Chapter 3
METHODOLOGY PHASE-I
METHODOLOGY

This chapter will deal with the materials and methods used in the study. This includes subject evaluation and consent, instructions given to the subjects, instrumentation and testing protocol.

3. Ethical approval Phase - I and II

Ethical approval of the study was obtained from the University Ethical Committee in 2013 before the commencement of the study (Ethical clearance number 107/2013).

3.1 Study design

Hospital based Cross sectional study

3.1.1 Study center

Kasturba Hospital, Manipal, Udupi district, Karnataka state, India.

3.1.2 Study duration

1 year (March 2013 to April 2014)

3.1.3 Study subjects

Subjects in the study consisted of 30 – 65 years with diagnosed T2DM with and without clinical signs of DPN.

3.1.4 Inclusion criteria

- Subjects with biochemically diagnosed T2DM
- Subjects with type 2 diabetes mellitus duration for more than 1 year
- Age group of 30-65 years
3.1.5 Exclusion criteria

- Subjects with hypothyroidism
- Subjects with retinopathy
- Subjects with history of cerebrovascular accident
- Subjects with history of Ischemic heart disease
- Subjects with history of cardiac arrhythmias
- Subjects with history of lower extremity fractures
- Subjects with arthritic conditions (Osteoarthritis, rheumatoid arthritis, septic arthritis etc.)
- Subjects with congenital and acquired foot deformities

3.1.6 Sampling Method

Systematic sampling method

3.1.7 Sample size calculation

The sample size was calculated based on the neuropathy symptom score prevalence 34% with confidence interval of 95%, absolute precision of 4% and 20% non-respondent rate using the below formula.

\[ N = Z^2 \frac{1-\alpha}{2} \times P \times (1-P) / d^2 \]

N = 539 subjects; N = 674 subjects with 20% non-respondent rate

N = Sample size

Z = Value of standard variant at given confidence interval (1.96)

P = sample proportion (34%)

d = absolute precision of 4%
3.1.8 Study Setting and sampling

Subjects with diagnosed T2DM were screened for inclusion/exclusion criteria before the enrollment in the study. Systematic random sampling was performed using equal probability method where every third subject from the list of subjects visiting the hospital were recruited in the study.

A total number of 724 subjects were screened and 539 subjects met the inclusion criteria and gave the consent. 185 subjects were excluded from the study, where 123 subjects were in exclusion criteria and 62 subjects did not agree to give consent to participate in the study.

3.1.9 Materials and procedure

After obtaining written informed consent from all the subjects. Detailed clinical evaluation was performed to identify foot complications (dry skin, callus, fissures, hammer/claw toes, bunions, ingrown nails, fungal infections, Charcot’s foot, and ulcers) using Michigan neuropathy screening instrument (MNSI) and musculoskeletal complications (shoulder adhesive capsulitis, low back pain, plantar fasciitis, early fatigue, carpal tunnel syndrome, deputryens contracture and flexor tenosynovitis) using short musculoskeletal function assessment questionnaire (SMFA)

3.1.10 Michigan neuropathy screening instrument (MNSI)

MNSI consists of two components, component ‘A’ is self-administered questionnaire and component ‘B’ is examination performed by the therapist. The MNSI component ‘A’ questionnaire is self-administered. Responses are added to obtain a total score. 'Yes' responses to questions 1–3, 5–6, 8–9, 11–12, 14–15 are each counted as one
point. ‘No’ responses to questions 7 and 13 each count as one point. Question 4 was considered to be a measure of impaired circulation and question 10 a measure of general asthenia and were not included in the published scoring algorithm. A score of ≥ 7 was considered abnormal and presence of peripheral neuropathy.

During the Michigan neuropathy screening instrument (MNSI) component ‘B’ examination, the subject’s foot was inspected for dry skin, callus, fissures, hammer/claw toes, bunions, ingrown nails, fungal infections, Charcot’s foot, and ulcers. Any foot with any abnormality was given a score of 1. In addition, each foot was inspected for ulcers, and any foot with an ulcer was given a score of 1. Ankle reflexes was also elicited; if present, the reflex was designated as present with reinforcement and was scored as 0.5. If the reflex was absent, it was scored as 1. Vibration sensation was tested using a 128Hz tuning fork placed over the dorsum of the great toe on the bony prominence of the DIP joint. Vibration is scored as 0 if the subject is able to sense the vibration sensation <10 seconds, score 0.5 and considered reduced when the subject is able to sense the vibration but taken >10 seconds and score 11 is given when the subject is not able to sense the vibration. The total potential score on the MNSI is 8 points and in the published score algorithm, a score ≥2.5 is considered to indicate the presence of peripheral neuropathy.

3.1.11 Short musculoskeletal function assessment (SMFA)

Short musculoskeletal Function Assessment (SMFA) questionnaire was administered. SMFA consists of 46 points under the components Dysfunction index and bother indexing. The dysfunction index consists of 34 items and the bother indexing with 12 items. The dysfunction index assesses the patients’ perceptions of the amount of difficulty they
sustain in the performing specific functions (25 items) and how often the subjects have trouble when performing specific functions (9 items). The dysfunction items are categorized into four categories: daily activities, emotional status, function of the arm and hand, and mobility. Each item consists of 5-point response format (1 point indicating better function and 5 points indicating poor function). The bother index asks the subjects to assess how much they are bothered by problems in various areas of life (e.g., recreation, work, sleep and rest). These items also have a 5-point response format (1 point indicates not at all bothered and 5 points indicates extremely bothered).

The scores of the dysfunction index and the bother index are calculated by summing up the responses to the items and then transforming the scores according to formula:

\[
\frac{(\text{Actual raw score} - \text{lowest possible raw score})}{(\text{Possible range of raw score})} \times 100
\]

This transformation gives the final scores, which is ranged from 0 to 100. The higher scores denote poorer function and lowest score indicating better function.
Study design: Hospital based cross sectional study

Sampling method: Systematic sampling

724 Subjects were screened

185 Subjects were excluded
123 subjects were in exclusion criteria
62 subjects did not agree to give consent

539 subjects were included based on inclusion criteria

After obtaining written informed consent from all the subjects, detailed clinical evaluation was performed to identify foot and musculoskeletal complications

Administration of Michigan Neuropathy screening instrument (MNSI)
Administration of Short Musculoskeletal Function Assessment (SMFA)

Musculoskeletal and foot complications data was collected and analyzed using descriptive statistics expressed in percentage.

*Figure 2. Flowchart representation of phase- I procedure*
METHODOLOGY PHASE-II
3.2 To develop and validate the structured foot health program for T2DM

3.2.1 Procedure

In depth literature search was carried out to find existing educational material on diabetes foot care. Data was extracted and limitations of those study material were studied. Foot care manual was developed based on the information retrieved through the literature and additional information was incorporated based on the cultural need.

After developing the foot care manual it was reviewed by subject experts in the field of diabetes and foot care, including one senior diabetes physician, three general medicine physicians, and one diabetes and diabetic foot care expert physiotherapist for content validation.

Content validation was done using a self-developed questionnaire which consisted of ten questions pertaining to the comprehension, utility, clarity, and legibility of the educational material. Experts rated these aspects on a 5 point Likert scale: strongly agree (5), agree (4), neutral (3), disagree (2), strongly disagree (1). Thereafter, content validity index was calculated for each item in the education material. The result showed excellent CVI (≥ 0.88) for all the items in the educational material in the foot care manual (Table 1).
Table 1

*Content validity scoring for Diabetic foot care manual*

<table>
<thead>
<tr>
<th>Item</th>
<th>Validator</th>
<th>No of agreement</th>
<th>CVI</th>
<th>% total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. This manual is presented in a simple understandable language</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>2. This manual provides information to the patient about diabetes and foot complications</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>3. Images presented in the manual are legible and clear</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>4. Self-foot care methods are made simple and clear</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>5. Exercises and what to do during and after exercises are well explained</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>6. Precautions to be taken during exercises are clearly mentioned</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>7. Exercises for the foot are explained with simple images</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>8. Do’s and don’ts are informative</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>9. Detection and type of footwear to be used is well explained</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>10. This manual is comprehensive and serves the objective of self-foot care management in diabetic population</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total score (%)</strong></td>
<td><strong>96</strong></td>
<td><strong>96</strong></td>
<td><strong>96</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
3.3 PHASE II B

3.3.1 Trial registry: CTRI/2016/01/006566

3.3.2 Study design: Randomized controlled trial

3.3.3 Study center: Kasturba Hospital, Manipal, Udupi District, Karnataka State, India.

3.3.4 Study duration: 2 year (January 2014 – December 2015)

3.3.5 Study subjects: Subjects in the study consisted of 30 – 65 years with diagnosed T2DM with and without clinical signs of DPN.

3.3.6 Inclusion criteria:

- Subjects with biochemically diagnosed T2DM
- Subjects with T2DM for more than 1 year
- Age group of 30-65 years with diagnosis of T2DM with and without clinical signs of DPN.
- ABI with value between 0.91 - 1.20

3.3.7 Exclusion criteria:

- Subjects with hypothyroidism
- Postural hypotension, foot ulcers
- Subjects with retinopathy
- Subjects with history of cerebrovascular accident, Ischemic heart disease, cardiac arrhythmias
- Subjects with history of lower extremity fractures, walking with assistive devices, part or complete foot amputation
- Subjects with arthritic conditions (Osteoarthritis, rheumatoid arthritis, septic arthritis)
- Subjects with congenital and acquired foot deformities
3.3.8 Sample size calculation:

The sample size was calculated based on the forefoot plantar pressure mean difference of 0.187, pool standard deviation 0.392, confidence interval of 80% with effect size of 0.47

\[ N = \frac{2\sigma^2}{d^2} \left( Z_{1-\alpha/2} + Z_{1-\beta} \right)^2 \]

N = 116 subjects; N = 58 subjects in each group

With 20% non-respondent rate: N= 140, 70 subjects in each group

N = Sample number

\[ Z_\alpha = 1.96 \] at 95% confidence interval

\[ Z_\beta = 0.87 \] (Power = 80%)

\[ \sigma = 0.392 \] (pooled standard deviation)

\[ d = \text{Estimated difference of 0.187 was considered clinically significance for the study with effect size 0.47} \]

3.3.9 Setting and Randomization

Biochemically diagnosed T2DM participants were screened for inclusion/exclusion criteria before the enrollment in the study. This was a parallel group randomized controlled trial (RCT), conducted in Kasturba Hospital, Manipal, Karnataka, India. Block randomization was used in the trial design to reduce the bias and achieve a balance in the allocation of subjects to the experimental and the control groups. Total of 219 subjects were screened and 186 subjects met the inclusion criteria for the study. Finally, 140 subjects gave their final consent. Randomization was carried out systematically with 10 blocks in each stratum with size of 14 in each blocks.
3.3.10 Blinding

Blinding was at a single level. After determining the eligibility criteria plantar pressure analysis, gait analysis was evaluated by assessor 1, and assessor 2 performed the musculoskeletal ultrasound of the foot for both the groups at baseline and at the end of the study duration (12 weeks). Whereas, blood glucose levels and glycosylated hemoglobin was evaluated in the hospital laboratory at baseline and 12th week of for the experimental and control groups.

3.3.11 Clinical evaluation

3.3.11.1 10gm monofilament test

Sensory examination was performed with subjects in the supine position and eyes closed. The 10-g/5.07 Semmes Weinstein monofilament (Diabetik Foot Care India, Chennai, India) was applied perpendicularly to eight locations on the plantar aspect of the foot, including the plantar surface of each toes, mid-foot, and near the calcaneum with sufficient force to cause the filament to bend or crumple (Figure 3). Outcomes were recorded as absent, reduced, or present depending on the subject’s answer. The presence of peripheral neuropathy was considered if the outcomes of monofilament test readings were recorded as absent.

3.3.11.2 Vibration perception Threshold

The vibration perception threshold (VPT) was tested using a biothesiometer with the subject in the supine position with or without eyes closed. The stem of the test probe head was placed perpendicular to the base of great toe. (Figure 4).
The intensity of the vibration to the probe head increased gradually and the subject was asked to respond once he/she felt the vibration. Reading range between 8V – 14V was considered normal, 15V – 24V was considered as risk for peripheral neuropathy and ≥ 25V was considered presence of peripheral neuropathy. For better understanding we have classified Peripheral neuropathy into mild (25V – 35V), moderate (36V – 45V) and severe (> 36V).

3.3.11.3 Plantar pressure analysis

The iStep® scanner (Aetrex Worldwide, Teaneck, NJ, USA) was used to record plantar pressures under the feet. istep scanner is a force plat platform which consists of 1050 barometric sensors that measure the pressure exerted under the feet and displayed on a computer screen through a software.

All the subjects were explained and made familiar with the procedure. All the subjects were asked to take off the footwear’s, socks and additional accessories in pockets like key chains, mobile phones, belts are asked to remove as this can alter the reading. Then the subjects were made to stand on the pressure sensor platform for 20 seconds with eyes open, gazing straight ahead and arms at their sides (Figure 5). Recordings were taken.
which was a quantifying measurements in kilograms per sensor (kg/sensor). Maximum pressures under the forefoot (Umax) and hind foot (Lmax), as well as average plantar pressure was measured.

![Plantar pressure analysis using static plantar pressure analyzer](image)

*Figure 5. Plantar pressure analysis using static plantar pressure analyzer*

### 3.3.11.4 Spatiotemporal gait analysis using foot pressure sensitive walkway

Gait characteristics analysis was performed using Win-Track (MEDICAPTEURS Technology France) – foot pressure sensitive walkway. Win-Track is a foot pressure sensitive walkway with length x Width x Height (1610 X 652 X 30mm) consists of 12,288 sensors that measures the spatiotemporal gait characteristics for the foot and displayed on the computer monitor through a software.

Test procedures were demonstrated and made familiar to all the subjects. Subjects were instructed to look ahead and walk on the platform at a comfortable speed (Figure 6). The data was transmitted to the computer through win-track software for analysis. Step
duration, step length, gait cycle length, gait cycle duration and stride duration were recorded through this procedure.

![Figure 6. Spatiotemporal gait analysis using pressure sensitive walkway](image)

3.4.11.5. Musculoskeletal ultrasonography of the foot

Musculoskeletal Ultrasonography was performed using a pure wave ultrasound system (PHILIPS HD15, Software Version 3.0.1; Philips Healthcare, Andover, MA, USA) (Figure 7). All the subjects underwent musculoskeletal ultrasonography of the foot performed by a radiologist (Figure 8), using an 8–15 Hz linear array real-time ultrasonic probe (Philips). During the procedure, the subjects were placed in the supine position with the ankle joint in neutral posture. The following procedure was used to measure the thickness and cross sectional area of the intrinsic foot muscles, plantar skin and fat pad thickness.
The ultrasound probe was placed perpendicular to the test site and, using generous amounts of ultrasound conductivity gel, moved gently along the skin to avoid any pressure-induced changes of the muscle tissue dimension.

The thickness of the extensor digitorum brevis (EDB; in cm) and CSA (in cm²) were determined by scanning transverse to the muscle fibers on the anterolateral aspect of the foot just anterior to the lateral malleolus (Figure 9). For evaluation of the EDB muscle, a line drawn perpendicular to the midpoint of a straight line between the lateral malleolus and the tuberosity of the fifth metatarsal bone defined the scanning plane. The exact position along this line for maximum cross-sectional muscle thickness differs between individuals and was defined at each scanning procedure.

The thickness of the abductor hallucis muscle was determined by landmark on the anterior aspect of the medial malleolus and a perpendicular scanning line was drawn directly inferiorly to palpate the abductor muscle belly and ultrasound transducer applied at a perpendicular angle to the aforementioned scanning line and long axis of the foot on the proximal aspect of the reference line to encompass the muscle fibers of abductor hallucis (Figure 10).

The thickness of the first lumbrical muscle was determined by scanning longitudinal to the muscle fibers between 1st and 2nd metatarsal space (Figure 12). The thickness of the plantar fascia was scanned at one-fifth of the plantar fascia length, going from the calcaneus insertion, on the medial aspect of the foot (Figure 11). Skin and fat thickness under the metatarsals was scanned placing the ultrasound probe perpendicular to the plantar surface of the corresponding metatarsal heads (Figure 13). All images were stored and transferred to a computer for measurement.
Figure 7. Musculoskeletal Ultrasound PHILIPS HD15

Figure 8. Musculoskeletal Ultrasound Performed by the Radiologist

Figure 9. Ultrasound scanning for Extensor Digitorum Brevis (EDB) muscle thickness and cross Sectional area

Figure 10. Ultrasound Scanning for Abductor hallucis muscle thickness
**Figure 11.** Ultrasound scanning for plantar fascia

**Figure 12.** Ultrasound scanning for first lumbrical muscle thickness

**Figure 13.** Ultrasound scanning for plantar skin and fat pad thickness
3.3.11.6 Assessment of quality of life (QOL) using WHOQOL-BREF

QOL was measured by the WHOQOL-BREF instrument. It is a brief 26-item questionnaire, derived from the WHOQOL-100 (Group, 1998), and mostly used in clinical and epidemiological studies. WHOQOL-BREF consists of 24 facets and provides a profile of scores on 4 domains such as (a) physical health and level of independence (7 items assessing areas such as presence of pain and discomfort, dependence on substances or treatments, energy and fatigue, mobility, sleep and rest, activities of daily living, perceived working capacity), (b) psychological wellbeing (8 items assessing areas such as negative and positive feelings, higher cognitive functions, body image and spirituality), (c) social relationships (3 items assessing the areas such as social contacts, family support and ability to look after family, sexual activity), (d) environment (8 items assessing areas such as freedom, quality of home environment, physical safety and security and financial status, involvement in recreational activity, health and social care: quality and accessibility)

Scoring the WHOQOL-BREF

After administering the questionnaire to all the subjects, each answers of each question were graded from 1 to 5. The raw scores were converted to transformed scores by using the WHOQOL-BREFF transformation scoring method. The first transformation converts scores to a range of 4 –20 and the second transformation converts domain scores to a 0–100 scale. Higher scores >60 is considered as better quality of life.
3.3.12 Protocols followed for two groups

3.3.12.1 Experimental group

3.3.12.1.1 Aerobic exercise (Mode of exercise: walking) prescription for T2DM subjects in experimental group

Aerobic exercise was prescribed based on American College of Sports Medicine (ACSM) guidelines. Exercise testing was carried out using Balkes treadmill testing in the range of 40–60 % of heart-rate reserve (HRR) as an adjunct to this rating of perceived exertion (RPE) (scale ranging from 6 to 20). Moderate intensity exercise with 40%-60% of HRR was prescribed for 6 weeks. Once patients became more conditioned to moderate intensity exercise program, exercise was progressed to higher intensity (70%-80%) of HRR using RPE to the point where they reach “somewhat hard” on the scale (scale range 6–20) and asked to continue the high intensity exercise for 6 weeks. The frequency of each exercise session was 5–6 days of the week of moderate to high intensity exercises.

Intensity of exercise was calculated using the Karvonen formula for target heart rate (THR)

\[
\text{Target HR} = [(\text{max HR} - \text{resting HR}) \times \% \text{Intensity}] + \text{resting HR}
\]

Special considerations during training regime was given to foot care, and steps were taken to prevent any episodes of hypoglycemia during and after the exercise sessions.

3.3.12.1.2 Intrinsic foot muscle strengthening

Following intrinsic foot muscle strengthening techniques were taught to T2DM subjects under supervision which was followed at home for duration of 12 weeks and asked to perform regularly for 15 minutes with 10 repetitions of each exercises. A log book was maintained to check the compliance of the home exercise program.
Figure 14. Step 1: Flexing the toes down towards the floor with ankle neutral.

Figure 15. Step 2: Dorsiflexing the ankle with toes held in flexed position

Figure 16. Step 3: extending the toes with dorsiflexion maintained at the ankle

Figure 17. Step 4: Dorsiflexion with toes in flexion

Figure 18. Step 5: Performing inversion and eversion with toes held in flexed position

Figure 19. Repeat each exercises for 10 repetitions per session
3.12.1.3 Self-foot care

The following self-foot care techniques were explained and taught to T2DM subjects in the experimental group:

**Figure 20.** With help of a mirror, inspect the feet every day for cuts, bruises, blisters and swelling.

**Figure 21.** Cut the nails and trim it regularly. Keep the skin soft with a moisturizing lotion, but do not apply it between the toes.

**Figure 22.** Wash the feet in lukewarm water daily and dry the feet well by a cotton cloth, especially between the toes.

**Figure 23.** Quit smoking. Do not perform bathroom surgeries.

**Figure 24.** Examine the shoes every day for cracks, pebbles, nails, or anything that could damage the feet.
3.3.12.2 Control group

At the beginning of the study standard medical care and diet (same as the intervention group) were given to all the subjects in the control group and followed up for the duration of 12 weeks.

At the end of data collection after 12 weeks in both control and experimental group, subjects in the control group were also given self-foot care and education.
Figure 25. Flowchart representation of phase II procedure

**Control Group**
- Allocated to intervention (n=70)
- Received allocated intervention (n=70)
- Received Standard physician care and Diet

**Experimental Group**
- Allocated to intervention (n=70)
- Received allocated intervention (n=70)
- Received standard Physician care along with structured foot health programme consists of:
  - Aerobic exercises
  - Intrinsic foot muscle strengthening
  - Self-foot care and education

**Follow-Up**
- 10 subjects lost to follow-up and 2 subjects discontinued the intervention due to 1 subject met with a bike accident
  - 1 subject moved abroad
- 8 subjects lost to follow-up and 4 subjects discontinued the intervention due to 2 subjects experienced angina while exercising
  - 1 subject developed Peripheral vascular disease
  - 1 subject developed foot ulcer under the great toe

**Analysis**
- Analyzed (n=58)
3.4 Data Analysis

**Phase I:**

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 16.0 for windows.

Continuous variables were summarized using mean and standard deviation and categorical variables were expressed as frequency and percentage. Prevalence of musculoskeletal and foot complications were expressed in percentage using descriptive analysis.

Stepwise multiple logistic regression analysis was used to analyze association between foot complications and duration of T2DM.

**Phase II:**

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 16.0 for windows.

Continuous variables were summarized using mean and standard deviation and categorical variables were expressed as frequency and percentage “Wilcoxon signed rank” test was used to analyze changes within the group and “Mann Whitney U” test used to analyze changes between the groups.