BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2016
VOL. 73, NO. 2, 84–86
http://dx.doi.org/10.1080/09674845.2016.1159047

BIOMEDICAL SCIENCE IN BRIEF

Aseptic meningitis after measles–mumps–rubella (MMR) vaccination

Setareh Mamishi[a,b], Maryam Sarkardeh[b], Babak Pourakbari[a], Sepideh Keshavarz Valian[c], Farah Sabouni[b] and Shima Mahmoudi[a]

[a]Pediatric Infectious Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran; [b]Department of Pediatric Infectious Disease, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; [c]School of medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE HISTORY Received 12 January 2016; Accepted 24 February 2016

KEYWORDS Aseptic meningitis; measles-mumps rubella (MMR); vaccination

There are several most commonly used mumps vaccine strains. The Jeryl Lynn strain was licensed in the USA in 1967, and by 1992 it had been administered to approximately 135 million children and adults around the world. The Leningrad-3 mumps attenuated strain was developed in the Soviet Union and nearly 8–11million doses of this vaccine are produced annually. Over the period 1976–1987, more than 10 million doses of Leningrad–Zagreb mumps vaccine were obtained by further attenuation of Leningrad-3 mumps virus by adaptation and passage on chick embryo fibroblast cell culture.[1] The Leningrad–Zagreb attenuated mumps viral strain was developed in the 1970s in Croatia, formerly Yugoslavia, and is used in a combined live vaccine against measles–mumps–rubella.[2]

One of the major concerns in any large-scale vaccination programme is the safety of the vaccine itself as well as the occurrence of unintended events.[3] In recent years, there has been growing controversy over the safety of the MMR vaccine.[3] One of the adverse events of MMR vaccination is the occurrence of aseptic meningitis, a complication of natural mumps infection. There have been several studies to investigate the association between the mumps vaccination and aseptic meningitis.[4] We sought to study the occurrence of aseptic meningitis as an adverse event of Leningrad–Zagreb MMR vaccine based on data from Children Medical Center Hospital, an Iranian referral Hospital in Tehran, Iran during 2006–2012.

This retrospective study (2006–2012) investigated the possible aetiologic relationship between MMR vaccination and aseptic meningitis in hospitalised Iranian children. A case was defined as a child hospitalised for aseptic meningitis syndrome with symptom onset within 6 weeks after MMR vaccination. Cerebrospinal fluid (CSF) was drawn for examination and diagnosis, and was preferentially collected prior to antibiotic treatment, consistent with the patient's condition and physician treatment. Aseptic meningitis syndrome was defined by clinical symptoms and characteristic changes in the CSF. At least one of the following clinical symptoms had to be present: neck stiffness (or other signs of meningism), altered level of consciousness or neurological symptoms. The course of the illness should also be compatible with aseptic meningitis syndrome. Indicative CSF changes were pleocytosis (≥5 leukocytes/µl, predominantly lymphocytes) with glucose within the normal range or a little increase and no bacterial isolate.[5] Definitive proof of causality between aseptic meningitis and MMR vaccination requires laboratory evidence of vaccine involvement. In this study, it was demonstrated by polymerase chain reaction (PCR) analysis of mumps virus from the CSF sample; therefore, we performed a reverse transcription (RT)-nested polymerase chain reaction for the detection of mumps virus RNA in CSF.

RNA was extracted using a QIAamp MinElute Virus Spin Kit and QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Total RNA extracted from the samples was used for the synthesis of the first-strand cDNA. cDNA synthesis was performed using random hexamers and a RevertAidTM M-MuLV (moloney-murine leukaemia virus) enzyme for the RT process (Fermentas, Thermo Fisher Scientific Inc.).[6] The first round of PCR amplification was carried out in a 50-µl reaction volume containing 20 µl of the cDNA product, 5 µl of the PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 6 µl of 25 mM $MgCl_2$, 20 pmol of each primer (NP7 and NP8)[7] and 1.5 U of *Taq* DNA polymerase. This reaction mixture was amplified for 35 cycles using the following conditions: 94 °C for 60 s, 47 °C for 60 s and 72 °C for 60 s, plus a final extension step at 72 °C for 5 min. One microlitre from the first PCR

CONTACT Shima Mahmoudi sh-mahmoudi@alumnus.tums.ac.ir

Table 1. The frequency of aseptic meningitis in hospitalised children in CMC Hospital during 2006–2012.

Year	All admitted children	All aseptic meningitis cases		Aseptic meningitis after vaccination	
	*N**	*N***	%	*N****	%
2006	9859	13	0.13	2	15
2007	10550	16	0.15	2	12
2008	11186	18	0.16	11	61
2009	16719	14	0.08	4	28
2010	14235	24	0.17	9	37
2011	15473	28	0.18	13	46
2012	17306	19	0.11	8	42

*Total number of admitted children according to CMC reports.
**Total number of cases with diagnosis of aseptic meningitis.
***Total number of cases who revealed aseptic meningitis after vaccination.

Table 2. The laboratory findings of hospitalised children with aseptic meningitis in CMC Hospital during 2006–2012.

Test*	Minimum	Maximum	Median	IQR
CSF glucose (mmol/L)	1.3	4.5	2.9	0.7
CSF protein (mg/dL)	9	204	38.1	35.27
CSF WBC (cells/μL)	0	1440	411.8	363.6
CSF RBC (cells/μL)	0	30000	1295.6	5294
Blood glucose (mmol/L)	3	15.5	5.9	2.2
ESR (mm/hr)	1	101	18.8	18.3

*The laboratory findings of hospitalised children with aseptic meningitis.

was further amplified with the inner pair of primers in a 50-μl reaction mixture containing 10 mM deoxynucleoside triphosphate mixture, 20 pmol of each inner primer (5′-CAGGATCCAATTCAAGCACA-3′; virion sense primer) and Mumps-4 (5′ AATCTTGGTGTTCCATCCCC-3′; virion antisense primer), 4 μl of 25 mM $MgCl_2$, 5 μl of the PCR buffer described above and 1 U of *Taq* DNA polymerase. The second round of amplification was performed as follows: 94 °C for 60 s, 60 °C for 60 s and 72 °C for 60 s, plus an additional final extension step at 72 °C for 5 min.[7] To monitor the PCR, negative controls (CSF from patients with proven non-mumps virus-related CNS infections) and positive controls (Jeryl Lynn vaccine strain diluted with CSF from healthy subjects to obtain a final dilution 0.1 PFU/ml) were included in each run.

During 7 years, 481 cases (0.5%) with meningitis were identified from the computerised hospital records and 125 cases of them (27%) had aseptic meningitis (13 per 10000 cases). RT-nested PCR analysis confirmed the presence of the mumps virus sequence in CSF of 49 patients (49 out of 125, 39%). The prevalence of aseptic meningitis as well as this complication after MMR vaccination is presented in Table 1. The highest frequency of aseptic meningitis was found during 2011 (28 cases, 0.18%) (Table1). Data on sex, age, treatments and season are shown in Table 1. Most case-patients were male (n = 87, 70%) and 1–5 years of age (n = 63, 50%). The highest frequency of aseptic meningitis following MMR vaccination was found during summer (47%). The laboratory finding of patients with aseptic meningitis is shown in Table 2. The incidence of mumps vaccine-associated aseptic meningitis was 2/10,000 in 2006 and 2007, 1/1000 in 2008, 2/10000 in 2009, 6/10000 in 2010, 7/10,000 in 2011 and 5/10,000 in 2012. The average period of stay in hospital for all cases was about six days. Symptoms of aseptic meningitis were observed within 10–33 days of MMR immunisation with the average period of 19 days.

This study confirms the risk of aseptic meningitis with Leningrad–Zagreb mumps vaccine. Among the available strains, the rates of vaccine-associated aseptic meningitis vary. It has been reported that natural mumps infection leads to aseptic meningitis in up to 10% of patients, and this also resolves spontaneously within a week without sequelae.[8] Following reports of aseptic meningitis cases temporally associated with the administration of MMR vaccine containing Urabe mumps virus strain, Canada initiated molecular studies, which showed that the Urabe vaccine is a mixture of viruses, with wild type A and variant G.[9]

A systematic review of published articles concerning the frequency of unintended events following immunisation with MMR showed that exposure to MMR is unlikely to be associated with aseptic meningitism following MMR vaccine containing mumps Jeryl Lynn strain.[3] However, there are some studies that reported the vaccine which contained the Jeryl Lynn mumps component did not show any evidence of an association with aseptic meningitis in the 15–35-day postvaccination period.[10,11]

It has been reported that L–Zagreb is more reactogenic than Urabe and Jeryl Lynn strains.[12] However, the scarcity of published data limits comparison with these or other estimates. Horizontal transmission of the L–Zagreb vaccine produced by the Institute of Immunology of Zagreb has been reported.[13,14] It has been reported that overcrowding due to mass campaign could have provoked the outbreaks of aseptic meningitis [15] and spread of some circulating virus seems to be responsible for high number of aseptic meningitis cases.[16] In our study, the time lag between MMR vaccination and aseptic meningitis

was 10–33 which was similar to Fujinaga et al.'s study ranging from 14 to 28 days in the 35 cases of meningitis.[17] In some cases, aseptic meningitis occurs up to 42 days after administration of mumps vaccination.[18]

In our study, the highest incidence of mumps vaccine-associated meningitis was found during 2008 (1/1000 vaccine recipients). According to Cizman et al. report, the incidence of mumps vaccine-associated meningitis was 1/1000 vaccine recipients during 1979–1986 [19] following vaccination with the Leningrad-3 strain of mumps virus.[19] This retrospective observational study has some limitations. Because we had no virus isolates from cases of aseptic meningitis in this study, we cannot definitively exclude the possibility that other circulating viruses may have caused the observed epidemic patterns. Since the time lag between MMR vaccination and aseptic meningitis was 10–33, we suggest aseptic meningitis as a probable result of vaccine strain. We agree that a comparison between unvaccinated and vaccinated individuals would be a better evaluation of the frequency of adverse events, and a randomised controlled trial with vaccine strains and an unvaccinated arm would be required to establish with more certainty whether these outbreaks were due to specific vaccine batches used in the immunisation programme. However, such evaluations are not feasible or ethical.[15]

In conclusion, we found that the large number aseptic meningitis after MMR vaccination; therefore, the mumps vaccine virus might be considered as one of the causative agents of aseptic meningitis in countries such as Iran. A mistake in the production of a vaccine lot can be ruled out as an explanation for these events. In addition, these events might be a consequence of a change in population susceptibility, rather than in the properties of the vaccine virus. The risk of vaccine side effects is the leading argument of groups opposing MMR vaccination. It is therefore important to inform the public about the relative safety of the vaccine and of possible complications of mumps. This work represents an advance in biomedical science because RT-nested PCR showed the presence of mumps virus in large number of patients with aseptic meningitis after MMR vaccination (39%); therefore, the mumps vaccine virus might be considered as one of the causative agents of aseptic meningitis in countries where Leningrad–Zagreb vaccine is used.

References

[1] Smorodintsev A, Nasibov M, Jakovleva N. Experience with live rubella virus vaccine combined with live vaccines against measles and mumps. Bull. WHO. 1970;42:283.

[2] Beck M, Welsz-Malecek R, Mesko-Prejac M, et al. Mumps vaccine L-Zagreb, prepared in chick fibroblasts. I. Production and field trials. J. Biol. Stand. 1989;17:85–90.

[3] Jefferson T, Price D, Demicheli V, et al. Unintended events following immunization with MMR: a systematic review. Vaccine. 2003;21:3954–3960.

[4] Park T, Ki M, Yi SG. Statistical analysis of MMR vaccine adverse events on aseptic meningitis using the case cross-over design. Stat. Med. 2004;23:1871–1883.

[5] Rezaei M, Mamishi S, Mahmoudi S, et al. Cerebrospinal fluid ferritin in children with viral and bacterial meningitis. Br. J. Biomed. Sci. 2013;70:101–103.

[6] Pourakbari B, Mahmoudi S, Movahedi Z, et al. Viral etiology of acute lower respiratory tract infections in hospitalized young children in a children's referral hospital in Iran. Turk. J. pediatr. 2014;56:354–359.

[7] Poggio GP, Rodriguez C, Cisterna D, et al. Nested PCR for rapid detection of mumps virus in cerebrospinal fluid from patients with neurological diseases. J. clin. microbiol. 2000;38:274–278.

[8] Galazka A, Robertson S, Kraigher A. Mumps and mumps vaccine: a global review. Bull. WHO. 1999;77:3.

[9] Brown EG, Dimock K, Wright KE. The Urabe AM9 mumps vaccine is a mixture of viruses differing at amino acid 335 of the hemagglutinin-neuraminidase gene with one form associated with disease. J. Infect. Dis. 1996;174:619–622.

[10] Farrington P, Rush M, Miller E, et al. A new method for active surveillance of adverse events from diphtheria/tetanus/pertussis and measles/mumps/rubella vaccines. Lancet. 1995;345:567–569.

[11] Barlow WE, Davis RL, Glasser JW, et al. The risk of seizures after receipt of whole-cell pertussis or measles, mumps, and rubella vaccine. N. Engl. J. Med. 2001;345:656–661.

[12] da Silveira CM, Kmetzsch CI, Mohrdieck R, et al. The risk of aseptic meningitis associated with the Leningrad-Zagreb mumps vaccine strain following mass vaccination with measles-mumps-rubella vaccine, rio grande do sul, brazil, 1997. Int. J. Epidemiol. 2002;31:978–982.

[13] Atrasheuskaya A, Kulak M, Fisenko EG, et al. Horizontal transmission of the Leningrad–Zagreb mumps vaccine strain: A report of six symptomatic cases of parotitis and one case of meningitis. Vaccine. 2012;30:5324–5326.

[14] Tešović G, Poljak M, Lunar MM, et al. Horizontal transmission of the Leningrad–Zagreb mumps vaccine strain: A report of three cases. Vaccine. 2008;26:1922–1925.

[15] da Cunha SS, Rodrigues LC, Barreto ML, et al. On "No definitive evidence for L-Zagreb mumps strain associated aseptic meningitis: a review with special reference to the da Cunha study", Vaccine 2005;23:5286–8, by Kulkarni PS, et al. Vaccine. 2006;24:4261–4262.

[16] Kulkarni PS, Phadke MA, Jadhav SS, et al. No definitive evidence for L-Zagreb mumps strain associated aseptic meningitis: a review with special reference to the da Cunha study. Vaccine. 2005;23:5286–5288.

[17] Fujinaga T, Motegi Y, Tamura H, et al. A prefecture-wide survey of mumps meningitis associated with measles, mumps and rubella vaccine. Pediatr. Infect. Dis. J. 1991;10:204–208.

[18] Al-Mazrou Y, Tumsah S, Khalil M, et al. Safety evaluation of MMR Vaccine during a primary school campaign in Saudi Arabia. J. Trop. Pediatr. 2002;48:354–358.

[19] Cizman M, Mozetic M, Radescek-Rakar R, et al. Aseptic meningitis after vaccination against measles and mumps. Pediatr. Infect. Dis. J. 1989;8:302–308.