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Research Article

The Correlation of Fc-gamma Receptor I (CD64) Expression and Procalcitonin in Early Onset Neonatal Sepsis

Zenia Angelina[1], Eko Sulistijono[1], Loeki Enggar Fitri[2]

[1]Department of Pediatric, Faculty of Medicine, Universitas Brawijaya/dr Saiful Anwar General Hospital, Malang-65122, Indonesia
[2]Department of Parasitology. Faculty of Medicine, Universitas Brawijaya Malang-65145, Indonesia

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ABSTRACT

Objective: The aim of this study is to prove the relationship of FcγRI (CD64) expression and Procalcitonin value in early-onset neonatal sepsis to assist in diagnosis of early-onset neonatal sepsis. Method: A descriptive and analytical case control study was conducted in dr. Saiful Anwar General Hospital Malang. There were 40 children divided into two groups: 1). Group of infants with neonatal risk factors who express signs of SIRS and proven by blood culture; 2). Group of infants with neonatal risk factors who showed no sign for SIRS. Both groups were performed examination of FcγRI (CD64) expression with flowcytometry and Procalcitonin value with ELISA. Data were statistically analyzed using normality test (Kolmogorov-Smirnov), chi square test, t test and Pearson correlation. We used SPSS 16 for this analysis. Results: The study showed that the FcγRI (CD64) expression and Procalcitonin value were higher in the infants group with proven early-onset neonatal sepsis ($p < 0.05$). There was a significant relationship between FcγRI (CD64) expression and the Procalcitonin value ($p=0.036$). Conclusions: We conclude that expression of FcγRI (CD64) and Procalcitonin value were higher in the infants group with proven early-onset neonatal sepsis. There is a positive relationship between FcγRI (CD64) expression and Procalcitonin value in early onset neonatal sepsis.

Keywords: neonatal, sepsis, early onset, FcγRI, CD64, Procalcitonin.

INTRODUCTION

Neonatal sepsis is neonatal response to various infections which can occur in early or late onset. Neonatal sepsis is the main cause of morbidity and mortality in developing countries. In those countries neonatal sepsis is predicted to be cause of death in newborns around 30-50% each year[1,2]. Early onset sepsis is a perinatal infection occurs immediately in the postnatal period (less than 72 hours) and transmitted transplacentally, during delivery or in utero. Early onset sepsis causes a high mortality rate of 15-50%[3]. Clinical appearance of neonatal sepsis and systemic inflammation response syndrome are often nonspecific and make a delay of diagnosis which increase mortality rate. The gold standard of sepsis is obtained from blood culture result which is positive for bacteria. Unfortunately, blood culture is only available to read in 48-72 hours[4]. Therefore, a diagnostic test is needed to differentiate between infected and not infected newborn, especially in early period rapidly[5].

Procalcitonin (PCT) is a peptide precursor from calcitonine (CT) hormone which consists of 116 amino acids and has a 13 kDa molecular weight[6]. In normal condition PCT only synthesized in C cell thyroid gland and spreads in circulation in low concentration (<o,o5 ng/mL)[7]. Procalcitonin is a biomarker which recently researched about its role as a hormone and cytokine in recognizing bacterial infection. A lot of studies have shown PCT as a biomarker in diagnostic, prognostic and as a guide in using and ceasing antibiotics[8]. A research stated that an increase in PCT value correlates with condition severity and mortality which show the ability of PCT as prognostic factor[9]. Unfortunately, from those researches, until recently there are no clear cut off points to determine PCT value increase.

FcγRI (CD64), known as Fc-gamma 1 receptor, bond with monomer IgG antibody in high affinity in the process of phagocytosis and intracelluler opsonised microbe destruction. This receptor is a integral glicoprotein membrane with molecular weight of 72 kDa. The structure of FcγRI (CD64) consists of peptide signal, three extracellular immunoglobulins where immunoglobulin attached, hydrophobic transmembrane area and short cytoplasmic tail. FcγRI (CD64) is commonly found only in macrophage and monocyte, however after stimulation from IFN-γ cytokine, this receptor is also found on polymononuclear cell[10]. FcγRI (CD64) regulation increased on neutrophile as physiological response to bacterial wall component such as lipopolysaccharide, complement release product and several cytokines (IFNγ, TNFα, IL-8 dan IL-12). The increased regulation of FcγRI (CD64) occurs in four to six hours after receiving stimulation from IFNγ, *granulocyte colony stimulating factor* (GCSF) andkinetic activator such as lipopolysaccharide[11,12]. Expression of neutrophile FcγRI

**Author for Correspondence: lovely_zenia@yahoo.com*

Table 1: Characteristic of The Subjects.

Subject Characteristic	EOS (n=20)	Without shown clinically sepsis (n=20)	P value
Gender			
Male, n	14 (14/20)	16 (16/20)	0.465
Female, n	6 (6/20)	4 (4/20)	
Birth weight			
LBW, n	11 (11/20)	9 (9/20)	0.527
Normal, n	9 (9/20)	11 (11/20)	
Gestational age			
Preterm, n	11 (11/20)	4 (4/20)	0.022
At-term, n	9 (9/20)	16 (16/20)	
Method of delivery			
Caesarean section, n	8 (8/20)	11 (11/20)	0.342
Spontaneous, n	12 (12/20)	9 (9/20)	
Infection risk factor			
PROM, n	11 (11/20)	4 (4/20)	
Preterm, n	3 (3/20)	6 (6/20)	
Mechanical ventilation, n	1 (1/20)	1 (1/20)	0.065
Leucorrhea, n	2 (2/20)	8 (8/20)	
Fever, n	3 (3/20)	1 (1/20)	

EOS=Early onset sepsis, LBW=Low birth weight, n=number, PROM=Premature rupture of the membrane

(CD64) by flowcytometry is used to diagnose bacterial neonatal sepsis. Several advantages of FcγRI (CD64) are fewer blood sample required (1 milliliter) and shorter time needed to calculate neutrophile FcγRI (CD64), less than 60 minutes. The flowcytometry itself has an advantage compared with other immunologic tests, which is the ability to localize active marker in a specific cell[13,14].

The aim of this study is is to prove the relationship of FcγRI (CD64) expression and IT Ratio value in early-onset neonatal sepsis to assist in diagnosis of early-onset neonatal sepsis.

MATERIALS AND METHODS

Study design

This study was a descriptive and analytical case control study of FcγRI (CD64) expression and Procalcitonin value. There were 40 children divided into two groups: 1). Group of infant who have risk factors for sepsis that showed signs of SIRS as proven by positive blood cultures; 2). Group of infants with neonatal risk factors with no sign of SIRS. Both groups were performed examination of FcγRI (CD64) expression with flowcytometry and Procalcitonin value with ELISA at Physiologic Laboratory/Faculty of Medicine and Biology Laboratory/Faculty of Math and Science. The study was conducted since May until July 2016 in pediatric ward dr. Saiful Anwar General Hospital Malang/Universitas Brawijaya Malang. This study had been approved by Ethical Committee of Saiful Anwar General Hospital Malang.

Population and Subject

Fourteen subjects were included in each group of this study. Inclusion criteria are all newborn babies with neonatal risk factor of infection who develop signs of SIRS will be performed blood culture, babies aged 0 until 72 hours, and if the parents able to give consent to volunteer in the research (informed consent). The exclusion criteria were babies aged more than 72 hours when SIRS signs first develop and babies with congenital malformation.

Statistical Analysis

We used Kolmogorov-Smirnov, chi square test, independent t test and Pearson correlation. Kolmogorov-Smirnov test is used to examine sample normality, if p value <0.05 then it is an abnormal sample distribution, however if p value ≥0,05 the sample is normally distributed. Research sample characteristics is analysed by chi square test. The difference of Procalcitonin and FcγRI (CD64) expression value between sample group was analyzed by independent t-test. Linier regression test with Pearson correlation is used to determine the correlation of FcγRI (CD64) and Procalcitonin value to early onset neonatal sepsis. We used SPSS 16 program to analyze the data. Statistical difference was set at $p<0.05$ for all the tests.

RESULTS AND DISCUSSION

The study was a case control study which recruited 40 research samples consists of 20 samples of early onset sepsis group and 20 samples of healthy babies. The following table 1 shows a basic research samples characteristics. It is shown that most babies with early onset sepsis were delivered spontaneously, with risk of infection in the mother was premature rupture of membrane. Most babies with early onset sepsis were born prematurely than term, male and low birth weight. Babies without sepsis were mostly delivered by term, male and normal birth weight.

The chi square result shows there was no significant relation between early onset sepsis, sex and birth weight ($p>0.05$) also delivery method and infection risk factor

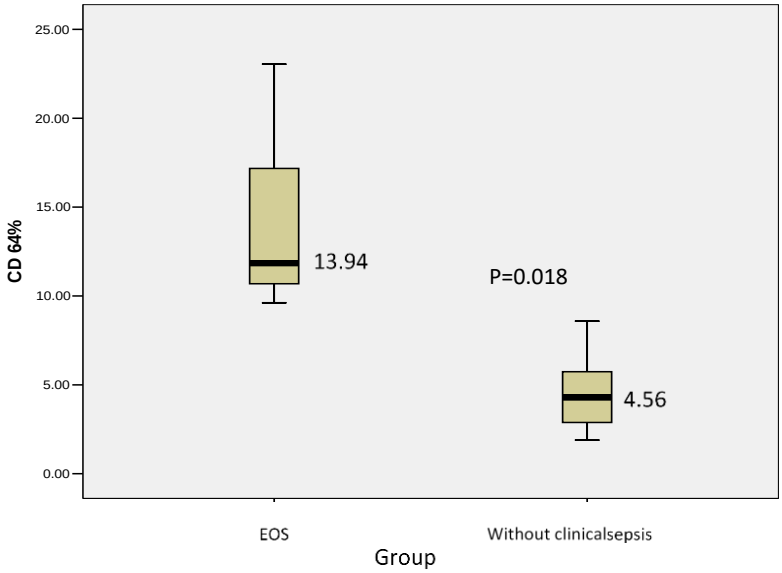

Figure 1: Comparison of CD64 expression between early onset sepsis and without clinical sepsis group showed significant difference (p=0.018). The number of each bar represents mean of CD64 expression.

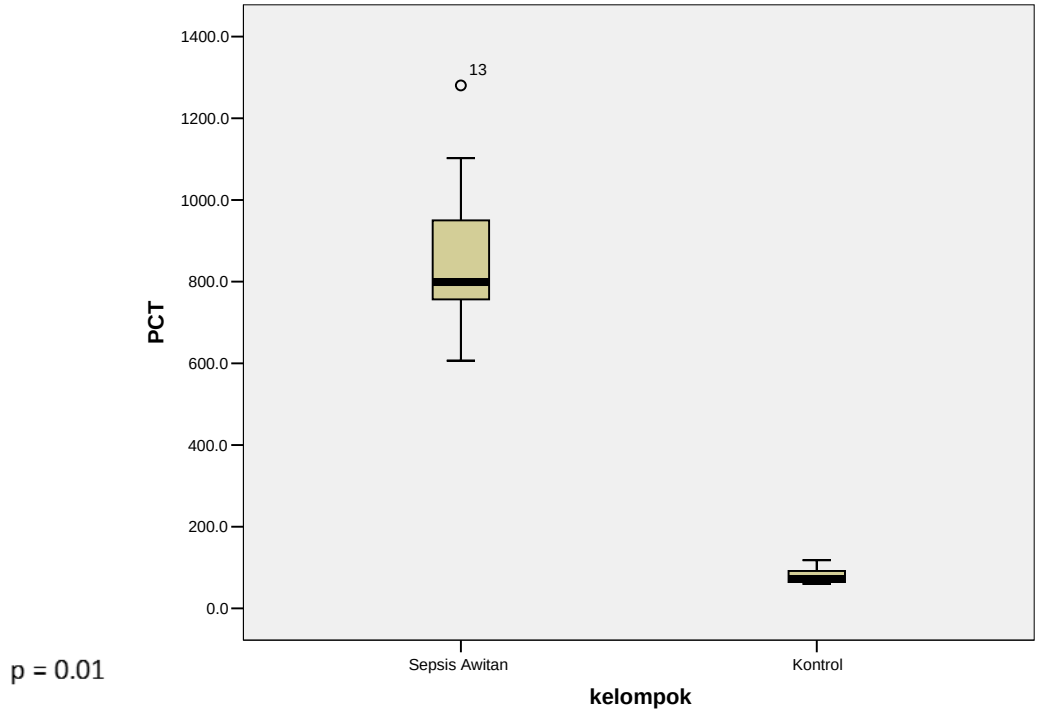

Figure 2: Comparison of Procalcitonin (PCT) value between early onset sepsis and without clinical sepsis group showed significant difference (p=0.01). The number of each bar represents mean of PCT value.

(p>0.05). However, there was a significant relationship between age during delivery and early onset sepsis (p=0,022). This is caused by lower immunity in premature babies compared to the term babies. Immunoglobulin transport through placenta mainly occurs during last half of third trimester. After delivery the serum immunoglobulin concentration continues to decline and leads to severe hypogamaglobulinemia. Skin immaturity also weakened the skin defense, so that premature babies are susceptible to infection. This condition is related to bactericidal activity, lower immunoglobulin, neutrophile and lymphocyte cytotoxic action[1,2].

The FcγRI (CD64) expression in early onset neonatal sepsis

Figure 1 shows the mean expression of FcγRI (CD64) in babies with early onset sepsis was 13.94%, those without sepsis is 4.56%. The p value of independent t test was 0.018 (p<0.05) which shows that there was a significant difference of FcγRI (CD64) between babies with early onset sepsis and without sepsis.

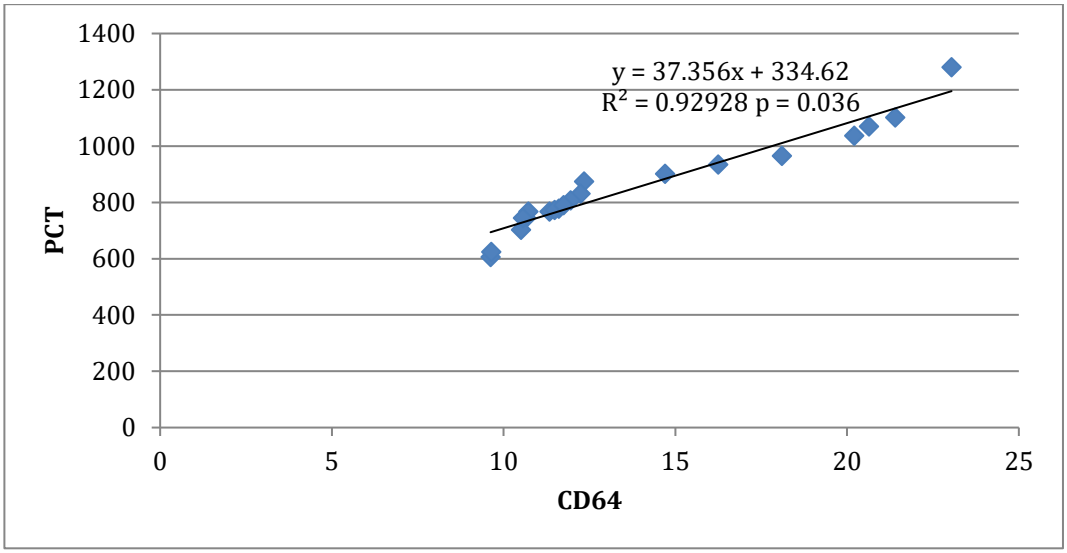

Figure 3: Correlation of CD64 expression and PCT value on early onset sepsis showed significant difference (p=0.036).

This result is similar to research by El-Mazary *et al.* which stated that the increase of FcγRI (CD64) expression has negative correlation with gestational age and birth-weight. That research also showed that the expression of FcγRI (CD64) significantly increase in neonates with sepsis, compared with healthy control group[15].

A research by Du *et al.* that focused on the usage of FcγRI (CD64) in diagnosing early onset sepsis of premature babies concluded that the expression of FcγRI (CD64) is a very sensitive as early onset sepsis marker, and independent to previous antibiotic exposure[16].

Research by Ng *et al.* stated that the expression of FcγRI (CD64) was increased in babies with sepsis whether early or late and this increase also occur in babies with very low birthweight during early sepsis evaluation and stayed high in the following 24 hours[17].

The Procalcitonin in early onset neonatal sepsis

Figure 2 shows the mean value of Procalcitonin on babies with early onset sepsis was 856,60 pg/ml, babies without sepsis was78,50 pg/ml. From the independent t test, the p value was 0.01 ($p<0.05$) which shows that there was a significant difference of Procalcitonin between the two groups.

The average procalcitonin value in this research is similar to result by Jeergal et al, which showed that procalcitonin in babies with clinically suspected early onset neonatal sepsis and according to positive blood culture is 2,1-1,0 ng/ml as positive result and >10 ng/ml as strong positive result[18]. Another cross sectional research by Hakeem et al in 2015 also showed an increase in PCT value of 1,1 pg/ml. Unfortunately this research used subjects suspected with sepsis according to risk factors in mothers without comparison to gold standard method which is positive blood culture result[19].

From the analysis it is obtained that the Procalcitonin value was higher in babies with early onset sepsis compared to those without clinical sepsis (p=0.01). In this research, sample with clinical sepsis is proven by positive result of blood culture. This result is supported by research done by Zahedpadhsa *et al.*, which showed an increase in Procalcitonin value in babies with positive blood culture result compared to those negative blood culture result[20].

The correlation of FcγRI (CD64) expression and Procalcitonin value on early onset neonatal sepsis

Figure 3 shows that there was a significant correlation of increasing FcγRI (CD64) and Procalcitonin value on early onset neonatal sepsis with p=0.036 ($p<0.05$).

FcγRI (CD64) is known as Fc-gamma 1 receptor, which bind monomeric IgG antibody with high affinity in the process of phagocytosis and destruction of intracellular-opsonised microbes. FcγRI (CD64) is commonly found only on macrophage and monocyte. However, IFN-γ stimulation can trigger this receptor on polymononuclear cells[10]. Increased regulation of FcγRI (CD64) occurs in four to six hours after receiving stimulation from IFNγ, granulocyte colony stimulating factor (GCSF) and kinetic activator such as lipopolysaccharide[11,12].

Microbial infections will lead to the release of a constitutive PCT[21]. Main pathophysiology of PCT increase is microbial infection both exogenous and endogenous. These microbial infections will then cause the release of proinflammatory mediators. Among the various proinflammatory mediators, TNF is a proinflammatory mediator that has been extensively researched and provide specific stimulation on increase of PCT[22].

This also might explain that the increased expression of FcγRI (CD64) correlated with increased levels of procalcitonin. As in sepsis condition, there is a release of proinflammatory mediators, one of them is IFNγ which leads to increased expression of FcγRI (CD64) and TNFα which triggered an increase in PCT.

This research has several limitations, first this research does not consider the equal timing of blood withdrawal. This might be caused by variation of onset age of blood samples, as some of the samples also come from referred patients more than 24 hours age. However, the research still includes samples with less than 72 hours of age as diagnostic criteria of early onset neonatal sepsis. Another limitation is the research is not followed by continued evaluation to examine how long the FcγRI (CD64) and

Procalcitonin value will be increased, so that the optimal cut off of those increase is not yet known. This research does also not consider the half time of increased expression of FcγRI (CD64). Peripheral blood samples taken also might not give the accurate result compared to the umbilical blood analysis, which consider vertical transmission as a maternal risk factor to early onset neonatal sepsis.

CONCLUSIONS

We conclude that the expression of FcγRI (CD64) and Procalcitonin value were higher in the infants group with proven early-onset neonatal sepsis. There is a positive relationship between FcγRI (CD64) expression and Procalcitonin value in early onset neonatal sepsis. Further study with better method is needed to get better result.

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