International Journal of Infectious Diseases 83 (2019) 163–166

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

Postmortem evidence of disseminated Zika virus infection in an adult patient

Giri Shan Rajahram[a,b,*,1], Gillian Hale[c,1], Julu Bhatnagar[c,1], Jessie Hiu[d], Ravindran Thayan[e], Timothy William[f], Kum Thong Wong[g], Paul Anantharajah Tambyah[h], Tsin Wen Yeo[i,j]

[a] Infectious Diseases Unit, Department of Medicine, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia
[b] Clinical Research Centre, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia
[c] Infectious Diseases Pathology Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA
[d] Department of Forensic Medicine, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia
[e] Virology Unit, Infectious Disease Research Centre, Institute of Medical Research, Kuala Lumpur, Malaysia
[f] Jesselton Medical Centre, Kota Kinabalu, Sabah Malaysia
[g] Department of Pathology, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia
[h] Department of Medicine, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore, Singapore
[i] Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
[j] Institute of Infectious Disease and Epidemiology, Tan Tock Seng Hospital, Singapore

ARTICLE INFO

Article history:
Received 13 December 2018
Received in revised form 28 January 2019
Accepted 28 January 2019

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Introduction

Fatal complications associated with the Zika virus (ZIKV) have rarely been reported in adults, and detailed postmortem reports are limited (Musso and Gubler, 2016). ZIKV infection is typically a mild uncomplicated febrile illness associated with arthralgia, rash, and conjunctivitis (Duffy et al., 2009). In adults, fatal and non-fatal neurological complications have been reported, including encephalitis and Guillain–Barré syndrome (Soares et al., 2016; Oehler et al., 2014). Non-neurological fatalities have also been reported sporadically in patients from Latin America, who have presented with an acute febrile illness and multi-organ failure (Sarmiento-Ospina et al., 2016; Zonneveld et al., 2016). This report describes the clinical features and postmortem findings of a 61-year-old Malaysian male with co-morbidities, who had a fatal outcome subsequent to an acute febrile and rash illness, with pre- and postmortem evidence of ZIKV infection.

Case report

A 61-year-old man with a history of hypertension, ischemic heart disease, and chronic kidney disease (stage 3A), presented to a local clinic in Kota Kinabalu, Sabah, with a 3-day history of fever, chills, rigors, myalgia, lethargy, diarrhea, and a petechial rash on both legs. He had a temperature of 38 °C, heart rate of 80/min, and a blood pressure of 110/70 mmHg. The patient was treated symptomatically, but returned the following day and was admitted with increased lethargy and weakness. In addition to the rash over the lower limbs, he had signs of fluid overload. His temperature was 37.5 °C, blood pressure 74/42 mmHg, heart rate 82/min, and oxygen saturation 92%. The laboratory results on and after admission are summarized in Tables 1 and 2.

Chest radiography demonstrated cardiomegaly and mild fluid overload, and an electrocardiogram showed new onset atrial fibrillation. Tests for dengue NS1, IgM, and IgG (Panbio Dengue Early Rapid and Panbio Dengue Duo; Panbio), blood smear for malaria parasites, and a rapid test for *Leptospira* IgM (Leptorapide; Linnodee Diagnostics) were all negative. He was started on intravenous ceftriaxone 2 g once daily and metronidazole 500 mg three times daily for presumed sepsis and transferred to the intensive care unit for worsening respiratory distress and rapid

* Corresponding author.
E-mail address: gsrajahram@gmail.com (G.S. Rajahram).
[1] Giri Shan Rajahram, Gillian Hale, and Julu Bhatnagar contributed equally to the manuscript.

https://doi.org/10.1016/j.ijid.2019.01.047
1201-9712/© 2019 The Author(s). Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 1
Laboratory results for the patient.

	Reference range	Admission	Day 1	Day 2	Day 3
Hemoglobin (g/dl)	(13.5–17.5 g/dl)	12.8	12.7	13.7	14.9
Leukocyte count (/l)	(4.5–11 $\times 10^9$/l)	7.58	11.3	13.79	17.85
Neutrophils (%)	(40–80%)	85%	84%	78%	87%
Lymphocytes (%)	(20–40%)	8%	25.5%	14%	8.2%
Hematocrit (%)	(40–50%)	38.7	40.2	43.1	45.5
Platelet count (/l)	(150–450 $\times 10^9$/l)	156	193	246	255
ESR (mm/h)	(0–14 mm/h)	ND	70	ND	ND
CRP (mg/l)	(0–5 mg/l)	ND	180	ND	ND
Sodium (mmol/l)	(136–145 mmol/l)	131	133	134	137
Potassium (mmol/l)	(3.5–5.1 mmol/l)	4.1	5.4	5.2	5.3
Urea (mmol/l)	(1.0–8.3 mmol/l)	13.7	10.4	11.8	12.4
Creatinine (mmol/l)	(63–133 mmol/l)	209	146	186	210
Chloride (mmol/l)	(98–107 mmol/l)	99	105	106	109
Calcium (mmol/l)	(2.1–2.5 mmol/l)	2.33	2.27	2.23	2.07
Phosphate (mmol/l)	(0.74–1.52 mmol/l)	0.85	1.07	1.25	1.53
Magnesium (mmol/l)	(0.66–1.07 mmol/l)	0.73	0.72	0.84	1.03
Total bilirubin (μmol/l)	(3.4–20.5 μmol/l)	ND	8.2	11	ND
Direct bilirubin (μmol/l)	(0–8.6 μmol/l)	ND	7.2	7.4	ND
Alanine aminotransferase (U/l)	(0–55 U/l)	ND	17	24	ND
Aspartate aminotransferase (U/l)	(5–34 U/l)	ND	36	82	ND
Alkaline phosphatase (U/l)	(40–150 U/l)	ND	126	117	ND
Total protein (g/l)	(64–83 g/l)	ND	63	58	ND
Albumin (g/l)	(35–60 g/l)	ND	27	23	19
Globulin (g/l)	(23–56 g/l)	ND	35	35	ND
Creatine kinase (U/l)	(30–200 U/l)	92	200	ND	ND
Troponin I (pg/ml)	(0–34.2 pg/ml)	ND	5884	ND	ND
Lactate (mmol/l)	(0.5–2.2 mmol/l)	ND	1.6	7.1	3.1

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ND, not done.

Table 2
Culture and serology test results for the patient.

Blood culture	Negative
Serology	
Herpes simplex virus 1 PCR	Negative
Herpes simplex virus 2 PCR	Negative
Cytomegalovirus PCR	Negative
Varicella zoster virus PCR	Negative
Adenovirus PCR	Negative
Influenza virus A and B PCR	Negative
Enterovirus PCR	Negative
Leptospira PCR	Negative
HIV antibody	Negative
Hepatitis B surface antigen	Negative
Hepatitis C virus antibody	Negative
Leptospira IgM	Negative
Leptospira MAT	1:200 (acute titer)
Urine culture	Negative
Tracheal aspirate for culture	Negative
Tracheal aspirate for acid-fast bacilli	Negative
Blood film for malaria parasite	Negative
Mycoplasma serology	Negative

MAT, microscopic agglutination test.

atrial fibrillation with hemodynamic instability, which required repeated cardioversion.

Due to clinical deterioration, antibiotics were escalated to intravenous meropenem 1 g twice daily and doxycycline 100 mg twice daily. An echocardiogram showed global hypokinesia with a left ventricular ejection fraction of 30%, dilated chambers, and elevation of troponin I. The patient went into asystole 41 h after admission and required multiple resuscitation attempts to maintain a cardiac rhythm. However, he died the following day despite maximal intensive care support. The patient's pre-mortem serum and urine returned a positive result for ZIKV in RT-PCR testing performed.

An autopsy was conducted 4 h after the patient's death. On gross examination, the heart was enlarged with biventricular hypertrophy and dilatation of the left ventricle, circumferential endocardial fibrosis, subendocardial pallor, and postero-lateral wall myocardial fibrosis. The coronary arteries were calcified with significant atheromata with no thrombi noted. Other abnormal findings included bilateral lung edema, a small and contracted right kidney with hypertensive changes of the left kidney, gastritis, splenic congestion, and a fatty liver. The brain, meninges, esophagus, intestines, pancreas, and adrenals were normal. Microscopic examination demonstrated features consistent with acute myocardial infarction in the apex, anterior, antero-septal, and posterior wall of the left ventricle. The epicardium, right ventricle, and the non-infarcted area of the left ventricle revealed a diffuse lymphocytic infiltrate with scattered foci of myocytolysis, and clusters of CD3-positive lymphocytes and macrophages. Other abnormal microscopic findings included pulmonary edema, diffuse hepatic steatosis, splenic hemorrhage and congestion, acute gastritis, and adrenal lymphocytosis. There was no inflammation noted in the brain, brain stem, and meninges, and changes in the kidney were consistent with chronic disease.

At the US Centers for Disease Control and Prevention (CDC), RNA was extracted from formalin-fixed paraffin-embedded (FFPE) brain, kidney, spleen, liver, and heart tissues using an optimized extraction protocol, as described previously (Bhatnagar et al., 2012). All of the samples were tested by ZIKV NS5 and ENV-gene RT-PCR (Bhatnagar et al., 2017). The positive amplicons were directly sequenced on a GenomeLab GeXP sequencer. The search for homologies to known sequences was done using the nucleotide database of the Basic Local Alignment Search Tool (BLAST). To localize genomic and replicative ZIKV RNA, all FFPE tissues were also analyzed by ZIKV in situ hybridization (ISH) assay using antisense and sense riboprobes, respectively, targeting multiple genes of ZIKV, as described previously (Bhatnagar et al., 2017). ZIKV RT-PCR assays performed on FFPE tissues were positive for kidney, spleen, liver, and heart tissues, and sequence analysis of positive amplicons (NS5 and ENV) confirmed the presence of ZIKV, showing highest identities (99–100%) with Asian genotype strains circulating in Asia. Focal intense staining was observed by both antisense

(detects genomic viral RNA) and sense (detects replicative RNA) probes in epithelial cells of kidney tubules (Figure 1A). Similar granular staining was also observed in the cardiomyocytes of the heart, using both antisense and sense probes (Fig. 1B). The spleen showed focal granular staining with the sense probe (negative by antisense probe), and the liver was negative by ISH.

Discussion

Recent studies from populations and returning travelers suggest that ZIKV may be endemic or re-emerging in Southeast Asia (Musso and Gubler, 2016; Musso and Lanteri, 2017). Animal models of ZIKV pathogenesis have demonstrated broad tissue tropism and virus accumulation in a variety of organs (Miner and Diamond, 2017). In this patient, ZIKV was detected by RT-PCR and sequencing analysis in FFPE liver, kidney, spleen, and heart tissues; however, except for the heart, there were no significant inflammatory responses or cytopathic effects in the major organs. Postmortem cardiac examination demonstrated extensive infarction but without clear evidence of any intramural thrombus or vasculitis. This suggests the infarction was most likely the result of coronary hypoperfusion from hypotension rather than atherosclerotic plaque rupture. The non-infarcted heart had scattered foci of myocytolysis with clusters of lymphocytic infiltrates and macrophages. The lymphocytes were CD3-positive, suggestive of acute lymphocytic myocarditis (Myocarditis, 1987). There was molecular evidence of ZIKV in the heart by both PCR and ISH – and these findings may suggest a role for ZIKV in the histopathological findings. Myocarditis has been reported in dengue virus-infected patients and the present study findings raise the possibility that this may also occur in ZIKV infection – possibly resulting in depressed myocardial function and refractory hypotension and a fatal outcome (Yacoub et al., 2014). This hypothesis is supported by the lack of inflammatory responses or cytopathic effects in any of the other organs.

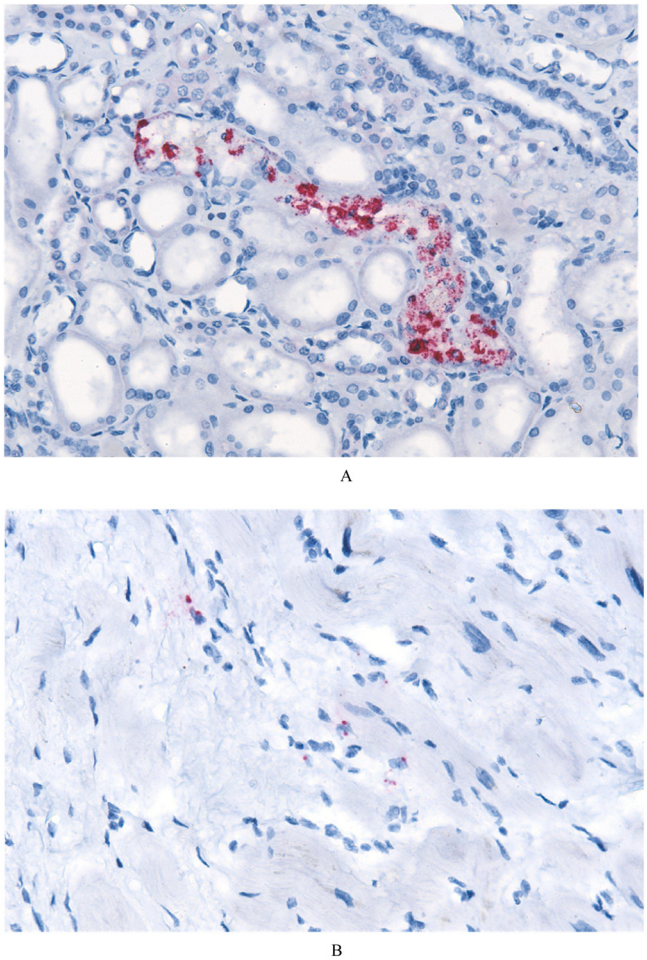

Figure 1. In-situ hybridization staining epithelial cells of kidney tubules and cardiomyocytes

In the case presented here, ZIKV genomic and replicating RNA was particularly localized to renal tubular epithelial cells, providing the first evidence for ZIKV replication in renal tubules of an adult patient, and raising the possibility that the kidneys could serve as a reservoir of ZIKV where the virus may persist. ZIKV RNA can be detected in urine as early as the first day of symptom onset, and as late as 20 days after symptoms first appear (de Campos et al., 2016).

In ZIKV renal pathogenesis, a viral cytopathic effect was observed in vitro in podocytes and renal glomerular endothelial cells and renal mesangial cells infected with ZIKV (Alcendor, 2017). A proposed model of ZIKV entry and persistence in the renal glomerulus involves viral dissemination across the glomerular endothelial cells to the parenchyma. Mesangial cells and podocytes have been shown to be permissive to ZIKV infection and may serve as amplification reservoirs resulting in persistent viruria (Alcendor, 2017).

Similar to the patient case presented here, six of the nine reported fatal cases were older (age range 59–73 years) with diabetes mellitus, hypertension, or cardiovascular disease (Sarmiento-Ospina et al., 2016; Zonneveld et al., 2016). All presented with a rapidly progressive febrile illness complicated by respiratory distress, refractory hypotension, and multi-organ failure, despite broad-spectrum antibiotics and respiratory and inotrope support (Sarmiento-Ospina et al., 2016; Zonneveld et al., 2016).

This report suggests ZIKV infection can result in fatal non-neurological complications in older patients with underlying co-morbidities. Large prospective studies are required to better delineate the effects of ZIKV infection in vulnerable populations.

Acknowledgement

The authors would like to thank the Director General of Health, Malaysia for permission to publish this report.

Funding

None.

Ethical approval

The study was registered and ethical approval was obtained from the Medical Research and Ethics Committee, Ministry of Health, Malaysia (NMRR-16-2049-32689). Written informed consent was obtained from the decedent's wife.

Conflict of interest

All authors declare no conflict of interest.

References

Alcendor DJ. Zika virus infection of the human glomerular cells: implications for viral reservoirs and renal pathogenesis. J Infect Dis 2017;216(July (2)):162–71.

Bhatnagar J, Blau DM, Shieh WJ, Paddock CD, Drew C, Liu L, et al. Molecular detection and typing of Dengue viruses from archived tissues of fatal cases by RT-PCR and sequencing: diagnostic and epidemiologic implications. AM J Trop Hyq 2012;86:335–40.

Bhatnagar J, Rabeneck DB, Martines RB, Reagan-Steiner S, Ermias Y, Estetter LB, et al. Zika virus rna replication and persistence in brain and placental tissue. Emerg Infect Dis 2017;23(March (3)):405–14.

Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536–43.
Miner JJ, Diamond MS. Zika virus pathogenesis and tissue tropism. Cell Host Microbe 2017;21(2):134–42.
Musso D, Gubler DJ. Zika virus. Clin Microbiol Rev 2016;29:487–524.
Musso D, Lanteri M. Emergence of Zika virus: where does it come from and where is it going to?. Lancet Infect Dis. 2017;17:255.
Aretz HT. Myocarditis:he dallas criteria. Hum Pathol 1987;18:619–24.
Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, et al. Zika virus infection complicated by Guillain-Barre syndrome–case report, French Polynesia, December 2013. Euro Surveill 2014;19.
Sarmiento-Ospina A, Vasquez-Serna H, Jimenez-Canizales CE, Villamil-Gomez WE, Rodriguez-Morales AJ. Zika virus associated deaths in Colombia. Lancet Infect Dis 2016;16:523–4.
Soares CN, Brasil P, Carrera RM, Sequeira P, de Filippis AB, Borges VA, et al. Fatal encephalitis associated with Zika virus infection in an adult. J Clin Micro 2016;83:635.
Yacoub S, Wertheim H, Simmons CP, Screaton G, Wills B. Cardiovascular manifestations of the emerging dengue pandemic. Nat Rev Cardiol 2014;11:335–45.
Zonneveld R, Roosblad J, Staveren JW, Wilschut JC, Vreden SG, Codrington J. Three atypical lethal cases associated with acute Zika virus infection in Suriname. ID Cases 2016;5:49–53.
de Campos MR, Cirne-Santos C, Meira GL, Santos LL, de Meneses MD, Friedrich J, et al. Prolonged detection of Zika virus RNA in urine samples during the ongoing Zika virus epidemic in Brazil. J Clin Virol 2016;77:69–70.