



## Short Communication

Dengue infection in pregnancy and its impact on the placenta<sup>☆</sup>

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## SUMMARY

A histopathological and immunohistochemical study was conducted in placental tissues and retained products of conception from 24 patients with confirmed dengue infection during pregnancy. The immunohistochemical assay was positive for dengue virus in 19 placental and three ovular remnants analyzed. The light microscopic findings were signs of hypoxia, choriodecidualitis, deciduitis and intervillitis and the viral antigens were found in cytoplasmic of the trophoblast, villous stroma and decidua. Our results suggest that immunohistochemistry could be used as a laboratory confirmation method for dengue in pregnant women, especially in endemic areas when embedded material is the only material available.

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## Introduction

The occurrence of dengue infection in pregnancy has been reported in the literature since 1948. A recent systematic review<sup>1</sup> reported several cases of antenatal complications associated with dengue, including miscarriage, stillbirth, and premature deliveries. In addition, maternal death due to dengue infection in pregnancy has been reported in Brazil.<sup>2,3</sup> In 2013, seven cases of vertical transmission were reported and confirmed by serology, RT-PCR, and immunohistochemistry.<sup>4</sup>

This study describes the fetal outcomes and the histopathological and immunohistochemical findings in placenta samples and retained products of conception in pregnant women with confirmed dengue infection.

## Materials and Methods

It is a descriptive study of fetal outcome and pathological alterations from 24 pregnant women with laboratory-confirmed dengue. Confirmation was defined as the presence of IgM by the method of antibody capture enzyme-linked immunosorbent assay and by the presence of nucleic acid by reverse-transcription polymerase chain reaction (RT-PCT) in maternal or neonatal serum, in the seasonal epidemics of dengue in the city of Rio de Janeiro, from January 2002 to May 2010.

Nineteen placentas and five ovular remnants were examined.

Pregnant women with seropositivity to HIV infection, hepatitis B, syphilis, toxoplasmosis, or rubella; preeclampsia and gestational diabetes were excluded.

Neonates were defined as premature if gestational age was inferior to 37 weeks and low weight if birth weight was less than 2,500 g.<sup>5</sup> Asphyxia was defined with Apgar score lower than 7 at the fifth minute of life.<sup>6</sup>

Placental weight and disk measurements and some of the characteristics of the fetal membranes, including color and transparency, were evaluated. The distribution and size of the chorionic vessels were evaluated in the fetus. The color and appearance of the placental lobes and tissue sections were examined. The color and thickness of the funicular vessels and

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Wharton's jelly were evaluated in the umbilical cord and the abnormalities recorded. After examination, the placental tissues were excised, and seven sections were obtained: from the umbilical cord, two cross-sections at opposite ends and a longitudinal section in the median region; from the membrane, a cross-section of the membrane roll; and from the placental disk, a sample of the cord insertion site and a sample from the middle and outer disks as well as of any other sites considered relevant. All of the tissues sections were stained with hematoxylin-eosin (HE).

The fetal membranes, umbilical cord, chorionic plate, villi, intervillous space, and decidua basalis were examined under a light microscope.

Immunohistochemistry was conducted with the murine IgG2A monoclonal anti-DENV complex (MAB8705; clone D3-2H2-9-21; Millipore, Billerica, Massachusetts) diluted 1:300 in phosphate-buffered saline and incubated overnight.

Paraffinized liver tissue from a patient who died from DENV was used as the positive control for the immunohistochemical analysis. For the negative immunohistochemical control,

2 different procedures were incorporated: (1) paraffinized liver tissue from the patient who had died of dengue, suppressing the monoclonal antibody, the primary antibody of the incubation step, using only the diluent solution (bovine serum albumin) and (2) paraffin-embedded placental tissue of patients without infectious disease on which the full technique was performed (without the deletion of the MAB).

## Results

The average gestational age at delivery was  $38 \pm 3$  weeks, and the average birth weight was  $2,881 \pm 543$  g. Damage to the fetus occurred as miscarriage in 5 cases (20,8%), fetal death in 2 cases (8,3%), prematurity in 3 cases (12,5%), and 7 symptomatic newborns (29%). Two newborns developed dengue shock syndrome (DSS) and were discharged without sequelae.

The pathological findings were described on Table 1. The light microscopic findings were shown in Figure 1. No changes were observed in the umbilical cord suggestive of maternal blood-borne

**Table 1**  
Clinical characteristics and outcomes of pregnant women with dengue, histopathological and immunohistochemical findings in the placenta and outcomes of the fetus.

Patient	Age	Period (days)	Gestational age (weeks) at birth	SEC <sup>***</sup> Mother	Laboratory confirmation		Signs of hypoxia <sup>****</sup>	Other changes on light microscopy	Immunohistochemistry <sup>*****</sup> – staining area	Outcome of the mother	Outcome of the fetus
					Mother	newborn					
1	17	58	39	Yes	IgM+		Yes	Choriodecidualitis, multifocal intervillitis	Villous stroma	Discharge	Discharge
2	38	1	12	Yes	IgM+		No	Choriodecidualitis	Trophoblast/villous stroma	Discharge	Miscarriage
3	16	8	34 <sup>**</sup>	Yes	IgM+		Yes	Deciduitis, PPA, sickled erythrocytes	Trophoblast	Discharge	Discharge
4	23	4	41	No	IgM+		No	Focal villitis	Decidua	Discharge	Discharge
5	33	13	34 <sup>**</sup>	Yes	IgM+		Yes	Choriodecidualitis, intervillitis, multifocal villitis, villous necrosis	Trophoblast/villous stroma	Discharge	Discharge
6	34	9	39	Yes	IgM+	DENV2	Yes	Choriodecidualitis, deciduitis, intervillitis, villitis, sickled erythrocytes	Trophoblast/villous stroma/decidua	Discharge	Discharge
7	17	11	40	No	IgM+		Yes	Choriodecidualitis, decidua basalis	Decidua	Discharge	Discharge
8	38	16	40	No	IgM+		Yes	PPA <sup>*****</sup>	Villous stroma/decidua	Discharge	Discharge
9	23	0	39	No	IgM–	IgM+	Yes	Deciduitis, villitis	Decidua/villous stroma	Discharge	Discharge
10	38	4	39	No	IgM+		Yes	Choriodecidualitis, intervillitis, villitis, infarction, intervillous thrombosis	Decidual/trophoblast	Discharge	Discharge
11	17	0	40	No	IgM+		No	Deciduitis	Decidua/villous stroma	Discharge	Discharge
12	19	3	38	No	IgM+		Yes	Villous hydrops	Trophoblast	Discharge	Discharge
13	26	3	39	No	IgM+	DENV2	Yes	Deciduitis, multifocal necro-proliferative villitis	Trophoblast/villous stroma/decidua	Discharge	Discharge
14	17	5	28	Yes	IgM+		Yes	Deciduitis, villitis, sickled erythrocytes	Trophoblast/decidua	<b>Death</b>	<b>Death</b>
15	19	13	11	Yes	IgM+		No	Deciduitis	Trophoblast	Discharge	Miscarriage
16	25	7	33 <sup>**</sup>	Yes	IgM+		Yes	Focal intervillitis	Villous stroma/decidua	Discharge	Discharge
17	21	1	40	No	IgM+		Yes	Irrelevant signs	Villous stroma/decidua	Discharge	Discharge
18	28	6	34	Yes	IgM+		Yes	Sickled erythrocytes	Villous stroma/decidua	<b>Death</b>	<b>Death</b>
19	36	3		Yes	IgM+		Yes	Choriodecidualitis, decidua basalis, multifocal villitis, subchorial intervillitis	Negative immunostaining	Discharge	Miscarriage
20	35	4	10	Yes	IgM+		No	Choriodecidualitis, intervillitis, diffuse villitis, decidua basalis	Negative immunostaining	Discharge	Miscarriage
21	17	6	42	Yes	IgM+		Yes	Choriodecidualitis	Trophoblasts/decidua	Discharge	Discharge
22	24	6	38	No	IgM+		Yes	Choriodecidualitis, PPA	Deciduous/trophoblast	Discharge	Discharge
23	32	3	12	No	IgM+		No	Multifocal intervillitis	Trophoblast/decidua	Discharge	Miscarriage
24	22	4	39	Yes	IgM+	IgM+	Yes	PPA	Villous stroma	Discharge	Discharge

\* Elapsed period between the onset of symptoms and childbirth.

\*\* Premature.

\*\*\* CAPILLARY LEAK SYNDROME IN THE MOTHER: hemoconcentration, hypotension, shock, pleural effusion, or ascites.

\*\*\*\* HYPOXIA: EDEMA OF THE VILLOUS STROMA, PRE-INFARCTION AREAS (INCREASE IN THE NUMBER OF SYNCYTIAL KNOTS AND REDUCED INTERVILLOUS SPACE), OR CHORANGIOSIS.

\*\*\*\*\* IHC: Immunohistochemistry.

\*\*\*\*\* PPA: Preterm placental abruption.

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