Effect of exercise and Yoga program on renin-angiotensin mechanism in cardiac autonomic neuropathy in Diabetes mellitus patients.

Sandip Meghnad Hulke
smh555@rediffmail.com

AIIMS BHOPAL  https://orcid.org/0000-0002-1351-9958

Sagar Khadanga
All India Institute of Medical Science - Bhopal

Ashwin Laxmikant Kotnis
All India Institute of Medical Science - Bhopal

Avinash Eknath Thakare
All India Institute of Medical Science - Bhopal

Rachna Parashar
All India Institute of Medical Science - Bhopal

Rajay Bharshankar
All India Institute of Medical Science - Bhopal

Santosh Wakode
All India Institute of Medical Science - Bhopal

Shweta Mishra
All India Institute of Medical Science - Bhopal

Research Article

Keywords: Diabetes mellitus, cardiac autonomic neuropathy, angiotensin, rennin

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Title

Title: Effect of exercise and Yoga program on renin-angiotensin mechanism in cardiac autonomic neuropathy in Diabetes mellitus patients.

Study design: single center open label 2 arm randomized control study done

Population: diagnosed case of cardiac autonomic neuropathy (CAN) in diabetes mellitus patients

Intervention:

Group 1 (Control)- supervised aerobic exercise program for 6 months.
Group 2 (Intervention) - to undergo a supervised YOGA program for 6 months.

Names protocol contributors

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Sandip M. Hulke*</td>
<td>Professor, Physiology Department, AIIMS Bhopal <a href="mailto:sandip.physiology@aiimsbhopal.edu.in">sandip.physiology@aiimsbhopal.edu.in</a>,</td>
</tr>
<tr>
<td>Corresponding author</td>
<td></td>
</tr>
<tr>
<td>Dr. Sagar Khadanga</td>
<td>Associate professor, Medicine Department, AIIMS, Bhopal. <a href="mailto:sagar.genmed@aiimsbhopal.edu.in">sagar.genmed@aiimsbhopal.edu.in</a></td>
</tr>
<tr>
<td>Dr. Ashwin Kotnis</td>
<td>Professor, Biochemistry Department, AIIMS Bhopal <a href="mailto:ashwin.biochemistry@aiimsbhopal.edu.in">ashwin.biochemistry@aiimsbhopal.edu.in</a></td>
</tr>
<tr>
<td>Dr. Avinash E. Thakare</td>
<td>Additional Professor, Physiology Department, AIIMS Bhopal <a href="mailto:avinash.physiology@aiimsbhopal.edu.in">avinash.physiology@aiimsbhopal.edu.in</a></td>
</tr>
<tr>
<td>Dr. Rachna Parashar</td>
<td>Professor, Physiology Department, AIIMS Bhopal <a href="mailto:rachna.physiology@aiimsbhopal.edu.in">rachna.physiology@aiimsbhopal.edu.in</a></td>
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<tr>
<td>Dr. Rajay Bharshankar,</td>
<td>Professor, Physiology Department, AIIMS Bhopal <a href="mailto:rajay.physiology@aiimsbhopal.edu.in">rajay.physiology@aiimsbhopal.edu.in</a></td>
</tr>
<tr>
<td>Dr. Santosh L. Wakode,</td>
<td>Professor and Head, Physiology Department, AIIMS Bhopal <a href="mailto:santosh.physiology@aiimsbhopal.edu.in">santosh.physiology@aiimsbhopal.edu.in</a></td>
</tr>
<tr>
<td>Dr. Shweta Mishra</td>
<td>Yoga Instructor, AYUSH department, AIIMS, Bhopal <a href="mailto:drshwetabnys@gmail.com">drshwetabnys@gmail.com</a></td>
</tr>
</tbody>
</table>

Abstract

• Background: Cardiac autonomic neuropathy (CAN) is one of the frequent complications of diabetes
mellitus. Renin-angiotensin mechanism play role in the development of CAN which may be affected by exercise as well as yoga. Aim of the study was to study the effect of a 6-month supervised exercise program/ Yoga program on cardiac autonomic neuropathy (CAN) status and renin angiotensin (RAAS) parameters in a diagnosed case of CAN in Diabetes mellitus patients.

**The context and purpose of the study**

- **Methods**: Diagnosed patients with CAN in diabetes mellitus would be randomly divided into two groups. One group will undergo a supervised exercise program and the other would follow a supervised YOGA program. CAN parameters and RAS mechanism parameters would be assessed before and after the start of the exercise/Yoga program. CAN parameters and RAAS parameters would be assessed using a cardiac autonomic function test battery and measurement of Plasma renin, Serum aldosterone, Angiotensin II, ACE, and Angiotensin (1-7) respectively.

- **Discussion**: The effect of exercise on cardiac autonomic function has been assessed, however, these studies are limited, the strength of evidence is low, and the need for RCT was stressed. How does exercise affect the RAS mechanism? There are limited studies in this area. Further various research has been recommended to study various components of the RAS mechanism. In India, work has been done mainly to assess the effect of the YOGA program on cardiac autonomic function in diabetes mellitus. There are no uniform findings. Further, literature regarding its effect on the RAS mechanism is limited. Internationally, work is focused on various exercise programs, while the focus was on the yoga program in India. There is a need to assess the effect of such a supervised program on the Indian population.

**Trial registration**: Registered with clinical trial registry of India and registration number is CTRI/2023/05/052268, registered retrospectively

**Keywords**

Diabetes mellitus, cardiac autonomic neuropathy, angiotensin, rennin

**Administrative information**

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see [http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/].)

<p>| Title {1} | Trial: Effect of exercise and Yoga program on renin-angiotensin mechanism in cardiac autonomic neuropathy in Diabetes mellitus patients. |</p>
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<thead>
<tr>
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</tr>
<tr>
<td>Trial registration set- trial is registered retrospectively</td>
</tr>
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</table>

<table>
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<td>NA-trial is registered-3rd May 2023</td>
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<th>Funding (4)</th>
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<tr>
<td>Reference no. R.11014/23/2023-GIA/HR (Grant in aid scheme of the department of Health Research), Indian Council of Medical Research V. RamalingaswamiBhawan, P.O. Box No. 4911 Ansari Nagar, New Delhi – 110029.</td>
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Introduction

Background and rationale {6a}

As per WHO, it is estimated that about 422 million people are suffering from diabetes mellitus. The majority of these are from low- or middle-income countries. (1) Diabetes mellitus is associated with various chronic complications like neuropathy, retinopathy, nephropathy and cardiac autonomic neuropathy. (CAN)

Hyperglycaemia induces oxidative stress and toxic glycosylation end products are responsible for neuronal dysfunction. (2,3) Besides hyperglycemia, the renin-angiotensin system (RAS) important role in the pathogenesis of CAN as mentioned below. (4, 5)
Various studies had shown favorable effect on autonomic function however strength of evidence is low and need for RCT is stressed. (6, 7, 8) Some of the mechanism through which exercise act is mentioned in diagram. Plasma renin level, angiotensin II and aldosterone has been shown to affected however findings are not uniform. (8,9, 10,11,12)

Internationally, there is a limited studies regarding the effect of Yoga on RAS mechanism, (13,14) however its effect on various component of autonomic function and heart rate variability parameters has been studied. (15) A significant effect was observed on the cardiac autonomic function test at the end of 6 months of yoga breathing program. (16)

American college of sports medicine had recommended a 3 to 7-day-per-week program for the diabetic population. (14) Various types of yoga program has been advised for diabetic population (17,18,19,20,21,22)

Thus, the effect of exercise on cardiac autonomic function has been assessed, however, these studies are limited, the strength of evidence is low, and the need for RCT was stressed. In India, work has been done mainly to assess the effect of the YOGA program on cardiac autonomic function in diabetes mellitus. There are no uniform findings. Further, literature regarding its effect on the RAS mechanism is limited.

Internationally, work is focused on various exercise programs, while the focus was on the yoga program in India. There is a need to assess the effect of such a supervised program on the Indian population.

**Objectives**

Primary objectives:
• To study the effect of a 6-month supervised exercise program/ Yoga program on cardiac autonomic neuropathy (CAN) status in a diagnosed case of CAN in Diabetes mellitus patients.

• To compare the effect of a 6-month supervised exercise program/Yoga program on Plasma renin, Serum aldosterone, serum ADH, Angiotensin II, ACE, and Angiotensin (1-7) in a diagnosed case of CAN in Diabetes mellitus patients with that baseline.

Secondary objectives

• To evaluate the effect of a 6-month supervised exercise program/ Yoga program on Serum insulin, Serum C peptide, HbA1c, and glucose level in a diagnosed case of CAN in Diabetes mellitus patients.

• To compare the effect of a 6-month supervised exercise program / Yoga program on fitness parameters in a diagnosed case of CAN in Diabetes mellitus patients with that of baseline.

**Trial design {8}**

**single centre open label 2 arm randomized control study**

**Methods: Participants, interventions and outcomes**

**Study setting {9}**

The study would be conducted by department of Physiology incollaboration with the Medicine and Biochemistry department, AIIMS, Bhopal India. This study would compare the effect of 6 months yoga program with that 6 months of aerobic exercise program. Enrollment the participants would be done from the Medicine department. Data collection and other processes would be conducted in the Physiology department.

**Eligibility criteria {10}**

**Inclusion criteria**

- Diagnosed case cardiac autonomic neuropathy in diabetes mellitus patients.
- Age group 30 to 50 years.

**Exclusion criteria**

- End-stage renal disease (by Cochroft-Gault formula )(23)
- Patient on ACE inhibitors (self-reported and validated by Showcard)
- Known case of heart disease (physician-diagnosed)
- Uncontrolled hypertension (physician-diagnosed)
- Uncontrolled diabetes mellitus (physician-diagnosed)
- Chronic alcoholics (CAGE questionnaire) - (24)
• Any neuromuscular disorder not allowing to participate in exercise/yoga program.

Who will take informed consent? {26a}
Informed consent will be taken by staff involved in the study. Assent form not needed.

Additional consent provisions for collection and use of participant data and biological specimens {26b}
In the informed consent it would be mentioned, however participant identity will not be disclosed. Biological specimen collected is only blood which will be collected with prior informed consent.

Interventions

Explanation for the choice of comparators {6b}
Diagnosed patients with CAN in diabetes mellitus would be randomly divided into two groups. One group will undergo a supervised exercise program and the other would follow a supervised YOGA program.

Comparator is supervised YOGA program where by it would compared with supervised exercise program.

we have come across the limited number of studies where CAN progression over 6 months as a result of YOGA and exercise program is studied.

The interplay between RAAS, yoga, and exercise programs and the progression of CAN is infrequently studied.

Longitudinal studies were very few in this area, hence the longitudinal study was planned.

Intervention description {11a}
Interventions for each group with sufficient detail to allow replication, including how and when they will be administered

Patients diagnosed with CAN would be randomly divided into two groups.-

Group 1- to undergo a supervised aerobic exercise program for 6 months

Group 2- to undergo a supervised YOGA program for 6 months.

Exercise Program-

The program will be based on ACSM guidelines for Exercise in the Diabetic population. The patient will be mainly advised to exercise like walking/ brisk walking/ running 5 times a week. Initially, duration, as well as intensity, would be less. Progression of exercise will be advised over 24 weeks/6 months.

Rest days may be altered depending on the convenience of volunteers.

Proper warm-up and cool-down would be ensured.
Advice would be given to increase the time and intensity of endurance exercise throughout the schedule.

A proper record would be maintained for all activities. In the record, time, frequency (per week), progression, and intensity based on the Borg scale would be asked.

The intensity would be judged using

- Subjective assessment by Borg scale and the Talk test

Talk test - If the patient can talk as well as sing comfortably then it would be mild, for moderate intensity, the patient will be able to speak but not sing. In severe exercise, the patient will not be able to talk continuously. There would be a frequent break for breathing.

Borg scale - Modified English/Hindi Borg scale would be used. Hindi Borg scale is considered to be valid in Indian adults. (25)

Yoga program-

Yoga program designs were based on the literature available about studies on the diabetic population. These sessions would be conducted by a yoga instructor. (18,19,21,22)

It would be done 30 min per day once a day, 5 days a week which would be subsequently increased slowly over 3 weeks to 60 minutes.

<table>
<thead>
<tr>
<th>Yogic Practices</th>
<th>Rounds</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prayer followed by some warm-up exercise</td>
<td></td>
<td>1-5 mins</td>
</tr>
<tr>
<td>Suryanamaskar</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Sithilikaran vyayama-Shoulder/elbow/wrist/finger/knee/Ankle joint movement</td>
<td></td>
<td>2 mins initially, subsequently increased</td>
</tr>
<tr>
<td>Forward/backward/sidewhat bend</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yogasanas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tadasana (The palm tree pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Katichakrasana (The half wheel pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Ushtrasana (The camel pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Exercise</td>
<td>Rounds</td>
<td>Duration</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Sasakasana (The hare pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Dhanurasana (The Bow pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Makarasana (The crocodile pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Viparit Karani (Leg up the wall pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Halasana (The plough pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Saral Matsyasana (The fish pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Shavasana (The Corpse Pose)</td>
<td>2 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Pranayama</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kapalbhati</td>
<td>2 rounds of 30 strokes</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>(Anuloma-Viloma/Nadishodhan)</td>
<td>5 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Bhramari</td>
<td>5 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Ujjai</td>
<td>5 rounds</td>
<td>2 mins initially, rounds would be increased</td>
</tr>
<tr>
<td>Dhyana (Breath awareness)</td>
<td>1 minute</td>
<td>To be increased subsequently</td>
</tr>
<tr>
<td>Omkaar naad meditation</td>
<td>1 minute</td>
<td>To be increased subsequently</td>
</tr>
<tr>
<td>Ending prayer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total initially</td>
</tr>
</tbody>
</table>

Criteria for discontinuing or modifying allocated interventions \{11b\}

These intervention are the part of healthy lifestyle, so they would be discontinued only if patient is not willing to continue.

Strategies to improve adherence to interventions \{11c\}

- Educating the participants and clearing their doubts.
• Regular fitness assessment would be done. (6 weeks to 8 weeks)
• Feedback- regular and timely feedback to all participants
• Maintenance of activity log
• Giving the SMART goal to participants (Specific, Measurable, Attainable, Realistic, Time based)
• Personal trainer who would be supervision
• Making the group so as to have social support
• Incentives in form prizes or certificates to participants achieving SMART goal.
• Use of technology in form of SMART phone
• Flexibility of schedule

Relevant concomitant care permitted or prohibited during the trial {11d}
Their routine activity is permitted and there is no prohibition of any activity.

Provisions for post-trial care {30}
Not expecting any adverse event

Outcomes {12}
Primary outcome parameters will be measured at the start and at the end of 6 months of intervention. These would assess how intervention has affected the status cardiac autonomic neuropathy and renin angiotensin mechanism.

• Plasma renin
• Serum aldosterone
• Serum ADH
• Angiotensin II
• ACE
• Angiotensin (1-7)
• Heart rate variability parameters
• Test for sympathetic function
• Test for parasympathetic function
• Resting heart rate
• Orthostatic hypotension
Secondary outcome parameters will be measured at the start and at the end of 6 months of intervention. These parameters would tell how intervention had affected the glycaemic control.

- Random Blood sugar
- Blood urea
- S. creatinine
- HBA1c
- C peptide
- Serum insulin

Fitness assessment parameters will be measured at the start and then at the interval of 6-8 weeks of intervention. Fitness assessment would act as a motivator for participants and it would be feedback for participant as well as investigator about exercise/yoga activity feedback about the participants.

- Spirometry parameters
- Body composition
- Flexibility
- Muscular strength Nauksan (boat pose)
- Abdominal/core strength
- Muscular endurance
- Cardiovascular endurance
- Static balance
- Handgrip strength

Participant timeline {13}

<table>
<thead>
<tr>
<th>TIMEPOINT**</th>
<th>Enrolment</th>
<th>Allocation</th>
<th>Post-allocation</th>
<th>Close-out</th>
</tr>
</thead>
<tbody>
<tr>
<td>start</td>
<td>1st week</td>
<td>3 M</td>
<td>6 M</td>
<td>9 M</td>
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<td>ENROLMENT:</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
<td>Informed consent</td>
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<td></td>
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<tr>
<td>Allocation</td>
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### INTERVENTIONS:

<table>
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<th>Intervention Yoga program 6 Months</th>
<th>For various participants start may vary from 0 to 6 months</th>
<th>allocation to be done in initial 1st week</th>
<th>Data analysis and writing report/publication</th>
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<td>For various participants start may vary from 0 to 6 months</td>
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<td>Data analysis and writing report/publication</td>
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### ASSESSMENTS:

<table>
<thead>
<tr>
<th>Fitness assessment parameters</th>
<th>Data analysis and writing report/publication</th>
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</thead>
<tbody>
<tr>
<td>Cardiac autonomic neuropathy and renin angiotensin mechanism parameters</td>
<td>Data analysis and writing report/publication</td>
</tr>
<tr>
<td>Glycemic control parameters</td>
<td>Data analysis and writing report/publication</td>
</tr>
</tbody>
</table>

Sample size {14}
Sample size was calculated using G power software assuming median effect size for difference between baseline and endline parameters for each intervention as follows:

- **t tests - Means:** Difference between two dependent means (matched pairs)
- **Analysis:** A priori:

  **Compute required sample size**

<table>
<thead>
<tr>
<th>Input:</th>
<th>Value</th>
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<tbody>
<tr>
<td>Tail(s)</td>
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</tr>
<tr>
<td>Effect size dz</td>
<td>0.5</td>
</tr>
<tr>
<td>α err prob</td>
<td>0.05</td>
</tr>
<tr>
<td>Power (1-β err prob)</td>
<td>0.95</td>
</tr>
<tr>
<td>Output Noncentrality parameter δ</td>
<td>3.6742346</td>
</tr>
</tbody>
</table>

  **Critical t** = 2.0057460
  **Df** = 53
  **Total sample size** = 54
  **Actual power** = 0.9502120

It was estimated to be 54 for each intervention, assuming a loss to follow-up of around 30%, we would enroll 75 patients in each group.

**Recruitment {15}**

Recruitment would be done from the patients visiting Medicine OPD as well as diabetic OPD. Patient would be explained about the role of intervention and study design. Further during follow patient would be constantly encouraged through the strategies mentioned in 11c so that there is less lost to follow up and desired sample size is achieved.

**Assignment of interventions: allocation**

**Sequence generation {16a}**

Through the random number generated through the computer.

**Concealment mechanism {16b}**

Open label RCT

**Implementation {16c}**

By Principal investigator and project staff

**Assignment of interventions: Blinding**

**Who will be blinded {17a}**

NA- open label RCT

**Procedure for unblinding if needed {17b}**
Data collection and management

Plans for assessment and collection of outcomes (18a)

Autonomic Function Assessment:
It will be done using Ewing’s battery of tests and the standard protocol was followed. (26)

- Immediate heart rate and blood pressure response to standing (30:15 R-R ratio)
- Blood pressure response to isometric exercise (Hand Grip test)
- Valsalva ratio
- Heart rate variation with respiration.

These parameters were assessed using Power Lab and Lab Chart 8 by Ad Instrument using Motorized tilt table (Medica Podium) and B.P measurement using Diamond model no. BPDG 124). (27)

Heart rate variability:
HRV recording and assessment were done in the Power lab (AD Instruments P Ltd, Castle Hill Australia). ECG was sampled at 1000 Hz for 5 min with the Power Lab acquisition system. The recording was started once the patient becomes stable in a quiet room with a comfortable temperature.

HRV recording was analyzed with both frequency-domain and time-domain analysis.

Before AFT testing and HRV recording, patients were instructed to abstain from any type of exercise, eating and drinking anything, or having caffeine, at least 2 h before the scheduled time for the test. AFT will be labeled as normal or abnormal based on the AFT test battery and HRV. Standardized guidelines will be followed for interpretation. (27)

Method for lab investigation -For all lab investigations, 20 ml of blood would be collected and stored in vials. Standard procedures would be followed for these.

Routine investigation and glycemic control parameters would be assessed as follows-

<table>
<thead>
<tr>
<th>Material and instrument</th>
</tr>
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<tbody>
<tr>
<td>Random Blood sugar</td>
</tr>
<tr>
<td>GOD-POD- Beckman coulter analyzer</td>
</tr>
<tr>
<td>Blood urea</td>
</tr>
<tr>
<td>Monooxime method- Beckman coulter analyzer</td>
</tr>
<tr>
<td>S. creatinine</td>
</tr>
<tr>
<td>Jaffe’s method- Beckman coulter analyzer</td>
</tr>
<tr>
<td>HBA1c</td>
</tr>
<tr>
<td>Chromatogrphic method- Birad (D10)</td>
</tr>
<tr>
<td>Seum ACE</td>
</tr>
<tr>
<td>ELISA</td>
</tr>
<tr>
<td>C peptide</td>
</tr>
<tr>
<td>CLIA</td>
</tr>
</tbody>
</table>
These investigations would be done in AIIMS, Bhopal Biochemistry department or it would be outsourced from an accredited lab. Standard procedures would be followed for these investigations. Serum ACE, Serum Insulin, and C peptide levels would be outsourced from an accredited lab in case not able to do at AIIMS, Bhopal.

Renin-angiotensin system mechanism assessment would be done as follows-

<table>
<thead>
<tr>
<th>Material and instrument</th>
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<tbody>
<tr>
<td>Plasma renin ELISA reader as per standard procedure</td>
</tr>
<tr>
<td>Serum aldosterone ELISA reader as per standard procedure</td>
</tr>
<tr>
<td>Serum ADH ELISA reader as per standard procedure</td>
</tr>
<tr>
<td>Angiotensin II ELISA reader as per standard procedure</td>
</tr>
<tr>
<td>ACE ELISA reader as per standard procedure</td>
</tr>
<tr>
<td>Angiotensin (1-7) ELISA reader as per standard procedure</td>
</tr>
</tbody>
</table>

For clinical examination and fitness-
These investigations would be done on all groups at the time of recruitment, then at the end of three months and six months for groups 1, 2, and 3.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Parameters</th>
<th>Method</th>
<th>Instruments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pulse and blood pressure</td>
<td>Clinical examination</td>
<td>Digital BP apparatus, MercuryBP, and stethoscope</td>
</tr>
<tr>
<td></td>
<td>Clinical examination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Spirometry parameters</td>
<td>Computerized spirometry</td>
<td>ndd Large TrueFlow (EasyOne) spirometer</td>
</tr>
<tr>
<td>3.</td>
<td>Body composition</td>
<td>Body mass index by measurement of height and weight, Fat-free mass by caliper and body composition analyzer.</td>
<td>Weighing machine, height meter (stadiometer), and Herpenden’s caliper, Body composition analyzer</td>
</tr>
<tr>
<td>4.</td>
<td>Flexibility</td>
<td>Sit and reach test</td>
<td>Sit and reach box</td>
</tr>
<tr>
<td>5.</td>
<td>Muscular strength</td>
<td>Boat pose</td>
<td>Cushioned mat, stopwatch</td>
</tr>
<tr>
<td></td>
<td>Naukasan (boat pose)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Abdominal/core strength</td>
<td>Partial curl up</td>
<td>Cushioned mat, stopwatch</td>
</tr>
</tbody>
</table>
For serial no. 1 standard guidelines would be followed. Spirometry would be done by standard guidelines. (28) For serial 3 to 9, standard guidelines as mentioned in fit India guidelines for the age group 18 to 65 years would be used. (29) Recommended procedure was followed for the measurement of remaining parameters (sr no. 10 to 13) (30–32)

**Plans to promote participant retention and complete follow-up {18b}**
It would be as per strategies as mentioned in 11c.

**Data management {19}**
Data would be entered by on the regular basis which would be cross checked by another person. In addition, principal investigator would randomly check set of data.

**Confidentiality {27}**
Data would be used only for research purpose and same would be mentioned participant information sheet.

**Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}**
Biological specimen is blood which would not be stored, it would be discarded once investigation is done.
Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Data would be checked for consistency and missing data. Missing data would be handled appropriately. We shall clean the data and then proceed for descriptive analysis in form of the generation of summary tables and graphs for each of the objectives.

Statistical analysis would be done using statistical software (Systac 13). The distribution of data will be explored by using density, Box, and Whisker plots. The categorical or nominal variable would be summarized by count or percentage, Numerical variable by mean and SD (normal distributed). If not normally distributed, then median interquartile range.

The difference between categorical variables would be tested by the chi-square test and their association by odds ratio. The numerical variable would be tested by paired t-test or Wilcoxon test (if not normally distributed)
Change in mean ± S.D. levels of Plasma renin, Serum aldosterone, serum ADH, Angiotensin II, ACE, Angiotensin (1-7), Serum Insulin, Serum C peptide, HbA1c, and glucose level will be tested using paired t-test / Wilcoxon signed rank test.
Change in the binary variable (baseline vs endline presence of CAN) will be tested by the McNemer test.
Change in the categorical variable (baseline vs endline frequency % of grades of CAN) will be tested by a chi-square test for trend.

Interim analyses {21b}

All these intervention are the part of healthy lifestyle, hence we won’t expect that trial would be terminated however funding agency has right to decide about termination of the trial.

Methods for additional analyses (e.g. subgroup analyses) {20b}

If subgroup analysis is to be done, statistical analysis mentioned in 20 a will be followed.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

Non adherence: Group 1 as well as Group 2 participant who were enrolled but failed to stick to intervention due to any reason, had followed intervention but their assessment were not done due to any reason.
Missing data: would be entered based on statistical software based on machine learning tools.

**Plans to give access to the full protocol, participant level-data and statistical code**

\{31c\}

No plans for granting public access to full protocol

**Oversight and monitoring**

Composition of the coordinating centre and trial steering committee \{5d\}

NA

Composition of the data monitoring committee, its role and reporting structure \{21a\}

There is no data monitoring committee, however timely progress report is to be submitted to funding agency which would act as a data monitoring.

**Adverse event reporting and harms** \{22\}

No adverse event is expected directly as a result of intervention

**Frequency and plans for auditing trial conduct** \{23\}

Can be done by funding agency

**Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees)** \{25\}

We have already done before the project was approved for funding.

**Dissemination plans** \{31a\}

Publication would be done and participant would be informed about the effect

**Discussion**

Cardiac autonomic neuropathy (CAN) is being observed in 17 to 90 percent in type 1 diabetes mellitus patients and 27 to 93 percent in type 2 diabetes mellitus patients. \(2\)

Oxidative stress and toxic glycosylation as a result of hyperglycemia are responsible for neuronal dysfunction. \(3\) Renin-angiotensin system (RAS) important role in the pathogenesis of CAN. Various component of RAS act through receptor like AT1, AT2, renin, mas and mas related g protein coupled receptor. \(4, 5\)

We had across various studies where the effect of exercise training on cardiac autonomic function has been studied. Some of the effect seen are increased heart variability, decreased
SDNN, rMSSD, LF power. Exercise training has been shown to improve cardiac autonomic function. However, the strength of evidence was low, and the need for RCT was stressed. (6, 7, 8)

The various mechanism by which Exercise is helpful in cardiac autonomic neuropathy is mentioned in the literature. The signaling mechanisms involved are phosphatidylinositol 3-kinase and protein kinase B. Aldosterone is also involved through insulin regulatory mechanism. Various markers of RAS mechanism like plasma renin level, angiotensin II and aldosterone has been shown to affected however findings are not uniform. (8,9, 10,11,12)

Internationally, there is limited data regarding the role of YOGA in modulating the RAS mechanism in CAN patients with diabetes mellitus. However, YOGA in any form is having a relieving effect on stress. Chronic stress-activated renin further activates the RAS mechanism. Thus effect of YOGA on RAS mechanism could not be ruled out. (13)

American college of sports medicine had recommended exercise program whereby aerobic, resistance and flexibility exercise are recommended for the diabetic population. (14)

In India, work has been done mainly to assess the effect of the YOGA program on cardiac autonomic function in diabetes mellitus. Heart rate variability parameters like SDNN, low frequency, high frequency, and total power had shown increasing trends. (15) A significant effect was observed on the cardiac autonomic function test at the end of 6 months of yoga breathing program. (16) Various types of yoga program has been advised which includes Shithilikarana Vyyama (loosening exercises), Surya Namaskar (sun salutation exercises), Asana (Yogic poses), Pranayama (breathing practices), and Dhyana (meditation) and relaxation practices. (17,18,19,20,21,22)

With this trial, attempt is being made to assess the effect of supervised exercise and or yoga program on CAN in diabetic population, further how RAS mechanism play role in development of CAN would be assessed.

**Trial status**

Recruitment of the participants is expected to start from January 2024 and anticipated to end by April 2025. The protocol is registered with clinical trial registry of India and registration number is CTRI/2023/05/052268, registered retrospectively. Protocol version 1, Date 19 Feb 2023

**Abbreviations**

ACE- angiotensin converting enzyme, ADH- antidiuretic hormone, AT1- angiotensin II receptor type 1, AT2- angiotensin II receptor type 2, CAN- cardiac autonomic neuropathy, ELISA- enzyme linked immunosorbent essay, GOD-POD- glucose oxidase-peroxidase, HBA1c- Glycosylated haemoglobin, IL- interleukin, LF- Low frequency, RAS- Renin angiotensin system, RCT- randomized control trial, rMSSD- root square of the mean of the sum of the squares of differences between adjacent RR interval, SDNN- standard deviation of NN (RR) interval

**Declarations**
Acknowledgements

I would like to acknowledge DHR for giving the fund and AIIMS, Bhopal research administration for support in every form. We used the SPIRIT checklist when writing our report (33).

Authors’ contributions {31b}

Corresponding author is principal Investigator in the study. Origin of idea, writing the protocol to funding agency and execution of the project will be done by corresponding author. Co-author (Dr. Sagar) is the Physician and would help in patient recruitment. Co-author (Dr. Ashwin) would help in biochemical investigation. All other co-author would help in execution of the project in terms of data collection, data analysis, writing and review of the research project.

Funding {4}

Reference no. R.11014/23/2023-GIA/HR (Grant in aid scheme of the department of Health Research), Indian Council of Medical Research V. RamalingaswamiBhawan, P.O. Box No. 4911 Ansari Nagar, New Delhi – 110029.

Availability of data and materials {29}

Principal investigator has access to data and once it is published in a form of data, respective journal/publisher would have access as copyright policy

Ethics approval and consent to participate {24}

the study is approved IHEC-LOP/2023/EL068 dated 15th Feb 2023

Consent for publication {32}

Informed consent material
PIS and Consent form
Participant Information Sheet- version 1.1 dated 22nd July 2022
Title: Effect of exercise and Yoga program on renin-angiotensin mechanism in cardiac autonomic neuropathy in Diabetes mellitus patients./ मधुमेह रोगियों में कार्डियक ऑटोनॉमिक न्यूरोपैथी में रेनिन- एंजियोटेंसिन तंत्र पर व्यायाम और योग कार्यक्रम का प्रभाव।

Introduction:

Diabetes mellitus is one of the commonest problems in India and Cardiac autonomic neuropathy (CAN) is one of the frequent complications of diabetes mellitus. Renin-angiotensin mechanism play role in the development of CAN which may be affected by exercise as well as yoga.
This project would help us to assess the effect of exercise and yoga program on the progression of CAN in diabetes mellitus. Further, the role of the RAAS mechanism would be evaluated thus it would help better management of diabetes mellitus patients.

Give your consent to participate in the study if you have completely understood the nature of the study.

Method: Diagnosed patients with CAN in diabetes mellitus would be divided into two groups. One group will undergo a supervised exercise program and the other would follow a supervised YOGA program for six months. CAN parameters and RAAS mechanism parameters would be assessed before and after the start of the exercise/Yoga program. This would be done through clinical examination, noninvasive investigation and biochemical assessment (through collection of blood)

Compensation for participation in the study: No compensation will be provided for participation.

Possible benefit: Exercise/Yoga in any form is considered to be beneficial for diabetes mellitus patients. CAN is one of the commonest complication in diabetes mellitus. How exercise/yoga would help benefit CAN through renin-angiotensin mechanism would be studied in this project? Thus it would help in better management of these patients.

.Any risk: there is minor increase over minimal risk or Low Risk .

Maintenance of confidentiality: Your data records, and research result would be kept strictly confidential. Only pooled data after analysis will be used for research.

Freedom of individual to participate and to withdraw from research at any time without penalty or loss of benefit: You have freedom to withdraw from the research at any point whenever you please without penalty or loss of benefit.

Report will not be provided unless asked.

Principle Investigator – Dr. Sandip Meghnad Hulke, Mobile no. 9039769805
Member secretory, IHEC- Dr. Abhijit R. Rozatkar, Phone no.- 8199001105

प्रतिवेदन

शीर्षक: व्यायाम और योग कार्यक्रम का प्रभाव।

This project would help us to assess the effect of exercise and yoga program on the progression of CAN in diabetes mellitus. Further, the role of the RAAS mechanism would be evaluated thus it would help better management of diabetes mellitus patients.

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Method: Diagnosed patients with CAN in diabetes mellitus would be divided into two groups. One group will undergo a supervised exercise program and the other would follow a supervised YOGA program for six months. CAN parameters and RAAS mechanism parameters would be assessed before and after the start of the exercise/Yoga program. This would be done through clinical examination, noninvasive investigation and biochemical assessment (through collection of blood)

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.Any risk: there is minor increase over minimal risk or Low Risk .

Maintenance of confidentiality: Your data records, and research result would be kept strictly confidential. Only pooled data after analysis will be used for research.

Freedom of individual to participate and to withdraw from research at any time without penalty or loss of benefit: You have freedom to withdraw from the research at any point whenever you please without penalty or loss of benefit.

Report will not be provided unless asked.

Principle Investigator – Dr. Sandip Meghnad Hulke, Mobile no. 9039769805
Member secretory, IHEC- Dr. Abhijit R. Rozatkar, Phone no.- 8199001105
Title of the research study: Effect of exercise and Yoga program on renin-angiotensin mechanism in cardiac autonomic neuropathy in Diabetes mellitus patients.

Aim: To assess the effect of exercise and Yoga program on renin-angiotensin mechanism in cardiac autonomic neuropathy in Diabetes mellitus patients

Explanation of procedure: I understand that the test that I will undergo has been fully explained to me. Fitness assessment would take approximately 30 minutes

Description of potential risk: there is minor increase over minimal risk or Low Risk

Name of Principal Investigator: Dr. Sandip Meghnad Hulke Tel. No(s). 9039769805

Informed consent form- version 1.1 dated 24th June 2022

The nature and purpose of the study and its potential risks / benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my record may be looked at by responsible individuals from AIIMS. I give permission for these individuals to have access to my records. I have been informed that I will be not be charged for carrying out these test and also I will not receive any payment for participating in the study.
I agree to take part in the above study.

(Signatures / Left Thumb Impression)  
Date:

Name of the Participant:

Son / Daughter / Spouse of:

Complete postal address: ______________________________________________________

This is to certify that the above consent has been obtained in my presence.

(Signatures of the Principal Investigator)  
Date:

Place:

Principle Investigator – Dr. Sandip Meghnad Hulke, Mobile no. 9039769805
Member secretory, IHEC- Dr. Abhijit R. Rozatkar, Phone no.- 8199001105

INFORMED CONSENT / अवगत सहमति

अध्ययन का उद्देश्य: Effect of exercise and Yoga program on renin-angiotensin mechanism in cardiac autonomic neuropathy in Diabetes mellitus patients. मधुमेह रोगियों में कार्डियक ऑटोनोमिक न्यूरोपेथी में रेनन-एंजियोटेंसिस तंत्र पर व्यायाम और योग कार्यक्रम का प्रभाव।

उद्देश्य – यह जानना की व्यायाम और योग कार्यक्रम का मधुमेह रोगियों में कार्डियक ऑटोनोमिक न्यूरोपेथी में रेनन-एंजियोटेंसिस तंत्र पर क्या प्रभाव होता है।

विधि की प्रक्रिया: मैं समझता/ समझती हूँ कि प्रक्रिया की जानकारी मुझे पूरी तरह दी गयी है। अपेक्षित अवधि 30 मिनट है।

जोखिम की जानकारी: इस जॉयंच में न्यूनतम जोखिम या कम जोखिम से थोड़ा ज्यादा जोखिम है।

मुख्य अध्ययनकारी- डॉ. संदीप मेघनाद हूलके, कोन न. 9039769805

जानकारी नोटिका ता. ______, जो मुझे दी गयी है, मैंने ध्यान से पढ़ लिया है। मुझे मेरी भाषा में विस्तार से समझाया गया है। मैंने सारी चीजों को पूरी तरह से समझ लिया है। मैं पुष्टि करता हूँ कि मुझे प्रश्न पूछने का अवसर मिला है। इस संदर्भ में सभी प्रश्नों को उत्तर मुझे दिया गया है मुझे यहं भी जानकारी दी गयी है मैं इस जानकारी से संतुष्ट हूँ।

अध्ययन का स्वरूप, उद्देश्य और इसके संबंधित जोखिम / लाभ, अध्ययन की अपेक्षित अवधि, और अध्ययन की अन्य जानकारी के बारे में मुझे विस्तार से बताए गया है। मैं समझता हूँ कि मेरी भागीदारी स्वैच्छिक है और मैं बिना कोई कारण बताए, मेरी चिकित्सा देखभाल या कानूनी अधिकार को प्रभावित किए बिना किसी भी समय वापस लेने के लिए स्वतंत्र हूँ।

मुझे यहं भी जानकारी दी गयी है कि पूरी जानकारी गोपनीय रखी जाएगी एवं ये जानकारी मेरे चिकित्सक के अलावा किसी को भी दी नहीं जाएगी। मैं समझता/ समझती हूँ कि यह जानकारी अनुसंधान के लिए इस्तेमाल
कि जाएगी। मुझे यहाँ भी जानकारी दी गयी है भागीदारी के लिए कोई मुआवजा प्रदान किया जाएगा एवं मुझसे कोई भुगतान प्राप्त नहीं करेगे।
अगर आप इन चीजों से सहमत हैं तो सहमति दें।
हस्ताक्षर/बाएं अंगूठा का निशान तारीख: मोबाइल न.
प्रतिभागी का नाम:____________________
पुत्र/पुत्री/पति/पत्नी:____________________
डाक का पूरा पता:____________________
यह प्रमाणित किया जाता है कि उपरोक्त सहमति मेरी उपस्थिति में प्राप्त की गई है।
मुख्या अध्ययनकारी के हस्ताक्षर- तारीख: मोबाइल न ६०३९६६८५०५
मुख्या अध्ययनकारी- डॉ. संदीप मेघनाद हलके – मोबाइल न. ६०३९६६८५०५
सदस्य सचिव, संस्थागत मानव आचार समिति- डॉ. अभिजीत रोज़टकर , मोबाइल न. ८९९००५०५०५

**Competing interests [28]**
There would be no financial or other interest of principal investigator.

**Authors’ information (optional)**

*Trials guidance: This section is optional.*

**References**

1. Diabetes [Internet]. [cited 2022 Mar 16]. Available from: https://www.who.int/westernpacific/health-topics/diabetes


18. 13966b829f-2081-4d1a-8aa2-d068dc66a1f7.pdf [Internet]. [cited 2022 Jun 16]. Available from: http://www.yogamdniy.nic.in/WriteReadData/LINKS/13966b829f-2081-4d1a-8aa2-d068dc66a1f7.pdf


