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The resuscitation effect of Ringer's lactate compared to a combination of Ringer's lactate/6% HES (130/0.4) with a ratio 2:1 on plasma syndecan-1 level as a marker of endothelial glycocalyx degradation in patients with hemorrhagic shock

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Abstract

***Objective*: This study aimed to compare the resuscitation effect of Ringer's lactate (RL) and the combination of Ringer's lactate/6% hydroxyethyl starch (HES) (130/0.4) with a ratio 2:1 (RLH) on plasma syndecan-1 (synd-1) level as a marker of endothelial glycocalyx (EG) degradation in patients with hemorrhagic shock (HS).**

***Design*: This prospective, experimental, analytic study with a single-blind trial design assessed the resuscitation effect of RL compared to RLH on the synd-1 level as a marker of EG degradation in patients with HS.**

***Setting*: We conducted this study in the operating room (OR) and the Intensive Care Unit (ICU) of Wahidin Sudirohusodo Hospital and Hasanuddin University Medical Research Center (HUMRC) from September 2022 to February 2023.**

***Patients and participants*: The patients underwent elective surgery with estimated HS class II and III.**

***Intervention*: The patients in the RL group were resuscitated with RL solution, and those in the RLH group were resuscitated with the RLH solution. The blood sample was collected to measure the synd-1 level.**

***Measurement and results*: The synd-1 level increased after HS. After resuscitation, the synd-1 level decreased in both groups at 6 and 24 hours in HS class II and 6 hours in HS class III, yet increased at 24 hours in HS class III. There was no significant difference between the two groups. However, the decrease in synd-1 level in the RLH group was higher than in the RL group, and the increase in synd-1 level in the RLH group was lower than in the RL group.**

***Conclusion*: The resuscitation effect of RL and RLH solution did not suggest a significant difference in improving the synd-1 level as a marker of EG degradation in HS. However, resuscitation with RLH solution provided a better profile of synd-1 level than RL.**

Key words: Resuscitation fluid, Ringer's lactate, HES 6% (130/0.4), endothelial glycocalyx degradation, syndecan-1.

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Introduction

Hemorrhagic shock (HS) is one of the significant causes of morbidity and mortality in trauma and surgical patients. Oxygen debt, acidosis, organ dysfunction, coagulopathy, and death resulted from hemodynamically significant hemorrhage when accompanied by the physiologic disorder associated with trauma and surgery if not treated aggressively. (1)

Plasma syndecan 1 (synd-1) and other proteoglycans and glycosaminoglycans are known to be indirect measures of endothelial glycocalyx (EG) shedding, which have been shown to correlate to the shedding activity at the level of the endothelium after injury and hemorrhage. Despite several isoforms in the syndecan and glypican families, the most cited shed marker in trauma and hemorrhagic shock is synd-1, a heparan sulfate proteoglycan. Synd-1 shedding appears to be primarily related to the severity of the microvascular injury and is selectively shed by heparinase. (2)

The type of fluid chosen for expanding blood volume or compensating for intraoperative blood loss, leading to acute normovolemic hemodilution (ANH), may alter EG components inconsistently. Colloid solutions may preserve EG components by maintaining the shear stress on endothelial due to a higher viscosity, osmotic pressure, and the presence of macromolecules. However, the degree to which EG shedding occurs in response to a stepwise ANH using different fluid types needs to be better described. In addition, the consequences of severe ANH on the vascular barrier permeability are unknown. (3)

Adverse safety signals are absent when using modern hydroxyethyl starch (HES) in surgery and trauma, which can indicate benefits in these clinical contexts. (4) In mice, 6% HES (130/0.4) exerts protective effects on EG integrity during systemic inflammation. (5) In a human study, EG degradation does not differ between using HES (130/0.4) and 5% albumin in major abdominal surgery. (6)

Muchtar et al. investigated the comparison of the resuscitation effects between Ringer's lactate (RL) and Ringer's lactate/6% HES (200/0.5) combination to serum lactate level in HS in Lepus nigricollis rabbits and concluded that resuscitation in both groups may decrease serum lactate level in HS, and resuscitation with RL/6% HES (200/0.5) with 2:1 combination provided better improvement than RL alone. (7) This study was designed to apply the 2:1 ratio of Ringer's lactate/6% HES fluid combination in humans. However, we used 6% HES (130/0.4) instead of 6% HES (200/0.5).

In another animal study, Uzawa et al. studied the protective effect of HES solution on the EG layer in an acute hemorrhage mouse model. They found that during the early stages of acute massive hemorrhages, an equal volume of saline fluid resuscitation impaired EG function, whereas 6% HES (130/0.4) inhibited the progress of EG injury and suppressed vascular permeability. The localization of 6% HES (130/0.4) to the inner vessel wall suggested that 6% HES (130/0.4) might have direct protective mechanisms for the EG. (8)

Material and methods

Settings and design

This prospective, experimental, analytic study compared the resuscitation effect between Ringer's lactate and a 2:1 combination of Ringer's lactate and HES 6% (130/0.4) to the synd-1 level as a marker of EG degradation in HS. This study was conducted in September 2022-February 2023 in the operating room and the Intensive Care Unit (ICU) of Wahidin Sudirohusodo Hospital Makassar, South Sulawesi, Indonesia. The blood samples were measured using a Human Syndecan-1 ELISA Kit (Assay Genie, Ireland) at Hasanuddin University Medical Research Center (HUMRC), Makassar, South Sulawesi, Indonesia. The Medical Research Ethics Committee of Hasanuddin University Makassar (377/UN4.6.4.5.31/PP36/2022) approved all experimental protocols employed in this study.

Study group

The patients enrolled were allocated into two groups:

- RL group: The patients were resuscitated with Ringer's lactate solution.
- RLH group: The patients were resuscitated with a 2:1 ratio of a combination of Ringer's lactate/6% HES (130/0.4) solutions.

Study protocol

The inclusion criteria for this study were the patients who underwent surgery with estimated hemorrhage of class II or III, American Society of Anesthesiologists Physical Status 1-2, aged 18-55 years old, and normal body mass index (BMI). The exclusion criteria were that the patient had a contraindication to the experimental fluid, the hemorrhage was under class II, the patient had severe respiratory and cardiovascular disease, and the patient had comorbidities (hypertension, diabetes mellitus, kidney disorder, liver disorder, and other uncontrolled comorbidities). The patient with uncontrolled bleeding during surgery or who died before the last sample collected was dropped out.

A total of 30 patients who met the inclusion criteria were included. These patients were randomly allocated into two groups (RL group and RLH group), with 15 patients in each group. The patients were prepared for elective surgery using the standard procedure. The first blood sample was collected before anesthesia (S0). When the bleeding reached HS class II (≥15% of estimated blood volume [EBV]), the second sample was collected (S1), and then the patients were resuscitated with the experimental fluid. Packed red cells (PRC) were transfused after the bleeding reached maximum allowable blood loss (MABL). After the surgery finished, the patients were admitted to the ICU. The following sample was collected 6 and 24 hours after resuscitation (S2 and S3, respectively).

Data analysis
The collected data was analyzed and presented as mean±SD or frequency and percentage. Statistical analysis was performed using the IBM® SPSS® Statistics version 29.0.1.1 for Macbook (SPSS Inc., Chicago, IL, USA). The normality test showed that the data was not normally distributed. Numerical variables were tested using the Mann-Whitney U test to compare the data between both groups and the Wilcoxon signed-rank test to compare data in each group. The categorical data presented as frequency (n) and percentage then were tested with a chi-square test. P-value<0.05 was considered as statistically significant.

Results

Homogeneity
Table 1 shows the demographic data of both groups. There were no significant differences in sex, age, body weight, hemorrhage volume, or class of HS. These data show that both groups were homogeneous and eligible to be compared.

Changes in synd-1 level after hemorrhage
Table 2 presents the changes in the synd-1 level when the bleeding reached HS class II (15% of EBV). The differences between the two groups were not statistically significant, so the data were eligible to be compared.

Changes in synd-1 level after resuscitation
- RL group

The changes in the synd-1 level in the RL group are presented in **Table 3**. There were no statistically significant differences between the changes in the synd-1 level based on the class of HS in the RL group. However, the synd-1 level was decreased 6 hours and 24 hours after resuscitation in patients with HS class II (-0.37±3.53 ng/ml and -0.03±2.05 ng/ml, respectively). The synd-1 level also decreased 6 hours after resuscitation in patients with HS class III yet increased 24 hours after resuscitation (-0.72±0.73 ng/ml and 1.58±2.85 ng/ml, respectively).
- RLH group

The changes in the synd-1 level in the RLH group are shown in **Table 4**. The difference between the synd-1 level at 6 and 24 hours was not statistically significant. The decreased synd-1 level was also seen 6 and 24 hours after resuscitation in the patients with HS class II (-1.74±1.68 ng/ml and -0.66±1.14 ng/ml, respectively). It also decreased 6 hours after resuscitation in the patients with HS class III, while it increased 24 hours after resuscitation (-0.82±1.61 and 1.34±2.05, respectively).
- Between RL and RLH group

The changes in synd-1 level between RL and RLH groups were not statistically significant at 6 and 24 hours after resuscitation. **Table 5** shows that the synd-1 level in both groups decreased 6 hours after resuscitation yet increased 24 hours after resuscitation. Despite not being significant, the decrease of synd-1 level was higher in the RLH group compared to the RL group at 6 hours (-1.74±1.68 ng/ml vs -0.37±3.53 ng/ml) and 24 hours (-0.66±1.14 ng/ml vs -0.03±2.05 ng/ml) after resuscitation in patients with HS class II. In the patients with HS class III, the decrease of synd-1 level at 6 hours after resuscitation was also higher in the RLH group compared to the RL group (-0.82±1.61 vs -0.72±0.73), while the increase of synd-1 level was lower in RLH group rather than RL group at 24 hours after resuscitation (1.34±2.05 vs 1.58±2.85).

Discussion

HS is one of the conditions that may cause EG degradation. Recent studies have shown that fluid resuscitation is an essential clinical therapeutic strategy for improving microcirculatory perfusion in critical illness patients. The fluid type used may benefit or harm the EG. (9)
There were many animal studies on EG degradation in HS. One of these studies suggested that the changes in endothelial cell permeability were associated with synd-1 shedding. (10) In another study, Filho et al. confirmed that monitoring plasma synd-1 or heparan sulfate as biomarkers of EG shedding was valid and might help guide resuscitation strategies following hemorrhage. (11) In this study, the synd-1 level in the RL and RLH groups increased when the HS class II occurred (the bleeding reached ≥15% of EBV). These results were consistent with the prior animal study that HS may cause EG degradation, as seen by the increased synd-1 level.
After resuscitation, the synd-1 level was decreased in both groups. However, the decrease was still above the baseline level. This result was similar to the study by Haywood-Watson et al. that after initial resuscitation, the synd-1 level significantly decreased, but the value was still elevated above the baseline level. (10)
Based on the HS class, the synd-1 level decreased until 24 hours after resuscitation in HS class II. However, in the HS class III, the decrease of the

synd-1 level was only seen at 6 hours after resuscitation and was increased again at 24 hours after resuscitation. This phenomenon occurred in both groups despite no significant differences between the two classes of HS in each group. Regardless of the type of resuscitation fluid, this result might be caused by hemodilution due to the replacement of more hemorrhage volume in HS class III rather than class II. Torres et al. showed that resuscitation with RL and Hextend solution was unable to achieve EG and coagulation repairs compared to fresh frozen plasma. (12)

The comparison of synd-1 level between the RL and the RLH groups was not statistically significant. It was similar to the study by Guerci et al. They showed that fluid resuscitation with balanced crystalloid and balanced HES significantly decreased the synd-1 level at the end of an animal experiment compared to the non-resuscitated HS group. (13)

Balanced crystalloids like Ringer's lactate are more physiological, similar in composition to human plasma, and more compatible with the EG layer. However, balanced crystalloids are hypotonic compared with extracellular fluid and are associated with metabolic alkalosis; hence, they are not entirely harmless. With a damaged EG layer, the distribution of the balanced salt solution changes, contributing to interstitial edema. (14) Besides, significantly more crystalloid than colloid solution was required in healthy pigs for the same hemodynamic effect. Furthermore, the volume-replacement ratio was very similar to that described by Starling. The Starling's "three-compartment model" requires an intact EG. Colloids may have a place in early resuscitation before the EG suffers impairment. (15)

Although the difference between both groups was not statistically significant, the decrease of synd-1 level in the RLH group tended to be higher than in the RL group at 6 and 24 hours after resuscitation in HS class II and 6 hours after resuscitation in HS class III. Moreover, the increase of synd-1 level in the RLH group tended to be lower than in the RL group at 24 hours after resuscitation in HS class III. Uzawa et al. found that fluid resuscitation with 6% HES (130/0.4) could preserve EG thickness, protect endothelial cell function, and improve hemodynamics, macrocirculation, and microcirculation via preservation of vessel integrity and prevention of capillary leak. (8,16,17)

Azumaguchi et al. showed that hemodilution with HES was associated with the localization of HES on the vascular endothelium and covering the vascular endothelium and EG, probably due to the high shear viscosity of HES. HES may act to protect rather than damage the vascular endothelium and EG. (18)

Ergin et al. concluded that using a balanced HES preserved the EG, maintained microcirculatory function, and improved total intravascular volume compared with both balanced and unbalanced crystalloids. (3) Zhao et al. showed that HES (130/0.4) infusion significantly decreased HS-induced pulmonary vascular leakage. The fluorescence exudation of fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) in lung tissue was significantly lower than in the RL group. The fluorescence density of FITC-BSA in lung tissue was also significantly lower than in the RL group. (19)

Further human investigation is needed to confirm the benefit of 6% HES (130/0.4) in EG, including the type of combined solution, the ratio of combination, and the effect on the other markers of EG degradation.

Conclusion

The resuscitation effect of RL and RLH solutions did not suggest a significant difference in improving the synd-1 level as a marker of EG degradation. However, resuscitation with RLH solution provided a better profile of synd-1 level than RL.

Declaration of competing interest

No potential conflict of interest relevant to this article was reported.

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Table 1. Demographic data

No.	Variables	Group		p-value
		RL	RLH	
1	Sex# • Male • Female	 6 (40%) 9 (60%)	 3 (20%) 12 (80%)	0.232
2	Age (years)^	33.3±12.5	37.5±7.7	0.217
3	Body weight (kg)^	61.6±11.8	57.2±9.5	0.137
4	Volume of hemorrhage (ml)^	1316.7±1006.2	1658.7±905.6	0.089
5	Class of HS# • Class II • Class III	 10 (66.7%) 5 (33.3%)	 5 (33.3%) 10 (66.7%)	0.068

Legend: HS=hemorrhagic shock; RL=Ringer's lactate; RLH=combination of Ringer's lactate/6% HES (130/0.4).
#Data was presented as frequency (n [%]) and was tested using the chi-square test; ^data was presented as mean±SD and was tested using the Mann-Whitney U test.

Table 2. Comparison of synd-1 level changes at initial HS (class II)

No.	Variable	Group		p-value
		RL	RLH	
1	Δ synd-1 at HS (S1-S0)	1.15±1.20	1.06±0.83	0.935

Legend: synd-1=syndecan-1; HS=hemorrhagic shock; RL=Ringer's lactate; RLH=combination of Ringer's lactate/6% HES (130/0.4).
Data was presented as mean±SD and was tested using the Mann-Whitney U test.

Table 3. Comparison of synd-1 level at 6 and 24 hours after resuscitation in the RL group

No.	Variables	Class of HS				p-value#
		Class II	p-value^	Class III	p-value^	
1	Δ synd-1 at 6 hours (S2-S1)	-0.37±3.53	0.767	-0.72±0.73	0.138	1.000
2	Δ synd-1 at 24 hours (S3-S1)	-0.03±2.05		1.58±2.85		0.310

Legend: synd-1=syndecan-1; RL=Ringer's lactate; HS=hemorrhagic shock
^Data was presented as mean±SD and was tested using the Wilcoxon signed-rank test; #data was presented as mean±SD and was tested using the Mann-Whitney U test.

Table 4. Comparison of synd-1 level at 6 and 24 hours after resuscitation in the RLH group

No.	Variables	Class of HS				p-value#
		Class II	p-value^	Class III	p-value^	
1	Δ synd-1 at 6 hours (S2-S1)	-1.74±1.68	0.345	-0.82±1.61	0.300	0.679
2	Δ synd-1 at 24 hours (S3-S1)	-0.66±1.14		1.34±2.05		0.129

Legend: synd-1=syndecan-1; RLH=combination of Ringer's lactate/6% HES (130/0.4); HS=hemorrhagic shock
^Data was presented as mean±SD and was tested using the Wilcoxon signed-rank test; #data was presented as mean±SD and was tested using the Mann-Whitney U test.

Table 5. Comparison of synd-1 level between both groups based on the class of HS

No.	Classes of HS	Variables	Group		p-value
			RL	RLH	
1	Class II	Δ synd-1 at 6 hours (S2-S1)	-0,37±3,53	-1,74±1,68	0,594
		Δ synd -1 at 24 hours (S3-S1)	-0,03±2,05	-0,66±1,14	0,594
2	Class III	Δ synd -1 at 6 hours (S2-S1)	-0,72±0,73	-0,82±1,61	0,679
		Δ synd -1 at 24 hours (S3-S1)	1,58±2,85	1,34±2,05	0,859

Legend: synd-1=syndecan-1; HS=hemorrhagic shock; RL=Ringer’s lactate; RLH=combination of Ringer's lactate/6% HES (130/0.4).
Data was presented as mean±SD and was tested using the Mann-Whitney U test.

References

1. Tanner CB, Boydston EA. Hemorrhagic shock. In: Abd-Elsayed A, editor. Advanced Anesthesia Review. New York: Oxford University Press; 2023.
2. Anand T, Reyes AA, Sjoquist MC, Magnotti L, Joseph B. Resuscitating the Endothelial Glycocalyx in Trauma and Hemorrhagic Shock. Ann Surg Open 2023;4:e298.
3. Ergin B, Guerci P, Uz Z, Westphal M, Ince Y, Hilty M, et al. Hemodilution causes glycocalyx shedding without affecting vascular endothelial barrier permeability in rats. J Clin Trans Res 2020;5(5):243-52.
4. Weiskopf RB, James MFM. Update of use of hydroxyethyl starches in surgery and trauma. J Trauma Acute Care Surg 2015;78:S54-9.
5. Margraf A, Herter JM, Kuhne K, Stadtmann A, Ermert T, Wenk M, et al. 6% Hydroxyethyl starch (HES 130/0.4) diminishes glycocalyx degradation and decreases vascular permeability during systemic and pulmonary inflammation in mice. Crit Care 2018;22:111.
6. Suzuki T, Koyama K. Open randomized trial of the effects of 6% hydroxyethyl starch 130/0.4/9 and 5% albumin on safety profile, volume efficacy, and glycocalyx degradation in hepatic and pancreatic surgery. J Anesth 2020;34:912-23.
7. Muchtar F, Arif SK, Tanra AH, Santoso A, Wiryana M, Bukhari A, et al. Comparison of the resuscitative effects between lactated Ringer's and lactated Ringer's/hydroxyethyl starch 6% (200/0.5) combination to serum lactate level in hemorrhagic shock in Lepus nigricollis rabbits. Crit Care Shock 2020;23:287-97.
8. Uzawa K, Ushiyama A, Mitsuda S, Ando T, Sawa M, Miyao H, et al. The protective effect of hydroxyethyl starch solution on the glycocalyx layer in an acute hemorrhage mouse model. J Anesth 2020;34:36-46.
9. Wang G, Zhang H, Liu D, Wang X, Chinese Critical Ultrasound Study Group. Resuscitation fluids as drugs: targeting the endothelial glycocalyx. Chin Med J (Engl) 2022;135:137-44.
10. Haywood-Watson RJ, Holcomb JB, Gonzalez EA, Peng Z, Pati S, Park PW, et al. Modulation of Syndecan-1 Shedding after Hemorrhagic Shock and Resuscitation. PLoS One 2011;6:e23530.
11. Filho IPT, Torres LN, Salgado C, Dubick MA. Plasma syndecan-1 and heparan sulfate correlate with microvascular glycocalyx degradation in hemorrhaged rats after different resuscitation fluids. Am J Physiol Heart Circ Physiol 2016;310:H1468-78.
12. Torres LN, Sondeen JL, Ji L, Dubick MA, Filho IT. Evaluation of resuscitation fluids on endothelial glycocalyx, venular blood flow, and coagulation function after hemorrhagic shock in rats. J Trauma Acute Care Surg 2013;75:759-66.
13. Guerci P, Ergin B, Uz Z, Ince Y, Westphal M, Heger M, et al. Glycocalyx Degradation Is Independent of Vascular Barrier Permeability Increase in Nontraumatic Hemorrhagic Shock in Rats**.** Anesth Analg 2019;129:598-607.
14. Hirata N. Fluid resuscitation with hydroxyethyl starch in perioperative acute hemorrhagic shock. J Anesth 2020;34:317-9.
15. Laszlo I, Demeter G, Oveges N, Erces D, Kaszaki J, Tanczos K, et al. Volume-replacement ratio for crystalloids and colloids during bleeding and resuscitation: an animal experiment. Intensive Care Med Exp 2017;5:52.
16. Uzawa K, Ushiyama A, Mitsuda S, Ando T, Sawa M, Miyao H, Yorozu T. The protective effect of hydroxyethyl starch solution on the glycocalyx layer in an acute hemorrhage mouse model. J Anesth 2020;34:36-46.
17. Ando T, Uzawa K, Yoshikawa T, Mitsuda S, Akimoto Y, Yorozu T, et al. The effect of tetrastarch on the endothelial glycocalyx layer in early hemorrhagic shock using fluorescence intravital microscopy: a mouse model. J Anesth 2023;37:104-18.
18. Azumaguchi R, Tokinaga Y, Kazuma S, Kimizuka M, Hamada K, Sato T, et al. Validation of the relationship between coagulopathy and localization of hydroxyethyl starch on the vascular endothelium in a rat hemodilution model. Sci Rep 2021;11:10694.
19. Zhao H, Zhu Y, Zhang J, Wu Y, Xiang X, Zhang Z, et al. The Beneficial Effect of HES on Vascular Permeability and Its Relationship With Endothelial Glycocalyx and Intercellular Junction After Hemorrhagic Shock. Front Pharmacol 2020;11:597.

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