CHAPTER 3
MATERIALS AND METHODS

A multi strategic preventive and treating measure is needed to face the challenge of elderly bone health care and its maintenance that involves a number of indicators to be handled carefully. One such indicator is calcium (Ca) and vitamin D supplementation. In old age a very high intake of Ca and vitamin D is required to fulfill the need and often the insufficient intake leads to bone mineral loss and development of osteoporosis. The treatment strategy may include supplementation of a high weekly dose of vitamin D for first two months to restore the loss and boost up the absorption of further regular small doses of Ca and vitamin D to maintain the optimum level of Ca and vitamin D in the blood to increase BMD and prevent further loss of bone mass. Impact of high doses of vitamin D_3 on bone health and serum vitamin D levels needs to be investigated. Such evidence will surely be of great use to formulate national health care policies for older person. Another indicator is dietary habits. It is important to have a look at the availability of dietary Ca and vitamin D of a particular community and prevalence of low BMD among the population.

PHASE I (Formative research)

Baseline assessment of diet, nutrition and health status of elderly subjects

Section 3.1.1: Ethical clearance
Section 3.1.2: Estimation of sample size
Section 3.1.3: Selection of study participants
Section 3.1.4: Inclusion and exclusion criteria for the study subjects
Section 3.2.5: Study protocol
Section 3.2.6: Assessment of bone mass density
Section 3.1.7: Anthropometric measurements
Section 3.1.8: Biophysical investigations
Section 3.1.9: Assessment of socioeconomic status

Section 3.1.10: Assessment of physical activity

Section 3.1.11: Assessment of major and minor illnesses

Section 3.1.12: Assessment of Dietary intake

Section 3.1.13: Assessment of knowledge and awareness

Section 3.1.14: Assessment of knowledge and awareness

PHASE II (Analytical/intervention research)

Supplementation and efficacy trial of two different doses of calcium and vitamin D and weight bearing exercise on osteopenic and osteoporotic elderly subjects

Section 3.2.2: Distribution of subjects and intervention strategy

Section 3.2.3: Stepwise execution of the therapy

Section 3.2.4: Study protocol

Section 3.2.5: Biochemical assessment

Section 3.2.6: Physical endurance test

Section 3.2.7: Monitoring the intervention

Section 3.2.8: Statistical tools and methods used in data analysis

Section 3.2.9: Tools and techniques used in data collection

PHASE I (Formative research)

The formative phase of this research included 1056 males and females above the age of 60 years from the free living population of urban Vadodara. Bone mineral density camps were organized across the city to enroll the subjects. The subjects were screened for BMD using an ultrasound based BMD machine. The machine was portable, reliable, and cost effective and was carried to the camps and suitable for initial screening (Stewart A. and Reid DM, 2014; Yang NP. *et. al.*, 2006; Marin F. *et. al.*, 2003). This study was a community based trial and was executed in the Vadodara city of Gujarat state. The city is divided in five zones i.e. East, West, North, South and Central. The study was designed with the aim of determining the pervasiveness of osteoporosis in geriatric males and females of urban Vadodara. To map the dominance of poor Bone Mass Density (BMD), a formative research had been

conducted. A number of BMD camps had been organized in places like senior citizen associations, hospitals, health clubs, gyms, old age homes, lions club, rotary clubs, temple, women's association, haveli etc. situated in all the five zones of urban Vadodara. Hand bills and pamphlets were distributed to bring the camp in notice of the elderly beneficiaries.

3.1.1: Ethical clearance

The study had been approved by the Institutional Medical Ethics committee of the Department of Food and Nutrition, The Maharaja Sayajirao University of Baroda and granted with the Institutional Medical Ethics Committee No. **IEHCR/2013/2**. Prior to enroll in the study informed written consent was taken from all the willing respondents on the consent from. The subjects were also informed about the requisite of their blood sample for the biochemical analysis (Appendix – I).

3.1.2: Estimation of sample size

The present study had the primary objective to map the prevalence of osteopenia and osteoporosis amongst geriatric population of the study region. Cross-sectional researches conducted among the Indian elderly population had reported the prevalence of osteoporosis as 50%, and the sample size was estimated by the formula (Mahajan, 2010):

$$n = \frac{4pq}{L^2}$$

Where, n = required sample size

p = approximate reported prevalence of the disease under study (Low BMD = 50%)

q = (1 - p)

L = permissible error in the estimate of p, which was taken as 5

On calculation, the sample size arrived at 800, and an extra buffer of 20% was set aside to balance the dropout or attrition rate and rounding up the calculated sample size. Thus, the final sample was calculated as 1000.

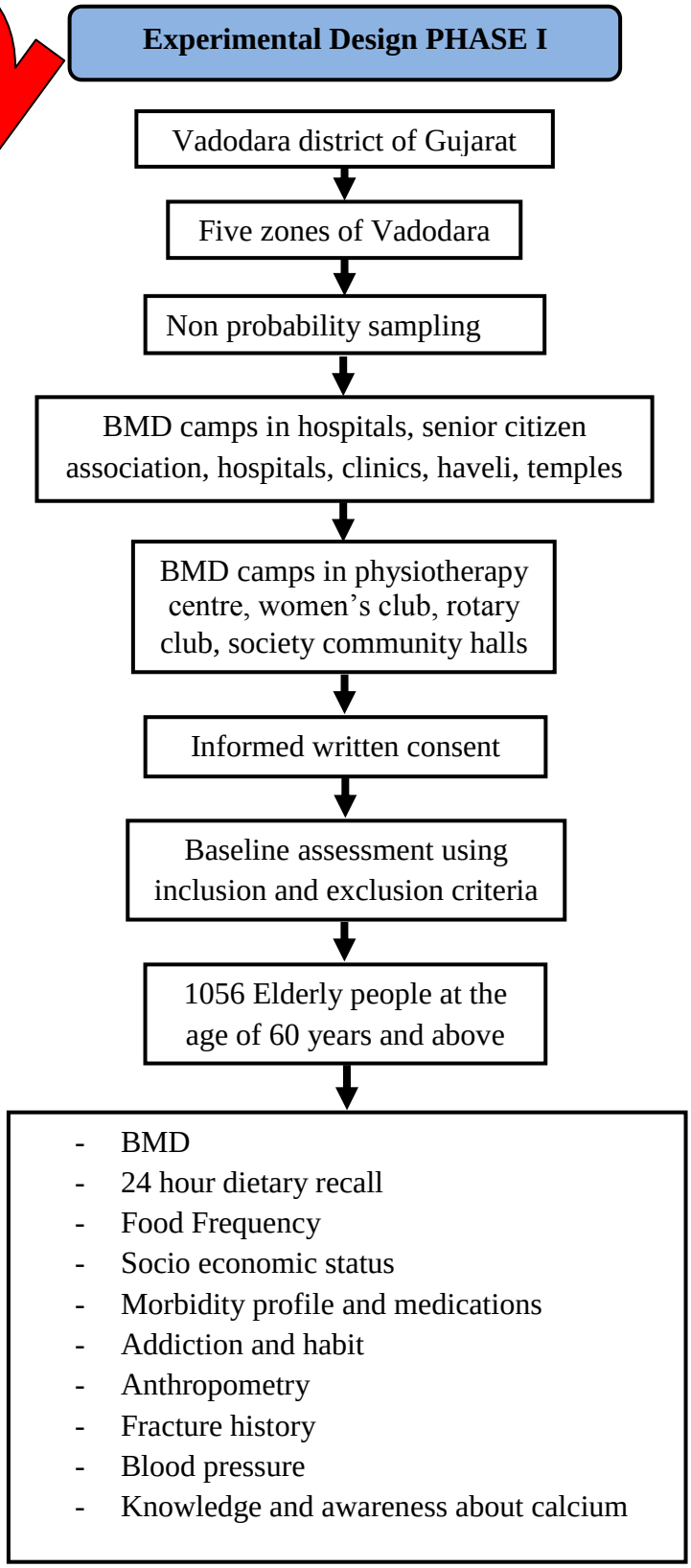
3.1.3: Selection of study participants

Selection of the subjects was done by a non-probability sampling method. Following the snowball sampling technique, members of the prior mentioned organizations (where the camps were organized) and relatives of the members and local people were encouraged to join the camp; and a total of 1056 subjects at the age of ≥60 years were enrolled in the study. Either a permission letter prior to organize the camps or a letter of appreciation after executing the camps was received from all the places wherever the camps were organized. Availability of BMD machine was made either by renting it or in collaboration with Pharmed Pharmaceuticals.

In order to give a clear picture of what the camp would exactly be about; all subjects were briefed on the procedures, purpose of the study, possible outcomes and benefits of the tests. All subjects were issued a detailed BMD analytical report. Also, the clarification was made that under any circumstances the participants will not bear any charges for BMD test or blood test or supplementation.

The willing participants then were provided with a detailed consent form and after reading the consent carefully and got all their queries satisfied the consenting participants returned the signed consent form to the study investigator. Those who were not able to read, were explained the consent form, objectives of the study, possible outcomes and benefits of the tests, requisite of blood sample before and after the intervention period etc. carefully and the return consent from such participants were collected in presence of their family members. It was clearly explained to the participants that disposable syringe will be used to collect blood samples and by an accredited laboratory's experienced phlebotomist (technicians trained to draw blood from patients for clinical research purpose). The willing subjects fulfilling the inclusion criteria according to the phase-wise requirements were enrolled in the study.

Figure 3.1.3.1 Experimental design



3.1.4: Inclusion and exclusion criteria for the study subjects

Phase I- Formative research
Inclusion criteria
Age above 60 years, but for the intervention age was restricted between 60-80 years.
Subjects 6 months less than 60 years of age (as they are likely to become 60 years of age during intervention period).
Willing to participate in the study.
Permanent resident of the study area.
Expects to remain in the area during the study period.
Exclusion criteria
Subjects with severe morbidity related to bone (Such subjects were referred to hospital).
Subjects with other illness required hospitalization.
Subjects who were likely to move away from study area during the study period.
Refused to participate.
Visible severe wasting or malnourished.
Subjects who were suffering from chronic diseases, which required therapeutic intervention.

3.2.5: Study protocol

Step 1: The pamphlets for BMD camps containing necessary brief information regarding the bone health problem, tests and its benefits were distributed amongst the geriatric population of urban Vadodara.

Step 2: BMD camps were organized in the all five zones of urban Vadodara. Participants willingly gathered in the camp to get their BMD assessed. Initial screening of BMD was executed by ultra sound based BMD machine. A printed BMD report was provided to the participants. Camps were conducted under the constant supervision of an orthopaedic doctor.

Step 3: A pre tested semi structured questionnaire was used for other baseline information (Appendix II) which include the following information.

- 24 hour dietary recall (mean of three days recall) (Appendix - III)
- Food Frequency (Appendix - IV) (consumption of vitamin D rich foods)

- Socio economic status
- Morbidity profile (Check list for major and minor illness, Appendix - V) and medications
- 24 hour activity recall (Check list, Appendix - VI)
- Addiction and habit
- Anthropometry
- Fracture history
- Blood pressure
- Knowledge and awareness about calcium

Primary variables: Bone mass density

Secondary variable: Age, BMI, WHR, biophysical parameters, dietary intake

3.2.6: Assessment of bone mass density

An Ultrasound based BMD machine was used to assess the BMD. Bone densitometry is a non-invasive technology that is used to measure bone mass. BMD is expressed as the amount of mineralized tissue in the area scanned (g/cm^2) or it may also be expressed as the amount per volume of bone (g/cm^3). Bone densitometers using ultrasonic measurements are used to assess bone density usually at the calcaneus or patella. The ultrasound reports calculate a T-score (the number of standard deviations above or below the mean for any young normal adults) based on the impedance and ultrasound attenuation. Since patients who have a low bone density have lost bone structure and a low T-score with ultrasound units is generally accurate.The tests were conducted using a QUS bone densitometer under the guidance of trained operator. To measure BMD the heel of the subject is placed in the ultrasound machine with the pre application of the ultrasound gel on the heel. The result of the ultrasound is indicated on the chart or a graph which is prepared according to the cut offs prescribed by WHO (2004). The heel bone is used because it is an accessible site to measure is the weight bearing capacity of the body and is often indicative of hip and spine health (Centre, 2012).

Table 3.2.6.1 BMD Cut offs by World Health Organization (WHO, 2004)

Range	BMD (young adult reference range)
Normal	> -1.0
Osteopenia	-1.0 to -2.49
Osteoporosis	< -2.5

3.1.7: Anthropometric measurements

Anthropometric assessment included parameters like height, weight, BMI, waist circumference, hip circumference and WHR.

3.1.7.1 Weight:

A bathroom scale was used to take the weight. The subjects were weighed barefoot and with minimal clothing on a standard portable bathroom scale. The scale was set to zero before each measurement.

3.1.7.2 Height:

A perpendicular wall against a flat floor was identified as the field and was marked using fibre tape to an accuracy of 0.1 cm. The subjects were asked to stand bare feet on the floor against the wall. The feet of the subjects were parallel and with heels, buttocks, shoulders and back of the head touching the wall. The head was held comfortably erect and marked for measuring height with a flat scale touching the top of the head horizontally and its vertical edge flat against the wall.

3.1.7.3 Body mass index (BMI):

The BMI was calculated with the following formula:

$$\text{BMI} = \text{weight (kg)} / \text{height (m}^2\text{)}$$

The classification for BMI has been shown in the following box.

Table 3.1.7.3.1 Asia pacific classification of BMI (WHO, 2004)

Presumptive diagnosis	BMI (kg/m^2)
Obese	≥25
Over weight	23-24.9
Normal	18.5-22.9
Underweight	<18.5

(Sharma K, 2008)

3.1.7.4 Waist circumference:

Waist circumference was measured around the narrowest point between ribs and hips when viewed from the front after exhaling, three consecutive recordings were made for each site to the nearest 1 cm using a measuring tape on a horizontal plane without compression of skin. The mean of three sets of value was taken.

3.1.7.5 Hip circumference:

Hip circumference was measured at the point where the buttocks extended the maximum, when viewed from the side. Three consecutive readings were made for each site to the nearest 1 cm using a measuring tape in a horizontal plane without compression of the skin, the mean of three values were used.

3.1.7.6 Waist hip ratio (WHR):

This was taken by using the following formula:

WHR = Waist circumference (cm) / Hip circumference (cm)

Table 3.1.7.6.1 Cut offs for WHR (WHO 2004)

Category	Male	Female
Waist (cm)	≥ 90	≥ 80
WHR ≥ 0.09	Obese	NA
WHR ≥ 0.08	NA	Obese

(Sharma K, 2008)

3.1.8: Biophysical investigations

Blood pressure: Blood pressure was measured to estimate the prevalence of hypertension amongst elderly subjects. Diamond mercurial sphygmomanometer was used to assess the blood pressure.

Table 3.1.8.1 Classification of blood pressure according to JNC VIII criteria:

Category	Systolic	Diastolic
Normal	<120	<80
Pre hypertension	120-139	80-89
Hypertension stage I	140-159	90-99
Hypertension stage II	≥160	≥100

Source: Joint National Committee (JNC 8), 2014

3.1.9: Assessment of socioeconomic status

A questionnaire was made which included information about age, marital status, education, occupation, religion, total family members, family income as well as per capita income.

3.1.10: Assessment of physical activity

A 24 hour recall and self-reported time spent in hours was conducted through a structured questionnaire to get information of routine activities which included leisure activities, exercise, yoga, social/religious activities, sleep/rest and idle time.

3.1.11: Assessment of major and minor illnesses

This included a checklist of questions regarding major (chronic) and minor illnesses (in last 15 days) experienced by the subjects. A detailed checklist was used for both major and minor problems like oral, gastrointestinal, respiratory, psychological, locomotors, cardiovascular, endocrine and hepatobiliary.

3.1.12: Addiction pattern

The subjects were asked if they were addicted to substances like cigarette/bidi, tobacco, gutkha, pan masala, snuff etc.

3.1.13: Assessment of Dietary intake

The data related to subject's dietary intake was obtained from 24 hour dietary recall, food frequency and general dietary aspects.

3.1.13.1 24-hour dietary recall:

24 hour dietary recall of three consecutive days was collected (avoiding fasting and feasting days). A mean of three days recall was included in the data. Standard measuring cups and spoons were used to indicate the intake amount. RDA was calculated for energy, protein, fat, iron, calcium, vitamin C, and β carotene.

3.1.13.2 Food frequency:

A 163 – item based food frequency questionnaire (FFQ) was constructed which included calcium and vitamin D rich raw and cooked food groups/items. All the food groups/items were divided into 12 categories such as cereals, pulses and legumes, roots and tubers, other vegetables, nuts and oil seeds, fruits, fish and other sea foods, meat and poultry, milk and milk products, sugars, cooked and readymade items like cakes and sweets and beverages. In order to know the frequency of consumption participants were asked to indicate how often, on average, they had consumed each food item over the past year, with eight predefined categories: <1 time/month, 1–3 times per month, 1–2 times per week, 3–4 times per week, 5–6 times per week, once a day, 2 times a day, and ≥3 times per day. Respondents were asked to indicate an average frequency of consumption and portion size (in grams, millilitres or household measurements) of the products consumed from FFQ list.

3.1.14: Assessment of knowledge and awareness

Questions to assess the awareness of subjects regarding osteoporosis, knowledge of Ca and vitamin D as two crucial nutrients for bone were included in the general questionnaire.

PHASE II (Analytical/intervention research)

In this phase, out of 1056 blood sample was collected from 328 randomly selected osteopenic or osteoporotic males and females. Then from 328 subjects 222 subjects

with either VDI or VDD were selected based on purposive sampling strategy. Osteopenia or osteoporosis and vitamin D insufficiency or vitamin D deficiency were the basic criteria to enroll subjects in this phase. The intervention included calcium and vitamin D_3 supplementation alone and along with physical exercise. Serum vitamin D was analyzed at the baseline as well as after the intervention to get the improvement in the period of six. Then, these 222 subjects were further divided into four small groups. The details of the groups and intervention in all four groups are discussed next.

3.2.1: Inclusion and exclusion criteria for the study subjects

Phase II- Analytical study
Inclusion criteria
Subjects who have low BMD.
Subjects with VDI or VDD.
Exclusion criteria
Subjects who were taking calcium and vitamin D supplementation or took the same in last 6 months.
Subjects having any history of calcium stones or similar health problems.

3.2.2: Selection, distribution of subjects and intervention strategy

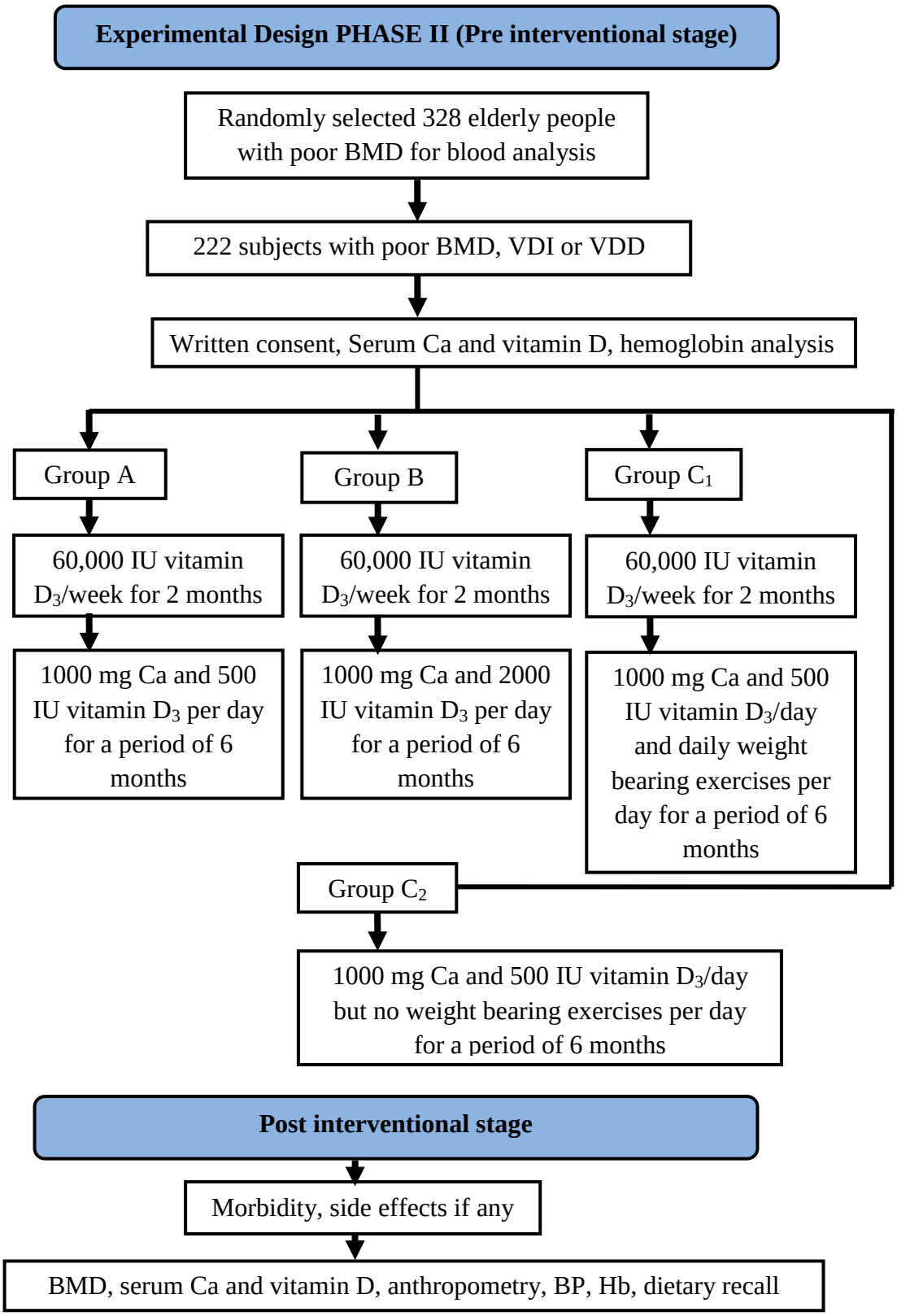
As per the inclusion criteria all eligible subjected were selected and randomly divided into four groups (n=222):

- **Group A (n=63, male=31 and female=32):** In this group, the subjects received a dose of 60,000 IU of vitamin D_3 per week for two months coupled with 1000 mg Calcium and 500 IU vitamin D_3 supplement per day for a period of six months.
- **Group B (n=59, male=29 and female=30):** In this group, the subjects received a dose of 60,000 IU of vitamin D_3 per week for two months coupled with 1000 mg Calcium and 2000 IU vitamin D_3 supplement per day for a period of six months.
- **Group C_1 (n= 50, male= 30 and female=20):** In this group, the subjects received a dose of 60,000 IU of vitamin D_3 per week for two months followed

by 1000 mg Calcium and 500 IU vitamin D_3 supplement per day coupled with daily weight bearing exercises (Details of the weight bearing exercises are mentioned in the Materials section).

- **Group C_2 (n=50, male= 30 and female=20):** In this group, the subjects received a dose of 60,000 IU of vitamin D_3 per week for two months along with 1000 mg Calcium and 500 IU vitamin D_3 supplement per day for a period of three months, but without weight bearing exercises. This group, therefore, was the control group to C1.

3.2.3: Stepwise execution of the therapy



3.2.4: Study protocol

Step 1: Subjects required immediate medical care (like severe osteoporosis) were referred to the doctor. After taking a written consent selected eligible participants for serum calcium and vitamin D analysis were given a date and venue to be gathered. Blood sample was collected to assess serum vitamin D, calcium and hemoglobin by a trained technician under constant super vision of a doctor.

Step 2: Objectives of the II phase of the study, importance and benefit of Calcium and vitamin D supplementation, exercise, expected outcome and possible side effects etc. were briefed (if any) to the subjects and their family members.

Step 3: The subjects in four groups were provided with an identification number. Subjects in four groups were given their respective doses separately with guidelines. A weekly distribution of doses was maintained. Intervention and compliance were recorded regularly in compliance sheet (Appendix-VII).

Step 4: Subjects were asked to perform the physical endurance tasks such as standing balance, grip strength, walking speed and rise from the chair and the performance scores were recorded. Then picture charts showing various weight bearing exercises were distributed among the subjects in group C_1 (Appendix-VIII). The subjects were guided and trained to perform the weight bearing exercises regularly along with oral Ca and vitamin D supplementation.

Step 5: Post interventional data was collected after a period of three months in group C_1 and C_2; and after six months in group A and B. Post interventional data included the following:

- BMD
- Serum calcium and serum vitamin D
- Anthropometric measurements
- Blood pressure
- Hemoglobin
- Morbidity
- Dietary intake

- Physical endurance tasks

  Physical endurance was reassessed after the intervention to analyze the impact of the intervention coupled with weight bearing exercises. The goal was to understand the efficacy of two different doses and exercise on poor bone health.

Step 6: After collecting the post interventional data, data was refreshed, and entered in a database.

3.2.5: Biochemical assessment

Venous blood sample was collected in vacutainers coated with EDTA by the using disposable syringes. These tubes may contain additional substances that preserve the blood until the assessment is finished. Serum was separated in vacutainer and stored at a temperature of -80^{o} C until they were analyzed. Following are the three estimations done.

3.2.5.1 Haemoglobin estimation:

The hemoglobin test is often used to check anemia, usually along with a hematocrit or as part of a complete blood count (CBC). Hemoglobin is the iron-containing protein found in all red blood cells that enables RBCs to bind to oxygen in the lungs and carry it to tissues and organs throughout the body. Hemoglobin was measured using the Cyanmet- haemoglobin method (Agarwal K. N. *et. al.*, 2006; Sari M. *et. al*., 2001; Gillispie, 1998).

Table 3.2.5.1.1 Cut offs for hemoglobin

Gender	Normal	Mild	Moderate	Severe
Male	≥ 13 gm/dl	11 – 12.9 gm/dl	8 – 10.9 gm/dl	<8 gm/dl
Female	≥ 12 gm/dl	11 – 11.9 gm/dl	8 – 10.9 gm/dl	< 8 gm/dl

Source: Khusun H. et. al. 1999

3.2.5.2 Blood calcium estimation:

Serum calcium levels were assessed using Cresolphthalein complexion method (Hovsepian S. *et. al.,* 2011; J. C. Reichert *et. al.,* 2010; Ikegame M. *et. al.,* 2004; Baginski E.S, 1973; Young S.D., 1975).

Table 3.2.5.2.1 Serum calcium cut offs

Serum level	Category
<9 mg/ dl	Deficiency
9 – 11 mg/ dl	Normal

Satyanarayana U, 2010

3.2.5.3 Blood vitamin D estimation:

For the assessment of serum vitamin D levels, the CLIA (Chemiluminescence Immunoassay) method was used (Wallace A.M. *et. al.,* 2010; Wagner D. *et. al.,* 2009; Rollins, 2009; Ersfeld E. L. *et. al.,* 2004).

Principle: The principle involves in the method that of Competitive protein binding assay.

***** In laboratory fully automated Chemileuminescence assay (CLIA) was used for serum vitamin D estimation.

Table 3.2.5.3.1 Serum vitamin D cut offs

Serum level	Category
≥ 30 ng/ ml	Normal
20 - <30 ng/ml	Vitamin D insufficiency (VDI)
<30 ng/ml	Vitamin D deficiency (VDI)
10 - <20 ng/ml	Mild VDD
5 - <10 ng/ ml	Moderate VDD
<5 ng/ ml	Severe VDD

(Source: Hollick MF, 2007, Lips P, 2001)

3.2.6: Physical endurance test

To measure the impact of calcium and vitamin D supplementation and daily weight bearing exercises on physical performance of the subjects, tests such as grip strength, standing balance, walking speed and time to raise one's own self from a chair were assessed. Each physical performance test was classified and scored accordingly. The four endurance tests are as follows:

3.2.6.1 Grip strength:

This test was performed using a dynamometer. Hand dynamometers are basically used for routine screening of grip strength. The subject was asked to hold the dynamometer with the elbow kept in a horizontal position. The base rested on first metacarpal, while the handle rested on the middle of other four fingers. Then the subject squeezed the dynamometer with maximum isometric effort and maintained it for at least 5 seconds. No other body movement or support was allowed. The scores below were given according to the performance:

Table 3.2.6.1.1 Grip Strength scoring

Score	Remark	Units
1	Good	>20
2	Average	11-20
3	Poor	<10

3.2.6.2 Standing balance:

Subjects were asked to put their feet side by side, semi tandem (heel of one foot directly in front of other foot) and full tandem positions and hold them in these positions for at least 10 seconds each. The subjects were given the following scores:

Table 3.2.6.2.1 Standing Balance scoring

Score	Time holding semi tandem position	Time holding full tandem position
1	<10 seconds	-
2	10 seconds	>2 seconds
3	-	3-9 seconds
4	-	10 seconds

3.2.6.3 Walking speed:

A 2.4 meter walk was performed by the subject at his/her own pace. The time taken to walk 2.4 meters was noted and scored according to the quartiles for length of time required. The shortest time of two such walks was used for scoring.

3.2.6.3.1 Walking Speed scoring

Score	Time (seconds)
1	>5.7
2	4.1-5.6
3	3.2-4
4	<3.1

3.2.6.4 Rise from the chair:

Subjects were asked to fold their arms across their chest and to stand up from a sitting position (without any support). If they successfully rose from the chair, they were asked to do the same five times and as quickly as possible. Quartiles for length of time required for this measure were used for scoring.

3.2.6.4.1 Rise from the chair scoring

Score	Time (seconds)
1	>16.7
2	13.7-16.6
3	11.2-13.6
4	<11

- Daily weight bearing and muscle strengthening exercises suggested by National Osteoporosis Foundation were referred and according to NOF the best exercises for building and bone density are:

- Weight bearing exercises, such as walking (either outside or on a treadmill machine) that makes the subject work against the gravity while staying upright.
- Muscle strengthening exercise, such as weight lifting, that would work against gravity in a standing, sitting or prone position.

3.2.7: Monitoring the intervention

Once the intervention was executed, monitoring it on a regular basis was an important and essential part of the study. The few points that were taken care of as a part of monitoring the intervention are as follows:

- All eligible enrolled subjects were given an identification number.
- Any side effect that occurred, if at all, was carefully followed on. Anything that indicated withdrawal of the subject was immediately taken care of.
- Supplements were given by undertaking weekly domiciliary visit. Compliance of the supplementation was checked regularly.
- Reinforcement of the prescribed exercises (using charts, pamphlets, leaflets etc.) was done by undertaking domiciliary visit every 15 days.

3.2.8: Statistical tools and methods used in data analysis

The data was entered in Microsoft Excel 2007 and transferred to STATISTICA 64 – version 12. Data was analysed in STATISTICA 64 and Diet soft (Version - 2).

- The quantitative (continuous) data has been presented as mean ± SD, median and range.
- Categorical data has been presented as frequencies and percentages.
- Analysis of variance and ANOVA Post – hoc LSD test have been performed to obtain p – value for continuous variables where there are more than two groups to be compared.
- Pearson correlation has been performed to determine the strength of the association between variables (+1 – perfect positive correlation, -1 – perfect negative correlation).

- Log normal has been performed to neutralise the extreme values from the continuous data set to follow normality, and Analysis of variance or ANOVA LSD test has been applied.
- Kruskal Wallis test has performed for the set of data, that neither follow normality nor log transformation is possible to perform (http://documentation.statsoft.com/STATISTICAHelp).
- Tukey HSD test was performed for continuous data where there are more than two groups with unequal N to be compared (http://documentation.statsoft.com/STATISTICAHelp).
- McNemar test was performed to predict the level of significance in paired nominal data set (http://www.statisticshowto.com/mcnemar-test/; http://documentation.statsoft.com/STATISTICAHelp.aspx?).
- Chi^2 test has been performed to determine association between two categorical variables.
- Students' t test has performed to compare continuous variables in two groups.
- Paired t test has been performed to compare the difference between the pre and post data for the outcome variables in the intervention phase.
- A confidence interval of 95% has been taken based on the sample observation.
- Bar charts have used to show frequency distribution of nominal and ordinal data.
- Histograms have been used to show frequency distribution of continuous data.
- Results are considered statistically significant with a 2 tailed $p<0.05$, <0.01, <0.001.

3.2.9 Tools and techniques used in data collection:

Parameters	Tools
Measurement of bone mineral density (BMD)	Ultrasound based BMD machine
Socio economic profile	Semi structured questionnaire
Life style pattern (activity and addiction)	Semi structured questionnaire
Nutritional status (anthropometry)	
Weight	Bathroom scale

Height	Fibre glass tape
BMI	By standard formula: weight(kg)/height (m^2)
Waist circumference	Fibre glass tape
Hip circumference	Fibre glass tape
WHR	By standard formula: WC/HC
Haematology	
Blood calcium estimation	Colorimetric method
Blood vitamin D estimation	CLIA(Chemiluminescence Immunoassay Technique)
Haemoglobin	Cyanmethemoglobin estimation method
Clinical parameters	
Blood pressure	Sphygmomanometer
Dietary intake	24 hour dietary recall method Food frequency questionnaire for calcium rich foods Diet Soft
Disease profile	- Check list of major and minor illnesses - General health profile
Physical endurance	- Grip strength (dynamometer) - Standing balance - Walking speed - Number of rises from the chair
Daily exercises	Selected exercises to improve BMD