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Assessment of the effect of unfractionated heparin administered either by intravenous infusion *vs.* subcutaneous injection on heparin-binding protein, and plasminogen activator inhibitor-1 in critically ill septic patients: a randomized controlled trial

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Abstract. – OBJECTIVE: The study compared the impact of unfractionated heparin (UFH) administered via two routes (infusion and subcutaneous injection) on heparin-binding protein (HBP) and plasminogen activator inhibitor-1 (PAI-1) levels in critically ill sepsis patients.

PATIENTS AND METHODS: Forty critically ill sepsis patients were randomly assigned to receive either a low-dose intravenous infusion of UFH (500 units/hour) or subcutaneous UFH (5,000 units/8 hours) for seven days. HBP and PAI-1 were measured at baseline and on days one, two, and seven.

RESULTS: Intravenous administration of UFH showed a significant reduction in percentage change of HBP compared to subcutaneous administration on days one [(-35% *vs.* -13%, $p = 0.03$*) (*indicates a significant result *$p < 0.05$, relative to the subcutaneous group)] and seven (-62% *vs.* -39%, $p = 0.02$*). Also, the percentage change of PAI-1 was significantly reduced in the infusion group compared to the subcutaneous group on days one (-28% *vs.* -3%, $p = 0.008$*), two (-42% *vs.* -3%, $p = 0.001$*), and seven (-62% *vs.* 27%, $p = 0.001$*), respectively. Furthermore, a significant improvement in the 14-day survival was observed in the infusion group compared to the subcutaneous group ($p = 0.008$*).

CONCLUSIONS: Intravenous infusion was the route of choice for UFH administration in critically ill septic patients, with a promising effect on HBP, PAI-1, and survival.

ClinicalTrials.gov: NCT0431379.

Key Words:
Heparin infusion, Subcutaneous heparin, Sepsis, Heparin binding protein, Plasminogen activator inhibitor 1, Critically ill patients.

Introduction

Sepsis was redefined by the third international consensus as a life-threatening organ dysfunction caused by a dysregulated host response to infection, which leads to cellular, metabolic, and circulatory abnormalities and raises the risk of morbidity and mortality, especially in critically ill patients[1,2]. Patient defense mechanisms during sepsis depend mostly on inflammation and coagulation pathways[3,4].

All critically ill patients with sepsis have coagulation abnormalities ranging from thrombocytopenia, prolongation of clotting time, and ending with disseminated intravascular coagulopathy (DIC)[5].

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Currently, organ dysfunction combined with sepsis is promoted by two main drivers, which are coagulation and inflammation[6]. Sepsis coagulopathy has commonly occurred and can progress to DIC, which is an independent predictor of mortality[7,8].

Coagulation and fibrinolysis are typically balanced, but during sepsis, pathogen invasion is perceived as a threat. This triggers a pathophysiological response that decreases tissue perfusion to block the pathogen's transmission route, leading to the formation of pathological thrombi[9].

Pathogen-associated molecular patterns (PAMPs) expressed by pathogens and death-associate molecular patterns (DAMPs) expressed by damaged host cells are both recognized by toll-like receptors (TLRs), which activate the release of sepsis proinflammatory mediators[10]. The activation of TLRs is considered the initial step in the onset of sepsis and the triggering of the proinflammatory response[10,11]. TLR activation also initiates tissue factors of extrinsic and intrinsic coagulation pathways and contributes to thrombus formation by increasing thrombin generation[8,10].

Plasminogen activator inhibitor-1 (PAI-1) is a human protein encoded by SERPINE1 gene[12]. Activation of PAI-1 and elevation of its concentration in blood increases the risk of thrombus formation[13]. The elevated PAI-1 levels contributed to the pathogenesis and inflammatory response of sepsis[14]. PAL-1 is regarded as an acute-phase protein during acute inflammation, and its primary role is fibrinolysis inhibition by inhibiting urokinase and tissue-type plasminogen activators[3,15]. In sepsis, fibrinolysis suppression occurs due to the overproduction of PAI-1, which facilitates the formation of thrombus in the microcirculation, which is associated with increased morbidity and mortality[16-18]. The higher concentration of PAL-1 is positively correlated with the immune response in sepsis[13].

Heparin-binding protein (HBP) is a promising biomarker in sepsis prognosis, which has higher sensitivity and specificity in sepsis prediction than procalcitonin and C-reactive protein[19,20]. HBP is also called Azurocidin or Cationic antibiotic protein 37 (CAP37)[21]. HBP is a stored protein in the neutrophile's secretory vesicles and azurophilic granules[22,23]. HBP is a potent inducer of inflammation as it promotes endothelial wall permeability[24]. Identification of sepsis biomarkers in early stages after intensive care unit (ICU) admission improves the disease prognosis[25].

HBP is released from neutrophils as a response to infection and is considered one of the earliest mediators of inflammation by modulating the inflammatory response of many cell types and inducing vascular leakage as in Acute respiratory distress syndrome (ARDS)[26,27]. Moreover, elevated HBP levels can predict renal and pulmonary dysfunction[21,23]. The level of HBP has a significant rise 24 hours before sepsis diagnosis, so it is an early marker of sepsis prediction and organ dysfunction prognosis[28-30].

Unfractionated heparin (UFH) is a heterogeneous mixture of negatively charged glycosaminoglycans with a molecular weight ranging from 3,000 to 30,000 Daltons[31]. UFH complexes with antithrombin, converting it to a more rapid thrombin inactivator. It also inactivates thrombin, factor Xa, platelet aggregation, and other clotting factors[32]. UFH not only has anticoagulant properties but also has an immunomodulatory effect, improving clinical outcomes in patients with sepsis-induced immune thrombosis[3,32-34]. Since UFH is negatively charged, it can bind to HBP and block its involvement in sepsis-induced organ failure[22,23]. However, the usage of UFH in sepsis treatment remains controversial. A recent meta-analysis[35] evaluated that UFH may have a role in the reduction of 28-day mortality and improvement of the clinical efficacy in sepsis patients without bleeding adverse effects.

The drug's pharmacokinetics behavior is greatly affected by the pathophysiological changes that occur in ICU patients with sepsis[36]. UFH is administered either by intravenous or subcutaneous routes. Subcutaneous heparin is frequently used for deep vein thrombosis (DVT) prophylaxis[37]. However, critically ill patients have special pharmacokinetics that make the intravenous route the most preferable[34]. Drug absorption in critically ill may be impaired by subcutaneous administration due to many contributing factors such as edema, peripheral vasoconstriction, and vasopressor administration[34,38,39].

We hypothesized that low-dose heparin infusion for thromboprophylaxis in critically ill patients with sepsis might have greater anti-inflammatory effects and reduce multi-organ damage more effectively than conventional subcutaneous heparin. Additionally, we aimed to compare the percent changes in HBP and PAI-1 in response to unfractionated heparin administered either subcutaneously or via infusion in the two study groups. The changes in HBP and PAI-1c are hypothesized to measure the treatment response in both study groups[40,41].

Patients and Methods

Study Design and Study Population

A randomized single-blind controlled clinical trial was conducted in a 33-bed ICU of a tertiary hospital between June 2020 and July 2022. All the study participants or their legal representatives signed a written informed consent before enrollment. This randomized controlled clinical trial was approved by the Research Ethics Committee of the Faculty of Pharmacy at Damanhour University (IRB, 320PP22). The study was registered on ClinicalTrials.gov under the identifier NCT0431379 (available at: https://clinicaltrials.gov/study/NCT04313790).

Critically ill patients aged 18-65 years diagnosed with sepsis/septic shock or developed sepsis/septic shock during their ICU length of stay were enrolled (inclusion criteria). Ninety-six patients with a new onset of sepsis were screened for eligibility. Sepsis was defined according to the Third International Consensus Definition for Sepsis and Septic Shock (Sepsis-3)[1]. 40 patients were eligible to participate in the study, while 56 others were excluded due to one or more exclusion criteria. Exclusion criteria were thrombocytopenia, intracerebral hemorrhage at the time of sepsis, bleeding tendency [International Normalized Ratio (INR), ≥ 1.5 or platelets (PLT) < 50 × 10^9/L], a medical condition requiring therapeutic anticoagulation, age < 18 years, and previous history of heparin-induced thrombocytopenia (HIT).

The eligible 40 patients were randomly assigned according to the administration route of heparin with a 1:1 allocation ratio, using a computer sheet available from randomizer.org. 20 patients were given subcutaneous heparin 5,000 unit/8 hours (the subcutaneous group), and the other 20 patients were given low-dose intravenous heparin continuous infusion 500 units/hour (the infusion group). The duration of heparin treatment was seven days or until patient death or discharge. Both groups were also given supportive measures and standard care management based on clinical conditions and the latest treatment guidelines[37,42].

Demographics and Baseline Assessment

At baseline, patients' variables, including sex, age, pre-existing medical conditions, cause of admission, source of infection, culture results, ventilation status, Acute Physiological and Chronic Health Evaluation (APACHE)-II score, Sequential Organ Failure Assessment (SOFA) score, and Glasco Coma Score (GCS) were recorded.

Outcome Measures, Study Endpoints, and Follow-Up

Patients with sepsis who met the criteria were monitored from the day they were admitted until 7 days later. The primary outcome was measuring the difference in the dynamic changes of HBP and PAI-1 on days one, two, and seven in the heparin subcutaneous group and the heparin infusion group during the study period.

All patients were closely monitored and followed up during the seven-day study period. Various parameters and measurements were assessed, including coagulation panel [INR, activated partial thromboplastin time (aPTT)], systemic inflammatory response syndrome (SIRS) criteria [temperature, respiratory rate (RR), total leukocyte count (TLC), heart rate (HR), C-reactive protein (CRP), renal function (creatinine, urea), liver function [aspartate transaminase (AST), alanine transaminase (ALT), albumin] and nor-epinephrine doses. These assessments were performed at baseline and on days one, two, and seven from the onset of sepsis.

Assessment for HPB and PAI-1 Using Enzyme-Linked Immunosorbent Assay (ELISA)

Plasma samples of HBP and PAI-1 were centrifuged at 5,000 x g for 10 minutes and stored at -80°C until assay within 60 minutes of sample withdrawal. Both HBP and PAI-1 antigens were measured by enzyme-linked Immunosorbent Assay (ELISA) (Innova Biotech Co., LTD, Beijing, China) according to the manufacturer's instructions.

The study evaluated dynamic changes in plasma levels of HBP and PAI-1, which were assessed on days 1, 2, and 7 of sepsis onset. The changes were defined as the difference between a certain time point and the baseline divided by the baseline and presented as a percentage. Mortality rates were calculated as all-cause mortality from the beginning of the enrollment.

Monitoring Adverse Effects

All participants underwent regular monitoring for potential adverse effects such as thrombocytopenia, bleeding, and deep venous thromboembolism. Heparin treatment was paused in the event of bleeding, prolonged aPTT (aPTT > 45 seconds) and resumed after normalization of coagulation tests[43].

Statistical Analysis

After data collection, results were entered into a Microsoft 365 Excel sheet. The study data were evaluated using (SPSS software for Windows, version 26; (IBM Corp., Armonk, NY, USA). The data were tested for normality using the Kolmogorov-Smirnov test. The normally distributed variables were represented as mean ± SD. Non-normally distributed variables were represented as median and interquartile range, whereas categorical variables were represented as numbers and percentages. The student t-test was used to compare the means of the normally distributed variables between the two groups. Nonparametric methods, including Mann-Whitney and Friedman statistical tests, were used for non-normally distributed variables. The Chi-square test was used to compare categorical variables between the study groups. A log-rank test was used to compare the survival curves between the two groups after the Kaplan-Meier analysis. 14-day survival prediction was calculated. A p-value < 0.05 was considered statistically significant.

Sample Size Calculation

The sample size was calculated using G*power software, version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Setting a power of 80%, an alpha error of 0.05, 1 as allocation ratio, and an effect size of 1.64 for two independent means of PAI-1 (two-tailed t-test)[44].

Results

Study Population

During the study period, a total of 96 critically ill patients with a new onset of sepsis or septic shock were screened for eligibility. 56 patients were excluded (seven-teen had thrombocytopenia, six had intracerebral hemorrhage at the time of sepsis, fourteen due to bleeding tendency INR ≥ 1.5 or PLT $< 50 \times 10^9$/L, twelve had a medical condition requiring therapeutic anticoagulation, two declined to participate, three refused the informed consent, and two due to age < 18 years). The flow chart for patient recruiting and follow-up, and the final analysis included 40 patients who were followed up for seven days, is represented in Figure 1.

Patients' Demographic Data and Clinical Characteristics

At baseline, there were no statistically significant differences between the infusion group and the subcutaneous group regarding sex, age, preex-

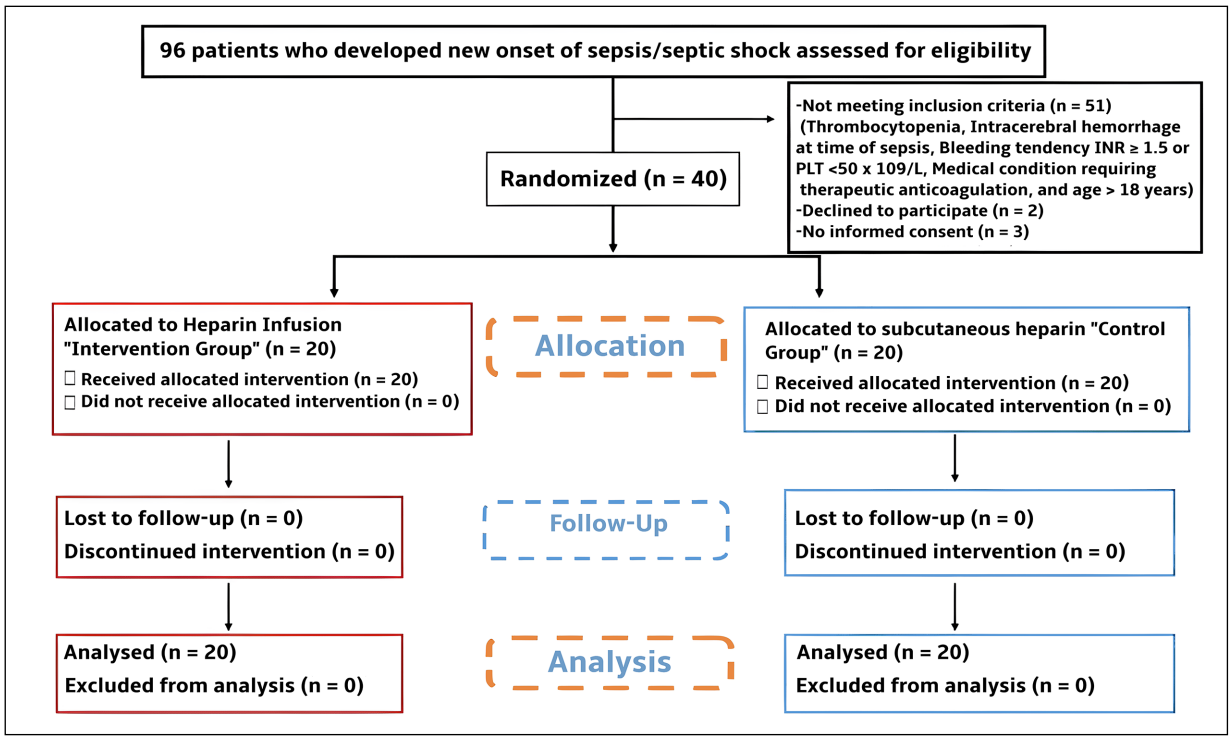

Figure 1. Flow chart of the enrolled patients.

isting conditions, cause of admission, SOFA score, APACHE II score, GCS score, PLTs count, arterial blood gas (ABG), prothrombin time (PT), INR, aPTT, liver function, renal function, medications used, vital signs and source of infection, Table I.

Outcome Measures

The change in sepsis biomarkers (HBP, PAI-1)

The intravenous administration of UFH showed a significantly higher median HBP percentage change on days one (-35% *vs.* -13%, p = 0.03) and seven (-62% *vs.* -39%, p = 0.02) compared to the subcutaneous administration. Also, a significantly higher median PAI-1 percentage change occurred in the infusion group compared to the subcutaneous groups on days one (-28% *vs.* -3%, p = 0.008), two (-42% *vs.* -3%, p = 0.001), and seven (-62% *vs.* 27%, p = 0.001), respectively (Table II and Figure 2).

Acute phase reactants (CRP, Albumin)

The infusion group showed a significant decrease in the median level of CRP on day two [108 (108) *vs.* 127 (367), p = 0.04] and on day seven [55 (56) *vs.* 110 (112), p = 0.005] compared

Table I. Baseline characteristics of all the enrolled patients in the two study groups.

Variable	Infusion group (N = 20)	Subcutaneous group (N = 20)	*p*-value
Demographic characteristics			
Age, mean ± SD, Years	44.4 ± 10.3	40 ± 12	0.22
Sex, n (%)			
Male, n (%)	8 (40)	9 (46)	0.749
Female, n (%)	12 (60)	11 (54)	
Preexisting conditions, n (%)			
Cancer	3 (15)	1 (5)	0.25
Ischemic stroke	4 (20)	2 (10)	0.66
Hepatic	2 (10)	1 (5)	1.00
Diabetes	4 (20)	3 (15)	1.00
COPD	0 (0)	1 (5)	1.00
Hypertension	6 (30)	8 (40)	0.74
Hemodialysis	5 (15)	2 (10)	1.00
CKD	2 (10)	1 (5)	1.00
IHD	2 (10)	2 (10)	1.00
Cause of admission, n (%)			
Septic shock	12 (60)	11 (55)	1.00
COPD exacerbation	2(10)	1 (5)	0.23
Trauma	2 (10)	2 (10)	1.00
Ischemic stroke	1 (5)	2 (10)	0.23
DKA	2 (10)	3 (15)	0.50
Hemorrhagic stroke	2 (10)	0 (00)	0.30
Source of infection, n (%)			
Lung	11 (55)	11 (55)	1.00
Abdomen	3(15)	6 (30)	0.45
Urinary	4 (20)	3 (15)	1.00
Blood culture organisms, n (%)			
Klebsiella sp.	1 (5)	0 (0)	1
MRSA	2 (10)	0 (0)	0.49
Coagulase Negative Staph.	1 (5)	0 (0)	1.0
Urine culture organisms, n (%)			
Acinetobacter sp.	0 (0)	1 (5)	1.00
E. coli	1 (5)	1 (5)	1.00
Enterococcus sp.	1 (5)	0 (0)	1.00
Klebsiella sp.	1 (5)	2 (10)	1.00
Candida	4 (20)	0 (0)	0.12

Continued

Table I *(Continued)*. Baseline characteristics of all the enrolled patients in the two study groups.

Variable	Infusion group (N = 20)	Subcutaneous group (N = 20)	*p*-value
Scores			
SOFA, median (IQR)	8.5 (5)	6 (7)	0.38
APATCHI, mean (SD)	17.2 ± 4.4	20.2 ± 0.5	0.170
GCS, median (IQR)	12.5 (6)	13.5 (7)	0.97
SIRS Criteria, median (IQR)			
Temperature, °C	37 (1.3)	37 (0.9)	0.59
Heart rate, bpm	90 (25)	94 (16)	0.73
Leucocyte count, $\times 10^9$/L	13 (8)	13.5 (9)	0.9
CRP, mg/L, median (IQR)	174 (83)	135 (376)	0.5
Complete blood picture, median (IQR)			
Hemoglobin, g/dL	10 (3.5)	9 (1.8)	0.01
Platelet count $\times 10^9$/L	220 (111)	152 (152)	0.07
Respiratory rate, cycles/minute, median (IQR)	29 (60)	20 (6)	0.06
MAP, mmHg median (IQR)	80 (52)	83 (20)	0.49
Arterial blood gases, median (IQR)			
PH	7.4 (0.04)	7.4 (0.1)	0.2
HCO_3, mean ± SD	20.3 ± 6	19.6 ± 3.3	0.83
pCO_2	31 (8)	35 (12)	0.024
Oxygen saturation	95 (12)	96 (20)	0.45
Coagulation panel, mean ± SD			
Prothrombin time, second	14.4 ± 1.2	16 ± 1.2	0.74
INR	1.1 ± 0.1	1.2 ± 0.1	0.06
aPTT, second	32.5 ± 5.3	41.7 ± 7.6	0.08
Liver function, median (IQR)			
AST, U/L	28 (18)	19 (22)	0.50
ALT, U/T	36 (60)	15 (13)	0.32
S. albumin gm/dL	2.5 (0.3)	2.1 (0.3)	0.75
Renal function, median (IQR)			
Urea, mg/Dl	72 (84)	56 (13)	0.89
SCr, mg/Dl	1.3 (4.9)	1.4 (1.1)	0.65
Medications, n (%)			
Hydrocortisone	7 (37)	9 (45)	0.75
Norepinephrine	12 (63)	11 (55)	0.75
Levofloxacin	13 (68)	10 (50)	0.33
Linezolid	5 (26)	4 (20)	0.71
Ceftazidime	3 (16)	4 (20)	1.000
Piperacillin/tazobactam	1 (5)	1 (5)	1.000
Meropenem	13 (68)	14 (70)	1.000
Acetylsalicylic acid	6 (32)	5 (25)	0.73
Atorvastatin	6 (320	5 (25)	0.73
Acetaminophen	14 (74)	16 (80)	0.71
Omeprazole	18 (95)	20 (100)	0.49

COPD = chronic obstructive pulmonary disease; CKD = chronic kidney disease; IHD = ischemic heart disease; DKA = diabetic ketoacidosis; MRSA = methicillin-resistant *staphylococcus aureus*; SOFA = sequential organ failure assessment score; GCS = Glasgow coma scale; SIRS = systemic inflammatory response syndrome; CRP = C-reactive protein; MAP = mean arterial pressure; INR = international normalized ratio; aPTT = activated partial thromboplastin time; AST = aspartate transaminase; ALT = alanine transaminase; SCr = serum creatinine; SD = standard deviation; IQR = interquartile range.

to the subcutaneous group. Regarding the mean albumin level, there was no significant difference between the two study groups at baseline [2.5 (±0.3) *vs.* 2 (± 0.3), $p = 0.75$] (Table I); however, on days one [2.7 (± 0.35) *vs.* 2.2 (± 0.2)], two [2.7 (± 0.36) *vs.* 2.1 (± 0.2)], and seven [2.7 (± 0.36) *vs.* 2.1 (± 0.5)] a statistically significant difference occurred ($p < 0.0001$, $p < 0.0001$, and $p = 0.001$) respectively, (Table III and Figure 3).

Mean arterial blood pressure (MAP) and the change in norepinephrine (NE) dose

At baseline, no statistically significant difference in MAP occurred between the two study

Table II. Comparisons of the dynamic change of heparin-binding protein (HBP), and plasminogen activator inhibitor-1 (PAI-1) between the infusion group and the subcutaneous group from baseline on days one, two, and seven of the study periods.

	Day one	Day two	Day seven
HBP change (%) median (IQR)			
Infusion	-35 (-57, -17)	-35 (- 67, -32)	- 62 (-71, -54)
Subcutaneous	-13 (- 37, 0)	- 25 (-53, -8)	-39 (-65, -21)
p-value	0.03*	0.1	0.02*
PAI-1 change (%) median (IQR)			
Infusion	-28 (-50, -7)	-43 (-78, -28)	-62 (-79, -49)
Subcutaneous	-3 (-28, 0)	-3 (-32, 0)	-27 (-53, -7)
p-value	0.008**	0.001**	0.001**

IQR = interquartile range. *$p < 0.05$ relative to subcutaneous group, **$p < 0.01$ relative to subcutaneous group.

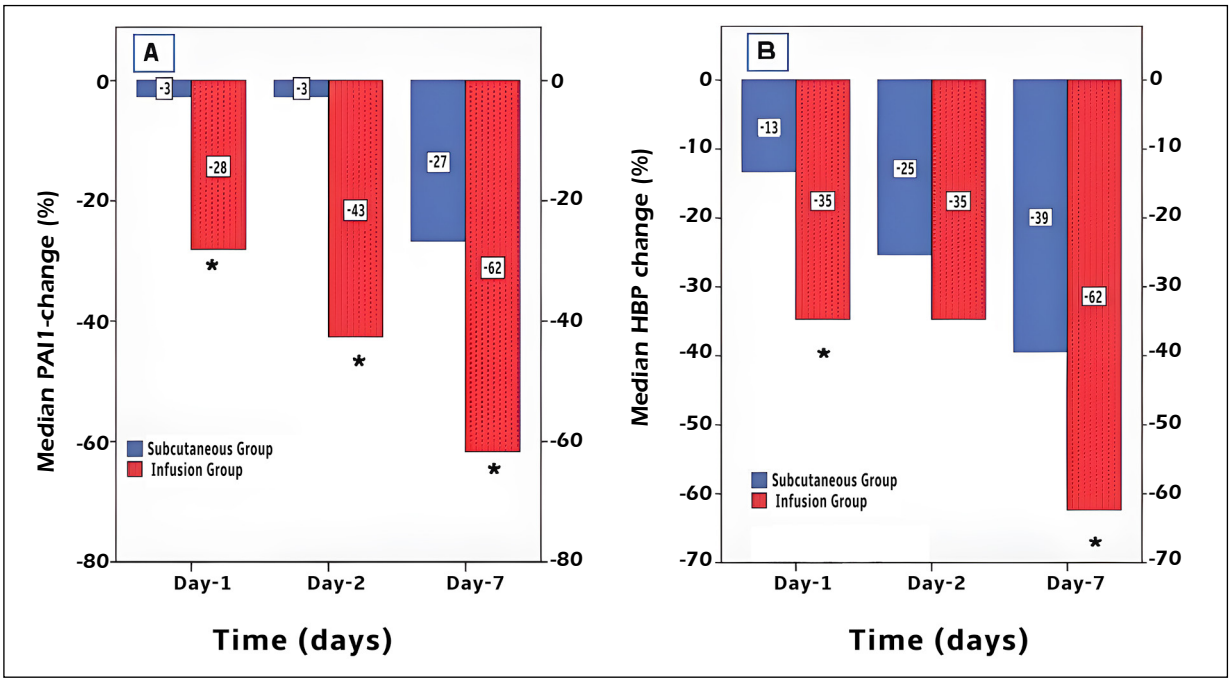

Figure 2. **A**, Dynamic change of PAI-1 on days one, two, and seven in the two study groups. *$p < 0.05$, relative to the subcutaneous group. **B**, Dynamic change of HBP on days one, two, and seven in the two study groups. *$p < 0.05$ relative to the subcutaneous group.

groups, but on days two and seven, there was an improvement in both groups. A significant increase in MAP occurred in the infusion group compared to the subcutaneous group on days two and seven (84 ± 7 *vs.* 74 ± 20, $p = 0.044$) and (83 ± 7 *vs.*76 ± 9, $p = 0.019$) respectively, (Table III). The infusion group showed a significant reduction in the norepinephrine dose represented as percent change in the dose compared to the subcutaneous group on days one [-30 (24) *vs.* -9.6 (28), $p = 0.012$], two [-60 (25) *vs.* -10 (88), $p = 0.03$], and seven [-80 (24) *vs.* -34 (65), $p = 0.013$], (Table III).

Median platelet count

There was no statistically significant difference in the median platelet count between the two groups at baseline ($p = 0.07$). While the infusion group showed a significant increase in the median platelet count compared to its corresponding

Table III. The measured study outcomes of all the enrolled patients during the study periods.

Outcomes	Infusion group (N = 20)	Subcutaneous group (N = 20)	*p*-value
CRP, median (IQR), mg/L			
CRP-D_1, mg/L	100 (35)	122.5 (107)	0.09
CRP-D_2, mg)/L	108 (108)	127 (367)	0.04*
CRP-D_7, mg/L	55 (56)	110 (112)	0.005**
Albumin, mean ± SD			
Albumin-D_1 gm/dL	2.7 ± 0.35	2.2 ± 0.2	< 0.0001***
Albumin-D_2 gm/dL	2.7 ± 0.36	2.1 ± 0.2	< 0.0001***
Albumin-D_7 gm/dL	2.7 ± 0.36	2.1 ± 0.5	0.001**
Platelet count, median (IQR), $\times 10^9$/Lc			
Platelet count-D_1 $\times 10^9$/L	204 (97)	108 (164)	0.009**
Platelet count-D_2 $\times 10^9$/L	220 (140)	130 (169)	0.01*
Platelet count-D_7 $\times 10^9$/L	220 (140)	140 (177)	0.005**
NE Dose Change (%), median (IQR)			
NE Dose change-D_1 %	-30 (24)	-9.6 (28)	0.012*
NE Dose change-D_2 %	-60 (25)	-10 (88)	0.03*
NE Dose change-D_7 %	-80 (24)	-34 (65)	0.013*
MAP, mean ± SD, mmHg			
MAP-D_1 mmHg	82 ± 9	73 ± 23	0.14
MAP-D_2 mmHg	84 ± 7	74 ± 20	0.044*
MAP-D_7 mmHg	83 ± 7	76 ± 9	0.019*
14-day survival, n (%)	19 (95)	11 (55)	0.008**
Mortality, n (%)	11 (55)	14 (70)	0.51
Ventilator days, median (IQR)	4 (7)	7 (12)	0.35
Vasopressor days, median (IQR)	3 (7)	4 (5)	0.44
DVT occurrence, n (%)	0 (0)	2 (10)	0.48

CRP = C-reactive protein; NE = norepinephrine; DVT = deep vein thrombosis; MAP = mean arterial pressure; D_1 = day one; D_2 = day two; D_7 = day seven; IQR = inter quartile range. *$p < 0.05$ relative to subcutaneous group, **$p < 0.01$ relative to subcutaneous group, ***$p < 0.001$.

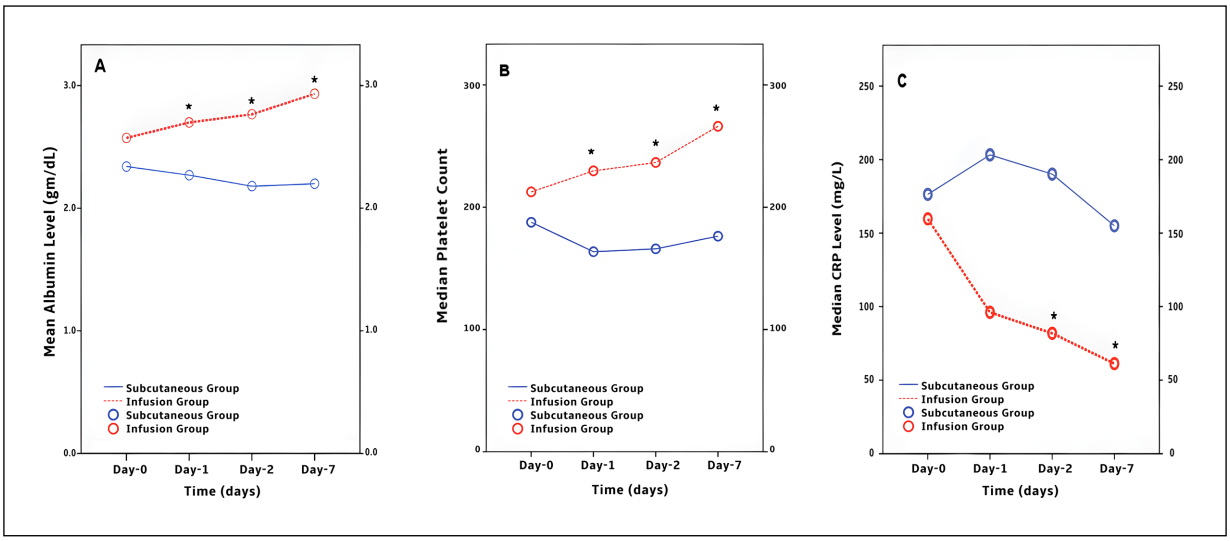

Figure 3. **A**, The mean albumin level at baseline, day one, day two, and day seven in the two study groups. **B**, The median platelet count level at baseline, day one, day two, and day seven in the two study groups. **C**, The median C-reactive protein level at baseline, day one, day two, and day seven in the two study groups. *$p < 0.05$ relative to the subcutaneous group.

subcutaneous group on days one [204 (97) *vs.* 108 (164), $p = 0.009$], two [220 (140) *vs.* 130 (169), $p = 0.01$], and seven [220 (140) *vs.* 140 (177), $p = 0.005$] respectively, (Table III).

The 14-days survival

The 14-day survival was significantly higher in the infusion group (95%) than in the subcutaneous group (55%) ($p = 0.008$) (Figure 4).

Adverse Effects Monitoring

No major bleeding events were observed in either of the two study groups. Two patients in the subcutaneous group incurred DVT compared to none in the infusion group but without a statistically significant difference [2 (10%) *vs.* (0%), $p = 0.48$] (Table III).

Discussion

Unfractionated heparin (UFH) and low molecular heparin are the most evidence-based pharmacological medications that are used for DVT prophylaxis in critically ill patients with sepsis[33]. The presence of infection increases the risk of venous thromboembolism (VTE) due to the release of inflammatory mediators, platelet activation, and activation of the coagulation cascade[6,7,35]. Critically ill patients have a higher risk of developing DVT due to ventilation, limited mobility, and vasopressors[17,45]. The risk of developing VTE in critically ill patients is double the risk compared to patients in the general medicine ward[46]. UFH is administered either by subcutaneous or intravenous route, and it is one of the oldest and most effective anticoagulants[33,47].

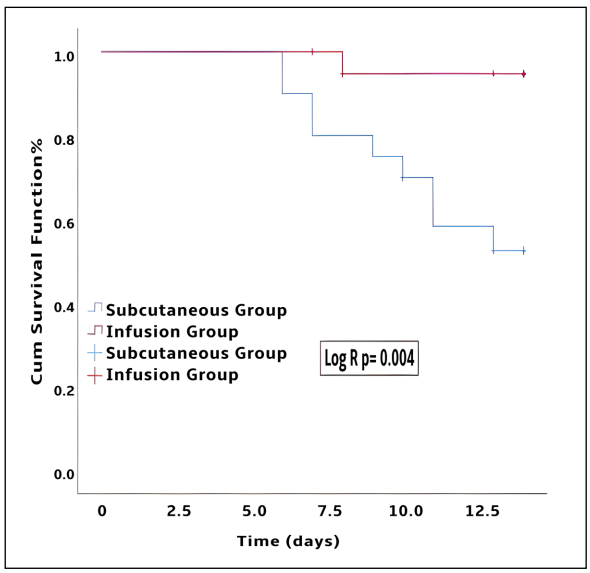

Figure 4. Kaplan-Meier survival curves: 14-day survival data on (log-rank $p = 0.004$).

Also, critically ill patients have special pharmacokinetics that impair subcutaneous absorption due to peripheral edema, vasoconstriction of skin and blood vessels caused by vasopressors, impaired renal clearance and elimination, and impaired fluid distribution[34,38,48]. All these contributing factors make the intravenous route of administration superior to the conventional subcutaneous route in critically ill patients[43,49].

PAL-1 plays a key role in fibrinolysis regulation as its high levels correlate with sepsis severity and mortality[50]. PAL-1 inhibits the activity of tissue-type plasminogen activator (TPA), an enzyme required for plasmin formation[16]. Inhibition of thrombin leads to microthrombus formation, tissue hypoperfusion, DIC, and organ failure in septic patients[16]. A meta-analysis[18] of 4,467 patients with sepsis/severe sepsis was conducted to assess the role of PAI-1 as a biomarker for predicting sepsis severity and mortality. The analysis showed that higher levels of PAI-1 can be used to compare the severity of sepsis among patients, as it significantly correlates with severe sepsis. Additionally, PAI-1 also showed a significant correlation with all causes of mortality. PAL-1 also acts as a proinflammatory mediator, resulting in the upregulation of the inflammatory state during sepsis[51].

UFH has an anticoagulant effect by binding to anti-thrombin (AT), which inhibits thrombin generation[52]. Heparin activates the arginine reactive site on the AT molecule, which blocks the serine active site on thrombin, which inhibits thrombin up to 1,000 times[32]. Thrombin has a role in coagulation cascade and inflammation by activating the production of inflammatory mediators (PAI-1)[4]. Heparin has an indirect inhibitory effect on PAI-1 by activating the heparin-antithrombin complex, which suppresses the endotoxin responsible for PAI-1 generation[53]. In our study a significant and rapid dynamic change of PAI-1 levels in the infusion group on days one, two, and seven compared to the subcutaneous group was demonstrated. This finding was consistent with a study conducted by Nouri et al[44], which showed a significantly lower level of PAI-1 in the heparin infusion group when compared to the subcutaneous heparin group on day seven of the study.

HBP is considered as a new promising sepsis prognostic biomarker, displaying multifunctional roles in sepsis[19]. It induced inflammatory response and organ dysfunction in septic patient[26,40]. HBP has a valuable role in the early diagnosis of sepsis, as its plasma levels have been elevated in sepsis patients hours before they experience hypotension or organ dysfunction[54]. Early diagnosis of sepsis is markedly reducing mortality, therefore sepsis biomarkers of early detection have a great role in reducing mortality[28,55]. Neumann et al[28] expressed the rapid release of HBP from neutrophil secretory granules after 15 minutes of incubation of healthy volunteers' blood samples with Gram-positive anaerobic cocci. A prospective multi-center study[24] of 355 children diagnosed with sepsis\severe sepsis showed a significantly higher median level of HBP in severe sepsis (170.5 ng/ml) than in sepsis (74.1 ng/ml). HBP induces vascular leakage and circulatory failure, which develops into septic shock[20,21,24,27]. Moreover, HBP was shown to have a significant role in the pathophysiology of severe bacterial infections, making it a possible diagnostic and target marker for sepsis treatment[56]. HBP presents in poly morphonuclear leukocyte granules[55]. In sepsis, the invasion of bacteria, toxins, and coagulation complexes activate the neutrophils to release HBP, which induces inflammation and tissue injury by increasing vascular permeability[24,57]. HBP increased oxidative stress by promoting macrophage M1 polarization and activated the NF-κB pathway in rats with sepsis[26]. HBP has a direct interaction with endothelium by binding to proteoglycan via glycosaminoglycan (GAGs), which leads to activation of protein kinase C (PKC)[26]. Activation of GAGs and PKC leads to gap formation between the endothelia, resulting in neutrophil extravasation and vascular leakage[22].

Heparin inhibits the inflammation induced by HBP[58]. The negative charge of heparin glycosaminoglycan binds to HBP and blocks HBP GAGs binding sites, which prevents the activation of endothelial surface GAGs by HBP[23]. This study demonstrated that UFH gradually reduced the HBP levels in both groups. However, a significant percent change in HPB was shown in the intravenous heparin administrative group compared to the subcutaneous heparin group on day one and day seven. This significant decrease in HBP in the infusion group was combined with a significant improvement in the circulatory system and mean arterial blood pressure (MAP) on day two and day seven. The improvement in the MAP in the infusion group significantly decreased the vasopressor requirement with a significant change in the norepinephrine dose at days one, two, and seven. This study is considered the first clinical study handling the effect of UFH on promising HBP markers. Recently, HBP and its dynamic change were identified as effective prognostic markers for patients with sepsis. It can be utilized to predict 28-day mortality and evaluate the efficacy of treatment in sepsis patients[59].

Many pre-clinical and clinical trials[4,32,35,60] have demonstrated the anti-inflammatory effect of UFH. One of the important mechanisms is the ability of UFH to impair the function and production of many inflammatory mediators (cytokines, interleukins, tissue necrotizing factors) and also preserve the endothelial cells and organ function[35]. Concurrently, UFH exerted its anti-inflammatory effect by enhancing the function of the endothelial barrier and angiopoietin (Agn) Tie2 axis. Also, the UFH therapeutic dose inhibits the inflammation by protecting the shedding of glycocalyx[44]. Moreover, the anti-inflammatory effect and the improvement of microcirculation may be attributed to the inhibitory effect of UFH on extracellular histones[61]. UFH was demonstrated to inhibit extracellular histones and diminish cytokine-induced inflammation in an animal model of sepsis[62]. Our study demonstrated a significant anti-inflammatory effect of UHF in addition to the superiority of heparin infusion over subcutaneous heparin, as IV UFH showed a significant decrease in CRP median levels on day two and day seven. These findings were supported by a prospective open-label pilot clinical trial[63] involving patients with ST-segment elevated myocardial infarction where the IV heparin group showed a significant reduction in CRP levels at 12, 24, and 48 hours after the intervention. Moreover, randomized controlled trials[64], including patients with atrial fibrillation, revealed a significant reduction of CRP in the heparin-treated group at day 2 and day 4. There is a positive correlation between the increase in the CRP level and the severity of sepsis and prognosis[15].

Sepsis-induced hypoalbuminemia occurs due to decreased albumin synthesis, increased utilization, and increased transcapillary leakage caused by heightened vascular permeability[65].

Hypoalbuminemia is considered a prognostic factor for mortality in sepsis[15,65]. In our study, the mean albumin level was significantly higher in the infusion groups on days one, two, and seven

than in the subcutaneous group. The significant increase in albumin levels may highlight the evident impact of IV heparin on albumin levels, which may serve as a spotlight for future research.

Early administration of unfractionated heparin (UFH) in septic patients with coagulopathy was linked to a reduced mortality rate. This was determined in a meta-analysis[33] of 6,646 adult septic patients. According to our study, 14-day survival significantly improved in the intravenous infusion of UFH compared to the subcutaneous group. This effect of UFH on mortality aligns with the results of a meta-analysis[35], which indicated that heparin reduced 28-day mortality in patients with severe sepsis. Additionally, a retrospective cohort study[66] has shown an association between early administration of heparin and a decrease in in-hospital mortality in patients with sepsis. These studies collectively highlight the potential benefits of heparin in improving survival outcomes in septic patients, which support our findings.

DVT prophylaxis was observed in the heparin infusion group compared to the corresponding subcutaneous group. Specifically, no patients in the infusion group developed DVT, while two patients in the subcutaneous group experienced DVT. This result was supported by Nouri et al[67], which demonstrated that low-dose heparin infusion (500 units/hour) resulted in changing the coagulation parameters using ROTEM analysis greater than the subcutaneous dose and decreased the thrombosis risk in septic patients. Platelets have a key role in infectious diseases and inflammation pathophysiology[68]. Sepsis-induced thrombocytopenia occurred because of decreased platelet production, platelet sequestration, and consumption[3]. Thrombocytopenia below 50,000/µL is a strong negative prognostic marker in sepsis[3,68]. Thrombocytopenia occurred to a greater extent in the subcutaneous group than in the infusion group, as we found that the infusion group had a significantly higher median platelet count on days one, two, and seven. None of the patients from the two study groups had major bleeding during the study period. This result was also supported by another study[67] that showed heparin treatment does not increase bleeding risk in patients with sepsis.

Limitations

Our study has some limitations. One limitation is the small sample size. Further larger studies are recommended to support our findings. Another constraint is the lack of financial resources, which hinders further investigation of promising serum histone biomarkers with anti-inflammatory properties. The study mainly focused on the effect of UFH on HBP and PAI as new sepsis biomarkers and the selection of a more effective administration route.

Conclusions

To the best of our knowledge, this is the first randomized prospective study comparing two administration routes of UFH, the subcutaneous and intravenous routes (10,000-15,000 units/day), on the promising sepsis biomarkers (HBP and PAI-1) in critically ill patients with sepsis/septic shock. The anti-inflammatory effect of heparin infusion was more prominent compared to the subcutaneous heparin. Low-dose continuous heparin infusion improved the clinical efficacy on circulatory function and 14-day survival without increasing the bleeding risk. Concerning our findings, low-dose continuous heparin infusion may be a promising route of administration, especially in critically ill septic patients. The small sample size and financial issues were limitations in this study. We recommend more research with further larger studies to support our findings.

Conflict of Interest

The authors declare that they have no competing interests.

Trial Registration

The study registered date was March 18, 2020, on ClinicalTrials.gov under the identifier NCT0431379 (available at: https://clinicaltrials.gov/ct2/show/NCT04313790), under the following title "Assessment of the Anti-inflammatory Effect of Heparin Infusion Versus Subcutaneous Injection in Septic Patients".

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Authors' Contributions

Nouran A. Elsheikh developed the hypothesis, participated in the study design, contributed to sample collection, carried out the experiment, discussed the results, and produced the manuscript with input from all authors. Ayman

Eltayar supervised the project, confirmed the diagnosis, reviewed the radiological reports, contributed to sample collection, reviewed the final manuscript, and gave critical feedback. Ahmed Salaheddin supervised the project, encouraged Amira B. Kassem to investigate the markers, supervised the biochemical analysis, discussed the results, and approved the final manuscript. Noha A. El-Bassiouny and Amira B. Kassem supervised the project, developed the theoretical formalism, discussed the results, approved the final manuscript, contributed results interpretation, gave critical feedback, shaped the research, produced the manuscript with input from all authors and gave critical feedback. Ahmed M. Hamdan performed the statistical analysis, revised the results, and revised the final manuscript.

Ethics Approval

The research was approved by the Research Ethics Committee of the Faculty of Pharmacy, Damanhour University (IRB,320PP22). It adhered to ethical standards set by the Institutional and National Research Committee and the principles outlined in the Declaration of Helsinki.

Informed Consent

All participants included in the study provided their informed consent prior to their involvement in the study and allowed permission for their information to be published.

Data Availability

Study data is available upon reasonable request by the corresponding author.

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AI Disclosure

We hereby disclose that no artificial intelligence or assisted technologies were used in the production of this study, including the creation of any figures or illustrations.

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