PHOSPHATIDYLGLYCEROL STUDY AS FETAL LUNG MATURATION PARAMETER AFTER DEXAMETHASONE ADMINISTRATION FOR WOMEN AT RISK OF PRETERM BIRTH

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ABSTRAK

*Fosfatidilgliserol merupakan indikator penting maturasi paru janin, yang berperan dalam stabilisasi komplek surfaktan lipoprotein. Antenatal kortikosteroid dapat menstimulasi sintesa surfaktan paru pada bayi dengan kelahiran prematur. Tujuan dari penelitian ini mengkaji kadar fosfatidilgliserol sebagai parameter pematangan paru janin pasca pemberian deksametason pada ibu dengan persalinan preterm dibandingkan dengan parameter rasio L/S. Jenis penelitian ini adalah longitudinal (kohort) prospektif. Sampel penelitian adalah ibu hamil beresiko persalinan preterm dengan usia kehamilan 28-34 minggu yang mendapatkan terapi deksametason antenatal 6 mg IM tiap 12 jam diberikan 4 kali dalam 48 jam. Besar sampel sebanyak 17 pasien. Penetapan rasio L/S dan kadar PG dilakukan dengan metode ELISA. Penelitian dilaksanakan mulai bulan Mei – November 2015 dan direview untuk mendapatkan izin kelaikan etik oleh komite etik penelitian RSUD Dr. Soetomo. Dari hasil penelitian menunjukkan bahwa nilai rasio L/S rata-rata adalah 2,28 dengan rentang 1,35 – 9,06 dan kadar PG rata-rata adalah 1,17 dengan rentang 0 – 3,79. L/S rasio dan PG menunjukkan tidak ada hubungan yang bermakna antara keduanya. Peningkatan kadar PG pada usia kehamilan 28 – 32 minggu belum menunjukkan perubahan yang bermakna secara klinis. Perubahan kadar PG terbesar terjadi pada usia kehamilan 32 – 34 minggu.**(FMI 2016;52:264-269)***

Kata kunci: *cortikosteroid, persalinan prematur, fosfatidilgliserol, maturitas paru janin*

ABSTRACT

*Phosphatidylglycerol is an important indicator of fetal lung maturation, which plays a role in stabilizing surfactant lipoprotein complex. Corticosteroid antenatal can stimulate the synthesis of pulmonary surfactant in infants with preterm birth. The objective of this study is to examine the phosphatidylglycerol levels as fetal lung maturation parameter after dexamethasone administration in women with preterm birth compared to L/S ratio parameter. This study was prospective longitudinal (cohort). The samples were pregnant women with preterm birth risk at 28-34 weeks gestation getting the therapy of antenatal dexamethasone 6 mg IM every 12 hours given 4 times in 48 hours. The samples were 17 patients. Determination of L/S ratio and PG levels was performed by ELISA. The study was conducted from May - November 2015 and reviewed to obtain ethics eligibility permit by the research ethic committees of Dr. Soetomo General Hospital. The results show that the mean value of L/S ratio is 2.28 with a range of 1.35 to 9.06 and the mean of PG level is 1.17 with a range from 0 to 3.79. L/S ratio and PG show no significant relationship between the two of them. Increased levels of PG on the gestational age of 28-32 weeks have not demonstrated clinically significant changes yet. The highest PG level occurs in the gestational age of 32-34 weeks. **(FMI 2016;52:264-269)***

Keywords: *corticosteroid, preterm birth, phosphatidylglycerol, fetal lung maturity*

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INTRODUCTION

Preterm birth has high level of mortality and morbidity. Problem found in preterm birth is due to the immaturity of organ systems. Respiratory Distress Syndrome (RDS) also called as Hyaline Membrane Disease (HMD), is a respiratory distress syndrome caused by surfactant deficiency, especially in infants born with shorter gestational age (Honrubia & Stark 2004). Pulmonary surfactant is a lipo protein complex synthesized and secreted by alveolar type II cells. Surfactant phosphorlipid consists of 60% phosphatidyl choline saturated mixture of which the 80% contains dipalmitofosfatidilkolin, 25% unsaturated phosphatidylcholine mixture, and 15% phosphatidylglycerol and phosphatidylinositol and a small amount of fosfatidiliserin, phosphatidyl ethanolamine, sphingomyelin and glycolipids (Poynter & mariee 2003).

Determination of L/S ratio is gold standard for the tests of lung maturity of amniotic fluid, this is because lecithin is the largest component of pulmonary surfactant (90% in the form of DPPC). PG is an essential component in human pulmonary surfactant by

increasing the adsorption of phospholipids to the surface of liquid - air and redistributed after the surfactant layer is compressed (Herbert & Chapman 1986). PG begins to be detected at the time of L/S ratio shows maturity and gradually the level increases with increasing L/S ratio (Spillman et al 1988). Glucocorticoids stimulates the surfactant production by alveoli type II cells and will accelerate the incorporation of protein surfactant so that it will spur structural change for the lung maturation, decrease the vascular permeability and increase pulmonary function by increasing the number of surfactant in alveolar (Maher et al 1994, Fakhory et al 1994, Bunt et al 2000). Through this research, it is expected to determine the levels of phosphatidyl glycerol after the administration of dexamethasone in women with preterm birth.

MATERIALS AND METHODS

Phosphatidylglycerol study as fetal lung maturation parameters after administration of dexamethasone in women with preterm birth had been conducted from May to November 2015 in the Delivery Room of Emergency Room, Dr. Soetomo General Hospital Surabaya. The inclusion criterion of this study was pregnant women with gestational age of 28 weeks to 34 weeks, supported by the history of antenatal and or ultrasound examination (patients with risks for preterm birth). From the results of research conducted during May - November 2015, it was obtained a total of 30 samples meeting the criteria for inclusion, getting antenatal dexamethasone 6 mg IM therapy every 12 hours given 4 times in 48 hours. Of the 30 patients, there were 13 research subjects stated dropout due to several reasons such as not birth that did not happen until the 7th day after the administration of last dose of antenatal dexamethasone 6 mg IM every 12 hours given 4 times in 48 hours, birth that occurred before the research subjects got a full dose and too little amniotic fluid sample. Therefore, it was obtained 17 research subjects, then performed an analysis on the level of L/S ratio and phosphatidylglycerol.

To determine whether there was a relationship between gestational age and levels of phosphatidylglycerol and ratio L/S, and it would be analyzed using SPSS Pearson correlation test.

RESULTS

From all the research subjects successfully following the research protocol, it was then performed data recapitulation based on maternal age, gravidity, risk of preterm birth, gestational age, mode of delivery, BWI (birth weight infant) and Apgar score (AS) (Table 1).

Table 1. Research sample characteristics

Characteristics of Samples / Patients	Number of Samples	%	Mean ± SD
Ages of Mother			
16 - 25	5	29.41	
26 – 35	10	58.82	29.76
36 – 45	2	11.77	± 5.14
Gravidity			
Primigravid	8	47	-
Multigravid	9	53	
Birth Risk			
KPP	8	47.06	
PPI	4	23.53	-
PEB	3	17.65	
Others	2	11.76	
Gestational Age			
28 - < 30 weeks	3	17.65	
30 - < 32 weeks	5	29.41	32.06
32 - 34 weeks	9	52.94	± 1.78
Mode of Delivery			
Perabdominal	7	41.18	-
Pervaginam	10	58.82	
Birth Weight Infant			
< 2000 gram	14	82.35	
2000- 2500 gram	3	17.65	1647.06
2500–3000 gram	-		± 269.53
AS minute 1			
<3	0	0	
3 – 6	15	88.23	4.88
>6	2	11.77	± 1.73
AS minute 5			
< 3	0		
3 – 6	7	41.18	6.59
>6	10	58.82	± 1.62
BMI			
BMI < 25	-	-	
BMI > 25	1	5.88	

Of the 17 amniotic fluid samples of the research subjects meeting the inclusion criteria examined by using ELISA at a wavelength of 450 nm, it was obtained absorbance values for lecithin, sphingomyelin and phosphatidylglycerol. From the absorbance values, it was known the content value of lecithin, sphingomyelin and phosphatidylglycerol.

In this study, it was performed the correlation test between gestational age on value of L/S ratio. Correlation analysis is a parametric statistical analysis that has the basic assumptions that have to be met, namely the data that should be normally distributed. Therefore, it was performed the normality test with

One-Sample Kolmogorov-Smirnov test. In all variables, the significance value was > 0.05 then it could be concluded that the data of gestational age, L/S ratio and PG levels were normally distributed, then it could be followed by correlation statistical test.

In correlation test between 28-34 weeks gestation with the L/S ratio, it was obtained the value of 0.013 (p <0.05), and from the correlation test between 28-34 weeks gestation and PG levels, it was obtained the value of 0.190 (p> 0.05). From the result of correlation test between L/S ratio and PG levels in 28-34 weeks gestation weeks, it was 0.5

DISCUSSION

Preterm birth is the birth less than 37 weeks gestation with birth weight variation, generally born as low birth weight infants. Less immaturity of pulmonary organ systems and surfactant deficiency result in changes in the physiology of lungs so that the pulmonary compliance decreases by 25% from normal, respiration is heavy, intrapulmonary shunting increases and it occurs severe hypoxemia, hypoventilation causing respiratory acidosis (Rennie & Roberton 2002). Determination of L/S ratio is the gold standard for pulmonary maturity test of amniotic fluid, this is because lecithin is the largest component of pulmonary surfactant (90% in the form of DPPC). PG is an essential component in human pulmonary surfactant by increasing the adsorption of phospholipids to the surface of liquid - air and flexibility after the surfactant layer is compressed (Yu & Possmayer 2003). Some studies indicate that PG constituting minor phospholipids in lung is powerful surfactant that has hydrogen bonding capacity greater than lecithin and plays a role in stabilizing the complex surfactant lipoprotein and have an important role in surfactant monolayer deployment on the alveoli surface (Akella & Deshpande 2013). PG begins to be detected at the time of L/S ratio shows the level of maturity and gradually increases with the increasing L/S ratio (Spillman et al 1988). Glucocorticoids stimulate surfactant production by the alveoli type II cells and will accelerate the incorporation of surfactant protein so that it will spur structural change for pulmonary maturation, decrease vascular permeability and increase lung function by increasing the number of surfactant in alveolar (Maher et al 1994, Fakhory et al 1994, Bunt et al 2000). In this study, the biggest preterm birth risk factor is rupture of membranes (KPP) of 47%. This is because in the premature rupture of membranes there is a direct contact between the outside world and space in the uterus, thus facilitating the occurrence of infection. Other risk factor for preterm birth in this study is PPI (Partus Prematurus Imminens) of 23.5%, PEB (Preeclampsia weight) of 17.5% and others 12% (Table 1).

In this study, it is found babies with the weight of 1000-3000 grams with the largest sample distribution on birth weight infant of 1000 - 2000 gr that is 94% (Table 1). It is consistent with the development of the infant weight based on gestational age. In the age of 28-34 weeks gestation, the infant weight is in the range of 1000-2100 grams (Cunningham et al 2010). Infant's clinical condition that can be observed is the birth weight infant and Apgar score. Babies born with the weight > 1750 gr likely to have small occurrence of RDS, either given antenatal steroid therapy or not (Amanullah et al 2002).

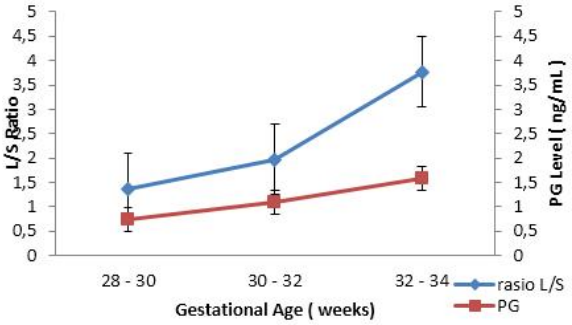

Fig. 1. Changes in level of L/S ration on 28 – 34 weeks gestation

In this study, it is used the groups of 28-34 weeks gestation with the goal that the population is at the same stage in the development of the lungs. RDS incidence is more common in under 34 weeks gestation because at this gestational age the fetus lungs are in the saccular stage of lung development. Preterm birth occurring during canalicular and saccular stages of lung development frequently leads to RDS because of poor development of airway peripheral and poor maturation of lung cells which are important in the maturation of the lung such as pneumocytes type 2 cell and inadequate antioxidant response on the increased oxygen suddenly (Amanullah et al 2002). From some references, it is stated that the older the gestational age, the less the RDS risk will be, this is because the higher amount of surfactant, because the biggest composition surfactant is lecithin, then the concentration of lecithin will also increase in accordance with increase in gestational age (Gluck & Kulovich 1973). Other studies have also suggested that the older the gestational age, the higher the value of L/S ratio and there is no significant difference in the use of dose of dexamethasone 4 mg and 6 mg x 4 x 4 in preterm birth women (Faizah et al, 2015). In this research, of the sample distribution based on the gestational age, it is obtained 17.65% of 28-30 weeks gestation, 29.40% of 30-32 weeks gestation and 52.95% of 32-34 weeks gestation (Table 1).

Table 2. Data distribution of gestational age, childbirth risks, fetal weight with L/S ratio and PG level

Patient Initial	Gestational Age	Birth Risk	PC Level ng/ml	SM Level ng/ml	L/S Ratio	PG Level ng/ml
NH	29-30	PPI	12	7.25	1.60	0.52
MS	29-30	KPP	20.92	14.25	1.47	0.61
ZH	29-30	PPI	33.85	22	1.54	3.62
JW	30-31	LL	18.61	10	1.86	0.307
EN	30-31	KPP	14.8	7.25	2.04	2.05
NR	31-32	KPP	21.1	13.75	1.53	0
SF	31-32	KPP	17.1	10.5	1.63	0.33
LS	31-32	KPP	24.62	8.75	2.81	0.85
EK	32-33	KPP	19.54	14.5	1.35	1.03
AE	32-33	KPP	23.4	16.25	1.44	3.79
DR	32-33	KPP	15.08	6.41	2.35	1.28
SA	33-34	PPI	25.85	12.5	2.07	0.9
MR	33-34	PEB	44.76	20.75	2.16	1.69
WS	33-34	KPP	25.38	8.5	2.98	0.67
SH	33-34	PPI	16	3.25	4.92	1.48
FT	33-34	PEB	18.92	2.5	7.56	2.69
SN	33-34	PEB	24.92	2.75	9.06	0.62
Mean			21,52	10.66	2.82	1.17
SD			7,28	5.79	2.27	0.96
Range			12,00– 44,76	2.50 – 20.75	1.35 – 9.06	0.00 – 3.79

In this study, it is performed parameter correlation SPSS statistical test of fetal lung maturity of L/S ratio and PG levels on gestational age at preterm birth after the administration of antenatal corticosteroids. From the results obtained, it shows that the higher the gestational age, the higher the L/S ratio will be, and vice versa, the lower the gestational age, the ratio lower the L/S will be (Table 3). From Table 3, it is also known that the significant value between gestational age and PG levels indicate that there is no significant relationship between gestational age of 28-34 weeks and PG levels ($p > 0.05$), although on average there is increase in PG levels at the gestational age of 28-34 weeks, but based on the results of the statistical analysis, the increase in PG levels is not significant in the gestational age of 28-34 weeks. This is according to some literature stating that the increased levels of PG occurs in pregnancy over 35 weeks (Jones & Ashwood, 1994), but many studies report that the detection of PG in amniotic fluid is a strong indicator of fetal lung maturation (Spillman et al, 1988; Farquharson et al, 1985; Kulovich et al, 1979). For instance, Halmann et al report that PG is not detected in pulmonary aspiration of infants with RDS and it is visible when the lungs mature (Hallman et al 1976).

Other studies have reported that RDS is rare when PG is in amniotic fluid, even when the L/S ratio is less than 2.0 (Obladen et al 1979). Hamilton et al report when PG is visible, 99% of infants born with mature lung function (Hamilton et al 1984). Of the examination of amniotic fluid in the gestational age before 34.5 to 35 weeks, PG appears only a little or nothing at all. PG levels increase between the gestational age of 35-36 weeks. Measurement of PG as a combination or a replacement L/S ratio can be used as an indicator of fetal lung maturity. In addition, blood contamination does not affect the results of PG analysis in amniotic fluid (Siegel et al 1983).

Table 3. Results of statistical test between L/S ratio and PG level

Patient Initial	Gestational Age (weeks)	L/S Ratio	PG Level (ng/mL)	Correlation Value	Significance Value (P)
NH	28-30	1.6	0.52	-0.997	0.048
MS		1.47	0.61		
ZH		1.05	1.07		
Mean ± SD		1.37 ± 0.29	0.73 ± 0.30		
Range		1.05 – 1.6	0.52 – 1.07		
JW	30-32	1.86	0.307	0.424	0.477
SF		1.63	0.33		
EN		2.04	2.05		
NR		1.53	0		
LS		2.81	0.85		
Mean ± SD		1.97 ± 0.51	1.10 ± 1.25		
Range		1.53 – 2.81	0 – 2.05		
SH	32-34	4.92	1.48	0.114	0.770
SN		9.06	0.62		
WS		2.98	0.67		
DR		2.35	1.28		
SA		2.07	0.9		
EK		1.35	1.03		
MR		2.16	1.69		
AE		1.44	3.79		
FT		7.56	2.69		
Mean ± SD		3.77 ± 2.81	1.57 ± 1.05		
Range		1.35 – 9.06	0.62 – 3.79		
Mean ± SD of Gestational Age of 28 – 34 weeks		2.82 ± 2.27	1.17 ± 0.96	0.148	0.572
Gestational Age Range of 28 – 34 weeks		1.05 – 9.06	0.52 – 3.79		
Significance of Gestational Age of 28 – 34 weeks		0.190	0.013		

From the analysis results of the relationship between L/S ratio and PG levels, it is obtained that in the gestational age of 30-32 and 32-34 weeks, it is showed significant relationship. It shows that on that gestational age, the higher value of L/S ratio is not followed by the increase in PG levels (Table 3). This study has provided data on PG levels in the gestational age of 28-34 weeks after the administration of examethasone with dose of 6 mg every 12 hours for 48 hours. Increased level of PG on the gestational age of 28-32 weeks has not demonstrated clinically significant changes yet. Changes in levels of PG appears on the gestational age of 32-34 weeks.

CONCLUSION

L/S ratio still becomes better parameter of fetal lung maturation on the gestational age of 28-34 weeks compared to PG levels.

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