

# Evaluation of the Effect of Curcumin and Zinc Co-Supplementation on Glycemic Measurements, Lipid Profiles, Inflammatory and Antioxidant Biomarkers in Overweight or Obese Pre-Diabetic Patients: A study Protocol for a Randomized Double-Blind Placebo-Controlled Phase 2 Clinical Trial

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## Study protocol

**Keywords:** curcumin, zinc, prediabetes, glycemic measurement, lipid profile, antioxidant biomarkers, inflammatory biomarkers

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**Evaluation of the effect of curcumin and zinc co-supplementation on glycemic measurements, lipid profiles, inflammatory and antioxidant biomarkers in overweight or obese pre-diabetic patients: a study protocol for a randomized double-blind placebo-controlled phase 2 clinical trial**

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**Abstract:**

**Background:** The prevalence of prediabetes is increasing worldwide. Unfortunately, prediabetes is related to non-communicable diseases. A high risk of developing type 2 diabetes mellitus (T2DM) is reported in people with prediabetes. Curcumin, a polyphenol, might lead to its therapeutic role in obesity and some obesity-related metabolic diseases. Zinc is a trace element that plays a key role in the synthesis and action of insulin, carbohydrate metabolism, and decreasing inflammation. There has been no clinical trial of zinc and curcumin co-supplementation in patients with prediabetes. In previous studies, the single administration of zinc or curcumin hasn't been conducted on many of the studied markers in pre-diabetic patients.

**Methods:** The propose of this randomized double-blind placebo-controlled clinical trial is to investigate the effect of curcumin and zinc co-supplementation on glycemic measurements, lipid profiles, inflammatory and antioxidant biomarkers among 84 pre-diabetic patients with body mass index (BMI) between 25 and 35. Also, liver enzyme, serum zinc, urine zinc, blood pressure, anthropometric parameters, quality of life, adherence to co-supplementation, the side effects of co-supplementation, physical activity, and dietary intake will be assessed. Women or men (18-50 years old, for women: 18 years - before menopause) will be followed for three months (90 days). This study will be conducted at Yazd Diabetes Research Clinic, Shahid Sadoughi University of Medical Sciences.

**Discussion:** A diet rich in antioxidants, polyphenols, and phytochemicals has been shown to have a beneficial role in prediabetes. According to the beneficial properties of curcumin or zinc and inadequate evidence, RCTs are needed to assess the effect of curcumin and zinc co-supplementation in native prediabetes patients. We hope the results of the present trial, negative or positive, fill this gap in the literature and facilitate the approach for a much larger, multi-center clinical trial. In conclusion, a synergic effect of co-supplementation along with a weight-loss diet may delay the progression to type 2 diabetes mellitus.

**Trial registration:** The project is a registered clinical trial (Registration number: IRCT20190902044671N1, Iranian Registry of Clinical Trials (IRCT), registered October 11, 2019.

**Key words:** curcumin, zinc, prediabetes, glycemic measurement, lipid profile, antioxidant biomarkers, inflammatory biomarkers.

## Introduction

### Background and rationale

Prediabetes or intermediate hyperglycemia (1) is the state referred to impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or glycated hemoglobin A1C (HbA1C) of 5.7% to 6.4% (39–47 mmol/mol). There is a difference in terms of the diagnostic criteria for illustrating a prediabetes state between the American Diabetes Association [ADA] (2), International Diabetes Federation [IDF] (1), and World Health Organization [WHO] (3) (Supplementary Material.1, Table S.1.). The prevalence of prediabetes is increasing worldwide (4).

Unfortunately, prediabetes is related to non-communicable diseases (the different types of obesity, dyslipidemia, and hypertension) (5). A high risk of developing type 2 diabetes mellitus (T2DM) is reported in people with prediabetes (6). However, it is preventable (7) by lifestyle interventions (8) (such as weight-loss strategies using dietary changes and/or physical activity (9)). The beneficial effects of a diet rich in antioxidants (10), polyphenols (11) and phytochemicals (12) have been shown in many studies.

Turmeric (*Curcuma longa*) is a plant-derived spice related to the ginger family (*Zingiberaceae*) with medicinal properties. The largest producer of turmeric is India. The bioactive yellow molecules in turmeric are called curcuminoids (13) that consist of curcumin (diferuloylmethane) (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BMC) (14). Curcuminoids are generally recognized as safe (GRAS) according to the US Food and Drug Administration (FDA) (15). Also, a maximum single oral dose (12g/day) of curcuminoids is well tolerated (16). In addition, the 6-month intervention of curcumin shows only slight adverse effects (17). Curcumin, a polyphenol (18), can improve metabolic syndrome (MeS) (19), diabetes, antioxidant capacity (20), cancer (14), and arthritis (21).

Curcumin modulates the several cellular transduction pathways and molecular targets (Advanced Glycation End-products (AGEs)-mediated induction of the receptor for AGEs gene expression, de novo synthesis of glutathione, PPAR $\gamma$  activity, NF-kB, STAT-3, Nrf2, TNF- $\alpha$ , IL-1 $\beta$ , resistin and leptin, adiponectin and etc...) which might lead to its therapeutic role in obesity and some obesity-related metabolic diseases such as T2DM (22-24). The effect of curcumin alone or combined with nutraceuticals in prediabetic patients was shown in three trials (25-27). In four studies, it was assessed in the patients with MeS (prediabetes, pre-hypertension or Dyslipidemia) (28, 29), MeS, and non-alcoholic fatty liver disease (NAFLD) (30) or prediabetic or controlled T2DM (31).

Zinc is a trace element that plays a key role in more than 300 enzymes (32) such as antioxidant enzymes, synthesis and action of insulin, carbohydrate metabolism (33), and decreasing inflammation (34). Zinc levels improve the glycemic status, lipid parameters (35) (total cholesterol (TC), serum low-density lipoprotein-cholesterol (LDL-C), and triglycerides (TG)) (36) and blood pressure (BP) (35). Some complications of diabetes may be related to oxidative stress and zinc can be an essential element in the cellular antioxidative defense (33). Also, there is a high concentration of zinc in the human pancreatic beta-cells (37). The effect of zinc alone or combined was assessed in prediabetic patients by two trials (38, 39) and it was investigated on MeS only in one trial (40).

The characterizations of the previous studies, which related to the title of this research, are shown in Table S.2 (Supplementary Material.1).

Both zinc and curcumin have antioxidant properties (20, 33). Co-supplementation of curcumin and zinc in patients with pre-diabetes is not yet studied. Also, in previous studies, the effect of single administration of zinc or curcumin hasn't been evaluated simultaneously on many of the studied markers in patients with pre-diabetes. These supplementations are remarkably free of toxicity (18, 41) and they been used as an available and inexpensive food condiment for human consumption. It is proposed that their usage may show synergistic effects in prediabetes. So, this double-blind, randomized placebo-controlled clinical trial (randomized clinical trial (RCT)) will start with the following objectives & hypothesis:

#### **Primary Objectives:**

Comparing the mean changes in serum levels of biochemical parameters (fasting blood glucose (FBG), 2 hour Post Prandial (2hpp), HbA1c, serum insulin, insulin resistance (IR), insulin sensitivity % (IS), beta-cell function% (BCF), TG, TC, LDL-C, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), total blood antioxidant capacity (TAC), malondialdehyde (MDA), serum zinc, urine zinc, serum interleukin- B (IL-1B), high sensitivity C-reactive protein (hs-CRP), serum alanine transaminase (ALT) and serum aspartate enzyme transaminase (AST)) between the groups.

#### **Secondary Objectives:**

Comparing the mean changes of anthropometric data (weight, height, waist circumference (WC), hip circumference (HC), body mass index (BMI), waist-height ratio (WHtR), waist-hip ratio (WHR), fat mass (FM), free fat mass (FFM), muscle mass (MM), and A Body Shape Index (ABSI)), physical activity (PA), Diastolic

Blood Pressure (DBP), Systolic Blood Pressure (SBP), health-related quality of life (HRQOL), and dietary intake between the groups.

### **Hypothesis:**

Curcumin and zinc co-supplementation will significantly improve the serum levels of biochemical parameters, anthropometric data, HRQOL, PA, and BP in prediabetic patients.

### **Trial design**

We designed a single-center, double-blinded, randomized placebo-controlled clinical trial among patients with pre-diabetes with a convenience sampling technique.

### **Method**

#### **Study design**

The flow chart of the study design is shown in Figure 1. The participant will be randomized in a 2x2 factorial design to four parallel treatment groups, 1) curcumin group (Cur/ P.Zn), curcumin supplement + placebo for zinc; 2) zinc group (Zn/P.Cur), zinc supplement + placebo for Curcumin; 3) zinc+ curcumin group (Zn/Cur), (zinc supplement + curcumin supplement); 4) placebo group (P.Zn/P.Cur), (placebo for curcumin and Zinc). The follow-up duration will be three months (90 days).

This trial dated 05/10/2019 was approved by the Medical Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; (Ethical code: IR.AJUMS.REC.1398.504; available on site: <http://ethics.research.ac.ir/IR.AJUMS.REC.1398.504>) and it is a prospectively registered clinical trial (Registration number: IRCT20190902044671N1, Iranian Registry of Clinical Trials (IRCT), available on site: <https://en.irct.ir>). The study will be reported according to the Consolidated Standards for Reporting Trials (CONSORT) statement (<http://www.consort-statement.org/>, accessed April 14, 2017). The present study will be conducted at Diabetes Research And Clinical Practice Center of Yazd, central Iran, where the prevalence rate of diabetes is higher than other parts of the world (42).

#### **Participants**

The subjects are introduced to the trial and those who are interested to know more are invited via email, phone call, SMS, or social media (telegram or WhatsApp) to the study center, where the study investigator explains the

study in detail to the subject. After the clarifications have been provided, the subject will be asked to voluntarily sign the consent form and the first appointment will be booked. In the first appointment, the consented subjects undergo screening procedures for the study under an endocrinologist according to ADA guidelines. The eligible subjects will be enrolled in the trial and randomized to appropriate the study groups. The screening period is planned for 12 months and will continue until 84 participants are randomized in the study.

### **Inclusion/exclusion criteria**

#### **Inclusion criteria**

Women or men (18-50 years old for men and 18 years - before menopause for women) with prediabetes, (FBG  $\geq 100$  and  $< 126$  mg/dl, IGT  $\geq 140$  and  $< 200$  mg/dl, 2-h plasma glucose concentration after a 75-g glucose load on the oral glucose tolerance test (OGTT)  $\geq 140$  and  $< 200$  mg/dl or/and HbA1c (5.7 - 6.4 %)), and BMI between 25 and 35. Voluntary written informed consent for all patients is mandatory before any study related procedures.

#### **Exclusion criteria**

The participants must not have any of the following: a diagnosis of any types of malignancies and cancers, chronic or acute hepatic disorders (hepatitis B, C, etc.), bile disease, autoimmune diseases, neurological diseases (such as epilepsy), neurological diseases, effective hereditary disorders of the liver (iron and copper storage disease), endocrine diseases (hypothyroidism, hyperthyroidism, and Cushing's syndrome), inflammatory diseases (rheumatoid arthritis), hypertension, cardiovascular, acute or chronic kidney disease, lung disease; taking blood pressure, glucose or lipid-lowering drugs (e.g. insulin, metformin, glucophage or atorvastatin); taking multivitamin-mineral supplements for three months before or during the intervention; a history of weight loss surgery in the last year, a weight-loss plan in the last 3 months; receiving from a weight-loss medicine or program; lactating, pregnant or planning to get pregnant; a history of any condition that the investigator may consider a contraindication to participation (such as the sensitivity to supplementation or simultaneously participating in another project), unwillingness to continue cooperation, non-compliance during the intervention, or no signed informed consent.

#### **Sample size**

The sample size was determined based on the ability to detect a mean effect of FPG between the supplement and placebo groups for each supplement (curcumin or zinc), separately.

By using the following formula, the maximum sample size for curcumin group based on trial by Chuengsamarn et al. (25) and zinc group based on trial by Ranasinghe et al. (39) was determined to be 8 ( $\bar{X}_1=3.64, \bar{X}_2=7.54, S_1=8.17, \text{ and } S_2=7$ ) and ( $\bar{X}_1=+16, \bar{X}_2=+3, S_1=12.8, \text{ and } S_2=15.3$ ) 19, respectively (( $\alpha = 0.05$ , significance), ( $\beta = 0.2$ , power)). Then, the maximum sample size was considered as final sample size, which is sufficient to evaluate the main effects. According to an expected 10% loss at follow-up, 84 subjects (21 participants in each group) will be recruited, finally.

$$n_1 = \frac{(S_1^2 + S_2^2)(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2}{(\bar{X}_1 - \bar{X}_2)^2}$$

In the formula above,  $S^2$  and  $\bar{X}$  show the variance and the mean effect for FBS in the placebo and treatment group, respectively. Also, for a more accurate calculation of sample size, WinPepi statistical program (Version 11.4: Abramson, 2011) was performed.

### **Randomization and blinding**

The randomization of the participants for this study will be conducted using the method of block randomization with a block size of 4 by a computer-generated random number sequence. Then, the allocation concealment will be conducted assigning the unique codes in order to prevent “selection bias”; and it will be maintained by the research staff (NN) during the study. After checking the inclusion and exclusion criteria and baseline measurements, participants will be given consecutive numbers, which will be forwarded to NN who will open the sealed envelope with the treatment code after the final data analysis and/or an emergency situation. All the assessments in the trial will be made by assessors blinded to the treatment allocation. In the event of an emergency situation where the knowledge of the treatment allocation is critical for further management of the participant, NN is given access to unblinding envelopes provided for each participant. The treatment code would be communicated to the medical personnel in charge of the treatment but should not be recorded or verbally disclosed in any of the study documents or to the patient. The emergency unblinding and the reason should be recorded in the case report form. Since this is a double-blinded study, the investigator, participants and those

involved in the study won't be informed about the type of the used supplement except NN. The supplements will be delivered to the participants according to the allocation in the 1<sup>st</sup>, 30<sup>th</sup>, and 60<sup>th</sup> day by a blinded assessor.

## **Intervention**

The dose of supplements was calculated based on the data from previous studies, where a dose of 30 mg/day zinc element as zinc sulphate significantly improve FBG, BCF, IS, and IR for 6 months (38) whereas, 20 mg/day of zinc element had a favorable effect on FPG, OGTT, HOMA-IR, TC and LDL-C, and BCF but for 12 months (39). According to less cytotoxicity of zinc gluconate than zinc sulphate (moderate vs. high) (43), a dose of 30 mg/day of zinc as zinc gluconate for 3 months will be prescript. Therapeutic effect of curcumin extract on the patients with IFG, IGT, and MeS been seen with different doses ranging from 20 mg to 2400 mg and follow-up duration between 2- 9 months (25-31).

Zinc supplement contains 30 mg zinc as zinc gluconate. Placebo will be identical in texture and appearance to its active supplement. The curcumin supplement (BCM95/ Curcugreen) used in the study is an extract of dried turmeric rhizomes which was identified by a qualified botanist as *Curcuma longa* and a voucher specimen is kept with herbarium ID HERB-ED-22. The turmeric rhizomes were extracted with ethyl acetate and each 500 mg capsule of curcumin supplement contained 475mg of curcuminoids and turmeric essential oil. The purity of the bio actives in capsule were tested by HPLC, residual solvents by GCHS, heavy metals by ICPMS and microbial parameters conforming to EU standards.

All participants will receive a tablet (zinc supplement or placebo for zinc) to be consumed before breakfast and a capsule (curcumin supplement or placebo for curcumin) to be taken after breakfast, daily. Also, the energy requirement of the participants will calculated by Report of a Joint FAO/WHO/UNU Expert Consultation, 2001 (44), individually. Given that the participants were overweight or obese, they will receive a hypo-caloric diet with at least a 7% weight loss (45).

In each visit (visit 1<sup>st</sup>: 1<sup>st</sup> day, visit 2<sup>nd</sup>: 30<sup>th</sup> day, visit 3<sup>rd</sup>: 60<sup>th</sup> day), the health information (diet and physical activity) will be recommended to all participants to improve healthy habits, individually.

The recommendations will be included 1) following a hypo-caloric diet; 2) avoiding excessive intake of high-fat products (whole dairy products, poultry fat (the skin on chicken), red meat, butter, cream, ice cream, processed meats (sausages), coconut oil, and palm oil); 3) avoiding excessive sugar consumption (candy, soda, syrups, sugar-loaded desserts, caramels, chocolate); 4) increasing PA by walking or cycling for 150 minutes per week (45).

The recall of the supplements intake and the routine recommendations will be given using messaging tools (Telegram and WhatsApp) daily; the telephone service will be used for the participants who are unable to get access to these tools, weekly. If supplements intake is missed in the morning, patients will be advised to take them during the same day.

### **Outcomes**

The primary outcomes include serum levels of biochemistry parameters. The secondary outcomes are body composition, PA, BP, HRQOL, and dietary intake. The primary and secondary outcomes will be conducted at baseline and the end of the study (90<sup>th</sup>) for all participants. Only adherence will be checked at the end of every month.

### **Outcome measurements**

All outcomes and time points are specified in Fig. 2.

### **Demographic and medical information**

Demographic and medical history data include gender, age, full address, postal code, marital status, income, occupation, ethnicity, educational background, smoking history, alcohol consumption, family history of diabetes or/ and hypertension, medication history (dosage and type of drugs) and the diagnosis time of pre-diabetes.

### **Anthropometric data**

Weight and height will be measured using a pre-calibrated electronic scale (Seca, Germany) to the nearest 0.5 kg and a wall-mounted stadiometer to the nearest 0.1 cm, respectively. BMI (kg/m<sup>2</sup>) will be calculated by this equation: weight (kg) /height (m<sup>2</sup>). A non-elastic and flexible tape will be used to measure WC (at the midpoint between the iliac crest and the rib cage in standing position at the end of normal expiration) and HC (the level of the greater trochanters) to the nearest 0.1 cm.

WHtR and WHR will be calculated by dividing WC by height and WC by HC, respectively. The ABSI was calculated using the formula:

$$\text{ABSI} = \text{WC (m)} / (\text{BMI}^{2/3} * \text{height}^{1/2} \text{ (m)}) \text{ (46)}.$$

Body composition will be estimated using the bioelectrical impedance analysis (BIA) method by a Body Composition Analyzer (InBody 270, Seoul, South Korea).

All anthropometric assessments will be conducted by a trained assessor in duplicate, with the mean measurement recorded.

### **Blood Pressure**

According to the American Heart Association protocol, BP will be measured at baseline and end of the study by an assessor under the following conditions:

After 5 minutes of rest in a quiet place, the participants will be sitting without crossed legs and unsupported back and arms. The BP of the participants will be measured in both arms by the Korotkoff sound technique with a calibrated mercury sphygmomanometer (Omron, Tokyo, Japan). If a consistent difference in BP measurement between arms is shown, the maximum pressure will be recorded. The mean of three readings will be recorded (1-minute interval between them) (47).

### **Laboratory investigations**

Blood samples (12 ml) of all participants will be collected after 12 hours of overnight fasting at the baseline and the end of study. The samples will be collected into two different tubes (in the tube without anticoagulant and in EDTA tubes) and centrifuged to obtain serum or plasma. The serum samples (3 ml) will be stored at -80° c. Plasma and the remaining serum will be used to determine the FBG, 2hpp, (glucose oxidase/peroxidase method, Biosystem, Spain), HbA1c (hplc, TOSOH, Japan), TG (Glycerol phosphate oxidase/peroxidase method, Bio system, Spain), TC (cholesterol oxidase/peroxidase method, Bio system, Spain), HDL-C (direct method, Bio system, Spain), SOD (colorimetric (420 nm) method, ZellBio GmbH, Germany), TAC (colorimetric (570 nm) method, , ZellBio GmbH, Germany), and MDA (colorimetric (535 nm) method, , ZellBio GmbH, Germany), ALT, and AST (IFCC method, Bio system, Spain), serum insulin (Enzyme-Linked Immunosorbent Assay (ELISA) kit, Monobind, USA), serum zinc and urine zinc (flame atomic absorption spectrometry method [Ziest Chem Diagnostics kit, Iran (normal range: 70 - 115 µg/dL (serum), 15-150 µg/dL (random urine)), serum IL-1B (Human IL-1β, (ELISA Kit, BE58011, IBL, Hamburg, Germany), and serum hs-CRP (ELISA Kit, EU59131, IBL, Hamburg, Germany). All biochemical tests except IL-1B, hs-CRP, SOD, TAC, and MDA will be conducted immediately after sampling.

The homeostasis model of assessment (HOMA) methods will be used to assess IR and BCF (48).

The following formula will be used to calculate VLDL and LDL-C:

VLDL = TG (mg/dL)/5 (This formula is valid only when TG are ≤400 mg/d)

$LDL-C = TC \text{ (mg/dL)} - HDL-c \text{ (mg/dL)} - TG \text{ (mg/dL)}/5$ ; Friedewald formula (49).

HOMA2 calculator will be downloaded from university of oxford and used to calculate IR, S%, and BCF%.

<http://www.dtu.ox.ac.uk/>.

### **Dietary intake**

Dietary intake will be recorded using 3-day food intake records (2 weekdays, 1 weekend day), which is a validated tool for diet analysis,(50) at the baseline and the end of the study by the participants (self-administered). Dietitian will train participants to record the amount of food consumed with a multiple homemade cutlery in order to enhance the accuracy of portion size and review all entries in food records. A blinded expert dietitian to study allocation will review all forms of the completed 3-day food intake records. Dietary intake data will be analyzed using Nutritionist IV software (Version I) to estimate the energy and the number of macronutrients.

Also, they will be qualitatively recommended the dietary intake to improve dietary habits by messaging tools or telephone, weekly. The qualitative dietary recommendations will include 1) follow her/his diet; 2) avoid excessive intake of high-fat products (whole dairy products, poultry fat (the skin on chicken), red meat, butter, cream, ice cream, processed meats (sausages), coconut oil, and palm oil); 3) avoid excessive sugar consumption (candy, soda, syrups, sugar-loaded desserts, caramels, chocolate).

### **Physical activity**

Short form of the International Physical Activity Questionnaire (IPAQ-SF), acceptable reliability and validity, will be used to assess total weekly PA (51) by a face-to-face interview. The IPAQ is suitable for adults between 15 and 69 years of age. IPAQ-SF include four generic items (walking, moderate (such as leisure cycling), vigorous activities (such as aerobics), and sitting) and reported as minutes per week (min/week) within each activity category by a Metabolic Equivalent of Task (MET) energy expenditure.

The following formula will be used to calculate the PA ( $MET \cdot min \cdot wk^{-1}$ ):

$MET \text{ level} \times \text{duration} \times \text{frequency per week}$ .

The results of PA will categorize into 3 levels (vigorous-intensity activity, moderate-intensity activity, low-intensity activity) (52). The participants will be interviewed by the blinded research staff to the study allocation to fill IPAQ-SF.

### **Health-related quality of life**

A validated and translated to Persian Short Form Health Survey (SF-36) questionnaire will be used to assess health-related quality of life (HRQOL) (53). The assessment of HRQOL will show the patients' overall health status, the impact of treatment, formulation of health policy and decision on resource allocation (54). Eight health-related dimensions will be measured by the SF-36 questionnaire that include physical functioning (10 items), role limitations due to physical problems (4 items) and emotional problems (3 items), bodily pain (2 items), general health perceptions (5 items), vitality (4 items), social functioning (2 items), and perceived mental health (5 items). Also, there is a single item for health transition that provides an indication of perceived change in general health status over a one-year period. The score of SF-36 will obtain from the sum of the questions in eight domains. Scores range from 0 to 100. The participants with more or less disability will have lower or higher scores of SF-36, respectively (55).

### **Participant adherence**

At each follow-up visit, 1) participants will be briefed on the study guidelines (dietary and PA recommendation), adherence to the dosing schedule, dose timing, storage, and missed dose; 2) participants will be asked to return the unused supplementation and bottle; 3) the importance of contacting the study staff will also be noted in case of any problems (e.g. unusual symptoms). Adherence to the intake of the supplements will be assessed good, moderate, or poor by pill counts (at 2, 3 and 4<sup>th</sup> visits). Participants are also contacted daily with the methods mentioned earlier.

### **Adverse events**

Serious adverse events (SAE) with curcumin (18) or zinc supplementation (41) at the determined dosage have not been reported in the last studies. However, all adverse events (AE) including SAE, Suspected Unexpected Serious Adverse Reaction (SUSAR) will be documented and reported to the Data Monitoring Committee (DMC) and Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences.

### **Statistical analysis**

Before statistical analysis, all data will be reviewed to check accuracy and completeness. The normality of data will be checked by the Shapiro-Wilk W Test. All variables will be reported as mean  $\pm$  SE. The percentage changes for each variable will be separately reported and calculated by the following formula:

$[(E-B)/B \times 100]$ ,

where “E” and “B” are the end value and the baseline value of variable, respectively.

According to normality assumption, the significant changes between the groups will be assessed through a one-way analysis of variance (one-way ANOVA) with post hoc (Tukey’s) analysis or Kruskal-Wallis. Changes from the base-line to post-intervention within the groups will be illustrated through a paired t-test or Wilcoxon signed-rank test. The qualitative and quantitative variables in the two groups will be compared using chi-square and t-test, respectively. If necessary, the Analysis of Covariance (ANCOVA) will be used to control the potential confounding variables.

All analyses will be performed using a statistical software package (SPSS), version 22.0 (SPSS, Inc, Chicago, Illinois, USA) or NCSS2020. Statistical significance will be determined at  $p < 0.05$ .

### **Monitoring**

The present trial will be supervised and monitored by a project manager (MK) and a research advisor (HM). They will examine trial procedures to ensure data quality and compliance with the trial protocol. The findings of the trial monitoring will be reviewed by Data Monitoring Committee (DMC).

### **Discussion**

A diet rich in antioxidants (10), polyphenols (11) and phytochemicals (12) has been shown to have a beneficial role in pre-diabetes. According to the beneficial properties of curcumin or zinc and inadequate evidence, RCTs are needed to assess the effect of curcumin and zinc co-supplementation on glycemic measurements, lipid profiles, inflammatory and antioxidant biomarkers in prediabetic patients. The several strengths of this trial are the evaluation of 1) the effect of zinc and curcumin co-supplementation on native prediabetic patients for the first time; 2) the adherence to co-supplementation; 3) the side effects of co-supplementation; 4) healthy lifestyle education (PA and dietary intake) weekly; 5) dietary weight-loss interventions, individually; 6) glycemic measurements (FBG, 2hpp, HbA1c, IR, IS, BCF, and insulin), lipid profiles (TG, TC, HDL-C, LDL-C, VLDL), inflammatory (IL-1B, and hs-CRP) and antioxidant (SOD, TAC, MDA) biomarkers, liver enzyme (ALT and AST), serum zinc, urine zinc, anthropometric measurement (weight, height, WC, HC, and BMI, WHtR, WHR, FM, FFM, MM, and ABSI), dietary intake, PA, HRQOL, DBP, and SBP, simultaneously.

This study will have several limitations. First, the prediabetes diagnosed duration won't be considered. A different duration may cause more bias in the results. Short prediabetes duration at baseline was associated with a higher probability of meeting optimal care goals such as remission of pre-diabetes and prevents type 2 diabetes. Second, the self-reported dietary intake in this study is subject to biases. Third, self-selection bias may occur; due to the recruitment of the participants is being achieved via voluntary participation by interested subjects. These participants may be potentially cautious and more health-conscious. However, the randomization will decrease this bias. Forth, a small sample size due to a lack of financial support. Finally, the results of this trial aren't generalizable to other populations (the participants with a BMI of normal or greater than 35 kg/m<sup>2</sup>, children, adolescents, and the elderly), due to only recruit prediabetes adult with a  $25 \leq \text{BMI} \leq 35 \text{ kg/m}^2$ .

We hope the results of the present trial, negative or positive, 1) fill this gap in the literature (the diverse recommendations are proposed to improve prediabetes such as lifestyle modification (diet plus exercise), taking the herbal medicine, or taking the pharmacological drug; however, the combination therapy may be a more benefit due to prevent for some complications, lower side effects and cost-effective (56); 2) facilitate the approach for a much larger, multi-center clinical trial.

## **Conclusion**

According to the studies, curcumin or zinc supplementation plays a positive role in the patients with prediabetes; it seems a synergic effect of co-supplementation along with a weight-loss diet can promote the prediabetes state.

## **Trial status**

The recruitment of this trial with ID number: NRC-9810 on October 20, 2019 was begun on January, 2020 and it seems the recruitment will be completed on December, 2020.

## **Supplementary Material**

**Table S.1:** Diagnostic criteria for prediabetes.

**Table S.2:** The characteristics of the previous studies about the effect of curcumin and/or zinc supplement on different markers in pre-diabetes status.

## **Abbreviations**

ABSI, A Body Shape Index; ADA, American Diabetes Association; AEs, Adverse Events; AGEs, Advanced Glycation End-products; ANCOVA, Analysis of Covariance; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; BCM, Beta Cell Function; BMC, Bisdemethoxycurcumin; BMD, Bone Mineral Density; BMI, Body Mass Index; BP, Blood Pressure; CONSORT, Consolidated Standards for Reporting Trials; CUR, curcumin; DBP, Diastolic Blood Pressure; DMC, Demethoxycurcumin; DLP, Dyslipidemia; FDA, Food and Drug Administration; FFM, Free Fat Mass; FM, Fat Mass; FO, Fish Oil; FPG, Fasting Plasma Glucose; F/M, Female/Male; GRAS, Generally Recognized As Safe; HC, hip circumference; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA, Homeostasis Model of Assessment; HRQOL, Health Related Quality Of Life; hs-CRP, high sensitivity C-reactive Protein; IDF, International Diabetes Federation; IGT, Impaired Glucose Tolerance; IL-1B, interleukin- B; IPAQ-SF, Short form of International Physical Activity Questionnaire; IQR, Interquartile range; IRCT, Iranian Registry of Clinical Trials; IR, insulin resistance; IS, Insulin Sensitivity; LDL-C, Low-Density Lipoprotein cholesterol; MDA, Malondialdehyde; MET, Metabolic Equivalent of Task; MM, Muscle Mass; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; One-way ANOVA, One-way Analysis of variance; Pre-D, prediabetes; pre-HTN, prehypertension; QUICKI, Quantitative Insulin Sensitivity Check Index; RCT, Randomized Clinical Trial; SAE, Serious adverse events; SBP, Systolic Blood Pressure; SD, Standard Deviation; SE, Standard Error; SF-36, Short Form Health Survey; SPSS, Statistical Software Package; sUA: serum Uric Acid; SUSAR, Suspected Unexpected Serious Adverse Reaction; TAC, Total Blood Antioxidant Capacity; T/C, Treatment/Control; TC, Total Cholesterol; TG, Triglycerides; T2DM, Type 2 Diabetes Mellitus; VLDL, Very Low-Density Lipoprotein; WC, Waist Circumference; WHO, World Health Organization; WHR: Waist-Hip Ratio; WHtR, Waist-Height Ratio; 2hpp, 2 hour Post Prandial;

## **Declarations**

### **Ethics approval and consent to participate**

This trial was approved by the Medical Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethical code: IR.AJUMS.REC.1398.504) in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments

or comparable ethical standards. A voluntary informed consent will be provided by all participants included in the study before initiating any study procedure. All collected data will be held confidential.

#### **Consent for publication**

Not applicable

#### **Availability of data and material**

Not applicable

#### **Competing interests**

The authors declare that they have no conflict of interest.

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#### **Authors' contributions**

MA, MK, and HM participated in study design and protocol development. HM and MK smoothed out the whole administrative process. MA collected data adhering to study protocol. BC provided statistical advice and input. SMM confirmed all participants. MA and SMM coordinated recruitment and participant management. MK, HM, BC, and SMM participated in the preparation of the manuscript by providing comments on drafts written by MA. All authors read and approved the final manuscript.

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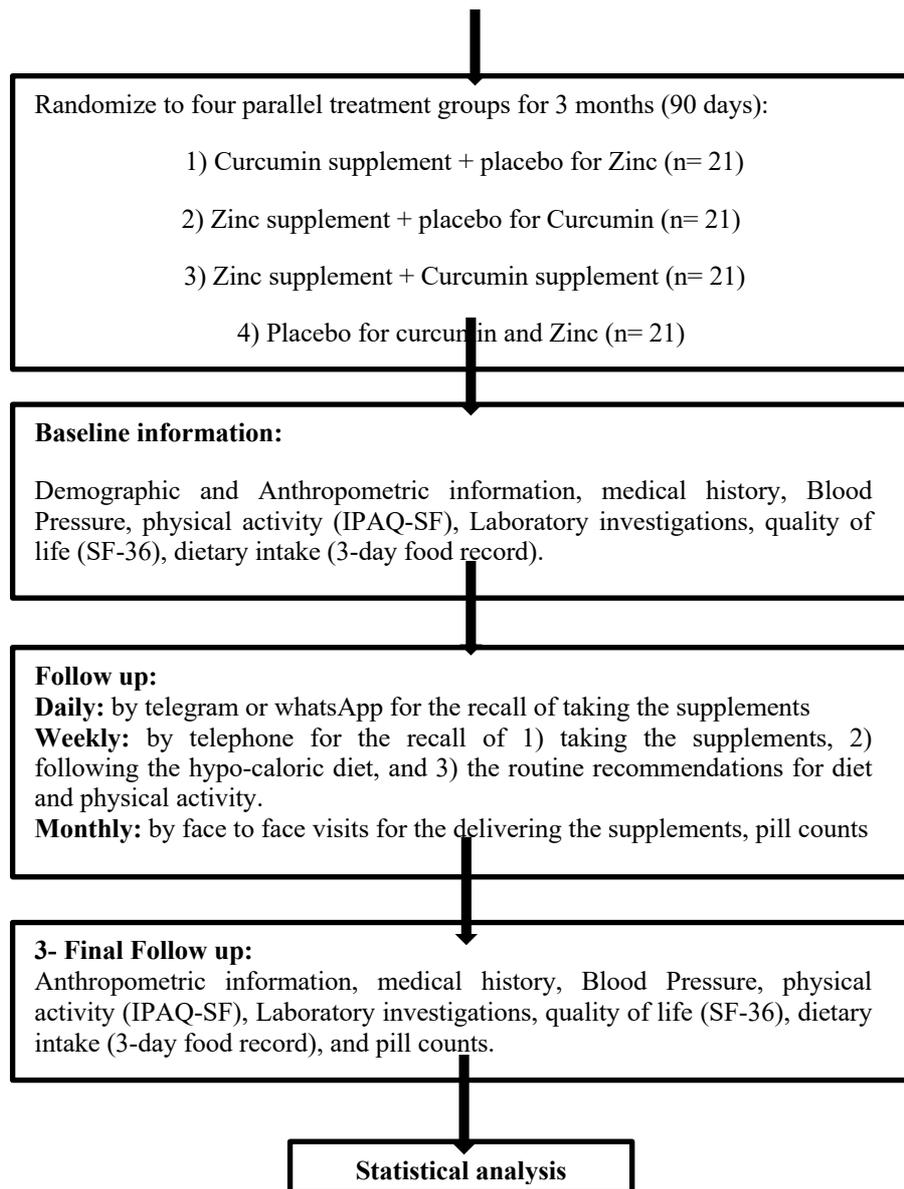
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Eligible pre-diabetic adults (women/men) with written informed consent recruited from Yazd Diabetes Research Clinic and enrolled in RCT (n=84)



**Figure 1. Study design flow chart**

RCT: randomized controlled trial; IPAQ-SF: short form of International Physical Activity Questionnaire; SF-36: short form-36 questionnaire.

TIMEPOINT**	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
	8 Months	0	Month 1	Month2	Month 3	-
<b>ENROLMENT:</b>						
Eligibility screen	X					
Informed consent	X					
Randomization and Allocation		X				
<b>INTERVENTIONS:</b>						
Curcumin group			←————→			
Zinc group			←————→			
Curcumin-zinc group			←————→			
Placebo group			←————→			
<b>ASSESSMENTS:</b>						
Demographic and medical history data: (gender, age, BMI, marital status, smoking history, alcohol consumption, family history of diabetes or/ and HTN, medication history (dosage and type of drugs) and the diagnosis time of pre- diabetes)	X					
<b>Primary outcomes</b> <b>Laboratory investigations:</b> FBG, 2hpp, HbA1c, insulin, HOMA(IR, IS, BCF), TG, TC, LDL, HDL, VLDL, TAC, MDA, serum and urine zinc, IL-1B, hs-CRP, ALT and AST		X			X	
<b>Secondary outcomes</b> <b>Anthropometric data</b> (weight, height, WC, HC, BMI, WHtR, WHR, FM, FFM, MM, ABSI)		X			X	
<b>IPAQ-SF</b>		X			X	
<b>Blood Pressure (DBP, SBP)</b>		X			X	
<b>HRQOL</b>		X			X	
<b>Dietary Intake</b>		X			X	
<b>Participant Adherence</b>					X	
<b>Adverse Events</b>					X	

**SPIRIT Figure.2. Schedule of enrolment, interventions, and assessments.**

ABSI, A Body Shape Index; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; BCM, Beta Cell Function; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; FFM, Free Fat Mass; FM, Fat Mass; FPG, Fasting Plasma Glucose; HC, hip circumference; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; HOMA, Homeostasis Model of Assessment; HRQOL, Health Related Quality Of Life; hs-CRP, high sensitivity C-reactive Protein; IL-1B, interleukin- B; IPAQ-SF, Short form of International Physical Activity Questionnaire; IR, insulin resistance; IS, Insulin Sensitivity; LDL, Low-Density Lipoprotein cholesterol; MDA, Malondialdehyde; MM, Muscle Mass; HTN, hypertension; SBP, Systolic Blood Pressure; TAC, Total Blood Antioxidant Capacity; TTC, Total Cholesterol; TG, Triglycerides; VLDL, Very Low-Density Lipoprotein; WC, Waist Circumference; WHR: Waist-Hip Ratio; WHtR, Waist-Height Ratio; 2hpp, 2 hour Post Prandial.

# Figures

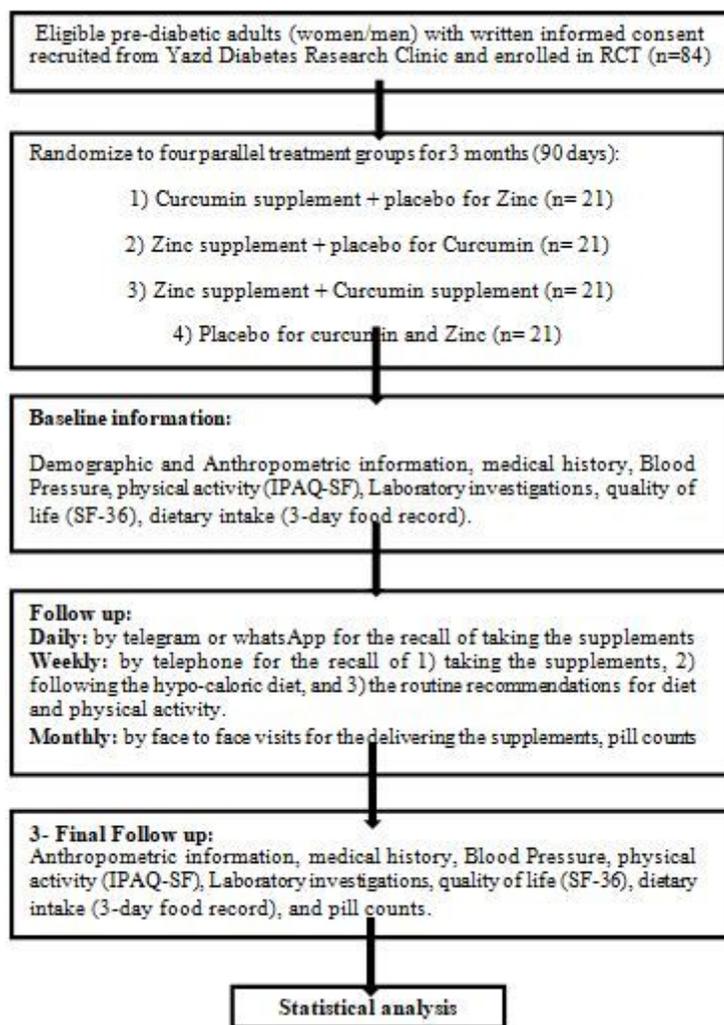


Figure 1

Study design flow chart RCT: randomized controlled trial; IPAQ-SF: short form of International Physical Activity Questionnaire; SF-36: short form-36 questionnaire.

TIMEPOINT**	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
	8 Months	0	Month 1	Month2	Month 3	-
<b>ENROLMENT:</b>						
Eligibility screen	X					
Informed consent	X					
<b>Randomization and Allocation</b>		X				
<b>INTERVENTIONS:</b>						
Curcumin group			←————→			
Zinc group						
Curcumin-zinc group						
Placebo group						
<b>ASSESSMENTS:</b>						
Demographic and medical history data: (gender, age, BMI, marital status, smoking history, alcohol consumption, family history of diabetes or/ and HTN, medication history (dosage and type of drugs) and the diagnosis time of pre-diabetes)	X					
Primary outcomes Laboratory investigations: FBG, 2hpp, HbA1c, insulin, HOMA(IR, IS, BCF), TG, TC, LDL, HDL, VLDL, TAC, MDA, serum and urine zinc, IL-1B, hs-CRP, ALT and AST		X			X	
Secondary outcomes Anthropometric data (weight, height, WC, HC, BMI, WHtR, WHR, FM, FFM, MM, ABSI) IPAQ-SF Blood Pressure (DBP, SBP) HRQOL Dietary Intake Participant Adherence Adverse Events		X			X	
		X			X	
		X			X	
		X			X	
		X			X	
					X	
					X	

Figure 2

Schedule of enrolment, interventions, and assessments. ABSI, A Body Shape Index; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; BCM, Beta Cell Function; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; FFM, Free Fat Mass; FM, Fat Mass; FPG, Fasting Plasma Glucose; HC, hip circumference; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; HOMA, Homeostasis Model of Assessment; HRQOL, Health Related Quality Of Life; hs-CRP, high sensitivity C-reactive Protein; IL-1B, interleukin- B; IPAQ-SF, Short form of International Physical Activity Questionnaire; IR, insulin resistance; IS, Insulin Sensitivity; LDL, Low-Density Lipoprotein cholesterol; MDA, Malondialdehyde; MM, Muscle Mass; HTN, hypertension; SBP, Systolic Blood Pressure; TAC, Total Blood Antioxidant Capacity; TTC, Total Cholesterol; TG, Triglycerides; VLDL, Very Low-Density Lipoprotein; WC, Waist Circumference; WHR: Waist-Hip Ratio; WHtR, Waist-Height Ratio; 2hpp, 2 hour Post Prandial.

## Supplementary Files

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