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Expression of Aquaporin 1 and Aquaporin 3 in Fetal Membranes and Placenta in Human Term Pregnancies with Oligohydramnios

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ABSTRACT

Objective: To explore the pathophysiology of oligohydramnios, the association between the expression of aquaporin 1 and aquaporin 3 in fetal membranes and placenta and oligohydramnios was investigated.

Methods: Sixty patients underwent elective cesarean sections at term were studied, 30 patients with isolated oligohydramnios and the other 30 with normal amniotic fluid volume (AFV). Real-time polymerase chain reaction and immunohistochemistry were employed to determine expression and localization of aquaporin 1 and aquaporin 3 in amnion, chorion and placenta, respectively.

Results: The expression of aquaporin 1 and aquaporin 3 was detected in amnion, chorion and placenta using real-time RT-PCR. By immunohistochemistry, aquaporin 1 and aquaporin 3 protein expressions in amnion epithelia and chorion cytotrophoblasts were identified. In placenta, aquaporin 1 was detected in placental vessels, while aquaporin 3 was found in trophoblast cells. In comparison to normal AFV group, there was a significant decrease of aquaporin 1 expression in amnion in oligohydramnios group, but no significant difference in chorion and placenta between the two groups. The expression of the aquaporin 3 in amnion and chorion in oligohydramnios group was significantly decreased, while expression in placenta was significantly increased compared with that in normal AFV group.

Conclusions: Alteration of aquaporin 1 and aquaporin 3 expression in fetal membranes and placenta may be important in the pathophysiology of isolated oligohydramnios.

1. Introduction

Normal amniotic fluid volume (AFV) is critical for normal fetal growth, movement and development. Reduction in AFV, especially oligohydramnios is associated with increased perinatal morbidity and mortality caused by umbilical cord compression, fetal distress, meconium staining, and meconium aspiration syndrome [1]. However, the detailed dynamics of the reduction in AFV during near-term and postdate pregnancy remain unclear.

The maintenance of AFV is a delicate balance between the amount of AF produced and the amount removed from the amniotic cavity. In the latter half of gestation, the primary sources of AF include fetal urine production and fluid secreted by the fetal lung, a result of maternal to fetal water flux [2]. The primary pathway for reabsorption of human AF is in the fetal gut after swallowing. An additional route of AF absorption is the intramembranous (IM) pathway; a critical regulatory pathway for AFV homeostasis [3]. The IM pathway describes the movement of water and solutes from the amniotic cavity, across the amnion and chorion overlying the placenta, directly into the fetal blood vessels beneath the placental surface [4].

Though the molecular mechanisms responsible for water and solute absorption across the fetal membranes by IM pathway are not well understood, studies showed one family of membrane proteins known as aquaporins (AQPs) appears to be particularly important in this pathway [5]. AQPs function as water channels and facilitate osmotically driven water movement across biological membranes. Up to now, thirteen members (AQP0–AQP12) have been found in mammals, and they have been detected in a wide variety of tissues [6,7].

Aquaporin 1 (AQP1), the first aquaporin to be named was originally found in human erythrocytes and since then has been found to have a wide distribution throughout the body including kidney, brain, heart and lung [8]. AQP1 mRNA has been demonstrated in murine and ovine placenta [9,10], while protein expression has been demonstrated in the fetal membrane at term in human pregnancies [11]. Unlike the exclusively water permeable AQP1,

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aquaporin 3 (AQP3) is also highly permeable to glycerol and urea in addition to water. AQP3 originally isolated from rat kidney has also been found to exist in numerous tissues including ear, eye, skin, gut and muscle [12]. Both AQP3 mRNA and protein were detected in human amnion [13]. AQP3 expression was also found in trophoblast cells in human placenta of normal term pregnancies [14]. Collectively, these evidences suggest that AQP1 and AQP3 may play an important role in regulating water flux across both fetal membrane and placenta.

Recently, several studies have explored the relationship between the expression of AQPs in fetal membrane and abnormal AFV [4,15,16]. Transgenic AQP1 knockout mice had a greater volume of amniotic fluid and lower amniotic fluid osmolality [4]. Therefore, authors hypothesized that the decrease of AQP1 expression caused idiopathic polyhydramnios. But in the following study, Mann et al. [15] found AQP1 was increased in fetal membrane derived from patients with idiopathic polyhydramnios, which suggested that alterations in AQP1 expression may be a compensatory response to and not a cause of idiopathic polyhydramnios. Shioji et al. [16] set up an oligohydramnios model using prostaglandin F2α receptor (FP)-deficient mice and demonstrated that AQP8 expression in the fetal membrane was decreased when oligohydramnios occurs. To the best of our knowledge, there is no report published to date about either the expression of AQP1 or AQP3 in fetal membranes and placenta in human term pregnancies with oligohydramnios.

In the present study, to explore the pathophysiology of oligohydramnios, real-time polymerase chain reaction (PCR) and immunohistochemistry were employed to determine expression and localization of AQP1 and AQP3 in human amnion, chorion and placenta at term, who presented with either oligohydramnios or normal AFV. The association between the expression of AQP1 and AQP3 and oligohydramnios was also investigated.

2. Materials and methods

2.1. Tissue specimens

2.1.1. Case selection

Between June 2006 and October 2007, 60 patients who requested and had elective cesarean deliveries were studied. Thirty patients had isolated oligohydramnios and the other 30 had normal AFV. All patients, whose age ranged from 22 to 36 years, had pregnancies between 37 and 40 weeks' gestational age. Excluded from both groups were pregnancies with echographically confirmed fetal abnormalities, premature rupture of the membranes, small for gestational age or growth restricted fetuses. Additionally, patients with any other maternal or fetal disorder that might have influenced AFV, such as gestational or pre-gestational diabetes, hypertensive disorders, cardiovascular diseases, autoimmune disease, etc. were excluded.

2.1.2. Diagnosis criteria for oligohydramnios

Before cesarean delivery, using real-time B-scan ultrasound, the amniotic fluid index (AFI) was measured by the summation of the vertical diameter of the largest amniotic fluid pocket in each of the four quadrants, with the linea nigra and umbilicus as landmarks, described initially by Phelan et al. [17]. The maximum vertical amniotic fluid pocket diameter in each quadrant not containing cord or fetal extremities was measured in centimeters; the sum of these measurements was the AFI. Oligohydramnios was defined as an AFI equal to or less than 5.0 cm [17,18], while an AFI of 8.0–18.0 cm was described as normal AFV.

The AFV was reassessed at abdominal delivery with the technique described by Horsager et al. [19]. Plastic drapes with large plastic pockets were used on all patients to facilitate the collection of the AF at time of cesarean section. After the bladder had been reflected away from the lower uterine segment, a 1-cm incision was made in the uterus, and the AF was aspirated into a suction collection device. The suction device used to collect the AF was in addition to the suction device used for the aspiration of blood or irrigating fluid from the operative field. The suctioning was continued until the AF no longer flowed freely. The fetus was then delivered, and any remaining AF in the uterus, operative field, and drape pockets was collected before delivery of the placenta. The collected fluid was measured, and a 3 ml specimen was collected in a tube containing ethylenediaminetetraacetic acid. The hemoglobin concentration was measured in the fluid specimen with a Sysmex XE-2100 automated hematology analyzer (Toa Medical Electronics, Kobe, Japan) in order to ascertain the volume of blood contamination. The patient's pre-operative hemoglobin was used for the calculation of the blood volume in the AF. This value was then subtracted from the collected fluid to calculate the corrected true AFV.

Oligohydramnios was defined as an AFV less than 300 ml, while an AFV 300–2000 ml was considered normal [20]. Eight patients in the oligohydramnios group were excluded from the study because the AFV measured directly at cesarean delivery was discordant with the pre-operative AFI determined by ultrasound.

2.1.3. Fetal membrane and placenta tissues

Immediately after delivery, placentas near the umbilical cord insertion were isolated by gently separating amnion, chorion and placenta. Half of the tissues were fixed in a 10% formaldehyde neutral buffer solution and embedded in paraffin. The other tissues were quick frozen in liquid nitrogen and stored at −80 °C until further analyses. All tissues were rinsed thoroughly in phosphate-buffered saline (PBS) solution to remove excess blood prior to fixing and freezing. Tissue specimens were obtained following the informed consent of all the patients and approval of the local research ethical committee.

2.2. Real-time PCR

Tissue was homogenized in Trizol reagent (Invitrogen Inc, Carlsbad, CA) and total RNA was isolated. The first-strand cDNA was synthesized from 2 μg RNA using random primers and M-MLV reverse transcriptase (200 U/μl, Fermentas, Hanover, MD). The reaction mix for cDNA synthesis was made up of 2 μl RNA (1 μg/μl) and 1 μl oligo (dT) 18 primer (0.5 μg/μl) and 9 μl DEPC water. This was heated at 70 °C for 5 min, snap cooled in ice for 1 min and the following were added: 5× reaction buffer – 4 μl, dNTP mix (2.5 mM each) – 2 μl, Rnase inhibitor (20 U μl) – 1 μl. This was kept at 25 °C for 5 min. Then 200 U of M-MLV reverse transcriptase (1 μl) were added. This reaction mix was kept in a thermal cycler with following cyclic conditions. 25 °C for 10 min, 42 °C for 60 min and 70 °C for 10 min. The cDNA synthesized was stored at −20 °C until further use.

PCR amplification was performed using a PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Primers and probes for human AQPs and β-actin were purchased from Applied Biosystems (ABI, Foster City, CA) and amplified in separate tubes. PCR reaction was run with a total volume of 25 μl in each well containing 1.25 μl of TaqMan primers and probes (AQPs or β-actin), 12.5 μl of TaqMan Universal PCR Master Mix (ABI), 5 μl of cDNA template and 6.25 μl of DEPC H_2O. Activation of DNA polymerase was carried out at 95 °C for 10 min, followed by 40 cycles consisting of denaturing at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. All assays were performed in triplicate.

Comparative threshold cycle (C_T) method was used for relative quantification. For amplicons designed to less than 150 bp and for which the primer and Mg^{2+} concentrations have been properly optimized according to ABI guidelines, the efficiencies of AQPs and β-actin were approximately equal. The plot of log input amount versus ΔC_T had a slope of 0.0306, approximately zero. Therefore, the amount of AQPs gene in oligohydramnios group, normalized to β-actin and relative to that in normal AFV group, was given by $2^{-\Delta\Delta C_T}$ [21].

In addition, the intra-assay and inter-assay coefficient of variation (CV) was calculated for each reaction to evaluate the reproducibility of the assay. Intra-assay precision was evaluated using 12 replicates of each gene, for two patients, in a single experiment. Inter-assay precision was evaluated on 8 experiments, using duplicate tests of each gene for two patients [22].

2.3. Immunohistochemistry

2.3.1. Microwave antigen retrieval

Paraffin sections (4 μm) were cut and mounted on 2% silanized slices, dried, dewaxed, rehydrated, and washed with PBS. Then, slides were heated in 10 mM citrate buffer (pH 6.0) in a commercial microwave oven (25 min at 750 W power setting). Then, the slides were cooled to room temperature and washed three times for 5 min in PBS.

2.3.2. Staining procedure

Tissue slices were incubated 15 min in 0.3% hydrogen peroxide in methanol to suppress endogenous peroxidase activity and then incubated with 1.5% non-immune goat serum for 15 min to prevent non-specific binding. After incubated overnight at 4 °C with either anti-AQP1 or anti-AQP3 polyclonal rabbit antibodies (sc-20810, sc-20811, Santa Cruz Biotechnology Inc, Santa Cruz, CA) at a dilution of 1:100, biotinylated goat anti-rabbit antibody was used as secondary antibody for 30 min at room temperature. Streptavidin peroxidase was used as label for 20 min and diaminobenzidine as chromogen for 10 min. The sections were washed with PBS after each step of the procedure, and counterstained with Mayer hematoxylin to enhance nuclear detection, dehydrated, and mounted in distrene dibutylphthalate xylene. Appropriate positive and negative control slides were stained in parallel. Human renal tissue was used as a positive control. Immunostaining of the negative control was incubated with PBS in the absence of primary antibody. The AQPs-positive cell showed yellowish brown staining of the cytoplasm and/or membrane under the microscope (Olympus-FM10).