JOURNAL OF NUTRITION FASTING AND HEALTH

The Effects of High-dose Selenium Supplementation on the Oxidative Stress Status and Inflammatory Markers in Critically Ill Pediatric Patients after Gastrointestinal Surgery: A Randomized Clinical Trial Protocol Study

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 22 Jun 2020
Accepted: 25 Jun 2020
Published: 20 Jul 2020

Keywords:
Selenium
Inflammation
Oxidative Stress
Intensive Care Unit
Pediatric

ABSTRACT

Introduction: Oxidative stress and inflammation could occur after major gastrointestinal surgeries. Selenium is a micronutrient with anti-inflammatory and antioxidant properties, which could improve the inflammatory markers in the children admitted to the intensive care unit (ICU) after gastrointestinal surgeries. Due to the lack of evidence on the potential effects of high-dose selenium on post-surgical critically ill children, the present study aimed to evaluate the effects of high-dose selenium supplementation on the levels of inflammatory markers and oxidative stress status in pediatric patients after gastrointestinal surgery.

Methods and analysis: We will conduct a single-blinded, randomized, parallel group superiority trial at Akbar Pediatrics Hospital in Mashhad, Iran. The sample population will consist of 70 patients undergoing gastrointestinal surgery, who will admit to the ICU at the selected hospital. The control group will receive the recommended dietary allowance (RDA) doses of selenium, and the intervention group will receive 20 µg/kg/d of selenium. The primary outcomes, (the pro-oxidant-antioxidant balance (PAB) status, interleukin-1 beta (IL-1β), and high-sensitivity C-reactive protein (hs-CRP)) will be measured before surgery and upon discharge time. The secondary outcomes, (serum glutathione peroxidase (GPX) level and serum and urine selenium levels), will be measured before surgery, after surgery, and upon ICU discharge time. We will perform the intra-group and inter-group data analysis in SPSS software, and we will consider the intention-to-treat approach, statistical significance level of <0.05, and 95% confidence interval in all the statistical analyses.

Trial Status: The study was initiated by enrolling eligible patients.

Article Summary
Strengths and Limitations of the Study
Strengths
- The effect of high-dose selenium supplementation will present in this study
- Block stratified randomization will be used for the allocation of the subjects
- Blinding the nursing system and patients' parents to the research procedures

Limitations
- The recommendation of an in-between range value for the supplementation dose, could elaborate a suboptimal effect

Non-blinding of the corresponding physicians and investigators

- *Please cite this paper as*:
Roudi F, Sezavar M, Rajabi O, Safarian M, Khademi Gh, Nematy M, Ranjbar G. Effects of High-dose Selenium Supplementation on the Oxidative Stress Status and Inflammatory Markers in Critically Ill Pediatric Patients after Gastrointestinal Surgery: A Randomized Clinical Trial Protocol Study. J Nutrition Fasting Health. 2020; 8(3): 159-168. DOI: 10.22038/jnfh.2020.48170.1261

Introduction

According to the literature, major gastrointestinal surgeries are associated with acute stress responses through metabolic and hormonal alterations, as well as increased inflammatory mediators in the body and production of free oxygen radicals mainly in the surgical site (1). It is believed that oxidative stress and inflammatory responses induce immunosuppression, cell necrosis, apoptosis, and insulin resistance, thereby increasing the length of hospital stay in surgical patients (2-4).

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Selenium plays a key role in the endogenous antioxidant defense mechanism through protecting the body against lipid peroxidation and regulation of glycolysis pathways and the activity of lymphocyte B and T cells and natural killer cells (2, 3, 5, 6). Notably, selenium is associated with cellular metabolism as an essential micronutrient due to its presence in selenoenzymes and selenoproteins(5). Several studies have confirmed the beneficial effects of selenium supplementation on diseases with inflammatory pathophysiology, such as rheumatoid arthritis, asthma, and inflammatory bowel disease. (6, 7).

According to the literature, serum selenium levels are directly correlated with the severity of oxidative stress and organ failure, while increased serum selenium levels are associated with improved clinical outcomes, such as the reduced duration of mechanical ventilation, length of ICU stay, and 28-day mortality (3, 8-10). According to previous pediatric studies which were conducted in critically ill children, the majority of critically ill children had low serum selenium levels upon admission to the intensive care unit (ICU) and low serum selenium may be linked to the increased incidence of multiple organ failure and deteriorated clinical outcomes in PICU patients (8).

Although serum selenium concentrations decrease as part of systemic inflammation and should be interpreted in combination with systemic inflammatory markers in patients with systemic inflammatory responses, serum selenium concentrations of less than 50 ng/mL are associated with the significant reduction of selenoproteins transcriptions under such circumstances (11). Therefore, even in acute phase response, serum selenium level for sufficient function must be > 50 ng/mL that this minimum level had not achieved in previous studies. For instance, in the study that was conducted by Leite and his colleagues, the mean serum level of selenium was reported to be 23.4 µg/L in 99 children with systemic inflammatory responses (3, 4). In addition,according to a recent clinical study by Broman et al., the majority of critically ill children had low serum selenium levels upon admission to the intensive care unit (ICU; 40% lower than the reference group). Low serum selenium may be linked to the increased incidence of multiple organ failure and deteriorated clinical outcomes in PICU patients (8). Moreover, according to the results of CRISIS study, which published by Carcillo et al. and Heidemann et al., 56% (156/278) of critically ill children had low serum selenium levels upon admission to the PICU (12, 13).

Since the metabolic stress state is associated with a short-term increase in the selenium requirement, the physiological doses may be insufficient for metabolic and antioxidative maintenance in these patients (14, 15).

Pediatric observational studies and interventional studies conducted on adults have indicated that high-dose selenium supplementation may improve the inflammatory and oxidative stress indices, as well as the clinical outcomes in patients with severe oxidative stress and inflammation (14, 16-18).

It is also notable that serum selenium levels could decrease in major gastrointestinal surgeries due to several factors, such as endothelial injury, redistribution, altered metabolic processes with varied consumption, and insufficient dose of selenium intake in such critical conditions for pediatric patients (2, 4, 8, 12, 19-23).

To the best of our knowledge, no prior studies have investigated the potential anti-inflammatory and antioxidative stress effects of high-dose selenium supplementation on critically ill pediatric patients after gastrointestinal surgeries. In the present study, we hypothesized that compared to the critically ill pediatric patients receiving only the RDA doses of selenium, the oxidative stress and inflammation status will be lower in the patients receiving high-dose selenium supplementation.

Materials and Methods

Study Design

The study protocol (version 2, 7/13/2019) describes the design of a single-blinded, randomized, parallel group superiority trial.

Research Objectives

Evaluation of Changes

We will evaluate the changes in serum concentrations of inflammatory markers (IL-1β, hs-CRP, and neutrophil-to-lymphocyte ratio) in the selenium supplementation and placebo groups, as well as between the groups and in the patients admitted to the PICU. We will measure the levels of IL-1β and hs-CRP in two stages of before surgery (day -1) and upon ICU discharge.

The other parameters will be the white blood cells (WBC), polymorphonuclear *leukocytes* (PMN), and lymphocytes (LYM), which will be measured in triplicate at the three intervals of day -1 (before surgery), day +1 (after surgery), and upon ICU discharge.

Changes in the severity of oxidative stress will be measured using the glutathione peroxidase (GPX) and PAB assay in the selenium supplementation and placebo groups, as well as between the groups. The PAB assay will be performed in two stages of before surgery (day -1) and upon ICU discharge. GPX will be measured in triplicate at the three intervals of day -1 (before surgery), day +1 (after surgery), and upon ICU discharge.

The serum levels of selenium will be measured in the selenium supplementation and placebo groups, while comparing between the groups. Moreover, serum and urine selenium will be measured in triplicate at the intervals of day -1 (before surgery), day +1 (after surgery), and upon ICU discharge.

Changes in the length of ICU stay, length of hospital stay, duration of ventilator dependency, incidence of hospital infections, and 28-day mortality will also be evaluated.

Inclusion Criteria

1. Age of 0-10 years;
2. Undergoing major gastrointestinal surgeries;
3. Admission to the ICU due to critical medical conditions;
4. Written informed consent provided by the parents or legal guardians

Exclusion Criteria

1. Non-eligible patients to receive nutritional support care within the first 24-48 hours postoperatively;
2. Baseline (before surgery) serum selenium concentrations of >106 µg/l (>95% confidence interval of Iranian children reference range) (24);
3. Diagnosis of autoimmune disorders, cancer, severe hepatic failure, renal failure, HIV infection, and severe sepsis upon admission;
4. History of chemotherapy and radiotherapy in the past month;
5. Severe and active bleeding;
6. Preterm neonates;
7. Earlier PICU discharge than five days after surgery

Subjects

The sample population of the present study will consist of the pediatric patients undergoing major gastrointestinal surgeries at Akbar Hospital in Mashhad, Iran, including esophageal atresia, intestinal atresia, biliary atresia, obstruction, gastroschisis, omphalocele, hirschsprung, gastric pull-up, and diaphragmatic hernia. The research coordinator will provide the details of the research procedures to the parents or legal guardians of the patients, and obtain the written informed consent from those willing to participate. Eligible patients will recruit and equally divide into two groups of selenium supplementation and placebo.

A customized step-by-step protocol will develop and implement in accordance with the recent guidelines of nutrition for the children admitted to the ICU (i.e.: ESPEN, ASPEN, and ESPGHAN guidelines)(25-29). Clinical nutritionists will visit and monitor the subjects daily. Anthropometric indices (weight, height/length, and head circumference) will be measured at the outset of the study. Additionally, we will measure weight every day during the ICU admission of the patients. Venous blood and urine samples will be obtained on day -1 (before surgery), day +1, and +10 (discharge from ICU; after surgery) in order to assess the oxidative stress status and inflammatory markers. Finally, Clinical nutritionists will monitor lack of administration with any of the antioxidant supplementations (e.g., vitamin C, vitamin E, and beta-carotene).

Sample Size

The primary outcome for the study is the mean of hs-CRP level at ICU discharge time.

Assuming a SD of 6 points for the hs-CRP, a mean difference of 4 or more points is clinically and practically important (15, 30). Therefore, the sample size has been calculated to have a 80% power of detecting this 4 point difference (equivalent to a standardised effect size of 0.7) in hs-CRP concentrations at ICU discharge time as being statistically significant at 5% (two sided) level.

Intervention and Randomization

We will use block stratified randomization to equally divide the patients into two groups, as follows:

1-*Intervention group*: Selenium supplementation will be continued with the dose of 20 µg/kg/day for 10 days or until discharge from the ICU/death

of the patient (minimum intervention duration: five days). High-dose selenium will be injected intravenously with 20 milliliters of 5% dextrose within 60 minutes. Afterwards, injectable selenium will be administered at the concentration of 50 µg/ml in the form of sodium selenite.

2-*Control group*: The placebo (serum: 5% dextrose) will administered with the same volume to the patients in the control group for 10 days or until discharge from the ICU/death.

All the patients receiving enteral and parenteral nutrition will receive the RDA dose of selenium, while the RDA dose in total daily intake of selenium will be considered at this stage. As such, the patients in the control group will receive the RDA dose of selenium, and the intervention group will receive 20 µg/kg/day of selenium considering the enteral/parenteral RDA dose.

The random allocation of the patients to the intervention or control groups based on their nutritional status classification, which encompasses parameters such as the WHO weight for length, height, and body mass index (BMI) z scores for the ages of 0-2, 2-5, and >5 year, respectively, surgery type (upper/lower gastrointestinal tract), and admission to the neonatal intensive care unit (NICU)/PICU. Firstly, we will apply the random numbers table to this end, and the subjects in the selenium supplementation group will be selected for the intervention. The subjects in the placebo group will be matched to the selenium supplementation group. With the exception of pharmacists, corresponding physicians, and researchers, the patients and nursing team will be blinded to the supplement/placebo assignments. F. R. and H. E. generated an online stratified sequential randomization plan, which will be used to develop the allocation sequence and assign the participants to the placebo and selenium supplementation groups by the researchers.

Dose Determination

We had concerns about the dose of selenium supplementation in the protocol and our research team established several expert panels on the dose determination in the study, and there were numerous concerns in this regard. After several discussion panels on the current literature, it was concluded that this dose will be safe and probably beneficial for short-term supplementation in the patients admitted in the pediatric intensive care unit (PICU).

In this study the major concern was about children < 1 year old because we could easily estimate the safe dose of the supplements for participants> 1 year, according to their body surface. Given that several studies in adults reported 1000 mg/day dose with no adverse effects (31, 32), by multiplying the ratio of the child's body surface (1-10 years) to adult body surface (mean= 1.73 m2) dose, where the range of estimated dose was between 28-20 mg/kg/d (1-10 year old children) (33).

The optimum dose of short-term selenium supplementation for children was estimated using the NAIR equation, which was applied to convert the lowest dose at which there was an observed toxic or adverse effect into the optimum dose of various drugs in human models (equivalent dose for children: 48 µg/kg/d) (34, 35).

Considering the estimated selenium dose of 48 µg/kg/d based on the NAIR formula in terms of the established RDA for selenium (2 µg/kg/d), as well as the lack of similar studies on high-dose selenium supplementation at higher concentrations than 10 µg/kg/d in children, the selenium dose was estimated to be within the range of 10-48 µg/kg/d. Therefore, 20 µg/kg/d of selenium was recommended for the children admitted to the ICU after gastrointestinal surgeries.

Safety Monitoring

Subspecialists of pediatric critical care, neonatologists, ICU pediatrician staff, and the corresponding researchers will be aware of the randomly allocated data. Selenium poisoning will beevaluated based on various clinical signs and symptoms, such as changes in the neurological and digestive systems, hair and nail structures, and blood selenium levels. The procedures would be discontinued in case the quantities were higher than 0.813 µg/mL(36).

Assessments and Measurements

Anthropometric Indices

Weight will be measured using a digital scale (Balas) with minimum clothing with the accuracy of 10 grams. The length of the children younger than 24 months and height of those aged more than 24 months and above will be measured using a portable infantometer and stadiometer, respectively. However, using the standard methods of length/height measurement are not possible in all the cases, and a predictive

equation will be applied to estimate the stature based on the ulnar lengths (37, 38).

Primary Outcomes

Table 1 shows the schedule of the enrollment, interventions, and assessments in the present study.

Primary outcomes will be compared between the groups in terms of the differences in the final values and changes in the PAB status, IL-1β), and hs-CRP. These variables will be measured before surgery and upon discharge from the ICU using the PAB assay, ELISA assay, and immunoturbidimetry tests, respectively. These three primary outcomes were selected since they reflect the pro-oxidant-antioxidant balance and inflammation status and could be used in the other clinical trials performed in ICUs. Moreover, the primary endpoint was selected upon the ICU discharge (day 10; minimum: five days) in order to determine the early benefits of the minimal post-surgical anti-inflammatory effects of routine ICU care.

Table 1. Content for the schedule of enrolment, interventions, and assessments.

	STUDY PERIOD					
	Enrolment	**Allocation**	**Post-allocation**		**Close-out**	
TIMEPOINT**	*-t1*	*0* *Day -1 (Pre operation)*	*t1* *Day +1 (Post operation)*	t_2 *ICU discharge time (up to 10 days)*	T_3 *Hospital discharge time(*	T_4 *Day +28*
ENROLMENT:						
Eligibility screen	X					
Informed consent	X					
Allocation		X				
INTERVENTIONS:						
[Intervention A]			X	X		
[Intervention B]			X	X		
ASSESSMENTS:						
Anthropometrics		X		X	X	
CBC, diff		X	X	X		
BUN,Creatinine		X	X	X		
Inflammatory factors		X		X		
Serum selenium		X	X	X		
Urine selenium		X	X	X		
Serum GPX		X	X	X		
PAB assay		X		X		
Length of ICU stay				X	X	
Length of hospital stay					X	
Duration of ventilator dependency				X		
Possible infections			X	X	X	
Mortality			X	X	X	X

Secondary Outcomes

Measurement of serum GPX and serum and urine selenium will be performed on day -1 (before surgery), day +1, and upon ICU discharge (day 10; after surgery) using the ELISA assay and atomic absorption methods, respectively. The final values and changes during the study will be compared between the intervention and control groups, and the secondary outcomes were selected to assess the efficacy of the two utilized approaches. Table 2 shows the devices used for laboratory analysis and time intervals of the tests.

Table 2. Laboratory measurements, time points and equipment.

Number	Test	Time points of measurement	Test kit or equipment	Country	Company
1	CBC, diff[1]	Day -1, +1, discharge time/+10	Hematology Analyzer	Germany	Sysmex Models: XS-800i, XN-350
2	BUN[2]	Day -1,+1, discharge time/+10	Biochemical auto analyzer machine	Japan	Premium Biolis 24i
3	Creatinine	Day -1,+1, discharge time/+10	Biochemical auto analyzer machine	Japan	Premium Biolis 24i
4	HS-CRP[3]	Day -1, discharge time/+10	Biochemical auto analyzer machine	Japan	Premium Biolis 24i
5	IL1β[4]	Day -1, discharge time/+10	ELISA kit	Germany	Zellbio
6	Serum selenium	Day -1,+1, discharge time/+10	Atomic absorption spectrometer	USA	Perkin Elmer HGA-700 Graphite Furnace
7	Urine selenium				
8	Serum GPX[5]	Day -1,+1, discharge time/+10	ELISA kit	Germany	Zellbio
9	PAB[6] assay	Day -1, discharge time/+10	Stat Fax 2100 Microplate Reader	USA	AWARENESS

[1]CBC: Complete Blood Count, [2]BUN, Blood Urea Nitrogen, [3]HS-CRP: High-Sensitivity C-Reactive Protein, [4]IL1β: Interleukin 1 beta, [5]GPX: Glutathione peroxidase, [6]PAB: prooxidant-antioxidant balance

Clinical Outcomes

We will record the clinical outcomes of all the patients, including the length of ICU and hospital stay, 28-day mortality, duration of ventilator dependency, possible infections (e.g., hospital-acquired and ventilator-associated pneumonia), and systemic inflammatory response syndrome. Figure 1 depicts an overview of the study process.

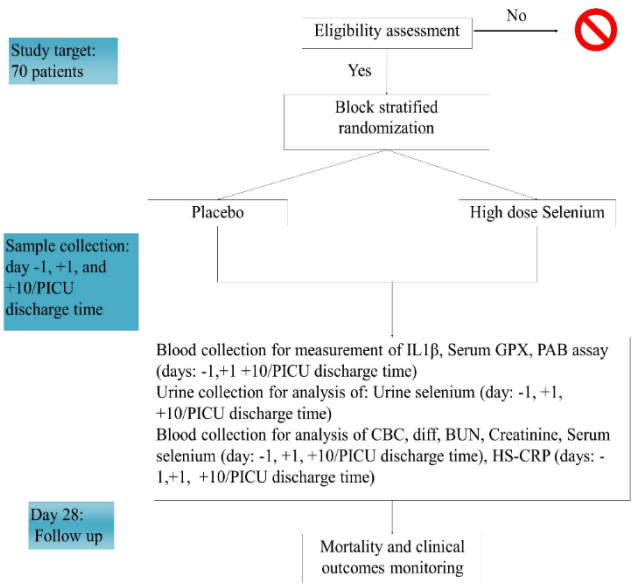

Figure1. Flowchart of study protocol.

Patients and Public Involvement

Six months prior to the research, face-to-face interviews were conducted with the parents or guardians of the children who underwent gastrointestinal surgeries and were admitted to the ICU regarding their expectations, experiences, and priorities, which was part of the teamwork in the current research to develop the research question, study's conception and design. Also, we plan to involve the participants and their parents in some aspects of the data collection and in creating and delivering the study dissemination plans.

Statistical Analysis

We will perform the data analysis in SPSS version 20 using the intention-to-treat approach at the significance level of <0.05 and 95% confidence interval. The demographic characteristics of the subjects, disease severity, lengths of PICU and hospital stay, mortality, and nutritional markers will be expressed using descriptive statistics in the form of appropriate tables and charts for each group. In addition, the normality of data will be examined using the Kolmogorov-Smirnov test. Intra-group comparison will be carried out to assess the normal distribution of the data using paired t-test.

In order to compare the quantitative variables between the two groups in case of parametric and non-parametric data and their homogeneity, independent t-test and Mann-Whitney U test will be applied, respectively. For the intra-group comparison of the variables that will be measured more than twice (GPX, serum and urine selenium, WBC, and PMN/LYM), we will use repeated measures ANOVA or Friedman's test. In addition, the analysis of covariance will be employed to control the confounding variables (nutritional status upon admission and length of ICU stay). Paired t-test will also be used to compare the intra-group variables that will be measured in both stages before and after the intervention (PAB assay, IL-1β, and hs-CRP).

Adherence Assessment

As the patients received the placebo/supplementation by nurses under the supervision of the researcher, adherence assessment was not required.

Ethics and dissemination

Ethical Approval, Protocol Amendments, and Dissemination Plan

This study was approved by the Ethics committee of Mashhad University of Medical Sciences (ethics code: IR.MUMS.MEDICAL.REC.1397.553). Any modification of the protocol that may affect the conduction of the study will be approved by MUMS Ethics Committee prior to implementation. Additionally, it will be announced to the IRCT registration dataset by the researchers. The primary RCT results will be submitted for publication to an international, peer-reviewed journal. Authorship eligibility will be based on the recommendations from the International Committee of Medical Journal Editors (ICMJE).

Patient Consent

The parents or legal guardians of the patients will provide the written informed consent, and after receiving their approval, the selenium/placebo supplementation will be initiated. Blood and urine samples will be collected, and the research coordinator will provide the details of the study to the parents or legal guardians of the patients.

Confidentiality

The identifiable information of the patients will be stored at the security site, and all the data collection and reports will be identified by a coded ID number.

Data Sharing Statement

The de-identifiable data will be available only to the researchers. The data could be obtained via email by Dr. Mohsen Nematy (Email: nematym@mums.ac.ir). Other researchers in academic institutions could also send their request to Dr. Mohsen Nematy via email, and they will receive the data after consultation and approval of the research team.

Data Monitoring

One pediatrician (academic professor) monitored the data and audited the trial monthly independent of the investigators without any competing interests.

Intervention Discontinuation

In case of intervention-related complications, the obtained results were presented to the Ethics Committee of Mashhad University of Medical Sciences (MUMS) for decision-making. The supplementary files contain the data on the ethical approval, protocol summary of the Iranian Registry of Clinical Trials, informed consent forms (in Persian), and the completed

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist.

Discussion

Selenium is an essential trace element for the body, which plays a pivotal role in antioxidant defense mechanisms through its association with selenoproteins, such as GPX, thioredoxin reductases, and iodothyronine deiodinases (29). The antioxidant enzymes that are involved in the defense mechanism of the body against oxidative stress (e.g., catalase superoxide dismutase and GPX) require essential cofactors, such as micronutrients (e.g., selenium) (30). Several experimental studies have confirmed the protective role of selenium against oxidative stress (29). Selenocysteine is a form of selenium that is involved in a large number of antioxidants enzymefunctions, as well as multiple steps of intracellular antioxidant defense mechanisms (31).

According to the literature, inflammation, surgeries, and sepsis are associated with low plasmatic levels of selenium (8, 14, 19). In children, serum selenium levels reflect the severity of inflammation and nutritional status (20). For instance, Leite et al. conducted a study on 99 children with systemic inflammatory responses, and the mean serum level of selenium was reported to be 23.4 µg/L, which was below the normal range (46 µg/L) in 90.9% of the patients (3).

Recent findings have indicated that the metabolic stress state is associated with increased selenium requirement, and the physiological dose administration of this micronutrient may be insufficient for the reduction of the oxidative stress status and inflammatory markers (8, 39). Interestingly, studies performed on adult patients have shown that high-dose selenium supplementation may improve the clinical outcomes in the patients admitted to the ICU (9). The literature search revealed that no studies have yet evaluated the effects of selenium supplements with higher doses than the physiological values in critically ill children after gastrointestinal surgery. Therefore, previous studies highlight the importance of selenium as an antioxidant and anti-inflammatory agent for the clinical improvement of these patients. The present study aimed to assess the effect of high-dose selenium supplements on the levels of inflammatory markers and oxidative stress status in critically ill pediatric patients after gastrointestinal surgery.

Trial Status

During the submission of this document, the sampling and assessments of the patients started.

Authors' Contributions

F. R., H. R., M. N., G. K., G. R., and H. E. were involved in the study design, and will contribute data collection, and data analysis. M. N., F. R., H. E., and G. R. contributed to development of the protocol. All the authors read and approved the final manuscript.

Acknowledgements

Hereby, we extend our gratitude to Dr. Omid Rajabi, Dr. Majid Sezavar, Dr. Abbas Boskabady, and all the medical staff at Akbar Hospital for assisting us in this research project.

Funding/Support

This study will financially be supported by the Deputy of Research and Technology at MUMS (grant #971045). (Qoreishi Center, Daneshgah Street, Mashhad, Iran; Email:(vcresearch@mums.ac.ir). All the study stages will perform under the supervision of the same institution.

Conflicts of interest

None declared.

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