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Associations BetweenFecalsIgA Level with Neonatal Sepsis

Jeanette I.Ch. Manoppo[1*],Dr. Nelly Mayulu[2], Fahrul Nurkolis[3]
[1]Department of Pediatrics, Faculty of Medicine, Sam Ratulangi University/Prof.dr.R.D.Kandou Hospital Manado.
[2]Nutrition and Food Department, Faculty of Medicine, Sam Ratulangi University.
[3]Biological Sciences, Faculty of Sciences and Technology, the State Islamic University of SunanKalijaga.
Email: [1]j.manoppo@yahoo.com

ABSTRACT

IntroductionSepsis is one of the leading causes of infant morbidity and mortality throughout the world. Early diagnosis and accurate treatment of neonatal sepsis is still challenging because as the gold standard, blood culture takes a long time. Secretory IgA (sIgA) holds an important part in mucosal integrity and mucosal homeostasis. Therefore, sIgA level can be considered as a predictor for neonatal sepsis.
Purpose This research is aimed to analyze the associations between fecalsIgAlevel with neonatal sepsis.
Methods This research uses analytic observational design with cross-sectional approach. All infants with suspected sepsis in the NICU of RSUP Prof. Dr. R.D. Kandou Manado from July to August 2019 is included in the study.
Results A total of 41 infants are included in the study, where 21 (51.22%) are confirmed neonatal sepsis. Secretory IgA levels are higher among the non-sepsis infants (1347.59 ± 503.24 µg/ml) than their counterparts (461.86 ± 297.28 µg/ml) which is statistically significant ($P < 0.001$) with r_{pb} = -0,742. This result is also confirmed by logistic regression, with regression coefficient of -0.005 ($P < 0.001$).
Conclusion There is a significant inverse association between fecalsIgA level and neonatal sepsis.

Keywords
FecalsIgA, Neonatal Sepsis

Introduction

Nowadays, sepsis is still a major health problem that is one of the main causes of infant morbidity and mortality in the world, hence requiring significant attention. Several factors play important roles in defining sepsis mortality in infant, such as host factor, microorganism, early diagnosis, and given management. Currently there is no single biomarker to establish the diagnosis of sepsis, which causes the work for improving the diagnostic criteria for sepsis is still ongoing.[1]

Neonatal sepsis is a clinical syndrome caused by microorganism invasion intu the bloodstream that happens during the first month of life. Neonatal sepsis remains one of the main causes of morbidity and mortality, whether in term or preterm infants. Sepsis of serious infection in the first four months of life takes the life of more than one million newborn infants globally per year.[2]

Morbidity rate for neonatal sepsis is different in each country. The incidence is varied from 1 to 5 per 1,000 live births is developed countries, but could reach up to 49 to 170 per 1.000 live births in developing countries. In Asia, the incidence of neonatal sepsis is 7.1 to 38.0 from 1.000 live births.[3,4]

Early diagnosis of neonatal sepsis is important in the management and affecting the prognosis of the patient. A delay in diagnosis may potentially threaten the patient's life and worsen the prognosis. A specific diagnosis of neonatal sepsis is hard to establish as its clinical manifestation is not specific. Classic sepsis manifestation that may be found in older children is rarely found in newborn. The signs and symptoms of sepsis in newbornis similar to other severe disease of non-infectious origin. There are currently no diagnostic modalities that can individually be used to diagnose neonatal sepsis.[2]

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The clinical diagnosis of neonatal sepsis has its own problem. "Suspected sepsis" is one of the most common diagnosis found in the neonatal intensive care unit (NICU) even though the manifestation is unspecific and may be mimicked by other non-infectious inflammation disorder. Blood culture is the gold standard in diagnosing neonatal sepsis, but proving the presence of an infection by culture is impractical and takes a long period of time. Blood culture results usually will only be available after approximately five days, which by then treatment delay would have happened that may cause worsening of the patient's condition. In the other hand, over treatment may increase the use of antibiotics and lengthen the duration of stay in the hospital, hence increasing healthcare cost. Therefore, there is currently a lot of researches that are done trying to develop a non-invasive and accurate diagnostic test.[5,6]

An immune response is needed for the host defence against bacterial growth and translocation that may cause sepsis. Secretory immunoglobulin A (sIgA) as the main immunoglobulin in the mucosal surface is one of the key factors in this process by interfering and preventing translocation of bacteria in a process called "immune exclusion" in the digestive, respiratory, and urogenital tract.[7]

Gastrointestinal tract plays an important role in maintaining immune homeostasis by producing antibody and maintaining a certain population of antibody-producing cells. Even though sIgA contained in the feces is highly associated with the ability to neutralize and excrete pathogenic microbes, researches on sIgA on sepsis and non-sepsis infants is rarely done.

Therefore, a research on the associations between sIgA level and neonatal sepsis is important. By finding an association between these two variables, sIgA may be considered to be used as a marker for the incidence of neonatal sepsis and be used as a basis for more researches around this topic. Therefore, this study is aimed to find the association between sIgA level and neonatal sepsis.

Methods

This research uses analytic observational method with cross-sectional design, that took place in the neonatal intensive care unit (NICU) of pediatric department RSUP Prof. dr. R.D. Kandou, Manado, North Sulawesi, Indonesia from July through August 2019. This study is done under the permission of Research Ethics Committee of RSUP Prof. Dr.Kandou, Manado.

Study Participants

The study population of this research is infants with suspected sepsis that are receiving treatment in the NICU of pediatric department RSUP Prof. dr. R.D. Kandou, Manado, North Sulawesi, Indonesia. Suspected neonatal sepsis is defined as the presence of two major or one major + two minor risk factor criteria for neonatal sepsis (see Table 1).[8,9] Inclusion criteria for this study is all infants with suspected sepsis that are receiving treatment in NICU and are given consent from their respective parents for inclusion in research. Subjects are excluded when born with prematurity or having necrotizing enterocolitis (NEC) which may interfere with study results. By using correlative sample size formula, it was calculated that the minimum sample size is 38 subjects, that are collected using consecutive sampling method.

Outcome Measures and Definitions

After consent is given by their respective parents/guardians, subjects with suspected neonatal sepsis will be put through physical and fecal examination, while being observed for three days awaiting their laboratory and blood culture results. Subjects will then be categorized into sepsis

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and non-sepsis group as a dependent variable. Neonatal sepsis is confirmed by the presence of four out of six clinical signs (see Table 2) and two positive hematologic profile (see Table 3) with or without a positive blood culture result.[9-11] Non-sepsis is defined as subjects that do not show clinical manifestations of sepsis and do not meet the criteria for confirmed sepsis after three days of observation. Independent variable of this research is fecalsIgA level. Fecal material is collected into sterile feces pot which will be stabilized in 15-30°C for 1 night, 2-8°C for 3 days, or -20°C for 4 weeks to inhibit bacterial growth. Fecal material will be sent to Prodia© clinical laboratory for examination of fecalsIgA level by ELISA method.

Statistical Analysis

Dependent variable (neonatal sepsis) in expressed as a binomial variable into sepsis and non-sepsis, whereas the independent variable (sIgA level) is a numeric variable expressed in means ± standard deviation (SD). Univariate analysis will be done to analyze the sample characteristics and fecalsIgA level and its distribution among sepsis and non-sepsis groups. Bivariate analysis will be used to find any correlation between fecalsIgA level and neonatal sepsis with point-biserial correlation coefficient and logistic regression analysis which will be expressed in P value. P <0.05 is considered as statistically significant for all analysis. All statistical analysis will be done using IBM SPSS (Statistical Product and Service Solutions) version 25.0 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY).

Results

This research is conducted between July and August 2019. A total of 41 infants with suspected sepsis in the NICU of RSUP Prof. Dr. R. D. Kandou, Manado are included in the study. Among these subjects, 25 are males (60.98%) while 16 are females (39.02%). After observation and examination, 21 subjects (51.22%) are confirmed neonatal sepsis while the other 20 subjects (48.78%) are categorized as non-sepsis. In the sepsis group, 13 (61.90%) are males and 8 (38.10%) are females; whereas in the non-sepsis group, 12 (60.00%) are males and 8 (40.00%) are females (see Table 4).

sIgA levels are higher among the non-sepsis infants (1347.59 ± 503.24 µg/ml) than among sepsis infants (461.86 ± 297.28 µg/ml), like seen on Figure 1. Point-biserial correlation coefficient was calculated, and it was found that r_{pb} = -0,742 with a P value < 0.001, which explains that sIgA levels are inversely correlated with the likelihood of neonatal sepsis. This result is also confirmed by logistic regression (see Figure 2), which has a regression coefficient of -0.005 with a P value of < 0.001 (see Table 5).

Discussion

Study Population Characteristics

In this study, 41 infants with suspected neonatal sepsis are admitted into the study population to find out the association between fecalsIgA level and neonatal sepsis. From those, 20 are males and the rest are females. After observation and examination, it was found that 21 of the study population have confirmed sepsis, where 13 among them (61.90%) are males. This finding shows that the in this study population, there are more male infants affected by neonatal sepsis than females, which is consistent with several researches.

A research done in by Wilar, et.al.[9,11] found that neonatal sepsis is found more common in male infants, which may happen as a result of sex related factors in host susceptibility to infection. The

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X chromosome may have genes that affect the function of thymus gland and immunoglobulin synthesis, in which the males only have one X chromosome. Vasantha, et.al.[12] also found that neonatal sepsis happens more common in male infants because male infants have a higher level of interleukin-6 (IL-6), which is a glycoprotein cytokine produced by monocytes, endothelial cells, and fibroblasts. These IL-6 functions as a signal to activate T-cell, increase B-cell antibody secretion, differentiation of cytotoxic T-cell, and stimulate secretion of other cytokines, especially tumor necrosis factor alpha (TNF-α) ang IL-1. Even so, several researches by Hayun, et.al.[13], Hematyar, et.al.[14], and Shehab, et.al.[15] concluded that there are no significant difference in the prevalence of neonatal sepsis within gender difference.

Associations BetweenFecalsIgA Level and Neonatal Sepsis

This study found that the median of fecalsIgA level in sepsis infants is 354 µg/ml with minimum and maximum value of 195.50 µg/ml and 1,050.00 µg/ml; in the other hand, the median of fecalsIgA level in non-sepsis infants is 1,232.25 µg/ml with minimum and maximum value of 500.00 µg/ml and 2,043.00 µg/ml. This result shows that there is a big difference between the fecalsIgA levels of sepsis and non-sepsis infants. Point-biserial correlation test shows a coefficient of r_{pb} = -0.742 with P < 0.001, which means that there is a statistically significant inverse correlation between fecalsIgA level and neonatal sepsis.

Logistic regression was also used to figure out the association between fecalsIgA level and neonatal sepsis, which resulted in a negative significant association between the two variables (regression coefficient = -0.005), with P value of less than 0.001. This result means that the lower the level of fecalsIgA, there is a greater likelihood for neonatal sepsis to happen.

This association may happen as several preclinical researches concluded that in the condition of sepsis or multiple organ dysfunction, there is a diminishing function of sIgA in mucous membrane integrity. Secretory IgA has been identified as a direct inhibitor of bacterial adhesion, toxin and pathogenic enzyme inactivation and neutralization, and an inhibitor of pro-inflammatory response. Rodriguez, et.al.[16] found that there is a diminishing total IgA concentration in mouse's mucous membrane under severe infection. Similar research by Watson, et.al.[17] found that IgA secretion is inhibited by severe infection. This decrease in IgA is found in tears, saliva, intestinal secretion, and serum. Another research by Corthesy, et.al.[18] found that sIgA production by intestinal mucous membrane is an adequate first-line of defence against pathogenic microorganisms and keeping host-agent homeostasis in intestines.[19,20] With this in mind, it is theoretical that when sIgA level decreases, infection may be present.

This research is a pilot study in analyzing the association between fecalsIgA level and neonatal sepsis by using point-biserial correlation test, that may later be used as a basis of determining a cut-off point for the diagnosis of neonatal sepsis.

There are several limitations to this study, such as the measurement of sIgA that is only done one time at the time the patient is admitted to NICU, where a diagnostic biomarker needs to be measured in serial to be able to be used as a neonatal sepsis predictor. Another limitation is that the etiology of sepsis in the subjects areindetermined to be caused by virus, bacteria, or fungi. As this is a pilot study, upcoming researches should do serial measurements of fecalsIgA level and classify them according to its etiology.

In conclusion, this research found that there is a strong and significant association between fecalsIgA level and neonatal sepsis. Furthermore, as determined by point-biserial correlation and logistic regression, the relationship between these two variables is inverse, which when fecalsIgA level is low, there is a high chance of neonatal sepsis.

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Conflict of Interests

The author declares that there are no financial support, sponsorship, or conflicts of interest regarding the writing and publication of this article.

Key Message

The prevalence of neonatal sepsis among this study population is 51.22%. Secretory IgA levels are higher among the non-sepsis infants (1347.59 ± 503.24 µg/ml) than their counterparts (461.86 ± 297.28 µg/ml) which is statistically significant ($P < 0.001$) with $r_{pb} = -0,742$. This result is also confirmed by logistic regression, with regression coefficient of -0.005 ($P < 0.001$).

Key Message
1. The prevalence of neonatal sepsis among this study population is 51.22%. 2. Secretory IgA levels are higher among the non-sepsis infants (1347.59 ± 503.24 µg/ml) than their counterparts (461.86 ± 297.28 µg/ml). 3. This finding is statistically significant by point-biserial correlation test and logistic regression test ($P < 0.001$ and $P < 0.001$) with rpb = -0,742 and regression coefficient = -0.005.

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Tables

Table 1. Criteria for suspecting neonatal sepsis [8,9]

Major risk factor
Premature rupture of membranes > 18 hours
Maternal intrapartum fever (>38°C)
Chorioamnionitis
Sustained fetal heart rate >160 bpm
Foul-smelling amniotic fluid
Minor risk factor
Premature rupture of membranes > 12 hours
Maternal intrapartum fever (>37.5°C)

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Low APGAR scores (<5 in 1'; <7 in 5')
Very low birth weight (<1500 g)
Untreated maternal leukorrhea
Untreated suspected/confirmed maternal UTI

UTI = Urinary Tract Infection

Table 2. Clinical signs of neonatal sepsis [9-11]

System	Signs
Respiratory	Tachypnea (RR >60 bpm), apnea attacks (>20 s or occurred >2x in 1 hour), severe apnea (apnea episode which needed mechanical ventilation), bradypnea (RR <30 bpm), oxygen saturation <85%
Cardiovascular	Bradycardia (HR <100 bpm), tachycardia (HR >160 bpm), decreased perfusion (CRT >3 s or cold cyanotic extremity)
Metabolic	Hypothermia (axillary temperature <36,5 ºC), glucose instability (serum glucose level <45 mg/dL or >125 mg/dL), metabolic acidosis (blood pH <7,35)
Neurologic	Lethargy, hypotonia, decreased activity level, seizures
Gastrointestinal	Vomiting, diarrhea, abdominal distention, ileus, low intake, disrupted nutritional intake tolerance (stomach residue >20% from 2x feeding in 24-hour period)
Hematologic	Anemia, icterus, petechiae, purpura

RR = Respiratory Rate; HR = Heart Rate; CRT = Capillary Refill Time

Table 3. Hematologic profile of neonatal sepsis [9,11]

Examination	Results
Hemoglobin	Anemia (<15 g/dL)
Leukocyte count	Leukocytosis (>25.000/mm^3) or leukopenia (<5.000/mm^3)
Thrombocyte count	Thrombocytopenia (<100.000/mm^3)
Acute phase reactant	Elevated CRP (>6 mg/dL)
I/T ratio	Elevated (>0.2)
Blood culture	Positive

I/T ratio = Immature-to-total neutrophil ratio

Table 4. Study participant's gender distribution

Gender	Sepsis	Non-Sepsis	Total
Male	13 (52.00%)	12 (48.00%)	25 (100.00%)
Female	8 (50.00%)	8 (50.00%)	16 (100.00%)
Total	21 (51.20%)	20 (48.80%)	41 (100.00%)

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Table 5. Study participant's gender distribution

Gender	N	Mean (µg/ml)	StandarDeviasi (µg/ml)	Median (µg/ml)	Minimum (µg/ml)	Maksimum (µg/ml)
Sepsis	21	461.86	297.28	354.00	195.50	1050.00
Non-Sepsis	20	1347.59	503.24	1232.25	500.00	2043.00
Total	41	2569.8100	604.48	867.00	195.50	2043.00

Figures

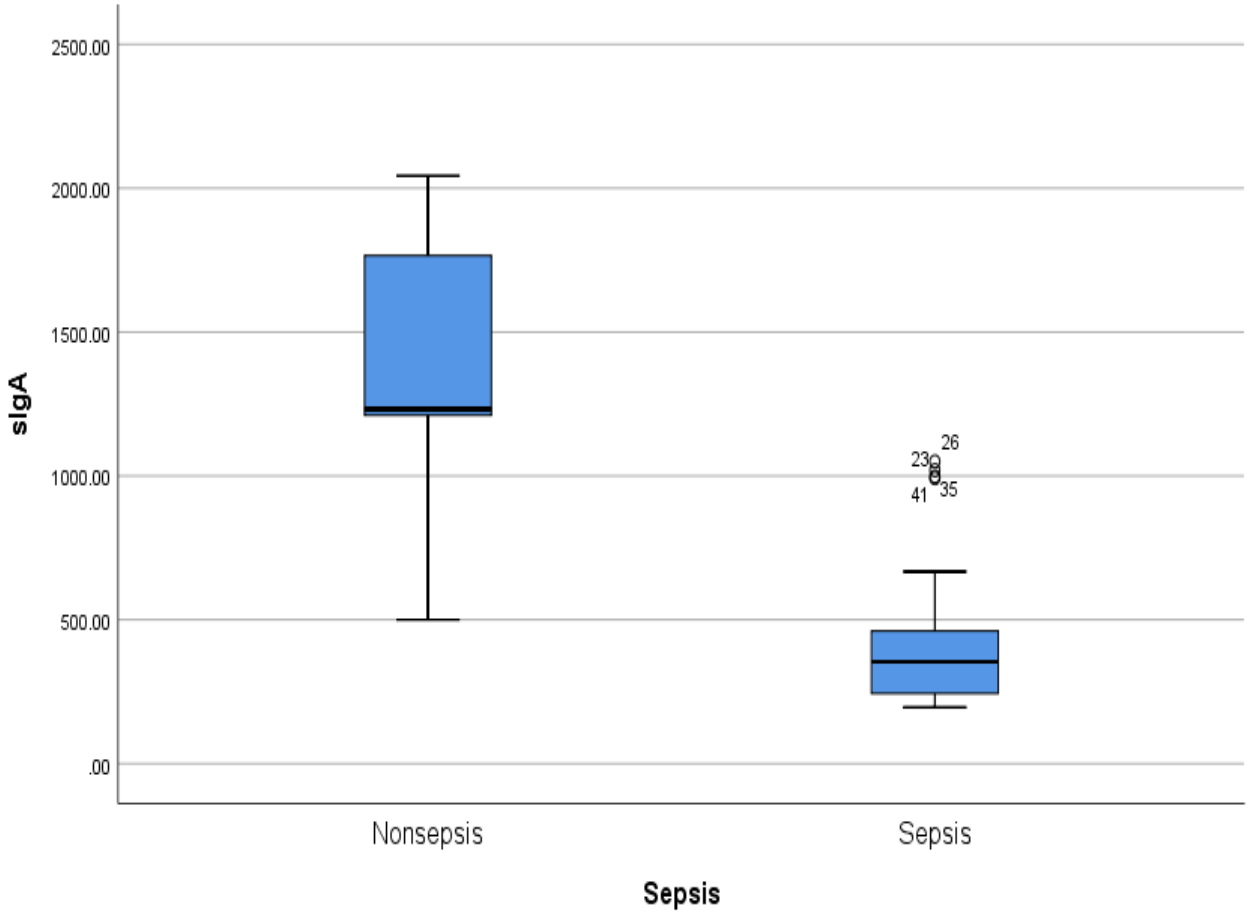

Figure 1. Association boxplot of fecal sIgA level and neonatal sepsis shows that sIgA levels are lower in sepsis group compared to non-sepsis group

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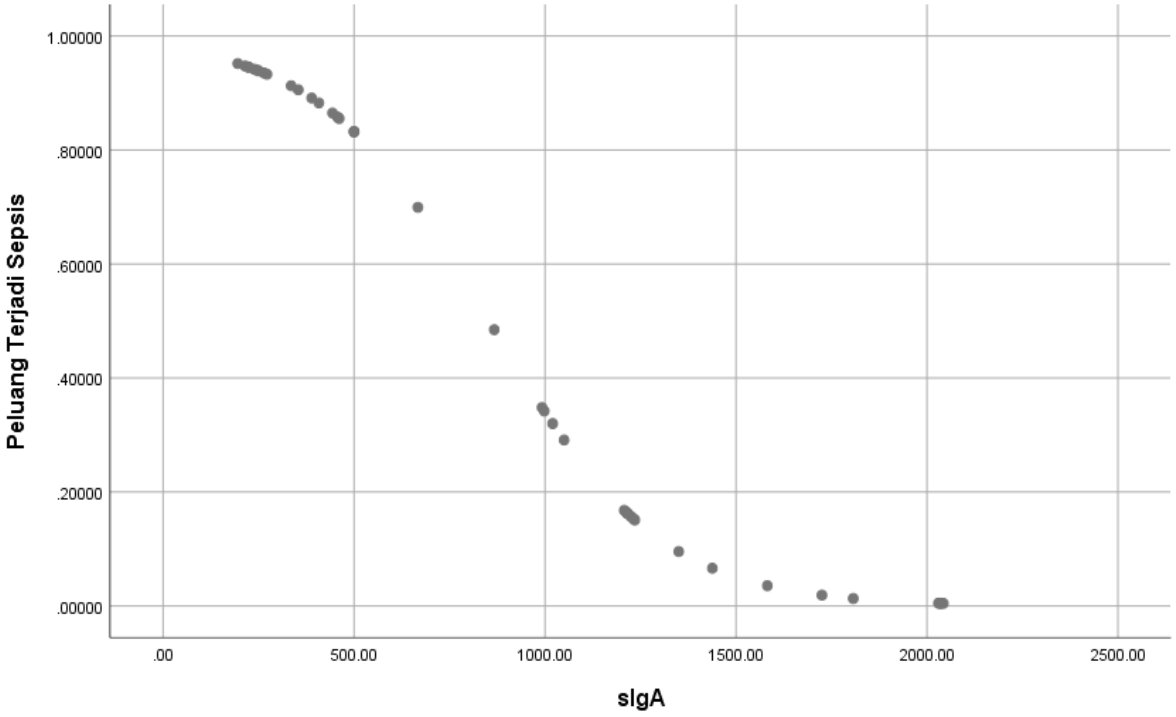

Figure 2. Logistic regression analysis of fecal sIgA level and neonatal sepsis shows an inverse relationship, which means that the lower the fecal sIgA level, the higher the probability of neonatal sepsis