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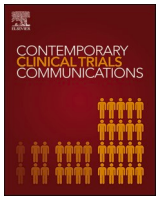

Effect of trehalose on mortality and disease severity in ICU-admitted patients: Protocol for a triple-blind, randomized, placebo-controlled clinical trial

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ABSTRACT

Background: Improvement in organ failure in intensive care unit (ICU) patients is accompanied by lower mortality rate. A disaccharide, trehalose is a candidate to improve organ failure and survival by autophagy induction and enhancing oxidative stress defense. The aim of this study is to assess the effectiveness of trehalose in improving clinical outcome and reducing mortality in ICU patients.

Methods: a triple-blind, randomized, placebo-controlled, two arm, parallel-group, superiority clinical trial will enroll 200 ICU-admitted patients at Imam Reza hospital, Mashhad, Iran. The patients will be randomly allocated to receive either a 100 ml solution of 15 % trehalose or normal saline intravenously. Primary outcomes include ICU mortality and 60-day mortality, while secondary outcomes focus on blood parameters on day 5 and length of hospital/ICU stay.

Conclusion: Trehalose has demonstrated beneficial effects in diverse patients; however, no study has evaluated its effect in all ICU-admitted patients. Consequently, this study provides an opportunity to investigate whether trehalose's anti-inflammatory effects, mediated by inducing autophagy and enhancing oxidative stress defense, can play a role in reducing mortality and improving clinical outcomes in the critically ill patients. If successful, trehalose could offer a potential therapeutic approach in the ICU setting.

1. Introduction

There is a high mortality rate among ICU-admitted patients which varies among different regions. The reported ICU mortality rate has ranged from 9.3 % in North America to 26.2 % in the Middle East and even higher numbers in Africa [1,2]. The high rate in developing countries is a result of constraints such as limited ICU beds, financial and infrastructural resources, and the burden of diseases such as trauma and tuberculosis. Longer ICU stay and more severe diseases lead to an increased burden on the ICU, which, in turn, are associated with higher mortality [3]. Therefore, it is crucial to endeavor towards shortening the duration of ICU stays and mitigate the severity of illness and mortality in patients admitted to the ICU.

One of the leading contributors to mortality among ICU patients is organ failure [4]. However, even without complete organ failure, these patients commonly experience varying degrees of organ dysfunction throughout the course of their illness [4]. Regardless of the severity of the organ failure at the time of admission, enhancements in organ function within the initial 24–48 h of ICU admission lead to lower mortality rates [5]. One of the major reasons for organ failure is the dysfunction of cell resulting from oxidative stress [6] and mitochondrial and endoplasmic reticulum dysfunction among ICU patients [5,7].

Oxidative stress is a problem in critically ill patients [8,9] and it has been proposed as a potential long-term mortality predictor in these patients [10]. Studies have shown the involvement of free radicals, specifically reactive nitrogen species (RNS) and reactive oxygen species

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(ROS), in multiple critical conditions [11,12]. Oxidative stress also activates nuclear factor-κB (NF-κB) [13,14]. Due to the highly reactive nature of the free radicals, these molecules damage cell structures such as proteins [15–17], lipids, and DNA [11,12].

Mitochondria play a role not only in energy production, but they are also involved in cell signaling, gene expression regulation, calcium modulation, and cell death pathways. Mitochondrial dysfunction impacts the development of various diseases and can lead to organ damage, as diseased mitochondria release toxic compounds such as mitochondrial DNA that eventually damage organs [18].

As mentioned earlier, cells in critically ill patients experience both oxidative stress and mitochondrial and endoplasmic reticulum dysfunction. Oxidative stress leads to mitochondrial dysfunction [19], while the latter itself induces oxidative stress [20–23]. Therefore, cell enters a vicious cycle leading to organ failure. The cell faces two options to break the cycle: removing impaired molecules and organelles in the process of autophagy or accepting the programmed cell death, apoptosis, which, if excessive, may subsequently lead to tissue damage and organ failure [24,25]. Hence, autophagy induction and antioxidant supplementation [26] may have the potential to reduce organ failure and enhance the clinical outcomes of ICU patients and lower both mortality and morbidity rates.

Autophagy, a vital cellular renovation process, fulfills an essential housekeeping function by clearing out impaired organelles like mitochondria [18], endoplasmic reticulum, and peroxisomes, as well as eliminating misfolded or aggregated proteins, and damaged DNAs, it also eradicates intracellular pathogens [27,28]. ICU-admitted patients show compromised autophagy levels [7]. Moreover, certain interventions measured in ICU-admitted patients, including insulin therapy and protein supplementation, inhibit autophagy [29]. Nevertheless, the value of autophagy is such that there is an ongoing debate about whether these interventions are more beneficial for the health of critically ill patients or preserving autophagy [29]. While mitochondrial dysfunction itself leads to impaired autophagy [30], autophagy induction is a critical defense mechanism to lower dysfunction of mitochondria and endoplasmic reticulum [7,18,28], resulting in lower cell dysfunction, organ dysfunction and mortality, respectively.

A naturally occurring sugar, trehalose, that has received growing attention in recent years, may offer a solution to the aforementioned complications by promoting autophagy and oxidative stress defense. Trehalose is a non-reducing disaccharide composed of two glucose units. The difference between trehalose and other commonly known saccharides, which consist of α-D-glucose including maltose and starch, lies in the glycosidic linkage. While maltose and starch have α-1,4 linkage, trehalose has α-1,1 linkage. Trehalose is a chemically stable molecule; it does not react with biological structures [31] and maintains its amorphous structure in dehydrated, low temperatures and other extreme conditions [32,33]. Despite vertebrates lack the ability to synthesize or store trehalose [32], they have the enzyme responsible for its metabolism, trehalase, in the brush border of the intestinal mucosa, kidney, liver, and blood plasma [34]. The metabolism of trehalose by trehalase in the human body produces two harmless glucose molecules.

The anti-inflammatory properties of trehalose are discussed [35] and confirmed in several in vitro and in vivo studies [36–40]. Trehalose administration has resulted in a significant decrease in the levels of inflammatory cytokines, including interleukin IL-6 and IL-8 in human eye cells [41]. Likewise, the administration of trehalose to rats with spinal cord injury decreased the IL- 1β, TNF-α, and nitric oxide levels [42]. It is suggested and confirmed by several animal studies that trehalose administration is effective in treatment of various conditions such as neurodegenerative disorders [43,44] including Parkinson's [45,46], Alzheimer's [47–49], and Huntington's diseases, atherosclerosis [50,51], stroke [52], fatty liver disease [53,54], diabetes mellitus type II [55–58], and pulmonary edema [37]. Although trehalose is composed of two glucose molecules, several clinical trials have shown its effect on lowering insulin resistance and preventing metabolic syndrome and type 2 diabetes [59–61]. Additionally, its effect on blood glucose levels, insulin secretion, and gastric inhibitory polypeptide (GIP) is significantly lower than that of glucose [62,63]. Moreover, trehalose has been demonstrated to improve microvascular artery resistance function, which is a risk factor for cardiovascular diseases [64].

There are various molecular mechanisms underlying the effects of trehalose [65]. Trehalose induces autophagy in a mTOR (mechanistic target of rapamycin)-independent manner [65–67]. It suppresses the activity of glucose transport proteins on the cell surface, leading to the phosphorylation of the autophagy-initiating kinase ULK1. This, in turn, enhances the activation of adenosine monophosphate kinase (AMPK), subsequently promoting autophagy initiation [53,68]. Additionally, trehalose activates transcription factor EB (TFEB) [69]. Trehalose reduces inflammation and protects cells against oxidative stress by inducing mitophagy [70], removing free radicals, inhibiting the nuclear factor NF-κB pathway and reducing inflammatory cytokines [37,71], higher expression of LC3 and P62 [72], regulation of the KEAP1-NRF2 pathway [73], shielding proteins from free radicals and preventing protein aggregation [71].

Investigations have shown that trehalose induces autophagy [66]. Additionally, trehalose promotes cellular defense against oxidative stress through an autophagy-independent manner [74]. It also stabilizes proteins and prevents protein aggregation and protects cell components from the effects of oxidative stress [65,75,76]. Furthermore, trehalose reduces inflammatory cytokines. These findings suggest that the potential therapeutic effects of trehalose in preventing cell dysfunction, cell death, organ failure, and eventually mortality in critically ill patients. However, to date, no studies have been conducted on the effectiveness of trehalose in improving the critically ill patients' outcome.

As explained, there is a need to improve clinical outcome and lower mortality rate in ICU-admitted patients. One of the major contributors to ICU-admitted patients' mortality is organ failure and lessening it lowers mortality rates. Organ failure occurs due to cell dysfunction or cell death caused by oxidative stress and mitochondrial and endoplasmic reticulum dysfunction. Trehalose is a sugar that is a candidate for lowering organ failure and improving ICU patient's clinical outcome and survival through inducing autophagy and strengthening cell defense against oxidative stress. This paper describes a randomized clinical trial protocol on administration of trehalose in ICU-admitted patients and evaluates its effect on clinical outcome and survival of ICU-admitted patients.

2. Objectives

We seek to investigate the efficacy of trehalose in mitigating the disease severity of ICU-admitted patients and improving their clinical outcomes. A positive outcome from this investigation may establish intravenous trehalose administration as a therapeutic option in the ICU setting.

3. Methods

The protocol for this triple-blind, randomized, placebo-controlled, two arm, parallel-group, superiority trial, was prepared in accordance with the SPIRIT and CONSORT guidelines and approved by the Medical Ethics Committee of the Mashhad University of Medical Sciences (IR.MUMS.REC.1402.026). Also, our study design is registered under the code IRCT20130829014521N22 in the Iranian Registry of Clinical Trials.

4. Recruitment and consent

All eligible patients admitted to the ICU wards of the Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, will be recruited. The recruitment process will involve a comprehensive explanation of the study's objectives and procedures to the patients or their legal guardians prior to signing written informed consent forms.

5. Eligibility criteria

We will consider patients for possible enrollment in the study if they have the following inclusion and exclusion criteria.

6. Inclusion criteria

- Patients at least 18 years old regardless of their gender.
- Patients who have been admitted to the ICU within 48 h prior to inclusion.
- Patients with an APACHE-II score higher than 15, calculated using values recorded from 24 h before inclusion up to the time of inclusion.
- Patients who have provided signed informed consent, either by themselves or through their legal guardians.

7. Exclusion criteria

- Referral ICU transferred from other facilities after spending one week in another ICU
- Pregnancy and breastfeeding

8. Withdrawal criteria

Patients or their guardians may withdraw from the study at any time point. Also, in case of any complications that result from the intervention or treating physician's decision to hold the intervention patients will be excluded from the study.

9. Study design and setting

A total of 200 eligible patients who admitted to the ICU wards of Imam Reza Hospital, Mashhad, Iran will be enrolled. Eligible patients will be randomly allocated to two groups with an allocation ratio of 1:1. In the intervention group, 100 ml of a 15 g/100 ml trehalose solution (manufactured by Dr. Rajabi Pharmaceutical Co) with an intravenous infusion rate of 4 ml/min will be administered. The placebo group will receive normal saline infusion with the same volume. All vials will have the same shape and contents of each vial will be indistinguishable, to ensure the blind condition. All patients will receive routine nutritional and medical support. Patients will be examined daily for presence of adverse effects and any alteration in underlying disease severity. Patients will be evaluated for clinical and laboratory parameters at baseline and day 5. Also, survival of patients will be followed on day-60 after enrollment (Fig. 1).

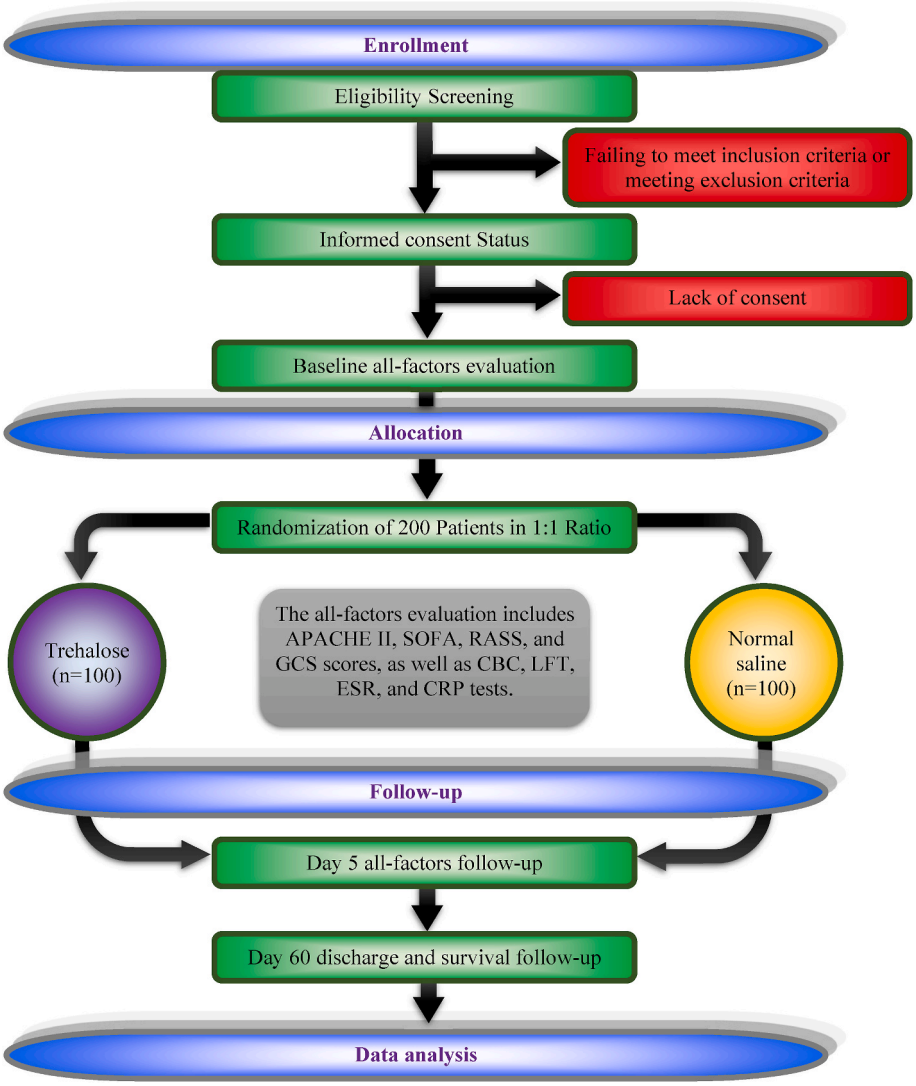

Fig. 1. - Flow diagram of the clinical trial protocol.

10. Power calculation and sample size estimate

Based on the outcome of the ICU-admitted patients in the 3 months before our study we estimated the mortality rate of the included patients to be 46 %. We postulated that the intervention will decrease the mortality rate by 20 % based on a 30 % mortality reduction in our pilot study on a sample of twenty ICU-admitted traumatic brain injury (TBI) patients [77]. Therefore, considering $\alpha = 0.05$ as a type 1 error and $\beta = 0.20$ as the type II error with a power of 80 % by using G * Power 3 software the sample size was calculated to be 91 patients in each study group. To adjust the sample size with presumed 10 percent dropout rate, we will aim to include 100 patients in each study group, allowing for potential dropouts while maintaining statistical power and significance for our investigation.

11. Randomization and blinding

Block randomization will be done using block sizes 4 and 6 with the randomization website (www.sealedenvelope.com). After randomization, the type of treatment is written A/B codes in separate papers. Using sequential numbering, a third party (who does not contact patients) will number the vials with specific numbers related to the randomized codes. Therefore, there will be 200 vials similar in shape and color with numbers 1 to 200. Neither patient nor the physician/nurse who will give the vials to the participants can distinguish any difference between vials other than numbers. Data from each patient will be recorded alongside the number of the vial administered to the patient, so the assessor will be blind. After entering data in the SPSS, a third party will change numbers 1 to 200 to codes A/B and data will be analyzed by comparing codes A and B by a blinded analyzer.

12. Patient and public involvement

This study exclusively focuses on ICU-admitted patients, and there will be no engagement of the general public in any aspect of its design, conduct, reporting, or dissemination.

13. Study objectives and endpoints

We aim to evaluate the effect of trehalose on 60-day mortality and the severity of disease in patients admitted to the ICU. We will also investigate the efficacy of trehalose administration on hospital and ICU mortality, length of hospital and ICU stay, and blood factors.

14. Primary and secondary endpoints

The primary outcomes are ICU mortality and 60-day mortality. For the secondary outcomes, we will evaluate the effect of trehalose on blood sugar, liver function tests, complete blood count (CBC), creatinine, and C-reactive protein (CRP). For this purpose, the blood sample will be taken before infusion and on day five. Also, clinical measures including blood pressure, Glasgow coma scale (GCS), acute physiology and chronic health evaluation (APACHE II) score, sequential organ failure assessment (SOFA) score, and Richmond agitation sedation Score (RASS) will be assessed at the study commencement and on day five. In case of discharging before the 5th day, follow-up assessments will be done before the discharge.

15. Physical examination and measurements

At baseline demographic and clinical status of patients will be recorded. The clinical status of the participants will be assessed by APACHE II score, SOFA score, GCS score, and RASS score. Compliance with the intervention and complications during treatment will be monitored daily until day five.

16. Laboratory analyses

The central clinical chemistry laboratory at the hospital will conduct all tests following a standardized protocol.

17. Safety

In this study, a single 15 g dose of trehalose will be administered to the patients. Several clinical trials have been performed [78–82] and are currently underway, investigating the intravenous administration of 15 g/week trehalose for 12 weeks in diverse patients and no adverse effect has been reported in these trials. Moreover, the enzyme accountable for trehalose metabolism, trehalase, occurs naturally in the brush border of the intestinal mucosa, kidney, liver, and blood plasma of humans [34]. The product of trehalose metabolism by trehalase is glucose, suggesting that no adverse effect is anticipated. Nonetheless, patients will undergo daily monitoring for the occurrence of any potential adverse effects. In cases with clinical signs of adverse drug reactions, trehalose administration will be discontinued and the patient will receive appropriate medical support.

18. Statistical analysis

The data analysis will be performed using SPSS (Version 16; SPSS Inc., Chicago, IL, USA) software. The normality of the distribution of continuous variables will be assessed using the Kolmogorov-Smirnov test. Normally distributed data will be summarized using mean and standard deviation and analyzed using parametric tests. On the other hand, data that are not normally distributed will be presented by the median and interquartile range and corresponding analysis will be accompanied by non-parametric tests. Within-group differences will be assessed using either the paired *t*-test (for normally distributed data) or the Wilcoxon sum-rank test (for non-normally distributed data). Meanwhile, between-group comparisons will be performed by either independent sample T-test (for normally distributed data) or Mann–Whitney *U* test (for non-normally distributed data). Frequencies and percentages will be used to present categorical variables. The comparison of 60-day mortality between groups will be conducted. Also, the Kaplan–Meier plot and Cox-hazard regression will be applied to evaluate the effect of trehalose on survival. A p-value less than 0.05 will be considered statistically significant.

19. Discussion

This paper outlines the methodology for a clinical study that follows a triple-blind randomized controlled trial design. The study aims to investigate the impact of a single intravenous 15 g dose of trehalose on critically ill patients admitted to the ICU. The anticipated outcome of this trial is to yield valuable insights regarding the efficacy of trehalose in relation to the clinical outcome and survival rates of the patients.

Declarations

Ethics approval and consent to participate:

The protocol of this clinical trial is approved by the Medical Ethics Committee of the Mashhad University of Medical Sciences (IR.MUMS.REC.1402.026).

Consent for publication

By participating in this clinical trial, participants or their guardians acknowledge the possibility of their data being used for publication purposes. However, all data presented in any publication will be anonymized to protect the participants' privacy and confidentiality.

Availability of data and materials

The current article does not involve the generation or analysis of any datasets; therefore, data sharing is not relevant for this article.

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CRediT authorship contribution statement

Mehrdad Sahranavard: Conceptualization, Writing – original draft. **Hesamoddin Hosseinjani:** Conceptualization, Writing – review & editing. **Maryam Emadzadeh:** Methodology, Writing – review & editing. **Tannaz Jamialahmadi:** Writing – review & editing. **Amirhossein Sahebkar:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

Not applicable.

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Abbreviation

(APACHE II): Acute physiology and chronic health evaluation
(CBC): Complete blood count
(CRP): C-reactive protein
(GCS): Glasgow coma scale
(ICU): Intensive care unit
(NF-κB): Nuclear factor-κB
(RASS): Richmond agitation sedation score
(RNS): Reactive nitrogen species
(ROS): Reactive oxygen species
(SOFA): Sequential organ failure assessment