


STUDY PROTOCOL

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# Effects of melatonin supplementation on metabolic parameters, oxidative stress, and inflammatory biomarkers in diabetic patients with chronic kidney disease: study protocol for a double-blind, randomized controlled trial

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## Abstract

**Introduction** Chronic kidney disease (CKD) is a pervasive disease of the current century that usually affects the adult population, especially people with diabetes and hypertension. According to the recent studies, inflammation, oxidative stress, apoptosis, and mitochondrial dysfunction are determining risk factors in the pathogenesis of CKD. Melatonin as a strong antioxidant is produced in various tissues including the kidneys. The present clinical trial aims to examine the efficacy of melatonin supplementation on metabolic parameters, oxidative stress, and inflammatory biomarkers in diabetic patients with CKD.

**Methods** This is a double-blind, randomized, placebo-controlled clinical study that will be investigated the impacts of melatonin supplementation in diabetic patients with CKD. Laboratory findings will be applied to diagnose diabetic CKD. Forty-eight eligible diabetic subjects with CKD will be selected and randomly assigned to receive 5 mg melatonin tablets or identical placebo twice daily for 10 weeks. Participants will be asked to remain on their usual diet and physical activity. The primary outcome of this study is changes in oxidative stress and inflammatory biomarkers. The secondary outcomes include changes in lipid profile, renal function indicators, fasting blood sugar and serum insulin, systolic and diastolic blood pressure (SBP and DBP), serum phosphorous concentration, sleep quality, body weight, body mass index (BMI), and waist circumference (WC). Statistical analysis will be conducted using the SPSS software (version 25).

**Discussion** We hypothesize that melatonin administration may be useful for treating diabetic CKD by modulating oxidative stress, inflammation, regulating lipid metabolism, and increasing insulin sensitivity through different mechanisms. The current trial will exhibit the effects of melatonin, whether negative or positive, on diabetic CKD status.

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**Ethical aspects** The current trial received approval from Medical Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.SHARIATI.REC.1402.072).

**Trial registration** This study had been registered in Iranian Registry of Clinical Trials. Registration number: IRCT20170202032367N9 on 11 August 2023. <https://www.irct.ir/trial/70709>.

**Keywords** Chronic kidney disease, Melatonin, Metabolic parameters, Oxidative stress, Inflammatory biomarkers

## Introduction

CKD is a pervasive disease of the current century that usually affects the adult population, especially people with diabetes and hypertension [1, 2]. Almost 40% of diabetic patients develop CKD as a typical microvascular complication [3, 4]. Epidemiological studies have shown that more than 700 million adults (9% of global population) suffer from CKD worldwide [5]. In Iran, 27.5% of the population (24% in male and 30.3% in female) are involved in this clinical pathological condition [6]. Since the prevalence of CKD is increasing, cardiovascular morbidity and mortality risk is growing rapidly, emphasizing early prevention and treatment of the CKD [7].

Due to the great importance of finding an appropriate strategy to manage this condition, several studies have been performed [8]. Recent studies have shown that inflammation, oxidative stress, apoptosis, and mitochondrial dysfunction are determining risk factors in the pathogenesis of CKD [9]. Considering the major role of oxidative stress in the progression of CKD, treatment using antioxidants can be effective in this field. Melatonin as a strong antioxidant is produced in various tissues including the kidneys and other tissues like pineal gland. Also, melatonin could exert intracrine, autocrine, or paracrine functions [10]. The results of the CREAM study indicate a direct relationship between disruption of melatonin secretion rhythm and kidney dysfunction [11]. Melatonin can play its antioxidant role directly by scavenging free radicals or indirectly by increasing the activity of antioxidant enzymes (SOD, catalase, GPX) and decreasing the activity of oxidant ones [12]. There are several studies that administered melatonin supplements as a strategy for modulating oxidative stress and inflammation and reported favorable effects [13–17].

Also, lipid profile abnormalities are common in CKD patients. In the early stages of kidney failure, changes in lipoprotein metabolism and activity of some key enzymes are the causes of dyslipidemia. In the final stages, metabolic abnormalities may further progress and negatively impact kidney function [18]. Since the most common lipid-lowering medications (statins and fibrates) may cause adverse effects including hepatotoxicity and myopathy, identification of supplemental agents with the ability to modulate lipid profile has attracted much attention. Melatonin is also effective in regulating lipid metabolism

and can improve dyslipidemia by increasing insulin sensitivity, elevating lipoprotein lipase (LPL) activity, reducing fat tissue lipolysis, increasing LDL receptor activity, inhibiting cholesterol absorption from the intestine, and converting cholesterol into bile acids. In a systematic review and meta-analysis of randomized controlled trials, Mohammadi et al. concluded that melatonin supplementation can induce dramatic favorable effects on triglyceride and total cholesterol levels [19].

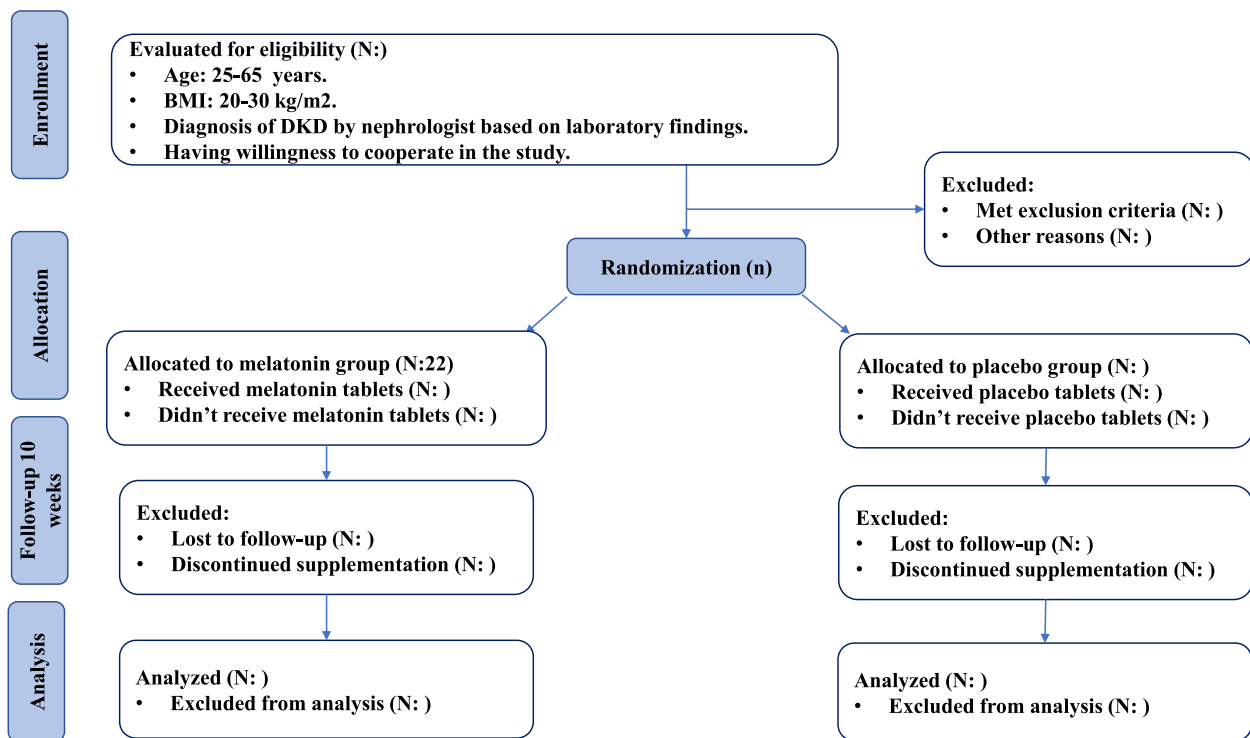
As mentioned above, the positive effects of melatonin in various metabolic disorders have been proven. However, there is no study about CKD patients exclusively. Therefore, we designed this randomized, placebo-controlled, parallel clinical trial to examine the effects of melatonin supplementation on oxidative stress, inflammatory biomarkers, and lipid profile in diabetic patients with CKD.

## Patients and methods

### Participants

The present study is a phase III parallel randomized double-blind placebo-controlled clinical trial (RCT) among diabetic patients with CKD in the stages before dialysis (stage 3–4). This clinical trial is registered at the Iranian Registry of Clinical Trials (ID: IRCT20170202032367N9). CKD will be confirmed by a nephrologist through the use of laboratory findings. This study is designed to be conducted at Shariati Hospital, affiliated with Tehran University of Medical Sciences, Tehran, Iran. All participants will read and sign the informed consent. We will request consent for review of participants' medical records and for the collection of blood samples to assess oxidative stress, inflammatory markers, lipid profile, renal function indicators, fasting blood sugar and serum insulin, and serum phosphorous concentration. This study has already been approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.SHARIATI.REC.1402.072). We developed the study protocol based on Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 checklist (Supplemental File 1). Timeline of the trial and study flow chart of enrolment, allocation, intervention, and assessment are presented in Table 1 and Figs. 1 and 2, respectively. Before the study is conducted, any changes to the current protocol that have to do with patient safety and welfare,





**Fig. 2** Timeline of the trial

**Table 1** Timeline of the trial

| Explanation of the trial activities        | Time (month) |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |
|--|--------------|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|
|  | 1            | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Material preparation                       | *            | * | * | * |   |   |   |   |   |    |    |    |    |    |    |    |
| Recruitment                                |              |   |   |   | * | * | * | * |   |    |    |    |    |    |    |    |
| Clinical assessments at baseline           |              |   |   |   | * | * | * | * |   |    |    |    |    |    |    |    |
| Nutritional assessments at baseline        |              |   |   |   | * | * | * | * |   |    |    |    |    |    |    |    |
| Biochemical assessments at baseline        |              |   |   |   | * | * | * | * |   |    |    |    |    |    |    |    |
| Intervention                               |              |   |   |   |   |   |   |   | * | *  | *  | *  | *  |    |    |    |
| Clinical assessments after intervention    |              |   |   |   |   |   |   |   | * | *  | *  | *  | *  |    |    |    |
| Nutritional assessments after intervention |              |   |   |   |   |   |   |   | * | *  | *  | *  | *  |    |    |    |
| Biochemical assessments after intervention |              |   |   |   |   |   |   |   | * | *  | *  | *  | *  |    |    |    |
| Data analysis                              |              |   |   |   |   |   |   |   |   |    |    |    |    | *  | *  |    |
| Writing the final report of the trial      |              |   |   |   |   |   |   |   |   |    |    |    |    |    |    | *  |
| The expected time                          | *            | * | * | * | * | * | * | * | * | *  | *  | *  | *  | *  | *  | *  |

**Study design**

Individuals who meet the inclusion criteria will be enrolled. After recruitment, participants will pass a 2-week run-in period, during which three 24-h food recalls (2 working days and 1 weekend day) and two 1-day physical activity records will be completed for each patient to collect demographic and dietary

information. To measure biochemical parameters, 10-mL overnight fasting venous blood samples will be obtained from each patient. At the end of run-in period, anthropometric measures, oxidative stress and inflammatory biomarkers, lipid profile, glycemic status, renal function, blood pressure, serum phosphorous level, and sleep quality will be evaluated.

### Randomization

After recruiting participants, we will use stratified block randomization, in which participants will be stratified based on age (under 30 and above 30) and sex (male/female) into different blocks. For each patient in a certain block, a matched person in terms of age and sex would be placed in other block. Then, the two patients in a single block would be randomly assigned into the intervention and control groups. We will employ a 1:1 allocation ratio to ensure equal representation in both groups. Furthermore, our study is designed as a superiority trial. A third person who is not aware of the study's aim will randomly allocate participants using a computer-generated random sequence (sequentially numbered).

### Blinding

All patients, researchers, nephrologist, and laboratory staff will all be blind to the intervention. The supplements used in both groups are identical in shape, color, and smell. The supplements will be given to both groups packing in similar containers. The supplement cans separated by the letters A or B. The blinding code will be unknown until the end of the study except in emergency cases. Study participants will take their bottles in two times: at their first visit and in the middle of the trial, at week 5.

### Study intervention

Patients in the experimental group ( $N=24$ ) will receive 5 mg melatonin supplements twice a day for 10 weeks. Patients in the control group ( $N=24$ ) will consume placebo tablets containing starch. Participants in both groups will be requested to take the tablets before bedtime for 10 weeks. Weight, size, shape, taste, color, smell, and lot number are the same in melatonin and placebo capsules. Even the patients in the placebo group will not be deprived of their main medical treatment during the study. There is no anticipated harm and compensation for trial participation; if any of the participants have a problem, necessary treatments will be done for them. Safety of melatonin supplementation was confirmed by study pharmacist. All subjects will be asked not to change their routine daily diet, physical activity, and medicines. There will be no special criteria for discontinuing or modifying allocated interventions. Melatonin or placebo will not require alteration to usual care pathways (including use of any medication) and these will continue for both trial arms. If any of the participants does not consume 10% of their capsules, this state is considered as a research dropout and is excluded from the study.

### Outcomes

#### *Primary and secondary outcomes*

The main primary outcome in the current study would be oxidative stress biomarkers including total antioxidant capacity (TAC), total oxidative stress (TOS), and malondialdehyde (MDA) as well as inflammatory markers including interleukin-6 (IL-6) and highly sensitive C reactive protein (hs-CRP). The study secondary outcome variables would be lipid profile (total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL)), renal function indicators (blood urea nitrogen (BUN), creatinine, uric acid), fasting blood sugar and serum insulin, systolic and diastolic blood pressure (SBP and DBP), serum phosphorous concentration, sleep quality, weight, BMI, WC, energy intake, and consumption of energy-contributing nutrients including carbohydrates, fat, and protein.

#### *Measurements and assessments*

##### *Dietary intake and physical activity*

To assess dietary intakes throughout the study, they will be asked to fill three 1-day dietary recalls at the beginning of the study, weeks 5 and 10 of intervention. Dietary recall format is presented in the online supplemental file. We will compute food and nutrient intake of study participants based on the examination of these dietary recalls. The Nutritionist 4 software, which was modified for Iranian foods, will be used to perform this calculation.

In addition, two 1-day physical activity records will be gathered from all participants to examine the difference in activity between two groups. In order to analyze physical activity records, we will use MET-h/day values for each type of physical activity, based on published guidelines [20], considering the time spent by each participant.

##### *Anthropometric measures*

Data on anthropometric indices including body weight, height, WC, and BMI will be collected at study baseline and end of the trial. Participants will be weighed in a fasting state, without shoes with minimal clothing to the nearest 0.1 kg accuracy, using a digital scale. Standing height will be measured using a standard stadiometer without shoes with an accuracy of 0.5 cm. WC will be measured to the nearest 0.1 cm accuracy by non-stretching tape measure around the abdomen at the distance between the suprailiac bone and the last rib. BMI will be calculated by the measured height and weight ( $\text{weight (kg)/height (m}^2\text{)}$ ).

##### *Clinical outcomes*

Systolic and diastolic blood pressures will be measured twice at the right arm using a mercury barometer



calibrated by the Institute of Standardization and Industrial Research, with a 15-min interval in between measurements, while the patient is sitting quietly for 5 min. The average of the two measurements will be analyzed to calculate the systolic and diastolic blood pressures. Before starting the intervention and at the end of trial, the sleep quality of the patients will be evaluated using a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval. Nineteen individual items generate seven “component” scores. The sum of scores for these seven components yields one global score: Pittsburgh Sleep Quality Index (PSQI) [21].

#### Other biochemical variables

At the study baseline and after the intervention, a 10-mL venous blood sample will be taken from each person after 12-h fasting. Then, serum will be isolated from whole blood by centrifugation for 10 min at 3500 rpm and for further analysis serum samples will be stored at  $-80^{\circ}\text{C}$ . The process of accessing serum concentrations of inflammatory factors and oxidative stress biomarkers (TAC, TOS, MDA, IL-6, hs-CRP) will be done by the enzyme-linked immunosorbent assay (ELISA) commercial kits.

#### Sample size calculation

Considering the type I error of 5% ( $\alpha=0.05$ ) and type II error of 20% ( $\beta=0.20$ , power = 80%) and total antioxidant capacity (TAC) as the key variable [13], we manually, without the use of any software, calculated required sample size using the following formula:

$$n = ((z_{(1-\alpha/2)} + z_{(1-\beta)})^2 (S_1^2 + S_2^2)) / \Delta^2$$

$n$  = sample size in each group  
 $\alpha$  = type 1 error = 0.05  
 $\beta$  = power = 80%  
 $S_1^2$  = variance of the intervention group = (173)<sup>2</sup>  
 $S_2^2$  = variance of the control group = (108)<sup>2</sup>  
 $\Delta^2$  = minimal clinically important difference = (86)<sup>2</sup>

Based on this formula, we reached a sample size of 22 people in each group. Considering a 10% likelihood of dropping out, the number of participants in each group increased to 24 people.

#### Data management and monitoring

A clinical trial monitor occasionally supervises the study progress, ensures patient rights and well-being are safeguarded, the protocol, ethical requirements, standards, and regulations are being followed, the essential documentation is available, and collected data are accurate as there were recorded. One of the investigators will check the coding, security, and storage of data. In addition, he/she will evaluate data entry and data values twice. If any participant

reports the occurrence of adverse events, more information is required to make decision about excluding the participants from the trial. Unblinding is permissible in this situation based on the Medical Ethics Committee criteria.

#### Adherence to the intervention

The study progress will be pursued by calling the patients once a week to ensure that they regularly consume the tablets. Adherence to the intervention will be checked by counting the returned tablets at the half and end of the trial visits. Compliance rate will be computed according to the following formula and poor compliance will be considered as less than 90% [22].

$$\text{Compliance rate} = (\text{Capsules taken} / \text{Capsules prescribed}) \times 100$$

#### Statistical analysis

Statistical analysis will be conducted using the SPSS software (version 25, SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test will be applied to examine the normality of data. Moreover, we will use chi-square test and Fisher’s exact test to compare categorical variables. Also, independent sample *t*-test and Wilcoxon rank-sum test will be applied to compare continuous variables within-group, whereas we will use paired sample *t*-test and Mann–Whitney *U* test for between group comparisons. Normally distributed variables will be reported as the mean and standard deviation, while the median and interquartile range (IQR) will be used for reporting non-normally distributed variables. To compare the differences in primary and secondary outcomes between the two study groups at the end of the trial and also adjust the final findings for potential confounders, we will apply the analysis of covariance (ANCOVA) test. Subgroup analysis or adjusted analysis will not be applicable. A *p* value less than 0.05 will be regarded as statistically significant. If we have missing data, statistical analysis will be done using the intention to treat (ITT) method by imputation.

#### Discussion

DM as the leading cause of diabetic kidney disease (DKD) is one of the most common diseases of the present century [2, 23]. People with DKD may develop ESRD more rapidly, compared to people with non-diabetic CKD [24, 25]. And since there is no definitive treatment for ESRD, preventing the disease from progressing to end stage can improve the patient’s quality of life, relatively [26]. Treatment with antioxidant agents is one of the main topics of interest for the management of DKD [27].

Melatonin or N-acetyl-5-methoxytryptamine is a natural antioxidant of the body, which belongs to the indolamines group [28]. A large number of experimental studies have investigated the favorable effects of

melatonin on human health. Melatonin supplementation may protect the body against several pathophysiological conditions by scavenging free radicals and modulating apoptosis and autophagy [29, 30]. Moreover, a growing body of evidence from animal studies found that melatonin supplementation not only increases the levels of SOD and catalase activity and prevents increasing MDA and MPO levels, but also reduces the excessive penetration of immune system cells into the kidney [31–34]. As a result, subsequent activation of inflammatory mediators such as TNF- $\alpha$  and IL-1 $\beta$  occurs less [35]. Furthermore, many experimental studies concluded that melatonin has some properties that make it able to prevent lipid peroxidation. For example, it has high fat solubility and easily passes through cell membranes [36], scavenges the hydroxyl and peroxy free radicals that play a major role in the initiation and spreading lipid peroxidation, respectively [37, 38]. There are some other studies that have reported the role of melatonin in improving mitochondrial morphology and function, reducing angiotensin 2 induced apoptosis [39], blocking the NF- $\kappa$ B and iNOS pathway in kidney tissue, reducing proteinuria [40], and downregulating the activity of peroxidase enzymes such as lipoxygenase [41]. Although many experimental studies have investigated the effects of melatonin on various metabolic disorders, there are not enough clinical trials assessing the efficacy of melatonin in patients with DKD. This gap can be addressed through conduction of relevant clinical trials with human subjects.

Regarding to the DKD pathophysiology, we hypothesize that melatonin may improve metabolic parameters, oxidative stress, and inflammatory biomarkers in diabetic patients with CKD. According to aforementioned hypothesis, the present trial is designed. One of the novel characteristics of the current clinical study is suggesting an endogenous antioxidant, which has less side effects than other exogenous antioxidants [42]. Furthermore, melatonin has not pro-oxidant properties and unlike other antioxidants, it does not participate in the oxidation–reduction cycles after scavenging free radicals [43].

## Trial status

The date of registration was 11 August 2023 (protocol version: 1.0). The recruitment started on 20 October 2023 and will be almost finished on 5 May 2024. The study will finish approximately on 3 May 2024.

## Abbreviations

|       |  |
|-------|--|
| BMI   | Body mass index                                    |
| BUN   | Blood urea nitrogen                                |
| CKD   | Chronic kidney disease                             |
| CREAM | Centre for Research and Education in Art and Media |
| DKD   | Diabetic kidney disease                            |
| DM    | Diabetes mellitus                                  |
| DBP   | Diastolic blood pressure                           |

|                |  |
|----------------|--|
| ELISA          | Enzyme-linked immunosorbent assay                                  |
| ESRD           | End stage renal disease  |
| GPX            | Glutathione peroxidase   |
| HDL            | High-density lipoprotein   |
| hs-CRP         | Highly sensitive C reactive protein                                |
| IL-6           | Interleukin-6  |
| iNOS           | Inducible nitric oxide synthase                                    |
| ITT            | Intention to treat   |
| LDL            | Low-density lipoprotein  |
| LPL            | Lipoprotein lipase   |
| MDA            | Malondialdehyde  |
| MET            | Metabolic equivalents  |
| MPO            | Myeloperoxidase  |
| NF- $\kappa$ B | Nuclear factor kappa B   |
| NSAIDs         | Non-steroidal anti-inflammatory drugs                              |
| PSQI           | Pittsburgh Sleep Quality Index                                     |
| RCT            | Randomized controlled trial  |
| SES            | Socio-economic status  |
| SBP            | Systolic blood pressure  |
| SOD            | Superoxide dismutase   |
| SPIRIT         | Standard Protocol Items: Recommendations for Interventional Trials |
| TAC            | Total antioxidant capacity   |
| TC             | Total cholesterol  |
| TG             | Triglyceride   |
| TNF- $\alpha$  | Tumor necrosis factor alpha  |
| TOS            | Total oxidative stress   |
| WC             | Waist circumference  |

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13063-024-08584-x>.

Additional file 1. SPIRIT checklist

## Acknowledgements

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## Code availability

Not applicable.

## Authors' contributions

SS, FN, MH, FP, HM, and HI conceived and developed the idea for the study. SS and MH contributed to data collection. SS and FN wrote drafts of the manuscript. HM advised on statistical analysis. HM and HI contributed to the final revision of the manuscript. The manuscript has been read and approved by all authors.

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## Data availability

The first and corresponding authors will have access to interim results and make the final decision to terminate the trial. The non-identifiable individual patients' data will become available to other researchers in academic institutions. The datasets analyzed during the current study and statistical code are available from the corresponding author on reasonable request, as is the full protocol.

## Declarations

### Ethics approval and consent to participate

The current trial received approval from Medical Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.SHARIATI.REC.1402.072). Written informed consent will be obtained from participants before participation in the research project.

**Consent for publication**

Not applicable—no identifying images or other personal or clinical details of participants are presented here or will be presented in reports of the trial results. The participant information materials and informed consent form are available from the corresponding author on request.

**Competing interests**

The authors declare that they have no competing interests.

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