Glomerular capillary tuft collapse and podocytopathic changes in a newborn with congenital Zika virus syndrome

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ABSTRACT

Introduction: In early 2015, several patients presenting with dengue-like symptoms were described in Brazil’s northeast region. Reverse transcription polymerase chain reaction (RT-PCR) results from patients’ sera revealed the Zika virus (ZIKV) infection. In parallel, an unusually high incidence of microcephaly in newborns was observed in the same region. Zika virus infection can induce microcephaly and congenital abnormalities.

Case Report: A male newborn (9 months of gestation, cesarean delivery) died within 20 hours. The mother had a confirmed viral infection in the third month of pregnancy. This report describes a case of ZIKV intrauterine infection associated with histologic alterations in the newborn kidney tissue. Immunohistochemistry, RNA extraction, and real-time RT-PCR were performed for the confirmation of ZIKV infection in tissues. Kidney samples were stained using conventional hematoxylin-eosin (H&E), methenamine silver, and periodic acid-Schiff with diastase digestion techniques. Analysis of brain tissue revealed severely affected gray and white matter sites of neuronophagy, gliosis, and calcium microdeposits. Immunohistochemistry (4G2 and specific anti-ZIKV monoclonal antibodies) showed diffusely distributed immunopositivity in glial cells. Aspects compatible with a focal segmental glomerular capillary tuft collapse associated with podocytopathic features with pseudo-crescent formation were observed in the kidney tissue.

Conclusion: This case suggests the ability of congenitally acquired ZIKV to produce alterations in renal cells and describes histological details of kidney involvement.

Keywords: Kidney glomerulus, Newborn, Podocytes, Zika virus

INTRODUCTION

Zika virus (ZIKV) is an emerging flavivirus that belongs to the same family as Dengue (DENV), West Nile, and Yellow Fever viruses [1]. In early 2015, several patients presenting with dengue-like symptoms were described in the northeast region of Brazil. Reverse
transcription polymerase chain reaction (RT-PCR) results from patients’ sera revealed the country’s first autochthonous Zika virus (ZIKV) infection [2]. In parallel, an unusually high incidence of microcephaly in newborns was observed in the same region. By May 2017, more than 2700 confirmed cases of microcephaly in newborns had been reported.

Since then, it has been consistently reported that congenital infection by ZIKV not only causes microcephaly but also induces alterations in other organs. A list of clinical findings was reported in fetuses with the congenital ZIKV syndrome, such as neurological, ophthalmological, auditory, articular, and urogenital alterations, intrauterine growth restriction (IUGR), and abortions. Postmortem examination of neonates with congenital Zika virus syndrome revealed microcephaly in addition to ventriculomegaly, microencephaly, polymicrogyria, lissencephaly, hydrocephalus, cerebellar dysgenesis, dystrophic calcifications, and neuronal depletion, arthrogryposis, and pulmonary hypoplasia. Findings confirmed the presence of ZIKV in the nervous system and systemic organs, including the kidney [3–6]; however, the virus’s presence in the tissue was not associated with pathological changes in these cases.

Viral infections are essential causative agents in kidney disease and are responsible for significant morbidity and mortality. Epstein–Barr virus, cytomegalovirus, adenovirus, and polyomavirus (type BK) cause specific diseases in the kidney [7]. Other viruses lack direct histological evidence of viral replication in the kidney, such as hepatitis C or human immunodeficiency virus (HIV) but are associated with distinct histologic changes patterns. Histological alterations of the kidney tissue in newborns from ZIKV-infected mothers need a more detailed study to support the affected. The authors aimed to report the histological changes in the kidney tissue detected in a newborn case of intrauterine infection by ZIKV and the involvement of other tissues from a mother with confirmed intrauterine ZIKV infection.

**CASE REPORT**

A male newborn (9 months of gestation, cesarean delivery) died within 20 hours. The mother was a 26-year-old woman who lived in northeastern Brazil. This was her first pregnancy, and she denied the use of medications during pregnancy. The mother had a confirmed viral infection in the third month of pregnancy. She was also tested for other causes of intrauterine infections, such as Toxoplasmosis, Other Agents, Rubella (also known as German Measles), Cytomegalovirus, and Herpes (TORCH), Human immunodeficiency virus (HIV), Parvovirus, and was negative for all these serologies. In the third month of pregnancy, ultrasonography revealed fetal microcephaly and malformations of the limbs and genitalia.

No clinical signs of renal disease were detected, and, as the newborn died within 20 hours, no renal function tests were performed, such as urinalysis and 24-hour proteinuria.

A neonatal necropsy was performed on the tissue samples taken for study.

The Molecular Virology Laboratory of Carlos Chagas Institute/Fiocruz-PR (ICC/Fiocruz-PR) is one of the official sentinel laboratories assigned by the Brazilian Ministry of Health to perform ZIKV diagnosis. In this context, we describe one case of a newborn with congenital ZIKV syndrome. As the formalin-fixed paraffin-embedded (FFPE) samples were received to diagnose congenital syndrome by ZIKV, the patient’s clinical records were simplified. The autopsy was performed at the place of origin, in northeastern Brazil. We received the approval of the Brazilian National Ethics Committee of Human Experimentation under the number CAAE: 42481115.7.0000.5248 (http://aplicacao.saude.gov.br/plataformabrasil/login.jsf) to perform necessary research concerning ZIKV pathogenesis with the unidentified samples.

**Immunohistochemistry for the identification of the virus**

Antigen retrieval was performed using the BioSB ImmunoRetriever (Santa Bárbara, USA). The flavivirus-specific monoclonal antibody (Mab) 4G2 (hybridoma D1-4G2-4-15, ATCC HB-112) and specific anti-ZIKV Mab (produced at ICC/Fiocruz-PR) were used as a primary antibody (dilution = 1/100), followed by the secondary antibody (Reveal Polyvalent HRP-DAB Detection System, Spring Bioscience) with a 30-minute incubation at room temperature. The specificity of immunohistochemistry staining was confirmed by omitting the primary Mab or using the non-related anti-chikungunya virus Mab (produced at ICC/Fiocruz-PR). The immunostained slides were observed using an optical microscope (Olympus BX50, Tokyo, Japan) [3, 4].

**Viral RNA extraction and real-time RT-PCR**

Reverse transcription polymerase chain reaction was performed to diagnose the ZIKV infection. Total RNA was extracted from 3 mm cores punched from the FFPE tissue blocks using the ReliaPrep FFPE Total RNA Miniprep System (Promega). Real-time RT-PCR for detection of ZIKV RNA was performed, RNA was eluted in 50 µL of elution buffer, and 5 µL of extracted RNA was amplified by real-time RT-PCR using two primer/probe sets specific for ZIKV as described by Lanciotti et al. [8] and using the GoTaq Probe 1-Step RT-qPCR System (Promega) or the SuperScript III Platinum One-Step qRT-PCR System (Invitrogen) with amplification in the LightCycler 96 instrument (Roche). The amplification runs contained negative and positive controls. The negative controls consisted of a blank reagent with water and a negative
human serum sample. For the positive controls, RNA extracted from a virus stock or acute ZIKV human serum samples was used. The same tissue samples were also tested for DENV, another flavivirus endemic in the Northeast Region of Brazil. Real-time RT-PCR was performed using a published method to detect DENV-1, 2, 3 [9], and/or an unpublished method to detect the four DENV serotypes (primer sequences available upon request). RNA extracted from the four DENV-serotype virus stocks were used as positive controls and a blank reagent with water and a negative human serum sample as negative controls. To confirm the identity of the ZIKV in the case, the amplicon was cloned in a pGEM-T Easy vector (Promega). Nucleotide sequencing was performed by the Macrogen Sequencing Service (Seoul, South Korea) with upstream and downstream primers of the cloning site [3, 4].

**Kidney special techniques**

Formalin-fixed paraffin-embedded tissue samples from the brain, liver, spleen, and kidney were stained using a conventional H&E technique [3, 4]. Samples from the kidney were also stained using methenamine silver and periodic acid-Schiff with diastase digestion. Sections from the kidney FFPE tissue blocks were recut. The immunohistochemical reactions were automated for the following antibodies: Flex Monoclonal Mouse Anti-Human CD68 (clone PG-M1) Ready-to-Use, Flex Polyclonal Rabbit Anti-Human IgM Ready-to-Use, and Polyclonal Rabbit Anti-Human C3c Complement Ready-to-Use (Dako Cytomation, Glostrup, Denmark). Envision Flex Target Retrieval Solution was used for the antigen recuperation, and Envision FLEX/HRP was used as a secondary antibody. The immunohistochemical reactions were performed in a Dako Autostainer 48 [10].

H&E (hematoxylin-eosin) slides from the brain, liver, and spleen showed several pathological alterations. Histology of brain tissue revealed severely affected gray and white matter regions with extensive destruction, clusters of microglia and macrophages marking neuronophagia sites, diffuse microglial hyperplasia, severe gliosis with reaction of gemistocytic astrocytes, and microdeposits of calcium. Liver tissue sections showed moderate extramedullary hematopoiesis, and spleen and heart tissue displayed moderate vascular congestion.

Immunohistochemistry analysis in the brain sections for 4G2 and specific anti-ZIKV monoclonal antibodies revealed diffusely distributed immunopositivity in glial cells. No reliable immunopositivity was observed in the heart, liver, spleen, and kidney tissue samples. Reverse transcription polymerase chain reaction assays yielded positive results for ZIKV RNA in brain tissue samples and negative for liver and kidney tissue samples [3, 4].

H&E-stained kidney samples showed aspects compatible with a focal segmental glomerular capillary tuft collapse associated with podocytopathic changes (podocyte hypertrophy and hyperplasia) and pseudo-crescent formation in two out of the ten glomeruli analyzed. Histologically, a glomerulus was characterized by an implosive collapse of the capillary loops with wrinkling of the basement membrane and hypertrophy and hyperplasia of the podocytes, which tend to fill the Bowman’s space resembling crescents (Figure 1). No endothelial proliferation, hyaline droplets, or lipids were noted. There were no prominent tubule interstitial changes (interstitial fibrosis or tubular atrophy) in this case. Segmental tuft necrosis was observed in one glomerulus. No significant inflammation was found in any of the compartments. Arteries and arterioles looked normal. No immunohistochemical evidence of immune complex or complement deposition was demonstrated.

**DISCUSSION**

The congenital Zika virus syndrome can present microcephaly (most commonly observed), facial disproportionality, cutis gyrata, brainstem dysfunction including feeding difficulties, hypertonia, hyperreflexia and irritability, arthrogryposis, pulmonary hypoplasia,
hearing and ocular abnormalities, paralysis of the diaphragm, premature closure of the anterior fontanelle, polyhydramnios, heart defects [11], fetal growth restriction, placental malformation phenotypes, and perinatal demise [12]. Neuroimages can show calcifications, cerebellar hypoplasia, ventriculomegaly, and lissencephaly [13–15]. This newborn’s main changes in the kidney tissue were compatible with a focal segmental glomerular capillary tuft collapse associated with podocytopathic changes and pseudo crescent formation.

Focal segmental glomerulosclerosis (FSGS) in children is associated with low birth weight and is responsible for 10–20% of nephrotic syndrome cases [16]. The causes of FSGS are primary idiopathic FSGS and genetic, familial, and secondary FSGS due to systemic diseases. In most patients, the causative agent is still unknown, but the viral association is evident in some cases. In adults, the most-established FSGS-inducing virus is HIV. Other viruses are also suspected, such as hepatitis B virus, parvovirus B19, and cytomegalovirus, although viral infections are a rare cause of FSGS in children.

In the pathogenesis of FSGS in viral infections, the podocyte is the key structure, and direct infection of these cells or immediate damage through the virus or viral components must be considered [17]. The histological variants described for FSGS include the classic cellular variant, tip lesion, FSGS with mesangial hypercellularity, perihilar FSGS, and collapsing FSGS, which were all observed in this case [18].

Podocytopathies are glomerular diseases related to damage to podocytes, leading to protein loss. The histological patterns of podocytopathies are minimal change nephropathy, diffuse mesangial sclerosis, collapsing glomerulopathy, and FSGS. Collapsing glomerulopathy is a variant of FSGS and is characterized by progressive obliteration of capillary lumina due to the basement membranes’ shrinking. It is relatively uncommon in children and was initially described in patients with HIV. Other causes of collapsing glomerulopathy include infections, such as malaria and visceral leishmaniasis, drugs, autoimmune diseases, and hematologic malignancies. Histologically, collapsing glomerulopathy is characterized by the collapse of the capillary loops with contraction of the basement membrane and hypertrophy and hyperplasia of the podocytes and pseudo-crescent formation. There was no endothelial proliferation, hyaline droplets, or lipids noted. Prominent tubule interstitial changes are a feature in this condition. No significant deposits are noted in the membrane besides some IgM and C3 in the collapsing glomerulopathy. Electron microscopy shows the wrinkling of the basement membranes with marked hypertrophy of the podocytes. There was also a foot process effacement. Patients with this condition progress rapidly to renal failure and show resistance to steroid therapy [18–23].

Natural ZIKV infection in the human renal compartment has not been reported, and proteinuria is not a part of ZIKV infection or congenital ZIKV syndrome. Alcendor (2017) [24] infected primary human podocytes, renal glomerular endothelial cells (GECs), and mesangial cells with ZIKV and showed that glomerular podocytes, renal GECs, and mesangial cells are permissive to ZIKV infection. Although the histopathological findings reported in this study appear to be very similar to those described in HIV collapsing nephropathy, there is not yet any known link between HIV-related nephropathy and ZIKV congenital syndrome [20, 24, 25]. Chen et al. (2017) have recently also demonstrated that ZIKV could infect renal proximal tubular epithelial cells in immunodeficient mice in vivo and immortalized and primary human renal proximal tubular epithelial cells in vitro [26].

The examination of renal tissue of stillborn or newborn babies who died in the first 37 hour of life in Brazil has observed the presence of ZIKV infection mainly in the renal tubular cells in four out of eight fetal kidneys examined [27]. In the same country, the PCR analysis of postmortem examination of 7 neonates with congenital ZIKV infection detected the virus in liver, lung, and kidney tissue, showing that the ZIKV may infect multiple tissues [4]. In Mexico, a study with necropsies material of a premature neonate identified the kidneys as a significant niche for viral replication, and demonstrated the ability of congenitally acquired ZIKV to produce disseminated infections and viral tropism toward epithelial cells [28].

Some authors have described renal involvement in other arboviruses. Regarding renal involvement in Dengue, which could be the cause of increased mortality, acute glomerulonephritis, rhabdomyolysis, and hemolytic uremic syndrome have been described. The renal involvement in chikungunya seems to reveal histopathological findings related to acute interstitial nephritis and acute tubular necrosis [29, 30].

A small FFPE sample mainly limits this study. Therefore, other studies with more extensive and frozen samples and using electron microscopy and immunofluorescence techniques would be interesting to confirm our findings. Further studies in a more extensive series of cases will be needed to confirm the congenital ZIKV syndrome’s kidney histologic characteristics. A prospective follow-up of survivors will determine the long-term renal consequences of these histological findings.

**CONCLUSION**

This is a detailed description of kidney histological alterations in a newborn with congenital ZIKV syndrome. Although congenital ZIKV infection has not been detected in the kidney, the presence of the virus in the kidney tissue is not necessary to link the consequences of the glomerular lesions. However, histologic alterations may
suggest the presence of podocytopathic changes, notably a collapsing focal segmental glomerulonephritis.

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Author Contributions

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Conflict of Interest
Authors declare no conflict of interest.

Data Availability
All relevant data are within the paper and its Supporting Information files.

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