Journal of the Neurological Sciences 387 (2018) 119–123

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

Study of the placentae of patients with neuromyelitis optica spectrum disorder

Yanyu Chang[a,1], Yaqing Shu[a,1], Xiaobo Sun[a], Tingting Lu[a], Chen Chen[a], Ling Fang[a], Dan He[b], Chengfang Xu[c], Zhengqi Lu[a], Xueqiang Hu[a], Lisheng Peng[a], Allan G. Kermode[a,d,e], Wei Qiu[a,*]

[a] Department of Neurology, The Third Affiliated Hospital of SUN Yat-sen University, Guangzhou, China
[b] Department of Pathology, The Third Affiliated Hospital of SUN Yat-sen University, Guangzhou, China
[c] Department of Obstetrics, The Third Affiliated Hospital of SUN Yat-sen University, Guangzhou, China
[d] Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Department of Neurology, Sir Charles Gairdner Hospital, Queen Elizabeth II Medical Centre, Perth, Western Australia, Australia
[e] Institute of Immunology and Infectious Diseases, Murdoch University, Perth, Western Australia, Australia

ARTICLE INFO

Keywords:
Neuromyelitis optica spectrum disorder
Pregnant
Placenta
AQP4-IgG

ABSTRACT

Previous studies have shown that circulating AQP4-IgG may lead to negative consequences during pregnancy in patients with neuromyelitis optica spectrum disorder (NMOSD). The objective of this study was to explore whether AQP4-IgG influences pregnancy by affecting AQP4 expression and inducing placental inflammation in patients with NMOSD. We prospectively collected clinical data from six pregnant AQP4-IgG-seropositive NMOSD patients and their infants, and investigated AQP4 expression and placental inflammatory infiltration by comparing hematoxylin and eosin and immunohistochemical (AQP1, AQP4, C5b-9, IgG, CD3, CD8, CD20, and CD68) staining results with three normal controls. Four patients were term pregnant and their infants were normal for development, serum AQP4-IgG was positive at the time of birth, and three infants were negative for AQP4-IgG after 3 months. Two patients underwent induced abortion; one because of NMOSD relapse and another because of fetal malformation. Histological investigation showed normal structure of the chorionic villi, and no significant difference in the intensity of the immunohistochemical staining for AQP1, AQP4, and inflammatory markers in placentae of patients and the controls. Our results showed that there was no significant decrease in placental AQP4 expression, and no obvious placental inflammation or signs of damage in term placentae of NMOSD patients seropositive for AQP4-IgG.

1. Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune disease of central nervous system (CNS) that primarily affects women of childbearing age. Circulating antibodies against aquaporin 4 (AQP4, AQP4-IgG) is a critically important feature of NMOSD [1]. AQP4-IgG specifically binding to AQP4 protein located in the foot process of astrocytes in CNS triggers a complement-dependent immune response that leads to astrocyte damage, inflammatory infiltration, and axonal injury. Certain non-nervous system organs can also be affected, including the thyroid, exocrine glands, intestinal tract, and skeletal muscle [2].

Several previous studies have shown that circulating AQP4-IgG may increase the risk of miscarriage in NMOSD patients [2–5]. Saadou et al. showed in animal experiments that AQP4-IgG can specifically bind to AQP4 in placental trophoblasts, activating complement-dependent immune reactions and causing inflammatory infiltration and tissue damage in the placenta, eventually leading to miscarriage [3]. Immunohistochemical staining of the placenta of a pregnant woman with NMOSD who suffered spontaneous miscarriage showed complete loss of AQP4 immunoreactivity and diffuse, mainly perivascular, deposits of membrane attack complexes in the syncytiotrophoblasts [6], suggesting that maternal AQP4-IgG may lead to negative consequences during pregnancy.

In this study, we investigated the pathological changes in the placentae of six AQP4-IgG-seropositive pregnant NMOSD patients, to evaluate the possible correlation between AQP4-IgG and pregnancy outcome.

* Corresponding author at: Department of Neurology, The Third Affiliated Hospital of SUN Yat-sen University, Guangzhou, Guangdong 510630, China.
E-mail address: qiuwei120@vip.163.com (W. Qiu).
[1] Yanyu Chang and Yaqing Shu contributed equally to this manuscript.

https://doi.org/10.1016/j.jns.2018.01.040
Received 8 November 2017; Received in revised form 7 January 2018; Accepted 31 January 2018
Available online 02 February 2018
0022-510X/

2. Patients and methods

2.1. Patient enrollment

We prospectively enrolled child-bearing age female AQP4-IgG-seropositive patients diagnosed by the Neurology Department of the Third Affiliated Hospital of SUN Yat-sen University, China, from June 2014 to June 2017. During this period, four patients (Patients 1, 2, 3, and 4) delivered at term, one patient (Patient 5) terminated her pregnancy voluntarily because of disease relapse at 9-weeks' gestation, and one patient (Patient 6) terminated pregnancy voluntarily because of fetal malformation (ectrodactyly) at 24-weeks'gestation. Three randomly-selected healthy mothers with term-delivered infants served as normal controls. All included participants were willing to donate placentae and undergo blood tests (of both mother and baby). Patients 1, 2, 3, 4, and the controls all chose caesarean delivery, patient 5 underwent induced abortion under general anaesthesia, and patient 6 underwent induced labour under general anaesthesia to terminate pregnancy. Blood and placental samples were delivered to the laboratory and processed within 6 h.

This study was approved by the Ethics Committee of The Third Affiliated Hospital of SUN Yat-sen University. All participants have signed informed consent.

Maternal peripheral blood and infants' umbilical cord blood was collected and tested for AQP4-IgG titres by indirect immunofluorescence assay on human AQP4-transfected cells (EUROIMMUN) both at pregnancy termination and 3 months after delivery.

2.2. Pathological investigation

Immunohistochemical staining was performed as described previously by De Falco et al. [7]. Placental samples (chorionic villi in Patient 5) were divided into sections along the widest diameter, washed with sterile saline solution, and fixed in formalin within 30 min. Then sections were embedded in paraffin, cut at 2 μm consecutively and stained with hematoxylin and eosin for verification. All sections were then deparaffinized in xylene, rehydrated through graded concentrations of alcohol, and washed in phosphate-buffered saline (PBS). Tissue sections were quenched sequentially in 3% hydrogen peroxide and blocked with PBS-6% non-fat dry milk (Bio—Rad, Hercules, CA, USA) for 1 h at room temperature. Slides were then incubated at 4 °C for 24 h with antibodies against AQP1 (Santa Cruz Biotechnology, Dallas, TX, USA; sc20810, 1:400), AQP4 (Santa Cruz Biotechnology, Dallas, TX, USA; sc20812, 1:300), C5b-9 (Santa Cruz Biotechnology, Dallas, TX, USA; sc58935, 1:400), CD3 (Dako Denmark, Glostrup, Denmark;L26, 1:400), CD8 (Dako Denmark, Glostrup, Denmark;DK25, 1:400), CD20 (Dako Denmark, Glostrup, Denmark; L26, 1:400), andCD68 (Leica Microsystems Inc., Deerfield, IL, USA;514H12, 1:400). After several washes by PBS (3 × 5 min) to remove excess antibody, the slides were incubated with diluted anti-goat biotinylated antibody (Vector Laboratories, Burlingame, CA, USA) for 1 h. All slides were then processed using the ABC method (Vector Laboratories) for 30 min at room temperature. Novared (Vector Laboratories) was used as the chromogen, and hematoxylin was used as the nuclear counter stain. All slides were stained in a single batch to receive equal staining. AQP1, AQP4, IgG, and c5b-9 staining were judged as positive by cytoplasmic staining of cytotrophoblasts or syncytiotrophoblasts. Staining intensity was weighted as described by Ma et al. [8]. Intensity of the immunohistochemical staining colour was graded as 0 (no colour), 1 (light yellow), 2 (light brown), or 3 (brown),and the percentages of coloured cells/area was graded as 0 (< 5%), 1 (5%–25%), 2 (25%–50%), 3(51%–75%), or 4 (> 75%). The two grades were then added as an indicator of overall staining intensity. CD3, −8, and −20 staining were judged as positive by cytoplasmic staining of lymphocytes, and CD68 staining was judged as positive by cytoplasmic staining of macrophages. Inflammatory infiltrate was graded as 0 (no inflammatory cell infiltration), 1 (mild inflammatory cell infiltration), 2 (moderate inflammatory cell infiltration or < 50 aggregated inflammatory cells), 3 (> 50 aggregated inflammatory cells as an organized “focus”), and 4 (more than one focus) [9]. A representative section was evaluated for each sample by two skilled pathologists at different times, averaging the scores.

2.3. Statistics

Statistical analysis was performed using SPSS version 19.0 software (IBM Inc., Armonk, NY, USA), and $P < 0.05$ was considered statistically significant. Differences between patients and the controls were compared using the Mann-Whitney U test.

3. Results

3.1. Study population

Peripheral blood of infants from Patients 1, 2, 3, 4, and 6 was collected, and their serum AQP4-IgG titres were 1:32, 1:32, 1:320, 1:10 and 1:10, respectively. Three months after delivery, AQP4-IgG titres were negative in the peripheral blood of infants from Patients 1, 2, and 4, but still positive in the infant from Patient 3, although the titre had decreased to 1:100. The weights of the four full-term infants were 3050 g, 3900 g, 3000 g, and 3100 g (Patients 1, 2, 3, and 4, respectively); all within the normal weight range for Chinese full-term newborns. Apgar scores 1 min after birth were 10, 10, 8, and 10 (Patients 1, 2, 3, and 4, respectively); the score for the newborn from Patient 3 was lower by 2 levels because of limb cyanosis and poor response to stimulating the sole of the foot. Apgar score 5 min after birth were 10 in all infants. Detailed information for the mothers and infants is shown in Table 1.

3.2. Pathological findings

All placentae were macroscopically normal, and histological evaluation of the placentae showed normal chorionic villus structure (Fig. 1A). Some of the NMOSD patients had normal AQP4 expression in trophoblasts, but two patients (Patients 1 and 3) had loss of AQP4 immunoreactivity (Fig. 1E). Membrane attack complex deposits were seen in the samples from Patients 5 and 6 (Fig. 1G), and different degrees of IgG infiltration in cytotrophoblasts and stroma of the chorionic villi were seen in samples from all patients (Fig. 1I). All samples had no or mild CD3+ and CD8+ lymphocyte infiltration (Fig. 1K and M), while moderate CD20+ lymphocyte and macrophage infiltration as well as cell aggregation were seen some patients (Fig. 1O and Q). However, the intensity of immunohistochemical staining for AQP1, AQP4, and inflammatory markers in placentae (and chorionic villi in Patient 5) of both patients and the controls were not statistically significantly different (Table 2).

4. Discussion

AQP4 is found in the cytoplasm of syncytiotrophoblasts, cytotrophoblasts, stroma of placental villi, and placental endothelial cells, playing roles in the regulation of maternal-fetal fluid exchange, as well as in the regulation of amniotic fluid volume homeostasis [7]. Several studies have evaluated the relationship between circulating AQP4-IgG and the risk of miscarriage in NMOSD patients. NMOSD patients were found to have a higher risk of miscarriage [2–4], and pregnancies after NMOSD onset were associated with a significantly increased risk of miscarriage compared with pregnancies before NMOSD onset [4,5], suggesting that AQP4-IgG may be a causative agent. Studies of animal models and placentae from NMOSD patients suffering miscarriage also showed that AQP4-IgG can cause placental inflammation and lead to negative pregnancy outcomes [3,6]. Theoretically, AQP4-IgG can cause