Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Application value of EV/EV71/CA16-SAT detection in children with hand-foot-mouth disease

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ARTICLE INFO

Original paper

Article history:
Received: April 15, 2023
Accepted: July 18, 2023
Published: November 30, 2023

Keywords:

Clinical value, Colloidal gold method, EV/EV71/CA16-SAT, Foot and mouth disease in children, Hand, RNA detection

ABSTRACT

The objective of this work was to explore the application value of a new type of fluorescent nucleic acid isothermal amplification (SAT) to detect EV/EV71/CA16-SAT in children with hand-foot-mouth disease (HFMD). For this purpose, from March 2017 to September 2019, Chengdu Children's Specialized Hospital collected throat swabs from children with clinical manifestations of hand, foot and mouth disease, and used SAT technology to screen and detect universal enterovirus (EV) nucleic acid (There were 1860 children with EV-RNA) positive. Patients who are EV-RNA positive at any time: first use the same throat swab specimen to detect EV71/CA16-RNA; secondly, collect venous blood and use the colloidal gold method to detect IgM antibodies in EV71/CA16 serum. The patients with positive EV71/CA16-RNA or EV71/CA16-IgM (or both) were repeated the above two methods 2 weeks and 4 weeks after standard treatment for review and comprehensive analysis. Results showed that 763 cases were enrolled for the first time: 59.76% were male and 40.24% were female; the age ranged from 1 month to 13 years, of which 69.06% were from 1 to 4 years old; CA16-RNA positive 56.23%, EV71-RNA positive 21.89%, CA16/EV71 -RNA were all positive in 1.57%; CA16-IgM was positive in 64.48%, EV71-IgM was positive in 54.26%, and CA16/EV71-IgM were both positive in 18.74%. After 2 weeks, 722 cases were reexamined: 26.73% were positive for CA16-RNA, 7.89% were positive for EV71-RNA, 0.28% were both positive for CA16/EV71-RNA; 66.21% were positive for CA16-IgM, 51.52% were positive for EV71-IgM, and IgM were all positive in 17.73%. Four weeks later, 489 cases were reexamined: among them, CA16-RNA positive 5.73% of which were positive for EV71 color RNA (0.005%), and 12.68% of them were all positive for EV71lym. The strategy of combining SAT technology and colloidal gold method to detect EV/EV71/CA16 nucleic acid (RNA) and serum IgM antibody in children HFMD can improve the early detection rate and accuracy of HFMD; According to the comprehensive analysis of the detection results of children with HFMD at the early stage, 2 weeks and 4 weeks of the present study, it is suggested that EV/EV71/CA16-SAT nucleic acid detection can be used to judge the prognosis, follow-up treatment, set isolation time, return students to school, and community management in children with HFMD. and prevention and control have more clinical application value.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.12.11

Introduction

Hand, foot and mouth disease (HFMD) is a common infectious disease caused by enterovirus (EV) infection in children under the age of 5. The common clinical manifestations of HFMD in children are fever with hands and feet, and oral, and buttocks rash, some cases may have no fever, but severe cases may have a series of clinical manifestations such as nervous system involvement, and respiratory and circulatory dysfunction (1). HFMD can be caused by more than 20 kinds of EV, among which coxsackievirus group 16 (CA16) and enterovirus 71 (EV71) are important pathogens of many large-scale outbreaks of HFMD around the world, and EV71 is also the absolutely dominant pathogen causing the death of severe cases of HFMD (2,3). According to data from the Chinese Center for Disease Control and Prevention in June 2018, the number of reported cases of HFMD and the number of deaths in China rank first among C infectious diseases (4). At present, there is no specific drug for HFMD treatment (2). From January 2017 to December 2019, a total of 3492 children with HFMD were diagnosed and treated in our hospital, ranking first among children's infectious diseases in our hospital. However, some children first presented with herpetic angina or other symptoms. The early symptoms of the rash on the hands and feet were not typical, and some cases had severe systemic symptoms, which were even life-threatening. In the clinic, only serum antibody is used to detect EV71/CA16 in children suspected of HFMD, because it is easy to be affected by many factors, and the test results will be false positive or false negative; in addition, the clinical symptoms of some children have been relieved and the serum antibody is still positive, which makes doctors, parents, schools and communities

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confused about whether children return to school or not. Therefore, early laboratory diagnosis of HFMD children and accurate identification of EV infection such as EV71 and CA16 are particularly important (4,5). This study will explore the application value of EV/EV71/CA16-SAT detection in children with hand-foot-mouth disease.

Materials and Methods

General information

From March 2017 to September 2019, Chengdu Children's (Specialized) Hospital selected 1860 cases of hand, foot and mouth disease in 1-3 days after diagnosis and treatment. Among them, all the cases that met the following conditions were included in the study. children with HFMD had direct or indirect contact history before onset; clinical manifestations of fever, hand, foot and mouth herpes, or in the early onset of hand, foot rash is not typical, but fever, herpes angina, or encephalitis cases; the results of pharyngeal swab nucleic acid RNA and serum IgM antibody were consistent: the general nucleic acid EV-RNA was positive, and any one of the two methods detected by EV71/CA16-RNA and EV71/CA16-IgM was positive (or the same positive). The included cases were re-examined with the same two methods at 2 and 4 weeks after treatment. Comprehensive comparative analysis and research on the three detection results. The research was carried out after being approved by the medical ethics committee of the hospital; all guardians of the children had signed the informed consent for the research. The included cases met the diagnostic criteria for HFMD in children in the "Guidelines for Diagnosis and Treatment of HFMD (2010 Edition)" (5) formulated by the Chinese Ministry of Health and the "Guidelines for Diagnosis and Treatment of HFMD (2018 Edition)" (1) prepared by the Expert Committee of the National Health Commission.

Supplementary examination

Blood analysis and C-reactive protein (CRP) routine examination were performed for each HFMD outpatient clinic. Blood biochemical tests, liver and kidney function tests, and myocardial enzymes were performed for children with severe hospitalization; if children with HFMD were complicated with severe respiratory tract infection, abnormal cardiac function, nervous system and other abnormal symptoms, relevant examinations and symptomatic treatment were given.

Exclusions

The study excluded children whose parents were uncooperative or lost to follow-up; children who were suffering from other serious diseases, diagnosed with severe immunodeficiency, severe congenital diseases, mental or neurological diseases; children with throat swabs at the first visit Children with sub-universal EV nucleic acid samples tested negative.

Selection of objects

Among 1860 pharyngeal swab samples, the SAT qualitative method was used to detect EV universal RNA nucleic acid. A total of 763 cases of universal EV-RNA positive and meeting the requirements of the subject were selected, and the same pharynx swab sample of the child was detected by CA16/EV71-SAT in time. At the same time, the serum samples were taken to determine the CA16/EV71-IgM antibody with the colloidal gold method. All cases with positive EV71/CA16-RNA and/or serum EV71/CA16-IgM antibodies in pharyngeal swabs were re-examined by the same two methods 2 and 4 weeks after onset after symptomatic treatment according to their clinical manifestations. If the clinical symptoms disappeared and the nucleic acid RNA and serum IgM antibodies were negative after 2 weeks, they would not enter the retest in the 4th week.

Real-time PCR

Fluorescence isothermal nucleic acid detection (SAT-RNA): Reagent manufacturer: Shanghai Rendu Biotechnology Co., Ltd. Kit name: Enterovirus (EV) universal nucleic acid detection kit (RNN constant temperature amplification); Enterovirus 71 (EV71) nucleic acid detection kit (RNA constant temperature amplification); Coxsackie virus A16 (CA16) Nucleic acid detection kit (RNA isothermal amplification). Testing PCR instrument: TL988 PCR instrument of Xi'an Tianlong Technology Co., Ltd. Testing unit: Huada Hospital affiliated to Sichuan Huada Institute of Genetics. The principle and method of EV-RNA extraction: The SAT-RNA qualitative method was used for detection. The nucleic acid detection of the kit was divided into nucleic acid extraction and isothermal amplification. In the nucleic acid extraction process, the nucleic acid released by viral cleavage interpretation specifically binds to the magnetic particles in the nucleic acid extraction solution by capturing the probe and obtaining pure target nucleic acid (RNA) by cleaning the magnetic particles. SAT-RNA detection was performed using mouse leukemia virus (M-MLV) reverse transcriptase, T7RNA polymerase and optimized probe technology. Reverse transcriptase is used to generate DNA copies of target nucleic acid (RNA), T7RNA polymerase generates multiple RNA copies from DNA copies, and optimized probes with fluorescent labels bind specifically to these RNA copies. Turn on the real-time fluorescent nucleic acid amplification instrument, and set the reaction program as 42oC 1min, 60 cycles, after completing a series of procedures, save the experimental results and complete the interpretation (the test kits for EV/EV71/CA16 are different but the operation process and principle are the same).

Colloidal gold method

The kit was purchased from Beijing Wantai Biopharmaceutical Co., Ltd. Kit name and use: Coxsackie virus A16 IgM antibody kit was used to detect CA16-IgM antibody in serum samples, and *in vitro* qualitative enterovirus 71 IgM antibody kit was used to detect EV71-IgM antibody in serum samples. Testing unit: Department of Laboratory Medicine, Chengdu Children's (Specialized) Hospital, Sichuan Province. Detection method: Using the principle of capture method, the anti-human IgM (anti-u chain) pre-coated on the microwell strip can be combined with the IgM antibody of the sample species. After washing the plate, add the antigen reagent and the enzyme labeling reagent for incubation. The serum samples of the children were detected with an in vitro qualitative kit, and the CA16-IgM antibody and EV71-IgM antibody were detected by immunochromatography combined with the principle of capture method.

Statistical processing

SPSS21.0 software was used for statistical analysis of the data; the enumeration data were expressed as positive percentages and clinical classification cases. The χ2 test and Fisher exact probability method were used for the comparison between EV-71 and CV-A16 throat swab RNA nucleic acids and serum antibodies. The rank sum χ2 test was used for the comparison of grade data. P<0.05 indicated that the difference was statistically significant.

Results

There were 456 male and 307 female HFMD763 children who met the requirements of the study (Table 1; Figure 1).

The age is from 1 month to 13 years old, the youngest is 1.8 months, the oldest is 12.9 years old, the median age is 2.8 years old, and 527 cases are 1 to 4 years old, accounting for 69.06% (Table 1). There were 536 cases (70.25%) in the urban area of Chengdu, and 227 cases (29.75%) in other areas (Table 2; Figure 2).

Clinical manifestations: fever (37.5-41.5%), and rash (hand, foot, mouth, pharyngeal isthmus or whole body) were found in all cases. Only fever and herpes angina were found in 9 cases (1.18%). 156 cases (20.45%) were accompanied by liver and kidney, heart function, nerve and other system damage or systemic complications, 36 cases (4.72%) were severe, and 0 cases died. Among the 763 cases of general EV-RNA included in the first diagnosis, 167 cases (21.89%) were positive for EV71-RNA, 429 cases (56.23%) for CA16-RNA, and 12 cases (1.57%) were positive for both EV71-RNA and CA16-RNA. EV71-IgM was positive in 414 (54.26%), CA16-IgM in 492 (64.48%), and both EV71-IgM and CA16-IgM were

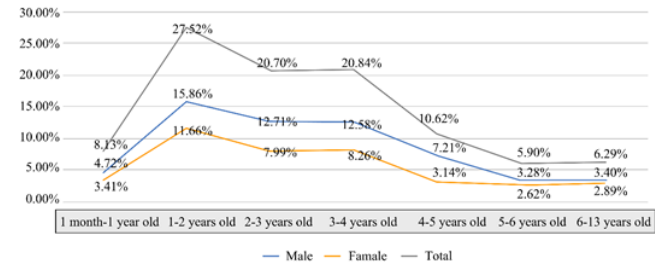

Figure 1. Distribution chart of the number of male and female cases in each age group included in the HFMD study at the first diagnosis.

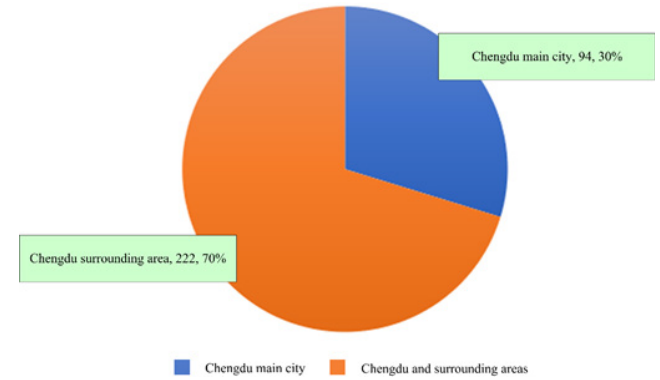

Figure 2. Distribution chart of Chengdu urban area and its surrounding areas included in HFMD study for the first diagnosis.

Table 1. The distribution table of the number of male and female cases in each age group included in the HFMD study at the first diagnosis (%).

Gender / age	Male	Rate(%)	Female	Rate (%)	Total	Total rate (%)
1 month-1 year old	36	4.72	26	3.41	62	8.13
1-2 years old	121	15.86	89	11.66	210	27.52
2-3 years old	97	12.71	61	7.99	158	20.70
3-4 years old	96	12.58	63	8.26	159	20.84
4-5 years old	55	7.21	26	3.41	81	10.62
5-6 years old	25	3.28	20	2.62	45	5.90
6-13 years old	26	3.40	22	2.89	48	6.29
Total	456	59.76	307	40.24	763	100.00

Table 2. Distribution table of Chengdu urban and surrounding areas included in the first HFMD diagnosis.

Region/Quantity	Quantity	Proportion (%)
Main urban area of Chengdu	94	30
The area around Chengdu	222	70

Table 3. Positive distribution table of EV/CA16/EV71-RNA and CA16/EV71-IgM (first diagnosis, 2 weeks, 4 weeks).

Name time	First diagnosis	%	2-week review	%	4-week review	%
EV-RNA	763	100				
CA16-RNA	429/763	56.23	193/722	26.73	28/489	5.73
EV71-RNA	167/763	21.89	57/722	7.89	4/489	0.82
Double positive	12/763	1.57	2/722	0.28	0/89	0
CA16-IgM	492/763	64.48	478/722	66.21	286/489	58.49
EV71-IgM	414/763	54.26	372/722	51.52	173/489	35.38
Double positive	143/763	18.74	128/722	17.73	62/489	12.68

positive in 143patients (18.74%) (Figures 3,4; Tables 3,4).

After 2 weeks (14-15d), 763 cases should be re-examined, and 722 cases (722/763, 94.63%) were actually re-examined: 57 cases (7.89%) were positive for EV71-RNA, 193 cases (26.73%) were positive for CA16-RNA, EV71-RNA and CA16-RNA were positive in 2 cases (0.28%); EV71-IgM was positive in 372 cases (51.52%), CA16-IgM was positive in 478 cases (66.21%), EV71-IgM and CA16-IgM were positive in both cases 128 cases were positive, accounting for 17.73%, (Figures 3,5; Tables 3,5).

After 4 weeks (28 to 29 days), the number of revisits was 523, and the actual number of revisits was 489 (489). Among them, 0 cases (0.005%) were positive for EV71-RNA, 28 cases (5.73%) were positive for CA16-RNA, and 0 cases were positive for both EV71-RNA and CA16-RNA. EV71-IgM was positive in 173cases (35.38%), CA16-IgM was positive in 286 cases (58.49%), and EV71-IgM and CA16-IgM were co-positive in 62 cases, accounting for 12.68% (Tables 3,6; Figures 3,6). According to the project plan, 400 cases were completed after 4 weeks, and 489 cases were actually completed.

Discussion

Among the 763 children with HFMD included in the first diagnosis.

(a) the sex: male: female is 1.49%. The result is significantly different from that reported in the literature (6). Considering that it is related to the physiological characteristics of boys who like to exercise, exercise more and

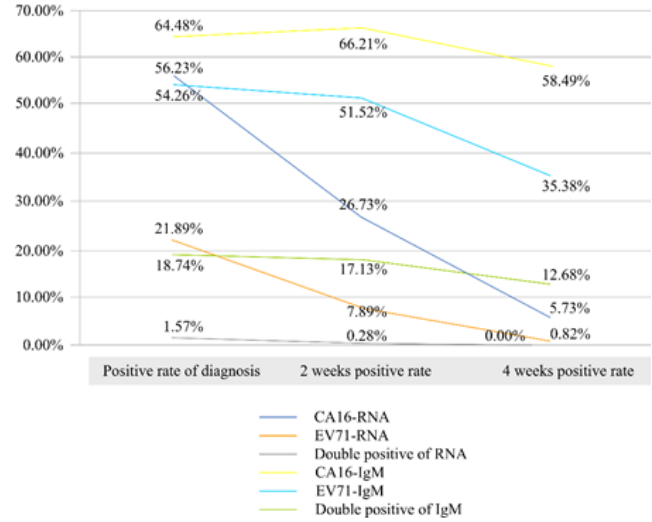

Figure 3. Curve of positive rate of EV/CA16/EV71-RNA and CA16/EV71-IgM (first diagnosis, 2 weeks, 4 weeks).

Table 4. EV/CA16/EV71-RNA and CA16/EV71-IgM positive distribution (%) between 1 month and 13 years old at first diagnosis.

Age Name	1 month-1 year old	1~2 years old	2~3 years old	3~4 years old	4~5 years old	5~6 years old	6~13 years old	Positive number
Total number of cases	62(8.13)	210(27.52)	158(20.71)	159(20.84)	81(10.62)	45(5.90)	48(6.29)	763
CA16-RNA	26(6.06)	103(24.01)	92(21.45)	87(20.28)	58(13.52)	28(6.53)	35(8.16)	429
EV71-RNA	13(7.79)	48(28.74)	41(24.55)	38(22.76)	14(8.38)	7(4.19)	6(3.59)	167
Double positive	1(8.33)	3(25.00)	4(33.34)	2(16.67)	1(8.33)	1(8.33)	0	12
CA16-IgM	33(6.71)	126(25.61)	107(21.75)	105(21.34)	58(11.79)	29(5.89)	34(6.91)	492
EV71-IgM	34(8.21)	110(26.57)	91(21.98)	90(21.74)	45(10.87)	24(5.80)	20(4.83)	414
Double positive	5(2.10)	26(6.99)	40(36.37)	36(32.16)	22(8.39)	8(9.79)	6(4.20)	143

Table 5. EV/CA16/EV71-RNA CA16/EV71-IgM positive distribution table between 2 weeks and 1 month to 13 years old (%).

Age Name	1 month-1 year old	1~2 years old	2~3 years old	3~4 years old	4~5 years old	5~6 years old	6~13 years old	Total
2 weeks follow-up	60(8.31)	195(27.01)	155(21.47)	152(21.05)	71(9.83)	43(5.96)	46(6.37)	722
CA16-RNA	8(4.15)	48(24.87)	36(18.65)	61(31.61)	27(13.99)	2(1.04)	11(5.69)	193
EV71-RNA	4(7.02)	11(19.30)	19(33.33)	16(28.07)	4(7.02)	3(5.26)	0	57
Double positive	0	1(50.00)	1(50.00)	0	0	0	0	2
CA16-IgM	32(6.70)	122(25.52)	106(22.18)	102(21.33)	54(11.30)	29(6.07)	33(6.90)	478
EV71-IgM	32(8.60)	99(26.61)	88(23.65)	76(20.43)	38(10.22)	21(5.65)	18(4.84)	372
Double positive	4(3.13)	26(20.31)	39(30.47)	26(20.31)	21(16.40)	7(5.47)	5(3.91)	128

Table 6. Positive distribution table between 4 weeks of EV/CA16/EV71-RNA and CA16/EV71-IgM between 1 month and 13 years old (%).

Age Name	1 month-1 year old	1~2 years old	2~3 years old	3~4 years old	4~5 years old	5~6 years old	6~13 years old	Total
4 weeks follow-up	53(10.84)	114(23.31)	105(21.47)	112(22.90)	50(10.22)	31(6.34)	24(4.91)	489
CA16-RNA	3(10.71)	6(21.44)	8(28.57)	6(21.43)	2(7.14)	2(7.14)	1(3.57)	28
EV71-RNA	0	2(50.00)	2(50.00)	0	0	0	0	4
Double positive	0	0	0	0	0	0	0	0
CA16-IgM	28(9.79)	55(19.23)	81(28.32)	72(25.17)	26(9.09)	10(3.50)	14(4.90)	286
EV71-IgM	25(14.45)	43(24.86)	31(17.91)	40(23.12)	21(12.14)	11(6.36)	2(1.16)	173
Double positive	0	11(17.74)	14(22.58)	20(32.26)	12(19.36)	4(6.45)	1(1.61)	62

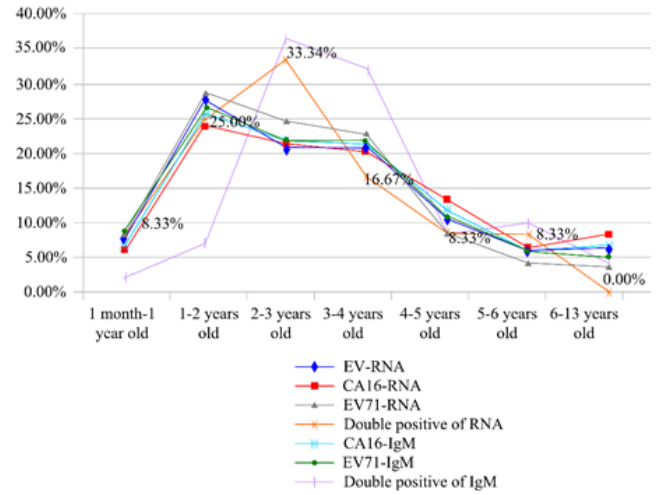

Figure 4. The positive distribution curve of EV/CA16/EV71-RNA and CA16/EV71-IgM detection between 1 month and 13 years old at the first diagnosis (%).

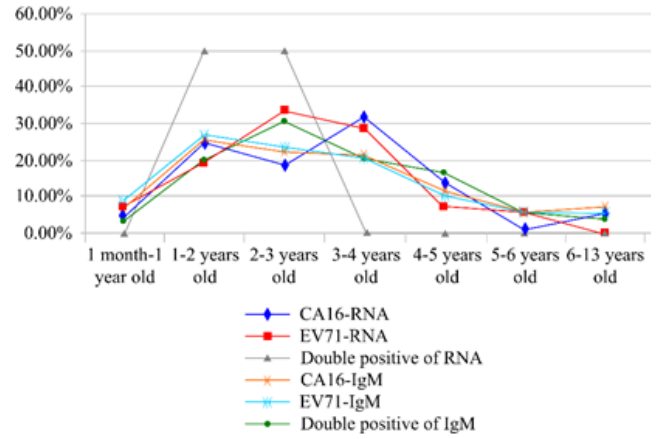

Figure 5. 2-week positive distribution curve of EV/CA16/EV71-RNA and CA16/EV71-IgM detection between 1 month and 13 years old (%).

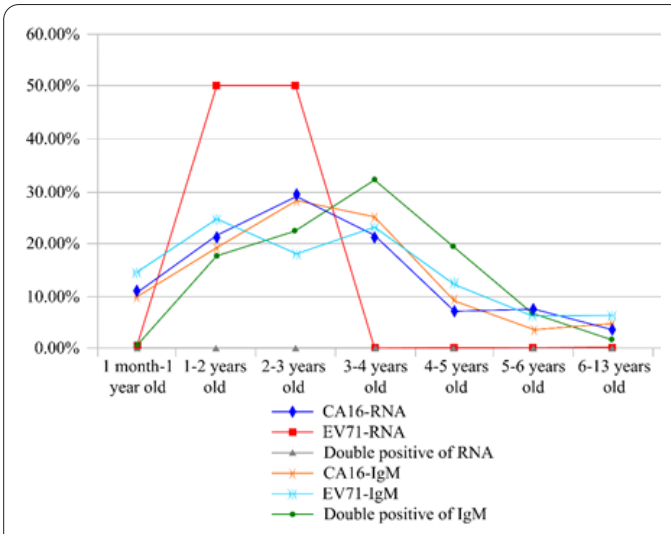

Figure 6. The positive distribution curves of EV/CA16/EV71-RNA and CA16/EV71-IgM between January and 13 years old after 4 weeks (%).

have a wider range of outdoor activities, there are more opportunities to come into contact with virus-contaminated items and suspected sources of infection (7). Therefore, attention should be paid to the management of boys.

(b) age: 1-4 years old accounted for 60.07 %, indicating that the younger the age was, the more HFMD occurred. Combined with the retest results of CA16/EV71-RNA and CA16/EV71-IgM at 2 and 4 weeks, the younger the age was, the slower the positive turn of nucleic acid RNA and serum IgM antibody was. This study shows that HFMD takes children under 5 years old as the high-risk age and high-risk group, and its conclusion is similar to the previous reports (8,9), so it is necessary to strengthen the key protection for young children.

(c) Environment: 70.25% of the cases are in the urban area of Chengdu. There are two main factors to consider: one is that our hospital is located in the city center, and it mainly treats cases from the urban area; and primary schools are relatively concentrated, which also shows that HFMD is a highly agglomerative childhood infectious disease.

(d) Percentage of EV types: There are more than 20 kinds of EV causing HFMD in children. Among the HFMD general EV-RNA positive cases, the positive rate of isolated CA16-RNA and EV71-RNA was 78.1%. It was considered that 21.9% were caused by other EV types. Ningbo, China, the EV71 was 63.7%, the EV was 24.0%, and the other EV was 12.3% (10). This suggests that there are regional differences in the proportion of EV types in children with HFMD infection.

(e) The results of nucleic acid RNA and serum antibody EV/CA16/EV71 at the first diagnosis were as follows: CA16-RNA > EV71-RNA (2.57 CA16-RNA), CA16-IgM > EV71-IgM (1.19 CA16-RNA), suggesting that CA16-RNA/CA16-IgM > EV71-RNA/EV71-IgM. CA16 was the main pathogen of HFMD in China from 1980s to 1990s, and EV71 became the main pathogen after 2007 (2,6), The main causative agent of HFMD in the 2013 Indian Andaman Islands outbreak was CA16 (11,12), It may be related to the following three points: In recent years, EV infection in children with HFMD may be mainly caused by CA16, may be regional, Since 2015, China has taken the lead in launching three EV71 vaccines in the world (13), and many children have been vaccinated, which has played a positive role in reducing HFMD in children caused by EV71, especially in reducing severe cases and deaths (14).

(f) Positive comparison of first diagnosis, 2 weeks and 4 weeks: EV71-RNA: first diagnosis (21.89%) > 2 weeks (7.89%) > 4 weeks (0.82%); cA16-RNA: first diagnosis (56.23%) > 2 weeks (26.73%) > 4 weeks (5.73%). EV71-IgM: first diagnosis (54.26%) > 2 weeks (51.52%) > 4 weeks (35.38%); cA16-IgM: first diagnosis (64.68%) 4 weeks (58.49%). The results were analyzed as follows. First, the positive and negative conversion rates of EV71/CA16-RNA were faster than those of EV71/CA16-IgM; Second, the positive and negative conversion rates of CA16-RNA and CA16-IgM were slower than those of EV71-RNA and EV71-IgM, respectively. In particular, the positive rate of CA16-IgM after 2 weeks was higher than that at the first diagnosis, and the positive rate of CA16-IgM after 4 weeks was still high. In recent years, it has been found that the age of severely ill children caused by CA16 is younger, and CA16 infection often causes recurrent infections, making the children persistently unhealed (15,16). The results of the first diagnosis in this study are basically consistent with those reported in the literature. Consistent, but no relevant research literature has been

found to use the two detection methods to re-examine larger data at 2 weeks and 4 weeks in children with HFMD.

(g) The comparison of nucleic acid RNA and serum antibody detection methods: the positive ratio of CA16-IgM to CA16-RNA was 1:0.87; the positive ratio of EV71-IgM to EV71-RNA was 2.48:1, There are differences in the results: this may be different from the usual EV71 antibody can be detected 1 day after the onset, and the detection rate can be higher than 90% after 3 days; while CA16 can be detected 4 days after the onset, and the detection rate can reach 100% after 7 days (17), however, in this study, the positive rate of CA16-IgM detected in children within 1 to 3 days of onset was 64.48%, and further research and discussion are needed.

(h) CA16/EV71 double-positive comparison detected at the first diagnosis, 2 weeks and 4 weeks: CA16-RNA and EV71-RNA: first diagnosis (1.57%) → 2 weeks (0.28%) → 4 weeks (0.00%); cA16-IgM and EV71-IgM: first diagnosis (18.74%) → 2 weeks (17.73%) → 4 weeks (12.68%). It indicated that the double positive rate of serum IgM antibody at the first diagnosis was significantly higher than the positive rate of nucleic acid RNA; the double positive rate of nucleic acid RNA turned negative after 2 weeks and 4 weeks was significantly faster than that of serum IgM antibody, considering that the homology of EV71 and CA16 gene level was 77%, and the amino acid homology is 86% (18).

80% of HFMD children have no obvious symptoms or signs but can be used as a potential source of infection to spread the virus (19). In the study, most of the clinical symptoms of common HFMD have been cured, and the symptoms of severe children have been relieved and discharged from the hospital, but some children still have detoxification after 2 or 4 weeks, suggesting that symptomatic children and asymptomatic convalescent children may become sources of infection (20,21), Causing the spread and prevalence of HFMD in families, communities, kindergartens and schools. From the comprehensive analysis of the results of this study and related literature: the detection of serum colloidal gold is suitable for the clinical auxiliary diagnosis of CA16 and EV71 HFMD in the acute infection stage. It has the advantages of simple operation, easy operation, and batch processing of specimens. During the process, there will be a high false positive rate due to the presence of antibody cross (double positive) reaction; It may also be affected by the type of antigen coated on colloidal gold nitrocellulose membrane for detection and antibody titer in vivo, and false negative results may also occur. Throat swab SAT technology for EV-RNA detection is fast and sensitive, enabling early and accurate diagnosis; high specificity, primers and molecular beacons specifically designed for target nucleic acid are RNA, and RNA is easily degraded in the environment outside the pathogen. The test results can be used as the basis for distinguishing between dead bacteria and live bacteria, effectively preventing false negative results, more accurately reflecting the results of clinical samples, and more conducive to monitoring and judging the efficacy of drugs after treatment (22,23); It has the advantages of high efficiency and strong coverage, can detect EV universal RNA and EV71/CA16-RNA at the same time, and can fully cover various common subtypes of EV pathogens of HFMD in children (9,24). The difference was statistically significant between the two detection methods in the positive detection rate of the children at the first diagnosis and after 2 weeks and 4 weeks ($P<0.05$), and the positive rate of virus serology EV71/CA16-IgM antibody detection was obvious It was higher than the positive rate of EV71/CA16-RNA detection in throat swabs, and the agreement and consistency were different. Taking into account the different courses of disease, different sample collection methods, virus replication, detoxification cycle, individual immune differences and other factors, a single detection method has some limitations and missed or false detected rate. Therefore, in the early stage of HFMD in children or when the immune function is relatively low, the combined detection of nucleic acid RNA and serum IgM antibody is helpful to improve the early detection rate (25,26) and accuracy. Two methods of detection and follow-up are helpful to evaluate the recovery of children: to prevent overtreatment to avoid secondary injury, and to eliminate the excessive worries and worries of children and their families about the disease. Therefore, the research on EV/EV71/CA16-SAT detection of HFMD in children has great clinical application value and prospects, and we should continue to expand the sample size for further research. Follow-up and re-examination of EV71/CA16-RNA and serum EV71/CA16-IgM were performed 2 weeks and 4 weeks after the recovery period of HFMD; Other types of EVs infected with HFMD other than EV71 and CA16 should also be tested as much as possible. Provide strong evidence for early accurate diagnosis and treatment of HFMD in children, determine prognosis, isolation time, and return to school on time, and provide reliable and more valuable literature for infectious disease prevention and control institutions.

Conclusion

The combination of SAT technology and the colloidal gold method for detecting EV/EV71/CA16 nucleic acid (RNA) and serum IgM antibodies can improve the early detection rate and accuracy of HFMD in children. Based on the results of tests conducted on HFMD patients at the early stage of illness, at 2 weeks, and at 4 weeks, the EV/EV71/CA16-SAT nucleic acid detection strategy is more clinically valuable for judging the prognosis, follow-up treatment, setting isolation time, resumption of studies, community management, and prevention and control of HFMD in children.

Fundings

The research is supported by the Scientific Research Project of Health and Family Planning Commission of Sichuan Province, China. Application value of EV/EV71/CA16-SAT detection in children with hand-foot-mouth disease (NO.17PJ267).

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