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Prostaglandin E_2 and patent ductus arteriosus in premature infants

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Abstract

Background Patent ductus arteriosus (PDA) is a congenital heart disease most commonly occurring in premature infants. Spontaneous ductus arteriosus (DA) closure in premature infants has been suggested to be associated with duct lumen maturity and the DA sensitivity to prostaglandin E_2 (PGE_2).

Objective To assess for a possible correlation between serum PGE_2 levels and PDA size in premature infants.

Methods This observational study using repeated measurements on premature infants with PDA detected at days 2-3 of life was undertaken in Cipto Mangunkusumo Hospital and Fatmawati Hospital, Jakarta, from April to May 2014. The PDA was diagnosed using 2-D echocardiography and PGE_2 levels were measured by immunoassay. Pearson's correlation test was used to evaluate a possible correlation between PGE_2 level and DA diameter.

Results Thirty-three premature infants of median gestational age 31 (range 28-32) weeks and median birth weight 1,360 (range 1,000-1,500) grams were enrolled. Almost two-thirds of the subjects were male. Almost all (30/33) subjects had spontaneous DA closure before the age of 10 days. Subjects' mean DA diameter was 2.9 (SD 0.5) mm with maximum flow velocity of 0.2 (SD 0.06) cm/sec, and left atrial-to-aortic root ratio (LA/Ao) of 1.5 (SD 0.2). Their mean PGE_2 levels at the ages of 2-3, 5-7, and after 10 days were 5,238.6 (SD 1,225.2), 4,178.2 (SD 1,534.5), and 915.2 (SD 151.6) pg/mL, respectively. The PGE_2 level at days 2-3 was significantly correlated with DA diameter ($r = 0.667$; $P < 0.001$), but not at days 5-7 ($r = 0.292$; $P = 0.105$) or at day 10 ($r = 0.041$; $P = 0.941$).

Conclusion There is a strong, positive correlation between the PGE_2 level and DA diameter in preterm infants at 2-3 days of age. However, there is no significant correlation between PGE_2 level and persistence of PDA. **[Paediatr Indones. 2016;56:8-14.]**

Keywords: *patent ductus arteriosus, prostaglandin E2, premature infants*

Patent ductus arteriosus (PDA) is a congenital heart disease (CHD) commonly found in premature neonates.[1-4] Spontaneous closure or persistency of the ductus arteriosus (DA) in premature neonates has been suggested to be associated with DA lumen maturity and sensitivity of DA to PGE_2. High levels of PGE_2 may cause a DA to remain open and persist.[5-10] Current treatment for PDA in premature neonates has focused on anti-prostaglandins (APG).[10,11] As PGE_2 maintains the persistency of the DA, APGs are used to block the cyclooxygenase enzyme (COX inhibitors) in the PGE_2 pathways, in order to induce DA closure.[7,10] However, in practice, APG treatment does not always successfully close the DA. If the DA fails to close or reopens after APG treatment, ligation surgery is indicated.[6,12,13] The failure of PDA closure after APG treatment and the fact that DAs may spontaneously close indicate that DA diameter may not always be related to PGE_2 levels.[13,14] There may be a particular PGE2 level, where APG is useful in closing the PDA.[5-7] Above normal PGE_2 levels usually are an indication for the immediate use of APG, while, below normal

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levels generally suggest conservative approach.[8] We aimed to assess for a possible correlation between PGE_2 level and DA size in premature neonates, by which PGE_2 level may guide the decision of APG treatment to close a PDA.

Methods

This observational study with repeated measurements was undertaken in preterm neonates with PDA at Cipto Mangunkusumo and Fatmawati Hospitals, Jakarta, from April to May 2014. More than two measurements per subject were done sequentially, and at different times.[15,16] Subjects were premature neonates 2-3 days of age, with gestational age of 28-32 weeks and birth weight 1,000-1,500 grams. All subjects' parents provided informed consent. This sudy was approved by the Ethics Review Board at the University of Indonesia Medical School, Jakarta. Premature neonates not meeting these criteria were excluded.

Blood specimens for PGE2 levels were taken at ages 2-3 days, 5-7 days, and > 10 days. Measurements of PGE_2 levels were done at the Prodia laboratory. Principle of this examination was based on competitive binding between PGE_2 serum with PGE_2 conjugate to specific antibody. Result of this quantitative test was based on measurement and compared to the standard value. Color degradation was inversely proportional with PGE_2 level in the sample and standard. Result of this test was categorized according the color. If the color was bright blue, there was no PGE_2, and if the color was bluish, there was little amount of PGE_2. If the color was pale blue, there was much of PGE_2 and if the color was white, there was plenty of PGE_2.[17]

Persistent ductus arteriosus (PDA) was diagnosed if ductus arteriosus was not closed and settled spontaneously after the chronological age of ≥ 10 days, confirmed with two-dimensional echocardio-graphy.[18-20] Echocardiographic examinations were done using a digital ultrasound machine (*Philips HD-11*, Netherlands 2010) with a *Philips S12* ultrasound transducer. Patency of the DA defined as persistence of DA after 2-3 days of life (chronological age). Subject classified as drop out if they were excluded from the study because of no appropriate acceptance criteria.

The diameter of the DA was measured on the high left parasternal long or short axis view. Color Doppler and 2-D images were used to assess the type and PDA size, which was measured three times and then averaged. The maximum velocity of trans-ductal Doppler continuous flow was measured on the left parasternal short axis view and the average of three measurements was taken. Pulse of transductal continuous flow and location of maximal contraction was measured by viewing restrictive and non-restrictive pattern, as well as DV max. The LA/Ao ratio was determined using M-Mode in the parasternal long axis view and the average of three measurements was taken. [18-20]

Data was first described as percentage (age, gender, and gestational age) or mean/median and range (PGE_2 level and DA diameter) as appropriate. Kolmogorov-Smirnov test was used to evaluate data normality. Correlation coefficient with its corresponding 95% confidence interval for PGE_2 level and DA diameter was calculated using the Pearson's or Spearman's test. A coefficient of 0.6 or above indicated a strong correlation and a two sided P value of less than 0.05 was considered statistically significant.[15-16] All statistical analyses were performed using *SPSS version 17.0 for Windows.*

Results

This study was conducted from April 2014 to May 2015. The subject recruitment flow is shown in

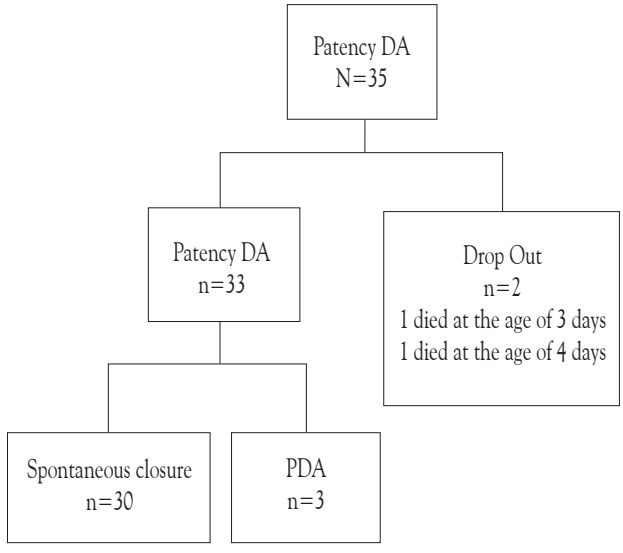

Figure 1. Subject recruitment pathway

Figure 1. Twenty-nine subjects were recruited from the Neonatal Intensive Care Unit, Department of Child Health at Cipto Mangunkusumo Hospital and 6 subjects were from the Perinatology Ward, Pediatric Health Unit at Fatmawati Hospital.

Of the 35 patients, 2 were excluded from the observation by the death at the age of 3 and 4 days, respectively, due to neonatal sepsis and respiratory distress syndrome. Three other subjects died between 9-11 days of age, but these subjects were still included in the analysis because spontaneous closure DA prior to death. By the 10th day of observation, 3 subjects still had open DAs, who hemodynamically significant and therefore they were then treated with intravenous APG.

Subjects' demographic and clinical features are shown in **Table 1**. Median gestational age was 31 weeks. Median birth weight was 1,360 g (range 1,000-1,500 g) and male babies outnumbered female. The majority of subjects (85%) had respiratory distress and required CPAP. None of 33 subjects received surfactant therapy. Five patients died: 2 during the observation period so they were excluded, while 3 later after their DA closed.

The clinical course of PDA is shown in **Table 2**. At the age of 2-3 day, all subjects were diagnosed with PDA. At the age of 5-7 day, 23 subjects had their duct closed. On the age of 10 day, of the 10 subjects with previously patent duct, 7 had their duct closed, while three patietns had their duct open. In total, 30 subjects had spontaneous closure.

Table 3 shows the mean echocardiography and PGE2 level results based on repeated measurements at the prescribed time points. For the 3 echocardiographic parameters, mean DA diameter was 2.9 mm on days 2-3, with transductal velocity (DVmax) of 0.2 cm/second and ratio of left atrium to aorta (LA/Ao) of 1.5. On days 5-7, the mean DA diameter

Table 1. Subjects' characteristics

Characteristics	Patency of DA (n= 33)
Median gestational age (range), weeks	31 (28-32)
Median birth weight (range), grams	1,361 (1,000-1,500)
Gender, n	
Male	21
Female	12
RDS*	
Yes	
No	6
CPAP** zero age	
Yes	27
No	6
Not given surfactant therapy	33
Subsequently died	3

*RDS: respiratory distress syndrome; **CPAP: continuous positive airway pressure.

Table 2. Echocardiographic results

Variables	Age of subjects (n = 33)			
	2-3 days	5-7 days	10 days	10-15 days
Echocardiographic results				
Spontaneous closure	0	23	7	0
PDA	33	10	3	3*
Reopened	-	-	0	0

*diagnosed to have PDA and continued with the PG inhibitors

Table 3. Results of echocardiographic parameters and PGE2 levels

Variables	Age of subjects		
	2-3 days	5-7 days	≥10 days
Echocardiography:			
Mean DA diameter (SD), mm	2.9 (0.5)	0.4 (0.1)	0.5 (0.1)
Mean DVmax (SD), m/minute	0.2 (0.06)	0.2 (0.02)	0.18 (0.04)
Mean LA/Ao ratio (SD)	1.5 (0.2)	0.2 (0.04)	0.2 (0.1)
Serum PGE_2			
Mean PGE_2* (SD), pg/mL	5,238.6 (1,225.2)	4,178.2 (1,534.5)	915 (151.6)

DA: ductus arteriosus; DVmax: ductus maximal velocity; LA/Ao: left atrium/aorta ratio. Data presented in the form of the mean (standard deviation).
*Reagent kit: *R&D System, Inc., Minneapolis, MN 55413, US product. sample value:* serum = *non detectable-2116 pg/ml* (standard range: 39-2500 pg/mL)

was very small at 0.4 (SD 0.1) mm, with transductal velocity (DVmax) of 0.2 cm/second and LA/Ao ratio of 0.2. On day 10, echocardiography showed a mean transductal velocity (DVmax) of 0.18 cm/second and LA/Ao ratio of 0.2.

On 2-3 day of life, subjects had a high mean PGE2 level of 5,238.6 (SD 1,225.2) pg/mL. On age of 5-7 day, the mean PGE_2 decreased to 4,178.2 (SD 1,534.5) pg/mL. On 10 days of age, the PGE2 measurements were done only in subjects with PDA, revealing a mean of 915.2 (SD 151.6) pg/mL.

Table 4 shows the correlation between DA diameter and PGE_2 level. On age of 2-3 day, there was a strong correlation between duct diameter and PGE2 level ($r = 0.67$, $P < 0.001$), with a determination coefficient of 44.5%. No correlations were found in the subsequent measurements on age of 5-7 day and 10 day.

The high percentage of subjects with spontaneous closure was probably influenced by the birth weight and gestational age in the inclusion criteria. Premature neonates with birth weight ≥ 1,000 grams, gestational age ≥ 28 weeks, and diagnosed with PDA, have a higher probability of spontaneous closure before 2 weeks of age. Furthermore, there is higher probability of PDA incidence in premature neonates of birth weight < 1,000 grams and gestational age < 28 weeks. A Texas study in 2006 concluded that only 34% of premature neonates with very low birth weight (≤ 1,000 g) and gestational age 26 (SD 2) weeks had spontaneous closure at the postnatal age of 4.3 (SD 2) days. [22]

Table 4. Correlation between PGE2 level and DA diameter

Variable	Diameter of DA based on age groups								
	2-3 days			5-7 days			10 days		
	r	R (%)	P value	r	R (%)	P value	r	R (%)	P value
PGE_2	0.667*	44.5	<0.001	0.292**	8.5	0.105	0.041**	0.16	0.941

*Pearson's correlation test; **nonparametric correlation Spearman's test; r = coefficient correlation; R= determination correlation

Discussion

The outcomes of PDA in premature neonates are varied and influenced by many factors. Previous studies have been inconclusive on the timing of DA closure, as many patients have patency of DA lasting until the 4th to 7th day of life.[1-5] However, in a small number of cases, the DA has not spontaneously closed even by days 7-10 of age. [5-7] Even some studies have reported that the delayed DA closure in preterm neonates could reach 3 months or even later.[1-4] Delayed DA closure impacts the cardiovascular and respiratory systems. As such, the condition can affect the course of primary illnesses in these premature neonates (RDS). [7-8]

In our study of 33 subjects with PDAs on days 2-3 of life, 30/33 subjects had spontaneous closure before the 15th day of life, and only 3 subjects (9.1%) had an open DA BY the end of study. Similarly, a 2007 prospective study in Belgium found that among 30 premature neonates with mean gestational age of 28 weeks and birth weight 1,010 g, 20 subjects had spontaneous resolution of their PDA. Those babies had no special treatment and were only subjected to restrictive fluid therapy.[21]

Prostaglandin E_2 (PGE_2) is a prostaglandin derivate and biosynthetic product of arachidonic acid. This derivate has strong, biological effects of smooth muscle stimulation and vasodepression. The PGE_2 is degraded in the lung through an active transport mechanism, involving 15-hydroxyprostaglandin dehydrogenase (HPGD). The metabolites of PGE_2 are excreted in urine (90%) and feces (10%).[7-10] The PGE_2 level was very high on the 2nd-3rd days of life, and then decreased on the 5th-7th days. On the 10th day, PGE_2 level was normal. Prostaglandin E2 level was measured by using repeated measurement. On the 5th-7th days, PGE_2 level was measured in all subjects regardless of patency of da at that time. However, on day 10, PGE_2 measurements were only done in the 3 subjects with patency of DA. There are two theories which may explain the decrease in PGE_2 level with age. Prostaglandins are produced by the placenta during the fetal period, decrease with the cutting of the umbilical cord, and the respiratory system function of prostaglandin metabolism in the lung.[4,23-25]

Statistical analysis revealed a strong and significant correlation between PGE_2 levels and DA diameter on the 2nd-3rd days. We observed a tendency of higher PGE_2 levels with wider DA diameter. This suggests that PGE_2 level has important role in PDA closure in premature neonate. Clyman *et al*.[8] had similar results in premature newborn lambs with PDA. They aimed to determine the PGE_2 level that could maintain an open DA in the first days of life. At 2 hours after birth, the blood PGE_2 level was twice that of lambs without PDA. They concluded that PGE_2 level has an important role in PDA occurrence. In our study on the second observation period at 5-7 days, 23 out of 33 subjects had spontaneous DA closure. Statistical analysis revealed no significant correlation between PGE_2 level and DA diameter. From this point onwards, PGE_2 level seemed to have a small impact on patency of DA. This result was also seen on the 10th day. However, the lack of a significant correlation between PGE_2 level and DA diameter at 10 days of age may result form the very small number of subjects (3 patients).

High PGE_2 levels in the first few days after birth may maintain patency of DA. The PGE_2 activity is mediated by the 3G-protein-coupled receptor (EP2, EP3 and EP4) in the brain, the kidney, thrombocytes, and vascular smooth muscle, including the DA vasculature. The PGE_2 binding to the 3G-protein-coupled receptor activates the adenylate cyclase enzyme and K-ATP channels through a cAMP mechanism, so that vasculature in the DA smooth muscle dilates.[26-29] Cyclic guanosine monophosphate (cGMP) can activate cGMP kinase which can cause K^+ cell membrane opened directly. This opening leads to K^+ efflux from the cell, depolarization of cell membranes, and blocking of the Ca^+ channels. Decreased Ca2+ influx and releasing Ca^{2+} can cause vasodilation. It has been theorized that phosphodiesterase-5 (PDE-5) activity, as a PDE enzyme that metabolizes cGMP and cAMP, is at a low level, hence, limiting its ability to degrade cGMP and cAMP. As such, sensitivity to PGE_2 is high so that the DA remains open.[26-29]

Based on basic mechanism of PGE_2, we know that decreased activity of one or all receptor PGE_2 (EP_2, EP_3 and EP_4) may trigger maturation process and spontaneous closure of DA. Decreased PGE_2 receptor (such as EP_4) or HPGD may also dramatically lower the patency of DA. In other word, loss of EP4, HPGD, or COX function, has a role in spontaneous closure of DA.[28] This may explain why 90% of our subjects had their DA closed spontaneously on day 5-7 of life. Spontaneous closure of DA in premature neonate basically occurs later than in term neonate, and as their age are getting older, spontaneous closure can be expected. This was possible if functional and anatomical of DA closure is expected to be normal. Under these conditions, DA smooth muscle cell contraction produce a hypoxic zone and simultaneous remodeling in lumen of the DA.[2,30,31]

Hammerman *et al.* had the similar results in a study on prostaglandin level and indomethacin therapy in premature neonates with PDA.[9] Prostaglandin E_2 measurement was done before indomethacin therapy. Nine out of 16 infants with PDA and treated with indomethacin had DA closure. Among the 9 infants, 8 infants had PGE_2 levels higher than normal. Of the 7 infants who did not respond to indomethacin and whose DAs remained open, 6 babies had PGE_2 level within normal limits from the beginning. They concluded that PGE_2 level may lead to PDAs, and anti-PGs are indicated in such situations. But some cases of PDA are not related to high PGE_2 levels.[9]

Patency of DA that lasts through 10 days of life without a discernible role for PGE_2 suggests that other factors affect PDA in premature neonates. One such factor may be the immaturity of the lumen DA. The ductus lumen functions as a conduit for nutrition in the DA, but some nutritional resources come from the vasa vasorum tissue. Vasa vasorum tissue can enter the lumen through the ductus outer wall and can grow inside to the lumen. They stop growing about 400-500 μm from the lumen. The zone between the vasa vasorum and the lumen is called the avascular zone.[30,31] This process can cause the ductus wall to become ischemic, inducing vascular remodeling. In premature neonates, the vasa vasorum thickness is about 200 μm, such that it is spread just at the surface of the vasculature around the tunica adventitia layer, without penetrating the tunica media of the ductus. If the lumen DA is large and the vasa vasorum is small, the avascular zone will not be very thick. Hence, the hypoxic zone does not form, leading to the failure of the vascular remodeling process, and leaving the DA open. The other factor is factor of "time". Probably

it is needed longer time to observe to explain that problem. Unfortunately, complication that can happen because of PDA in premature neonate can affect primary illness.[31]

Since we had only 3 subjects with a PDA diagnosis at day 10, the small sample size did not allow us to determine a correlation between PGE2 with PDA incidence. Further study is needed with a larger sample size and longer research period.

In conclusion, there is a strong correlation between PGE_2 level and patency of DA in premature neonates at the 2^{nd}-3^{rd} days of life. However, patency of DA has no correlation with PGE_2 level in the 3 PDA cases at 10 days of age. In premature neonates with PDA, gestational age $\geq$30 weeks, and birth weight $\geq$ 1,000 grams, early use of PG inhibitors may not be necessary, as 90% of PDAs close spontaneously before 10 days of life.

Conflict of interest

None declared.

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