM in serum samples). These samples were taken during the first week after the onset of the disease for all patients except two of them (respectively 15 days and 30 days after the onset of the disease).

The studied samples consisted in 49 throat swabs (10 from encephalitis cases and 39 from patients with rash and fever), 21 CSF and 27 serum samples, all from encephalitis cases.

3.2.2. Virus detection and sequencing

Viral RNA was extracted from 140 μl of sample using the commercial QIAamp viral RNA Mini Kit (QIAGEN, Germany), according to the supplier's protocol. The presence of rubella virus RNA was first assessed by real-time PCR, using the Superscript™ III Platinium® One-Step Quantitative RT-PCR system from Invitrogen (France) and WHO-recommended primers and probes, provided by CDC-Atlanta-USA and amplifying a 185-nucleotide region in the rubella envelope protein 1 (E1) coding region (Kit Catalog #KT0128). The assay is presently recommended by the WHO as a standard method for qualitative detection of rubella virus genome in clinical specimens.

3.2.3. Genotyping

Samples showing positive results by real-time PCR were assessed for virus genotyping by a standard reverse transcription/PCR test, amplifying two overlapping DNA fragments in the E1 region. Reverse transcription and PCR amplifications were performed on 5 μl of RNA extract, using the One-Step RT-PCR kit from QIAGEN and WHO-recommended reverse and forward primers: RV8633 (Forward 1: 5′-AGCGACGCGGCCTGCTGGGG-3′), RV8945 (Forward 2: 5′-TGGGCCTCCCCGGTTTG-3′), RV9112 (Reverse 1: 5′-GCGCGCCTGAGAGCCTATGAC-3′) and RV9577 (Reverse 2: 5′-CGCCCAGGTCTGCCGGGTCTC-3′). PCR products were then sequenced using the BigDye terminator method according to the manufacturer's protocol (Applied Biosystems) and reaction products were analyzed in an automated sequencer (ABI 3130). Sequence data were analyzed on a 739-nucleotide-long fragment derived from the two overlapping DNA fragments, using Mega software version 5.10. Dendrograms were drawn using the maximum parsimony and the maximum likelihood methods and confirmed with 1000 bootstrap replicates. The obtained sequences were compared to the WHO reference sequences representing the different rubella virus genotypes recognized up-to-date [3]. Tunisian rubella sequences detected from previous years were also included in the dendrogram. The Tunisian rubella sequences reported herein were submitted to the GenBank international sequence database under accession numbers KF029640 to KF029643 and KF018680 to KF018689.

3.3. Storage condition

All samples were kept frozen at −70 °C until further investigation.

3.4. Statistical analysis

The binomial test was used to determine whether the proportion of subjects in each one of the two groups is different from a specific value. Difference was considered significant if p-value <0.05.

4. Results

4.1. Epidemiological and clinical study

Among the 5000 patients investigated during two years, 280 were laboratory-confirmed rubella cases including 143 in 2011 and 137 in 2012. Fig. 1 shows the monthly distribution of rubella cases diagnosed in our laboratory during 2011 and 2012 in comparison to the previous years (unpublished data). An obvious increase in the number of cases was found, especially when compared to 2010 (24 cases). Fig. 1 also shows the beginning of the rubella epidemic in April 2011 with peaks from May to July 2011 and from February to April 2012.