

The correlation between the mother's vaginal bacterial colonization and incidence of early onset neonatal sepsis

by Andi Dwi Bahagia Febriani

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TIME SUBMITTED	08-NOV-2020 02:36PM (UTC+0700)	WORD COUNT	4187
SUBMISSION ID	1439399147	CHARACTER COUNT	21963

The correlation between the mother's vaginal bacterial colonization and incidence of early onset neonatal sepsis.

A Dwi Bahagia Febriani^{1,2}, Andi Handriyati¹, Ema Alasiry¹, Dasril Daud^{1,3}

¹Department of Pediatrics, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

²Neonatology Division, Department of Pediatrics, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

³Hematology Division, Department of Pediatrics, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

Abstract

Introduction: Neonatal sepsis is one of the major causes of mortality and morbidity in neonates. Early onset neonatal sepsis (EONS) is a severe condition with high mortality rate. EONS commonly caused by maternal microorganism before or during the delivery process. Moreover, mother's genital tract microorganisms also contribute in EONS incidence.

Objective: To identify the relationship between the mother's pathogenic vaginal colonization and occurrence of EONS.

Method: A prospective cohort study was conducted in Dr. Wahidin Sudirohusodo hospital and its network hospital from April until August 2015. Samples included inpartu mothers who met the inclusion criteria, and the babies who were followed up for the development of EONS.

Results: Out of 90 samples, there were 56 (62.2%) mothers with vaginal pathogenic bacterial colonization. The bivariate analyses result showed that there was no significant difference for EONS occurrence in babies born from mother with vaginal bacterial colonization and those who were not ($p=0.399$). The frequency of newborn from mothers with vaginal pathogenic bacteria colonization who had EONS was 4 (7.1%), while the frequency of newborns from mother without vaginal pathogenic bacteria colonization was 1 (2.9%). There was no significant correlation between the kind of vaginal pathogenic bacteria colonization and EONS incidence ($p=0.163$). The pathogenic vaginal colonization was dominated by negative gram bacteria (77.97%).

Conclusion: There was no correlation between vaginal bacterial colonization and EONS incidence.

Keywords: Early onset neonatal sepsis, Pathogenic bacteria colonization.

Accepted January 23, 2017

Introduction

Until now, neonatal sepsis is a leading cause of mortality and morbidity of newborns. In developing countries, most of the neonates who were hospitalized concerned with infection. The same thing was found in developed countries on neonates who were hospitalized in neonatal intensive care unit [1]. The incidence of sepsis in developing countries is higher (1.8-18/1000) than the incidence in developed countries (1-5/1000). WHO reports that 5 million babies do not survive until 28 days age per year and 98% of mortality are from developing countries. Neonatal deaths in developing countries were caused by infections (42%), asphyxia and birth trauma (29%), preterm babies

and low birth weight (10%), congenital abnormalities (14%) and other causes (4%) [2]. Although the infection can be caused by viruses, fungi, and parasites, bacterial infection is the leading cause in neonatal sepsis. Exposure can occur during gestation (in utero), during labor, and after birth. The exposure that occurs during pregnancy or during childbirth is classified into early onset sepsis (early onset) and the exposure that occurs after birth is classified into slow-onset sepsis (late onset) [3].

The bacteria that most commonly cause prenatal sepsis which found on rectovaginal are group B streptococcus (GBS), *E. coli* and *Klebsiella*, while in the environment are *staphylococcus aureus*, *E. coli* and *Klebsiella* [4].

GBS colonization can be found in the genital tract and lower gastrointestinal of healthy pregnant women as an asymptomatic colonization. Meanwhile, in urinary tract during pregnancy, this bacterium is associated with asymptomatic bacteriuria caused by ascended recolonization. Approximately 5-30% of pregnant women have GBS colonization and 29-72% of their infants will get the same colonization through the ascending transmission which is through the maternal genital tract on delivery process [5].

The data about pathogenic bacteria that contained in mother's vagina that possible to be transmitted prenatally in pregnant women in Makassar is still unknown because screening check up was never done so, the distribution of prophylactic antibiotics does not appropriate.

Based on the description above, it is necessary to conduct a study to determine the relationship between the mother's vaginal bacterial colonization with early-onset neonatal sepsis. By determine their correlation, hopefully we can take a fast and precise action, so mortality due to neonatal sepsis can be reduced. Study on the relationship of pathogenic bacteria colonizing the vagina of pregnant women with early-onset neonatal sepsis has not been done in South Sulawesi yet.

Materials and Methods

This study was a prospective cohort study that conducted at Dr. Wahidin Sudirohusodo Hospital in Makassar and its network hospital (Faisal Islamic hospital and Ibnu Sina hospital) from April until August 2015. The examinations were conducted at the Nehri laboratory of Hasanuddin University Teaching Hospital. Samples are all affordable population that met the inclusion criteria. The inclusion criteria of the mother are inpartu regardless of gestational age, prim gravid and multi-gravid, willing to follow the study/ gets a permission from her husband or family, While the inclusion criteria for infant babies born vaginally are aged ≤ 3 days and get parental consent. This study use consecutive sampling with the subject of the study based on the entry sequence to the hospital. All the data were obtained from the primary data group by its type of data, and then analyzed with statistical; univariate analyses, unpaired Student's t test, Mann Whitney test and X^2 test (Chi square).

Results

In this study, the characteristics of pregnant women that assessed are maternal age, gestational age, gravid, education, and employment. Out of 90 samples, there are 56 mother (62.2%) with vaginal bacterial colonization and 34 (37.8%) with no vaginal bacterial colonization. On mother's age category, the group with vaginal bacterial colonization mean ± 29.16 and SD is ± 5.91 , while in the group without vaginal bacterial colonization is 26.68 ± 4.54 . Gestational age of mother with vaginal bacterial colonization is the same with the group of mother without

colonization. The median is 38 weeks with range of exist groups is 37-41 and 36-40 on none group. Prim gravid subject in vaginal bacterial colonization group is the same with the group without colonization which is 50%, while the multi-gravid are 77.5% in the group with bacterial colonization and 22.5% in the group without colonization. On education status, basic 12 (80.0%), intermediate 14 (63.6%) and high 30 (56.6%) in vaginal bacterial colonization group, while in the group without colonization, basic education 3 (20.0%), intermediate 8 (36.4%), and high 23 (43.4%). unemployed mothers (housewives) are 33 (63.5%) in bacterial colonization group and 19 (36.5%) in the group without colonization. Meanwhile, employed mother are 23 (60.5%) in the group with colonization and 15 (39.5%) in the group without colonization (Table 1).

Table 2 shows the relationship between mother's ages with the number of pregnancies with mean ± 32.08 and SD $\pm 4,854$ on multi-gravid group, while on prim gravid group ± 25.14 and SD ± 3.897 .

Out of 90 subjects (infants), 39 (43.3%) are male and 51 (56.7%) are female. Out of 39 (43.3%) male subjects, there were 4 (10.3%) with EONS and 35 (89.7%) without EONS. Meanwhile, out of 51 (56.7%) female subjects, there is 1 (2.0%) with EONS and 50 (98.0%) without EONS. In gestational age category, there is no significant difference between outcomes of EONS and not EONS with median 38 weeks and range 37-38 weeks on EONS group and 36-41 weeks in not EONS group, $p=0.354$. Likewise, in newborn baby category, there is no significant difference between EONS group and not EONS group with median (range) in EONS group was 2800 g (2400-3200) and not EONS group was 2900 g (2300-4100), $p=0.332$ (Table 3).

Table 4 shows characteristic of babies born from mother with vaginal bacterial colonization and without bacterial colonization. The result shows that there is no significant correlation on between sex, gestational age and birth weight with the presence or absence of bacterial colonization on mother's vagina.

Analyses of the relationship between mothers with vaginal bacterial colonization and EONS incidence can be seen in Table 5. Statistical analyses showed there is no significant correlation between the presence of bacterial colonization with EONS or not EONS with $p=0.399$ ($p>0.05$).

Table 6 provided an overview of 43 mothers who had colonization of gram-negative bacteria, EONS incidence are 2 (4.7%) and not EONS are 41 (95.3%). Out of 10 mother who have colonization of gram positive, there is 1 (10.0%) with EONS and 9 (90.0%) without EONS, while 3 mothers who have a combined of gram negative and gram positive, there is 1 (33.3%) with EONS and 2 (66.7%) without EONS. Statistical analyses showed no significant correlation between the incidences of bacterial colonization types with EONS incidence.

Table 1. Characteristics of maternal pregnancy

Characteristics	Vagina bacterial colonization		P
	Exist	None	
Mother's age (year)			
Mean	29.12	26.68	0.038*
Median	30.00	26.00	
SD	5.91	4.53	
Range	19-41	17-37	
Gestational age (week)			
Mean	38.16	37.74	0.037**
Median	38.00	38.00	
SD	0.97	0.86	
Range	37-41	36-40	
Gravid, n (%)			
Primigravid	25 (50.0)	25 (50.0)	0.007***
Multigravid	31 (77.5)	9 (22.5)	
Education level, n (%)			
Basic	12 (80.0)	3(20.0)	0.253***
Intermediate	14 (63.6)	8 (36.4)	
High	30 (56.6)	23 (43.4)	
Occupation, n (%)			
Housewife	33 (58.3)	19 (36.5)	0.777***
Work	23 (60.5)	15 (39.5)	

* Student t test; ** Mann-Whitney test; *** Chi-square X²

Table 2. Relationship of the mother's age with number of pregnancies

Mother's age (year)	Number of pregnancy	
	Multi-gravid	Prim gravid
Mean	32.08	25.14
Median	32	25
SD	4.854	3.897
Range	19-41	17-32

Student t test $p=0.000$ ($p < 0.05$)

Table 3. Characteristics of infants with EONS and not EONS

No.	Variable	EONS (n=5)	Not EONS (n=85)	P
1	Sex, n (%)			0.089*
	Male	4 (10.3%)	35 (89.7%)	
	Female	1 (2.0%)	50 (98.0%)	
2	Gestational age (week)			0.354**
	Mean	37.60	38.02	
	Median	38.00	38.00	
	Standard deviations	0.547	0.963	
	Range	37-38	36-41	
3	Newborn infant (gram)			0.332**
	Mean	2770	2965.88	
	Median	2800	2900	
	Standard deviation	327.10	432.25	
	Range	2400-3200	2300-4100	

* Chi-square X² test; ** Mann-Whitney test

Table 7 showed the frequency of the type of bacteria in mother's vaginal bacterial colonization examination. Gram-negative bacteria (77.97%) were more than the gram-positive (22.03%). Gram-positive bacteria that most commonly found was *S. aureus* which are 11 (18.64%),

while streptococcus is only 2 (3:39%). Meanwhile, gram-negative bacteria that most commonly found were *Enterobacter agglomerans* as many as 11 samples (18.64%). *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Proteus*

Table 4. Characteristics of babies born from mother with bacterial colonization of the vagina and without bacterial colonization

No.	Variable	Bacterial colonization		P
		Exist	None	
1	Sex, n (%)			0.578*
	Male	23 (59.0%)	16 (38.5%)	
	Female	33 (70.6%)	15 (29.4%)	
2	Gestational age (week)			0.367**
	Mean	38.16	37.74	
	Median	38.00	38.00	
	Standard deviation	0.968	0.864	
	Range	37-41	36-40	
3	New-born infant (g)			0.842**
	Mean	2991.96	2894.12	
	Median	2895	2930	
	Standard deviation	485.36	308.68	
	Range	2400-4100	2300-3400	

* Chi-square X² test ** Mann-Whitney test**Table 5.** The relationship between maternal vaginal bacterial colonization with EONS

Maternal vaginal bacterial colonization	Group		Total
	EONS (n=5)	Not EONS (n=85)	
Exist	4 (7.1%)	52 (92.9%)	56 (100%)
None	1 (2.9%)	33 (97.1%)	34 (100%)
Total	5 (5.56%)	85 (94.4%)	90 (100%)

X² Chi-square test; p=0.399 (p>0.05)**Table 6.** Relationship of the maternal vaginal bacteria type with EONS

Colonization	EONS	Not EONS	Total
Gram Negative	2 (4.7%)	41 (95.3%)	43 (100%)
Gram Positive	1 (10%)	9 (90.0%)	10 (100%)
Gram Positive and Negative	1 (33.3%)	2 (66.7%)	3 (100%)
Total	4 (6.67%)	52 (93.3%)	56 (100%)

X² Chi-square test; p=0.163 (p>0.05)**Table 7.** Frequency of bacteria types in the vaginal swab culture of pregnancy examination

	Bacteria types	N	%
1.	Gram-positive bacteria		
	<i>Streptococcus</i>	2	3.39
	<i>Staphylococcus aureus</i>	11	18.64
	Gram-negative bacteria		
	<i>Escherichia coli</i>	3	5.08
	<i>Klebsiella pneumoniae</i>	3	5.08
	<i>Enterobacter cloacae</i>	2	3.39
	<i>Enterobacter agglomerans</i>	11	18.64
	<i>Enterobacter aerogenes</i>	8	13.56
	<i>Proteus vulgaris</i>	3	5.08
	<i>Providencia alkalifaciens</i>	9	15.25
	<i>Pseudomonas aeruginosa</i>	1	1.69
	<i>Alkaligenes faecalis</i>	3	5.08
	<i>Acinetobacter calcoaticus</i>	3	5.08
	Total	59	100

vulgaris, *Providencia alcalifaciens*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Acinetobacter calcoaticus* was found in smaller amounts.

Discussion

Neonatal sepsis causes about 718.000 deaths each year or about 23.4% of the 3.1 million deaths in 2010 [2]. Neonates are particularly vulnerable to infection in the first week of life. There are 42% of deaths in the first 5 week of life caused by infection [7]. EONS is generally caused by microorganisms that obtained from the mother before or during the birth process. Maternal genital tract organisms have a important role in EONS incidence [8]. Rectovaginal colonization of pregnant women was suspected to be a risk factor for neonatal sepsis within the first seven days of life [9]. This study is a prospective cohort to identify a relationship between the vaginal bacterial colonization with incidence of EONS.

Statistically, there is a significant difference on maternal age category between the group of vaginal bacterial colonization and the group without vaginal bacterial colonization ($p=0.038$), but these results are contrast with the study conducted by Chan and, which indicates that there are no significant differences based on maternal age in the group of rectovaginal colonization and group without rectovaginal colonization, $p=0.085$ [10].

Besides, analyses of the relationship between maternal ages with the number of pregnancies were done. The result shows that there are significant differences in maternal age with the number of pregnancies with $p=0.000$. Multi-gravid have an older age compared with prim gravid, so it can be concluded that the significance correlation of the age of the mother with maternal vaginal colonization was also affected by the number of pregnancies.

The gestational age associated with the presence or absence of bacterial colonization in mother's vagina with median 38 weeks and range 37-41 weeks in group with colonization and 36-40 weeks in group without colonization ($p=0.037$). It could be caused by mechanical and hormonal changes that occur in pregnancy. These changes reach the peak at the end of the second trimester and early third trimesters which are can cause urinary tract infection (UTI) in pregnancy. *Escherichia coli* are the bacteria that cause UTIs in pregnancy were found in 80-90% cases. These bacteria will spread from the urethra to the vagina. In addition, the level of hygiene will decrease along with increasing of gestational age.

Based on frequency of pregnancy, there is significant difference between the groups of prim gravid and multi-gravid to the presence or absence of vaginal bacterial colonization ($p=0.007$). Women with multi-gravid is 1.7 times more risk to have the colonization of pathogenic bacteria in the rectum and vagina than the prim gravid. Multi-gravid has a high contact with health system (such as visits to the clinic, medical action) that can increase

the chances of transmission and colonization of *S. aureus* and increase the occurrence of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) [11].

Mother's education level and occupation were not significantly different in the group with vaginal bacterial colonization and the group without bacterial colonization with p value 0.253 and 0.777. It is relevant to a study by Chan et al. [10] which showed that the level of education and employment status (working or not working) are not related with the colonization of streptococcus group B in rectovaginal of pregnant women, $p=0.88$. In contrast, according to Karina et al. [11] Socioeconomic is relate to rectovaginal colonization with $p=0.003$. Low levels of hygiene facility make the growth of colonization and transmission of *S. aureus* easier in population with low socioeconomic and minimum level of education.

This study also analyzed the characteristics of the baby, sex, gestation and the newborn infant [13-16]. Sex dimorphism from immune response of human is quite obvious; women produce a more active cellular and humoral immune reaction. Therefore, they are more resistant to infections like bacterial and have a higher incidence of autoimmune disease than men [12]. This study found no relationship between sexes and EONS ($p=0.089$). It is relevant with the report by Shah et al. [16] Hayun [13] and Amalia [14] with p value 0.203 and 0.067 [13,14]. Study in India also reported that there was no difference between the sexes with the rate of infection between male (2:05%) and female (2:08%) [15].

Report from Nepal stated that baby who born <37 weeks are 4.85 times more risk to have EONS on BKB [16]. In Mexico, term infants 2.91 times more risk having EONS but it is not significantly related on LONS [3]. Hayun also reported that gestational age is a risk factor which is 13.45 (95% CI 3.91-46.26) times [13]. It was because of the physical barriers of term infants began to mature in 32-34 weeks of gestational age [17]. term infants also have limited capacity to increase the production of neutrophils in response to infection and the dysfunction in all neutrophil function. In our study, we found that the gestational age was not associated with EONS incidence ($p=0.354$). It is because all samples in this study are term infants with a median 38 weeks.

According to Shah et al. [16] LBW are statistically significant with odds ratio 4.85. There are also another study that showed low birth weight (≤ 2500 g) was not a risk factor for both EONS and LONS. In our study, birth weight was not significantly relate to EONS incidence ($p=0.332$). It is probably because birth weight of the samples in this study dominated by infants with birth weight 2400-3200 grams which is birth weight of late preterm and term infant, while the immunity of infant is low on LBW especially in UG<32 weeks or in the range of birth weight <1500 g.

According to Poupolo et al. [18] EONS risk on newborn from mother with vaginal bacterial colonization was 30.14 % (95% CI 23.92-36.36). Meanwhile, the results of our study showed that mothers vaginal bacterial colonization do not significantly relate to EONS incidence with $p=0.399$ ($p>0.05$). These results are relevant with a study by Matsubara, Niduvaje, Amavar, Buckler and Faro, with successive OR 0.00 (95% CI -0.01-0.01), 0.00 (95% CI -0.01-0.01), 1.08 (95% CI -1.02-3.17), 0.00 (95% CI -0.01-0.01) and 0.54 (95% CI -0.07-1.16) [10].

Rectovaginal bacterial colonization of pregnant women and chorioamnionitis has a positive correlation with the incidence of early-onset neonatal sepsis. Babies who born from mothers with chorioamnionitis lead to bacterial colonization and sepsis faster [10].

Correlation of the EONS incidence and not EONS with the types of bacteria is also analyzed (Table 6), but it shows no significant difference in the types of bacteria with outcome EONS and not EONS $p=0.163$ ($p>0.05$). It is probably because there are only a few samples that have EONS while there is various kinds of bacteria.

In this study, mother's vaginal pathogenic bacteria colonization dominated by gram-negative bacteria (77.97%) and gram-positive bacteria in small amounts (22.03%). *Enterobacter agglomerans* is gram-negative bacteria which commonly found (18.64%), while gram-positive bacteria dominated by *Staphylococcus aureus* (18.64%). Schuchat A in Miami reports the kind of bacteria which commonly found are GBS (1.4 cases out of 1000 births) and *E. coli* (0.6 cases in 1000 births) [19] Research by Guida in Philadelphia found that most of the gram-positive bacteria are *Staphylococcus aureus*, *Enterococcus*, while the gram-negative bacterium is *Escherichia coli* [20]. At Dr. Cipto Mangunkusumo hospital during 2002, most commonly found bacteria are *Enterobacter* sp., *Acinetobacter* sp. and *Colis* [21].

Based on these results we can conclude that there is no correlation between vaginal bacterial colonization and EONS incidence. The results obtained in this study maybe because of the study excluded mother with chorioamnionitis. Moreover, it can also be influenced by the type and number of bacteria, while this study only identified the type of bacteria but not the number of bacteria. If that kind of analysis was done, we could conclude that to cause pathogenicity, mother's vagina bacteria must be at $\geq 10^6-10^7$ in management of the patient. In this study, we found that out of five EONS incidence four are from the mother with pathogenic vaginal bacteria colonization and one without colonization, while from the results of blood cultures of infants with EONS, there are three infants with positive cultures and the rest are negative. There is only one who has similar kind of bacteria between the mother with vaginal bacterial colonization and the baby's blood culture. From these results, it can be assumed that there are other factors that influence EONS incidence besides mother's vaginal pathogenic bacteria colonization.

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Correspondence to:

A Dwi Bahagia Febriani
Department of Pediatrics
Faculty of Medicine
Hasanuddin University
Makassar
Indonesia
Tel: 6281355524595
Email: Bahagiadwi@yahoo.com

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