Abstract

Background A relationship between Zika virus (ZikV) infection in pregnancy and the occurrence of microcephaly was established during the Zika outbreak in Brazil (2015–2016). Neuropathological findings in congenital Zika syndrome helped to understand its pathogenetic mechanisms.

Results The most relevant postmortem findings in the central nervous system (CNS) of fetuses and neonates infected with ZikV early in gestation are microcephaly with ex-vacuo ventriculomegaly and large head circumference associated with obstructive hydrocephalus due to severe midbrain and aqueduct distortion. Babies with severe brain lesions are born with arthrogryposis. Histologically, there is extensive destruction of the hemispheric parenchyma, calcifications, various disturbances of neuronal migration, reactive gliosis, microglial hyperplasia and occasional perivascular cuffs of lymphocytes, also in the meninges. Hypoplastic lesions secondary to the lack of descending nerve fibers include small basis pontis, pyramids and spinal corticospinal tracts. Cerebellar hypoplasia is also common. Severe nerve motor nerve cell loss is observed in the anterior horn of the spinal cord.

Conclusion A spectrum of neuropathological changes, from severe microcephaly to obstructive hydrocephalus was observed. The severity of the lesions is directly related to the gestational age, the most severe occurring when the mother is infected in the first trimester. Infection of progenitor cells at the germinal matrix was demonstrated. The lack of spinal motor neurons is responsible for fetal acynesia and consequent arthrogryposis.

Keywords Congenital Zika virus infection · Neuropathology · Microcephaly · Ventriculomegaly · Calcifications · Migration disturbances

Introduction

Zika virus (ZIKV) infection during pregnancy causes serious brain anomalies as has initially been demonstrated with neuroimaging methods in fetuses [1] and then in neonates [2]. These include calcifications and microcephaly, but also ventriculomegaly associated with normal or large head circumference, abnormalities of the corpus callosum and cerebellar hypoplasia. Calcification is specially seen at the gray matter/white matter junction, but also at the basal ganglia and thalamus. There are few and small series of postmortem analysis of the central nervous system (CNS) in congenital ZIKV (CZIKV) infection [3–8]. All observed microcephaly with ventriculomegaly. Histologically, a diffuse severe brain damage with extensive destruction and calcifications were seen mainly at the gray and white matter junction, besides perivascular cuffs of lymphocytes, microglial nodules, and reactive gliosis. Cerebellar hypoplasia and meningeal and parenchymal inflammation varied in intensity and distribution, with predilection for a periventricular and perivascular distribution [5].

Chimelli and cols [9] reported the largest series of a neuropathological study involving ten cases, including three stillborn and seven neonates who survived from 15 min to 37 h. Six babies were born at term and four were premature (between 32 and 36 weeks of gestation). Nine mothers had ZIKV symptoms between the 4th and 18th and one in the 28th
gestational week. All babies had arthrogryposis, except one whose mother was infected at 28 weeks gestation. A large head circumference due to obstructive hydrocephalus related to severe midbrain distortion was observed in five cases; ex vacuo ventriculomegaly associated with microcephaly was present in four; the baby infected at 28 weeks gestation had normal head circumference and ventricles. The authors defined three patterns of CNS lesions with various degrees of destructive, calcification, hypoplasia, and migration disturbances [9].

Macroscopic findings

The first pattern is represented by severe ventriculomegaly due to midbrain damage with aqueduct stenosis/ataresia. The ventriculomegaly is usually asymmetric, especially in the occipital lobes where the parenchyma is very thin with agyria, sometimes acquiring a cystic appearance with abundant cerebrospinal fluid (Fig. 1). This finding corresponds to Blake’s pouch cyst observed on the ultrasound image [2].

The second pattern corresponds to small brains associated with mild/moderate (ex-vacuo) ventriculomegaly. In these cases, the cranial bones may be overlapped or fused with occipital prominence. Brain surface shows shallow sulci or agyria (Fig. 2), sometimes with a cobblestone appearance. In these cases, the aqueduct is patent (Fig. 3a) and even dilated.

Calcifications are easily detected in the cortical mantle, junction between gray and white matter, deep gray nuclei, and in the brainstem, especially in cases with

![Fig. 1](image1.png) Collapsed brains (a, b) due to severe ventriculomegaly. The parenchyma is very thin (c) without gyri. The cerebellum is hypoplastic and smooth (b, arrow).

![Fig. 2](image2.png) A very small and smooth cerebral hemisphere does not fill the cranial cavity. Congested veins are seen along the leptomeninges and dura mater.

![Fig. 3](image3.png) a Midbrain showing patent aqueduct (second pattern). b Distorted midbrain where the aqueduct is not visible (first pattern) and shows calcifications (c—H & E). In both patterns, the pons is small and flat due to the lack of descending fibers (d).
severe ventriculomegaly, when the midbrain is distorted and the aqueduct is not visible (Fig. 3b, c), a characteristic of the first pattern.

The hippocampus is frequently not well identified and may be vertically oriented or malformed. The corpus callosum is either absent (particularly when there is severe hydrocephalus) or very thin. The basal ganglia and thalami are small, malformed, or not well recognized. There is cerebellar hypoplasia with irregular or smooth cortical surface (Fig. 1b) and the fourth ventricle is enlarged. The whole brainstem is small, particularly the basis pontis which is flat (Fig. 3d), and the pyramids may not be detected.

The third pattern, observed when the infection occurs late in gestation, is a well-formed brain with mild calcification observed in deep hemispheric white matter.

In the three patterns, the leptomeninges are transparent, congested, or focally thickened.

**Histopathological findings**

In cases with severe ventriculomegaly, the cerebral parenchyma is very thin, sometimes consisting of remnants of germinal matrix and leptomeninges only, where hemosiderin-laden macrophages can be seen. Disturbances of neuronal migration in cerebral and cerebellar hemispheres and in the brainstem are frequent and include polymicrogyria, meningeal glioneuronal heterotopia (Fig. 4), and cortical dysplasia (Fig. 5). Large and irregular clusters of immature cells are usually seen along the ventricular surface and towards the
cortex, sometimes intermingled with fine or coarse calcification in various levels of the cerebral parenchyma (Fig. 6). The germinal matrix has various thicknesses, may be disorganized, and the ependyma may show multifocal erosions, granulations, and subependymal rosettes. Apoptotic bodies are occasionally observed along the ependymal surface and in some remnants of the germinal matrix. Myelination is practically absent in hemispheric white matter, while in deep gray nuclei and posterior fossa structures, very few myelinated fibers are seen in the deformed internal capsule and cerebellum, while in the brainstem and spinal cord, they are evident. There is also Wallerian degeneration and axonal spheroids in deep gray nuclei and the brainstem, associated with coarse and filamentous calcification, gliosis, and aqueduct stenosis/atrophy (particularly in cases with important ventriculomegaly).

The spinal cord has abnormal shape due to the lack, or very small corticospinal tracts (Fig. 7a), and also, motor nerve cell degeneration and loss, gliosis and calcification (Fig. 7b), which explain the arthrogryposis. Ventral roots are abnormally thinner than the dorsal ones, while the dorsal column, dorsal nerve roots, and ganglia are preserved and myelinated.

Inflammatory reaction represented by mild-to-moderate lymphocytic meningitis and perivascular parenchymal CD8+ T lymphocytes, CD68+ histiocytes, microglial hyperplasia, and gliosis are observed in some cases. Microglial nodule and mild lymphocytic infiltration in the choroid plexus are rare.

ISH (in situ hybridization) for Zika virus in the CNS may demonstrate the viral RNA in the meninges, and occasionally in the neocortex and in the germinal matrix, the latter only when the interval between infection and birth is short.

Conclusions

Histopathological findings are based on autopsy cases from babies with severe and dramatically abnormal cerebral lesions, whose mothers were infected by ZIKV in the first or beginning of the second trimester of gestation. When the infection occurs late in gestation, brain damage is mild, suggesting that the timing of infection during gestation is one of the most relevant risk factor for the development of congenital Zika virus syndrome.

The spectrum of lesions can be summarized as follows:

1) Disturbances of migration in cerebral, cerebellar hemispheres, and brainstem represented by abnormal clusters or bands of germinal matrix towards the cortex, meningeal glioneuronal heterotopias, polymicrogyria, and cortical dysplasia, in those who were infected earlier in pregnancy.

2) Destructive lesions with nerve cell degeneration; apoptosis; coarse and filamentous calcification in the hemispheres, basal ganglia, thalami, brainstem, and spinal cord; and spinal motor nerve cell loss.

3) Hypoplastic lesions defined by lack of descending fibers leading to small basis pontis, pyramids, and spinal corticospinal tracts.

4) Inflammation is mild with predominance of T lymphocytes CD8+ and no active necrotizing lesions.

Compliance with ethical standards

Conflict of interest

I would also like to inform that neither Dr. Avvad Portari nor myself have any conflict of interest concerning the publication of this manuscript.

References


