

Acute encephalitis and encephalopathy associated with human parvovirus B19 infection in children

Toru Watanabe, Hideshi Kawashima

Toru Watanabe, Hideshi Kawashima, Department of Pediatrics, Niigata City General Hospital, Niigata 950-1197, Japan

Author contributions: Watanabe T designed the aim of this mini-review and performed the majority of the writing; Kawashima H coordinated the writing of the manuscript.

Conflict-of-interest statement: We declare that we have no conflicts of interest in the manuscript, including financial, consultant, institutional or other relationships.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CCBY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Toru Watanabe, PhD, Department of Pediatrics, Niigata City General Hospital, 463-7 Shumoku, Chuoku, Niigata 950-1197, Japan. twata@hosp.niigata.niigata.jp
Telephone: +81-25-2815151
Fax: +81-25-2815169

Received: June 24, 2015

Peer-review started: June 26, 2015

First decision: August 10, 2015

Revised: August 11, 2015

Accepted: September 10, 2015

Article in press: September 16, 2015

Published online: November 8, 2015

Abstract

Reports of neurologic manifestations of human parvovirus B19 (B19) infection have been on the rise. Acute encephalitis and encephalopathy is the most common, accounting for 38.8% of total B19-associated neurological manifestations. To date, 34 children with B19

encephalitis and encephalopathy have been reported, which includes 21 encephalitis and 13 encephalopathy cases. Ten (29%) were immunocompromised and 17 (39%) had underlying diseases. Fever at the onset of disease and rash presented in 44.1% and 20.6% of patients, respectively. Neurological manifestations include alteration of consciousness occurred in all patients, seizures in 15 (44.1%) patients, and focal neurologic signs in 12 (35.3%) patients. Anemia and pleocytosis in cerebrospinal fluid (CSF) occurred in 56.3% and 48.1% of patients, respectively. Serum Anti-B19 IgM (82.6%) and CSF B19 DNA (90%) were positive in the majority of cases. Some patients were treated with intravenous immunoglobulins and/or steroids, although an accurate evaluation of the efficacy of these treatment modalities cannot be determined. Nineteen (57.6%) patients recovered completely, 11 (33.3%) patients had some neurological sequelae and 3 (8.8%) patients died. Although the precise pathogenesis underlying the development of B19 encephalitis and encephalopathy is unclear, direct B19 infection or NS1 protein of B19 toxicity in the brain, and immune-mediated brain injuries have been proposed.

Key words: Encephalitis; Neurological manifestation; Human parvovirus B19; Encephalopathy; Pathogenesis; Complication

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Reports of acute encephalitis and encephalopathy associated with human parvovirus B19 (B19) infection have recently increased. B19 DNA has been detected in cerebrospinal fluid samples in approximately 4% patients with etiologically undiagnosed encephalitis. Some patients were treated with intravenous immunoglobulins and/or steroids. More than half of the patients with B19 encephalitis and encephalopathy recovered completely, but some patients developed severe neurological sequelae or died. Although the precise pathogenesis underlying the development of B19

encephalitis and encephalopathy is unclear, direct B19 infection or NS1 protein of B19 toxicity in the brain, and immune-mediated brain injuries have been proposed.

Watanabe T, Kawashima H. Acute encephalitis and encephalopathy associated with human parvovirus B19 infection in children. *World J Clin Pediatr* 2015; 4(4): 126-134 Available from: URL: <http://www.wjgnet.com/2219-2808/full/v4/i4/126.htm> DOI: <http://dx.doi.org/10.5409/wjcp.v4.i4.126>

INTRODUCTION

Human parvovirus B19 (B19) is a member of the erythrovirus genus in the family Parvoviridae^[1], first discovered by Cossart *et al*^[2] in 1975. B19 is a small, single-stranded DNA virus that binds the cellular receptor P antigen on erythrocytes^[3]. The viral genome encodes only three proteins of known function, including nonstructural protein 1 (NS1), and two viral capsid proteins, viral protein 1 (VP1) and viral protein 2 (VP2)^[1]. NS1 promotes multiple replicative functions and is cytotoxic to host cells^[1]. Since Shneerson *et al*^[4] reported the first symptomatic febrile patients with B19 infection in 1980, various clinical manifestations of B19 have been identified. These include erythema infectiosum (EI)^[5], arthropathy, non-immune hydrops fetalis and congenital anemia, thrombocytopenia, hepatitis, myocarditis and neurologic diseases in healthy hosts, chronic pure red cell aplasia in immunodeficient hosts, and transient aplastic crisis in patients with increased red cell turnover^[1,6]. Of these, neurologic manifestations of B19 infection have been increasingly reported, especially in children^[7,8].

B19-associated neurologic manifestations include encephalitis, encephalopathy, meningitis, cerebellar ataxia, transverse myelitis, stroke, and peripheral neuropathy^[7,8]. Of these, encephalitis and encephalopathy are the most common with 38.8% of total B19-associated neurological manifestations^[8]. Furthermore, recent reports reveal that specific forms of encephalopathy also are associated with B19 infection, which include chorea encephalopathy^[9-11], mild encephalitis/encephalopathy with reversible splenic lesion (MERS)^[12] and posterior reversible encephalopathy syndrome (PRES)^[13]. The following is a review of B19 associated encephalitis and encephalopathy (B19 encephalitis and encephalopathy) in children.

DEFINITION OF B19 ENCEPHALITIS AND ENCEPHALOPATHY

Because encephalitis is defined as inflammation of the brain parenchyma, pathologic examination of brain tissue (brain biopsy) is necessary for its diagnosis. However, brain biopsy is rarely done pre-mortem with its potential risk for patients^[14]. In this review, we used

the Pillai encephalitis definitions^[15], as follows. Encephalopathy was defined as an altered or reduced level of consciousness and a change in personality or behavior or confusion lasting ≥ 24 h. Encephalitis was defined as an acute encephalopathy with 2 or more of the following: fever ≥ 38 °C, seizures or focal neurologic signs, cerebrospinal fluid (CSF) pleocytosis (≥ 5 white blood cells/ μ L), electroencephalographic (EEG) findings consistent with encephalitis, and neuroimaging suggestive of encephalitis^[15]. B19 infection was based on the detection of either B19 DNA or anti-B19 IgM specific antibodies in serum or CSF^[8]. Two patients with encephalitis and encephalopathy without laboratory tests confirming B19 infection were included in this review on the basis of clinical presentation of EI^[16,17].

EPIDEMIOLOGY

Balfour *et al*^[16] reported an 8-year-old boy who developed encephalitis associated with EI in 1970. Seven years later, Breese *et al*^[17] reported a 9-month-old boy with severe encephalopathy during the course of EI who developed permanent neurologic sequelae. In the era of molecular and serological diagnostics for B19 infection, cases of encephalopathy and B19 DNA in sera and in CSF are accruing^[18,19]. To date, 34 patients less than 19 years of age with B19 encephalitis or encephalopathy have been reported (Table 1)^[9-11,16-31]. This includes 21 patients with encephalitis, five with encephalopathy, four with chorea encephalopathy, three with MERS and one with PRES. Eleven patients have been reported from United Kingdom, eight from Japan, six from Jamaica, three from United States, two from Italy, two from Brazil, one from Germany and one from Turkey. The age of patients ranges from 1 to 15 years (mean of 6.7 years, median of 7 years). Fifteen patients were male, and the male to female ratio was 0.79. 24 (71%) were immunocompetent individuals, and 10 were immunodeficient. Seventeen (39%) patients had underlying diseases, including sickle cell disease, hereditary spherocytosis (HS), congenital heart diseases, acute lymphocytic leukemia, Crigler-Najar syndrome, Cockayne syndrome, Turner syndrome, and post-renal transplantation for nephropathic cystinosis^[12,23-26,28,29]. One patient suffered from ulcerative colitis eight years after B19 encephalopathy^[20].

Although the prevalence of B19 infection for all encephalitis and encephalopathy is unknown, two studies reported that B19 DNA has been detected in CSF samples in 2 of 43 (4.7%) and 7 of 162 (4.3%) patients with etiologically undiagnosed encephalitis^[22,23].

CLINICAL MANIFESTATIONS

Fever at the onset of diseases presented in 15 of 34 (44.1%) patients (Table 2). Rash was reported in seven (20.6%) patients^[13,16,17,19,21,29,31]. Rash developed before the onset of CNS manifestations in two patients, simultaneously with the onset in two patients, and

Table 1 Basic characteristics of children with B19 encephalitis and encephalopathy

Case No.	Ref.	Age	Gender	Diagnosis	Underlying disorders	Immunodeficiency
1	[16]	8 yr	M	Encephalitis		-
2	[17]	9 mo	M	Encephalitis		-
3	[19,20]	5 yr	F	Encephalitis		-
4	[23,24]	2 mo	F	Encephalitis		-
5	[23,24]	2 yr	M	Encephalitis	Acute lymphocytic leukemia	+
6	[23,24]	2 yr	F	Encephalitis		-
7	[23,24]	6 yr	F	Encephalitis	Cockayne syndrome	+
8	[23,24]	9 yr	M	Encephalitis		-
9	[23,24]	13 yr	F	Encephalitis	Crigler-Najar syndrome	-
10	[23,34]	13 yr	M	Encephalitis		-
11	[23,34]	15 yr	F	Encephalitis		-
12	[23,24]	1 d	F	Encephalitis	Necrotizing enterocolitis Patent ductus arteriosus Respiratory distress syndrome	+
13	[23,24]	1 d	F	Encephalitis	Ventricular septal defect Atrial septal defect Patent ductus arteriosus Turner syndrome Obstructive jaundice	+
14	[25]	8 yr	F	Encephalitis	Sickle cell disease Nephrotic syndrome	+
15	[25]	8 yr	M	Encephalitis	Sickle cell disease Nephrotic syndrome	+
16	[25]	12 yr	F	Encephalitis	Sickle cell disease Aplastic crisis	+
17	[25]	14 yr	M	Encephalitis	Sickle cell disease Aplastic crisis	+
18	[27]	10 yr	F	Encephalitis		-
19	[28]	9 yr	M	Encephalitis	Nephropathic cystinosis Renal transplant	+
20	[30]	4 yr	F	Encephalitis		-
21	[31]	5 yr	F	Encephalitis and cerebellitis		-
22	[18]	8 yr	F	Encephalopathy		-
23	[19]	5 yr	F	Encephalopathy		-
24	[21]	5 yr	M	Encephalopathy		-
25	[22]	4 yr	M	Encephalopathy	Prader-Willi syndrome	-
26	[26]	13 yr	F	Encephalopathy	S β + thalassemia	+
27	[9]	8 yr	F	Chorea encephalopathy		-
28	[10]	1 yr	M	Chorea encephalopathy		-
29	[11]	1 yr	M	Chorea encephalopathy		-
30	[11]	1 yr	F	Chorea encephalopathy		-
31	[29]	9 yr	F	MERS	Hereditary spherocytosis	-
32	[12]	11 yr	M	MERS	Hereditary spherocytosis	-
33	[12]	10 yr	M	MERS	Hereditary spherocytosis	-
34	[13]	9 yr	F	PRES	Acute glomerulonephritis	-

MERS: Clinically mild encephalitis/encephalopathy with reversible splenic lesion; PRES: Posterior reversible encephalopathy syndrome; F: Female; M: Male.

after the onset in three patients. All patients with rash had normal immune function. Three (8.8%) patients complained of arthralgia.

Acute kidney injury occurred in three patients with sickle cell disease, and in one patient with Cockayne syndrome as a part of multiple organ failure^[23-25]. Patients experiencing acute B19 infection sometimes develop renal complications^[32,33]. These include acute glomerulonephritis, thrombotic microangiopathy and Henoch-Schönlein purpura nephritis, probably due to direct cytopathic effects on glomerular epithelial cells and endothelial cells, and an immune complex mediated mechanism^[32,33].

One of the neurological manifestations was a reduced

level of consciousness, which occurred in all patients. Seizures developed in 15 (44.1%) patients and two had statue epilepticus^[19,27]. Twelve (35.3%) patients exhibited focal neurologic signs (Table 2), which included neurogenic bladder, chorea, loss of vision, ataxia, dysarthria, hemiparesis, and focal seizures^[9-11,13,16,22,23,25,30,31].

LABORATORY DATA

B19 infection causes various blood diseases and cytopenias affecting several blood cell lineages, such as red cell aplasia, pancytopenia and hemophagocytic lymphohistiocytosis (HLH)^[34]. In patients with B19 encephalitis and encephalopathy, anemia developed in

Table 2 Clinical manifestations of children with B19 encephalitis and encephalopathy

Case No.	B19 related symptoms			Neurological manifestations			Complications	Sequelae or death
	Fever	Rash	Arthralgia	Unconsciousness	Seizure	Focal neurologic signs		
1	+	+	+	+	-	+Neurogenic bladder		Left weakness and clonus
2	+	-	-	+	+	-		Psychomotor retardation
3	+	+	-	+	+	-	Status epilepticus	Epilepsy Mental retardation
4	+	-	-	+	+	-		-
5	+	-	-	+	-	-		Cognitive deficit
6	+	-	-	+	-	+Ataxia		-
7	-	-	-	+	-	-	Multiple organ failure Acute renal failure	Died
8	-	-	-	+	+	-		Cognitive deficit Epilepsy
9	-	-	-	+	-	-		Not determined
10	-	-	-	+	-	+ Right hemiparesis Ataxia		-
11	-	-	-	+	-	-		Cognitive deficit
12	-	-	-	+	-	-		Died
13	-	-	-	+	-	-		Died
14	-	-	-	+	+	+ Right hemiparesis Transient blindness	Acute renal failure Aplastic crisis	-
15	-	-	-	+	+	+ Cortical blindness	Acute chest syndrome Acute renal failure Aplastic crisis	-
16	+	-	-	+	+	+ Focal seizures	Acute chest syndrome Acute renal failure Acute chest syndrome	-
17	+	-	-	+	+	-		-
18	+	-	-	+	+	-	Status epilepticus	Epilepsy
19	-	-	+	+	+	-		-
20	+	-	-	+	+	+ Cerebellar syndrome		Cerebellar syndrome
21	+	+	-	+	+	-		Cerebellar syndrome
22	-	-	+	+	+	-		-
23	-	+	-	+	+	-		-
24	-	-	-	+	-	-	Pancytopenia Liver dysfunction	-
25	-	-	-	+	-	-		Spastic quadriplegia
26	+	-	-	+	-	-	Aplastic crisis	-
27	-	-	-	+	-	+ Chorea		-
28	-	-	-	+	-	+ Chorea		-
29	-	-	-	+	+	+ Left hemiparesis Athetosis		-
30	-	-	-	+	+	+ Left hemiparesis Choreo-athetosis		Global developmental delay
31	+	+	-	+	-	-	Aplastic crisis Pancytopenia	-
32	+	-	-	+	-	-	Aplastic crisis HLH Pancytopenia	-
33	+	-	-	+	-	-	Aplastic crisis HLH Pancytopenia	-
34	+	+	-	+	+	+ Loss of vision	Transient SLE symptoms Hypertension	-

HLH: Hemophagocytic lymphohistiocytosis; SLE: Systemic lupus erythematosus.

18 of 32 (56.3%) patients (Table 3). Aplastic crisis was recorded in five patients with sickle cell disease and three patients with HS. Pancytopenia and HLH occurred in 4 patients and 2 patients, respectively.

CSF examination showed pleocytosis in 13 of 27 (48.1%) patients and increased protein levels in 8 of 20 (40%) patients (Table 3).

Anti-B19 IgM antibodies and B19 DNA were detected

in the sera of 19 out of 23 (82.6%) patients and 12 out of 20 (60%) patients, respectively. Anti-B19 IgM antibodies and B19 DNA were detected in the CSF of two out of 11 (18.2%) patients and 18 out of 20 (90%) patients, respectively (Table 3). These results suggest that serum anti-B19 IgM and CSF B19 DNA examinations are useful tools for diagnosing of B19 infection in patients with encephalitis and encephalopathy.

Table 3 Laboratory data and treatment of children with B19 encephalitis and encephalopathy

Case No.	Anemia	Serum B19 markers			CSF B19 markers			CSF		IVIG and/or steroids
		IgM	IgG	DNA	IgM	IgG	DNA	Pleocytosis	Increased protein	
1	-							+	-	-
2	-							-	+	-
3	-	+	+	+			-	+	+	-
4		-	-	-	-	-	+	-		IVIG
5					+	-	-	-	-	-
6	+				-	-	+	-	-	-
7	-				-	-	+			-
8	+	+	-	+	-	-	+			-
9	-						+			-
10	-						+	-		-
11	-						+	+		-
12	+	+	-	+	+	-	+			-
13	+	-	-	-	-	-	+			-
14	+	+	+	+				+		-
15	+	+	+	+				+		-
16	+	+	+	+				+		-
17	+	+	+	+				+		-
18	-	+	-		-	-		+	+	IVIG and steroids
19	+	+		+				+	+	-
20	-	-	-	+	-	-	+	+	-	IVIG and steroids
21	-	+	+	+			+	+	+	Steroids
22	+	+		+			+	-		-
23	-	+	+	+			+	-	+	-
24	+	+	+	+			+	-	+	-
25	-	+	+	+	-	-	+	+	+	-
26	+	+	+	-	-	-		-	-	Steroids
27	+			+			+	-	-	-
28	-	+	+				+	-	-	IVIG and steroids
29	-	+	-							-
30	+	+	-					+	-	-
31	+	-	+	+			+	-	-	Steroids
32	+							-	-	IVIG
33	+							-	-	IVIG
34	+	+	+	+			+	-	-	-

IVIG: Intravenous immunoglobulin; CSF: Cerebrospinal fluid.

IMAGING STUDIES AND EEG

Six of 13 (46.2%) patients had abnormal brain computed tomography findings, which included an enlarged ventricle, brain edema, frontal and occipital vasogenic swelling, and lesions in right parietal, temporal or occipital areas^[13,22,23,25]. Brain magnetic resonance images showed abnormal findings in 10 of 15 (66.7%) patients. These findings included high signal intensity in the white matter^[13,23,26,30], basal ganglia, and cerebellum in T2 weighted images, high signal intensity in the splenic lesion of corpus callosum in diffusion-weighted images or T2 weighted images, and enlarged ventricles^[12,23,26,29,31].

EEG revealed abnormal findings in 15 of 19 (78.9%) patients, which included diffuse or focal slowing of the background activity, encephalopathic changes or epileptic abnormality^[10,12,16,17,24,26,27,29,30].

TREATMENT

As discussed elsewhere, direct B19 infection or NS1 toxicity in the brain, and immune-mediated brain injuries have been proposed as pathogenic causes that result in the development of B19 encephalitis and encephalopathy^[7,8,35].

Some patients with B19 encephalitis and encephalopathy were treated with intravenous immunoglobulin (IVIG) and/or steroids (Table 3). IVIG and steroids are often used in post-infectious or immune-mediated encephalitis as immuno-modulatory therapies^[36]. In addition, IVIG is used for many clinical conditions associated with B19 infection such as chronic anemia, because immunoglobulin preparations are a good source of neutralizing antibodies against B19^[6].

Both IVIG and steroids were administered to three patients^[10,25,30], two of them showing neurologic sequelae (epilepsy and cerebellar syndrome). Three patients were treated with IVIG only, and they all recovered completely. Steroids only were administered to two patients, one of whom exhibited neurological sequelae (cerebellar syndrome)^[12,23,26,31]. Since there have been only a few reports of B19 encephalitis and encephalopathy with IVIG and/or steroids, an accurate evaluation of the efficacy of these treatment cannot be determined. However, due to the lack of other effective treatments for B19 encephalitis and encephalopathy, Barah *et al*^[8] recommended that treatment of severe cases might benefit from a combined regimen of IVIG and steroids.

OUTCOME

Nineteen of 33 (57.6%) patients recovered completely from neurologic disturbances, whereas 11 (33.3%) patients had some neurological sequelae and three (8.8%) patients died (Table 2). Patients with B19 encephalitis had worse prognosis (8 of 20 patients had neurological sequelae, and 3 patients died) than patients with B19 encephalopathy, MERS or PRES (2 of 13 patients had neurological sequelae, and no patient died). Neurological sequelae included psychomotor retardation^[11,17,19], cognitive deficit, epilepsy, cerebellar syndrome, hemiparesis and spastic quadriplegia^[16,19,22,23,27,30,31]. All of the patients that died were immunodeficient (two neonates and one patient with Cockayne syndrome)^[23].

SPECIFIC FORMS OF B19 ENCEPHALOPATHY

Recent reports have revealed that specific forms of encephalopathy are associated with B19 infection, which include chorea encephalopathy, MERS and PRES^[9-13].

Chorea encephalopathy

Chorea encephalopathy is characterized extrapyramidal features in the context of self-limiting neurological illness with CSF oligoclonal IgG bands, probably caused by immune-mediated (possibly autoantibody) pathogenic mechanisms^[37]. Four patients with B19 associated chorea encephalopathy have been reported to date^[9-11]. Grillo *et al*^[38] pointed out the clinical similarity between chorea encephalopathy and anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis, which is an autoimmune encephalitis caused by autoantibodies against the NR1 subunit of NMDAR^[39], and suggested that some cases of anti-NMDAR encephalitis could be triggered by B19.

Clinically mild encephalitis/encephalopathy with reversible splenic lesion

Clinically MERS is clinically mild encephalitis/encephalopathy characterized by an MRI finding of a reversible lesion with reduced diffusion in the corpus callosum, first proposed by Tada *et al*^[40] in 2004. Delirious behavior, impaired consciousness and seizure often occur as neurological symptoms, all of which completely recover within a month^[41]. Although a majority of patients with MERS are associated with viruses or bacteria^[41], some cases of Kawasaki disease associated with MERS have been reported^[42]. The precise pathogenesis of MERS is unknown, intramyelinic edema, interstitial edema in tightly packed fibers and transient inflammatory infiltrate have been postulated^[41].

Three patients with B19-associated MERS have been reported^[12,29]. Oshima *et al*^[29] reported a 9-year-old girl with HS developed MERS and aplastic crisis. They followed the plasma and CSF concentrations of B19 by real-time polymerase chain reaction and found that the symptoms of encephalopathy had occurred at

the peak viral load, suggesting that B19 MERS might occur as a result of direct B19 invasion. Suzuki *et al*^[12] also reported that two brothers with HS developed B19 MERS and HLH. HLH is due to dysregulated lymphocyte activation, which leads to macrophage hyperactivation and over-expression of pro-inflammatory cytokines, including IL-6^[43]. HLH has been reported in 28 patients with B19 infection, most of whom had HS as an underlying disease^[43]. Moreover, increased IL-6 levels in CSF have been reported in patients with MERS^[44,45]. These findings suggest that B19-induced IL-6 overproduction may lead to MERS and HLH in patients with HS^[12].

PRES

PRES is characterized clinically by headaches, impaired consciousness, visual disturbances and seizures, and radiologically by posterior (parieto-occipital) imaging changes predominantly occurring in the sub-cortical white matter^[46]. Although the pathophysiology underlying the development of PRES is multifactorial, hypertension plays a major role in the development of PRES through disruption of autoregulation in cerebral blood vessels^[46].

Cugler *et al*^[13] reported a 9-year-old girl with severe acute glomerulonephritis and PRES associated with B19 infection mimicking systemic lupus erythematosus. Only adequate control of blood pressure and diet led to complete resolution of the patient's symptoms.

PATHOGENIC MECHANISMS

Although the precise pathogenesis underlying the development of B19 encephalitis and encephalopathy is unclear, direct B19 infection or NS1 toxicity in the brain, and immune-mediated brain injuries that include cytokine dysregulation and autoantibody production directed against brain antigens, have been proposed^[7,8,35].

Direct B19 infection or NS1 toxicity

Only erythroid progenitor cells are permissive for B19 replication because these cells have both high concentrations of P blood type antigen, the receptor for B19^[3] and $\alpha 5\beta 1$ integrin, the co-receptor for B19^[47]. P antigen is also expressed on other cell types including megakaryocytes, granulocytes, lung, heart, liver, kidney, endothelium, and vascular smooth muscle^[48]. However, P antigen is necessary for B19 binding but not sufficient for virus entry into cell, which requires $\alpha 5\beta 1$ integrin as a cellular co-receptor^[47]. Because non-erythroid progenitor cells with P antigen do not have $\alpha 5\beta 1$ integrin, B19 can bind, but cannot infect them. However, the NS1 exerts a cytotoxic effect on the cells it binds without any B19 replication or virion accumulation^[49,50]. Therefore, NS1 may directly damage the cerebrovascular endothelium during B19 infection.

B19 DNA has been detected in the brain of two intrauterine deceased fetuses in mothers with recent B19 infection^[51,52]. Hobbs *et al*^[53,54] reported that B19

DNA was detected in 42% of dorsolateral prefrontal cortex and in 100% of cerebella, of postmortem samples taken from 104 subjects without recent acute B19 infection. Manning *et al.*^[55] also reported that B19 DNA was detected in 69% of frontal or occipital lobe of postmortem brain tissue samples taken from 29 subjects without recent acute B19 infection.

Although B19 DNA was frequently detected in the CSF of children with B19 acute encephalitis and encephalopathy, B19 detection in the brain has never been reported in children^[35]. Kerr *et al.*^[24] reported that postmortem brain tissue from a child with B19 acute encephalitis was negative for B19 in using *in situ* hybridization method. Skaff *et al.*^[56] also reported negative immunohistochemistry results for detecting B19 in brain biopsy tissue taken from an immunocompetent adult patient with B19 encephalitis. Meanwhile, B19 DNA was detected in brain biopsy tissues taken from adult patients with B19 encephalitis by PCR^[57,58]. There is still controversy regarding whether B19 has direct infection or NS1 toxicity in the brain cells or not^[59].

Cytokines dysregulation

B19 infection induces cytokine dysregulation. Wagner *et al.*^[60] studied concentrations of interleukin (IL)-1 β , and IL-6 concentrations and interferon (INF)- γ messenger RNA (mRNA) in peripheral blood mononuclear cells (PBMC) from patients with acute B19 infection and reported that these cytokine genes were activated in PBMC, suggesting systemic monocyte and T cell activation. Moffatt *et al.*^[61] demonstrated that IL-6 production caused by the B19 NS1 protein was mediated by an NF- κ B binding site in the IL-6 promoter region. Kerr *et al.*^[62,63] reported that serum IL-6, tumor necrosis factor (TNF)- α , IL-1 β , IL-4, IL-8, INF- γ , macrophage chemoattractant protein-1(MCP-1), granulocyte-monocyte colony stimulating factor (GM-CSF) levels were increased during acute B19 infection.

Kerr *et al.*^[24] reported that increased levels of TNF- α , INF- γ , IL-6, INF- γ , GM-CSF and MPC-1 in the serum and CSF of patients with B19 encephalitis and suggested that over-production of inflammatory cytokines might play a role in B19 encephalitis.

Autoantibody production

B19 infection often develops clinical features similar to autoimmune disease and has been associated with the production of antibodies against self-antigens, including nuclear antigens, rheumatoid factor, neutrophils cytoplasmic antigens, and phospholipids^[64,65]. Molecular similarities between host and B19 proteins and the induction of inflammatory cytokine production encourage the development of autoimmune reactions during B19 infection^[64,65]. B19 chorea encephalitis has been suggested to be caused by autoimmune reactions against brain autoantigens^[38].

common B19-associated neurological manifestations. Some patients were immunocompromised or had underlying diseases. Impaired consciousness, seizures and focal neurologic signs are the main neurological features. Serum Anti-B19 IgM and CSF B19 DNA examinations are useful tools for diagnosing B19 infection in patients with B19 encephalitis and encephalopathy. Two studies reported that B19 DNA has been detected in CSF samples in approximately 4% of patients with etiologically undiagnosed encephalitis. Serum Anti-B19 IgM and CSF B19 DNA should be examined in patients with etiologically undiagnosed encephalitis and encephalopathy. Some patients with B19 encephalitis and encephalopathy were treated with IVIG and/or steroids; however, an accurate evaluation of the efficacy of these treatment modalities cannot be determined. Although more than half of B19 encephalitis and encephalopathy patients recovered completely, some patients developed severe neurological sequelae or died. While the precise pathogenesis underlying the development of B19 encephalitis and encephalopathy is unclear, direct B19 infection or NS1 toxicity in the brain, and immune-mediated brain injuries have been proposed.

REFERENCES

- 1 Young NS, Brown KE. Parvovirus B19. *N Engl J Med* 2004; **350**: 586-597 [PMID: 14762186 DOI: 10.1056/NEJMra030840]
- 2 Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. *Lancet* 1975; **1**: 72-73 [PMID: 46024 DOI: 10.1016/S0140-6736(75)91074-0]
- 3 Brown KE, Hibbs JR, Gallinella G, Anderson SM, Lehman ED, McCarthy P, Young NS. Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). *N Engl J Med* 1994; **330**: 1192-1196 [PMID: 8139629 DOI: 10.1056/NEJM199404283301704]
- 4 Shneerson JM, Mortimer PP, Vandervelde EM. Febrile illness due to a parvovirus. *Br Med J* 1980; **280**: 1580 [PMID: 7427176 DOI: 10.1136/bmj.280.6231.1580]
- 5 Anderson MJ, Jones SE, Fisher-Hoch SP, Lewis E, Hall SM, Bartlett CL, Cohen BJ, Mortimer PP, Pereira MS. Human parvovirus, the cause of erythema infectiosum (fifth disease)? *Lancet* 1983; **1**: 1378 [PMID: 6134148 DOI: 10.1016/S0140-6736(83)92152-9]
- 6 Heegaard ED, Brown KE. Human parvovirus B19. *Clin Microbiol Rev* 2002; **15**: 485-505 [PMID: 12097253 DOI: 10.1128/CMR.15.3.485-505.2002]
- 7 Douvouiannis M, Litman N, Goldman DL. Neurologic manifestations associated with parvovirus B19 infection. *Clin Infect Dis* 2009; **48**: 1713-1723 [PMID: 19441978 DOI: 10.1086/599042]
- 8 Barah F, Whiteside S, Batista S, Morris J. Neurological aspects of human parvovirus B19 infection: a systematic review. *Rev Med Virol* 2014; **24**: 154-168 [PMID: 24459081 DOI: 10.1002/rmv.1782]
- 9 Fong CY, de Sousa C. Childhood chorea-encephalopathy associated with human parvovirus B19 infection. *Dev Med Child Neurol* 2006; **48**: 526-528 [PMID: 16700948 DOI: 10.1017/S0012162206001101]
- 10 Grillo E, da Silva RJ. Childhood chorea-encephalopathy and unremarkable MRI: an association suggesting parvovirus B19 infection. *Dev Med Child Neurol* 2009; **51**: 759-761 [PMID: 19583739 DOI: 10.1111/j.1469-8749.2009.03388.x]
- 11 Chambers RH, Gooden MA, Gilbert TD, Jackson ST. Childhood chorea-encephalopathy associated with recent parvovirus B19 infection in two Jamaican children. *Ann Trop Paediatr* 2010; **30**: 339-344 [PMID: 21118630 DOI: 10.1179/146532810X1285895592]

CONCLUSION

Acute B19 encephalitis and encephalopathy are the most

- 1438]
- 12 **Suzuki H**, Kusaka T, Okada H. Clinically mild encephalitis/encephalopathy with a reversible splenic lesion caused by human parvovirus b19 infection: a case of two brothers with hereditary spherocytosis. *Pediatr Neurol* 2014; **51**: 470-472 [PMID: 25035141 DOI: 10.1016/j.pediatrneurol.2014.05.021]
 - 13 **Cugler T**, Carvalho LM, Facincani I, Yamamoto AY, Silva GE, Costa RS, Ferriani VP. Severe glomerulonephritis and encephalopathy associated with parvovirus B19 infection mimicking systemic lupus erythematosus. *Scand J Rheumatol* 2012; **41**: 79-81 [PMID: 22043904 DOI: 10.3109/03009742.2011.617315]
 - 14 **Venkatesan A**, Tunkel AR, Bloch KC, Lauring AS, Sejvar J, Bitnun A, Stahl JP, Mailles A, Drebrot M, Rupprecht CE, Yoder J, Cope JR, Wilson MR, Whitley RJ, Sullivan J, Granerod J, Jones C, Eastwood K, Ward KN, Durrheim DN, Solbrig MV, Guo-Dong L, Glaser CA. Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the international encephalitis consortium. *Clin Infect Dis* 2013; **57**: 1114-1128 [PMID: 23861361 DOI: 10.1093/cid/cit458]
 - 15 **Pillai SC**, Hachoen Y, Tantsis E, Prelog K, Merheb V, Kesson A, Barnes E, Gill D, Webster R, Menezes M, Arden-Holmes S, Gupta S, Procopis P, Troedson C, Antony J, Ouvrier RA, Polfrity Y, Davies NW, Waters P, Lang B, Lim MJ, Brilot F, Vincent A, Dale RC. Infectious and autoantibody-associated encephalitis: clinical features and long-term outcome. *Pediatrics* 2015; **135**: e974-e984 [PMID: 25802349 DOI: 10.1542/peds.2014-2702]
 - 16 **Balfour HH**, Schiff GM, Bloom JE. Encephalitis associated with erythema infectiosum. *J Pediatr* 1970; **77**: 133-136 [PMID: 4317884 DOI: 10.1016/S0022-3476(70)80059-2]
 - 17 **Breese C**, Horner FA. Encephalopathy with erythema infectiosum. *Am J Dis Child* 1977; **131**: 65-67 [PMID: 835523]
 - 18 **Zerbini M**, Musiani M, Venturoli S, Gallinella G, Gibellini D, Gentilomi G, La Placa M. Different syndromes associated with B19 parvovirus viraemia in paediatric patients: report of four cases. *Eur J Pediatr* 1992; **151**: 815-817 [PMID: 1468455 DOI: 10.1007/BF01957931]
 - 19 **Watanabe T**, Satoh M, Oda Y. Human parvovirus B19 encephalopathy. *Arch Dis Child* 1994; **70**: 71 [PMID: 8110015 DOI: 10.1136/adc.70.1.71-a]
 - 20 **Watanabe T**. Ulcerative colitis that developed 8 years after human parvovirus B19 encephalopathy. *Eur J Pediatr* 2004; **163**: 341-342 [PMID: 15346923 DOI: 10.1007/s00431-004-1433-x]
 - 21 **Yoto Y**, Kudoh T, Asanuma H, Numazaki K, Tsutsumi Y, Nakata S, Chiba S. Transient disturbance of consciousness and hepatic dysfunction associated with human parvovirus B19 infection. *Lancet* 1994; **344**: 624-625 [PMID: 7914998 DOI: 10.1016/S0140-6736(94)92014-1]
 - 22 **Haseyama K**, Kudoh T, Yoto Y, Suzuki N, Chiba S. Detection of human parvovirus B19 DNA in cerebrospinal fluid. *Pediatr Infect Dis J* 1997; **16**: 324-326 [PMID: 9076823 DOI: 10.1097/00006454-199703000-00013]
 - 23 **Barah F**, Valley PJ, Chiswick ML, Cleator GM, Kerr JR. Association of human parvovirus B19 infection with acute meningoencephalitis. *Lancet* 2001; **358**: 729-730 [PMID: 11551584 DOI: 10.1016/S0140-6736(01)05905-0]
 - 24 **Kerr JR**, Barah F, Chiswick ML, McDonnell GV, Smith J, Chapman MD, Bingham JB, Kelleher P, Sheppard MN. Evidence for the role of demyelination, HLA-DR alleles, and cytokines in the pathogenesis of parvovirus B19 meningoencephalitis and its sequelae. *J Neurol Neurosurg Psychiatry* 2002; **73**: 739-746 [PMID: 12438480 DOI: 10.1136/jnnp.73.6.739]
 - 25 **Wierenga KJ**, Serjeant BE, Serjeant GR. Cerebrovascular complications and parvovirus infection in homozygous sickle cell disease. *J Pediatr* 2001; **139**: 438-442 [PMID: 11562626 DOI: 10.1067/mpd.2001.117070]
 - 26 **Bakhshi S**, Sarnaik SA, Becker C, Shurney WW, Nigro M, Savaşan S. Acute encephalopathy with parvovirus B19 infection in sickle cell disease. *Arch Dis Child* 2002; **87**: 541-542 [PMID: 12456562 DOI: 10.1136/adc.87.6.541]
 - 27 **Erol I**, Alehan F, Yalçın K. Refractory status epilepticus owing to human parvovirus B19 encephalitis in a child. *J Child Neurol* 2006; **21**: 820-822 [PMID: 16970896 DOI: 10.1177/08830738060210092301]
 - 28 **Laurenz M**, Winkelmann B, Roigas J, Zimmering M, Quersfeld U, Müller D. Severe parvovirus B19 encephalitis after renal transplantation. *Pediatr Transplant* 2006; **10**: 978-981 [PMID: 17096771 DOI: 10.1111/j.1399-3046.2006.00599.x]
 - 29 **Oshima K**, Kikuchi A, Mochizuki S, Arai T, Oishi T, Hanada R. Acute encephalopathy with human parvovirus B19 infection in hereditary spherocytosis. *Pediatr Infect Dis J* 2008; **27**: 651-652 [PMID: 18520445 DOI: 10.1097/INF.0b013e3181694fcf]
 - 30 **Greco F**, Barbagallo ML, Chiodo DC, Guglielmino R, Sorge G. Severe ataxia as a complication of human parvovirus B19 acute encephalitis in a child. *J Child Neurol* 2008; **23**: 1078-1080 [PMID: 18487517 DOI: 10.1177/0883073808315420]
 - 31 **Uchida Y**, Matsubara K, Morio T, Kawasaki Y, Iwata A, Yura K, Kamimura K, Nigami H, Fukaya T. Acute cerebellitis and concurrent encephalitis associated with parvovirus B19 infection. *Pediatr Infect Dis J* 2012; **31**: 427 [PMID: 22418658 DOI: 10.1097/INF.0b013e3182481e24]
 - 32 **Watanabe T**. Renal involvement in human parvovirus B19 infection. *Pediatr Nephrol* 2003; **18**: 966-967 [PMID: 12883967 DOI: 10.1007/s00467-003-1232-z]
 - 33 **Waldman M**, Kopp JB. Parvovirus B19 and the kidney. *Clin J Am Soc Nephrol* 2007; **2** Suppl 1: S47-S56 [PMID: 17699510 DOI: 10.2215/CJN.01060307]
 - 34 **Kerr JR**. A review of blood diseases and cytopenias associated with human parvovirus B19 infection. *Rev Med Virol* 2015; **25**: 224-240 [PMID: 25962796 DOI: 10.1002/rmv.1839]
 - 35 **Barah F**, Valley PJ, Cleator GM, Kerr JR. Neurological manifestations of human parvovirus B19 infection. *Rev Med Virol* 2003; **13**: 185-199 [PMID: 12740833 DOI: 10.1002/rmv.388]
 - 36 **Shulte DJ**. Human parvovirus B19. In: Cherry JM, Demmler-Harrison GJ, Kaplan SL, Holtez PJ, Steinbach WJ. Feigin and Cherry's textbook of pediatric infectious diseases. Philadelphia: Elsevier Saunders, 2014: 1843-1857
 - 37 **Hartley LM**, Ng SY, Dale RC, Church AJ, Martineza A, de Sousa C. Immune mediated chorea encephalopathy syndrome in childhood. *Dev Med Child Neurol* 2002; **44**: 273-277 [PMID: 11995896 DOI: 10.1017/S0012162201002055]
 - 38 **Grillo E**, da Silva RJ. Anti-N-methyl-D-aspartate receptor encephalitis and parvovirus B19: A possible link? *J Pediatr* 2013; **163**: 1233-1234 [PMID: 23919909 DOI: 10.1016/j.jpeds.2013.06.079]
 - 39 **Wright S**, Hachoen Y, Jacobson L, Agrawal S, Gupta R, Philip S, Smith M, Lim M, Wassmer E, Vincent A. N-methyl-D-aspartate receptor antibody-mediated neurological disease: results of a UK-based surveillance study in children. *Arch Dis Child* 2015; **100**: 521-526 [PMID: 25637141 DOI: 10.1136/archdischild-2014-306795]
 - 40 **Tada H**, Takanashi J, Barkovich AJ, Oba H, Maeda M, Tsukahara H, Suzuki M, Yamamoto T, Shimono T, Ichiyama T, Taoka T, Sohma O, Yoshikawa H, Kohno Y. Clinically mild encephalitis/encephalopathy with a reversible splenic lesion. *Neurology* 2004; **63**: 1854-1858 [PMID: 15557501 DOI: 10.1212/01.WNL.0000144274.12174.CB]
 - 41 **Takanashi J**. Two newly proposed infectious encephalitis/encephalopathy syndromes. *Brain Dev* 2009; **31**: 521-528 [PMID: 19339128 DOI: 10.1016/j.braindev.2009.02.012]
 - 42 **Takanashi J**, Shirai K, Sugawara Y, Okamoto Y, Obonai T, Terada H. Kawasaki disease complicated by mild encephalopathy with a reversible splenic lesion (MERS). *J Neurol Sci* 2012; **315**: 167-169 [PMID: 22129937 DOI: 10.1016/j.jns.2011.11.022]
 - 43 **Ansuini V**, Rigante D, Esposito S. Debate around infection-dependent hemophagocytic syndrome in paediatrics. *BMC Infect Dis* 2013; **13**: 15 [PMID: 23324497 DOI: 10.1186/1471-2334-13-15]
 - 44 **Miyata R**, Tanuma N, Hayashi M, Imamura T, Takanashi J, Nagata R, Okumura A, Kashii H, Tomita S, Kumada S, Kubota M. Oxidative stress in patients with clinically mild encephalitis/encephalopathy with a reversible splenic lesion (MERS). *Brain Dev* 2012; **34**: 124-127 [PMID: 21576007 DOI: 10.1016/j.braindev.2011.04.004]

- 45 **Kometani H**, Kawatani M, Ohta G, Okazaki S, Ogura K, Yasutomi M, Tanizawa A, Ohshima Y. Marked elevation of interleukin-6 in mild encephalopathy with a reversible splenial lesion (MERS) associated with acute focal bacterial nephritis caused by *Enterococcus faecalis*. *Brain Dev* 2014; **36**: 551-553 [PMID: 23978488 DOI: 10.1016/j.braindev.2013.07.012]
- 46 **McCoy B**, King M, Gill D, Twomey E. Childhood posterior reversible encephalopathy syndrome. *Eur J Paediatr Neurol* 2011; **15**: 91-94 [PMID: 21074464 DOI: 10.1016/j.ejpn.2010.10.002]
- 47 **Weigel-Kelley KA**, Yoder MC, Srivastava A. Alpha5beta1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of beta1 integrin for viral entry. *Blood* 2003; **102**: 3927-3933 [PMID: 12907437 DOI: 10.1182/blood-2003-05-1522]
- 48 **Cooling LL**, Koerner TA, Naides SJ. Multiple glycosphingolipids determine the tissue tropism of parvovirus B19. *J Infect Dis* 1995; **172**: 1198-1205 [PMID: 7594654 DOI: 10.1093/infdis/172.5.1198]
- 49 **Ozawa K**, Ayub J, Kajigaya S, Shimada T, Young N. The gene encoding the nonstructural protein of B19 (human) parvovirus may be lethal in transfected cells. *J Virol* 1988; **62**: 2884-2889 [PMID: 2969055]
- 50 **Srivastava A**, Bruno E, Briddell R, Cooper R, Srivastava C, van Besien K, Hoffman R. Parvovirus B19-induced perturbation of human megakaryocytopoiesis in vitro. *Blood* 1990; **76**: 1997-2004 [PMID: 2146978]
- 51 **Salimans MM**, van de Rijke FM, Raap AK, van Elsacker-Niele AM. Detection of parvovirus B19 DNA in fetal tissues by in situ hybridisation and polymerase chain reaction. *J Clin Pathol* 1989; **42**: 525-530 [PMID: 2732347 DOI: 10.1136/jcp.42.5.525]
- 52 **Isumi H**, Nunoue T, Nishida A, Takashima S. Fetal brain infection with human parvovirus B19. *Pediatr Neurol* 1999; **21**: 661-663 [PMID: 10513695 DOI: 10.1016/S0887-8994(99)00055-7]
- 53 **Hobbs JA**. Detection of adeno-associated virus 2 and parvovirus B19 in the human dorsolateral prefrontal cortex. *J Neurovirol* 2006; **12**: 190-199 [PMID: 16877300 DOI: 10.1080/13550280600827351]
- 54 **Grant JK**, Yin NC, Zaytoun AM, Waseem H, Hobbs JA. Persistent adeno-associated virus 2 and parvovirus B19 sequences in post-mortem human cerebellum. *Cerebellum* 2009; **8**: 490-498 [PMID: 19585179 DOI: 10.1007/s12311-009-0126-4]
- 55 **Manning A**, Willey SJ, Bell JE, Simmonds P. Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. *J Infect Dis* 2007; **195**: 1345-1352 [PMID: 17397006 DOI: 10.1086/513280]
- 56 **Skaff PT**, Labiner DM. Status epilepticus due to human parvovirus B19 encephalitis in an immunocompetent adult. *Neurology* 2001; **57**: 1336-1337 [PMID: 11591864 DOI: 10.1212/WNL.57.7.1336]
- 57 **Druschky K**, Walloch J, Heckmann J, Schmidt B, Stefan H, Neundörfer B. Chronic parvovirus B-19 meningoencephalitis with additional detection of Epstein-Barr virus DNA in the cerebrospinal fluid of an immunocompetent patient. *J Neurovirol* 2000; **6**: 418-422 [PMID: 11031695 DOI: 10.3109/13550280009018306]
- 58 **Nolan RC**, Chidlow G, French MA. Parvovirus B19 encephalitis presenting as immune restoration disease after highly active antiretroviral therapy for human immunodeficiency virus infection. *Clin Infect Dis* 2003; **36**: 1191-1194 [PMID: 12715316 DOI: 10.1086/374603]
- 59 **Hobbs JA**. Parvovirus B19-brain interactions: infection, autoimmunity, or both? *J Clin Virol* 2007; **38**: 364-365 [PMID: 17337241 DOI: 10.1016/j.jcv.2007.01.005]
- 60 **Wagner AD**, Goronzy JJ, Matteson EL, Weyand CM. Systemic monocyte and T-cell activation in a patient with human parvovirus B19 infection. *Mayo Clin Proc* 1995; **70**: 261-265 [PMID: 7861814 DOI: 10.4065/70.3.261]
- 61 **Moffatt S**, Tanaka N, Tada K, Nose M, Nakamura M, Muraoka O, Hirano T, Sugamura K. A cytotoxic nonstructural protein, NS1, of human parvovirus B19 induces activation of interleukin-6 gene expression. *J Virol* 1996; **70**: 8485-8491 [PMID: 8970971]
- 62 **Kerr JR**, Barah F, Matthey DL, Laing I, Hopkins SJ, Hutchinson IV, Tyrrell DA. Circulating tumour necrosis factor-alpha and interferon-gamma are detectable during acute and convalescent parvovirus B19 infection and are associated with prolonged and chronic fatigue. *J Gen Virol* 2001; **82**: 3011-3019 [PMID: 11714978 DOI: 10.1099/0022-1317-82-12-3011]
- 63 **Kerr JR**, Cunniffe VS, Kelleher P, Coats AJ, Matthey DL. Circulating cytokines and chemokines in acute symptomatic parvovirus B19 infection: negative association between levels of pro-inflammatory cytokines and development of B19-associated arthritis. *J Med Virol* 2004; **74**: 147-155 [PMID: 15258981 DOI: 10.1002/jmv.20158]
- 64 **Lehmann HW**, von Landenberg P, Modrow S. Parvovirus B19 infection and autoimmune disease. *Autoimmun Rev* 2003; **2**: 218-223 [PMID: 12848949 DOI: 10.1016/S1568-9972(03)00014-4]
- 65 **Lunardi C**, Tinazzi E, Bason C, Dolcino M, Corrocher R, Puccetti A. Human parvovirus B19 infection and autoimmunity. *Autoimmun Rev* 2008; **8**: 116-120 [PMID: 18700174 DOI: 10.1016/j.autrev.2008.07.005]

P- Reviewer: Anselm CWL, Sangkhathat S
S- Editor: Qiu S L- Editor: A E- Editor: Jiao XK





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

