CHAPTER 3

METHODOLOGY

In this chapter, the materials and methods used in the current research entitled **"Metabolic syndrome in relation to body composition and lifestyle adaptation among selected adults residing in Bengaluru"** are explained below under different phases.

PHASE I:

3.1 Selection of subjects and development of research tools to collect data and validating equipment to be used in the research

3.1.1 Selection of locale for the research study

3.1.2 Sampling method and sampling criteria for the research study

3.1.3 Framing and checking the validity and reliability of the equipment and questionnaires used for performing body composition analysis and collecting the data

3.1.4 Obtaining the Institutional Human Ethical Committee (IHEC) clearances to conduct the research study

3.1.5 Conceptual framework of the research study

PHASE II:

3.2 Collection of socio- economic and medical history information from the research subjects

* Age, (year)

* Gender (male or female)

* Education (illiterate, primary schooling, secondary schooling, technical/ diploma/ certificate courses, graduate, professional or honours)

* Occupation (un-employed, semi-routine and routine occupations, lower supervisory and technical occupations, small employers and own account workers, intermediate occupations, higher managerial, administrative and professional occupations)

* Socio- economic class (upper, upper middle, lower middle, upper lower, and lower)

  - Total Income of the family
  - Income of the head of the family
  - Education of the head of the family

* Family structure (single member, nuclear family, joint family, and extended family)

* Marital status (unmarried, married, divorcee, and widowed)

PHASE III:

3.3 Evaluation of participants' characteristics and determination of participant's metabolic syndrome

3.3.1 Assessment of anthropometric measurements

3.3.2 Assessment of Biochemical Parameters

3.3.3 Assessment of lifestyle habits of dietary pattern, physical activity level, exercise, smoking and alcohol habits of the subjects

3.3.4 Assessment of BMR (Basal Metabolic Rate), TEE (Total Energy Expenditure) and Energy Balance

3.3.5 Metabolic Syndrome criterion prevalence in the participants

PHASE IV:

3.4 Assessment of body composition and imparting intervention to the sub sampled metabolic syndrome subjects

3.4.1 Estimating the study's sample size and sampling in accordance with the prevalence

3.4.2 Performing body composition analysis for the enrolled sub-sampled of subjects

3.4.3 Imparting lifestyle counselling intervention for the sub-sampled

3.4.3.1 Medical Nutrition Therapy (MNT), Nutrition Education for the sub-sampled subjects

3.4.3.2 Formulating Physical activity routines for the sub-sampled subjects

3.4.3.3 Developing Healthy habit routines for the sub-sampled subjects

3.4.3.4 Assessing Knowledge, Attitude, and Practice (KAP) of the sub-sampled subjects to plan effective education modules

3.4.3.5 Validating the developed lifestyle education modules to use as counselling materials during the intervention study period

PHASE V:

3.5 Evaluation of the effects of lifestyle counseling on the metabolic syndrome, body composition metrics, and the subsampled individuals' Knowledge, Attitude, and Practice (KAP)

3.5.1 Assessing anthropometric measurements, biochemical parameters, Lifestyle parameters of Dietary pattern, Physical activity level, Exercise, smoking and alcohol habits in sub-sampled subjects post lifestyle counselling intervention

3.5.2 Assessing the Metabolic Syndrome Criteria sub-sampled subjects post lifestyle counselling intervention

3.5.3 Assessing Body Composition Analysis parameters among the sub-sampled subjects post lifestyle counselling intervention

3.5.4 Assessing KAP (Knowledge, Attitude and Practice) of the sub-sampled subjects post lifestyle counselling intervention

PHASE VI:

3.6 Statistical analysis and interpretation

PHASE I:

3.1 Selection of subjects and development of research tools to collect data and validating equipment to be used in the research

3.1.1 Selection of locale for the research study

Sakra World Hospital, a well-known reputed hospital in Bengaluru was selected as the study area and the current study enrolled the individuals. Bengaluru is the largest city of South India and capital of Karnataka. Bengaluru is third most populus and fourth most urban population in South India. Bengaluru is India's major metropolis growing fast and demographically it is a diverse city. Geographical area of Bengaluru is 2196 sq. km with population of 96,21,551 and 87.67% literacy rate. People in and around Bangalore visit this hospital for regular preventive health check-ups and the Clinical Nutrition outpatient department. Figure 3.1 shows the political map of India with state of Karnataka marked. Figure 3.2 shows the Bengaluru district. This was a longitudinal research study that screened a total of 2250 who visited from July 2021 to February 2023 to the Preventive Health Checkup (PHC), Clinical Nutrition Out Patient Department (OPD). The research project was conducted in five phases and statistical analysis was done in the sixth phase. The study was conducted by complying with relevant national and applicable international guidelines for conducting the study.

Figure 3.1: Political map of India with the state of Karnataka marked

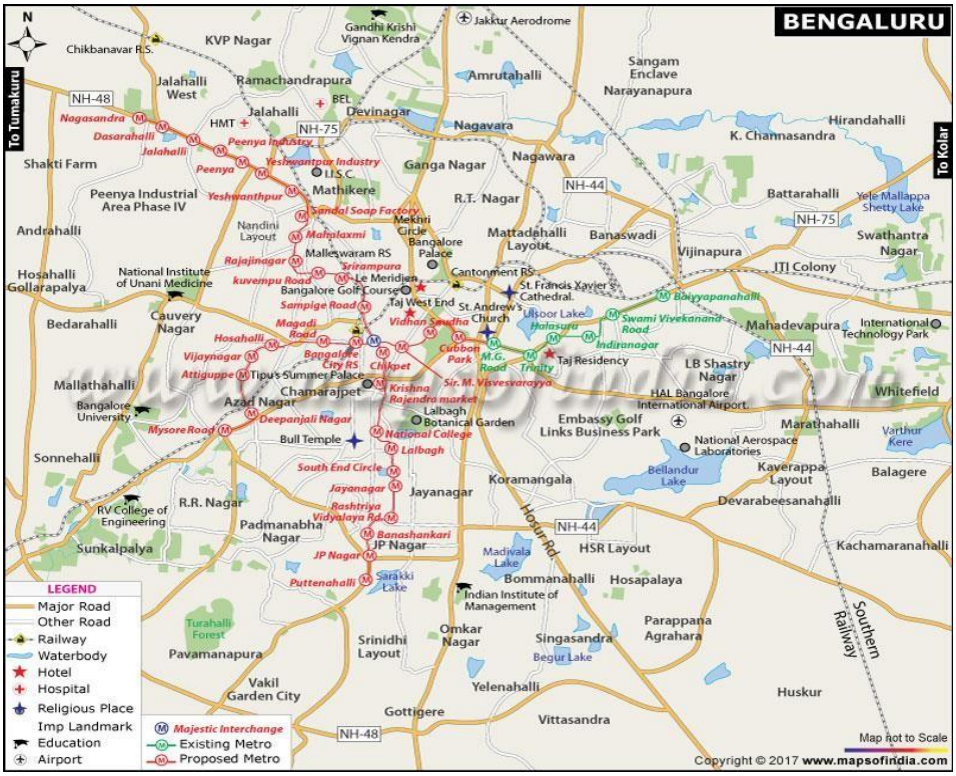

Figure 3.2: Bengaluru District

3.1.2 Sampling method and sampling criteria for the research study:

3.1.2.1 The sampling method for the current study is random as the subjects were enrolled into the study randomly and it is purposive sampling also as the research study is framed to research the subjects with defined inclusion criteria, the sample size is pre-calculated based on the prevalence rate in India. The study design is cross-sectional as the initial enrolment of subjects is done in the research study and data is collected and compared among the subjects. The research study is a Longitudinal study as the subset of sampled subjects were studied with intervention over a definite period and the data from these subsampled subjects is collected pre and post-intervention with a specific time gap.

3.1.2.2 Sampling criteria: Criteria for Inclusion and Exclusion are:

3.1.2.2.1 Inclusion criteria: The following subjects were included in the study:

- Young and Middle-aged 20–50 years old male and female residing in Bengaluru
- Undergoing Preventive Health Checkup in Sakra World Hospital, Bengaluru
- Willing to provide written consent and take part in the study

3.1.2.2.2 Exclusion criteria: The following subjects were not included into the study:

- Non-residents of Bengaluru
- Pregnant and Lactating women
- Adults who were differently abled challenged.

The present study employed the following methodology given in the Figure 3.3.

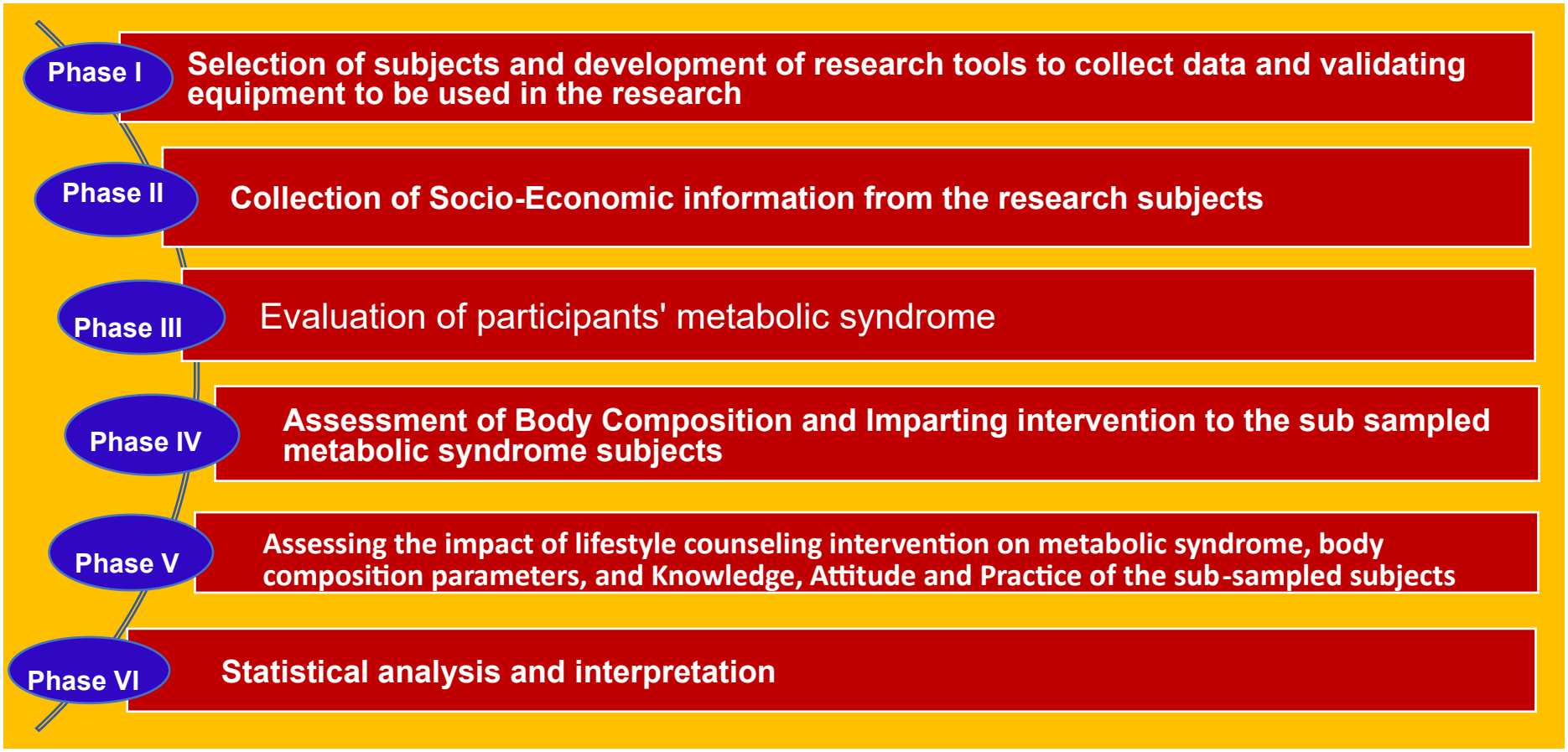

Figure 3.3: Methodology of Research Study

3.1.3 Framing and checking the validity and reliability of the equipment and questionnaires used for performing body composition analysis and collecting the data

3.1.3.1 Various tools were used to collect the data from the research subjects. A part of the questionnaire had Nutritional assessment questions (Annexure vi- ix) which were framed based on research studies of Bhattacharya *et al*., (2019) and Khursheed (2000). The questionnaires were developed and pretested to collect the information (Annexure: vi) from the enrolled research subjects after obtaining consent from them. The research participant information sheet (Annexure: iv) and consent form (Annexure: v) were developed in English and Kannada.

3.1.3.2 To avoid inter-observer variations, the researcher herself did all the measurements.

3.1.3.3 In the current research study, Body Composition Analysis (BCA) was performed using InBody 770 equipment which analysed body composition through the Bioelectrical Impedance Technique. The validity and reliability of this equipment were checked against gold standard body composition analysis of the Dual-energy X-ray Absorptiometry (DEXA) scan.

To perform this validity and reliability test, 20 subjects were selected, randomly, 10 males and 10 females of age between 21-50 years. For each subject, Body Composition Analysis was performed using InBody 770 equipment and a dual-energy X-ray absorptiometry scan simultaneously. Bujang *et al.,* (2018) gave instructions that researchers will find helpful, particularly in the medical profession where the suggested tables will make it easier for them to estimate the sample size needed for reliability studies. The assumption that "the CA'0' equals to zero" for one coefficient of the Cronbach's alpha (CA) test will typically result in a reduced sample size, which is suitable for pilot investigations.

Later the data was tested using the Intraclass correlation coefficient, agreement analysis and Cronbach's Alpha. The statistical analysis of the data showed that the InBody 770 is a valid and reliable equipment to perform the Body Composition Analysis. Hence in this research, Body Composition Analysis was done using InBody 770 equipment. DEXA scanner – Lunar of GE company is shown in

Plate 3.1 shows the DEXA equipment and Plate 3.2 shows the performance of the DEXA scan using the DEXA equipment.

Plate 3.1: Dual- Energy X-ray Absorptiometry Scanner – Lunar of GE company

Plate 3.2: Performing Dual- Energy X-ray Absorptiometry scan

3.1.4 Obtaining the Institutional Human Ethical Committee (IHEC) clearance to conduct the research study

The Institutional Human Ethics Committee (IHEC) of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore**,** approved the present research study (IHEC17-18/FSN/50, Annexure I). Later the research study was proposed and clearance was obtained from the Sakra World Hospital Institutional Ethics Committee (IEC/SWH/CN/26619, Annexure II).

The research study proforma was designed and validated using a small set of subjects in the study area. Minor alterations were suggested which were incorporated and accordingly study proforma was modified and questionnaires were finalized. The study was enrolled in the Clinical Trials Registry- India (CTRI) (CTRI/2021/07/035254) of ICMR.

3.1.5 Conceptual framework of the research study

A conceptual framework is a theoretical framework that offers a means of comprehending a specific issue or occurrence. It can be applied to formulate theories, direct empirical study, or offer a structure for assessing and deciphering evidence. A conceptual framework demonstrates how the study's variables relate to one another. It includes a narrative explanation of the model as well as a visual diagram, or model, as provided for the current research study in Figure 3.4, which summarizes the study's themes.

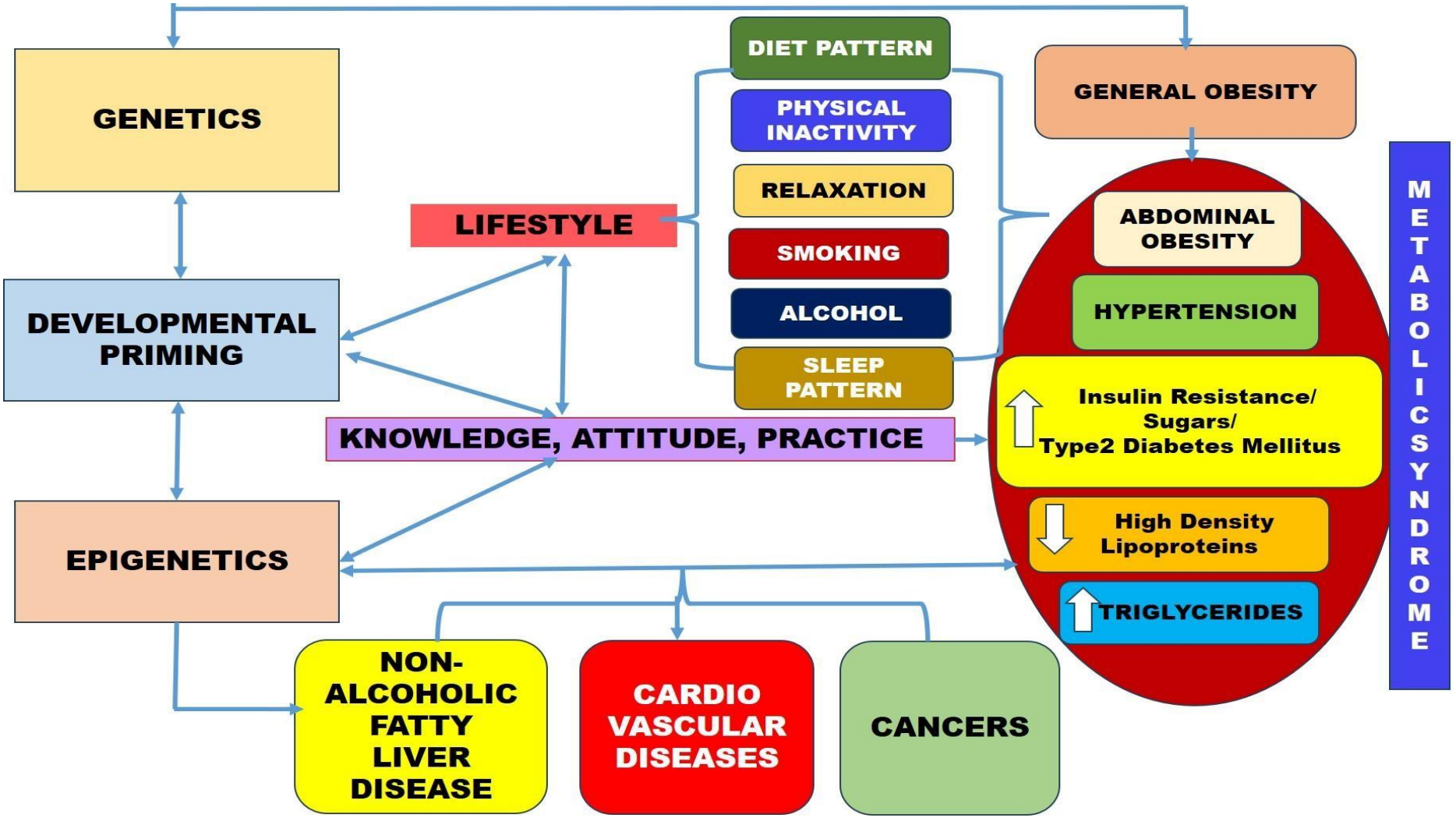

Figure 3.4: Conceptual framework of the research study

PHASE II:

3.2 Collection of socio-economic and medical history information from the research subjects

The questionnaire had ten major questions to collect the following information:

3.2.1 Socio-economic information:

Research subject profile included the age in year, gender (male or female), education (illiterate, primary schooling, secondary schooling, technical/ diploma/ certificate courses, graduate, professional or honours), occupation (un-employed, semi-routine and routine occupations, lower supervisory and technical occupations, small employers and own account workers, intermediate occupations, higher managerial, administrative and professional occupations). The Ministry of Human Resource Development, Department of school education & Literacy Statistics Division- Educational statistics at a glance report 2018 was considered.

Socio- economic class (upper, upper middle, lower middle, upper lower, and lower) was determined by using a modified Kuppuswamy socio- economic scale updated for the year 2021 (Kumar *et al.,* 2022; Sood and Bindra 2022, Saleem *et al*., 2021 and Misra *et al*., 2003) To derive at socio- economic class, the scores were marked after collecting the total monthly income of the family, occupation, and education of the head of the family. The socio- economic class was marked based on the score; if the score is 26-29 Upper, score 16-25 Upper Middle, score 11-15 Lower Middle, score 5-10 Upper Lower, and score <5 Lower.

Family structure (single member, nuclear family, joint family, and extended family), and marital status (unmarried, married, divorcee, and widowed) of the subject were collected [International Institute for Population Sciences (2007), National Family Health Survey 3 (2007), Singh (2003) and Saggurti *et. al.,* (2005)]

3.2.2 Medical history information:

3.2.2.1 The current and previous medical history of the subject was noted down with the duration of the disease if any present.

3.2.2.2 Family history regarding abdominal obesity, diabetes, hyperlipidemia, dyslipidemia and hypertension was captured.

PHASE III:

3.3 Evaluation of participants' metabolic syndrome

Various physical measurements and biochemical parameters testing were done as mentioned below. All the measurements were taken using standard techniques as defined by the CDC (Centers for Disease Control and Prevention) and, NHANES (National Health And Nutrition Examination Survey NHANES, 2007).

3.3.1 Assessment of anthropometric measurements

Anthropometric measurements were the non-invasive quantitative measures of the body. The Centers for Disease Control and Prevention state that anthropometry offers a useful evaluation of an adult's nutritional condition. Anthropometric measures are a basic and important tool that can be used to evaluate an adult's nutritional status, overall health, and future risk of disease. Height (cm), weight (kg), waist circumference (cm), and hip circumference (cm) are examples of anthropometric measures that were recorded.

All the equipment used for measuring was calibrated periodically. The entire anthropometric measurements were done by the researcher herself and she obtained adequate training.

3.3.1.1 Height:

The height is one of the standard measures and most common in all assessments of clinical research and assessments about lifestyle. The subjects were asked to remove their footwear and the height was recorded using an In-Body BSM 170 stadiometer. The subjects were asked to step onto the footplate and it was made sure that their feet should be flat, heels together and touching the back, Legs straight and knees together, arms at sides, shoulder blades and buttocks touching the measurement surface, and the subjects were looking straight ahead without lifting their head and shoulders relaxed. The height measuring step was repeated twice and a consistent reading was obtained. The Height is recorded precisely in cm to the nearest 0.1 cm. Plate 3.3 shows In-Body BSM 170 stadiometer and Plate 3.4 shows height measurement.

Plate 3.3: In-Body BSM 170 stadiometer **Plate 3.4: Measurement of Height**

3.3.1.2 Weight:

Weight is a measure that is widely used and a simple measure of the Nutritional status of the person. Managing the ideal body weight contributes to good health. The weight was taken in the morning (12 hours after eating) to ensure reliability.

Before checking body weight, the subjects were asked to void the urine and then assessed. The subjects were asked to wear minimal clothing with no ornaments/jewellery/ accessories removing their footwear. The weight measurement was recorded using Omron digital body weighing scale (Model- HN-283(HN-283AP). The Weight was recorded in kg to the precision of g. The measurement was taken twice to attain a consistent reading of the selected subject. Plate 3.5 shows Omron digital body weighing scale and Plate 3.6 shows the weight measurement.

Plate 3.5: Omron digital body weighing scale **Plate 3.6: Measurement of Weight**

3.3.1.3 Waist Circumference:

Waist circumference is measured to estimate potential disease risk. Obesity related conditions, such as cardiovascular diseases, Type 2 diabetes, and high blood pressure might develop due to excessive abdominal fat. The higher the waist circumference, higher is the risk of developing obesity-related conditions. It is not a diagnostic tool for body fat or health index of a person; but can be used as a screening tool (Report of a WHO Expert Consultation Geneva, 2008, Alberti *et al*., 2009)).

The research participants were instructed to stand upright and set their feet 25–30 centimeters apart, with equal weight distribution on each foot. There was a mark on the lower rib edge. A mark was made and the iliac crest at the mid-axillary line felt. The waist circumference in centimeters, measured to the nearest 0.1 centimeter, was obtained by passing the measuring tape around the waist horizontally, halfway between the iliac crest and the lowest rib border. Abdominal obesity was classified as having a waist circumference of more than 90 cm for all adult males and more than 80 cm for women (IDF, 2006; Zimmet and Alberti, 2005). The inch tape used to measure hip and waist circumference is displayed in Plate 3.7.

The waist and hip circumference measurements are displayed on Plate 3.8.

3.3.1.4 Hip Circumference:

The hip circumference was measured at the spot that yielded the maximum circumference in centimeters, up to the nearest 0.1 centimeter, using a measuring tape that was placed horizontally over the buttocks.

Plate 3.7: Measuring Inch Tape **Plate 3.8: Measurement of Waist and Hip Circumference**

3.3.1.5 Body Mass Index:

Body Mass Index is an easy and inexpensive tool. It cannot diagnose the body fatness or health of an individual accurately; but can be used as a screening tool. Many researches have shown that Body Mass Index has a significant positive correlation with body fat. Body Mass Index is a screening method for weight categories like underweight, normal/ healthy weight, overweight, and obesity. Using the Height and weight measures the Body mass index was calculated. It is the most widely used height weight index. Body Mass Index is calculated by taking weight in kilograms and dividing it by the square of the person's height in meter (kg/ m^2). An altered Body Mass Index can caution the risk of comorbidities and the individual can take the primary precautionary steps to attain normal Body Mass Index. Body Mass Index is the primary index to determine the Nutritional status of a person. WHO (World Health Organisation, 2000), the IASO (International Association for the Study of Obesity), and IOTF (International Obesity Task Force) in their publication - The Asia-Pacific Perspective: Redefining Obesity and its Treatment proposed the revision of cut-off values to prevent the risk of developing health issues. Table 3.1 Shows Asian adult body mass index classification proposed by WHO, IASO and IOTF (2000). In the current study, we have used these cut-off values.

Table: 3.1

Asian Adult's Body Mass Index proposed Classification

(WHO, IASO and IOTF, 2000)

Body Mass Index	Nutritional Status	Risk of Comorbidities
<18.5	Underweight	Low (but increased risk of other problems)
18.5 – 22.99	Normal	Average
23- 24.99	Overweight	Increased
25- 29.99	Obesity class I	Moderate
Above 30	Obesity class II	Severe

Compared to white Caucasians, Asians may be more at risk for health issues due to their body composition. Asian Indians were found to have higher amounts of body fat and abdominal obesity at lower or similar Body Mass Index levels compared to white Caucasians. Lower cut-offs for obesity and abdominal obesity have been

recommended for Asian Indians in light of these facts. (Body Mass Index: overweight >23 to 24.9 kg/m^2 and obesity ≥ 25 kg/m^2; and Waist Circumference; men ≥ 90 cm and women ≥ 80 cm, respectively) (Misra and Khurana 2011).

3.3.1.6 Waist – Hip Ratio (WHR):

Waist Hip Ratio is a measure or indicator of an individual's health and likelihood of acquiring serious medical disorders. Numerous academic research demonstrate that folks with apple-shaped bodies are more likely to experience health problems than those with pear-shaped bodies. The waist-hip ratio was used by the World Health Organization (2008) to quantify obesity and as a potential marker of other, more significant medical disorders. A measure of central obesity and metabolic issues, the waist-to-hip ratio (WHR) was computed by dividing the waist circumference (in centimeters) by the hip circumference (in centimeters). According to the World Health Organization's cut-off points and risk of metabolic problems, a person is considered obese if their waist-hip ratio is 0.90 or higher in men and 0.85 in women. This indicates a significant risk of metabolic issues.

3.3.1.7 Blood pressure measurement:

Readings of blood pressure (BP) consist of Diastolic blood pressure is the pressure on the blood vessels while the heart muscle relaxes. Systolic blood pressure is the pressure when the heart beats, when the heart muscle is contracting and pushing oxygen-rich blood into the blood vessels. There is usually a difference between the diastolic and systolic pressures.

Blood pressure is measured in units of millimetres of mercury (mmHg). The readings are always given in pairs, with the upper (systolic) value first, and followed by the lower (diastolic) value. The subject‘s BP levels were recorded in the morning by using a digital automatic BP monitor as shown in Plate 3.9 (HEM- 8712 Model, Omron Health Care Manufacturing Vietnam Co. Ltd., Singapore). The subjects were asked to be in a seated position while recording their Blood pressure. The measurement was taken on the Left hand. The subject was asked to sit and relax and rest for 3-5 minutes before taking the blood pressure measurement. Measurements were recorded two times, and the average was taken as a recorded measurement. Plate 3.10 shows the blood pressure measurement.

Plate 3.9: Omron Digital BP monitor **Plate 3.10: Measurement of BP**

3.3.2 Assessment of Biochemical Parameters

Fasting Blood Sugar, Serum Triglycerides and Serum High Density Lipoprotein (HDL) Cholesterols were to be analyzed for the current research project. As the subjects were coming for Preventive Health Checkup (PHC); blood sample was collected and analyzed as a part of the PHC.

3.3.2.1 Blood Sample:

Instructions for the subjects coming to give fasting blood sugar and lipid profile test: A first morning blood sample was required for analyzing Fasting blood sugar and Lipid profile. The subjects were instructed not to eat or drink anything (except water-no juice, tea, or coffee) overnight for at least 8-12 hours before coming for blood investigations. The subjects were asked not to smoke or drink alcohol. If they were on any previous medications, they were asked to continue medicines (unless the doctor specifies not to take the medicines). The Blood Samples were collected by a trained Phlebotomist (Plate 3.11) of Sakra World Hospital Laboratory and 6ml Blood was drawn for analysis. Standardized biochemical analysis was performed on blood samples to reduce the possibility of bias or error when obtaining the analytical data for this investigation. To measure the levels of high density lipoprotein (HDL), triglycerides, and fasting blood glucose, blood samples were taken. The consent was acquired before any blood was drawn for examination.

First-morning blood sample required for analyzing Fasting blood sugar and Lipid profile was drawn. The methods of analysis of the biochemical parameter and the standard reference range is as given in the Table 3.2. VITROS XT 7600 (Plate 3.12) equipment was used for testing FBS and lipid profiles. The reference values were specified in reagent kit inserts given by the manufacturer Ortho Clinical Diagnostics of VITROS XT 7600. Calibration was done once in six months or whenever the reagent lot was changed or if any Quality Control (QC) variations were observed. The test results were run in Biored unity software for QC purposes and LJ- Levey-Jennings graphs were observed. West guard rules (six rules) were observed for QC check. The coefficient of variation used was ±2SD.

Plate 3.11: Blood collection for Analysis

Plate 3.12: VITROS XT 7600 equipment

Table: 3.2

Standard reference range of blood tests using analysis method

Test	Method	Standard reference range
Haemoglobin	SLS-Haemoglobin method	Males- 12.8-16.4 g/dL Females- 11.2-14.4 g/dL
Fasting Blood Sugar	Glucose Oxidase peroxidase	Hypoglycemia: < 70 mg/dL Normal: 70 - 99 mg/dL Pre-diabetes: 100 - 125 mg/dL Diabetes: ≥ 126 mg/dL
Post Prandial Blood Sugar	Glucose Oxidase peroxidase	Hypoglycemia: < 70 mg/dL Normal: 70 - 139 mg/dL Pre-diabetes: 140 - 199 mg/dL Diabetes: ≥ 200 mg/dL
Glycosylated Haemoglobin (HbA1C)	HPLC	4 - 6 % (Non - Diabetics) 6 - 7 % (Good Control) 7 - 8 % (Fair Control) >8.0 (Poor Control)
Thyroid Stimulating Hormone (TSH)	ECLIA	21 – 54 years: 0.4-4.2 µIU/ml
Total Cholesterol	Cholesterol oxidase	Low risk: <200 mg/dL Borderline risk: 201-239 mg/dL High risk: ≥ 240 mg/dL
Triglycerides	Glycerphosphate oxidase	Normal: <150 mg/dL Borderline high: 150-199 mg/dL High: 200-500 mg/dL Very high: >500 mg/dL
High- Density Lipoproteins (HDL)	Non-HDL precipitation	40 - 60 mg/dL
Low- Density Lipoproteins (LDL)	Enzymatic Selective Protection	Optimal: <100 mg/dL Near-optimal: 100 – 129 mg/dL Borderline High: 130 – 159 mg/dL High: 160 – 189 mg/dL Very High: ≥ 190 mg/dL

Reference ranges source: Haemoglobin- Derived Sakra laboratory reference range; FBS, PPBS & HbA1C- American diabetic association reference range and TSH, Total cholesterol, Triglycerides, HDL and LDL cholesterol- reference range as per kit manufacturer's Instructions For Use (IFU).

3.3.3 Assessment of lifestyle habits of dietary pattern, physical activity level, exercise, smoking and alcohol habits of the subjects

3.3.3.1 Diet History and Dietary Intake:

3.3.3.1.2 The dietary history including eating patterns, the amount and kind of food eaten when dining out, and the amount of water drunk per day in general during their participation in the study were gathered.

3.3.3.1.3 The Dietary pattern (vegetarian-1 those who consume no milk and milk products, vegetarian-2 those who consume milk and milk products, ovo- vegetarian those who consume milk and egg, predominantly vegetarian and at times non-veg, predominantly non-vegetarian and at times vegetarian

3.3.3.1.4 Consumption of nutrient supplements if any was noted down.

3.3.3.1.5 The day before the interview and a regular weekend were the two days of a 24-hour food recall (Annexure viii). Standardized cups, spoons, and measurements were employed to improve communication and quantify recollection.

In Plate 3.13 Standardized measures and cups are shown

Plate 3.13: Standardized Cups, Spoons and Measures

3.3.3.1.6 The frequency of food consumption was recorded using a food frequency questionnaire (Annexure: vii). Calorie consumption per day is computed. The information gathered above was used to determine the macronutrients (fat, protein, and carbohydrates). IFCT NIN ICMR (Indian Food Composition tables, National Institute of Nutrition, Indian Council of Medical Research, 2017) were used to calculate the daily intake of selected macro and micronutrients (Longvah *et al.,* 2017).

The social habits of exercise routine, physical activity level, smoking and alcohol habits were collected.

3.3.3.2 Exercise routine and physical activity level:

The exercise routine was collected from the subjects under four headings-

(i) Never (0) - Not attempted any exercise routine,

(ii) Former (1) - stopped exercise routine for >1 year and if any exercise is performed; Then duration of activity and frequency of activity were obtained and classified under

(iii) Moderate activity (2) (3- 6 metabolic equivalents- 3.5 to 7 kcal/min) and

(iv) Vigorous activity (3) (Greater than 6 metabolic equivalents- more than 7 kcal/min).

The metabolic equivalents are the best to derive at the total energy expenditure of the subjects (Ainsworth *et al.,* 2011).

3.3.3.3 Smoking habit, tobacco chewing, and alcohol consumption were obtained under three major headings-

(i) Never (0), (ii) Former (1) (if the habit is left since > 1 year) and (iii) Current (2)

3.3.4 Assessment of Basal Metabolic Rate (BMR), TEE (Total Energy Expenditure) and EB (Energy Balance):

The BMR was calculated using the Harris-Benedict equation [Harris and Benedict (1918) and Schofield (1985)] and InBody 770 BCA also gives the value.

In men, BMR=66.4730 + 13.7516 x weight in kg + 5.0033 x height in cm – 6.7550 x age in year.

In women, BMR=655.0955 + 9.5634 x weight in kg + 1.8496 x height in cm – 4.6756 x age in year.

The Total Energy Expenditure (TEE) was estimated by multiplying the physical activity level (PAL) factor with BMR. PAL factor considered for Sedentary (little or no exercise) 1.2, Lightly active (light exercise/sports 1-3 days/week) 1.375, Moderately active (moderate exercise/sports 3-5 days/week) 1.55, Very active (hard exercise/sports 6-7 days a week) 1.725 and if vigorous activity (very hard exercise/sports and a physical job) 1.9 (WHO, 2010 and Jetté *et al*.,1990). Energy balance was calculated by subtracting total energy expenditure from total calories consumed. The output obtained is a negative or positive energy balance.

3.3.5 Metabolic Syndrome criterion prevalence in the participants:

NCEP (National Cholesterol Education Program, Adult Treatment Panel III proposed criteria for identifying the metabolic syndrome risk in adults in 2001 and 2002. American Heart Association and the National Heart Lung and Blood Institute in 2005 revised the earlier defined criteria (Grundy *et* al., 2005) as metabolic syndrome criteria which is currently used in the study. To assess the metabolic syndrome among subjects, the waist circumference, FBS, Triglycerides, HDL cholesterol, and Blood pressure of the subjects were recorded. Based on the criteria of Metabolic Syndrome as stated by NCEP ATP III (of 2002 revised in 2005); mentioned in below Table 3.4; the subjects were checked if they have Metabolic Syndrome. If they were having then the observer proceeded to the next phase and the procedure was

followed as per Phase IV. If the subject is not willing to participate; then the subject was given relevant counselling and not continued further in the research study.

Table: 3.3

Criteria of Metabolic Syndrome as stated by the NCEP ATP III (2005 revision)	
Absolutely required	**None**
Criteria	**Any Three Of The Five Criteria Below**
1. Obesity	Waist Circumference >90 cm Male; >80cm Female
2. Hyperglycemia	Fasting Glucose >= 100 mg/dl or on medicines
3. Dyslipedemia (Triglycerides- TG)	TG >= 150 mg/dl or on medicines
4. Dyslipedemia (2^{nd} separate criteria)	HDL <40 mg/dl Male Or <50 mg/dl Female Or on medicines
5. Hypertension	>130 Systolic Or >85 Diastolic- mmHg or on medicines

PHASE IV:

3.4 Assessment of body composition and imparting intervention to the sub sampled metabolic syndrome subjects

3.4.1 Estimating the study's sample size and sampling in accordance with the prevalence

Based on NCEP ATP III (2002 revised in 2005), the Metabolic Syndrome subjects were enrolled in the research study. As per the previous research studies the prevalence rate of Metabolic Syndrome is found to be around 15-20% in South Indian Population. Using the statistical formula given by Daniel proposed in 1999 (Daniel and Cross, 2018).

$$n = Z^2 P(1-P) / d^2$$

n - Sample Size Z = Level of confidence

P – Expected prevalence d – precision

In the current research, Z was considered 90% (1.645) confidence level, with P as 17% prevalence (mid value of 15-20%), and d as 0.05 precision, the expected population size was 153.

Sampling criteria: Inclusion and Exclusion criteria as explained below were used in the current sub-sample enrolment in this research study:

Inclusion criteria:

- Having NCEP ATP III (2005 revision) defined metabolic syndrome criteria
- Willing to participate in the study and give written consent **Exclusion criteria:**
- Type 1 diabetes mellitus adults
- Adults who have implants

A total of 2250 visited Clinical Nutrition Out Patient Department. Subjects in the age group of 20-50 year were 1359; after obtaining consent they were screened and the inclusion and exclusion criteria was followed. 180 subjects had Metabolic syndrome as per NCEP ATP III (2002 revised in 2005). Following inclusion and exclusion criteria, after obtaining consent 150 subjects were enrolled into the further study.

3.4.2 Performing Body composition analysis for the enrolled sub-sample of subjects

The Metabolic Syndrome subjects were explained about the research project in brief. The subjects were informed regarding the research project‘s title, the main objective, the duration of the project and that every 15 days a call conversation regarding the lifestyle to be followed will be carried. Written consent is obtained if they were willing to participate. If they said —No‖ then; the subject was thanked for the time and diet counselling was given to be followed, clarifying doubts if any. Before obtaining the written consent from those willing to participate, the confidentiality statement and research participant information sheet was explained to them. Then proceeded to the next phase of the research study.

Body Composition Analysis (BCA) in the current study is performed by the Bioelectrical Impedance Analysis (BIA) method with the help of InBody770 equipment. Changes in muscle mass, body fat percentage, and fat mass can all be

clearly displayed by body composition analysis. BCA provides a much more accurate depiction of health and description of weight than Body Mass Index.

Withers *et* al., (1998), Wattanapenpaiboon *et al*., (1998) and Tyrell *et al*., explained in their study about different methods of BCA- two, three and four compartmental models. In the current study, the analysis was done based on fourcompartment models- Fat mass of Body, Mass of Body Fat, Protein, Minerals, and Total Body Water. In order to deliver research-level results, InBody770 has multiple certifications, including Non-automatic Weighing Instruments (NAWI) and Conformite Europeenne (CE). Body Composition Analysis was medically approved because these certifications were accepted all over the world.

According to the study, InBody and Dual Energy X-ray Absorptiometry (DEXA) have a strong association. Since its commercial release in the middle of the 1980s, BIA has grown in popularity as a less labor-intensive substitute for traditional reference tools like densitometry. The InBody measures the body as five distinct segments/cylinders: the right arm, left arm, trunk, right leg, and left leg. This is done using a proprietary method called the Direct Segmental Multi-frequency Bioelectrical Impedance Analysis Method (DSM-BIA). When compared to the accuracy and validity of other two-compartment reference methods, SMF-BIA is a valid tool for estimating Body Composition Analysis in healthy euvolemic individuals (BosyWestphal and Schautz et al., 2013).

The analysis was done by the researcher herself. She underwent prior training for taking measurements in a body composition analyzer. Precautions to be followed before performing Body composition analysis were explained to the subjects.

- The Body composition Analysis for the research subjects was conducted before a meal or after two hours after the last meal. This precaution was taken so that the mass of the food is counted as weight, and thus, may result in measurement errors.
- To avoid biological error, the subjects were asked to void urine before conducting Body Composition Analysis.
- Research subjects were instructed to refrain from exercise as it can cause temporary changes in body composition.

- Female research subjects Body composition Analysis was performed only during the non-menstrual cycle as the body water tends to increase during menstruation.
- Body Composition parameters might change in cold or hot weather. So the test was conducted at 20 – 24 ℃ as the human body is stable at this optimal temperature and the body composition measurements were not susceptible. Plate 3.14 shows the InBody 770- BCA equipment and Plate 3.15 shows the BCA measurement.

Plate 3.14: InBody 770- BCA Equipment

Plate 3.15: Measurement of BCA

InBody 770 body composition analysis results considered into the study were explained below. The main components of body composition analysis include:

- Total Body Water is measured by using a multi-frequency technique that separates total body water into Intracellular water (ICW) which is the quantity of water within the cell membrane and Extracellular water (ECW) which is the total quantity of water in the blood and other interstitial fluids. In the case of a healthy body, 3:2 is the desirable ratio of ICW and ECW that is healthy to maintain. If Extra Cellular Water is more then it indicates acute inflammation.
- The solid organic compound consisting of nitrogen is Protein and is found in body cells. Intra-cellular water and Protein are related directly. That is why poor cell nutrition indicates a lack of protein.

- InBody770 analyses Minerals- osseous minerals and non-osseous minerals.
- Body Fat Mass is obtained when Fat free mass is subtracted from Total weight
- Total weight is Fat mass + Fat free mass (Total Body Water, Protein, and Minerals).
- The body fat percentage represents the proportion of body fat to total weight. Males and women have different ranges of body fat percentages; this is because women typically carry more body fat than males do because of biology and the reproductive system. Body Fat Percentage ranges according to ACE and ACSM recommendations for men and women. For men, it is between 10 and 20 percent; for women, it is between 18 and 28 percent.
- InBody score reflects the evaluation of body composition. A healthy individual scores more than 70 for 100 points. A muscular person might score over 100 points.
- Body fat can be classified into two basic categories: visceral and subcutaneous. The estimated amount of fat surrounding the abdomen's interior organs is known as the visceral fat region. It's critical to keep your visceral fat area around 100 cm 2 in order to maintain a healthy fat balance.
- Weight Control has sub- headings as the Target weight, Weight Control, Fat control, and Muscle control measures in kg are stated as values to target and attain.
- BMR: The number of calories needed for the fundamental daily processes is known as the basal metabolic rate. In order to develop a nutritional plan which is essential for achieving the appropriate body composition goals this value is important.
- Obesity degree is the ratio of present weight to standard weight
  It's an index that assesses a person's degree of obesity based on their weight and height. A 90–110% obesity degree is a normal value, 110–120% obesity degree is overweight and >=120% obesity degree is obese.

- Whole Body Phase Angle ranges from 3 to 15 degrees, with an average of 5-7 degrees.

3.4.3 Imparting lifestyle counselling intervention for the sub-sampled subjects exhibiting metabolic syndrome

3.4.3.1 Medical Nutrition Therapy- Nutrition Education for the sub-sampled subjects

NCEP ATP III and American Dietetic Association guidelines were used to frame the factsheets for the research subjects. The foods were classified based on Food groups and explained which were to be used daily, restricted, and avoided categories. Indian council for Medical Research, National Institute of Nutrition RDA values were considered and the Meal plans were planned for the subjects. ICMR (2018) guidelines were used for counselling on management of diabetes mellitus.

3.4.3.2 Physical activity routines for the sub-sampled subjects

World Health Organisation (WHO), American Diabetes Association (ADA) guidelines were used to tailor make the suggestions for the enrolled subjects based on the goals of the enrolled subjects.

3.4.3.3 Healthy habit routines for the sub-sampled subjects

Smoking and Alcohol cessation were advised and stress relief techniques were counselled to the sub-sampled subjects.

3.4.3.4 Knowledge, Attitude, and Practice (KAP) of the sub-sampled subjects were assessed.

The KAP (Knowledge, Attitude, and Practice) of the research subjects on lifestyle modification and body composition analysis with metabolic syndrome were collected before lifestyle educational intervention (Annexure ix). The subjects were explained to answer what they were aware of about lifestyle, body composition, and metabolic syndrome. The KAP assessed initially was utilized to develop the lifestyle education modules and lifestyle counselling was given to the subjects after their enrolment. The questions were to assess:

3.4.3.4.1 Knowledge: The Knowledge score was obtained under four major heads- Knowledge regarding the Healthy Diet/ General Nutrition, Knowledge regarding the

benefits of exercise and weight loss, Knowledge regarding the Body Composition and Knowledge regarding the Metabolic syndrome and diet to be followed. The subsampled subjects were assessed and scored.

Knowledge regarding the Healthy Diet/ General Nutrition had 12 questions in it and 1 score was given for each right answer. Score 0-5 were scaled as Very basic/ Poor, 6-9 were scaled as Average and 10-12 score were scaled Good. Six questions were present in Knowledge regarding the benefits of exercise and weight loss, Knowledge regarding the Body Composition and Knowledge regarding the Metabolic syndrome and diet to be followed. Score 0-2 Poor, 3-4 Average and 5-6 Good.

3.4.3.4.2 Attitude: The attitude score for lifestyle modification was assessed with six questions and 1 score was given for each right answer. Score 0- Strongly Negative, 1- Negative, 2- Neutral, 3- Positive and 4-6 Strongly positive.

3.4.3.4.3 Practice: The practice score according to lifestyle modification was assessed with six questions and 1 score was given for each right answer. Score 0- Very poor, 1- Poor, 2- Neutral, 3- Good and 4-6 Very good.

3.4.3.5 Validating the developed lifestyle education modules for using as counselling materials in the interventional study period

The subjects' assessment and KAP data were used to identify and develop the lifestyle education modules. The presentations and factsheets were developed with ICMR (2018) guidelines for diabetes, American Diabetes association guidelines, American Dietetic Association guidelines (Seagel *et* al., 2009), Diet in weight management for Indian Diabetics (Shanshank 2016), Kasper (2015), Passmore (1985), and Katz (2001) inputs for managing dyslipedemic condition, to create awareness on nutritional aspects, exercise routines and stress relief techniques. Smoking and alcohol cessation was advised by intimating the adverse effects of the habits (Bjorklund, 2022; Hurley, 2012; Mukamal, 2006). For the validating study, sixty subjects (30 males and 30 females) who knew (Healthcare) and didn‘t know (Nonhealthcare) about nutrition, exercise and yoga were selected and the developed education modules were validated using Cronbach‘s alpha (CA) test. Thus, validated Lifestyle education modules and factsheets were used in the research study. The education modules used to impart lifestyle counselling are as tabulated in Table 3.5 and screenshot of the education modules are in Figure 3.5.

Table: 3.4

Education Modules to impart lifestyle counselling

Sno.	Education Module	Contents of the module	No. of slides
1	Basics of Nutrition	Definitions of Food and Nutrition, Macronutrients and Micronutrients, Food pyramid, Food groups	10
2	Nutrition for Healthy living	My plate, Basal Metabolic Rate, Energy Expenditure, Energy Intake, Energy Balance, Recommended Dietary Allowances, Blood markers concerning health	12
3	BMI_BCA_Nutritional Status	Anthropometric measurements, Body Mass Index, Body Composition Analysis and nutritional status	6
4	Lifestyle to be followed_ Metabolic syndrome	Definitions of Metabolic syndrome, Diabetes Mellitus, Hypertension, Dyslipidemia and abdominal obesity, health markers normal range, abnormal values leading to metabolic syndrome, lifestyle to be followed to maintain normal health	15
5	Exercise as a part of Healthy Lifestyle	Physical Activity, Exercise, The right way of exercising-frequency, intensity, time and type of exercise	10
6	Stress relief tips for relaxing to lead a Healthy life	Stress Busters- Brainstorming, Yoga, Relaxation, Anlom Vilom, Smoking and Alcohol cessation	10
7	Mighty Millets	Introduction and Benefits of Millets, recipes and their nutritive values	12
8	Break the fast without skipping the main meal of the day	Importance of breaking fast, timely meals, Breakfast options and their nutritive values	36
9	Fresh Fruits in our day to day consumption	Fruits as snack options, Seasonal fruits available with their health benefits and their nutritive values	36
10	Antioxidants	Free radicals, antioxidants, minerals and vitamins under antioxidants, benefits of antioxidants	10

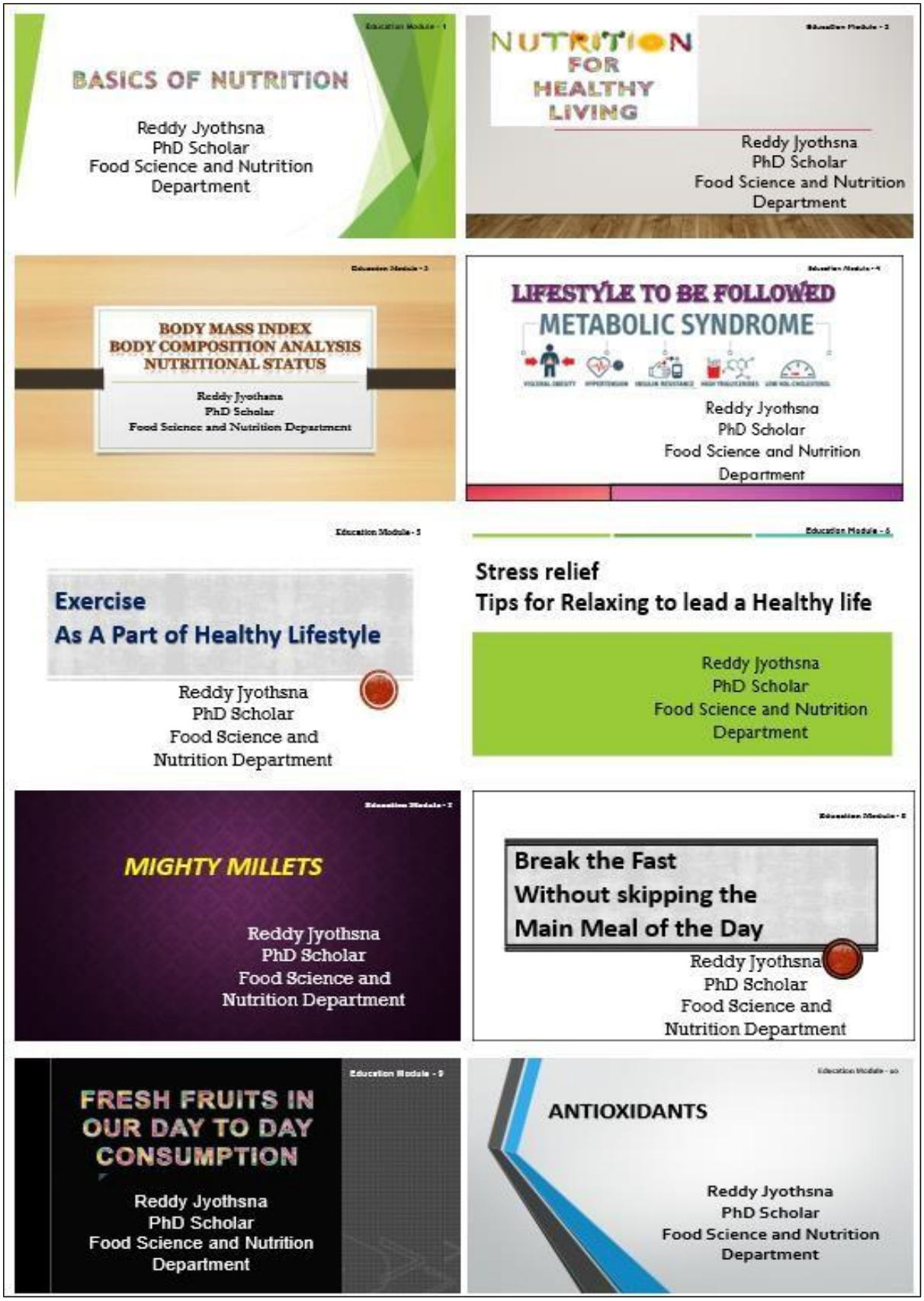

Figure 3.5: Lifestyle education module presentation screenshots to impart lifestyle counselling

PHASE V:

3.5 Evaluation of the effects of lifestyle counselling intervention on metabolic syndrome, body composition parameters, and Knowledge, Attitude and Practice (KAP) of the sub-sampled subjects

Following a lifestyle modification consultation, changes in anthropometric, biochemical, body composition, metabolic syndrome markers, and physical activity patterns were used to assess the intervention's effectiveness.

Initially screening of the subjects were done and with the consent enrolled them into the research study. The subjects were assessed as explained in chapter 3.1, 3.2. After obtaining consent the subjects were a part of follow-up study and the procedure explained in 3.3 and 3.4 is carried out. Every enrolled subject was given lifestyle modification counselling with nutrition fact sheet and meal plan on the initial day. The subjects enrolled for follow-up study were counselled both directly with factsheets as handouts and the presentation were once explained directly or oncall. Later the presentation modules were shared to the subjects for their easy reference and repeated learning.

The first six lifestyle education modules were designed based on the KAP, background data of the subjects. These were power point presentations made in simple language for understanding better. The topics were Basics of Nutrition, Nutrition for Healthy living, Body Mass Index (BMI), Body Composition Analysis (BCA) and nutritional status, Lifestyle to be followed in metabolic syndrome, Exercise as a part of healthy lifestyle and stress relief tips for relaxing to lead a healthy life.

As the researcher wanted to encourage the breakfast consumption, millet and fruit usage by the subjects a detailed display of the items were planned as a display stall and the subjects were invited to the stalls. Each stall was kept for three consecutive days at display for the subjects. For those who couldn‘t attend the display stalls soft copy of the breakfast items with their nutritive values, millet recipes with their nutritive values and health benefits and fruits with nutritive values and health benefits were shared. As the researcher observed the immunity levels of the subjects weren't that evident on the discussion with 30% of the subjects a power point presentation on Antioxidants was prepared and delivered. One more food

safety and food security stall was organised on the request of few subjects. This stall also was kept for subjects to visit for three days. For those who couldn‘t attend the soft copy was shared. The contents of the presentation and number of slides are given in Table 3.5 and Figure 3.5. All the queries raised by the subjects were cleared and encouraged to follow the lifestyle modifications explained to them. The subsampled metabolic syndrome subject was contacted, over call, every 15 days and re-counselling was done. Guidance was provided regarding diet, exercise and yoga/ relaxing techniques. Doubts were cleared after each session. The Plate 3.16 shows the researcher giving group presentations, explaining in the displayed stalls.

Plate 3.16: Snaps shots of the researcher giving the lifestyle education counselling by oral presentations and displays

3.5.1 Assessing anthropometric measurements, biochemical parameters, Lifestyle parameters of dietary pattern, physical activity level, exercise, smoking and alcohol habits in sub-sampled subjects post lifestyle education counselling intervention:

Height, weight, hip circumference, biochemical parameters- Fasting blood sugar, triglycerides, HDL cholesterol, Lifestyle parameters of Dietary pattern, Physical activity level, Exercise, smoking and alcohol habits; in the sub-sampled subjects. Using the standard methods mentioned above the measurements were recorded post-intervention.

3.5.2 Assessing Metabolic Syndrome criteria post lifestyle education counselling intervention:

The NCEP ATP III (2005) was used to categorise the subjects. So the measurements of waist circumference, fasting blood sugars, triglycerides, HDL cholesterol and blood pressure were observed post-intervention.

3.5.3 Assessing Body Composition Analysis parameters among the subsampled subjects was carried out post lifestyle education counselling intervention. The BCA assessment is done as mentioned in 3.4.2

3.5.4 Assessing Knowledge, Attitude and Practice of the sub-sampled subjects was done post lifestyle counselling education intervention.

All these assessments stated above were done as indicated in detail in Chapter 3.3.1.

PHASE VI:

3.6 Statistical analysis and interpretation

To look at the prevalence of metabolic syndrome and, significance/ relation between metabolic syndrome, body composition, and lifestyle adaptations, the statistical analysis and interpretations were carried out with the available entire research subjects (N=1359) data and subjects continued in the follow-up study (n=150) of metabolic syndrome pre and post-intervention data. The data collected was analyzed using R software Version 4.3.1 (R Core Team, Vienna, Austria, 2021). Descriptive Statistics were done for categorical data by presenting using frequencies and percentages. Depending on their distribution, continuous measurable data were described using the mean ± standard deviation or the median and interquartile

range. To evaluate the normal distribution assumption, the Shapiro-Wilk test was employed.

- As part of the Intervention study; the impact of health style modification was assessed by comparing pre and post- clinical, exercise, and Body Composition Analysis parameters. Changes in measured parameters were evaluated depending on the parameter distribution, the Wilcoxon signed-rank test or the Paired Sample T-test. Variations in the category parameters' levels were checked using the Stuart-Maxwell marginal homogeneity test.
- Social, Diet, Clinical, Exercise, and Body Composition Analysis parameters were compared between those who recovered from Metabolic syndrome and individuals who did not experience recovery from it. For continuous measured parameters based on distribution, the comparison was performed using independent sample T-tests or Mann-Whitney U tests; for categorical observations based on expected frequency, it was performed using Fisher's exact test or Chi-Square test.
- To compare the social, diet, clinical, exercise, and body composition analysis factors between patients with and without metabolic syndrome, a cross-sectional study was carried out. Based on the anticipated frequency, the chi-square test or Fisher's exact test were used for categorical observations. Once the normality assumption was verified, continuous measurements were assessed using independent sample t-tests or Mann-Whitney U tests.
- Univariate and Multivariate Logistic regression analyses were carried out to study the unadjusted and adjusted impact of risk factors on Metabolic syndrome. The Odds ratio was reported as the measure of association between the risk factors and metabolic syndrome. Parameters significant at a 5% level of significance were included in the adjusted regression model.
- In the Knowledge Attitude and Practice (KAP) study, the levels of KAP on Lifestyle modification were recorded before and after the intervention. The change in levels of KAP was tested using the Stuart-Maxwell marginal homogeneity test.
- For every comparison p values were deemed significant at 5% level or below
  *Significant correlation if P<0 .05 and ** Highly significant correlation if P <0.001