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# Correlation of Sodium Epithelial B-Subunit and Aquaporin-5 Level with Alveolar Fluid Clearance Assessed by Ultrasound in Early-Term Neonates

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**Abstract**

Alveolar fluid clearance (AFC) is an essential part of the transition process from intra to extra-uterine life, especially for normal respiratory function. Beta subunit channels (β-ENaC), and aquaporin-5 (AQP5), plays an essential role in fetal lung fluid absorption and maintaining pulmonary fluid homeostasis. Cesarian Section (CS) delivery is associated with an increased incidence of transient tachypnea of the newborn due to alveolar fluid retention and significantly impacts neonatal morbidity. This study aims to determine the feasibility of assessing β-ENaC and AQP5 levels in the umbilical artery, and it's association with AFC presented on lung ultrasonography (LUS) in early-term neonates born with elective CS. The study had a prospective cohort design. The blood samples from the umbilical artery of 60 subjects were obtained immediately after birth, and β-ENaC and AQP5 levels were determined. AFC evaluated by LUS at 30-45 minutes, 60-90 minutes, and 120-150 minutes of neonatal's age. AFC on LUS divided into three types. Type 1 as no AFC was seen, type 2 as partial AFC and type 3 as signs of no fluid retention (normal lung). The mean β-EnaC and AQP5 level were 6.68 ± 2.95 ng/ml and 6.37 ± 2.17 ng/ml, respectively. There was a significant difference (*P*-value = 0.000, <0.05) between β-ENaC and AQP5 levels with AFC type on LUS. At 120-150 minute old, 77% of the neonates had type 3 on their LUS. A significant association found between β-ENaC and AQP5 levels with the time of AFC assessed by LUS. β-ENaC and AQP5 level measured from umbilical artery feasible and can assist in predicting and diagnosing neonatal lung physiology.

**Keywords:** β-ENaC and AQP5, Alveolar fluid clearance, Cesarean section, Early-term infant

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## 1. Introduction

The respiratory epithelium secretes ions and water into the lumen of the airways, contributing to the growth, differentiation, and maturation of the respiratory tree during intrauterine life. Alveolar fluid clearance (AFC) in the form of ions and water absorption via β-ENaC and AQP5, started shortly before birth in preparation for gas exchange after birth. AFC occurs in response to the increased concentrations of glucocorticoids and catecholamines associated with term birth (Guglani et al 2009), (Castorena-Torres et al., 2018). During vaginal birth, thoracic compression by "vaginal squeeze" also contributes to the AFC process. After birth, AFC progressively increases via β-ENaC. Intraalveolar fluid will be released into the interstitial space and cleaned by the lymphatic channels and pulmonary capillaries (*Guglani et al 2009*). AFC failure at birth is a significant cause of neonatal morbidity and mortality, especially in very preterm infants. Cesarean section (SC) delivery is associated with a high incidence of respiratory distress due to alveolar fluid retention. It has a significant impact on neonatal respiratory distress morbidity (Alhassen et al., 2020). TTN and RDS are the two most common respiratory disorders in neonates. They have been associated with changes in respiratory epithelial gene expression of AQP5 and β-ENaC. RDS, mainly in preterm infants, is due to relative surfactant deficiency, which is superimposed on AFC inability and is strongly associated with decreased β-ENaC expression. In contrast, TTN, mainly in term infants due to delayed AFC, has been associated with increased AQP5 expression in tracheal aspiration. The

severity of RDS in preterm infants is related to plasma AQP-5 level. The more severe the disease, the higher the plasma AQP-5 levels (Wu et al., 2015).

Besides the AQP5 and β-ENaC, lung ultrasound could be used to detect a number of lung pathologies. Previously, lung ultrasound was not widely used due to the inability of the ultrasound wave to penetrate air-filled tissues. (Koegelenberg et al., 2009) However, nowadays, lung ultrasonography was reported to have an accurate diagnostic value to detect RDS and other lung pathologies. (Lichtenstein & Mezière, 2008).

Data of β-ENaC and AQP5 levels in early-term neonate born by elective SC have never been reported, even though it is more practical to use in daily practice than gene expression. This study aims to determine the feasibility of measuring β-ENaC and AQP5 levels in the umbilical arteries obtained immediately after birth in early-term newborns through elective CS delivery and their correlation with AFC assessed by lung ultrasound.

## 2. Methods

### *2.1. Samples*

This cohort study was conducted from May 2020 to December 2020 in Women and Children's Hospital in North Jakarta, Indonesia. The target population was healthy neonates born at an early term (gestational age $37^{0/7}$-$38^{6/7}$ weeks). The sampling method was carried out sequentially, which met the inclusion and exclusion criteria (taking samples from consecutive admissions) until it reached the samples size. Sixty neonates born by elective CS were recruited and stay in the hospital for three to four days after delivery. The inclusion criteria were healthy neonates born by elective SC at $37^{0/7}$-$38^{6/7}$ weeks and the mothers had no history of drug usage (such as corticosteroids, catecholamines or diuretics), no history of asthma, preeclampsia or any other hypertensive disorders, urinary tract infections, chorioamnionitis, and premature rupture of membranes. The neonates have appropriate weight for gestational age, and during birth do not require supplementary oxygen support. The parents' agreement was taking by signing the informed consent. Exclusion criteria were congenital anomalies, poor quality plasma umbilical artery samples and the mother is hospitalized a week before giving birth. The institutional human research review agency approved this study at Hasanuddin University, South Sulawesi, Indonesia, with Protocol number: UH19111017 (April 271.2020)

We recorded maternal age, gestational age, parity, blood pressure, and uterine contractions from the mothers. From neonatal data, we collected information on birth weight and gender. The subjects then observed for 4 hours of age.

### *2.2. Sample collection*

An umbilical artery blood sample was taken immediately after birth by an obstetrician. After the neonate examined by a paediatrician and diagnosed as a healthy neonate, the plasma was taken from the blood umbilical artery and collected for AQP5 and β-ENaC measurement by enzyme-linked immunosorbent assay.

### *2.3. Measurement of plasma level of β-ENaC and AQP-5*

All of the blood samples (@3ml) were centrifuged directly for 6 min at 3,000 g, and the serum obtained was immediately frozen in sterile tubes at -80°C. All selected good quality samples were measured for plasma levels of β-ENaC and AQP-5 using commercially available ELISA methods (LSBio: human SCNN1B / ENaC Beta ELISA Kit Catalog No.LS-F8497 and human AQP5 / Aquaporin 5 ELISA Kit Catalog. No.LS-F10879), according to the manufacturer's protocol. The laboratory technicians who carried out the assays were utterly blind to the clinical information.

### *2.4. Lung ultrasound*

After umbilical artery blood samples being taken, lung ultrasound was done at 30-45 minutes, 60-95 minutes, and 120-150 minutes of age. Samsung/Medison H60 ultrasound system and linear 3-16 MHZ probe was used at 3.5 cm depth. The examination was using B-mode in three lung areas for each lung sides. After birth, neonates were put on a mattress, in supine, left and right lateral positions. Both of the lungs were scanned as each lung were divided into three area: anterior (1): between the sternum and anterior axillary line, lateral (2): between the anterior and posterior axillary line and posterior (3): the area between the posterior axillary line and the vertebra (Figure 1). The probe was oriented sagitally as the 3.5 cm wide surface could show accurate lung images between two or three intercostal spaces (Figure 2).

Six captured and real-time (2-to-3-second) images were obtained and saved in the ultrasound machine storage. A senior pediatric radiologist with 15 years of experience, and a junior radiologist with five years of experience, interpreted all images and recordings and blinded by the sample's data. Samples were excluded if there were any inconsistencies regarding the results obtained by the two examinators. According to studies by Raimondi et al., and Blank et al., AFC was classified according to the amount of fluid retention (Blank et al., 2018; Raimondi et al., 2012) shown on LUS. Type 1 or white-out lung depict fluid retention (no AFC yet); white images were predominant in intercostal spaces due to the coalescence of B lines and thick pleural lines. Type 2 is partial AFC. Vertical hyperechoic B lines were found from the pleural lines, expanded, and blurring the A-line. Type 3 is normally aerated lung, A-lines observed as the hyperechoic pleural echo artefact and face to the air (Figure 3).

LUS image type classification for the subjects was made as follows: No AFC, if there was minimal 1 area in each lung still depict type 1 pattern. Partial AFC, if there was minimal 1 area in each lung depict type 2 pattern and no type 1 in all areas of both lung. Complete AFC, if all (3 areas) in both lungs depict type 3 pattern.

### *2.5. Statistical analysis*

Values expressed as means and standard deviations (SD); Categorical data expressed as frequencies and proportions. Analysis of the relationship between AQP5 levels and lung ultrasound images using Independent T-Test and ANOVA test. A comparison test of β-EnaC levels with lung ultrasound images performed using the Mann-Whitney test and the Kruskal-Wallis test. P values <0.05 considered being statistically significant. The statistical analysis was done with IBMSPSS Statistics software (ver. 25; IBM Corporation, Armonk, NY, USA).

## 3. Results

A total of 60 subjects, 53.3% was male with a mean bodyweight of 3.15 ± 0.17 kg. The demographic and clinical characteristics of the subjects are presented in Table 1 and the level of β-EnaC and AQP5 are presented in Table 2.

The AFC on LUS was evaluated at 30 minutes to 150 minutes after birth. At 30-45 minutes, there was no subjects has type 3, while at 120-150 minutes, there was no type 1 anymore, but type 2 still presents in 23% of subjects (table 3).

### *3.1. Association of AQP5 and β-ENaC levels with AFC assessed by ultrasound.*

AQP5 and β-EnaC levels in different type of LUS image at 30-45 minutes, 60-90 minutes, and 120-150 minutes of age were shown in table 4, 5, and 6 for AQP5 and in table 7,8,9 for β-EnaC level. At 30-45 minutes no complete AFC was found, while at 120-150 minutes there was no type 1 (No AFC). β-EnaC levels in different types of LUS image at 30-45 minutes, 60-90 minutes, and 120-150 minutes of age were shown in table 7,8,9.

The distribution of β-ENac and AQP5 levels and their correlation with LUS is presented in the box plot diagram below. β-ENaC levels positively correlated with AFC, and levels were higher on type 3 ultrasound, while AQP levels negatively correlated with AFC.

## 4. Discussion

Lower airway system development begins on day 22 until complete maturation at eight years old (Rehman & Bacha, 2020). The cellular transition between the conduction and alveoli and epithelial alveolar formation occurs through differentiation of AT1 into AT2 (Themes, 2016), (Rehman and Bacha, 2020). The fetal pulmonary epithelium starts secreting alveolar fluid at around six weeks of gestational age. The chloride ion ($Cl^-$) in the interstitium enters by active transport of sodium via β-ENaC, potassium, and $Cl^-$ into cells (transporter $Na^+$-$K^+$-$2Cl^-$). $Na^+$ follows the $Cl^-$ via a paracellular pathway, and water will transport through AQP5 across the epithelial cell (Jha et al., 2020). During intrauterine life, the secretion of water and ions by the AT1 and AT2 cells into the lumen of the airways contributes to the respiratory tree growth, differentiation, and maturation. Near the time of birth, in preparation for pulmonary gas exchange, the airway must be clean, and fluid should be reabsorbed(Castorena-Torres et al., 2018). Figure 4.

Elective CS at 38–39 weeks was associated with increased incidence of TTN, and NICU admission was significantly higher than birth after 39 weeks gestation (Pirjani et al., 2018), (Nakashima et al., 2014). Zhang et al. reported that more than 50% of pregnant women in Australia believe that 37-38 weeks of gestation is the earliest time for safe delivery (Zhang et al., 2015). Based on the 2017 report, an increasing trend of planned and unnecessary SC in Indonesia and information on planned elective CS are still limited in Indonesia (Prasetyoputra et al., 2020). In our hospital in North Jakarta, Indonesia, many patients undergo elective CS at 37-38 weeks of gestation. At the onset of labour, there will be hormonal changes in the mother and the fetus to prepare the fetus for transition. The concentration of glucocorticoids and catecholamines near the delivery time at term increased. $Na^+$ absorption occurs through the β-ENaC, and the AFC process begins in preparation for lung gas exchange (Castorena-Torres et al., 2018). Rapid AFC of fetal ion and lung fluid is an essential part of this process. Reabsorption of ion through the β-ENaC and H2O through aquaporin-5 (AQP5) plays a significant role in the transition of alveolar epithelial function from fetal lung fluid secretion to absorption, as well as maintaining postnatal lung fluid homeostasis (Hillman et al., 2012) (Graves & Haley, 2013). During childbirth, the physiological stress makes hormones such as catecholamines increase and accelerate AFC (Süvari et al., 2019). Disruption of AFC causes retention of the lung fluid and is one of the pathophysiological factors and neonatal respiratory problems. Babies born by elective SC carried out before starting the delivery process so that the physiological mechanisms underlying AFC will be delayed. The morbidity of neonatal respiratory disorders will increase (Ramachandrappa & Jain, 2008). The failure of the transition process can be life-threatening and often requires supportive care. Most of the data on neonatal transition studies obtained from animal studies; therefore, data for studying the human perinatal period, especially the transition period, is very lacking. As previously mentioned, neonatal respiratory distress was more common in babies born by CS. In recent years the tendency for mothers to chose birth by SC has increased (Kristensen & Henriksen, 2016), (Ahmed & Mohammad, 2019), (Diema Konlan et al., 2019), (Prasetyoputra et al., 2020).

Orwoll et al. reported several biomarkers that could be used in assessing respiratory disorders in children. The biomarker can use a blood sample or an alveolar fluid (bronchoalveolar lavage/BAL). These biomarkers have potential as biological markers and are grouped based on their pathogenesis, such as inflammation, coagulation, epithelium, endothelium, surfactants, and non-protein markers. One of the protein biomarkers is AQP5. Proteins and other biomarkers measured in the blood (plasma or serum) are preferred in most cases to biomarkers from BAL because of the relatively easy availability of blood samples (Orwoll & Sapru, 2016). This study assessed the AQP5 and β-ENaC levels as protein biomarkers. Both proteins play a significant role in the secretion and absorption of lung fluid before, shortly after, and after birth. Both are not water-soluble and bound in cells. It assumed that, in theory, if there is no cell damage, that this protein is not present in the alveolar-tracheal-gastric fluid. The location of AQP5 in the respiratory tree can found in the nasal cavity, submucosal glands, trachea, bronchi and lung (apical membrane AT1 cells) (Direito et al., 2016). ENaC, as an ion channel, is found in apical epithelial cells in almost all organ systems. This channel serves as the first mediator of active $Na^+$ reabsorption, which is essential for maintaining body water and salt homeostasis, controlling $Na^+$ reabsorption, especially in the respiratory tract, kidneys, large intestine, and sweat glands(Hanukoglu & Hanukoglu, 2016).

Several research reports have found various techniques and sample materials used to assess biomarkers of the respiratory system, particularly in children. Sample can use from gastric fluid and nasal epithelium (Castorena-Torres et al., 2018), from the aspirated tracheal fluid through an endotracheal tube (Li et al., 2009) and radial artery ("Wu 2015.pdf," n.d.).

**Table 1:** Demographics characteristics of early-term neonates (n=60)

| Demographics | Value |
|---|---|
| Gender | |
| - Male [a] | 32 (53.3%) |
| - Female [a] | 28 (46.7%) |
| Maternal age (years) [b] | 30±5 |
| Parity [a] | |
| 1 | 37 (61.7%) |
| 2 | 18 (30%) |
| 3 | 4 (6.7) |
| 4 | 1 (1.7) |
| Clinical characteristics | |
| - Birth weight (kg) [b] | 3.15 ± 0.17 |
| Apgar score [C] | |
| 1 minute (7-9) | 60 |
| 5 minutes (9-10) | 60 |

Annotations: [a] = Values expressed as frequency and (percentage). [b] = Values expressed as mean and (standard deviation). [c]= Median (minimum - maximum).

**Table 2:** β-ENaC and AQP5 level in umbilical artery of early-term neonates (n=60)

| **Variable** | **Min-Max (value, ng/ml)** | **Mean ± SD (value, ng/ml)** | **95% CI** |
|---|---|---|---|
| β-EnaC | 1.077 – 10.477 | 6.68 ± 2.95 | 5.92 - 7.44 |
| AQP-5 | 3.065 – 10.807 | 6.37± 2.17 | 5.81 - 6.94 |

Annotations: AQP5 = Aquaporin 5; b-ENaC = Sodium epithelial channel, beta fraction. Values are level and standard deviation.

**Table 3:** Lung ultrasonography image type at different time after birth

| Lung ultra-sound image type | Neonate Age | | |
|---|---|---|---|
| | **30-45 minutes** | **60-90 minutes** | **120-150 minutes** |
| | **N(%)** | **N(%)** | **N(%)** |
| No AFC | 17 (28) | 7 (12) | 0(0) |
| Partial AFC | 43 (72) | 19 (31) | 14 (23) |
| Complete AFC | 0 (0) | 34 (57) | 46 (77) |
| Total | 60 (100) | 60 (100) | 60 (100) |

**Table 4:** AQP5 level and lung ultrasonography image type at 30-45 minutes of age

| Lung US image | Min | max | Mean | SD | Nilai P* |
|---|---|---|---|---|---|
| No AFC | 5,477 | 10,807 | 8,96741 | 1,615171 | 0,000 |
| Partial AFC | 3,065 | 7,720 | 5,35077 | 1,360156 | |

*Note : * Independent T-Test*

**Table 5:** AQP5 level and lung ultrasonography image type at 60-90 minutes of age

| Lung Ultrasound image | Min | max | Mean | SD | Nilai P* |
|---|---|---|---|---|---|
| No AFC | 8,681 | 10,720 | 9,47371 | ,766509 | 0,000 |
| Partial AFC | 3,521 | 10,807 | 7,37463 | 2,207062 | |
| Complete AFC | 3,065 | 7,720 | 5,17926 | 1,264702 | |

*Note: *ANOVA Test*

**Table 6:** AQP5 level and lung ultrasonography image type at 120-150 minutes of age

| Lung Ultrasound image | Min | max | Mean | SD | Nilai P* |
|---|---|---|---|---|---|
| Partial AFC | 7,871 | 10,807 | 9,57236 | ,945141 | 0,000 |
| Complete AFC | 3,065 | 7,720 | 5,40252 | 1,338008 | |

*Note: * Independent T-Test*

**Table 7:** β-EnaC level on Lung ultrasonography aged 30-45 minutes

| Lung Ultrasound image | Min | max | Mean | SD | Nilai P* |
|---|---|---|---|---|---|
| No AFC | 1,077 | 7,582 | 2,73382 | 2,211042 | 0,000 |
| Partial AFC | 5,912 | 10,477 | 8,24281 | 1,245050 | |

*Note: * Mann-Whitney Test*

**Table 8:** β-EnaC level on Lung ultrasonography aged 60-90 minutes

| Lung Ultrasound image | Min | max | Mean | SD | Nilai P* |
|---|---|---|---|---|---|
| No AFC | 1,103 | 2,927 | 1,70857 | ,607055 | 0,000 |
| Partial AFC | 1,077 | 10,309 | 5,44495 | 3,028013 | |
| Complete AFC | 6,206 | 10,477 | 8,39712 | 1,149316 | |

*Note: *Kruskal-Wallis Test*

**Table 9:** β-EnaC level on Lung ultrasonography aged 120-150 minutes.

| Lung Ultrasound image | Min | max | Mean | SD | Nilai P* |
|---|---|---|---|---|---|
| Partial AFC | 1,077 | 2,964 | 1,77871 | ,640577 | 0,000 |
| Complete AFC | 5,912 | 10,477 | 8,17422 | 1,235791 | 0,000 |

*Note: *Mann-Whitney Test.*

**Table 10:** Table of β-ENaC and APQ5 in correlation with AFC

| AFC | β-ENaC (ng/ml) | | | AQP5 (ng/ml | | |
|---|---|---|---|---|---|---|
| | 30-45 | 60-90 | 120-150 | 30-45 | 60-90 | 120-150 |
| No AFC | 2,73382 | 1,70857 | - | 8,96741 | 9,47371 | - |
| Partial AFC | 8,24281 | 5,44495 | 1,77871 | 5,35077 | 7,37463 | 9,57236 |
| Complete AFC | - | 8,39712 | 8,17422 | - | 5,17926 | 5,40252 |

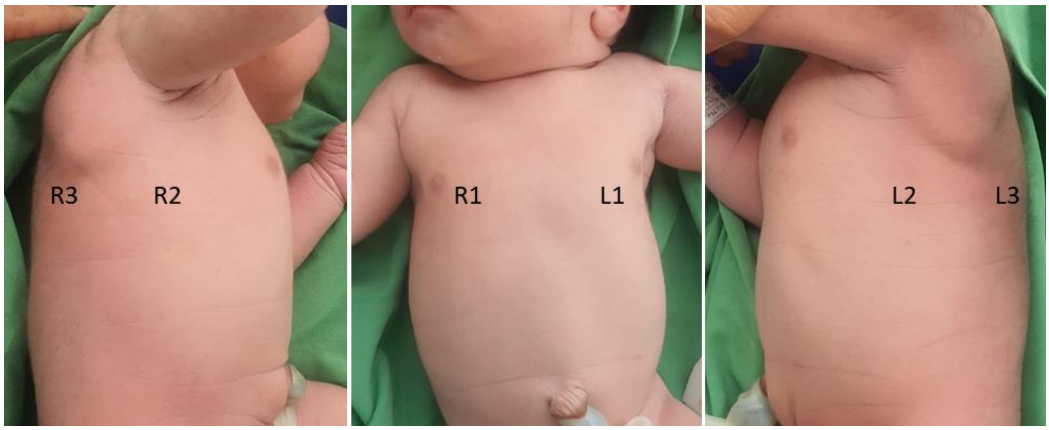


**Figure 1.** Lung ultrasonography scanning position
Remarks: R: right, L: left, 1: anterior, 2: lateral, 3: posterior

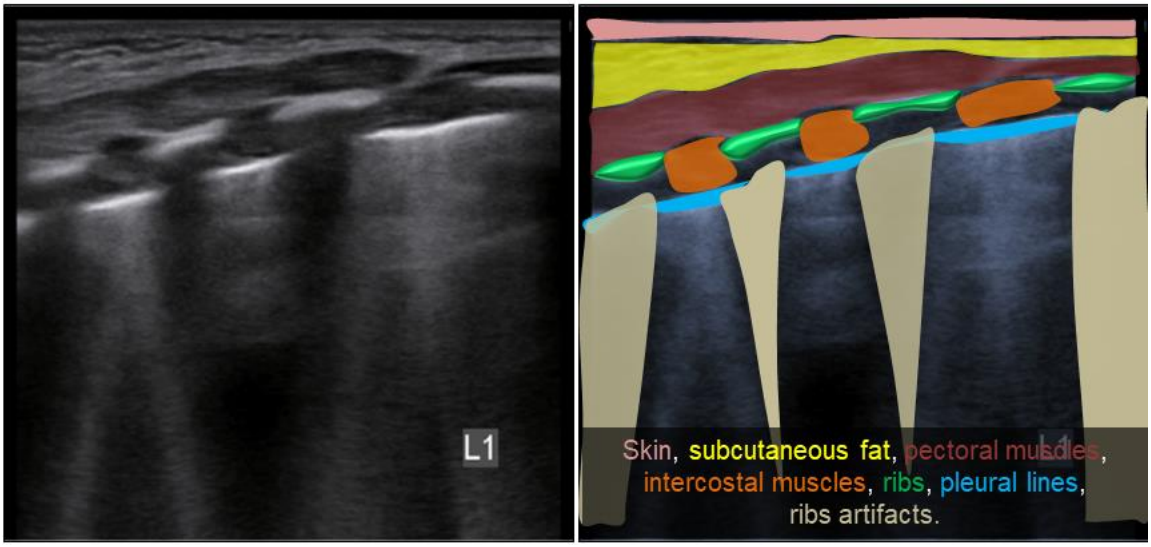


**Figure 2:** Lung ultrasonography image of left anterior area

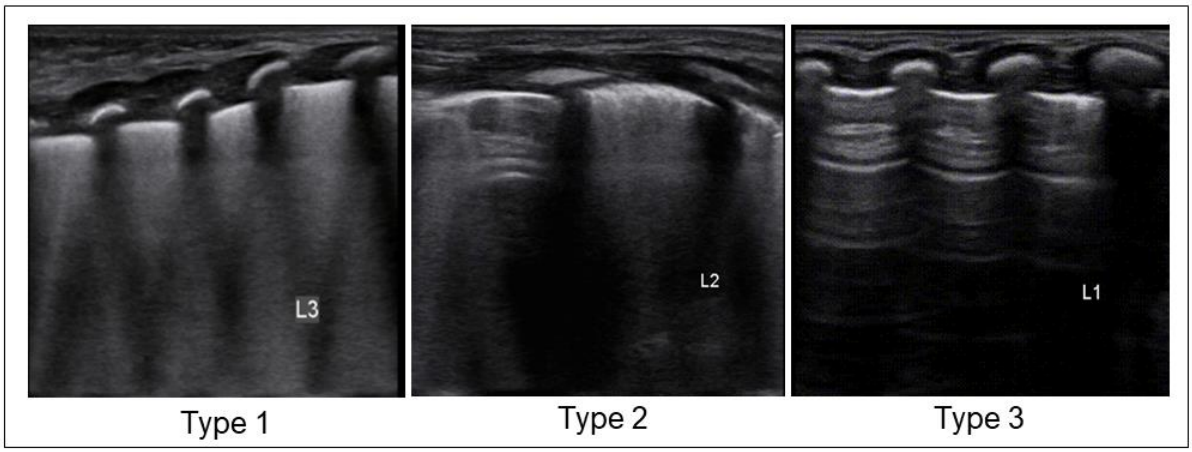


**Figure 3:** Ultrasound appearance of neonate lungs based on type:Type 1, 'white-out' lung, significant fluid retention. Type 2: partial retention of lung fluid (partial AFC). Type 3, lungs with normal aeration (complete AFC)

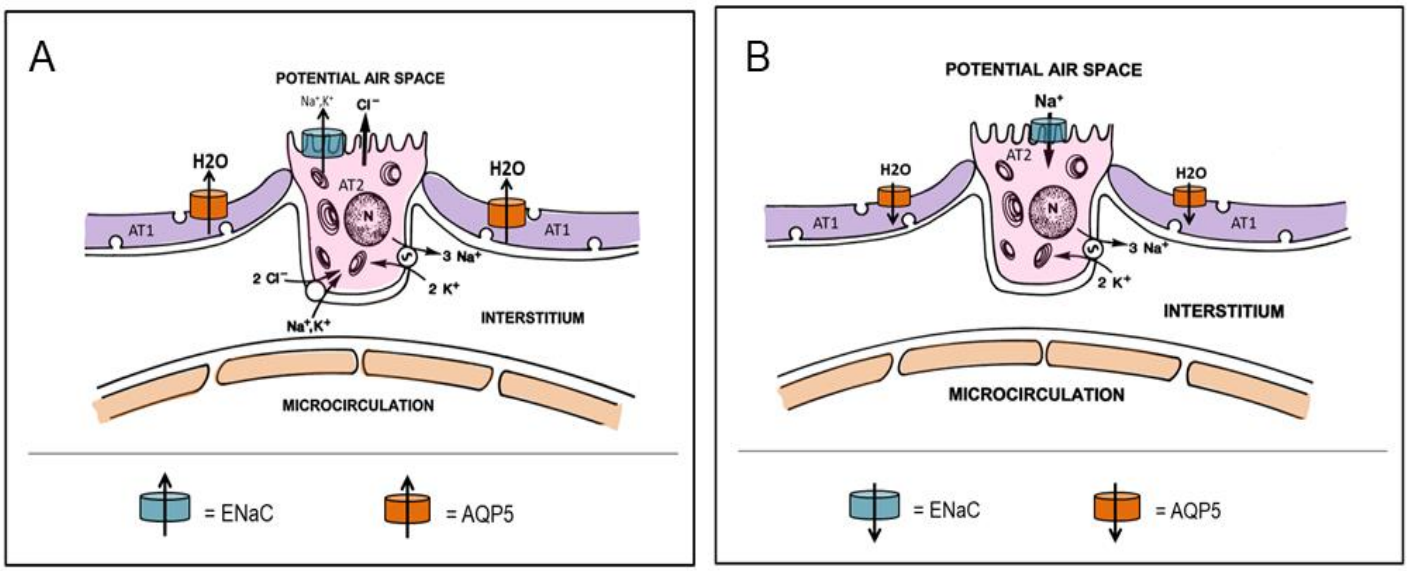


**Figure 4:** (A). During fetal life, alveolar epithelial cells secrete chloride ($Cl^-$) by a process that involves $Na^+$,$K^+$-$2Cl^-$ cotransport and $Na^+$,$K^+$-ATPase (Na pump) activity so the alveoli filled with chloride ($Cl^-$)-rich fluid. (B). In term fetus following birth, air enters the alveoli, and lung fluid is absorbed secondary to sodium ($Na^{+)}$ transport via the epithelial sodium channel (ENac) (Bland, 2005).

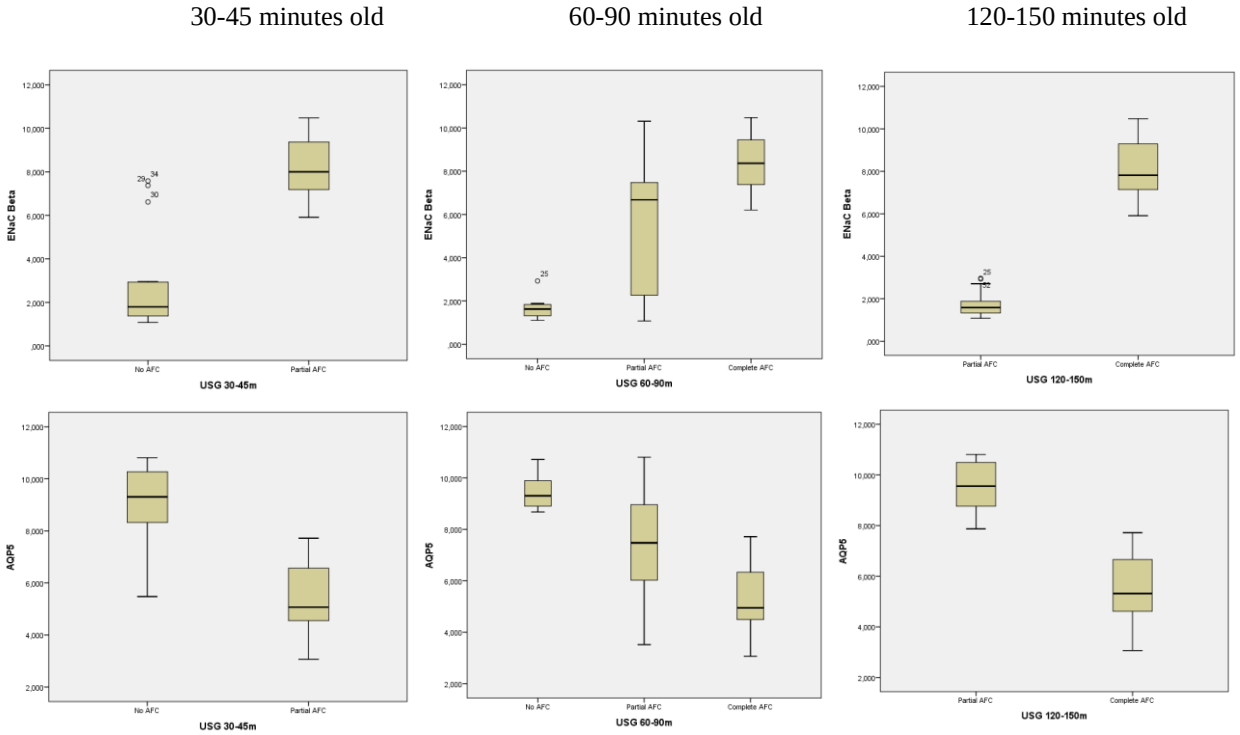


**Figure 5:** Box plot diagram of β-ENaC, APQ5 and Lung ultrasonography image result

A sampling of gastric juice is possible and easy for a sample. It can represent most of the fluid in the airway or stomach because the oro-nasopharynx is relatively straight in infants. The liquid that comes out of the alveoli trachea is swallowed mainly and enters the stomach. In our opinion, it is not always appropriate to assess proteins such as AQP5 and ENaC using gastric juices. As we know, gastric juices contain other elements such as pepsin, whose function is to break down protein (until now, we have not found a report on whether AQP5 and β-ENaC proteins can be broken down by pepsin. As pepsin can break down protein, it can be assuming that AQP5 and β-ENaC are also likely to break down. When there is lung epithelial cell damage, AQP5 and β-ENaC proteins can be found in gastric juices but are likely to be broken down by pepsin. In this study, direct assessment of protein biomarker levels using the ELISA technique, not through the expression of messenger RNA (m-RNA) genes, because in our opinion, data evaluation of protein level content is more useful in the application of daily clinical practice.

### *4.1. β-EnaC*

Towards delivery, the hormones cortisol, thyroxine and catecholamine are increase. Cortisol is the primary regulator of maturation, especially lung, as preparation for neonatal adaptation at birth. Cortisol rises more rapidly until it peaks a few hours after term delivery. Cortisol activates the sodium pump that plays AFC at birth. In neonates born with elective SC, the increase in cortisol is less due to a lack of stress response (Hillman et al., 2012). Cortisol activates β-ENaC in the apical AT2 cells, thus initiating the AFC process. During labour: pulmonary fluid is absorbed more rapidly in the presence of "vaginal squeeze" or thoracic compressions. After birth, ventilation and oxygenation further increases AFC via β-ENaC. Water exits the alveolus via AQP5 to the interstitial spaces, lymphatic channels and pulmonary capillaries ("Guglani et al. - 2009 - Risk factors and management of transient tachypnea.pdf," n.d.).

Castorena-Torres et al. Reported the expression of the β-ENaC gene in gastric fluid samples like those in the nasal epithelium. Relative values of nasal expression of the β-ENaC gene: neonates born PV, 1.3 (1.1), SC birth 0.6 (0.6) and gastric fluid: PV birth, 0.5 (0.5), SC birth 0.8 (1.0). B-ENaC expression was twofold greater in nasal samples of neonates born with PV, while there was no difference in gastric fluid samples (Castorena-Torres et al., 2018). Lie et al. Reported that neonatal respiratory distress was associated with altered β-ENaC expression. Lower β-

ENaC expression is one factor influencing the development of RDS (Li et al., 2009) Süvari et al. Reported β-ENaC gene expression in nasal epithelial cell samples assessed at birth age VG and elective SC: 2 minutes, 1 hour, and 24 hours. They reported the results of Rt-PCR expressed β-ENaC at 2 minutes (1.13-1.77), 1 hour (1.17-1.75) and 24 hours (1.35-2.00)(Süvari et al., 2019).

The results of this study from 60 samples of neonates born with elective SC, the lowest β-EnaC umbilication artery level was 1,077, and the highest was 10,477 with an average value of 6.68 (lower limit of 5.92 and upper limit of 7.44) or an average value of 6.68 (6.68 ± 2.95). The current study measured the expression of the mRNA gene, while this study assessed protein levels by ELISA examination. Until now, we have not found reports of studies assessing β-ENaC levels by ELISA technique, especially in neonates.

### *4.2. AQP5*

AQP5 plays an essential role in water regulation of the body's systems, especially in: the digestive tract, kidneys, respiratory tract, integumentary system, reproductive system, and sense organs. AQP5 of the respiratory system is present in the apical membrane of alveolar epithelial cells and the apical membrane (Direito et al., 2016). In the alveolus, AQP5 found in apical AT1 cells("King et al. - 2000 - Aquaporins and the respiratory system advice for .pdf," n.d.). ENac as ion transport creates an osmotic gradient and drives AFC, water (H2O) to move through the AQP5 and the intracellular pathways. This AQP5 activity will only increase before delivery(Song et al., 2000), (Direito et al., 2016). Castorena-Torres et al. Reported the expression of the AQP5 gene by rt-PCR examination of gastric fluid samples similar to those in the nasal epithelium. They reported nasal AQP5 gene expression: PV birth, 0.3 (0.3), SC 1.2 (1.1) birth, and gastric fluid: PV birth, 1.1 (1.4), SC 2.5 (2.7) birth. They concluded: the expression of the AQP5 gene detected to be higher in gastric fluid than in nasal epithelium and higher in the group of neonates born with SC (Castorena-Torres et al., 2018).

Yanhong Li et al. conducted a study of AQP5 gene expression by rt-PCT examination using tracheal fluid samples in neonates RDS, TTN and control groups. This study reported AQP5 gene expression in the control group = 0.38 (0.35, 0.39), abnormal chest X-ray = 0.29 (0.22, 0.40), TTN = 0.46 (0.39, 0.56) and RDS 0.29 (0.14, 0.36). The expression of APQ5 on TTN was higher than in the RDS group, the group with abnormal chest X-ray and the control group. There was no significant difference between AQP5 in the control group and RDS neonates (Li et al., 2009).

The study of Süvari et al. Reported the results of the rt-PCR examination of nasal mucosa samples of term neonate, PV and elective CS. It found, postnatal AQP5 gene expression related to birth stress in neonates 2 minutes: 1.43-2.78, 1 hour: 0.88-2.06, 24 hours: 1.46-3.072 minutes (Süvari et al., 2019). The study of Wu et al. Reported that the severity of RDS in preterm infants was related to plasma AQP5 levels: the more severe the disease, the higher the plasma AQP5 levels. Radial artery plasma AQP5 levels in preterm neonates at three different times, at neonatal age (24 hours, 48 hours and 72 hours) neonates with RDS and control groups were not significantly different. They reported AQP5 levels in "normal" preterm neonates (age 24 hours: 3.93 ± 1.09 03 ng/ml, age 48 hours: 4.19 ± 0.69 03 ng/ml and age 72 hours: 3.95 ± 0.92 03 ng/ml ("Wu 2015.pdf," n.d.).

There have been no reports of studies assessing AQP5 levels from umbilical artery samples from previous studies. The research results that may use as a comparative and "approaching" reference are the research conducted by Wu et al. The sample material for the examination is the same from the arteries. The analysis also uses the ELISA technique, but the artery location is different. Wu et al. study used the radial artery, and these studies using the umbilical artery, also the difference in gestational age of the neonates the time of collection. Wu et al. This study sample, gestational age $28\text{-}36^{+6}$ weeks birth PV and SC with a blood sample taken at neonate aged 24 hours, 48 hours and 72 hours in this study the gestational age was $37^{0/7}\text{-}38^{6/7}$ weeks birth elective SC with a blood sample taken at immediately before umbilical cord-cutting.

In this study, the average AQP5 level in the umbilical artery shortly after birth was 6.38 ± 2.17 ng/ml. Wu et al. study reported radial artery AQP5 levels of "normal" preterm neonates at 24 hours of age: 3.93 ± 1.09 03 ng/ml, 48 hours of age: 4.19 ± 0.69 03 ng/ml and 72 hours of age: 3.95 ± 0.92 03 ng/ml. This study found that AQP5 levels were slightly higher than what Wu et al. reported. In our opinion, this was probably due to differences in gestational age at birth. This study was $37^{0/7}\text{-}38^{6/7}$ weeks gestation (saccular stage), while Wu et al. study was $28\text{-}36^{+6}$ weeks gestation (alveolar stage). The difference in gestational age is quite a lot around nine weeks; of course, this will significantly impact the embryogenesis process and the maturation of alveolar epithelial cells, especially AT1 cells, which are the locations of AQP5.

### *4.3. Lung ultrasound*

Partial AFC (type 2) found in 72% of the neonates at 30-45 minutes. In 60-90 minutes, 88% of them have type 2 and 3, and only 12% of the neonates

still having type 1. In 120-150 minutes, Type 3 found in 77% of the neonates, type 2 found in 23% of them and type 1 no longer found. According to this result, 100% of the partial and complete AFC have achieved the neonates at 120-150 minutes. The result obtained was similar to the previous study conducted by Askin et al. and Hooper et al., in which they divided the physiological transitional respiratory system into three types. In the first phase (0-30 minutes), lung filled with more fluid, the second phase (30 minutes to 3 hours) AFC process was observed and completed in the third phase (2-8 hours). (Askin, 2009; Hooper & Wallace, 2006)

### *4.4. Correlation between AQP5 and β-ENaC with lung ultrasonography result*

Our findings prove for the first time the feasibility of quantifying the level of β-ENaC and AQP5 in umbilical artery plasma shortly after birth from early-term newborns elective CS. This finding may significantly contribute to future studies, and the umbilical artery collection is more accessible than other peripheral arteries in neonates. This study also correlates AQP5 and β-ENaC levels with AFC using LUS at different age in minutes. It hoped that further research could be carried out to make β-EnaC and AQP5 as predictor factors for respiratory disorders guided by LUS immediately after birth.

In conclusion, we demonstrated that quantifying the mean level of β-ENaC and AQP5 in umbilical artery plasma shortly after birth from early-term newborns elective CS is feasible. Umbilical artery sample allows the practitioner to avoid more invasive approaches, such as radial artery and bronchoalveolar lavage. This study also found a significant relationship between AQP5 and β-EnaC levels with AFC assessed by ultrasound, therefore lung ultrasound could be used to assess neonatal lung physiology.

This study has limitations. First, the sample is only at one phase of gestational age (early-term). Secondly, there was no control group for standard vaginal delivery. Third, this study conducted during the Covid-19 pandemic; it should also measure the blood stress hormone levels of the mother and child.

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