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Impact of Hypertonic Lactated Saline Resuscitation on Serum Interleukin-6 (IL-6) Level in Pediatric Severe Sepsis/Septic Shock in Developing Country

Herdiana Elizabeth Situmorang*, Dadang Hudaya Somasetia, Heda Melinda Nataprawira

Department of Child Health, Hasan Sadikin General Hospital-Universitas Padjadjaran, Bandung, Indonesia
*Corresponding author: herdiana1983@gmail.com

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Abstract **Background:** Fluid rescucitation with normal saline (NS) could aggravate IL-6 production. Our objective was to compare impact of small volume resuscitation hypertonic lactated saline (HLS) versus NS in pediatric severe sepsis/septic shock in developing country hospital setting. The primary endpoint was the decrease of serum IL-6 level after 6 and 12 hours fluid resuscitation. The secondary endpoint was fluid overload. **Methodology and principal findings:** A pre- and post-design, repeated measure study including 30 severe sepsis/septic shock children was conducted in Hasan Sadikin Hospital Bandung, Indonesia. Newly diagnosed severe sepsis/septic shock children (>12−168 months old) were eligible. Patients were resuscitated with either HLS (bolus of 5 mL/kgBW, repeated if no response and followed with 1 mL/kgBW/hour for 12 hours), or NS (bolus of 20 mL/kgBW, repeated if no response and followed with maintenance fluid requirement). If shock persisted inotropes and/or cathecolamine were commenced. There were no significant difference of serum IL-6 levels between groups over time (p=0.183). HLS group had significant lower fluid balance than NS group (p<0.001). **Conclusions:** There was no impact of HLS on serum IL-6 levels after 6 and 12 hours fluid resuscitation. As lower fluid overload observed in HLS group, HLS solution may likely to be a promising fluid for resuscitation in severe sepsis/septic shock children.

Keywords: *children, hypertonic lactated saline, interleukin-6, normal saline, septic shock, severe sepsis*

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1. Introduction

Sepsis is the main cause of mortality among infants and children worldwide, particularly in developing countries. Shock is an important risk factor and main predictor of mortality in septic children [1]. Imbalance of pro-inflammatory and anti-inflammatory response, dominated by pro-inflammatory response occurs in sepsis. [2] Important cytokines in early phase of sepsis are TNF-α, IL-1ß, and IL-6 [3]. Production of IL-6 is associated with vascular leakage, hypoperfusion, and organ dysfunction [4]. In experimental sepsis, IL-6 at 6 hours predicted mortality over 3 days [5]. Studies of [6] and [4] found high IL-6 on adult sepsis at day 1 admission. High serum IL-6 is correlated with severity of pediatric septic shock [7,8].

Fluid resuscitation is the main management of severe sepsis/septic shock. Severe sepsis/septic shock management was in accordance with Surviving Sepsis Campaign 2012 guidelines which used normal saline (NS) as resuscitation fluid could aggravate IL-6 production and risk children in fluid overload. Other fluid resuscitation choice i.e. hypertonic lactated saline (HLS) has been used in pediatric shock due to dengue shock syndrome [9] and severely burn [10]. Small volume resuscitation using HLS, may avoid risk of fluid overload [9].

Hypertonic lactated saline has hemodynamic [9] and immunomodulation impact [11]. Its hemodynamic impact had been studied in children [9], while to our knowledge, no available study of knowing its immunomodulation impact in septic shock children. We hypothesize that there are significant decrease of serum IL-6 levels on 6 and 12 hours after fluid resuscitation between severe sepsis/septic shock children treated with HLS compared with NS. The primary endpoint was decrease of serum IL-6 level after 6 and 12 hours fluid rescucitation. The second endpoint was fluid overload between groups.

Developing countries with inadequate health facilities and infrastructures limitation may cause delay in medical treatment, irrational antibiotics prescriptions, and finally lead to poor outcome in pediatric severe sepsis/septic shock [12]. Until now, there were limited studies related to severe sepsis/septic shock management in developing countries.

2. Methods

2.1. Patients Population

A pre- and post-design, repeated measure study including 30 severe sepsis/septic shock children were conducted in Hasan Sadikin Hospital, Bandung, Indonesia. Children fulfilled criteria of International pediatric sepsis consensus conference for severe sepsis/septic shock [13] (>12−168 months old) and had never received fluid rescucitation were eligible. Chronic diarrhea (may influence assessment of urine output), liver failure (liver aspartate aminotransferase-AST and alanin aminotransferase-ALT >20 times normal value), severe malnutrition, immunodeficiency diseases, and receiving long term corticosteroid were excluded based on history taking, physical examination, and/or laboratory tests. Simple randomisation with sealed opaque envelopes sequentially numbered was used to allocate patients into each groups. The enrollment and resuscitation of patients was performed by authors and four trained senior residents. Informed consent were obtained from the patients'parents and or guardian simultaneously while performing procedures of oxygenation, intravenous access, as shock condition required immediate management. The Ethics Committee of Hasan Sadikin Hospital had approved the protocol before the study. The intervention period ran for the first 12 hours of treatment and followed by a 12-hour observation period. The outcomes, complications, adverse events, and concomitant treatments were recorded throughout hospital stay until discharge.

2.2. Study Protocol

Patients were randomly assigned to receive intravenous bolus infusion of either HLS (5 mL/kgBW) or NS (20 mL/kgBW) for 10 minutes. Table 1 shows the comparison of both solutions composition.

If shock did not recover with the first bolus, a second bolus with same solution and dosage was infused once again. If the second bolus failed, then inotropes according to shock type was administered until shock reversed. If inotropes failed to reverse shock, then cathecolamine was administered according to shock type according to our hospital guidelines for severe sepsis/septic shock and maintenance fluid HLS (1 mL/kgBW/hr) or NS (according to fluid requirement/kgBW) for 12 hours was given. If shock reversed, patients received maintenance dose of HLS (1 mL/kgBW/hr) or NS (according to fluid requirement/kgBW) for 12 hours. After 12 hours, solution was changed to dextrose 2.25%/NaCl 0.45%. Antibiotics were administered within 1 hour of shock septic. Blood samples for IL-6 level baseline measurement were drawn from patients simultaneously with fluid resuscitation.

Table 1. Comparison of HLS and NS Composition

Content	Solutions	
	HLS	NS
Na^+ (mEq/L)	504	308
Cl^- (mEq/L)	6.7	154
K^+ (mEq/L)	4	-
Ca^{2+} (mEq/L)	1.36	-
Lactate (mEq/L)	504.15	-
Total osmolarity (mOsm/L)	1,020	308

2.3. Studied Parameters

Glasgow Coma Scale (GCS), blood pressure, shock index, were measured before and after boluses. Shock recovery time, inotrope-vasoactive index, fluid intake and urine output were observed. Laboratory parameters of blood including haemoglobin, hematocrit, leukocytes, thrombocyte, C-reactive protein,glucose, before and after bolus according to patient's condition. Interleukin-6 values were measured before (T0), 6 hours (T6), and 12 hours (T12) after fluid boluses with ELISA method using Bender MedSystem GmbH.

2.4. Study Endpoints

The primary endpoint was the decrease of IL-6 level at 6 and 12 hours after fluid resuscitation. Secondary endpoint was fluid overload between groups.

2.5. Sample-size Determination and Statistical Analysis

To calculate the sample size for three-repeated measure ANOVA with an alpha error of 0.05, 80% power, a correlation among repeated measures of 0.25, and an effect size of 0.25, we used G*Power 3.1 and obtained minimal 14 patients for each group. We included 15 patients for each group. The impact of solution on IL-6 levels over time in each group was analysed by repeated measure ANOVA. Numeric data of clinical evaluation after fluid boluses exhibiting non-normal distribution were analysed with Mann-Whitney tests. P value <0.05 was considered as statistically significant.

3. Results

3.1. Studied Population

Since December 2013 to October 2014, 82 patients were assessed for eligibility, 18 patients were excluded due to inclusion criteria and 2 patients declined to participate. Of the 62 randomised patients, each 31 patients were allocated to HLS or NS group. Only 15 patients in each group could be analyzed as death occurred. One patient in HLS group experienced hypernatremia >160 mEq/L, treatment was discontinued, and hypernatremia protocol was given. Figure 1 depicts participants' flow diagram

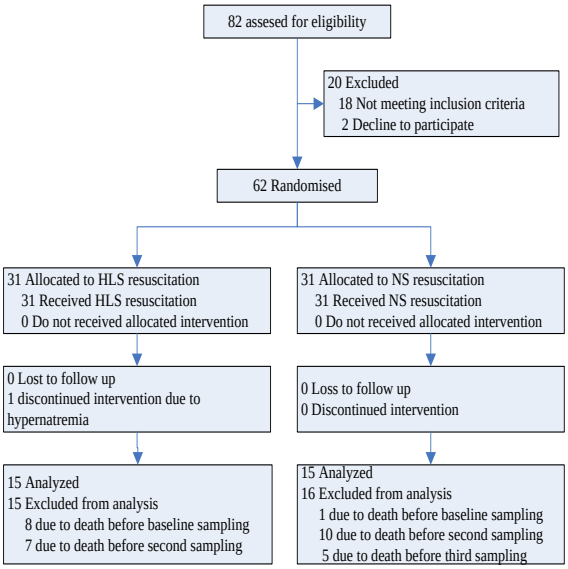

Figure 1. Participants' Flow Diagram

Patients consisted of 29 septic shock and 1 severe sepsis. As shown in Table 2, despite randomisation, higher number of patients with high PELOD score were in HLS group and moderate PELOD patients were in NS group.

Table 2. Characteristics of Study Patients

	HLS (n=15)	NS (n=15)
Age (months)	33 (12−156)	33 (12−120)
Sex		
Male	10	8
Female	5	7
Nutritional status		
Normal	9	8
Moderate malnutrition	6	7
Hemodynamic parameters		
Glasgow Coma Scale	11 (5−14)	9 (4−15)
Systolic (mmHg)	94.9 ±20.1	77±16.7
Diastolic (mmHg)	40 (0−80)	45(0−60)
Heart Rate (x/mnt)	167 (140−283)	160 (132−195)
Shock Index	2 (1.2−2.9)	2 (1.44−5.6)
Comorbidities		
Oncology	6	1
Neuromuscular	2	4
Respiratory	1	1
Gastrointestinal (GIT)		1
Genetic		1
Site of infection		
Central nervous System (CNS)	1	4
Lung		
Isolated lung infection	7	4
Lung and CNS	1	0
Lung + Abdomen/GIT	3	2
Lung+ENT	1	1
Abdomen/GIT:		
Isolated abdomen/GIT infection	1	1
Abdomen/GIT + Urogenital	1	1
CNS + ENT	1	0
Infection source		
Community infection (%)	12	13
Hospital infection (%)	3	2
Shock severity type		
Severe sepsis		1
Cold shock	7	11
Warm shock	8	3
PELOD score		
Low (<10)	3	4
Moderate (10−19)	3	9
High (≥20)	9	2
Laboratory parameters		
Haemoglobin (g/dL)	9.3±2.7	10.1±2.4
Hematocrit (%)	27.9±7.9	30,5±6.6
Leukocytes (cells/mm^3)	12,153.3±9,327.3	12.700 (500−55.600)
Thrombocyte (cells/mm^3)	241,133.3±156,702.6	227,933.3±182,731.1
C-reactive Protein (mg/dL)	30.3 (0.3−262.9)	93.7 (0.6−237.8)
Serum glucose level (mg/dL)	93 (46−147)	99 (30−294)
Onset of infection (hours)	72 (24−3600)	72 (5−720)
Shock reversal	13	11
48 hours survival	7	12
Survival	6	7
Mode of dying		
Respiratory failure	2	0
Not reversed shock	2	4
Reccurent shock not reversed	3	4
High intracranial pressure	1	0
Disseminated intravascular coagulation	1	0

3.2. Impact on Serum IL-6 Level

Logarithmic transformation was used to meet normal data distribution. Baseline serum IL-6 levels were not significantly different between groups. Repeated measure ANOVA demonstrated no significant difference of serum IL-6 level means between groups (p=0.183), even though there was a decreasing trend of serum IL-6 level over time in both groups (Table 3). Sequential serum IL-6 levels were depicted by Figure 2.

Table 3. Impact of Fluid Resuscitation on Serum IL-6 Levels

Serum IL-6 levels	HLS (n=15)	NS (n=15)
T0		
Mean±s.d	163.4±176.6	168.1±114
Median	94,9	191.1
Range	6.6−505.3	10.2−326.4
T6		
Mean±s.d	156±188.2	127.6±106.9
Median	47.1	90.2
Range	11.9−509.6	11.7−291
T12		
Mean±s.d	156.1±198.4	83.2±103.7
Median	35.2	42.1
Range	6.6−489.2	5.1−296.7

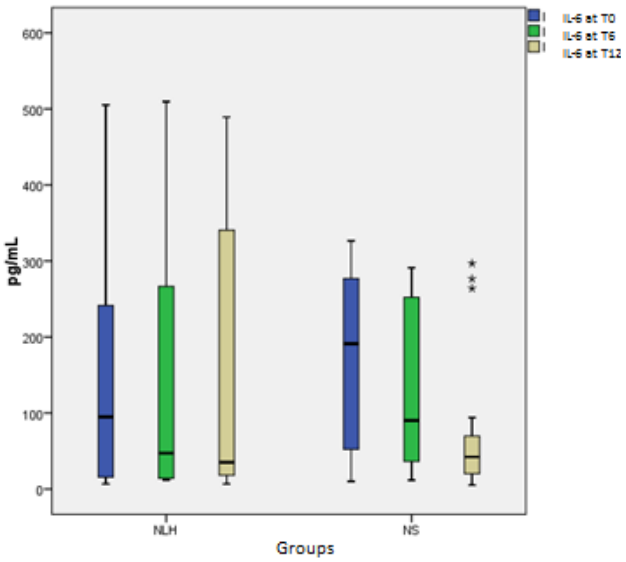

Figure 2. Sequential serum IL-6 level in groups. Asterisks represent outliers. Median of both groups decreased over time

Table 4. Impact of Fluid Resuscitation on Clinical Parameters

	HLS (n=15)	NS (n=15)	p value
Delta GCS	0 (0−2)	0 (0−2)	0.378*)
Delta systolic (mmHg)	0 (0−35)	5 (-10−30)	0.300*)
Delta diastolic (mmHg)	0 (0−50)	10 (-10−60)	0.896*)
Delta shock index	0.08 (-0.7−2.07)	0.29 (-0.24−1.32)	0.329*)
Shock recovery (minutes)	40 (20−360)	50.9 (15−90)	0.467*)
Inotrope-Vasoactive index	30 (6−40)	30 (5−40)	0.913*)
Total fluid intake (mL)	388 (130−1046)	1428 (592−2352)	0.000*)
Fluid overload (%)	0.4 (-6.7−7.7)	6.9 (2.4−17.2)	0.000*)

*) Mann-Whitney Test.

3.3. Impact on Fluid Overload

There was no statistically significant difference of clinical parameters changes between groups, except for total fluid intake and fluid overload ($p<0.001$). Total fluid intake was calculated in 12 hours as some patients could not survive for 24 hours. Fluid overload was calculated in similar way. Fluid intake and overload were significantly lower in HLS group. Time of achieving shock recoveries were not statistically different between groups (Table 4).

4. Discussions

Our patients' median age was 49.4 months old and boys were preponderance. Lung was the most common site of infections. Several comorbidities found in both groups were hematology/oncology disease (4 ALL, 1 CNS tumor, 1 solid tumor in HLS groups, and 1 ALL in NS group) and neuromuscular (1 CP, 1 epilepsy in HLS group, and 2 CP, 1 epilepsy, and 1 congenital hydrocephalus in NS group). These results were in accordance with severe sepsis epidemiology in children in the US [1].

In this study almost all of the patients had septic shock, with cold shock as prominent pattern. Other study [14] found that nearly 50% septic shock in children was cold shock. This was due to untreatable community-acquired infection, whereas warm shock was identified as hospital-acquired infection. In our study, community-acquired infection was the main origin of cold and warm shock. Warm shock was detected mainly during hospitalization. Possibilities of distinct shock pattern were earlier recognition/resuscitation of shock in hospitalised children and different bacterial aetiology [14].

Our study identified limited bacterial aetiology from four blood cultures i.e. *Enterobacter cloace*, *Achromobacter xylosoxidans*, *Acinetobacter baumanii*, and *Pseudomonas aeruginosa*. *Eschericia coli* was found from peritoneal fluid and *Staphylococcus warneri* from bone marrow culture. These were among the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) [15]. Actually, only 10% pneumonia cases yielded bacterial growth in blood culture [16]. Amoeba was found in faeces of two cases, demonstrated that parasite had a role in sepsis in developing country [17]. These polymicrobial pathogens were identified in different patients. Our patients in both groups received relatively similar antibiotics, which was third generation of cephalosporines (ceftriaxone or cefotaxime) with or without other antibiotics combination. *Acinetobacter baumanii* was resistant to all tested antibiotics, *Enterobacter cloace* was sensitive to aminoglycoside, and *Pseudomonas aeruginosa* was resistant to ceftriaxone.

Fluid resuscitation with hypertonic crystalloids aimed to reverse shock, decrease serum IL-6 level, and achieve lower fluid balance. No statistically significant difference of clinical response after fluid resuscitation was found between groups, even though lower changes were found in HLS group. Inotrope-Vasoactive index [18] could not precisely represent myocardial dysfunction in our study. It might be caused by limited availability of inotrope and vasoactive drugs, such as vasopressin and milrinone.

Median of baseline serum IL-6 level in this study was 140.2 pg/mL (6.6−505.3) which was quite similar with other study 213.1 pg/mL (10.85−396.7) [7], but lower than [8] 287 pg/mL (195.1−316.9). In contrary with our hypothesis, decreasing trend of serum IL-6 level was equal in both groups. We assumed that the possible reasons for this results were significantly high PELOD score patients in HLS group, pathogens virulence, and individualized complex immune response.

PELOD score is a valid measure of multiple organ dysfunction syndrome and marker of disease severity. Higher PELOD score reflected more severe the organ dysfunction. The more severe organ dysfunction, the higher serum IL-6 [19]. PELOD score could not be determined on admission before treatment as patient needed immediate management. Daily PELOD (dPELOD) score gave us information about organ dysfunction deterioration [20]. Unfortunately, dPELOD score could not be done because of resource limitation.

Virulence of pathogens are related to IL-6 production. A retrospective study demonstrated that Gram-negative bacterial sepsis induce higher serum IL-6 level than Gram-positive [21]. Gram-negative bacteria has different cell wall composition from Gram-positive bacteria. Lipopolysaccharides (LPS) induce macrophages, endothelial cells, and neutrophil to produce IL-6. Outer membrane protein of Gram-negative bacteria i.e. porin, could activate Janus Kinase and p38 Mitogen Activation Protein Kinase then released TNF-α and IL-6. Four of our Gram-negative septic patients had high baseline serum IL-6 level (191.1−423.8 pg/mL). Limited data of pathogens in our study did not enable us to compare serum IL-6 levels in Gram-negative and Gram-positive sepsis.

Immune response is a complex and dynamic process. During sepsis, pro-inflammatory and anti-inflammatory cytokines are produced simultaneously to maintain balance of immune response. Hosts' immune response to pathogens are varied. High IL-6 serum and persistent decreasing of monocyte Human Leukocyte Antigen-DR (mHLA-DR) was related with sepsis development in adult severe trauma [22]. Our study did not measure other pro-inflammatory and antiinflammatory cytokines to observe balance pattern of both responses. We suggested a possibility of immunoparalysis in our patients.

Lower fluid overload was achieved in HLS group. Smaller volume of fluid was administered in HLS group, but larger urine output. This might be a volume expander effect of HLS [9].

To our knowledge, this is the first study in comparing immunomodulation impact of HLS and NS in pediatric severe sepsis/septic shock.

Our setting was very different from developed countries, as several critically ill children were treated in wards with inadequate facilities. High mortality of sepsis came from developing countries was partly due to limited resources [23]. Our condition showed limited capacity of pediatric intensive care unit, lack of medications, monitors, ventilators, invasive measurement of fluid adequacy and laboratory.

This study is subject to several limitations. First, we could not control the PELOD score as patients need immediate resuscitation, including dPELOD score as well. Second, we could not establish microbial diagnosis due to the limitation of blood culture such as lack of rapidity and

low sensitivity, particularly for polymicrobial infections. Third, we could not rule out viral and fungal pathogens.

Further investigation is needed with larger sample size, multicenter approach along with invasive or noninvasive fluid adequacy monitoring. We suggest to perform well-designed molecular assays for sepsis.

5. Conclusions

There are no difference of serum IL-6 level at 6 and 12 hours and shock reversal time between groups along with significant smaller volume intake and lower fluid overload in HLS group. Hypertonic lactated saline is a promising fluid for resuscitation in severe sepsis/septic shock children who were at risk of fluid overload.

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Competing Interests

The authors declare no competing interests.

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