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RESEARCH

ANALYSIS OF LABORATORY PARAMETERS AS SEPSIS MARKERS IN NEONATALS WITH HYPERBILIRUBINEMIA

(Analisis Tolok Ukur Laboratorium Sebagai Petanda Sepsis di Neonatus dengan Hiperbilirubinemia)

Bachtiar Syamsir, Rachmawati Muhiddin, Uleng Bahrun

ABSTRAK

Hiperbilirubin di neonatus merupakan kondisi peningkatan bilirubin total (> 5mg/dL) di bayi baru lahir. Angka kejadian di bayi cukup bulan mencapai 60%. Kejadian hiperbilirubin di bayi baru lahir salah satu penyebab terbanyak bayi harus mendapat perawatan. Kejadian hiperbilirubinemia di neonatus yang disebabkan oleh sepsis 3–8%. Tujuan penelitian ini untuk mengetahui hubungan peningkatan petanda sepsis C-Reaktif Protein (CRP), Procalcitonin (PCT), rasio jumlah imature to total neutrofil (IT ratio) dan jumlah trombosit di neonatus dengan hiperbilirubinemia dan menilai kenasabannya. Penelitian retrospektif dengan pendekatan potong lintang dengan mengambil data rekam medis pasien yang diperiksa kadar bilirubin total di RSUP Dr. Wahidin Sudirohusodo Makassar masa waktu Januari-Desember 2015. Patokan kesertaan, pasien dengan pemeriksaan petanda sepsis CRP, PCT, IT Ratio dan trombosit, dengan diagnosis akhir sepsis. Data dikelompokkan menjadi kelompok hiperbilirubin dan non-hiperbilirubin, diuji dengan Uji Mann Whitnney atau Uji T Independen untuk melihat hubungan antara keempat petanda sepsis dengan kelompok bilirubin. Data kelompok hiperbilirubinemia kemudian diuji kenasaban Spearman untuk melihat kenasaban keempat petanda sepsis dengan kadar bilirubin. Diperoleh total 92 sampel yang memenuhi patokan kesertaan, kelompok hiperbilirunemia 67,4% dan non-hiperbilirubinemia 32,6%. Hasil penelitian menunjukkan tidak terdapat perbedaan bermakna nilai keempat petanda sepsis diantara kedua kelompok (p>0,05), terdapat kenasaban negatif jumlah trombosit dengan sepsis hiperbilirubinemia (p<0,05; r.-0,261). Tidak dapat disimpulkan adanya hubungan CRP, PCT, IT ratio dan trombosit terhadap kejadian hiperbilirubinemia. Pada subjek dengan hiperbilirubinemia, makin tinggi kadar bilirubin makin rendah jumlah trombosit.

Kata kunci: *Sepsis neonatorum, hiperbilirubinemia, CRP, procalcitonin, IT ratio, trombosit*

ABSTRACT

Hyperbilirubinemia in neonates is a common increasing of total serum bilirubin (>5 mg/dL) in the newborn. The incidence of hyperbilirubinemia in aterm infants is 60%. Hyperbilirubinemia in neonates is one of the most common causes of hospitalized babies. The prevalence of hyperbilirubinemia in neonates caused by sepsis is 3–8%. This study aimed to see and assess the correlation of increasing sepsis marker C-Reactive Protein (CRP), Procalcitonin (PCT), Immature to total neutrophil ratio (IT ratio) and platelet count in neonates with hyperbilirubinemia. A retrospective cross-sectional study by taking the medical record data of patients that ware tested for total bilirubin value at the Dr. Wahidin Sudirohusodo Hospital, Makassar in January-December 2015. The inclusion criterias were patients who were tested for sepsis marker CRP, PCT, IT ratio and platelet count which were diagnosed as sepsis. Data were divided into hyperbilirubinemia group and non-hyperbilirubinemia group. Mann-Whitney test and T-independent test was used to see the correlation of four sepsis markers with the bilirubin group. Data of the hyperbilirubinemia group then were tested with Spearman correlation test to see the correlation between the four sepsis marker with the bilirubin levels. A total of 92 samples were obtained that matched the inclusion criterias, 67.4% was the hyperbilirubinemia group and 32.6% was the non-hyperbilirubinemia group. This study showed that there was no significant difference of the four sepsis marker levels in both groups (p>0.05) and there was a negative correlation between platelet count with hyperbilirubinemia sepsis (p<0.05; r -0.261). It could not be concluded that there were correlations between CRP, PCT, IT ratio and platelet count with the incidence of hyperbilirubinemia, in subjects with hyperbilirubinemia however, the higher serum bilirubin the lower the platelet count.

Key words: Sepsis neonatorum, hyperbilirubinemia, CRP, procalcitonin, IT ratio, platelet

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INTRODUCTION

Hyperbilirubinemia in neonates is a condition in which the total bilirubin in the newborn reaches more than 5 mg/dL. In aterm infants, it reaches 60%, while in premature infants, it reaches 80%. In infants aged less than one month, it could also occur. The incidence of hyperbilirubinemia in newborns, thus, can be considered as one of the most common factors, then making the newborns be hospitalized. The prevalence of hyperbilirubinemia caused by sepsis even range from 3–8%.[1-3]

Neonatal sepsis, furthermore, is a clinical syndrome of systemic disease associated with the prevalence of bacteremia in the first month of birth. The incidence of neonatal sepsis is 1–5 per 1000 live births and can increase in Very Low Birth-Weight Babies (VLBW) weighing less than 1500 grams. Mortality rate caused by neonatal sepsis even reaches 13–25% and increases in premature babies and infants with early-onset severe illness. Sepsis can also lead to hyperbilirubinemia and play a role in increasing mortality rate in neonates (52.8%) due to sepsis + hyperbilirubinemia condition. For instance, the incidence of hyperbilirubinemia in neonatal sepsis in Jakarta, according to a research conducted by Bachtiar in 2007, reached 65.9%.[4,5]

Some of tests that can be used as sepsis markers, are C-reactive protein (CRP), Procalcitonin (PCT) and Immature to platelet ratio (IT ratio). Decreased platelets are also common in septic patients. Increased CRP is found in infections, autoimmune diseases, post-surgery, meconium aspiration and early vaccination. CRP value, nevertheless, does not increase significantly until 14–48 hours after the onset of infection. Procalcitonin (PCT), on the other hand, is a precursor of calcitonin and 116 protein amino acids. Procalcitonin has a longer half-life (25–30 hours). Procalcitonin concentration begins to increase within 3–4 hours after exposure to endotoxin, reaches its peak after 6 hours and keeps increasing for more than 24 hours.[6-9] A ratio of immature to total neutrophils (IT ratio) is a ratio of immature neutrophils to total neutrophils in peripheral blood smears. IT ratio can be used as a marker of sepsis. If the value of IT ratio is more than 0.2 (20%), it will indicate sepsis, accompanied by leukopenia, increased sedimentation and thrombocytopenia.[10-11]

In addition, a state of severe hyperbilirubinemia can be found in neonates with a total bilirubin concentration of more than 340 mol/L 28 days after the birth with symptoms of sepsis. However, severe hyperbilirubinemia is rarely found in infants. Voora et al[7], reported that in 1% of the prevalence of fever patterns in infants, there were 10% fevers at a rectal temperature of 37.8°C with culture results of sepsis. Besides, necrotizing enterocolitis (NEC) condition, a state of acute inflammatory necrosis of the gastrointestinal tract with an unusual feature of abdominal symptoms, also may be considered as the cause of neonatal sepsis. Chaaban et al[7], reported that 12 out of 51 infants with non-specific abdominal symptoms had a positive culture result in sepsis. Neonatal sepsis is a common cause of morbidity and mortality in neonates.[6,7,12]

This research aimed to determine and analyze the correlation of the enhancement of sepsis markers, such as C-reactive protein (CRP), Procalcitonin (PCT), IT ratio and platelet counts in neonates to hyperbilirubinemia.

METHODS

This research was a retrospective study with cross-sectional approach. This research was conducted by taking the medical records of patients examined for their total bilirubin levels in the Dr. Wahidin Sudirohusodo Hospital, Makassar from January to December 2015. Patients with both sepsis marker tests, such as CRP, PCT, IT ratio and platelet counts, as well as late diagnosis of sepsis were included in this research. C-Reactive Protein (CRP) test was performed using a latex-enhanced immunoturbidimetric assay (ABX pentra C400, Horiba Ltd., Japan). Meanwhile, procalcitonin test was conducted using ELFA (Vidas, USA). An IT ratio was assessed in peripheral blood smear, while platelet counts were measured using impendance method (Hematology autoanalyzer Sysmex XN-1000, Japan). On the other hand, patients with incomplete medical records and non-sepsis diagnosis were excluded from this research.

Furthermore, data analysis was performed using SPSS. Descriptive method was performed by calculating frequency distribution. Next, Kolmogorov-Smirnov test was conducted to analyze the distribution of the data. Comparison analysis on the mean laboratory parameters was then carried out between the sepsis + non-hyperbilirubinemia group and the sepsis + hyperbilirubinemia group by using Mann-Whitney test if the data were not normally distributed. Meanwhile, T Independent test was performed if the data were normally distributed. Afterwards, Spearman correlation test was carried out on the sepsis + non-hyperbilirubinemia group to analyze the correlation of the total bilirubin to some sepsis markers, such as CRP, procalcitonin, IT ratios and platelet counts. The test results would have been significant if the p-value had been <0.05.

RESULTS AND DISCUSSION

Ninety-two subjects of those 158 infant patients who underwent bilirubin examinations were diagnosed with sepsis based on clinical symptoms and laboratory results of sepsis markers. Of those 92 patients, 62 patients had hyperbilirubinemia, while 30 patients had normal total bilirubin values. Based on the total bilirubin values, those research subjects were divided into two groups, namely 62 patients in the sepsis + hyperbilirubinemia group and 30 patients in the sepsis + non-hyperbilirubinemia group.

In this research, the incidence rate or prevalence of sepsis in the neonatal patients, moreover, was 67.4%. This is in line with a research conducted by Tiker et al[3] showing that of 42 neonates who have hyperbilirubinemia, 15 neonates (53.7%) had sepsis. Similarly, a research conducted by Maamouri et al[13] stating that bacterial infection is considered as the main cause of increased bilirubin in neonates.[3,13]

Actually, there have been several types of research on sepsis in Indonesia showing that hyperbilirubinemia is mostly found in sepsis patients experiencing increased total bilirubin. For instance, a research conducted by Oswari et al[14] showed that the incidence of hyperbilirubinemia in 47 neonatal sepsis patients in NICU and neonatological wards was retrospectively 74.5%. Bachtiar's research also showed that the incidence of hyperbilirubinemia in 138 clinical sepsis samples was 65.9%.[4,14]

Table 2 showed a lower mean value of CRP in hyperbilirubinemia (25.9 mg/L) than in normobilirubinemia (35.7 mg/L). However, the results of the statistical test showed that there was no significant difference (p> 0.05). Unlike the mean value of CRP, the mean value of PCT was higher in hyperbilirubinemia (19.2 ng/mL) than in normobilirubinemia (15.3 ng/mL). Nevertheless, the results of the statistical test indicated that there was also no significant difference (p> 0.05). The mean value of IT ratio, furthermore, was lower in hyperbilirubinemia (13.3%) than in normobilirubinemia (15.6%). The results of the statistical test showed that there was no significant difference (p> 0.05). Similarly, the mean value of platelets was lower in hyperbilirubinemia (206.1 x 10^3/mm^3) than in normobilirubinemia (221.0x10^3/mm^3). However, the results of the statistical test indicated that there was also no significant difference (p> 0.05).

These above results indicated that sepsis conditions can affect both the increase and decrease of the values of the sepsis marker. Increased total bilirubin value, on the other hand, had no effect on sepsis parameter value. However, the increased total bilirubin is one of sepsis results. Sepsis is a clinical spectrum as a result of the continuation of the inflammation process

Table 1. Characteristics of the research subjects

Variables		Sepsis + Non-hyperbilirubinemia n (%) 30 (32.6)	Sepsis + Hyperbilirubinemia n (%) 62 (67.4%)	Total n (%) 92 (100)
Sex	Males	22 (23.9)	42 (45.7)	64 (69.6)
	Females	8 (8.7)	20 (21.7)	28 (30.4)
Age	1 day	26 (28.3)	15 (16.3)	41(44.6)
	2–7 days	35 (38)	14 (15.2)	49 (53.3)
	>8 days	1 (1.1)	1 (1.1)	2 (2.2)

Table 2. The comparison of the mean values of the laboratory parameters between the sepsis group and the sepsis-induced hyperbilirubinemia group

Variables	Diagnosis	N	Mean ± SD	p
CRP (mg/L)	Sepsis + Non-hyperbilirubinemia	30	35.7±42.3	0.103*
	Sepsis + Hyperbilirubinemia	62	25.9±36.9	
PCT (ng/ml)	Sepsis + Non-hyperbilirubinemia	30	15.3±19.9	0.957*
	Sepsis + Hyperbilirubinemia	62	19.2 ± 30.0	
IT Ratio (%)	Sepsis + Non-hyperbilirubinemia	30	15.6 ± 9.8	0.371*
	Sepsis + Hyperbilirubinemia	62	13.3 ± 6.2	
Platelet (10^3/mm^3)	Sepsis + Non-hyperbilirubinemia	30	221.0 ± 150.8	0.557**
	Sepsis + Hyperbilirubinemia	62	206.1 ± 91.6	

* Mann-Whitney Test

** T Independent Test

through the immune response with characteristics of systemic inflammation and coagulation factors. Hyperbilirubinemia, on the other hand, is triggered by impaired synthesis and/or bile acid secretion.[2,15,16]

Several types of research have concluded that sepsis may cause hepatic dysfunction. This occurs because of the release of sepsis cytokines that inhibit expression of the transporter protein genes, such as NTCP, OATP, MRP-2 and BSEP as the pathogenesis of hyperbilirubinemia, leading to sepsis. Endotoxin interferes with the Na + / K + -ATPase enzyme responsible for keeping the intracellular more negative, disrupting the tight junction of the hepatocytes and the signal transduction pathway, such as calcium, and triggering biliary canal contraction under normal circumstances.[2,17-21]

Next, The Spearman test was performed on the sepsis + non hyperbilirubinemia group to analyze the correlation of total bilirubin values to the sepsis markers, such as CRP, procalcitonin, IT ratios and platelets (see Table 3).

Table 3. The correlation of laboratory parameters to total bilirubin levels in the sepsis + non hyperbilirubinemia group

Laboratory parameters	Statistics	Total bilirubin
CRP	R	0.015
	P	0.910
PCT	R	0.233
	P	0.068
IT Ratio	R	0.138
	P	0.284
Platelet	R	-0.261
	P	0.041

Based on Table 3, there was a significant negative correlation between total bilirubin and platelet count. Therefore, the higher total bilirubin, the lower the platelet count was ($p < 0.05$). The correlation between these two variables was 26.1% ($R = 0.261$). Besides, there was no significant correlation of CRP, PCT and IT ratio to total bilirubin level ($p > 0.05$). This condition corresponded to the sepsis pathomechanism that can lead to hepatic dysfunction. Increased total blood bilirubin can signify hepatocyte damage, making the liver functions disturbed, one of which is the formation of thrombopoietin. In other words, the decreased thrombopoietin production is directly related to the disruption of thrombopoiesis. With the presence of thrombocytopenia, the death rate of neonatal sepsis will increase.[22]

In addition, the results of this research also showed that there was no significant correlation between the sepsis markers and sepsis + hyperbilirubinemia condition although there was an increase in the mean values of CRP and PCT. Increased CRP found in infection, nevertheless, did not increase significantly until 14–48 hours after the infection. Procalcitonin, on the other hand, had a longer half-life (25–30 hours). PCT concentration usually began to increase within 3–4 hours after exposure to endotoxin, reached its peak about 6 hours and kept increasing for more than 24 hours. Lipopolysaccharide (LPS) bacteria actually have been known to be a strong factor triggering the production of PCT into the systemic circulation. As a result, the mean value of PCT in the sepsis + hyperbilirubinemia group was elevated into 19.2 ng/mL, higher than in the sepsis + non-hyperbilirubinemia group (15.3 ng/mL). This suggested that increased bilirubin was not a direct result of sepsis although sepsis may lead to hepatic dysfunction. However, this research still had some limitations. For instance, this research was an observational study using medical record data, thus, it was difficult to avoid bias, especially related to the onset of infection.[6-9]

CONCLUSION AND SUGGESTION

In conclusion, there was no correlation of CRP, Procalcitonin, IT ratio and platelet counts to the occurrence of hyperbilirubinemia. In subjects with sepsis and hyperbilirubinemia, the higher the bilirubin level, the lower the platelet counts were. As a result, clinicians had better consider the risk of bleeding in sepsis patients with hyperbilirubinemia. Nevertheless, further researches were suggested to focus more on the role of platelets further in patients with neonatal sepsis and hyperbilirubinemia condition. Besides, further researches were also suggested to determine the correlation of increased sepsis markers to sepsis and hyperbilirubinemia and sepsis + non-hyperbilirubinemia conditions.

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