

### III. METHODOLOGY

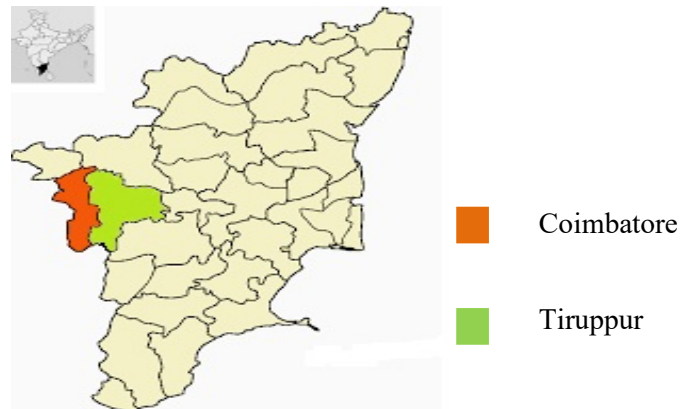
After identifying the study problem and conducting preliminary research about the problem, the present study titled “**Bone Health and Risk Assessment of Menopausal Women for Osteoporosis and Strategic Diet Care Practices using Calcite Enriched Recipes**” was framed. The study was carried out through three phases as mentioned below.

- Phase I** : Collection of Data on Demographic Profile, Anthropometric Measurements, Physical Activity, Exposure to Sunlight and Dietary Assessment
- Phase II** : Association of Risk Factors of Osteoporosis among Selected Subjects
- Phase III** : Development of Calcite (eggshell) Incorporated Recipes and Promotion of Bone Health through Awareness

**Phase I : Collection of Data on Demographic Profile, Anthropometric Measurements, Physical Activity, Exposure to Sunlight and Dietary Assessment**

#### **Selection of Study Area:**

Areas in Coimbatore and Tiruppur districts including urban and rural areas, were selected based on easy accessibility and availability of menopausal stage women to perform the study. Tiruppur or Tirupur is a city located in the state of Tamil Nadu, India. “Tiruppur is located at 11.1075°N 77.3398°E on the banks of the Noyyal River. It covers an area of 159.6 km<sup>2</sup>”. Coimbatore is one of the major metropolitan cities in Tamil Nadu, India. “Coimbatore lies at 11°1’6”N 76°58’21”E in South India at 411 meters (1349 ft) above sea level on the banks of the Noyyal River, in the northwestern part of Tamil Nadu. It covers an area of 642.12 km<sup>2</sup>”.



**Figure I - Tamil Nadu State Map**

### **Selection of the Sample:**

Women at various menopausal stages were selected for the study. Menopausal stage includes perimenopause, menopause and post menopause. Perimenopause begins about eight to 10 years before menopause. Menopause is a period where women experience no menstrual periods for a year and post menopause is when women experience more than one year of no menstrual periods (WHO, 1996 and Cleveland, 2021).

### **Sample Size Determination:**

The sample size was determined using the formula by Vishwakarma (2017). Based on the formula, the sample size was determined as 1036 numbers, hence the same number of subjects were selected to perform the study.

$$\text{Sample Size} = (Z\text{-score})^2 * \text{Std Dev} * (1 + \text{Std Dev}) / (\text{margin of error})$$

The subjects were selected using the purposive random sampling method. Judgmental or subjective sampling method is the other term for purposive sampling method, is a form of non-probability sampling. In purposive sampling method, the participants among the population are selected based on the judgment of the researcher itself (Kothari and Garg, 2019). The subjects were selected considering the inclusion and exclusion criteria mentioned below.

### **Inclusion Criteria:**

- Female subjects of age above 40 years.
- Those who are willing to participate in the study.

**Exclusion Criteria:**

- Without any chronic illness and disabilities
- Under regular calcium supplementation

The study was approved by the Institutional Human Ethical Committee (IHEC/21-22/FSMD-28) of Avinashilingam Institute of Home Science and Higher Education for women (Appendix I).

The significance of the study and its purpose was delivered to the selected subjects in the local language and their written consent was obtained (Appendix II). It was also informed that they can withdraw from the study whenever necessary.

**Data Collection Using Tools:**

In order to collect data on demographic profile, physical activity, exposure to sunlight, menopausal stage, symptoms and dietary pattern (24 hrs dietary recall), a well structured interview schedule was developed (Appendix III). The Interview schedule covered the following information.

**Demographic Data:**

Demographic data including age, education, marital status, religion, occupation and monthly income were collected. The educational status of the subjects were assessed based on their level of education which includes professional degree, graduate or postgraduate, intermediate or post-high school diploma, high school, middle school, primary school and Illiterate. The occupation was assessed based on the following criteria which include professional (white-collar), semi-professional, clerical, shop owner/farm, skilled worker, semi-skilled worker, unskilled worker, unemployed and other occupations.

Monthly income was collected under the following classifications according to their income per month  $\leq 2640$  rupees, 2641-7,886, 7887-13160, 13161-19,758, 19759-26354, 26355-5273,  $\geq 52734$ . The classification was based on the “Modified Kuppuswamy” scale (Sheikh, 2018) and based on the same scale various demographic data were collected and socio-economic status was gathered. The socio-economic class was represented as upper, upper-middle, lower-middle, upper-lower, and lower class.

**Physical Activity and Sun Exposure:**

Adequate physical activity and exercises like weight lifting, walking, running, jumping, step climbing and even swimming helps in prevention and in the treatment of osteoporosis among menopausal women (Parschau *et al.*, 2014).

The physical activity of subjects was studied according to the “International Physical Activity Questionnaire” (Winzenberg *et al.*, 2003) which includes data on the job related physical activity, transport-related physical activity, housework, house maintenance, and caring for family-related physical activity, recreation, sport, and leisure-time physical activity and time spent sitting based on which scores were given and subject’s physical activity was assessed.

Vitamin D plays a major role in maintenance of peak bone mass for which sun exposure serves as a great source of vitamin D (Holick, 2004 and Laird *et al.*, 2010). Sunlight exposure pattern of subjects were analysed and presented as adequate and inadequate exposure, which was classified based on the findings of Harinarayan (2018).

**Menopausal Stages and Symptoms:**

Menopause is the end of the menstrual cycle in women’s life. There are three stages perimenopause, menopause, and post-menopause (Khani *et al.*, 2021). The onset of menopausal symptoms differs from person to person it may start before four months or it may even begin two to three year menopause.

The most common menopausal symptoms like joint and muscular discomfort, physical and mental exhaustion, sleeping problems, hot flushes, sweating, irritability, dryness of vagina anxiety, depressive mood, sexual problems, heart discomfort, bladder discomfort were listed among the symptoms, the most common symptoms experienced by selected subjects were assessed (Santoro *et al.*, 2015 and Agarwal *et al.*, 2019).

**Diet Pattern:**

There is no single method available that would be suitable enough to gather all the information. Thus, a combination of various methods was used in the present study. Food frequency questionnaire and 24-hour dietary recall methods were used to obtain data on dietary pattern and intake of selected subjects.

**Food Frequency Questionnaire (FFQ):**

It is the measure of long term dietary intake and its frequency, retrospectively i.e., food consumed each day, week, month, or any other specific period is recorded by the interviewer or through a self-administered questionnaire, consisting of a list of specific foods or food types associated with frequency of consumption (Welch, 2021; Thornton and Villamor, 2016).

This technique is often employed along with 24-hour diet recall to enhance the proximity of dietary data collected. In order to obtain information on food consumption both qualitatively and descriptively along with the quantity of food or nutrient intake, frequency questionnaire was designed (Shim *et al.*, 2014).

In the present study, the food frequency questionnaire was formulated to gather qualitative information on the frequency of the consumption of various calcium-rich food items covered under major food groups.

**24-hour Dietary Recall Method:**

It is one of the most commonly used methods of dietary assessment (Kweon *et al.*, 2014). It is a retrospective and quantitative method (Vossenaar *et al.*, 2020). In this method, the subject is asked to recall household measures of all the food and beverages that they consumed over the past 24 hours.

A day's recall may also be appropriate for assessing the average intake of food and nutrients. This method was selected for the study considering the advantages such as its an easy and relatively inexpensive method, a large sample can be covered and it can be used for illiterate individuals, the respondent burden or effort is low and therefore the compliance is high.

Though, it could have been ideal to conduct three days of 24 hours of dietary recall; due to pandemic situation and a large number of the subjects in the study, a dietary recall was done only for one typical day. The subjects were asked to recall the previous day food intake and report in details of all the consumed food items along with their ingredients and the same was recorded.

**Calculation of Nutritive Value:**

Raw data collected from 24 hours recall method are converted into nutrient intake per day. Food items were converted into nutrients obtained by using 'Indian Food Composition Tables' by Longvah *et al.*, (2017). Nutrients calculated for each meal were summed up and specific nutrients like calories, carbohydrates, protein, fat, fiber, calcium, and phosphorous per day were calculated and compared with the "Recommended Dietary Allowances" by ICMR, 2010.

**Diet Diversity:**

Intake of various food groups in an individual's diet is associated with adequate intake of energy and essential nutrients. Therefore increasing variety in one's diet is recommended in most dietary guidelines globally and mainly it is important in a country like India where the staple food is rice and wheat which are high in starch (Ruel, 2003).

Hence the present study aimed at collecting the diet diversity of the subjects among the following food groups. i] Cereals; ii] White roots and tubers; iii] Vitamin A-rich vegetables and tubers; iv] Dark green leafy vegetables; v] Other vegetables, vi] Vitamin A-rich fruits, vii] Other fruits, viii] Organ meat, ix] Flesh meats, x] Eggs, xi] Fish & seafood, xii] Legumes, nuts & seeds, xiii] Milk & milk product, xiv] Oils & fats, xv] Sweets, xvi] Spices, Condiments, and Beverages.

**Osteoporosis Risk Assessment:**

In the present study the tools used to assess the osteoporosis risk were OST (Osteoporosis Self-assessment Tool), ORAI (Osteoporosis Risk Assessment Instrument) and SCORE (Simple Calculated Osteoporosis Risk Estimation).

**OST (Osteoporosis Self-assessment Tool):**

The Osteoporosis Self-assessment Tool (OST) is a simple formula that predicts the risk of osteoporosis. It is a rapid assessment and an useful tool to prompt a further diagnosis of skeletal health.

The subject's weight and age were collected and applied in the formula  $(\text{Weight} - \text{Age}) \times 0.2 = \text{score}$  and the obtained scores were compared with the table I and the risk of osteoporosis was assessed (Koh *et al.*, 2001).

**Table I - Osteoporosis Self-Assessment Tool**

Variable	Scores
OST>1	Low Risk
-3<OST<1	Moderate Risk
<-3	High Risk

**ORAI (Osteoporosis Risk Assessment Instrument):**

The Osteoporosis Risk Assessment Instrument (ORAI) is used to assess the risk of osteoporosis from which women could be identified for bone densitometry test, by which this tool reduces the screening time and cost as it is resourceful for people with risk of osteoporosis (Cadarette *et al.*, 2000).

Certain parameters like age in years, weight in kilograms and their current estrogen usage were collected. Age, weight, and estrogen usage are given scores as per the classification shown in the table II. Then the scores of three parameters are added together to get the final score, when it is above 9 it is considered as at the risk of osteoporosis.

**Table II - Osteoporosis Risk Assessment Instrument**

Variables		Scores
a	Age	
	75 $\geq$	15
	65-74	9
	55-64	5
	44-54	0
b	Weight	
	<60	9
	60-69	3
	$\geq$ 70	0
c	Estrogen usage	
	No	0
	Yes	2

### SCORE (Simple Calculated Osteoporosis Risk Estimation):

Parameters like gender, affected with rheumatoid arthritis, have undergone any non-traumatic fractures, age above 45, estrogen intake are considered. And the scores were given to each parameter as given in the table III. Finally, a sum of all the scores gives the estimation of osteoporosis risk. When the total score is above 6 it was considered as at risk of osteoporosis (Lydick *et al.*, 1998).

**Table III - Simple Calculated Osteoporosis Risk Estimation**

Variables		Scores
a	Every gender other than black	5
b	Rheumatoid arthritis	4
c	Non traumatic fractures	4
d	-12 scores for >45 years including wrist, rib and hip fracture	3
e	Every 10 years without taking estrogens	1

### Anthropometry Data:

Anthropometry is the systematic measurement of the physical properties of the human body (Russell and Steve, 2019). The future ill health, or mortality can be strongly predicted from individuals anthropometric characters; in turn, they may be used in many contexts to screen for or to monitor diseases (WHO, 1995). The measurements vary with age and nutritional status and the results are particularly useful in circumstances where a chronic imbalance of protein and energy are likely.

Anthropometry has the additional advantage of individual's past nutritional history by providing information which can be obtained with high confidence among other assessment techniques. According to Annamalai and Lal (2018) anthropometric measurements can be considered as a risk of osteoporosis. The risk of osteoporosis and its related complications can be assessed through weight and BMI.

### Measurement of Height:

The height of subjects was noted by making them stand wide their feet against a wall with marking on it. A wooden plank was placed at the head such that it makes contact with the top of the head and the height was measured in centimeters. Further, the height was measured to the nearest of 0.1 cm zero error was corrected every time before taking the actual measurement.

### Measurement of Weight:

Body weight is the key component of nutritional assessment. Weight generally reveals short-term nutritional status. The weighing balance is a useful and accurate tool for investigating the nutritional status. Weight was recorded using a portable weighing balance.

The subjects were asked to remove footwear and stand on the weighing scale with one foot on either side of the scale without holding on to anything with possibly minimum clothing and they were asked to stand with their head comfortably erect. The weight was recorded in kilograms and to the nearest of 0.1 kg, zero error was corrected every time before actual weighing.

### Body Mass Index:

Body Mass Index is also known as Quetelet's Index. It is considered a good indexing tool for measuring body fat (Gonzalez *et al.*, 2017). BMI is a mathematical formula, which correlates with the body fat of an individual than any other indicator like height and weight. The body mass index is a good measure, which reflects on the nutritional status of a person. Body mass index is defined as weight divided by Height in meter square and is a better indicator of nutritional status.

$$\text{BMI} = \text{Weight in kg} / \text{Height in meter square}$$

In the present study, BMI was calculated and categorized based on Asia pacific guidelines, 2017 as shown in the table IV.

**Table IV - Body Mass Index Classification based on Asia Pacific Guidelines**

Category	Cut-off points
Underweight	<18.5
Normal	18.5-22.9
Overweight	23-24.9
Obese	≥25

\*Asia Pacific Guidelines, 2017

### Family History and Self History of Morbidity:

Apart from the data discussed above, family history and self-history of the subjects were collected. Common morbidities like Obesity, Diabetes, High or Low Blood Pressure, Cardio Vascular Disease (CVD), Thyroid dysfunction, Epilepsy,

Osteoporosis were listed in the interview schedule. With this subject's co-morbidities and the history of subject's family members including father, mother, paternal grandparents, maternal grandparents, maternal relatives, and paternal relatives were collected.

**Assessment of Knowledge on Osteoporosis:**

To carry out assessment of knowledge on osteoporosis, OKAT was used (Appendix IV) and data were collected and analysed.

**Pilot Study:**

A pilot study was conducted among 60 selected subjects with the same inclusion and exclusion criteria to validate the interview schedule and osteoporosis risk assessment tool. The Bone Mineral Density (BMD) was assessed using bone densitometer for the selected subjects. The three osteoporosis risk assessment tools like OST, ORAI, and SCORE were used and compared statistically with the actual measured BMD of the selected subjects.

As the result of the pilot study, ORAI was found to be the most relevant tool compared with other tools and hence ORAI tool was used to assess the osteoporosis risk and to carry out further study.

**Collection of Data:**

Using the above-described tools, the data were collected from the selected subjects (n=1036) after getting their consent and explaining the significance. Questions were well explained in the local language for their better understanding and their responses were noted carefully. If there were any vague answers by subjects, the concept was explained again and their responses were subsequently recorded accurately.



**Plate 1- Collection of Data among Selected Subjects**

**Analysis of Data:**

Data collected from the interview schedule was coded and was analyzed using SPSS 21 (Statistical Package for Social Sciences). Baseline data were analyzed using descriptive statistics like cross tabs, percentages, mean and standard deviations. To assess the significance of different categories for all dependent variables, ANOVA was used and qualitative variables were analyzed using the chi-square test. The strength of association and relationship were analyzed using coefficient correlation.

**Phase II : Association of Risk Factors of Osteoporosis among Selected Subjects**

From the result of phase I, at risk subjects (678) were categorized based on age group into 40 to 45 years, 46 to 50 years, 51 to 55 years and 56 to 60 years and their association with risk factors were compared statistically. Bone Mineral Density (BMD) screening were performed for sub samples (416) and their result were analysed.

**Bone Mineral Density (BMD) screening:**

From the at risk subjects 416 sub samples had been selected based on the following inclusion criteria to perform Bone Mineral Density (BMD) screening,

**Inclusion Criteria:**

- Subjects willing to participate in awareness session
- Subjects willing to undergo BMD assessment
- Subjects having a smart phone

Bone Mineral Density (BMD) was measured by a bone densitometer. A bone densitometry is used to measure bone density most accurately which is a painless image scanning test; in other terms it can be a useful tool in diagnosing, preventing and treating osteoporosis. It is a small portable device with dry gel system that measures Broadband Ultrasound Attenuation (BUA) in dB/MHz and Speed of Sound (SoS). An ultrasound beam is passed through heel. The calcaneous BMD is calculated in  $\text{g.cm}^2$  (Ejaz *et al.*, 2012 and Kim, 2015).

Subjects were made to place their bare feet on the machine for scanning. As a result, BMD assessment machine gives a t-score value with which the bone mineral density is assessed. The criteria for assessment is given in the table below:

**Table V - Bone Mineral Density Classification based on WHO**

<b>T Score</b>	<b>Bone Mineral Density</b>
>-1	Normal
-1 to -2.5	Osteopenia
<-2.5	Osteoporosis

\*WHO,2007



**Plate 2 - Screening of Bone Mineral Density of Selected Subjects**

#### **Analysis of Data:**

Further analysis was done comparing BMD with the risk factors of osteoporosis like physical activity, sun exposure, calcium intake, knowledge on osteoporosis and BMI using SPSS 21 (Statistical Package for Social Sciences).

### **Phase III Development of Calcite (eggshell) Incorporated Recipes and Promotion of Bone Health through Awareness**

#### **A. Development of Egg Shell Calcite Powder:**

Calcium is the essential mineral required to maintain bone health. About 99 percent of absorbed calcium is stored in bones and teeth. About 600 mg of calcium is required per day for adults to maintain bone health. After a certain age, mainly in women after menopause causing osteoporosis (low bone density) where the bones become weak and brittle.

At this stage of low bone density, calcium supplements are suggested to maintain bone health and prevent severe osteoporosis and fractures. Most of the calcium supplements will be in the form of calcium carbonate or calcium due to their high elemental calcium. Chicken eggshell is claimed to have a high source of calcium.

Chicken egg is a crucial source of high-quality human nutrition. Massive numbers of eggs are produced annually with a significant proportion (30 percent) being processed in industrial breaking plants, leading to the accumulation of eggshell and eggshell membrane waste. Egg Shell can be utilized for various applications as a source of calcium carbonate (CaCO<sub>3</sub>), calcium oxide, and after transformation, hydroxyapatite. Eggshell applications include food supplements, adsorbents, antimicrobial agents, soil amendments, catalysts, Guided Tissue Regeneration (GTR), and CaCO<sub>3</sub>-based interventions (Ahmed *et al.*, 2019). Eggshells are roughly 40 percent of calcium approximately half of an eggshell contains the recommended daily intake of calcium (Brun *et al.*, 2013 and Schaafsma *et al.*, 2000).

#### **Collection of Eggshells:**

The chicken eggshells have been collected from the egg product industry from the same batch of white leghorn hen, where hens were uniformly fed. The collected eggshells were washed properly and then dried. The eggshells have been crushed using mortar and pestle into pieces as the eggshells were hard to grind into fine powder. And the eggshells have been powdered using the mixer grinder. The eggshell powder was stored in an airtight container.

#### **Processing of Eggshell Powder:**

The eggshell powder have been washed thoroughly for the removal of eggshell membrane and other impurities if any. The eggshell powder was washed with the purified water with less than 10 ppm and left for an hour in the water for the eggshell powder to settle down, so other impurities and membrane would be floating and it would form froth and the impurities were poured down and egg shell powder have been filtered. This procedure was performed again several times until all the impurities were washed out and the water remains pure.

Water or moisture greatly affects the shelf life of food. Spoilage of food mostly occurs due to excessive moisture content, let it be microbial spoilage or alteration in the chemical reactions (Lee and Robertson, 2021). The wet eggshell

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powder was spread in a sterilized tray and dried in a hot air oven at 135 degrees Celsius for about 3-4 hours till the eggshell powder had completely dried. And the dried eggshell powder is again stored in the sterilized container till further processing.

#### **Development of Eggshell Calcite Powder:**

Almost 94-96 percent of an eggshell was reported to be in the form of  $\text{CaCO}_3$  (Zaman *et al.*, 2018 and Sathiparan, 2021). The dried powder was weighed. Then the eggshell powder was taken in 6 crucibles as 6 samples and heated in the muffle furnace. The powdered and washed eggshell was heated in a muffle furnace to  $900^\circ\text{C}$  at half an hour interval for every  $200^\circ$  rise in temperature. The eggshell powder was maintained at  $900^\circ\text{C}$  for four hours and the final sample was taken out. At every  $200^\circ\text{C}$  raise in temperature, one sample among six samples was taken out for understanding the changes in eggshell powder during the process.

#### **Characterization of Developed Eggshell Powder:**

Characteristics like size, shape, nature of developed eggshell calcite powder, calcium, heavy metals, moisture and ash content were analyzed using FESEM (Field Emission Scanning Electron Microscope) analysis and AOAC Methods of calcium content analysis (Appendix VII), heavy metals analysis (Appendix VIII), moisture content analysis (Appendix IX) and ash content analysis (Appendix X).

### **B. Development of Recipes:**

#### **Selection of Ingredients:**

The recipes have been developed based on the requirements of menopausal women. After the age of 50 years, bone density begins to decrease subsequently (Dennison *et al.*, 2017). Due to lack of protein intake there is muscle deterioration (Bauer *et al.*, 2013). Less production of estrogen hormone is one of the main transitional changes during menopause, which is very essential to maintain bone health. Phytoestrogens are functional compounds present in plants that act as estrogen commonly used for the treatment of menopause-related conditions like osteoporosis (Rowe and Baber, 2021).

Adequate intake of calcium and vitamin D is essential for maintenance of bone mineral density, thereby preventing osteoporosis and fractures in older adults have been discussed in various studies. Calcium is one of the most important

minerals to maintain bone health. Considering all these nutrient requirements, recipes were prepared with ingredients that are rich in protein, calcium, omega 3 fatty acid, and vegetables rich in phytoestrogens.

### **Categorisation and Selection of Recipes:**

Recipes were developed based on five categories like starters, main course, desserts, snacks and ready-to-eat products with 10 recipes under each category. Around 50 recipes were prepared by incorporating the developed calcite powder in three variations as 250 mg, 350 mg, 450 mg (Trailokya *et al.*, 2017).

**Table VI -Categorisation and Selection of Recipes**

Variations of recipes based on Incorporation of Calcite powder	Starters	Main course	Desserts	Snacks	Ready to Eat Products
	Number of Recipes				
Variation 1 -250mg	10	10	10	10	10
Variation 2 -350mg	10	10	10	10	10
Variation 3 -450mg	10	10	10	10	10

**Starter:** In this category recipes like Garlic soup, Horse gram dhal (*Macrotylomauniflorum*) soup, Agathi leaves (*Sesbaniagrandidiflora*) soup, Soya chunks kebab, thoothuvalai (*Solanumprocumbens*) soup, vallarai (*Centellaasiatica*) soup, Egg fingers, Modakathan (*Green cardiospermumhalicacabum*) soup, Greengram dhal sprouts salad, Strawberry cucumber salad were prepared.

**Main course:** In this category recipes like Spanish omelet, Mughlai paratha, Cabbage ragi (finger millet) adai, Wheat garlic pasta, Ragi (finger millet) roti, Methi (fenugreek leaves) pulav, Amaranth (*Amaranthus*) leaves dosa, Mint rice, Manathakkali leaves (*Solanumnigrum*) rice, Bajra (pearl millet) kichdi were prepared.

**Dessert:** Desserts like Dates and almond brownie, Carrot kheer, Almond rabdi, Fruit custard, Fig laddoo, Sweet yogurt, Ragi (finger millet) semolina payasam, Shrikhand, Banana honey dessert, Sweet bajra porridge were prepared.

**Snacks:** Egg patties, Flaxseed laddoo, Multigrain bread paneer, Soyabean vada, Groundnut chikki, Kothimbir vadi, Paneer cutlet, Sesame seed balls, Ragi

(finger millet) banana pancake, Wheat flour appam were prepared as snacks recipes.

**Ready to Eat Products:** Ready to eat products like Drumstick (*Moringaoleifera*) leaves podi, Curry leaves podi, Groundnut chutney podi, Garlic podi, Thoor dhal podi, Sesame seed podi, Horse gram dhal (*Macrotylomauniflorum*) podi, Sundaikkai vathal (*Solanumtorvum*) podi, Amla (*Phyllanthusemblica*) murabba and Pumpkin seeds podi were prepared (Podi=Powder) (method of preparation of recipes are enclosed as Appendix V).

**Calcite Incorporation:**

These 50 recipes were prepared by incorporating developed calcite powder. The maximum dose of elemental calcium taken at a time is 500mg (Trailokya *et al.*, 2017) hence calcite powder is incorporated only upto 450gm as the recipe may already contain certain amount of calcium. The three variations are 250mg, 350mg and 450mg as mentioned in the table to assess the acceptability of the recipe.

**Preparation of Recipes:**

Recipes were prepared in the laboratory with fresh ingredients procured on the day of preparation and by following the keys of safer food handling as suggested by WHO (2006). The prepared recipes were displayed for sensory evaluation.



**Plate 3 - Preparation of Calcite Incorporated Recipes**

**Sensory Evaluation:**

Sensory evaluation have been performed to assess the quality of food in terms of its organoleptic properties. The characteristics of food materials includes, senses of sight, smell, taste, touch, and sound which have been measured, analysed and interpreted using a scientific tool known as sensory evaluation (Lawless *et al.*,2010).

Sensory evaluation is important to determine the degree of acceptability of the prepared recipe by screening them. So, whether one product is liked more than another product (Stone, 2020). Considering the importance of sensory evaluation, recipes were examined by 15 semi-trained panel numbers. Based on the score

obtained during the evaluation, the dishes are graded from most acceptable (highest score) to least acceptable (lowest score).

The '9 points hedonic scale' was considered for its reliability, acceptability, and discriminative ability. The scale has been proven in food acceptance tests with soldiers in the field, in the laboratory, as well as in large-scale food preference surveys. A '9 point hedonic scale' was used for the sensory evaluation of the study. The test was conducted to evaluate characteristics such as color, taste, appearance, flavor, and overall acceptance. Recipes were presented to each of the panelist. Each of the panelist tasted the recipes and gave scores from 1 to 9, where 9 indicates the best acceptability and 1 represent the least acceptance,

**Table VII - Nine Point Hedonic Scale for Sensory Evaluation of Formulated Recipes**

Score	Grade
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like nor Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

The sensory evaluation was done and scores were analyzed statistically. The nutritive value of each recipe was calculated using Indian Food Composition Table by Longvah *et al.*, (2017).



**Plate 4 - Sensory Evaluation of Formulated Recipes**

### **Design of Experiments:**

After the nutritive value calculation of recipes, recipes were analysed based on design of experiments. Design of experiments is statistical method which is a systematic way to analyse the data with multi input variables. Nutrient contribution of all the recipes developed, calcite powder's effect on the nutrients of the recipes prepared, nutrient contribution of the significant ingredients for the prepared recipes were analysed using 'Design of Experiments' method.

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**Creating Awareness on Calcite and its Role in the Prevention and Management of Osteoporosis:**

Based on the findings both at the national and international level it was concluded that awareness of osteoporosis was very less among middle-aged women (Sarita *et al.*, 2020 and Xu *et al.*, 2013), and in India mainly postmenopausal women have a great deficit in awareness level of osteoporosis (Gopinathan *et al.*, 2016). Hence pre and post osteoporosis knowledge assessment and awareness on osteoporosis and bone health was done.

**Selection of Subjects:**

The same set of sub samples were selected to carry out the e-awareness session using the same inclusion criteria.

**Pre-awareness knowledge assessment on osteoporosis and bone health:**

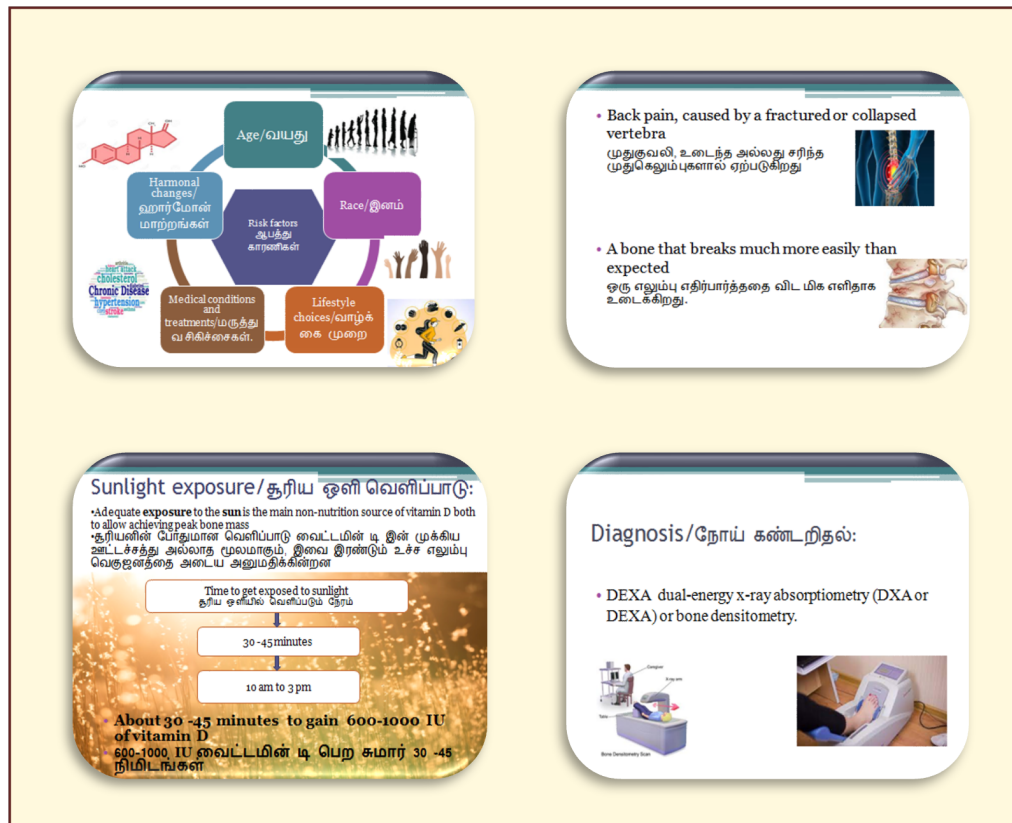
To carry out pre-awareness knowledge on osteoporosis, OKAT (Osteoporosis Knowledge Assessment Tool) was used. OKAT is a valid and reliable tool to assess knowledge about osteoporosis (Winzenberg *et al.*, 2003). OKAT consisted of 20 questions. The first 12 questions was about osteoporosis knowledge and the following questions from 13 to 17 were to assess their awareness on risk factors of osteoporosis and the remaining questions assessed about the prevention of osteoporosis and its treatment in India.

The questions were expected to be answered as either 'true', 'false', or 'I don't know. But the response "I don't know" was considered incorrect and the same was followed for unanswered questions too. Correct answers were awarded a point and no points were given to the wrong answer. The total score of the participant was the indication of the state of awareness. Scores 1 to 10 indicate awareness below 50 percent level and a score of 11 to 20 as above 50 percent of the total score.

Before the assessment, subjects were clearly explained about the assessment technique, assessment tool, type of questions asked in the tool, and confidentiality of their scores. A random question and answer were given as an example. Then the subjects were asked to fill the answers and answers were analyzed statistically.

## Development of Awareness Material:

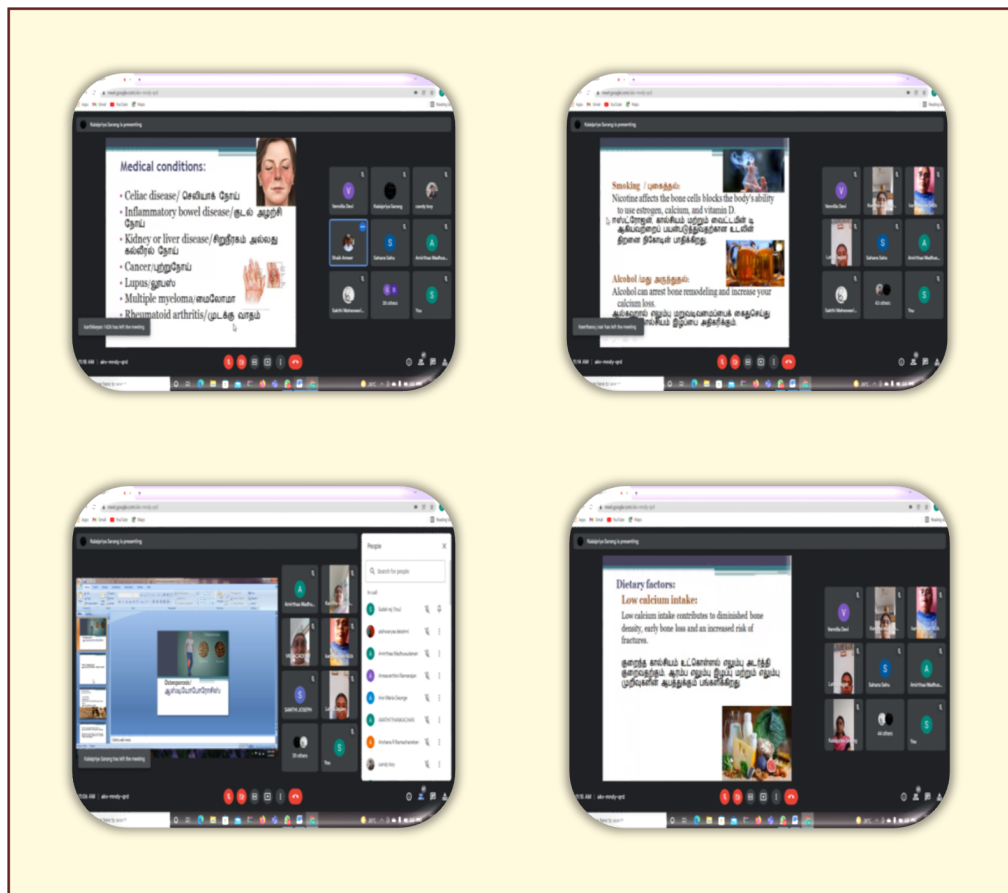
After the initial assessment of knowledge of the subjects, awareness material containing information on osteoporosis, the calcite's role in the management of bone health and prevention of osteoporosis, eggshell calcite powder incorporated recipes, prevention, causes and risk factors of osteoporosis and its prevention were prepared in the form of a power point presentation (Appendix VI).



**Plate 5 - Awareness Material on Calcite and Osteoporosis**

## Conduct of E-Awareness Session:

E-awareness sessions were conducted for the selected subsamples in different groups based on their locale. The sessions were interactive. After the session, the participants were provided a soft-copy of the awareness material and e-booklet on calcite incorporated recipes for their future use.



**Plate 6 - Conduct of E-Awareness Sessions**

### **Post-awareness knowledge assessment on osteoporosis and bone health:**

After the e-awareness session, the same OKAT was sent to the subjects for assessing their knowledge on osteoporosis and bone health. After receiving the answers, scoring was done based on the same criteria as pre-awareness knowledge assessment. Then pre-awareness and post-awareness knowledge on osteoporosis was compared and the effectiveness of awareness was assessed statistically.

