A multicentric, randomized, controlled phase III study of centhaquine (Lyfaquin®) as a resuscitative agent in hypovolemic shock patients

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Abstract:

Centhaquine is a novel, first-in-class resuscitative agent for the treatment of hypovolemic shock. Efficacy of centhaquine for the treatment of hypovolemic shock as an adjuvant to standard of care (SOC) was evaluated in a prospective, multi-center, randomized, double-blind, controlled phase III study. Key inclusion criteria were, systolic blood pressure (SBP) of \( \leq 90 \) mm Hg, blood lactate levels of \( \geq 2 \) mmol/L and patients receiving SOC in a hospital or ICU setting. Primary endpoints of the study were change in SBP, diastolic blood pressure (DBP), blood lactate levels and base deficit. Mortality through day 28 was the key secondary endpoint. A total of 197 patients were screened, of which 105 patients met eligibility criteria and were included in the study. Patients were randomized in a 2:1 ratio, 71 patients to centhaquine (0.01 mg/kg, IV infusion) group and 34 patients to control group. SOC was provided to both centhaquine and control groups. Demographics were similar in both groups, except patients in saline group were few years younger. Difference between the mean in control and centhaquine groups of hemoglobin level was 0.769 ± 0.590 (P=0.0984) and in hematocrit level was 2.419 ± 1.899 (P=0.1036) at the time of inclusion. Centhaquine substantially reduced 28-day all-cause mortality. In control group, mortality rate was 11.76% compared to 2.94% in centhaquine group (odds ratio: 4.4; 95% CI 0.9651 to 23.74 and P=0.037). At 24 hours of resuscitation, SBP of

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
more than 110 mmHg was in 60.61% patients of control and 79.69% patients of centhaquine group (P=0.0222; OR=2.55); while DBP of more than 70 mmHg was in 51.52% patients of control and 76.56% patients of centhaquine group (P=0.0061; OR=3.075). The number of patients with blood lactate levels of 1.5 mmol/L or less were 46.88% in control group compared to 69.35% in centhaquine group (P=0.0168; OR=2.565). The number of patients with base-deficit of less than minus 2 were 43.75% in control group compared to 69.84% in centhaquine group (P=0.0069; OR=2.977). Shock index improved in centhaquine but not in control group in the first 6 hours of resuscitation. Centhaquine improved acute respiratory distress syndrome (ARDS) and multiple organ dysfunction score (MODS). No drug related adverse event was reported. Centhaquine (Lyfaquin®) is a highly efficacious resuscitative agent for the treatment of hypovolemic shock as an adjuvant to SOC.
INTRODUCTION

Hypovolemic shock is a life-threatening condition of inadequate tissue blood perfusion and oxygenation due to a decrease in circulating blood volume. Severe loss of blood due to trauma, gastrointestinal bleeding, major surgeries, post-partum, diarrhea, or vomiting can cause hypovolemic shock. About 61,000 in the United States and 1.9 million people worldwide die due to hemorrhagic shock every year, with a large percentage of patients dying within the first six hours. Main features of hypovolemic shock include hypotension, increased blood lactate levels and base deficit. Hypovolemia decreases cardiac pre-load to a critical level ensuing a dramatic drop in cardiac output that results in low tissue blood perfusion, ultimately leading to multiple organ dysfunction and death. Urgent management is needed to prevent multi-organ failure and death. Patients in intensive care units (ICUs) across the world are treated with fluid therapy to restore blood volume and tissue blood perfusion. Although the goal is to increase the intravascular circulating volume, however, fluid tends to move out into the extravascular space. An ideal resuscitation fluid should be able to rapidly and effectively increase intravascular volume. Damage control resuscitation was developed to restore intravascular volume, prevent dilutional coagulopathy and preserve tissue oxygenation. An ideal replacement to whole blood is use of blood products in a balanced ratio of 1:1:1 for units of plasma to platelets to red blood cells and this remains an area of active research with strong evidence for use of 1:1:1 ratio. When volume resuscitation alone fails vasopressors are administered. The most common vasopressor used is norepinephrine, distantly followed by phenylephrine, epinephrine, and dopamine. Catecholamine infusion enhances cardiac contractility as well as vascular tone including arteriolar, venous and renovascular beds and thus influences overall arterial, venous, and capillary pressures and blood flow. Common adverse effects of vasopressors as a class
include arrhythmias, fluid extravasation, and ischemia\textsuperscript{11,12}. Current standard of care (SOC) is not adequate and is based on resuscitative agents developed more than 5 decades ago and no new agent has been developed to treat this life-threatening condition. There has been a vigorous debate about optimal methods of resuscitation\textsuperscript{13} and clearly there is a need for novel resuscitative agents.

Over five decades numerous attempts have been made to develop an effective resuscitative agent without success. Agents that could decrease metabolic activity to reduce oxygen demand were studied. Histone deacetylase inhibitors such as valproic acid and suberoylanilide hydroxamic acid\textsuperscript{14,15}, hydrogen sulfide and its donor sodium sulfide\textsuperscript{16,17}, mitochondria targeted hydrogen sulfide donor AP39\textsuperscript{18}, formulation consisting of d-beta-hydroxybutyrate and melatonin\textsuperscript{19} and other hibernation based approaches have been tried\textsuperscript{20} but none has shown any promise clinically.

Hemoglobin-based blood substitutes were prepared as resuscitative agents. Diaspirin crosslinked hemoglobin found effective in animal models of hemorrhagic shock\textsuperscript{21,22}, failed in phase III clinical trial\textsuperscript{23,24}. Polymerized hemoglobin effective in experimental models\textsuperscript{25,26}, were not successful clinically\textsuperscript{27-29} and were dropped from further development. Numerous other approaches to develop hemoglobin based resuscitative agent were made but none has been successful\textsuperscript{30}.

Centhaquine is a novel, first-in-class resuscitative agent for the treatment of hypovolemic shock. It acts on $\alpha_{2B}$ adrenergic receptors to produce venous constriction and increase venous return to the heart, resulting in an increase in cardiac output and improved tissue perfusion. Centhaquine also acts on central $\alpha_{2A}$ adrenergic receptors to reduce sympathetic drive and decrease systemic vascular resistance contributing to an improved tissue blood perfusion\textsuperscript{31}. Mechanism of action of centhaquine makes it an ideal candidate for the treatment of patients with hypovolemic shock.
Enhancing tissue blood perfusion is a significant advantage in reducing the volume of resuscitation and preventing extravasation of fluid and adverse effect of lung edema. Centhaquine does not act on beta-adrenergic receptors, and therefore the risk of arrhythmias is mitigated. Safety and efficacy of centhaquine was evaluated extensively in preclinical models, healthy volunteers, and in patients. Centhaquine was found to be effective in a phase 2 study with significant improvement in blood lactate levels, base deficit and blood pressure. Based on these highly encouraging data, a phase III study was undertaken, the results of which are described here.

METHODS

Trial Design: This was a prospective, multi-center, randomized, placebo-controlled, double-blinded phase III clinical study of centhaquine in patients with hypovolemic shock receiving the best SOC (NCT04045327; Clinical Trials Registry, India CTRI/2019/01/017196). Because centhaquine was found to be efficacious in a phase 2 study with statistically significant improvements in blood lactate levels (p=0.0012), base deficit (p= <0.0001) and blood pressure (p=<0.0001) and a trend towards reduced mortality, in consultation and agreement with the regulatory authorities, patients were randomized in a 2:1 ratio either to the centhaquine group receiving centhaquine dose of 0.01 mg/kg by IV infusion along with SOC or to the control group receiving SOC plus saline. The duration of study for an individual patient was 28 days, which included two study visits: visit 1 on day 1 included screening/randomization/baseline/treatment visit and visit 2 at the end of study (day 28 + 7).

The study was conducted in compliance with ICH-GCP Guidelines, the Helsinki Declaration, and local regulatory requirements. The study protocol (PMZ-2010/CT-3.1/2018) was approved by the Drugs Controller General of India (DCGI), Directorate General of Health Services,
Ministry of Health & Family Welfare, Government of India and Institutional Ethics Committee of all 14 participating institutions. A Data and Safety Monitoring Board (DSMB) was convened and its responsibilities were determined before the initiation of the study. The members included a biostatistician and senior practicing physicians with extensive experience in critical care medicine. The DSMB had access to SAEs as well as any other AEs that the investigator or the medical monitor deemed important. At pre-determined intervals, the DSMB reviewed the study data on safety and critical efficacy endpoints. They were able to recommend changes to the study design or to request suspension of the study if there were any safety concerns.

Patients were screened for study eligibility based on the following criteria: 1) patients aged 18 years or older; 2) systolic blood pressure (SBP) of ≤90 mm Hg; 3) blood lactate levels of ≥2 mmol/L that are indicative of hypovolemic shock; 4) patients receiving SOC in a hospital or ICU setting. SOC generally included endotracheal intubation, fluid resuscitation and vasopressors according to the treatment guidelines in the local hospital setting. Female patients with standing pregnancy and patients who were participating in other clinical trials were excluded from the study. Patients with pre-existing systemic diseases such as cancer, chronic kidney failure, liver failure, decompensated heart failure or AIDS were also excluded.

The patients randomized in this study, were in a state of severe life-threatening shock. Those patients who were not fit to give consent themselves at the time of initiation of treatment, informed consent was taken from their legally authorized representative (LAR). The patient/LAR was informed by the investigator both in writing and orally about all aspects of the study relevant to taking a decision on whether to participate or not. The informed consent form included all the elements required as per the ICH-GCP recommendations and schedule Y. Informed consent form in English and other regional languages were approved by respective
Ethics Committee and DCGI. The entire consenting process was recorded through an audio-video recording, labeled and stored at the site in a secured place. To ensure medical confidentiality and data protection, the signed informed consent forms were stored in the investigator's study file according to the requirements.

At baseline a variety of demographic data (age, gender captured including, weight, height), chest X-ray, ECG, vital signs were recorded. Baseline blood tests included CBC, blood lactate, base deficit, serum chemistry, liver and kidney function tests. Patient’s physical examination was done, information about their medical history, concomitant illness, concomitant medications were recorded. In addition, patient’s initial Glasgow coma scale (GCS) and Adult Respiratory Distress Syndrome (ARDS) scores was recorded.

**Treatment Regimen**: The study drug (1.0 mg of centhaquine citrate in a 10 mL vial) was manufactured by Pharmazz India Private Limited at Gufic Biosciences Limited and supplied to the investigators at the participating sites. Patients who met the eligibility criteria were randomized 2:1 to centhaquine group or control group, respectively. Throughout the study all patients in both groups received the best SOC for hypovolemic shock according to local institutional standard practice, including fluid resuscitation with crystalloids/colloids, blood products and vasopressors such as norepinephrine. Centhaquine or normal saline was administered intravenously after randomization to hypovolemic shock patients, and all patients continued receiving standard treatment for hypovolemic shock. In centhaquine group, centhaquine was administered at a dose of 0.01 mg/kg body weight as an intravenous infusion over 1 hour in 100 mL normal saline. The next dose of centhaquine was administered if SBP fell below or remained below 90 mmHg, but not before 4 hours of previous dose and total number of doses did not exceed three per day. Centhaquine administration if needed was continued for two
days post randomization. A minimum 1 dose and a maximum of 6 doses of centhaquine were administered within first 48 hours post randomization. In control group, single dose of equal volume of normal saline was administered as intravenous infusion over 1 hour in 100 mL of normal saline post randomization.

**Safety Evaluation**: All patients who received treatment were included in safety analysis. Safety was assessed during treatment period and during follow-up period post-treatment based on adverse events, physical examination, vital signs (pulse rate, heart rate, SBP and diastolic blood pressure (DBP), body temperature, and respiratory rate), ECG; and clinical laboratory parameters as per protocol. A variety of biochemical tests, serum chemistry tests, hematological variables, coagulation variables, urine output, organ function tests such as kidney and liver function tests were assessed. Adverse events that occurred or worsened during treatment or post-treatment were recorded. All AEs were coded by systems organ class and preferred term using the latest version of MedDRA. All patients were followed up for safety assessment till the end of study on day 28.

**Efficacy Assessments**: The primary objectives of this study were to determine: 1) change in SBP and DBP, 2) change in blood lactate levels and 3) change in base-deficit. For all these endpoints, changes were Mean through 48 hours [Time frame: first 48 hours]. Key secondary objectives of the study included proportion of patients with all-cause mortality and safety and tolerability of centhaquine. In addition, patient’s ARDS scores and multiple organ dysfunction score (MODS) were recorded.

**Sample Size and Statistical Analysis**: Sample size for this study was calculated based on the results of our phase 2 trial (CTRI/2017/03/008184, NCT04056065). In the phase 2 study, SBP in the centhaquine group was 7.19% higher than the control group, at 48 hours (25.39% increase...
from the baseline in control group compared to 39.51% increase from the baseline in the centhaquine group). The statistical power of the study was 80%. Because centhaquine was found to be efficacious in a phase 2 study with statistically significant improvements in blood lactate levels (p=0.0012), base deficit (p<0.0001) and blood pressure (p<0.0001) and a trend towards reduced mortality, in consultation and agreement with the regulatory authorities, patients were randomized in a 2:1 ratio either to the centhaquine group (SOC + centhaquine) or to the control group (SOC + saline) in the phase III study. The power was set to 90% (beta, 0.1), enrollment ratio of 2:1, and the level of significance (alpha) used was 0.05. To achieve this, we estimated that a sample size of a minimum 69 patients (46 in centhaquine group and 23 in normal saline group) was enough to achieve a power of 90%, when the level of significance alpha was 0.05. To increase the power of the study (>90%, beta, 0.05) approximately 84 patients (56 in centhaquine group and 28 in control group) were required to be enrolled. Considering discontinuation rate of approximately 20%, it was decided to enroll a total of 105 patients (70 in centhaquine group and 35 in control group) in this study. The results of the trial are presented as mean ± SEM. Unpaired t test with Welch's correction was used to analyze two sets of data with equal variances. The Unpaired t-test was used to compare the discrete variables between the two sets of data at baseline and at follow-ups. Non-parametric analysis was carried out using one-way ANOVA without assuming equal variances. This test was used for multiple comparison, significance of differences was estimated by Tukey's multiple comparisons test. Chi-square test was used to compare the groups. Baptista-Pike method was used to calculate Odds ratio. A P value of less than 0.05 was considered significant at 95% confidence level and 0.10 at 90% confidence level. Demographic variables and patient characteristics were summarized descriptively by treatment assignments. Demographic variables include age, weight, height, and body mass index. Variables
that are measured on a continuous scale, such as the age of the patient at the time of enrolment, the number of non-missing observations (n), mean and SEM were tabulated by treatment assignments. All available data was used in the analyses. Each group was summarized individually. Data not available was assessed as “missing values” and the observed population only were evaluated. The statistical analysis was processed with GraphPad Prism 8.1.1 (GraphPad, San Diego, CA).

Blinding/unblinding Procedures and Randomization Process: In this double-blind study, the patient and all relevant personnel involved with the conduct and interpretation of the study (including investigator, investigational site personnel, and the sponsor or designee’s staff) were blinded to the identity of the study drug (centhaquine/normal saline) assigned and the randomization codes. The biostatistician/unblinded pharmacist was independent of study team. The pharmacist was unblinded of the randomization to ensure double blinding of the study. The pharmacist has signed the undertaking of not disclosing the study treatments to study team. Dispensing activity was carried out by unblinded pharmacist independent of monitoring team. Final randomization list was kept strictly confidential and accessible only to authorized persons per sponsor until the completion of the study. Block randomization was used for patient randomization into the 2 treatment groups. The randomization list was prepared by statistician using a validated computer program, statistical analysis system SPSS. An IWRS method containing randomization codes was used to randomize/unblind the eligible patient to the treatment groups. Emergency unblinding through IWRS was available. As per study protocol, the investigator or his/her designee was permitted to unblind the code when medically needed, without identifying patient’s treatment. For those patients, where unblinding was done, the date, time and the reason for emergency unblinding was recorded in patient’s medical record. Any AE
or SAE that required unblinding the treatment was recorded and reported as specified in the protocol. Treatment unblinding was not done for any of the patients enrolled in this study.

RESULTS

Patient Enrollment and Demographics

This study was conducted in 14 Emergency Room/Intensive Care Units across India (Table 1). Patients with hypovolemic shock due to blood loss or fluid loss with SBP ≤ 90 mmHg and lactate level indicative (≥2 mmol/L) of shock at presentation and who continued receiving standard shock treatment were enrolled in the study. A total of 197 patients were assessed for eligibility, out of which 105 patients were enrolled in the study and 92 patients did not meet the eligibility criteria and were excluded. Out of 105 patients 71 were randomized in centhaquine group and 34 in control group. In centhaquine group 1 patient withdrew consent and 2 patients were excluded by the investigator (one patient was diagnosed with fulminant tuberculosis and another patient was diagnosed with refractory septic shock). A total of 34 patients (22 male and 12 female) in control and 68 patients (41 male and 27 female) in centhaquine group completed the study (Figure 1). In both treatment groups, patients were provided the best SOC. Both treatment groups were well matched in terms of demographics and baseline characteristics (Table 2). Majority of patients were below 65 years of age and there were more males in the study than females in both groups. Total number of patients with hemorrhagic shock were 45 in centhaquine group and 20 in the control group. Similarly, patients with hypovolemic shock due to fluid loss were 23 in the centhaquine group and 14 in the control group. In general, majority of the patients included were hypovolemic patients due to hemorrhagic shock. The number of doses of study drug administered in control group averaged 1.47 per patient and in centhaquine group it was 1.27 per patient. Comparison of hemoglobin and hematocrit levels (difference between
means -0.769 ± 0.590, P=0.0984 and -2.419 ± 1.899, P=0.1036 between control and centhaquine, respectively) between the groups suggest that the blood loss was similar in control and centhaquine groups. Similarly, total amount of fluids infused, total amount of blood products infused and urine output during the first 48 hours was similar in both groups suggesting that the fluid loss and subsequent fluid resuscitation was comparable in both groups. A total dose of vasopressors administered in the control group (4.402 mg) appeared to be higher compared to the centhaquine group (2.757 mg), however this difference (difference between means of control and centhaquine 1.645 ± 2.728, 95% CI 7.119-3.829, P=0.274) was not statistically significant. Percent time patient spent in the intensive care unit during their stay in the hospital was similar in both the groups 46.61 ± 7.95 in control vs 46.03 ± 5.72 in centhaquine.

**Centhaquine Improves Systemic Hemodynamics**

Centhaquine significantly increased both SBP and DBP in a greater number of patients compared to control (Table 3). SBP increased following resuscitation in both control and centhaquine groups, however, significantly greater number of patients treated with centhaquine had higher SBP compared to control group. At 12 hours of resuscitation, SBP of more than 90 mmHg was recorded in 87.50% patients in control and 96.92% patients in centhaquine group (P=0.0350). At 24 hours of resuscitation, SBP of more than 110 mmHg was in 60.61% patients of control and 79.69% patients of centhaquine group (P=0.0222) (Figure 2 (upper panel)). At 48 hours of resuscitation, SBP of more than 120 mmHg was recorded in 46.88% of control and 56.25% of centhaquine group, the difference was not statistically significant at 48 hours (P= 0.1928) (Figure 2 (upper panel)). Similarly, DBP increased above 65 mmHg in both groups however, significantly higher number of patients treated with centhaquine had DBP greater than control group. At 12 hours of resuscitation, DBP of more than 65 mmHg was in 60.61% patients in
control and 72.31% patients in centhaquine group (P=0.1196). At 24 hours of resuscitation DBP of more than 70 mmHg was in 51.52% patients in control group and 76.56% patients in centhaquine group (P=0.0061). At 48 hours of resuscitation, DBP of more than 80 mmHg was in 31.25% patients in control group and 50.00% patients in centhaquine group (P=0.0404) (Figure 2 (lower panel)). An increase in pulse pressure was noted in patients treated with centhaquine compared to control. The difference between means ± SEM at 0 vs 48 hours was 11.41 ± 1.572 mmHg (95% CI 8.20 to 14.61) in control compared to 14.76 ± 1.743 mmHg (95% CI 11.28 to 18.25) in the centhaquine group. Similarly, a decrease in heart rate was noted in control and centhaquine patients. Difference between means ± SEM at 0 vs 48 hours was 35.78 ± 3.576 beats/min in control group compared to 25.52 ± 3.063 beats/min in centhaquine group. Shock index (SI) defined as heart rate divided by SBP, has a normal range of 0.5 to 0.7 in healthy subjects. SI ≥ 1.0 has been associated with significantly poorer outcomes in patients with acute circulatory failure. Mean SI at the time of inclusion (0 hours) was 1.390 and 1.320 in control and centhaquine groups, respectively (difference between means 0.070 ± 0.066; 95% CI -0.2022 to 0.06165) indicating that degree of shock was similar in both groups and was moderate to severe in nature. At one hour of resuscitation SI decreased to 1.171 and 1.020 in control and centhaquine groups, respectively, and was significantly lower in centhaquine group compared to control (difference between means 0.1505 ± 0.0682; P=0.016). SI significantly improved (P<0.0001) in centhaquine group in the first 6 hours of resuscitation (Figure 3).

**Centhaquine Decreases Blood Lactate Levels**

As expected the blood lactate levels in hypovolemic shock patients were high on day 1, ranging from 2.04 to 11.90 mmol/L. Mean value ± SEM of blood lactate levels on day 1 in the centhaquine group was 4.50 ± 0.29 mmol/L. Treatment with centhaquine led to a significant
decrease in blood lactate levels as evidenced by blood lactate levels on day 3 that ranged from 0.6 to 4.82 mmol/L. Except for one out of 68 patients, every patient treated with centhaquine had lower levels of blood lactate on day 3 compared to day 1. In that patient, blood lactate levels were 2.69 and 4.82 mmol/L on day 1 and day 3 respectively. This patient was the only outlier where there was no decrease in blood lactate levels upon treatment with centhaquine. Mean value ± SEM of blood lactate levels on day 3 in the centhaquine group was 1.43± 0.09. In the control group two out of 34 patients had higher levels of blood lactate on day 3 compared to day 1. One patient had blood lactate levels of 4.80 and 5.30 mmol/L on day 1 and day 3, respectively and the other patient had blood lactate levels of 2.12 and 2.48 mmol/L on day 1 and day 3, respectively. In control group, mean value ± SEM of blood lactate levels were 4.13 ± 0.40 and 1.91 ± 0.26 mmol/L on day 1 and day 3 respectively. Blood lactate levels at day 3 of resuscitation were found to be significantly lower in centhaquine group compared to control group receiving standard treatment (P=0.046; 95% CI -1.048 to 0.08253). The number of patients with blood lactate levels of 1.5 mmol or less were 46.88% in the control group compared to 69.35% in centhaquine group (P=0.0168) (Figure 4).

**Centhaquine Improves Base Deficit**

Base deficit ranged from -1.60 to -21.8 in patients from the centhaquine group on day 1. Only 4 out of 68 patients (5.88%) treated with centhaquine had lower base deficit on day 3 compared to day 1. Mean value ± SEM of base deficit was -7.58 ± 0.56 on day 1 and -1.84 ± 0.50 on day 3 in centhaquine group (Difference between means 5.744 ± 0.749; 95% CI 4.261 to 7.226). In the control group 7 out 34 patients (20.59%) had lower base deficit on day 3 compared to day 1. Mean value ± SEM of base deficit was -7.36 ± 1.07 on day 1 and -3.33 ± 0.92 on day 3 in control group (Difference between means 4.029 ± 1.212; 95% CI 1.212 to 6.846). Base-deficit improved
in patients treated with centhaquine by 1.495 ± 1.045 mmol/L compared to control patients receiving standard treatment (P=0.0794). The number of patients with base-deficit of less than minus 2 were 43.75% in standard treatment group compared to 69.84% in those receiving centhaquine (P=0.0069) (Figure 5).

**Centhaquine Improves ARDS and MODS**

Acute Respiratory Distress Syndrome (ARDS) was compared between day 1 (before resuscitation) and day 3 of resuscitation. In control group of patients receiving standard treatment the difference between means from day 1 to day 3 was -0.06850 ± 0.06497 (P=0.1480). On the other hand, in centhaquine treated group of patients the ARDS difference between means from day 1 to day 3 was -0.09927 ± 0.04891 (P=0.0224). These results indicate that centhaquine treatment significantly improved ARDS following resuscitation, whereas in control group there was insignificant improvement (Figure 6). MODS was compared between day 3 and day 7 of resuscitation. There was no improvement in MODS in the control group and the difference between means was 0.5893 ± 0.9370 (P=0.2701), whereas in centhaquine group the difference between means was -0.5485 ± 0.3421 (P=0.0566). Although P values do not indicate difference, however the change in control was towards worsening (MODS from 1.138 to 1.727) whereas in centhaquine patients it was towards improvement (MODS from 1.367 to 0.8182). Centhaquine treatment decreased MODS whereas in control it increased and worsened. Centhaquine treated group showed statistically significant decrease in MODS which was not observed in control group (Figure 7). Number of patients with 2 or more MODS score on day 7 were 54.55% in control and 13.64% in centhaquine groups (P=0.0064; Odds ratio 7.60, 95% CI 1.37 to 33.11).

**Centhaquine Decreases Mortality**
Centhaquine treatment led to decreased 28-day all-cause mortality in hypovolemic shock patients that was statistically significant. In the control arm, 28-day all-cause mortality was 11.76% compared to 2.94% in centhaquine arm (odds ratio: 4.40; P=0.0371; 95% CI 0.9651-23.74) (Figure 8).

**Centhaquine is Safe and Well Tolerated**

Centhaquine was well tolerated and a repeat dose if needed was administered to every patient without any sequel. Adverse events of any grade were reported in 3 patients in the centhaquine group. Two patients had elevated levels of serum creatinine (moderate severity) and one patient had vomiting (mild severity). None of these AEs were related to drug treatment. A total of six serious adverse events (deaths) were reported in the study, 4 deaths in the control group and 2 deaths in centhaquine group. No other serious adverse events were reported in the study. None of these SAEs were related to drug treatment (Table 4).

**DISCUSSION**

Treatment of blood loss resulting in hemorrhagic shock has been guided by traditional practices and not on randomized clinical trials conducted in multiple centers. In the past 10 years a decrease in use of crystalloids and an increase in use of blood products in ratios that depict blood transfusion has been observed to improve outcome. However, fluids and vasopressors are still recognized as essential part of resuscitation and are associated with undesired effects such as fluid responsiveness, extravasation of fluids and cardiac complications. There is a need for the development of novel resuscitative agents that either work as single agent or assist in improving the outcome of existing therapeutics.
Centhaquine is a resuscitative agent acting as an $\alpha$-adrenergic receptor agonist. It acts on venous $\alpha_{2B}$ adrenergic receptors to produce constriction and increase venous return to the heart, resulting in an increase in cardiac output and tissue perfusion. It also acts on central $\alpha_{2A}$ adrenergic receptors to reduce sympathetic drive and decrease systemic vascular resistance leading to improved tissue blood perfusion 31,32. The resuscitative effect of centhaquine is completely blocked by $\alpha_2$ adrenergic receptor antagonists, yohimbine or atipamezole 43. Centhaquine does not act on beta-adrenergic receptors, therefore the risk of arrhythmias is mitigated. We have received approval from World Health Organization (WHO) of the International Nonproprietary Name (INN) of previously used names (centhaquin, PMZ-2010) as centhaquine 44.

In hypovolemic conditions, there is a drop in cardiac pre-load to a critical level resulting in a dramatic drop in cardiac output that in turn results in low tissue and organ perfusion, ultimately leading to multiple organ dysfunction and death. Clinical outcome of patients is predominantly monitored using biomarkers, blood pressure and blood lactate levels. Vasopressors tend to increase blood pressure by causing arterial vasoconstriction and increasing heart rate. An increase in heart rate will augment cardiac output. However, the force or rate of contraction cannot explain large increases in cardiac output 45. About two-third of blood volume is stored in the venous system serving as a reservoir that is adjustable 46. An increase in venous return from systemic veins into the right atrium will lead to a significant increase in cardiac output by Frank-Starling mechanism resulting in increased arterial blood pressure and tissue perfusion. Based on the mechanism of action, centhaquine increases venous return to the heart, increases cardiac output and tissue perfusion, making it an ideal candidate for use as a resuscitative agent in the treatment of patients with hypovolemic patients.
In pre-clinical models centhaquine significantly decreased blood lactate, and increased mean arterial pressure, pulse pressure and cardiac output. It was also found to decrease mortality and increase the survival time of hemorrhaged rats compared to Ringer's lactate, hypertonic saline or blood. Similarly, centhaquine improved survival rates in rat, rabbit and swine models of hemorrhagic shock that closely mimic the human condition. Based on this encouraging preclinical data, further clinical development of centhaquine was undertaken. Safety and efficacy of centhaquine was evaluated in healthy volunteers and hypovolemic shock patients in phase 1 (NCT02408731) and phase 2 studies respectively. In the phase 2 study (NCT04056065), centhaquine demonstrated significant efficacy in the improvement of key clinical features.

Hallmark features of hypovolemic patients are hypotension, increased blood lactate levels and increased base deficit. Reduction in blood pressure and increase in blood lactate are the main biomarkers that are used in identifying the risk and progress of clinical outcome in hypovolemic shock patients. Under conditions of shock, inadequate blood flow to the tissues, results in an increase in blood lactate levels. High blood lactate levels and an increase in base deficit in patients are suggestive of poor outcomes and high mortality rates. Early lactate clearance has been associated with decrease in mortality, shorter ICU length of stay and duration of mechanical ventilation. Results of this phase III study (NCT04045327) have confirmed previously observed efficacy in preclinical studies and in patients with hypovolemic shock. In the present study, centhaquine was found to be highly efficacious with statistically significant improvements in blood lactate levels, base deficit and blood pressure. Since time is of the essence in hypovolemic shock patients, it was observed that within 12 hours of resuscitation blood pressure was significantly improved in centhaquine compared to control group suggesting faster recovery towards normalization of blood pressure with centhaquine. SI also indicated a
rapid improvement of left ventricular function in centhaquine compared to control group. More importantly, treatment with centhaquine resulted in reduced 28-day all-cause mortality (odds ratio: 4.4; 95% CI 0.9651 to 23.74 and P=0.037). To our knowledge this is the only late stage clinical study that has demonstrated a statistically significant survival advantage. Centhaquine was found to be safe and well tolerated with no drug related adverse events. Based on the data from this study, centhaquine obtained Marketing Authorization from the regulatory authorities in India for the treatment of hypovolemic shock patients in May 2020.

SI, an important prognostic indicator, is linearly inversely related to physiologic parameters, such as cardiac index, stroke volume, left ventricular stroke work, and mean arterial pressure. SI significantly improved (P<0.0001) in centhaquine group in the first 6 hours of resuscitation. A difference between centhaquine and control groups was observed within the first hour of resuscitation where a decrease in SI was significant (0.1505 ± 0.0682; P=0.016) in centhaquine compared to control group. Studies in a swine model of hemorrhagic shock showed that centhaquine significantly improved Horowitz index (327 ± 10 and 392 ± 16 in control and centhaquine group, respectively) and reduced pulmonary edema. In addition, centhaquine significantly improved ARDS and MODS. An improvement in all the above clinical and biological markers appear to contribute towards improved outcomes and reduced deaths when centhaquine was added to the SOC. Shock can occur due to various conditions, however, irrespective of the cause it results in circulatory collapse which can lead to life-threatening multi-organ failure. Although, clinical studies are warranted, the therapeutic potential of centhaquine for other forms of shock associated with hemodynamic instability or refractory hypotension resulting in multi-organ failure and ultimately death may be explored. Some of these conditions may include sepsis, and in fact a significant number of COVID-19 patients die from sepsis.
Effect of centhaquine on systemic hemodynamics of patients with hypovolemic shock will depend on the fluid status at the time of its administration. A limitation of this study is that we have not examined the effect of centhaquine on volume status of patients with hypovolemic shock. Centhaquine was administered in a total volume of 100 mL over 60 minutes, this is small volume and not likely to cause any volume overload. Moreover, the total volume of fluids administered in control and centhaquine groups in the first 48 hours was similar. Blood products administered in the first 48 hours of resuscitation were also similar in control and centhaquine groups. Moreover, urine output in the first 48 hours was also not different in control and centhaquine groups. Another limitation of this study is that although it is a multi-center study, it was conducted exclusively in patients from India. We recognize that the demographics and SOC for the treatment of hypovolemic shock across the world varies significantly and that the efficacy of centhaquine needs to be established in population across the world.

**Conclusion:** Centhaquine (Lyfaquin®) is a highly efficacious resuscitative agent for the treatment of hypovolemic shock as an adjuvant to SOC.

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Legend to figures:

**Figure 1**: Assessment and enrollment of patients

**Figure 2**: Changes in systolic and diastolic blood pressure following resuscitation of patients with hypovolemic shock in control and centhaquine groups.

**Figure 3**: Shock index (heart rate divided by systolic blood pressure), an important indicator of cardiac performance (left ventricular stroke work) in early hemorrhage was significantly improved by centhaquine in the first 6 hours of resuscitation.

**Figure 4**: Changes in blood lactate levels following resuscitation of patients with hypovolemic shock in control and centhaquine groups.

**Figure 5**: Changes in base-deficit following resuscitation of patients with hypovolemic shock in control and centhaquine groups.

**Figure 6**: Acute Respiratory Distress Syndrome (ARDS) was compared between day 1 (before resuscitation) and day 3 of resuscitation. Centhaquine treatment significantly improved ARDS following resuscitation, whereas in control group there was insignificant improvement.

**Figure 7**: Multiple Organ Dysfunction Score (MODS) was compared between day 3 and day 7 of resuscitation. In control group worsening of MODS from 1.138 to 1.727, whereas in centhaquine group an improvement in MODS from 1.367 to 0.8182 was observed.

**Figure 8**: All cause 28-day mortality hypovolemic shock patients in control and centhaquine groups.
| S. No. | Study Site                                                                 | Site Initiation         | Assessed Patient | Enrolled Patients |
|-------|-----------------------------------------------------------------------------|-------------------------|------------------|-------------------|
| 1     | Institute of Post Graduate Medical Education and Research and SSKM Hospital, Kolkata, West Bengal | 31st January 2019       | 36               | 25                |
| 2     | King George’s Medical University, Lucknow, Uttar Pradesh                    | 26th March 2019         | 09               | 04                |
| 3     | Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh | 12th April 2019         | 21               | 11                |
| 4     | Seven Star Hospital, Nagpur, Maharashtra                                   | 18th April 2019         | 13               | 06                |
| 5     | Rahate Surgical Hospital, Nagpur, Maharashtra                              | 19th April 2019         | 13               | 05                |
| 6     | New Era Hospital, Nagpur, Maharashtra                                     | 20th April 2019         | 01               | 01                |
| 7     | Sidhu Hospital, Doraha, Ludhiana, Punjab                                   | 2nd May 2019            | 32               | 09                |
| 8     | Ganesh Shankar Vidyarthi Memorial Medical College, Kanpur, Uttar Pradesh   | 21st May 2019           | 04               | 02                |
| 9     | KLE’s Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum, Karnataka | 22nd May 2019           | 09               | 02                |
| 10    | Criticare Hospital and Research Institute, Nagpur, Maharashtra             | 18th June 2019          | 08               | 02                |
| 11    | Christian Medical College & Hospital, Ludhiana, Punjab                     | 19th June 2019          | 08               | 06                |
| 12    | Jawahar Lal Nehru Medical College, Ajmer, Rajasthan                       | 2nd July 2019           | 08               | 02                |
| 13    | ACSR Government Medical College & Hospital, Nellore, Andhra Pradesh       | 4th July 2019           | 14               | 10                |
| 14    | Chiranjeev Medical Centre, Jhansi, Uttar Pradesh                          | 9th August 2019         | 21               | 20                |
|       | **Total**                                                                  | **197**                 |                  | **105**           |
Table 2: Patient demographics (Mean ± SEM)

| Group                             | Gender   | Age (Years) | Body Weight (Kg) | Height (Cm) | BMI (Kg/m²) |
|-----------------------------------|----------|-------------|------------------|-------------|-------------|
| Standard Treatment + Saline (N=34) | (22M/12F)| 36.50±2.81  | 56.74±1.62       | 162.35±1.60 | 21.56±0.59  |
| Standard Treatment + PMZ-2010 (N=68) | (41M/27F)| 42.81±2.31  | 58.90±1.37       | 161.31±1.46 | 22.72±0.51  |

Table 3: Primary and key secondary efficacy endpoints

| Endpoint                                      | Centhaquine % of Patients | Placebo % of Patients | P Value | Odds Ratio (95% CI) |
|-----------------------------------------------|---------------------------|-----------------------|---------|---------------------|
| **Primary Efficacy Endpoints**                |                           |                       |         |                     |
| Systolic Blood Pressure >110 mmHg @ 24 hr     | 79.69                     | 60.61                 | 0.0222  | 2.550 (1.033 to 6.391) |
| Diastolic Blood Pressure >70 mmHg @ 24 hr    | 76.56                     | 51.52                 | 0.0061  | 3.075 (1.215 to 7.266) |
| Blood Lactate Levels ≤ 1.5 mmol/L            | 69.35                     | 46.88                 | 0.0168  | 2.565 (1.047 to 5.873) |
| Base Deficit < -2                            | 69.84                     | 43.75                 | 0.0069  | 2.977 (1.223 to 6.907) |
| **Key Secondary Efficacy Endpoint**          |                           |                       |         |                     |
| Mortality                                    | 2.94                      | 11.76                 | 0.037   | 4.4 (0.9651 to 23.74) |
Table 4: Safety of centhaquine and incidence of adverse events

| Event                          | Centhaquine (N=71) | Placebo (N=34) |
|-------------------------------|--------------------|----------------|
| **Adverse Events of any grade** |                    |                |
| Increase in blood Creatinine  | 2 (2.8%)           | 0 (0%)         |
| Vomiting                      | 1 (1.4%)           | 0 (0%)         |
| **Serious Adverse Events**    |                    |                |
| Deaths                        | 2 (2.94%)          | 4 (11.76%)     |
Figure 1: Patient enrollment
Figure 2: Centhaquine Increases Systolic and Diastolic Blood Pressure
Figure 3: Shock index was significantly improved by centhaquine in the first 6 hours of resuscitation
Figure 4: Centhaquine Decreases Blood Lactate Levels

![Figure 4: Blood Lactate Levels](image)

- **Lactate**
  - Control: P = 0.04599
- **Blood Lactate (mmol/L)**
  - Control: 15 patients
  - Centhaquine: 17 patients

| Difference between means ± SEM | -0.4826 ± 0.2794 |
|--------------------------------|------------------|
| 95% confidence interval        | -1.048 to 0.08253|
| P value (significant at 90% CI)| 0.0460           |
| Welch-corrected t, df          | t = 1.728, df = 38.34 |

Odds ratio: 2.565
95% confidence interval: 1.047 to 5.873
P value: 0.0168
Chi-square, df: 4.514, 1

Figure 5: Centhaquine Decreases Base Deficit

![Figure 5: Base Deficit](image)

- **Base Deficit**
  - Control: P = 0.07937
- **Base Deficit (mmol/L)**
  - Control: 14 patients
  - Centhaquine: 18 patients

| Difference between means ± SEM | 1.495 ± 1.045 |
|--------------------------------|---------------|
| 95% confidence interval        | -0.804 to 3.595|
| P value                        | 0.0794        |
| Welch-corrected t, df          | t = 1.431, df = 50.08 |

Odds ratio: 2.977
95% confidence interval: 1.223 to 6.907
P value: 0.0069
Chi-square, df: 6.075, 1
Figure 6: ARDS in patients receiving centhaquine versus control.

Figure 7: MODS in patients receiving centhaquine versus control.
Figure 8: Centhaquine demonstrates a significant improvement over standard of care in reducing mortality.
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