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A case of intracranial molluscum contagiosum virus infection diagnosed by metagenomic sequencing of cerebrospinal fluid

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Summary. – Molluscum contagiosum is a common, self-limiting infectious disease of the skin caused by molluscum contagiosum virus (MCV). The disease primarily affects children, sexually active adults, and immunocompromised individuals. Transmission of the virus occurs by direct skin contact. Therefore, the virus is usually detected in the skin and genitals of patients. However, the diagnosis of intracranial infection by the virus is difficult if the skin/mucosa lessons are atypical or absent, and the presence of the virus in the cerebrospinal fluid has not been reported. We report a very rare case of intracranial infection by molluscum contagiosum virus. A 25-year-old girl was admitted to our hospital due to severe headache but no fever or other symptoms. Upon examination, some small flesh-colored flattened papules on both arms were noticed. Blood tests showed slightly reduced levels of CD3 and CD4 T lymphocytes. Three-dimensional time-of-flight magnetic resonance angiography (3D-TOF-MRA) and head magnetic resonance (MR) were both normal. Lumbar puncture was performed, and metagenomic sequencing was applied to the spinal fluid. The unique sequences of the molluscum contagiosum virus were identified in the fluid. The patient was then diagnosed with intracranial molluscum contagiosum virus infection. No special treatment was given. The headache gradually disappeared, and the patient was discharged. During our quarterly follow-up, the girl appeared normal, and her skin lesions disappeared. However, her CD3 and CD4 T lymphocyte counts were still slightly lower than the normal level. Our case shows that the application of metagenomic sequencing to cerebrospinal fluid is a sensitive and powerful means to detect pathogens causing intracranial infection.

Keywords: Molluscum contagiosum; intracranial infection; metagenomics sequencing

Molluscum contagiosum is a benign viral papular eruption characterized by small, discrete, waxy, flesh-colored papules with central umbilication (Chen *et al.*, 2013). The disease primarily affects school-going children (grade 1–5) and sexually active or immunocompromised adults (Chen *et al.*, 2013). Lesions are frequently observed on the skin of the face, trunk and extremities in children or on the genitals of young adults and rarely on the palms, soles and mucous membranes (Chen *et al.*, 2013; Zhuang *et al.*, 2015). Therefore, the virus that causes this disease is usually found on the skin and mucosa. Intracranial infection by the virus is very rare, and the detection of the virus in cerebrospinal fluid has not been previously reported. We present a case of intracranial molluscum contagiosum infection, comprising the symptoms, diagnosis, treatment and outcome.

A 25-year-old girl presented to our outpatient department due to severe headache. The headache had started 7 days prior and gradually intensified. She presented with no fever, vomiting, cough, unconsciousness or any psychoneural symptoms. On examination, several small, flesh-colored, flattened papules on both arms were found; these papules

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Abbreviations: MCV = molluscum contagiosum virus; NGS = next generation sequencing

Table 1. The counts of T lymphocyte subsets were less than the lower limits of normal in the patient, while the cerebrospinal fluid appeared normal

Items	Result		Normal range
T lymphocyte subsets			
Absolute count of CD3+ cells	745	cells/μl	955-2600
CD3+T cells/lymphocytes	70.27%		50-84
Absolute count of CD3+CD4+ cells	337	cells/μl	550-1440
CD3+CD4+T cells/lymphocytes	31.83		27-51
Absolute count of CD3+CD8+ cells	357	cells/μl	320-1250
CD3+CD8+T cells/lymphocytes	33.73%		15-41
CD3+CD4+/CD3+CD8+	0.94		0.71-2.78
Cerebrospinal fluid			
Color	colorless		
Clarity	hyaline		
Clot	no		
pH value	8.0		
Proportion	1.020		
Pandy test	positive		
Erythrocyte microscopic examination	0/HP		
Leucocyte microscopic examination	0/HP		
Chloridion	123.00	mmol/l	
Glucose	3.80	mmol/l	
LDH	11.00	U/l	
Albumin	0.415	g/l	

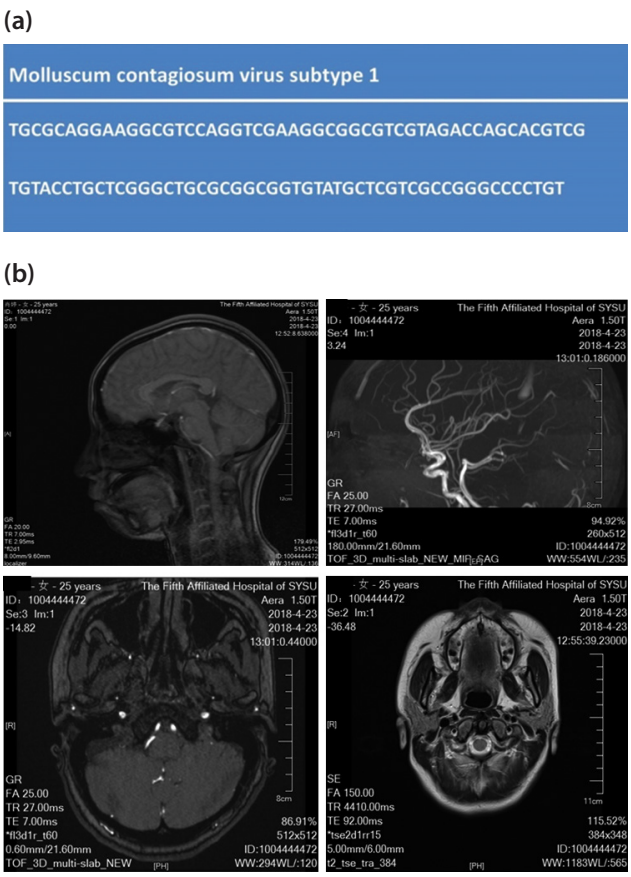

Fig. 1

(a) The gene sequence of MCV in the cerebrospinal fluid. (b) Three-dimensional time-of-flight magnetic resonance angiography (3D-TOF-MRA) and head magnetic resonance (MR) were performed on the patient and were both normal.

appeared at approximately the same time as her headache. The lesions were not pruritic or painful. The patient was a virgin and denied recent trauma or skin contact with persons who had similar skin lesions.

The patient was then admitted to our Infectious Diseases Department. Three-dimensional time-of-flight magnetic resonance angiography (3D-TOF-MRA) and head magnetic resonance (MR) imaging were performed. The results of both examinations were normal. However, blood tests showed reduced CD3 and CD4 T lymphocyte counts (Table 1). We therefore performed a lumbar puncture. The physical and biochemical analyses showed a positive Pandy test (i.e. proteins were present in the cerebrospinal fluid) and otherwise normal results (Table 1)

In addition to the physical and biochemical examinations, metagenomic sequencing was applied to the cerebrospinal fluid to detect the presence of pathogens. DNA was extracted from the fluid using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions, and next-generation sequencing (NGS) was performed on the BGISEQ-50 platform. Low-quality reads and reads from human hosts were excluded for further analysis. Finally, the remaining reads were classified by simultaneous alignment to a microbial genome database (in house) consisting of viruses, bacteria, fungi, and parasites. Two sequence reads were uniquely mapped to the molluscum contagiosum virus genome (Fig. 1a).

We then diagnosed the patient with an intracranial molluscum contagiosum virus infection. No special treatment was prescribed, but treatments to alleviate the symptoms were given. The headache disappeared 10 days after admission, and the skin lesions recovered 15 days after admission. The patient was then discharged from the hospital. Our quarterly follow-up showed that the girl remained healthy, except that her CD3 and CD4 T lymphocytes were still slightly lower than the normal level.

Molluscum contagiosum is a common benign infectious skin disease caused by the molluscum contagiosum virus, a member of the linear double-stranded DNA poxvirus family (Chen *et al*., 2013). The skin lesions are characterized by small, discrete, waxy, flesh-colored papules, an average of 3–5 mm in diameter and are usually solitary or in clusters

(Bandino *et al*., 2011). When the lesions are squeezed or traumatized, a white curdy-type material can be extruded (Chen *et al*., 2013). The skin lesions are usually asymptomatic, although a minority of patients have complained about tenderness or pruritus (Bandino *et al*., 2011). The most commonly affected skin sites are the trunk, arms, and face in children and the genitals in sexually active adults (Chen *et al*., 2013). The disease can develop at any age but predominantly affects three distinct patient populations: children, sexually active adults, and immunocompromised individuals. In immunocompetent individuals, the number of molluscum contagiosum lesions is generally less than 20 (Chen *et al*., 2013). However, immunocompromised patients with HIV infection or those with atopic dermatitis may develop widespread, persistent and large lesions of molluscum contagiosum (Ianhez *et al*., 2011). Our patient was sexually inactive but had reduced CD3 and CD4 lymphocytes, indicating that she was immunocompromised, although she had no symptoms or signs suggestive of any other infection. The virus is transmitted by direct contact with an infected person or contaminated objects; evidence also indicates occasional transmission via fomites such as bath sponges and towels. Our patient could not recall having contact with any infected people, which is consistent with the usual difficulty in tracing the origins of the infection in immunocompromised individuals.

The clinical diagnosis of molluscum contagiosum is based on typical skin lesions and umbilicus papules. Histological and microbiome examinations are often unnecessary (Hoy *et al*., 2013). The differential diagnosis considers extensive papules and benign adnexal tumor, condyloma accuminatum, chickenpox (infection with varicella zoster virus), plane warts, cutaneous fungal infections, keratoacanthoma, leiomyoma, verruca vulgaris, papilloma, papular granuloma annulare, Paget's disease and other possibilities (Bandino *et al*., 2011; Hanson and Diven, 2003). The skin lesions in our patient were local, mild and atypical, and her predominant complaint was severe headache. However, she had no signs or symptoms of other diseases that may have caused the clinical picture. Metagenomic sequencing of her cerebrospinal fluid detected unique sequences of the virus. Together with the skin lesions and the exclusion of the other differential diagnoses, the metagenomic result enabled us to diagnose the patient with intracranial molluscum contagiosum virus infection. These findings show the power of metagenomic sequencing in detecting pathogens that cause intracranial infections. To the best of our knowledge, this is the first report of the molluscum contagiosum virus identified in cerebrospinal fluid. As metagenomic sequencing has not been used in most cerebrospinal fluid examinations and because molluscum contagiosum is self-limiting, the rate of infection in the central nervous system may be high. Metagenomic sequencing is a reliable method for detecting this virus and other pathogens, such as amoeba protozoa (Wang *et al*., 2018), in the cerebrospinal fluid.

The medical treatment of molluscum contagiosum is somewhat controversial. The lesions are benign and usually self-limiting. The average duration of a single lesion in immunosuppressed patients is approximately 2 months (Gottlieb and Myskowski, 1994; Chen *et al*., 2013); however, as the infection can be transmitted by autoinoculation via scratching or trauma, the mean duration of the infection is approximately 8 months, and several cases may persist for 12 months or longer (Gur, 2008). Our patient strictly followed the doctor's instructions after diagnosis. Her infection was quickly self-limited in 1 month. No evidence-based consensus has been reached on the best treatment for immunocompetent patients with molluscum contagiosum (Chen *et al*., 2013). In immunocompromised individuals with HIV infection, the presence of molluscum contagiosum lesions seems to indicate advancing immunosuppression (Valentine and Diven, 2000). For patients with HIV, the evidence suggests that systemic immune-modulating and antiviral drugs can be more effective than local ablative therapies. We did not give our patient any special treatment, but we did prescribe symptom-alleviating treatments, and the patient recovered, which further confirms our diagnosis. Our diagnostic approach, cerebrospinal fluid coupled with unbiased next-generation sequencing (NGS), provided a rapid and sensitive diagnosis of pathogens causing intracranial infection that eluded conventional testing for weeks after the initial presentation. NGS enables to identify both common and rare pathogens without any hypothesis. Especially in cerebrospinal fluid sample, some normal pathogens or non-pathogenic microbes were detected, like neurobrucellosis (Fan *et al*., 2018)2016 and June 1, 2017. The clinical characteristics and NGS results of patients with the diagnosis of NB were reviewed in this study. Results Four patients were rapidly diagnosed with NB using NGS of the CSF in patients with clinically suspected CNS infections, although the clinical manifestations varied dramatically between these patients. NGS of the CSF revealed that the sequence reads identified that corresponded to Brucella species ranged from 11 to 104, with genomic coverage ranging from 0.043% to 0.4%. Rapid diagnosis led to prompt treatment with the appropriate antibiotics. Conclusions This study demonstrates the power of NGS of the CSF coupled with a bioinformatic pipeline in the diagnosis of NB.”,”author”:[{“dropping-particle”:””,”family”:”Fan”,”given”:”Siyuan”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Ren”,”given”:”Haitao”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Wei”,”given”:”Yanping”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Mao”,”given”:”Chenhui”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:

”Ma”,”given”:”Zhenzi”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Zhang”,”given”:”Lu”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Wang”,”given”:”Li”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Ge”,”given”:”Ying”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Li”,”given”:”Taisheng”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Cui”,”given”:”Liying”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Wu”,”given”:”Honglong”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Guan”,”given”:”Hongzhi”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””}],”container-title”:”International Journal of Infectious Diseases”,”id”:”ITEM-1”,”issued”:{“date-parts”:[[“2018”]]},”title”:”Next-generation sequencing of the cerebrospinal fluid in the diagnosis of neurobrucellosis”,”type”:”article-journal”},”uris”:[“http://www.mendeley.com/documents/?uuid=1772b866-febd-43a5-8cd4-fb447ae6032b”]}],”mendeley”:{“formattedCitation”:”(Fan et al. 2018 or *Propionibacterium acnes* (Ye *et al*., 2016)current diagnostic methods are of limitation in many aspects, such as detecting range, time-consuming, specificity and sensitivity. In this report, we apply our new-developing pathogen detection method to clarify that Propionibacterium acnes is the causative agent of a two-year-old boy with juvenile myelomonocytic leukemia presenting clinical symptoms including serious rash and hyperpyrexia while traditional clinical methods of diagnosis fail to detect the pathogenic agent and multiple antimicrobial drugs are almost ineffective Propionibacterium acnes is confirmed to be the infectious agent by quantitative real-time polymerase chain reaction.\\n\\nCASE PRESENTATION: After haploidentical hematopoietic stem cell transplantation, a two-year-old boy with juvenile myelomonocytic leukemia presented to a pediatrist in a medical facility with hyperpyrexia and red skin rash which later changed to black skin rash all over his body. Traditional diagnostic assays were unrevealing, and several routine antimicrobial treatments were ineffective, including the vancomycin, meropenem, tobramycin, cefepime and rifampin. In this case, pediatrist resorted to the next-generation sequencing technology for uncovering potential pathogens so as to direct their use of specific drugs against pathogenic bacteria. Therefore, based on the BGISEQ100 (Ion Proton System. These were supposed to be abnormal in cerebrospinal fluid in the past, but more and more cases were reported by metagenomics sequencing. What's more, the pseudorabies virus was detected in endophthalmitis (Ai *et al*., 2018) by NGS. That is unbelievable if there was no prompt NGS. In our case, molluscum contagiosum is also a normal pathogen but it is rare to detect the infection in the cerebrospinal fluid. Besides, the NGS results can provide information about abundance of pathogen, it can be viewed like a semi-quantitive sequencing platform that dynamically monitors the disease progression (Ai *et al*., 2017)”type”:”article-newspaper”},”uris”:[“http://www.mendeley.com/documents/?uuid=3fdc8cbd-8105-3f96-9c57-280c18abe938”]}],”mendeley”:{“formattedCitation”:”(Ai et al. 2017. Because the unbiased characteristic, even if the NGS is negative, it also might help to identify a non-infectious disease like tumor.

Conclusion

We report a very rare case of intracranial molluscum contagiosum virus infection. The patient presented with headache, atypical skin lesions of molluscum contagiosum and reduced CD3 and CD4 cell counts. Metagenomic sequencing applied to the cerebrospinal fluid detected sequences unique to the molluscum contagiosum virus. We diagnosed the patient with an intracranial molluscum contagiosum virus infection. The patient recovered with no special treatment, and this finding confirmed our diagnosis. Our experience showed the usefulness and power of the application of metagenomic sequencing to cerebrospinal fluid for detecting pathogens that cause intracranial infections.

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