Rash after measles vaccination: laboratory analysis of cases reported in São Paulo, Brazil

Exantema após vacinação do sarampo: análise laboratorial de casos notificados em São Paulo

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Keywords
Measles vaccination. Human parvovirus B19. Human herpes virus 6. Exanthema. Rubella. Measles.

Abstract

Objective
The clinical differential diagnosis of rash due to viral infections is often difficult, and misdiagnosis is not rare, especially after the introduction of measles and rubella vaccination. A study to determine the etiological diagnosis of exanthema was carried out in a group of children after measles vaccination.
Methods
Sera collected from children with rash who received measles vaccine were reported in 1999. They were analyzed for IgM antibodies against measles virus, rubella virus, human parvovirus B19 (HPV B19) using ELISA commercial techniques, and human herpes virus 6 (HHV 6) using immunofluorescence commercial technique. Viremia for each of those viruses was tested using a polimerase chain reaction (PCR).
Results
A total of 17 cases of children with exanthema after measles immunization were reported in 1999. The children, aged 9 to 12 months (median 10 months), had a blood sample taken for laboratory analysis. The time between vaccination and the first rash signs varied from 1 to 60 days. The serological results of those 17 children suspected of measles or rubella infection showed the following etiological diagnosis: 17.6% (3 in 17) HPV B19 infection; 76.5% (13 in 17) HHV 6 infection; 5.9% (1 in 17) rash due to measles vaccine.
Conclusions
The study data indicate that infection due to HPV B19 or HHV 6 can be misdiagnosed as exanthema due to measles vaccination. Therefore, it is important to better characterize the etiology of rash in order to avoid attributing it incorrectly to measles vaccine.

Descritores
Vacinação do sarampo. Parvovírus humano B19. Herpes vírus humano 6. Exantema. Rubéola. Sarampo.

Resumo

Objetivo
O diagnóstico diferencial de doenças exantemáticas causadas por vírus é geralmente difícil, e equívocos não são raros, especialmente depois da introdução da vacina contra o sarampo e a rubéola. Um estudo laboratorial foi conduzido com o objetivo de estabelecer o diagnóstico etiológico de casos de exantema em crianças que receberam a vacina contra o sarampo.

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Métodos

Soros de casos de exantema em crianças que receberam vacina contra o sarampo, em 1999, foram analisados para anticorpos IgM contra os vírus do sarampo, da rubéola e do parvovírus humano B19 (HPV B19), por técnicas comerciais de Elisa, e o herpes vírus humano tipo 6 (HHV 6), por técnica comercial de imunofluorecência. A viremia para cada um desses vírus foi testada pela reação em cadeia da polimerase (PCR).

Resultados

Foram notificados, em 1999, 17 casos de crianças com exantema pós-vacinal. A idade das crianças era de nove a 12 meses (mediana, dez meses). Uma amostra de sangue colhida para investigação laboratorial foi obtida para cada criança. O tempo decorrido entre a aplicação da vacina e o aparecimento do exantema variou de um a 60 dias. Os resultados da sorologia das 17 crianças sugeriram o seguinte diagnóstico etiológico para o exantema: 17,6% (três em 17) infecção pelo HPV B19; 76,5% (13 em 17) infecção pelo HHV 6; 5,9% (um em 17) exantema originado pela vacina do sarampo.

Conclusão

Os resultados indicaram que a infecção pelo HPV B19 ou pelo HHV 6 pode ser diagnosticada como sarampo de origem vacinal. Portanto, é fundamental incluir esses vírus no diagnóstico laboratorial para corretamente apontar a etiologia das doenças exantemáticas, evitando, assim, atribuir à vacina do sarampo efeito colateral.

INTRODUCTION

The scarcity of clinical disease, as an expected outcome of an effective measles vaccination program, makes it more difficult for clinicians to diagnose correctly the etiology of exanthematic diseases. Also, atypical cases may occur due to other viruses and be mistaken as measles. Studies on immune responses after measles vaccination showed clinical reactions, such as fever and rash, observed 10 days after immunization. This has led to the hypothesis that an abnormally intense cellular immune response may account for vaccine-induced measles infection.[13] The classic measles is typically a childhood infection with high fever for 2 to 3 days, and a rash that coincides with the appearance of the immune response.[6,7] But many childhood viral diseases may present rash and fever, namely: rubella, human herpes 6 (HHV 6) and human parvovirus B19 (HPV B19), among others.

HHV 6 was recognized as the agent responsible for exanthema subitum/roseola, which occurs at an early age, between the ages of 6 and 18 months. It is the most common infectious exanthema during the first two years of life; 82% of all cases occur during this period, and it is characterized by a febrile episode that lasts 3 to 5 days, skin rashes developing after fever, and the rashes continuing to affect pigmentation. This leads to antibody prevalence of up to 80-100% in adults. Although low prevalence was found in some areas, further comparisons are necessary to determine the differences between various racial and ethnic groups and the geographic distribution of the virus.[15]

HPVB19 infection can occur in all age groups. The reported seroprevalence ranges from 15% to 60% in children aged 5-19 years. The level of transmission in a community seems to vary from year to year. A facial rash is characteristic of the illness, but it also occurs on the torso and extremities. Recurrence of the rash may take place several weeks following nonspecific stimuli such as change in temperature.[2]

The disease caused by the rubella virus is more important when the infection occurs in pregnant women during the first trimester, causing defects in organ formation of the fetus. Rubella virus can be congenitally acquired. It can be recovered from nasopharyngeal secretions of 50-60% infants by the age of 3 months and from about 10% by the age of 9-12 months.

Typical rubelliform rashes may be a result of enterovirus, HPVB19, and some arboviruses infections, which may cause both rubelliform rashes and arthralgia. Rubella vaccines are well tolerated, although rash, lymphadenopathy and arthropathy may occur between 10 days to 4 weeks after vaccination.[3]

Differential diagnosis among measles virus (MV), rubella virus (RV), HPV B19 and HHV 6 infections has been associated with a variety of other exanthemas, particularly if the patient shows a rash after the classic prodromic phase, which increases the difficulty of an accurate diagnosis based on clinical symptoms.[7]

The present study aims to establish the etiology of rash after measles vaccination by laboratory analysis of sera taken from cases reported in the state of São Paulo, Brazil.

METHODS

Patients and samples

There were 109 cases of measles confirmed in 1999, reported to the Health Department of the state of São Paulo as part of the Measles Surveillance Program. Seventeen cases of children with manifested symptoms after measles immunization were studied. The vaccinated children aged 9 to 12 months and presented exanthema 1 to 60 days after measles vaccination with clinical hypotheses of measles or rubella infection (Table).

Blood samples were collected and their sera were tested for IgM specific following the manufacturer instructions. Polimerase chain reaction (PCR) was used to detected measles virus (MV), rubella virus (RV), HHV 6 and HPV B19.

Measles virus (MV)

ELISA assay

Measles virus specific IgM were tested with three different kits:
1) Measles IgM capture enzyme immunoassay – CDC, Atlanta, GA;
2) Measles IgM capture enzyme immunoassay;
3) Enzygnost® anti-masern – virus/IgM.

PCR assay

The detection of MV RNA was from serum RNA isolated using protocols of RNA isolation for acid guanidinium isothiocyanate-phenol-chloroform extraction and reverse transcriptase (RT-PCR). The methods and primers were described by Rota.[11]

Rubella virus (RV)

ELISA assay

The assay used for the detection of RV antibodies was the Rubenostika IgM II micro Elisa system**.**

PCR assay

The detection of RV in sera was done from total RNA extracted using the guanidinium isothiocyanate method and reverse transcriptase-PCR (RT-PCR) followed by Nested-PCR. The methods and primers were described by Jennifer.[3]

Human herpes virus 6 (HHV 6)

Imunofluorescence assay

HHV 6 IgM immunofluorescence assay was used.

PCR assay

The detection of HHV 6 in the sera was done using phenol and chloroform treatment and alcohol precipitation. The Nested-PCR assay with primers and methods were described by Huang.[15]

Human parvovirus B19 (HPV B19)

ELISA assay

HPV B19 sera samples were tested using ELISA to

Table - Results of IgM serology and PCR for MV, RV, HPV B19 and HHV6, as well as the final diagnosis report.

Patient N	Gender	Age in months	Time between vaccination and onset the rash (days)	Time between vaccination and blood collection (days)	Time between onset of the rash and blood collection (days)	PCR and IgM				Final diagnosis
						HPV-B19	HHV6	MV	RV	
1	F	12	2	7	3	-	+	+	-	HHV6
2	F	9	1	6	5	-	+	+	-	HHV6
3	M	9	14	15	1	-	-	-	-	MV
4	F	10	2	15	13	-	+	+	-	HHV6
5	F	12	15	29	4	-	+	+	-	HHV6
6	F	9	4	11	7	-	+	+	-	HHV6
7	F	11	3	60	3	-	+	+	-	HHV6
8	F	10	3	60	2	-	+	+	-	HHV6
9	M	9	5	16	12	-	+	+	-	HHV6
10	F	10	60	9	1	-	+	+	-	HHV6
11	F	10	15	15	0	-	+	+	-	HHV6
12	M	9	-	60	-	-	+	+	-	HHV6
13	F	11	24	26	2	-	+	+	-	HHV6
14	M	10	29	29	1	+	-	-	-	HPV-B19
15	M	11	9	9	0	+	-	-	-	HPV-B19
16	F	9	21	24	3	+	-	-	-	HPV-B19
17	F	12	60	60	3	-	+	+	-	HHV6

PCR – polimerase chain reaction MV – meales virus RV – rubella virus HPV – human parvovirus
HHV – human herpes virus

detect IgM antibodies specific to parvovirus B19.

PCR assay

The detection of HPVB19 DNA in sera was done by using the method of detergent and proteinase K for DNA extraction. Oligonucleotides for Nested-PCR assay were described by Durigon.[5]

RESULTS

The following results were obtained from 17 children aged 9 and 12 months with exanthema after measles immunization.

In 17 sera were tested to detect specific IgM antibody and viremia for each virus using PCR assay. The results were positive for measles virus, and negative for rubella virus. The time of vaccination and blood collection was between 6 to 60 days.

In addition, 17.6% (3 in 17) were positive for HPV B19, and 76.5% (13 in 17) tested positive for HHV 6. The time of rash onset and blood collection was between 0 to 13 days.

Therefore, the possibility of rash due to measles vaccine could be suspected only in one child (5.9%), given that his/her serum tested negative for three virus infections: HHV 6, RV, and HPV B19. The time between vaccination and blood collection was 15 days, and the time between vaccination and rash onset rash was 1 day. In 3 children positive for HPV B19, the infection occurred in the months of July, August, and September. HHV 6 infections occurred throughout the year.

The final diagnosis for each child is shown in Table.

DISCUSSION

A clinical diagnosis of rash causing infections is not always possible and reliance has to be placed on serological evidence of infection, especially in the presence of specific immunoglobulin IgM. In patients presenting a history of rash the least to consider is the hypothesis of measles, rubella, HPVB19, and HHV 6 infection. Vaccine induced exanthema has also to be considered.[12]

The immune responses to MV are important for the virus clearance and recovery from infection, and are directly responsible for several clinical manifestations of measles.[6]

A previous study on individuals who developed detectable measles IgM after natural measles infections demonstrated that 77% developed IgM 4 to 11 days after the onset of the rash. Moreover, more than 90% of the individuals remained IgM positive for 28 days. The interpretation of positive IgM results from individuals with suspected natural measles infections becomes more difficult if these individuals have been recently vaccinated. The timing of the rise and decline of measles IgM in the first 4 to 8 weeks after primary vaccination has not been well documented.[1,8] In the present study this time was 6 to 29 days after vaccination. MV was positive in all samples. In 4 samples this time was 60 days and more studies with other samples should be performed.[8]

The recommended age of immunization has varied from 6 to 15 months of age and remains a subject of discussion. Children between 9 to 12 months of age were studied, wherein the probability of seroconversion is determined by the level of persisting MV-specific maternal antibodies in the infant. In addition, some investigators have reported decreased rates of seroconversion in children.[1]

HHV 6 is uncommon in infants younger than 6 months of age. This is suggestive of the protective effect of maternal antibodies. The disease shows a distinct seasonal variation: most cases occur during springtime.[15] Cases of virus circulating were seen all year round. Of all cases, 76.5% were HHV 6, confirming the clinical manifestation seen in 82% during the first 24 months of life.[15] For HPV B19, it was 17.6%.

HPVB19 occurs most commonly in the 5-to-14-year-old age group during winter and springtime. The 3 children with confirmed HPV B19 infection showed symptoms during this period.[2]

For HPVB19 the viremia occurs approximately one week after inoculation and lasts for 3 to 5 days. There is no evidence of persistent viremia at the time of the rash, which occurs 4 to 8 days later.[2,4] HPV B19 IgM and viremia using PCR were detected when the time between the rash onset and blood collection was 0 to 3 days.

The incubation of HHV6 infantum is 7 to 15 days. The disease does not have a prodromic phase. The onset is heralded by high fever, at least 38.8°C and often as high as 40.5°C. The fever typically lasts 3 days and may be intermittent. It is accompanied by a characteristic rash.[15] The blood-collection time was between 0 to 13 days after the rash onset and during this time IgM and viremia were detected using PCR.

Only one serum in 17 was reactive to measles

virus (specific IgM and RT-PCR) and the time between vaccination and the rash onset was 16 days. A possible alternative to reach a definitive diagnosis would be to sequence MV to verify if it was a wild measles infection or a measles vaccine virus.[14] Another possibility is that the rash is due to a second infection not tested.

The study results confirm that there is a simultaneous IgM reactivity against more than one virus in measles, HPVB19 and HHV 6 infections. In recent vaccination 3 in 17 sera were also reactive to HPVB19 specific, and 13 in 17 sera were also reactive to HHV 6.

Early in the 1980s reports showed cases of simultaneous appearance of specific IgM against rubella virus and HPVB19.[3,5] In 1997, Jensen[9] demonstrated the simultaneous existence of IgM against measles and rubella with antibodies against other viruses such as Epstein Barr virus (EBV), cytomegalovirus (CMV), and HPV B19.

The study results indicate to the need to include in the differential diagnosis HHV 6 and HPV B19, both infections with rash as a clinical manifestation. The simultaneous occurrence of these two infections is important for epidemiological surveillance, mainly because etiological misdiagnosis of rash can put into question the safety of measles vaccine and decrease the public adherence to immunization programs.

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