Syphilitic placentitis: demonstration of Treponema pallidum by immunoperoxidase staining

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Summary. We report a case of early congenital syphilis in which the placenta showed diffuse proliferative villitis and Treponema pallidum was identified by indirect immunoperoxidase stain in formalin-fixed paraffin-embedded placental tissue. This is the first report demonstrating T. pallidum in placental tissue using an immunohistochemical method.

Key words: Congenital syphilis – Villitis – Immunoperoxidase

Introduction

Since the advent of penicillin, the reported incidence of congenital syphilis has fallen to such an extent that it has become an almost forgotten disease. However, it is anticipated that the resurgence of syphilis in women of child-bearing age (Hira et al. 1985) will renew the interest of medical personnel, especially those involved in perinatal medicine. We report a case of early congenital syphilis confirmed by the direct demonstration of Treponema pallidum by a immunoperoxidase method in formalin-fixed, paraffin-embedded placental tissue.

Materials and methods

A female infant weighing 960 g was born at 28 weeks gestation to a 19-year-old primiparous woman. At birth the baby was asphyxiated and required intubation for resuscitation. She was transferred to the neonatal intensive care unit of the Kanagawa Children's Medical Center. On admission the infant had severe respiratory distress without hepatosplenomegaly or petechiae. The chest roentgenogram showed a reticular pattern with air-bronchograms. C-Reactive protein was 3.6 mg/dl and non-specific immunoglobulin M (IgM) was elevated at 340 mg/dl. She was mechanically ventilated and given cefotaxim sodium and amikacin sulphate with a diagnosis of congenital pneumonia. Bacterial cultures, including Ureaplasma urealyticum, and viral cultures of the nasopharynx were negative. Posterior nasal smear specimens were negative for Chlamydia trachomatis by both enzyme immunoassay and the direct fluorescein antibody method. Specific IgM antibodies to rubella virus and cytomegalovirus were not elevated by enzyme immunoassay. One month after admission, the attending neonatologist was informed that the mother had untreated syphilis. Subsequent studies of both the mother's and infant's serum revealed strongly positive results for the serological test for syphilis (STS). The infant's serum was positive for T. pallidum haemagglutination (TPHA)-IgM by the passive haemagglutination method. Her STS and TPHA-IgM became negative following two courses of ampicillin sodium. Bone roentgenographic series on the 33rd day of life showed a bone-in-bone appearance suggesting intrauterine malnutrition. She had two episodes of transient ileus on the 30th and 45th days of life. She developed a conjugated hyperbilirubinemia during the 2nd week of life with its peak values occurring in the 2nd month of life. After 4 months the patient was discharged from the neonatal intensive care unit without neurological or respiratory abnormalities.

The placenta, submitted 5 h after delivery, was fixed in neutral-buffer-formalin for 4 days and prepared for histological sections. After paraffin embedding, 3-μm-thick sections were prepared for the immunoperoxidase method. The positive control used was a syphilitic skin lesion (condylomata lata). Negative controls consisted of three placentas with villitis of unknown aetiology, three normal placentas, and autopsied materials of lung, adrenal and liver tissues without pathological findings.

The sections were deparaffinized, and incubated with methanol containing 0.3% hydrogen peroxide for 20 min, followed by incubation in 5% normal goat serum (Dako, Denmark) for 10 min. The effect of proteinase-K was checked with serial preparations of slides with 0.1% proteinase-K/0.01 M phosphate buffered saline (PBS) pH 7.4 at 37°C for up to 20 min. The anti-T. pallidum rabbit serum (The Japan Lyophilization Laboratories, Kiyose, Tokyo) was diluted 1:300, 1:900, and 1:2700 with 0.01 M PBS pH 7.4 containing 0.3% hydrogen peroxide for 20 min, followed by incubation in 5% normal goat serum (Dako, Denmark) for 10 min. The effect of proteinase-K was checked with serial preparations of slides with 0.1% proteinase-K/0.01 M phosphate buffered saline (PBS) pH 7.4 at 37°C for up to 20 min. The anti-T. pallidum rabbit serum (The Japan Lyophilization Laboratories, Kiyose, Tokyo) was diluted 1:300, 1:900, and 1:2700 with 0.01 M PBS pH 7.4 containing 1% bovine serum albumin. After soaking with the above-mentioned anti-T. pallidum serum for 60 min, each section was washed and incubated with a peroxidase-labelled anti-rabbit immunoglobulin (Fab fragment, MBL, Nagoya, Japan, diluted 1:50). Counterstaining was performed with haematoxylin.

Results

The 335-g placenta was moderately enlarged with a pale maternal surface. The fetal surface was cloudy and had
Fig. 1. The placenta. The villous stroma was hypercellular. Fetal vessel lumens showed varying degrees of narrowing with endovascular and/or perivascular proliferation. Haematoxylin and eosin, original magnification ×172.

Fig. 2. High power view of the placenta. A focus of necrotizing villitis is shown. Note infiltration of mononuclear cells and polymorphonuclear leucocytes. Haematoxylin and eosin, original magnification ×343.

Fig. 3. Immunoperoxidase stain with anti-Treponema pallidum antibody demonstrated numerous spirochaetes in the villous stroma. Original magnification ×343.