Jpn. J. Infect. Dis., 65, 430-432, 2012

Short Communication

Detection of Five Rash-Associated Viruses Using Multiplex Real-Time PCR during 2006–2011

Atsushi Kaida*, Hideyuki Kubo, Jun-ichiro Sekiguchi, Minori Ohyama, Kaoru Goto, Atsushi Hase, and Nobuhiro Iritani

Department of Microbiology, Osaka City Institute of Public Health and Environmental Sciences, Osaka 543-0026, Japan

(Received March 12, 2012. Accepted June 11, 2012)

SUMMARY: Many viruses have been reported to be associated with rash development. Multiplex real-time PCR was used to investigate the presence of 5 viruses associated with rashes: measles virus (MV), rubella virus (RV), human parvovirus B19 (PVB19), human herpes virus 6 (HHV-6), and HHV-7. A total of 187 clinical specimens from 169 patients with erythema were collected between January 2006 and December 2011. Virus-positive specimens were as follows: MV (n = 23), PVB19 (n = 8), RV (n = 2), HHV-6 (n = 5), HHV-7 (n = 1), MV and PVB19 (n = 1), and HHV-6 and HHV-7 (n = 1). All of the MV-positive specimens were collected in 2007 and the strains whose sequence were available (21/24, 87.5%) were of genotype D5. The results indicate that multiplex real-time PCR might be a useful screening method for detecting and differentiating rash-associated viruses in clinical specimens.

Many viruses are associated with erythema (1). It is difficult to differentiate causative viruses based on clinical symptoms alone. We investigated the presence of 5 viruses associated with rashes—measles virus [MV], rubella virus [RV], human parvovirus B19 [PVB19], human herpes virus 6 [HHV-6], and HHV-7—in clinical specimens from patients with erythema. Simultaneous detection of the viruses was performed using multiplex real-time PCR to reduce the detection time.

From January 2006 to December 2011, 187 clinical specimens (123 throat swabs, 29 serum samples, 26 nasal mucus samples, 4 whole blood samples, 2 urine samples, 2 mouth wash samples, and 1 conjunctival swab) were collected from 169 patients with erythema as part of a virus surveillance program in Osaka City, Japan (2). There were 92 men and 77 women patients aged 0 to 450 months (mean 48.0 ± 71.7 standard deviation). Peripheral blood mononuclear cells were separated from whole blood specimens using Mono-Poly Resolving Medium (DS Pharma Biomedical Co., Osaka, Japan) and were used in the subsequent tests. RNA and DNA were extracted simultaneously using a QIAamp Viral RNA Mini kit (QIAGEN Inc., Valencia, Calif., USA) (3) and cDNA was synthesized using the PrimeScript RT reagent kit (Takara Bio Inc., Shiga, Japan). Multiplex real-time PCR detection of 5 viruses (MV, RV, PVB19, HHV-6, and HHV-7) was performed using the QuantiTect Multiplex PCR kit (QIAGEN), according to the manufacturer's instructions. Sequence information for primers, TaqMan probes, and their combinations for target viruses are shown in Table 1. MV, RV, and PVB19 were detected simultaneously in a single well as Set 1, and HHV-6 and HHV-7 were detected in another single well as Set 2 (Table 1). Previously reported primers and probes were modified for the detection of RV, PVB19, HHV-6, and HHV-7 (4–6). Primers and probes for the detection of MV were designed for this study. The presence of HHV-6 and HHV-7 was only investigated in serum samples. The PCR conditions were as follows: 95°C for 15 min, followed by 45 cycles at 94°C for 60 s, and 60°C for 90 s, using a 7500 Real-Time PCR System (Life Technologies Corp., Carlsbad, Calif., USA).

The results showed that 41 specimens (21.9%) from a total of 37 patients tested positive for virus (Table 2) as follows: MV (n = 23), PVB19 (n = 8), RV (n = 2), HHV-6 (n = 5), HHV-7 (n = 1), MV and PVB19 (n = 1), and HHV-6 and HHV-7 (n = 1). Epidemiological data suggests that 3 of the 8 PVB19-positive specimens were from 2 patients (2-year- and 4-year-old sisters) in the same family. Two MV-positive specimens were determined to be from brothers (aged 2 years and 5 years). The other virus-positive specimens were all from sporadic cases. Results of multiplex real-time PCR for MV were compared to those for RT-PCR of the hemagglutinin gene (7) and showed 100% sensitivity and 98.8% specificity (data not shown). All the MV-positive cases were detected in 2007 and the strains (21/24, 87.5%) whose sequence were available were demonstrated to be of genotype D5 based on the phylogenetic analysis of the nucleoprotein gene (7,8). The Western Pacific Regional Office of the World Health Organization planned to eliminate measles by 2012 from 37 countries and areas of the Western Pacific Region, including Japan (9). To determine the accurate number of MV-positive patients, it is important to be able to differentiate MV from other rash-associated viruses. Detection of HHV-6 and HHV-7 DNA are not always associated with a rash as both viruses cause persistent infections (10). Moreover, IgM and IgG tests for HHV-6 and HHV-7 were not done. Therefore, it is unclear whether

*Corresponding author: Mailing address: Department of Microbiology, Osaka City Institute of Public Health and Environmental Sciences, 8-34 Tojo-cho, Tennoji-ku, Osaka 543-0026, Japan. Tel: +81-6-6771-3147, Fax: +81-6-6772-0676, E-mail: a-kaida@city.osaka.lg.jp

Table 1. Sequence information on primers and probes used in multiplex real-time PCR

Virus	Name	Sequence (5′–3′)	Nucleotide position	Strain (GenBank no.)	Target gene	
Set 1						
Measles virus	MV_P F	GCAGCATGGTCAGAAATATCAGAC	1906–1929	Leningrad-16 (JF727650)	phosphoprotein	designed in this study
	MV_P R	GCACCGCCTTCAGTTGATCC	2030–2011			
	MV_P Probe	(FAM)-TTGCTGAGACCCGAACTGCCTGCCT-(BHQ1)	1991–1967			
Rubella virus	Forward	CCTAHYCCCATGGAGAAACTCCT	32–54	RVi/Bismarck.ND.USA/23.08/2B (JN635296)	5′UTR + NS gene	changed fluorescence from FAM to VIC compared with original paper
	Reverse	AACATCGCGCACTTCCCA	160–143			
	Probe	(VIC)-CCGTCGGCAGTTGG-(MGB)	93–106			
Parvovirus B19	PVB19 F	CCCCGGGACCAGTTCAGG	2241–2258	F27 (AB550331)	NS	substituted from original nucleotide to mix oligonucleotide in reverse primer and probe sequences compared with original paper
	PVB19 R	CCCCTYACACCRTCCCACAC	2393–2374			
	PVB19 Probe	(Cy5)-ATCATYTGTCGGAAGCYCAGTTTCCTCCG-(BHQ3)	2262–2290			
Set 2						
HHV-6	HHV-6 U31 Forward	TTTGCAGTCATCACGATCGG	46661–46680	Z29 (AF157706)	U31	changed fluorescence and quencher from Cy5-BHQ3 to FAM-BHQ1 compared with original paper
	HHV-6 U31 Reverse	AGAGCGACAAATTGGAGGTTTC	46883–46862			
	HHV-6 U31 Probe	(FAM)-AGCCACAGCAGCCATCTACATCTGTCAA-(BHQ1)	46753–46780			
HHV-7	HHV-7 U57 Forward	CGGAAGTCACTGGAGTAATGACAA	88307–88330	RK (AF037218)	U57	changed fluorescence and quencher from JOE-BHQ1 to Cy5-BHQ3 compared with original paper
	HHV-7 U57 Reverse	ATGCTTTAAACATCCTTTCTTTCGG	88412–88390			
	HHV-7 U57 Probe	(Cy5)-CTCGCAGATTGCTTGTTGGCCATG-(BHQ3)	88332–88355			

Table 2. Results of rash-associated viruses detection in patients with erythema between 2006 and 2011

	2006	2007	2008	2009	2010	2011	Total
No. tested specimens	9 (9)[1)]	49 (41)	18 (15)	28 (26)	44 (43)	39 (35)	187 (169)
Measles virus	0	23 (20)	0	0	0	0	23 (20)
Rubella virus	1 (1)	0	0	0	1 (1)	0	2 (2)
Human herpes virus 6	0	0	1 (1)	3 (3)	1 (1)	0	5 (5)
Human herpes virus 7	0	0	0	0	0	1 (1)	1 (1)
Parvovirus B19	0	2 (2)	0	0	2 (2)	4 (3)	8 (7)
Measles virus + Parvovirus B19	0	1 (1)	0	0	0	0	1 (1)
Human herpes virus 6 + Human herpes virus 7	0	0	1 (1)	0	0	0	1 (1)
Total	1 (1)	26 (23)	2 (2)	3 (3)	4 (4)	5 (4)	41 (37)

[1)]: Number of patients is shown in parenthesis.

detection of HHV-6 and HHV-7 DNA is directly associated with rash in this study. The results of this study indicate that the multiplex real-time PCR might be a useful screening method for the detection and differentiation of rash-associated viruses in clinical specimens. However, the detection rate was low (11.0%, 18/164) when MV-positive specimens were excluded, and testing for other rash-associated viruses, such as enterovirus, should also be included. Further examination and analysis are needed to evaluate this assay system.

Acknowledgments The authors are grateful to all clinical doctors who attended the virus surveillance in Osaka City, Japan.

This work was supported in part by a Grant-in-Aid for Young Scientists (B) (21790600) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Conflict of interest None to declare.

REFERENCES

1. Weber, D., Cohen, M., Morrell, D., et al. (2010): Chapter 52. The acutely III patient with fever and rash. *In* Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases: Expert Consult Premium Edition—Enhanced Online Features and Print. p. 791–807.
2. Kaida, A., Kubo, H., Shiomi, M., et al. (2008): Evaluation of real-time RT-PCR compared with conventional RT-PCR for detecting human metapneumovirus RNA from clinical specimens. Jpn. J. Infect. Dis., 61, 461–464.
3. Neske, F., Blessing, K., Tollmann, F., et al. (2007): Real-time PCR for diagnosis of human bocavirus infections and phylogenetic analysis. J. Clin. Microbiol., 45, 2116–2122.
4. Okamoto, K., Fujii, K. and Komase, K. (2010): Development of a novel TaqMan real-time PCR assay for detecting rubella virus RNA. J. Virol. Methods, 168, 267–271.
5. Shibata, Y., Kitajima, N., Kawada, J., et al. (2005): Association of cytomegalovirus with infantile hepatitis. Microbiol. Immunol., 49, 771–777.
6. Wada, K., Kubota, N., Ito, Y., et al. (2007): Simultaneous quantification of Epstein-Barr virus, cytomegalovirus, and human herpesvirus 6 DNA in samples from transplant recipients by multiplex real-time PCR assay. J. Clin. Microbiol., 45, 1426–1432.
7. Tashiro, M., Okabe, N., Mizuta, K., et al. (2008): Laboratory Diagnostic Manual of Measles Virus Infection. 2nd ed. (in Japanese).
8. Kaida, A., Kubo, H., Sekiguchi, J., et al. (2011): Molecular epidemiology of measles virus strains detected in Osaka City, Japan. Annu. Rep. Osaka City Inst. Public Health Environ. Sci., 73, 1–6 (in Japanese).
9. World Health Organization, Western Pacific Regional Office (2007): Monitoring Measles Surveillance and Progress Towards Measles Elimination. Measles Bull., 13, 1–6.
10. Yamanishi, K., Mori, Y. and Pellett, P. (2007). Human herpesviruses 6 and 7. p. 2819–2843. *In* D. M. Knipe and P. M. Howley (ed.), Fields Virology. 5th ed. Wolters Kluwer Health, Lippincott Williams & Wilkins, Philadelphia.