Original Article

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Role of Nebulised Heparin as an Adjunct in Critically-ill COVID-19 Patients: A Randomised Controlled Trial

Anaesthesia Section

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ABSTRACT

Introduction: Coronavirus Disease 2019 (COVID-19) is associated with an increased risk of Venous Thromboembolism (VTE) and coagulopathy. The available studies have shown the anticoagulant and mucolytic effects of nebulised heparin in non COVID-19 patients. Hence it was decided to conducted to study the efficacy of nebulised heparin in patients suffering from COVID-19 pneumonia requiring mechanical ventilation.

Aim: To evaluate the safety and efficacy of nebulised heparin administered to patients with COVID-19.

Materials and Methods: A double-blinded randomised controlled trial was conducted at Basaveshwara Medical College and Hospital in Chitradurga, Karnataka, India among 100 patients with COVID-19 who required mechanical ventilation from February 2021 to May 2021. They were randomly assigned to two equal groups of 50 patients each. One group received nebulised heparin, and the other group received a placebo. The patients were compared for baseline characteristics, coagulation characteristics, and Oxygen Saturation (SpO_2). Data were analysed using Statistical Package for Social Sciences (SPSS) version 22.0, expressed as frequency and percentages, and displayed in tables and figures. The association between two variables was determined using the Chi-square test and paired t-test.

Results: The two groups did not differ significantly in terms of age, sex, respiratory failure, vasopressin use, and severity score. Respiratory failure was present in 54% of the heparin group and 38% of the placebo group. Vasopressin was used in 64% of the heparin group and 56% of the placebo group. The severity score was 4.44 in the heparin group and 4.42 in the placebo group. Activated Partial Thromboplastin Time (APTT) levels did not differ significantly between the groups. None of these parameters showed significant differences between the heparin and placebo groups. However, both groups showed a significant difference in Thrombin Antithrombin (TAT) complex levels from baseline to follow-up ($p<0.05$). D-Dimer levels decreased during follow-up, and SpO_2 improved significantly in the nebulised heparin group compared to the placebo group.

Conclusion: Nebulised heparin used as an adjunct in critically ill COVID-19 patients was shown to decrease TAT and D-Dimer levels. Nebulised heparin also significantly improved oxygenation levels. Importantly, heparin nebulisation was not associated with any adverse events, even when administered with systemic heparin.

Keywords: Anticoagulants, Coronavirus disease-2019, D-dimer, Placebo, Thrombin antithrombin complex, Viral pneumonia

INTRODUCTION

A new coronavirus called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) developed in the Chinese city of Wuhan in December 2019 [1] and was the source of an atypical viral pneumonia that resulted in cases of Acute Respiratory Distress Syndrome (ARDS) [2]. Some probable clinical manifestations of COVID-19 include asymptomatic or mild respiratory symptoms, pneumonia with respiratory failure, and mortality [3]. COVID-19 is associated with an increased risk of VTE and coagulopathy, especially in patients who are very sick. Endothelial injury caused by SARS-CoV-2 is now understood to be a major pathogenetic mechanism for the development of issues during the acute stage of the illness, as well as for a number of postdischarge sequelae [4].

Additionally, a large number of patients displayed coagulation abnormalities: elevated D-dimer concentration upon hospital admission, a decrease in platelet count, and an extension of the prothrombin time, all suggesting that COVID-19 was hypercoagulable, which could increase the risk of thromboembolic complications [5,6]. In fact, VTE has become a frequent consequence, especially in critically unwell patients [7]. A recent study in antemortem and postmortem cohorts of critically ill COVID-19 patients revealed an increased occurrence of VTE and an improvement in prognosis following a change in anticoagulant treatment [8].

Heparin's biological effects, known as its pleiotropic effects, include anti-inflammatory, antiapoptotic, and anticancer properties, in addition to its primary function of preventing clotting [9-11]. Despite previous anticoagulant failures in critical illness, the high incidence of VTE in COVID-19 and strong evidence of coagulopathy suggest that heparin may improve patient outcomes. A retrospective report of 449 COVID-19 patients from Wuhan, China, where prophylaxis in medical patients is comparatively uncommon due to a low incidence of VTE, first suggested the usefulness of heparin as an anticoagulant in COVID-19 [12]. In this cohort, 99 patients received low-dose prophylactic heparin doses, while 350 patients received no heparin therapy (neither low-dose prophylactic nor high-dose therapeutic). Receiving prophylactic heparin reduced mortality in patients with elevated D-dimer (>6-fold over the upper limit of normal) or raised sepsis-induced coagulopathy scores by almost 20% [13]. Another finding that supports the clinical importance of thrombosis in severe disease is that intravenous tissue plasminogen activator, a strong thrombolytic, can momentarily enhance oxygenation in acute respiratory distress syndrome linked to COVID-19 [14].

The present study was conducted to study the efficacy of nebulised heparin in patients suffering from COVID-19 pneumonia requiring mechanical ventilation.

MATERIALS AND METHODS

A double-blinded randomised controlled study was conducted among patients admitted with COVID-19 aged over 18 years at Basaveshwara Medical College and Hospital in Chitradurga, Karnataka, India. The study took place for a period of three months,

from February 2021 to May 2021. IEC clearance (BMCH/IEC/2020-2021/97) was obtained before initiating the study.

Sample size calculation: The sample size was determined in consultation with a statistician and based on initial pilot observations. For a difference of 1 in TAT levels between the two groups (d), the Standard Deviation (SD) was calculated as 1.7, indicating that approximately 46 patients should be included in each group. Considering a 5% dropout rate, the authors fixed 50 patients for each group.

Sample size calculation:

Sample size=$(SD)^2/(d)^2$

$=(1.7)^2/(1)^2$

=46.24

Inclusion criteria:

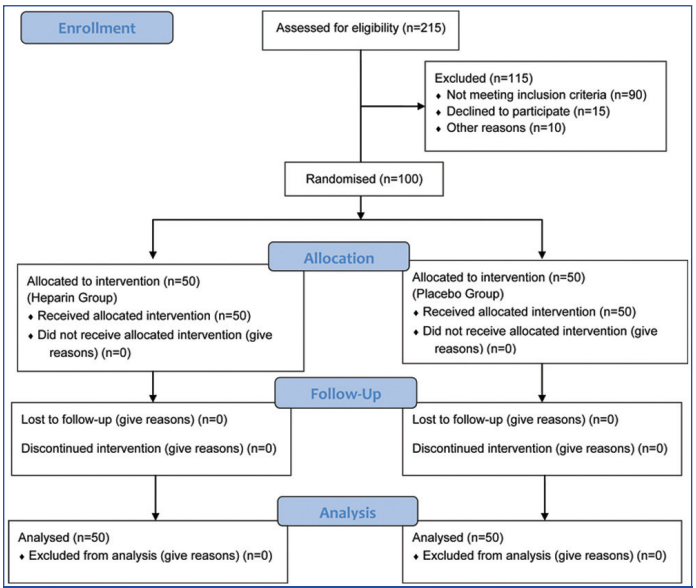
1. Patients aged over 18 years.
2. Reverse Transcription Polymerase Chain Reaction (RT-PCR) positive within the last 21 days.
3. Computed Tomography (CT) severity score greater than 15 [15].

Exclusion criteria:

1. Patients with heparin allergy.
2. Patients with an Activated Partial Thromboplastin Time (APTT) exceeding 120 seconds.
3. Platelet count less than 20,000.
4. Pregnant patients.
5. Patients with brain injury and myopathy.

Study Procedure

Informed written consent was obtained from the patients. Those admitted to the intensive care unit due to ARDS were randomly divided into two groups using computer-generated random numbers [Table/Fig-1]. The intervention group consisted of 50 patients suffering from ARDS due to COVID-19, who were administered nebulised Heparin (25,000 IU). The control group, also consisting of 50 patients, received treatment with other drugs, including parenteral heparin. The heparin and placebo were presented in identical 5 mL plastic ampules: heparin sodium (porcine mucous) 25,000 U/5 mL and placebo (0.9% sodium chloride) [16]. Double-blinding (both investigator and patient) was implemented. Patients received 5 mL of the study medication every four hours, or if they were less than 165 cm in height, every six hours. Heparin and placebo were nebulised using a standard nebuliser for 30 minutes.



[Table/Fig-1]: Consolidated Standards of Reporting Trials (CONSORT) flow diagram for case enrollment.

The nebuliser was placed in the inspiratory limb just before the Y piece. An active humidification system was used.

A mechanical ventilation system with pressure control was utilised. The target tidal volume was set at no more than mL/kg of estimated body weight, following standard procedures during the study period. Demographic information was collected upon study admission, including breathing parameters, clinical data, sputum characteristics, medication usage, and adverse events such as red cell transfusions and blood-stained sputum, including frank blood. TAT levels (normal <3.0 ng/mL) and D-dimer levels (normal <1.0 mcg/mL) were assessed at baseline and followed-up daily until day 4 as the primary outcome of the study [17]. Spo_2 levels were also recorded at baseline and monitored for four days as a secondary outcome of the study. The data was collected using a proforma specifically designed for the study. CT severity scores were employed for the study [Table/Fig-2] [15].

CTSS (CT severity score)	Significance
< 8	Mild disease
9-15	Moderate disease
16-25	Severe disease

[Table/Fig-2]: CT Severity Score (CTSS) employed in the study [15].

STATISTICAL ANALYSIS

The data was analysed using SPSS version 22. It was entered into an Excel sheet for further analysis. The outcomes were presented in tables and figures, accompanied by frequency and percentage explanations. To assess the relationship between two variables, the paired t-test and the chi-square test were employed.

RESULTS

In the present study, the two groups did not show significant differences in terms of age, sex, respiratory failure, vasopressin use, and CT severity score [Table/Fig-3].

Variables	Heparin group	Placebo group	p-value
Age in years, Mean (±SD)	46.2 (±11.47)	46.0 (±13.7)	0.962
Sex, Male n (%)	26 (52.0)	27 (54.0)	0.841
Respiratory failure n (%)	27 (54.0)	19 (38.0)	0.108
Vasopressin use	32 (64.0)	28 (56.0)	0.414
CT Severity score (Mean±SD)	16.44 (±0.99)	16.42 (±0.67)	0.906
Activated Partial Thromboplastin Time (APTT) (Mean±SD)	37.78 (±7.65)	38.0 (±5.93)	0.873

[Table/Fig-3]: Baseline characteristics of the study groups*.
*paired t-test, chi-square test

The thrombin antithrombin complex exhibited significant differences at baseline and follow-up, with notable changes observed in both groups. D-Dimer levels decreased on follow-up compared to baseline in both groups, with a more pronounced decrease in the heparin group. Mean SPO_2 levels also improved during the four-day follow-up period [Table/Fig-4].

Variables	Heparin group	Placebo group	p-value
TAT Baseline (mcg/L)	15.9 (±8.35)	20.24 (±9.28)	0.016
TAT Day 1	10.1 (±4.77)	16.5 (±8.0)	<0.01
TAT Day 2	10.5 (±4.36)	9.0 (±3.5)	0.049
TAT Day 4	11.1 (±4.45)	7.22 (±2.7)	<0.01
D-Dimer Baseline (mcg/mL)	2.5 (±1.1)	4.1 (±1.2)	<0.01
D-Dimer Day 1	2.0 (±0.9)	5.7 (±1.2)	<0.01
D-Dimer Day 2	1.3 (±0.7)	1.4 (±1.1)	0.402
D-Dimer Day 4	0.56 (±0.5)	0.6 (±0.5)	0.842
SPO_2 Baseline (%)	74.0 (±8.3)	73.7 (±7.8)	0.872
SPO_2 Day 1	90.1 (±1.2)	85.56 (±3.1)	<0.01

SPO_2 Day 2	92.1 (±1.4)	86.4 (±3.2)	<0.01
SPO_2 Day 4	93.7 (±1.3)	89.5 (±3.5)	<0.01

[Table/Fig-4]: TAT, D-Dimer and SpO_2 levels in heparin and placebo groups.
*paired t-test, p<0.05 statistically significant; Values presented in Mean±SD

The study demonstrated that heparin nebulisation was not associated with any adverse events, even when administered alongside systemic heparin. The drug was well-tolerated by the patients. The occurrence of blood-stained sputum in the heparin group was significantly lower in the present study.

DISCUSSION

The present study focused on investigating the efficacy of nebulised heparin in COVID-19 pneumonia patients requiring mechanical ventilation. The study included 50 cases in both the Heparin group and the placebo group. The mean age of patients in the Heparin group was 46.2 years, while it was 46.0 years in the placebo group. The majority of patients in both groups were male. In a study conducted by Gupta B et al., which examined the role of nebulised heparin in reducing COVID-19-induced acute lung injury, they reported a mean age of 54.5 years, with the majority of patients being male (79.0%) [17].

The authors observed that mean SPO_2 levels improved over the four-day follow-up period, with a more significant improvement in the nebulised heparin group compared to the placebo group. These findings align with Gupta B et al.'s study, where they also reported a statistically significant improvement in oxygenation (pO_2/FiO_2 ratio) over seven days (mean=184.96, p=0.00) [17]. Additionally, they found a significant improvement in PaO_2 (84.17±33.82) and SO_2 (92.30±3.49). Compared to the present study, the heparin group demonstrated a notable daily change in oxygenation levels over the first three days. There was a significant clinical improvement in terms of ventilation-free days in patients receiving nebulised heparin.

Reduced fibrin deposition in the pulmonary microcirculation and alveolar sacs, known as hyaline membranes, may be the underlying mechanism [18]. A study has shown that intravenous heparin significantly reduced histological signs of pulmonary microvascular thrombosis in individuals with acute inflammation following heart surgery [19]. Fibrin deposition causing a barrier to gas exchange has been associated with reduced alveolar perfusion and ventilation [20]. Pulmonary microvascular thrombosis may lead to ischaemic damage to alveolar tissue and strain on the right heart by increasing the right ventricular afterload [21]. In addition, leukocyte infiltration of lung tissue mediated by fibrin may cause further harm [22]. Nebulised heparin has been associated with fewer days of mechanical ventilation in a study by Dixon B et al., [18].

In the present study, significant differences were observed between baseline and follow-up thrombin antithrombin complexes, with notable changes in both groups. D-Dimer levels were lower on follow-up compared to baseline in both groups, with a more pronounced decrease in the heparin group. This finding is similar to a study by Gupta B et al., where D-dimer levels did not show a statistically significant change [17].

The systemic anticoagulant effect of nebulised heparin is often reflected in higher Activated Partial Thromboplastin Time (APTT) readings compared to placebo [18]. The absence of a discernible difference in APTT values between the groups in this investigation may be due to delayed heparin clearance from the lungs [23].

The study demonstrated that heparin nebulisation, even after systemic heparin dosing, did not result in any adverse side-effects. The medication was well tolerated by the patients. The number of patients with blood-stained sputum in the heparin group was significantly reduced. The increased prevalence of Venous Thromboembolism (VTE) in COVID-19 patients and its impact on mortality, particularly in Intensive Care Unit (ICU) patients, has been confirmed by data from various studies. Additionally, there was an increased risk of bleeding [24-27].

Limitation(s)

In the present study, the authors were unable to evaluate the duration of hospitalisation as it was fixed for 14 days of mechanical ventilation. Long-term follow-up assessments could be conducted.

CONCLUSION(S)

The administration of nebulised heparin as an adjunct in COVID-19-induced lung injury resulted in reduced coagulant markers (TAT and D-dimer levels) and increased oxygen concentration. The present study also demonstrated that systemic heparin dosing, combined with heparin nebulisation, did not lead to any side-effects. The study further showed no noticeable difference in APTT values between the groups. Additional trials are needed to confirm these study findings with other variables.

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