· 病例报告 ·

A case report of varicella-zoster virus infection associated glomerulonephritis and encephalitis

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SUMMARY A 15-year-old boy was admitted with nephritic and nephrotic syndrome, renal dysfunction and decreased serum C3, who suffered from varicella for two months. His renal histopathology revealed endocapillary proliferative glomerulonephritis with podocytes proliferation and severe tubular injury by light microscopy. Direct immunofluorescence showed global granular deposition of IgG, IgA, IgM, C3, C1q and fibrinogen in mesangium and along glomerular capillary wall. Electron microscopic examination showed electron-dense deposits in multiple sites of glomeruli. Furthermore, specific serum IgM antibodies against varicella-zoster virus (VZV) were detected. VZV antigen and mRNA were demonstrated in glomerular and tubular epithelial cells by immunohistochemical staining and *in-situ* hybridization. Virus particles and virus inclusions were identified by electron microscopy and special staining (Methylene Blue and Eosion staining or Mann staining). The patient also experienced epileptic episodes and his brain MRI and electroencephalogram indicated herpes encephalitis with secondary epilepsy. Therefore, the diagnosis of VZV-associated glomerulonephritis and encephalitis was established. This is the first case of VZV-associated glomerulonephritis with renal histopathological evidence using *in situ* hybridization technique.
KEY WORDS Herpesvirus 3, human; Glomerulonephritis; Encephalitis

Chickenpox is a common infectious disease in children. It is caused by primary varicella-zoster virus (VZV) infection. Besides typical cutaneous injury, other organs, including the brain, lung, heart, kidney, liver, gut, hematological system, and so on, were also be involved in severe cases[1-4]. Although several patients suffering from VZV-associated kidney injury had been reported since 1884[5], it was not well investigated due to the low incidence and lack of pathologic diagnostic methods. Recently, we have treated a young boy who suffered from VZV-associated glomerulonephritis and encephalitis. Solid evidence of VZV-associated glomerulonephritis, in his renal biopsy tissue, was identified using immunopathologic and molecular pathologic techniques.

To our knowledge, this is the first case of VZV-associated glomerulonephritis with renal histopathological evidence using *in situ* hybridization technique.

1 Case report

The patient was a 15-year-old boy. Two months before, disseminated bubbles-like herpes had developed on his face, trunk and extremities with fever up to 39.5 ℃ and hematuria. He was diagnosed as chickenpox and treated with ganciclovir and ribavirin. Five days later, he had gross hematuria (red cells in full field of vision), proteinuria (+ + to + + + +), edema and hypertension (145/100 mmHg) (1 mmHg = 0.133kPa), urinary volume decreased to 500 mL per day, serum creatinine level 212 μmol/L and albumin 16.8 g/L. The diagnosis of nephritic / nephrotic syndrome and acute renal dysfunction was made. Twelve days passed, his serum creatinine increased to 451.9 μmol/L and albumin decreased to 16 g/L. He received first intravenous methylprednisolone 200 mg/d for 6 consecutive days, then hemodialysis and prednisone 60 mg/d orally for 3 weeks, and finally orall lamivudine 20 mg/d was addede. His renal function recovered gradually but his edema and hematuria were not relieved. He had experienced epilepsy twice during hemodialysis 50 days before being transferred to our hospital for definite diagnosis and treatment.

The patient was admitted to our hospital and allround check performed. His blood pressure was 148/114 mmHg and temperature 36.7 ℃. Numerous small scabs on face, trunk and extremities were noticed (Figure 1). Pitting edema was found on face, both lower extremities and scrotum. Laboratory investigation showed hemoglobin was 141 g/L and urinary protein 3.85 g/d. Urinary sediments showed deformed RBC and RBC casts. Serum albumin was 17 g/L and cholesterol 9.3 mmol/L. Urea was 17.09 mmol/L. serum creatinine 65 μmol/L, and creatinine clearance rate 47.6 mL/min. The level of serum complement C3 was 451 mg/L (normal range: 700 to 1 280 mg/L). Antistreptolysin O titer and C reactive protein were normal. Autoantibodies (ANA, ANCA, anti-GBM antibodies) were negative. Specific serum IgM antibodies against VZV were positive. Other anti-virus antibodies were negative (Cytomegalovirus, Rubella virus, Herpes simple virus, etc). Ultrasonography displayed normal size of both kidneys and a great quantity of ascites

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appeared. The percutaneous renal biopsy was performed on the fifth day of hospitalization.

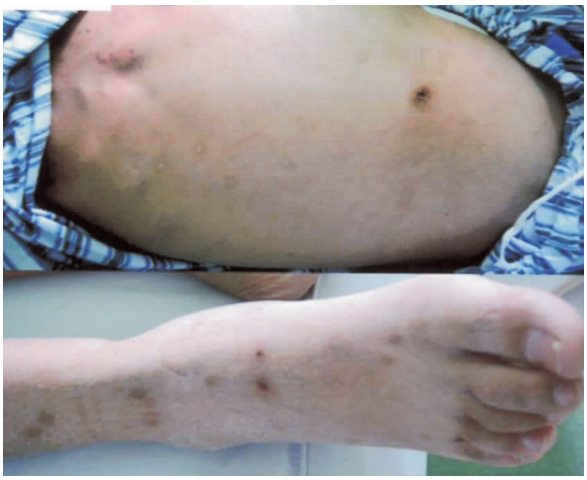


Figure 1　Numerous small scabs on ftrunk and lower limbs

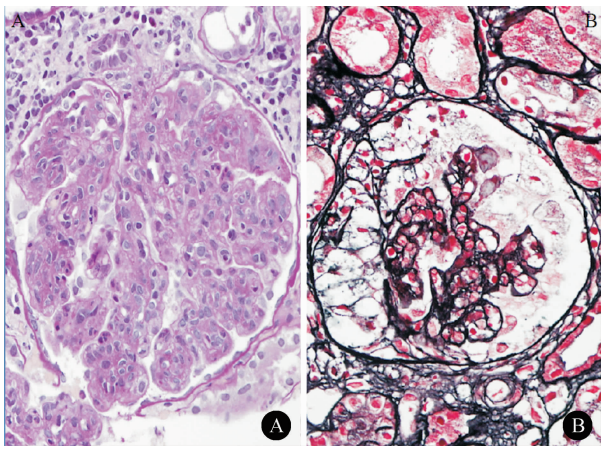
The pathologic findings were as following: Light microscopic examinations (3 μm section, HE, PAS, PASM and Masson stain) showed that glomeruli were enlarged and glomerular hypercellularity as a result of glomerular endothelial cells and mesangial cells proliferation with polymorphonuclear leukocytes and monocytes infiltration. Partial glomerular capillaries were obstructed. Podocytes were found in proliferation, hypertrophy and vacuolar degeneration, with cellular crescent-like structure (Figure 2). There was also severe vacuolar degeneration of the tubular epithelium. Multiple focal brush border loss of the tubular epithelium and enlarged lumen were seen. Some tubular epithelial cells were broken. Some smudgy-like viral inclusions were observed in the tubular epithelium by Methylene Blue and Eosion staining. There were diffuse edema and focal lymphocytes, monocytes infiltration and fibrosis in the interstitium.

Immunofluorescence examination (DAKO company) demonstrated diffuse granular deposits of immunoglobulins and components in the glomerular mesangium and along the capillary wall. The intensities of IgG (++), IgA(++), 1gM(++), C3(++++), Clq (+) and FRA(+) (Figure 3).

Electron microscopy showed diffuse glomerular endothelial cell and mesangial cell proliferation, podocytes also proliferated with extensive foot process effacement. Electron dense substance was found in the intrabasemetmembrane, subendothelial, paramesangial and mesangial regions (Figure 4). Virus particles were observed in cytoplasm of podocytes. There was severe vacuolar degeneration of the tubular epithelium where virus inclusions were found (Figure 5).

Mann staining (Methylene Blue and Eosion staining) showed some inclusions in the cytoplasm or nuclei of the glomerular cells and the tubular epithelial cells.

Immunohistochemistry staining(Vector company, Pk-6103) for VZV antigen was demonstrated in some glomeruli and tubular epithelia (Figure 6).



A, endocapillary proliferative pattern; B, podocytes proliferative and vacuolar degenerative pattern.

Figure 2　LM reveals (PAS ×400)

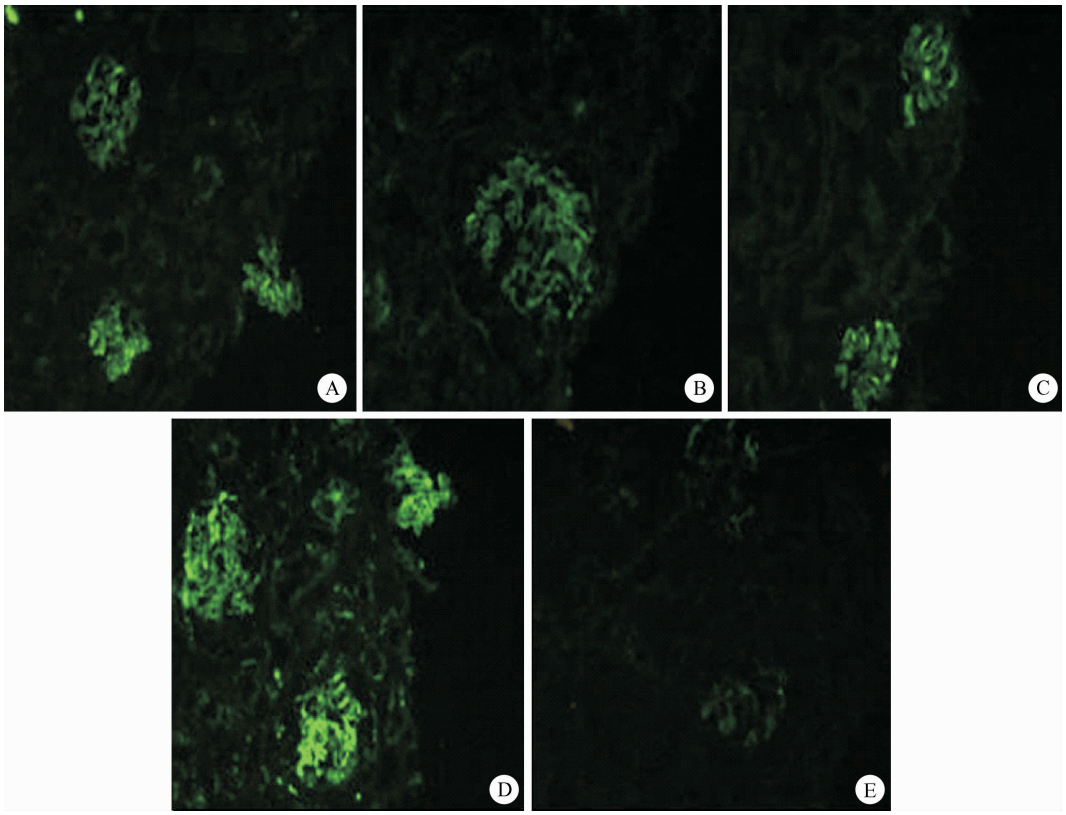
In-situ hybridization for VZV RNA was observed in some glomeruli and tubular epithelial cells (probe: 5′-CTACAQCGCCATGGGGGGGCGGTATATCATGCC-3′) (VZV Gene63, product is RNA) (NCBI Gen Bank:X02132.1) (Figure 7).

The patient experienced twice epileptic episodes on the 4th day of hospitalization and improved after antiepileptic treatment. Further investigation just revealed a high pressure of spinal fluid at 95 mmH_2O (1 mmH_2O = 0.009 8 kPa) without any other abnormality in routine spinal fluid examination. Then he was performed with brain magnetic resonance imaging (MRI) (axial view: T2WI/ FLAIR sequences), hyper-signal lesions were identified at both his occipital lobes and left frontal lobe (Figure 8). Electroencephalogram showed continuous widespread slow waves at the occipital regions and sharp waves at the frontal regions occasionally. The neurologist's consultation indicated secondary epilepsy due to VZV infection. The epileptic seizures were controlled by antiepileptic drugs. His brain MRI became normal after two months.

The diagnosis of this patient was: Varicella-zoster virus infection associated glomerulonephritis and encephalitis

During his hospitalization, oral prednisone 60 mg/d was given with gradual tapering. Intravenous cyclophosphamide was administered at a total dosage of 3.5 g, and was replaced by oral mycophenolate mofetil 1.5 g/d due to hepatic dysfunction.

His edema and ascites were resolved gradually, proteinuria decreased to 1.77 g/d, blood pressure was 110/70 mmHg, serum creatinine 66 μmol/L, creatinine clearance rate 111.7 mL/min, and serum complement C3 636 mg/L.



A, IgA; B, IgG; C, IgM; D, C3; E, C1q.

Figure 3 Immunofluorescence pattern (×200)

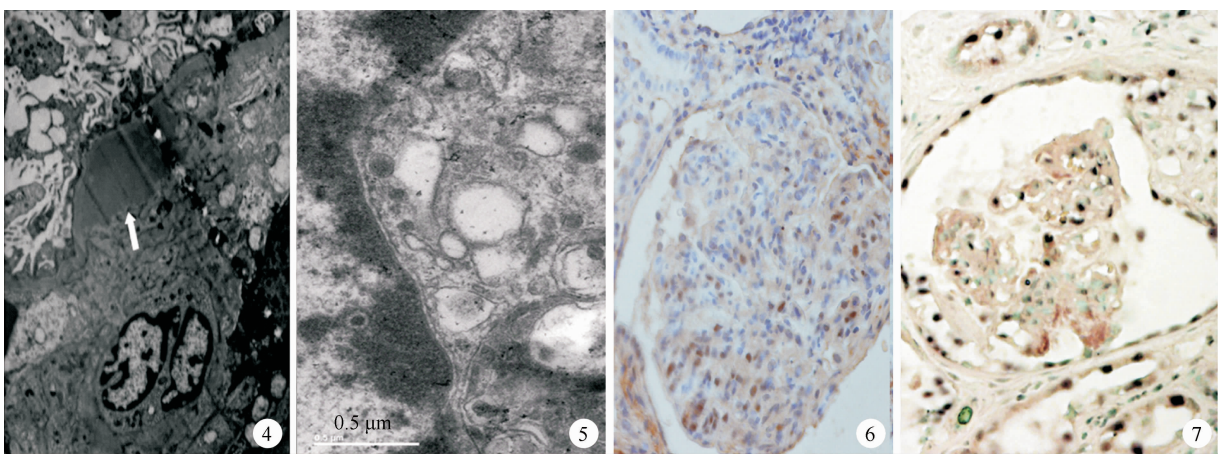


Figure 4 EM reveals intra-GBM, subendothelial and mesangial electron dense deposits (↑) (EM ×8 000) **Figure 5** Viral inclusions in tubular cells (EM ×30 000) **Figure 6** Anti-VZV antibody positive in glomerular cells and tubular cells (immunohistochemistry ×400) **Figure 7** VZV-RNA positive in glomerular cells and tubular cells (*in situ* hybridization ×400)

2 Discussion

Varicella-zoster virus (VZV) belongs to double-stranded DNA viruses and the infection is via respiratory tract transmission or direct contact with vesicle fluid of patients. Primary infection causes varicella which mainly occurs in children[6]. However, the virus can still stay in scabs after varicella recovery and recurrence will cause herpes zoster. The virus can induce glomerulonephritis and encephalitis occasionally[1-2].

The earliest report on VZV infection associated nephritis dated in 1884, which reported four children who were infected with VZV and manifested as nephrotic syndrome with hematuria. Then many cases of VZV infection with acute glomerulonephritis were reported[1-2,7-8]. In 1968, a statistical material of 2 534 inpatients with VZV infection and the clinical diagnosed glomerulonephritis accounted for 0.1%[7].

In the literature, it was reported that the renal involvement usually occurred after VZV infection appeared or after skin lesions disappeared[2], the duration between VZV infection appearing and onset of nephritis varied from 3 to 21 days[1-2,7-8]. Clinical manifestations included acute nephritic syndrome and/or

nephrotic syndrome[1-2,7-8], and rapidly progressive glomerulonephritic syndrome occasionally[9]. Renal biopsy and autopsy pathologic expression: the glomeruli were mainly proliferative lesions similar to endocapillary proliferative glomerulonephritis, which manifested as proliferation of endothelial cells and mesangial cells with polymorphonuclear leukocyte and monocytes infiltration[1-2,7-9], segmental fibrin-like necrosis could be seen occasionally[8], furthermore, podocyts proliferation, swelling and degeneration, and viral inclusion body could be found in the swelling cells[7]. The renal tubular epithelial cells were with degeneration and even necrosis, and viral inclusion body could also be seen[7]. The renal interstitium had edema, leukocytes and monocytes infiltration[9]. Lin et al reported that, by immunofluorescence examination of renal biopsy showed that global intense staining for IgG, IgA, gM, C3 and C1q in the glomerular mesangium and capillary wall[9]; electron microscopic examination showed electron dense deposit was seen in glomerular mesangial, subendothelial and subepithelial regions[9]. These results suggested that the glomerular disease was immune complex mediated[9].

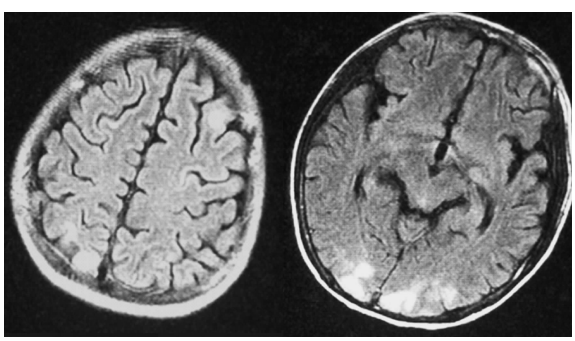


Figure 8 Hyper-signal lesions on both occipital lobes and left frontal lobe (MRI)

Our patient manifested as the following: (1) well-defined Varicella-zoster virus infection; (2) acute nephritic syndrome with nephrotic syndrome and renal function damage for short-term, ASO and CRP were normal; (3) hypocomplementnemia ; (4) The renal histopathology indicated endocapillary proliferative glomerulonephritis with podocyte swelling and severe renal tubular injury by light microscope. Immunofluorescence showed "full-house" positive, and electron microscopic examination showed electron dense deposits in multiple sites; (5) Virus-like particles and inclusion bodies were found in glomerular cells and renal tubular epithelial cells using special staining (Methylene Blue and Eosion staining) and electron microscopic examination; (6) VZV antigens and RNA were demonstrated in glomerular and renal tubular cells by immunohistochemical staining and *in-situ* hybridization of renal tissues. Therefore the patient was diagnosed as VZV-associated nephritis. To our knowledge, this is the first case of VZV-associated nephritis confirmed using *in situ* hybridization technique.

The pathogenesis of VZV-associated nephropathy is not clear. The possible pathogenesis includes: (1) renal deposition of circulating VZV antigen that contained immune complex; (2) renal deposition of virus antigen that induced *in-situ* immune complex formation; (3) direct VZV infection of renal cells that induced host inflammatory response; (4) VZV-induced autoimmunity[10-11]. In this case, the hypocomplementnemia, the "full-house" positive of glomerular immunofluorescence, electron dense deposits in glomeruli and the successful demonstration of VZV antigen and RNA in renal cells indicate that the renal lesion might be immune complex mediated, which is similar to the case reported by Lin et al[9].

There are some theory about VZV infection relationship with glomerulonephritis. Can not be dependent on VZV infection and glomerulonephritis simultaneous or in succession in same patients. The pathologic immunohistochemistry method proved VZV antigen in kidney, but this method is a protein level method only, can not remove adhered to kidney of VZV antigen in blood. We used molecular pathologic method (*in situ* hybridization) first verified that VZV is growing and reproduce in kidney, and result in glomerulonephritis.

The similar case of VZV-associated glomerulopathy, reported by Lin et al[9], achieved a successful remission after 14 days of treatment with corticosteroid [prednisolone 2 mg/(kg·d)]. However, our patient acquired only incomplete curative effect, using the combination of corticosteroid and cytotoxic or immunosuppressive agents. The reason for the discrepancy is not clear and further study is needed.

The patient experienced epileptic seizure for two times and MRI showed there were multiple lesions in his brain. Encephalitis secondary to VZV infection was diagnosed. In the literature, although a few reports indicated VZV infection involved central nervous system, few VZV infection induced encephalitis which was reported with an incidence of 0.04%[12]. The pathogenesis can be either virus duplication in the brain or immune-mediated damage[2, 12]. Encephalitis usually appeared within a week or several weeks after varicella, even before varicella[2,12-13]. Epileptic seizure was common[12-13]. Examination of cerebrospinal fluid could be absolutely normal or just increasing of pressure. The levels of protein and lymphocytes might slightly increase[12]. Electroencephalogram usually showed abnormal cortex function and diffuse slow wave[12]. CT and MRI examination were usually abnormal[2, 12]. The prognosis is good in majority of patients and pathological changes can be absorbed completely

in a short time. However, some patients may have permanent brain damage and 5% or more cases even died[2, 12-13]. The manifestations of our case are similar to those reported about VZV-associated brain lesions.

In conclusion, we reported a case with VZV-associated glomerulonephritis and encephalitis. The demonstration of VZV antigen and RNA in renal tissue confirmed the diagnosis. However, the appropriate treatment of renal disease needs further investigation.

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水痘病毒感染伴肾小球肾炎和脑炎 1 例报告

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[摘 要] 患者 15 岁,男性,因水痘、肾炎和肾病综合征、肾功能损伤、补体 C3 下降 2 个月而入北京中日友好医院肾内科治疗,肾活检证实为毛细血管内增生性肾小球肾炎伴足细胞增生和肾小管损伤,直接免疫荧光显示 IgG、IgA、IgM、C3、C1q 和纤维蛋白原沿肾小球毛细血管壁和系膜区颗粒状和团块状沉积,透射电子显微镜检查显示电子致密物在肾小球多部位沉积,血清中抗水痘病毒抗体(IgM) 阳性,免疫组织化学和原位杂交显示水痘病毒抗原和 mRNA 存在于肾小球和肾小管上皮细胞,特殊染色和透射电子显微镜显示病毒颗粒和病毒包涵体存在于肾小球和肾小管上皮细胞内。患者住院期间尚有癫痫发作,脑电图及核磁共振证实为病毒性脑炎导致的癫痫,故确诊为水痘病毒感染伴发肾小球肾炎和脑炎。这是 1 例通过分子病理学方法证实水痘病毒在肾内感染导致的肾小球肾炎。

[关键词] 疱疹病毒 3 型,人; 肾小球肾炎; 脑炎
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