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Aquaporin-5, Subunit Beta in the Sodium Channel Epithelium, Lung Ultrasonography Examination in Transient Tachypnea of the Newborn

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ABSTRACT

Studies have identified several isoforms of aquaporin that expressed in many types of cells and located in different locations in human body. Aquaporin plays an important role in water regulation system by providing the water channels to facilitating water transport through the barriers. In neonates, aquaporin helps pulmonary fluid clearance as quick as after the birth time by absorption mechanism to allows the air exchange normally and avoid the respiratory distress as in transient tachypnea of the newborn (TTN). Subunit beta in the sodium channel epithelium also plays a role in respiratory distress of the newborn. Some pathological condition affect the aquaporin expression. Study showed that aquaporin 5 expression was higher in TTN group than the control and respiratory distress syndrome group. The primary findings of lung ultrasonoghraphy in diagnosing TTN are double lung point and alveolar interstisial syndrome.

Keywords: Aquaporin 5, Subunit beta in the natrium channel epithelium, X-ray, Lung ultrasonography, Transient tachypnea of the newborn.

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INTRODUCTION

The internal surface of lung is formed by two types of cells known as epithelial type 1 and type 2, both are have different cell shapes and functions.[1] Most lung surface covered by type 1 cells squamous shaped for air exchange during the respiration process, their performance is identified by phenotypic specific markers, one of them is aquaporin 5. Type 2 cells are cuboid-shaped, they play important role in the synthesis of surfactant proteins, such as SFTPA.[2]

The process of fluid transfer between the airspace to the capillaries occurs by osmosis and across several barriers (i.e. epithelial cells, interstitial and endothelial spaces).[3,4,5,6] Aquaporin is an important protein in water regulation system by providing the water channels that facilitating the water transport through high permeability epithelial cells and lung microvascular. [7,8,9,10] Although it facilitates the water transport, aquaporin selectively prevent the passage of ions and other solute materials through the membrane by a cluster of amino acids as a selective filter, called as arginine or ar/R.[11,12,13]

Horsefield et al examined the structure of aquaporin 5 in humans (HsAQP5) with high-resolution X-ray. HsAQP5 contains several phosphorylation sites and has terminal C and N that shaped similarly to those that found in aquaporin 1. The shape of crystal aquaporin 5 look like a stacked two-dimentional membranes. The results of this study also showed the structure of aquaporin 5 tetramer and its overlays 4 protomers. All of these protomers showed the same water channel profiles when measured by using HOLE. There is a cavity that narrows in the extracellular surface area of aquaporine 5, it will be filled by lipids and cause occlusion. The occlusion will prevents the passage of air and ions through the center of tetramer.[14]

There are six alpha helical domains that stretch along the membrane, accompanied by carboxylates and amino terminals on both sides of the aquaporin.[15] Aquaporin is also involved in the physiological human body response to pulmonary edema condition after acute lung injury (ALI).[16,17] Whereas in neonates, aquaporin plays a major role in maintaining the stability of lung fluid by quick absorption process of lung fluid. All roles in the physiological and pathophysiological processes in the lungs are carried out by several types of aquaporin that located in different locations.[18] The first type, aquaporin 1, its expression upregulated near the delivery time of neonates, it's located in the peribronchial endothelium, visceral pleura, and some pneumocytes.[19,20] The second type, aquaporin 3 is located on the basolateral surface of the bronchial epithelium.[21] The third type, aquaporin 4, located in basolateral epithelial membrane of bronchial and trachea epithelial, just like the first type its expression also upregulated near the delivery time of neonates.[22] The fourth type, aquaporin 5, located in

apical membrane of type 1 alveolar epithelial cells, trachea and bronchial submucosa, its expression increases after the baby delivery time.[21,23,24]

Each aquaporin protein is located in a different gene in the human body. Aquaporin 1 is in gene 7p14, aquaporin 3 in gene 9p21 - 12, aquaporin 4 in gene 18q11.2-12.1, and aquaporin 5 in gene 12q13. If a deletion occurs in aquaporin, it will causes a decrease the ability of water permeability and various other effects that vary according to the type of protein.[18,21] The deletion of some type of aquaporin protein in type 1 pneumocytes will be replaced by another type of aquaporin protein.[21] People with aquaporin 1 deficiency will still look healthy, but decrease the body ability to extract various solutes in urine and conserve water in low water intake condition.[25,26] Deletion of aquaporin 5 will not cause damage to the microscopic picture of the lung and do not affect other types of aquaporin.[27] However, it will caused 10-fold decreases the osmotically water transport between airspace and the capillary.[18,24]

Aquaporin 5 in the human body is not only found in the lungs, but also in the salivary glands and lacrimals gland.[28] In salivary glands, acetylation of histones of H4 and DNA methylation act as regulators of aquaporin 5 work. Research by Flodby et al on rat lungs as subjects, showed an increase in aquaporin 5 expression by histone deacetylation (HDAC) inhibitor suberoylanilide hydroxamide acid (SAHA).[29]

The results of Tonghui et al's study of the knockout mice showed that aquaporin 5 plays an important role in regulating the water transportation through the apical membrane. However, the occurrence of hydrostatic pulmonary edema is not caused by aquaporin 5 deletion.[24] Type 1 pneumocytes has excellent fluid osmosis permeability and supports massive absorption of fluid because aquaporin 5 is most commonly found in this epithelials type. This ability is necessary in drowning cases that causes cerebral edema and hemolysis condition.[30]

Human body will response the infection by releasing the immune cells as protection to fight against the pathogens. It will induces the increase of leukocytes number and leads to leukocytosis. One of leukocytes type that released is neutrophils, it becomes the first migrated cell to the target location in bacterial infection.[31,32] In a state of acute viral infection, aquaporin 1 and 5 expression will decreased.[18] Other studies have shown that migration of neutrophils to lung is influenced by the expression of aquaporin 5 and the type of its genotype. Neutrophils migration is greater and faster in people with AA genotype.[33] Therefore, the type of genotype plays an important role in the process of lung inflammation and participate in determining the prognosis.

In neonates, pulmonary fluid clearance must occur immediately after the birth time by absorption mechanism of air cavity fluid and the exchange of oxygen and carbon dioxide gas can occur normally. If this does not work well, it will cause respiratory distress conditions, some pathological conditions in neonates that often occur are transient tachypnea of the newborn (TTN) and respiratory distress syndrome.[34] Lack of oxygen supply to cells under physiological and pathological conditions called hypoxia. Hypoxic conditions can lead to pulmonary edema, it will get worse in people with low amiloride-sensitive Na^+ channel (ENaC) expression. Because, ENaC plays an important role in fluid clearance from the respiratory tract.[35]

Kawedia et all performed a research on mice and shows that hypoxia causes a significant decrease in aquaporin 5 in the lungs up to 70%.[28] Deletion of aquaporin 5 in neonates will reduce the effectiveness of alveolar airway clearance, which can lead to transient tachypnea condition.[36] Furthermore, subunit beta in the sodium channel epithelium also plays a role in respiratory distress in neonates.[34,37]

X-ray findings of transient tachypnea on the newborn may be include pulmonary hyperexpansion, density in the perihilum with fissure filling fluid, pleural effusion.[38] X-ray become a traditional method now and concludes radiation exposure to patients.[39] Lung ultrasound (LUS) is considered safer and become the first-line tools, especially in neonatal critical care. Moreover, in some cases x-ray is able to detect pathological conditions better than CT, for example in intersisial syndrome it has 93% of specificity.[40] LUS finding in TTN that are often seen are intersisial syndrome, abnormalities in the pleural borderline, loss of A-line and double lung point (DLP). The DLP sign refers to a border that clearly seems different between upper lung field and the lower field. According to Liu et al, DLP has a 100% specificity as TTN marker.[41,42]

AQUAPORIN 5 RESEARCHES

Below is the comparison of aquaporin 5 expression researches based on subject, subject's underlying respiratory condition, the delivery method of neonates, and its genotypes.

Table 1: Comparison of Aquaporin 5 Expression Researches

No	Title (Authors)	Subject	Method	Results	
1	The Expression of Aquaporins 1 and 5 in Rat Lung after Thoracic Irradiation (Cheng-Ying Sun, Yu-Xia Zhao, Wen	21 male Sprague Dawley rats (divided into 2 groups: control group (6 rats) and irradiation group (15 rats)	The rats were anesthetized, placed in prone position and given single dose of 17 Gy of radiation therapy in their both lungs. Observed on	Treatment	Aquaporin 5
				control	99.55 ± 10.05
				7 days	233.93 ± 29.42
				14 days	131.56 ± 18.73
				28 days	54.66 ± 8.03
				Conclusion: Pathological conditions caused changes in aquaporin expression after irradiation. Aquaporin 5 increased after 1 to 2 weeks irradiation and then decreased 2 weeks	

	Zhong, Da-Wei Liu, Yan-Zhi Chen, Li-Li Qin1, Lu Bai, and Liu)		7th, 14th and 28th day of irradiation.	thereafter. Increased expression of aquaporin 5 is characterized by exudation of proteins into the alveolar space and increase in permeability that caused mild edema conditions in the intra alveolar. Aquaporin 5 protein plays an important role in the pathogenesis of pneumonia and fibrosis due to radiation-induced.
2	Expression of water and ion transporters in tracheal aspirates from neonates with respiratory distress (Yanhong Li, Marie-Odile Marcoux, Martine Gineste, Mireille Vanpee, Marina Zelenina, Charlotte Casper)	32 neonates with ventilator were divided into 4 groups: control, diagnosed with respiratory distress syndrome, diagnosed with TTN and neonates with abnormal chest X-ray imaging.	Tracheal aspirate samples from each neonates were collected. Samples were frozen until protein expression analysis was performed.	Group / n / Aquaporin 5 Control / 6 / 0.38 (0.35, 0.39) Abnormal chest radiograph / 8 / 0.29 (0.22, 0.40) Takipneu transient (TTN) / 8 / 0.46 (0.39, 0.56) Respiratory distress syndrome (RDS) / 10 / 0.29 (0.14, 0.36) Conclusion: Aquaporin 5 expression at TTN group was higher than the control and RDS group. There was no significant difference in aquaporin 5 expression between the control and RDS group.
3	Aquaporine-5 and epithelial sodium channel b-subunit gene expression in gastric aspirates in human term newborns (Fabiola Castorena-Torres, Mario Rene Alcorta-García, Víctor Javier Lara-Díaz)	Term newborns (37 weeks or more gestational age) delivered vaginally (24 samples) and cesarean section (35 samples), females and males.	Samples collected from nasal scrapings and gastric aspirate that taken by suction. The samples collected in a sterile vial that contains 3 mL of phosphate buffer saline, then centrifuged for 5 minutes, and stored at -80 degrees celsius until extraction DNA performed.	Group / How to give birth: Vaginal / Abdominal Mean Aquaporin Nasal: 0.3 (0.3) / 1.2 (1.1) Gastric: 1.1 (1.4) / 2.5 (2.7) Mean sodium epithelial beta subunit Nasal: 1.3 (1.1) / 0.6 (0.6) Gastric: 0.5 (0.5) / 0.8 (1.0) Conclusion: Aquaporin 5 expression was detected higher in babies delivered by cecarian section. And it's also found higher in gastric aspirates than nasal scrapings. Gastric aspirates can be used as samples in neonatal pulmonary maturity testing, especially in babies with membrane rupture or amniotic fluid loss.[32] Whereas sodium channel beta subunits detected higher in nasalis scrapings of babies delivered vaginally.
4	Aquaporin 5 – 1364A / C Promoter Polymorphism Is Associated with	136 Caucasian patients diagnosed with acute respiratory distress syndrome due to bacteria.	Peripheral blood and bronkoalveolar rinses taken within 24 hours of treatment at the ICU were taken as the	Component / Serum / Alveolar Bronko rinses Genotype: A A / AC / CC / A A / AC / CC TNF alpha pg / ml: 4.3 / 3.9 / 9.9 / 8.3 IL-6 pg / ml: 1,750 / 1,485 / 681 / 329

	Pulmonary Inflammation and Survival in Acute Respiratory Distress Syndrome (Tim Rahmel, MD, Katharina Rump, Jürgen Peters, MD, Michael Adamzik, MD)		samples. The aim of analysis was to determine the effect of aquaporin 5 genotype to the level of inflammation and prognosis of respiratory distress syndrome.	IL-10 pg / ml	9.4	6.0	10.9	5.5
				Neutrophil count / ml	-	-	336	142
				Conclusion: Different genotypes produced different survival abilities and levels of inflammation too. People with AA genotype had higher concentrations of leukocytes and proinflammatory cytokines, than those with AC/CC genotype. And those immune cells were detected higher in bronkoalveolar samples than in blood (serum). The survival rate of people with AA genotype is lower than the another people with AC/CC genotype (62% and 86%, respectively)				

LUNG ULTRASONOGHRAPHY RESEARCHES IN TRANSIENT TACHYPNEA OF THE NEWBORN

Below is the comparison of ultrasonography findings in transient tachypnea of the newborn.

Table 2: Comparison of Ultrasonography Findings in Transient Tachypnea of the Newborn.

No	Title (Authors)	Subject	Method	Results		
1	A Multicenter Lung Ultrasound Study on Transient Tachypnea of the Neonate (Francesco Raimonde, Nadya Yousef, Javier Rodriguez Fanjul, Daniele De Luca, Iuri Corsini, Shivani Shankar-Aguilera, Carlo Dani, Vito Di Guardo, Old Silvia, Fabio Mosca, Fiorella Migliaro, Angela Sodano, Gianfranco Vallone, Letizia Capasso)	65 neonates with gestational age 34-40 weeks and diagnosed with TTN.	Subjects underwent pulmonary ultrasonography in the first 60-180 minutes of life, repeated every 6-12 hours if symptoms of respiratory distress continued. Subjects were divided into 2 groups based on the presence and absence of double lung point (DLP).	Aspects	With DLP	Without DLP
				n	31	34
				Duration of respiratory distress	32 ± 38.6	18 ± 15.4
				LUS score at onset	7.6 ± 2.6	5.6 ± 3.8
				Silverman score at onset	4.0 ± 1.5	4 ± 2.1
				The need for CPAP	24/32 (75%)	24/32 (75%)
				Without a consolidated picture		99.5%
				With a consolidated picture		0.5%
				Conclusion: Statistically there was no significant difference in Silverman or LUS score between group with and without DLP sign. It was considered DLP sign is not essential to diagnose TTN. The consistent finding in 99.5% of study subjects is a regular pleural line without consolidation.		
2	Lung ultrasound in early diagnosis of neonatal transient tachypnea	65 neotatus experienced respiratory distress symptoms (73.8% among those subjects	Subjects underwent pulmonary ultrasonography in the first 12-24 hours of	LUS examination results in TTN patients		
				Findings in lung ultrasonography	Sensitivity (%)	Specificity (%)
				Loss of pleural lines	93.5	88.9

	and its differentiation from other causes of neonatal respiratory distress (M. Ibrahim, A. Omrana, NB AbdAllah, M. Ibrahim and S. El-Sharkawy)	were diagnosed with transient tachypnea of the newborn).	admission in NICU.	DLP	69.6	100
				B-lines	28.3	88.9
				Loss of A-lines	91.3	77.8
				Conclusion: DLP had the highest specificity (100%) in LUS finding to diagnose the TTN.		

DISCUSSION

Sun et al performed a research on mice with acute pneumonitis due to radiation-induced lung toxicity, the results showed that pathological conditions caused changes in aquaporin expression after irradiation. Aquaporin 1 decreased while aquaporin 5 increased after 1 to 2 weeks irradiation and then decreased 2 weeks thereafter. Expression increases of the aquaporin 5 is characterized by exudation of proteins into the alveolar space and increase in permeability that caused mild edema conditions in the intra alveolar.[27]

Rahmel et al conducted a study to observe the differences of inflammation level and prognosis of survival in 30 days of treatment in the ICU between people with AA genotype and AC/CC genotype of aquaporin. The study was performed to 136 patients that diagnosed with acute respiratory distress syndrome due to bacteria. The results showed people with AA genotype had higher concentrations of leukocytes and proinflammatory cytokines, such as IL-1, IL-6 and TNF alpha,[43] than those with AC/CC genotype. And those cells were detected higher in bronkoalveolar samples than in blood (serum). The survival rate of people with AA genotype is lower than the another people with AC/CC genotype (62% and 86%, respectively).[44] This result indicates that different genotypes produced different survival abilities and levels of inflammation.

Fabiola et al examined the differences of aquaporin 5 and sodium epithelial beta subunits expressions in nasal scrapings and gastric aspirates of the newborn by cesarean section and vaginal delivery. The results showed a higher aquaporin 5 expression in babies delivered by cecarian section. Aquaporin 5 expression was detected higher in gastric aspirates than nasal scrapings. This results indicates that delivery method affects the aquaporin 5 expression on the newborn and gastric aspirates can be used as samples in neonatal pulmonary maturity testing, especially in babies with membrane rupture or oligohydroamnion (small amount of amniotic fluid).[37] Whereas subunit beta in the sodium channel epithelium detected higher in nasalis scrapings of babies delivered vaginally. In the previous, the works of sodium channel beta subunits was thought detectable and assessed since the embryonic period, but now it's known only detectable during 17th-24th week of gestational (the canalicular phase of lung formation).[37,45,46]

A research of aquaporin expression in transient tachypnea of the newborn was performed by Yanhong Li et al of 32 neonates with ventilation. They were divided into 4 groups: control group with normal lung X-ray (six people), diagnosed with respiratory distress syndrome (eight people), diagnosed with transient tachypnea of the newborn/TTN (eight people) and a group with abnormal lung X-ray (ten people). The result showed that expression of aquaporin 5 in TTN group was higher than the control and the neonates with respiratory distress syndrome.[34]

Ibrahim et al showed the lung ultrasonography findings of TTN patients: disrupted pleural line, double lung point (DLP) sign, positive scattered B-lines, partially or completely disappearance of A-line and interstitial syndrome were seen 93.7%, 68.8%, 29.2%, 89.6% and 25%, respectively. Among all of the signs that appeared in LUS imaging, DLP had the highest specificity (100%) in the diagnosis of TTN.[47] However, Raimondi et al studied of 65 neonates with transient tachypnea of the newborn, the result showed DLP was seen in only 47.6% in the lower lung field, statistically there was no significant difference in Silverman or LUS between group with DLP and without DLP. It was considered DLP sign is not essential to diagnose TTN. The consistent finding in 99.5% of study subjects is a regular pleural line without consolidation.[48,49,50] Regardless of the various result of the studies, the main characteristic of TTN is pulmonary edema and its primary radiographic signs are DLP and alveolar intersisial syndrome.[34,51,52]

CONCLUSION

Aquaporin, especially aquaporin 5, helps pulmonary fluid clearance as quick as after the birth time by absorption mechanism to allows the air exchange normally and avoid the respiratory distress as in transient tachypnea of the newborn (TTN). That condition affected by subunit beta in the sodium channel epithelium that also plays a role in respiratory distress of the newborn. TTN induces higher expression of aquaporin 5 and markes by several signs by radiography imaging, i.e. double lung point sign in lung ultrasonography.

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