Chapter-3

Materials and Methods

The chapter discusses various methods and techniques used for collecting data for present research. It includes information regarding the duration of research, the instruments involved in measurements, the sample size and statistical procedures followed for analysis and presentation of data.

MATERIALS

The present study is an attempt to investigate cross-sectionally the age related changes in body composition and bone density on the sample size of 723 subjects (248 rural and 475 urban) ranging in age from 30 to 70 years belonging to Bania community residing in the rural and urban regions of District Panchkula. The rural sample was selected from villages of District Panchkula namely, Shyamtu, Rattewali, Kot, Bhanu, Ramgarh, Barwala, Raipur Rani, Morni, Mallah, Toda and Mauli. However, the urban sample was randomly selected from the urban towns of Panchkula, Kalka and Pinjore, collectively.

Detailed information regarding Bania families residing in the rural areas of District Panchkula was obtained beforehand from the block office. It is pertinent to note that the availability of rural Bania families in District Panchkula was limited due to migration of families to the urban areas and the subjects approached included all the available females in the respective villages during multiple visits made by the investigator. Therefore, the age matched sample of rural and urban females could not be obtained.

METHODS

Sampling method:

The method of sampling used for data collection in the present study was Purposive or Judgement sampling method of non-probability sampling used in Research. Although, the non probability method of sampling is subjective in approach, but the purposive sampling involves great effort from the researcher to find a most appropriate sample for his/her research. This method is useful when a research is focussed on a specific population and need is to reach a target sample quickly or in definite time span. In this
method the probability of selection of data items increases and indeed it helps in giving better estimates.

**Age recording:**

Age in years has been obtained from date of birth which most of the urban females could recall and also provide a documentary proof of the same in the form of aadhar card, voter ID, etc, where as in rural females age had to be ascertained in majority of cases by association with some important event like age at marriage, age at first child birth, any important festival, etc. With this cross-questioning, it was possible to ascertain nearly the correct age of the subject.

The data were arranged in eight age groups, each of five years duration except for first age group, which is of six year duration for both rural and urban Bania females, using decimal age calendar given by Tanner *et al.* (1966). Age group 30-35 years includes all the subjects from 29.500 to 35.499, the age group 36-40 years from 35.500 to 40.499 and so on. The distribution of sample and mean age of urban and rural Bania females of District Panchkula has been shown in table 3.1.

**Table-3.1:** Distribution of sample and mean age of urban and rural Bania females of District Panchkula

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Rural N</th>
<th>Mean</th>
<th>S.D.</th>
<th>Urban N</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.500-35.499</td>
<td>34</td>
<td>33.06</td>
<td>1.59</td>
<td>60</td>
<td>32.52</td>
<td>1.91</td>
</tr>
<tr>
<td>35.500-40.499</td>
<td>34</td>
<td>38.15</td>
<td>1.56</td>
<td>60</td>
<td>37.93</td>
<td>1.65</td>
</tr>
<tr>
<td>40.500-45.499</td>
<td>30</td>
<td>42.86</td>
<td>1.57</td>
<td>64</td>
<td>42.87</td>
<td>1.40</td>
</tr>
<tr>
<td>45.500-50.499</td>
<td>30</td>
<td>47.54</td>
<td>1.47</td>
<td>63</td>
<td>47.58</td>
<td>1.47</td>
</tr>
<tr>
<td>50.500-55.499</td>
<td>30</td>
<td>52.47</td>
<td>1.65</td>
<td>60</td>
<td>53.16</td>
<td>1.32</td>
</tr>
<tr>
<td>55.500-60.499</td>
<td>30</td>
<td>57.78</td>
<td>1.68</td>
<td>60</td>
<td>58.24</td>
<td>1.45</td>
</tr>
<tr>
<td>60.500-65.499</td>
<td>30</td>
<td>62.67</td>
<td>1.43</td>
<td>60</td>
<td>63.21</td>
<td>1.39</td>
</tr>
<tr>
<td>65.500+</td>
<td>30</td>
<td>71.63</td>
<td>4.83</td>
<td>48</td>
<td>70.81</td>
<td>4.24</td>
</tr>
</tbody>
</table>

Field work was conducted in different phases from year 2013 to 2014, covering the rural and urban areas of District Panchkula separately. The subjects included only the apparently healthy individuals, who were not suffering from any chronic disease or physical deformity.

The data were collected using personal interviews, by taking relevant anthropometric measurements and by measuring bone density. Before the data collection the entire
Materials and Methods

A questionnaire was explained in Hindi language to the subjects with the aims and objectives of the study. The procedure for data collections was also explained to them. An informed consent duly signed by the subject was taken before obtaining the data.

A. **Personalised interview:** The subjects were interviewed using structured and semi-structured questions in the form of schedule for the personal information that included age, caste, sub-caste, family composition, educational status, occupational status, income, place of birth, age at menarche, age at menopause, etc. The women were inquired about the continuation or the cessation of menstrual cycle to analyse reproductive senescence, i.e., menopause. Women who had either attained menopause (had no menstruation in the last twelve months) were categorised as postmenopausal and perimenopausal women were those who were passing through the phase to attain menopause. The term perimenopause describes the phase immediately before the menopause—from the time when the hormonal and clinical features of approaching menopause commence till the end of the first year after menopause (World Health Organisation, 1996b). The third category of females were premenopausal i.e. those females who were experiencing their regular menstruation. The subjects were also inquired about the menopausal symptoms experienced by them depending on their menopausal status. The reproductive history, diseases associated with menopause, involvement in any type of physical activity, sun exposure, diet preferences and dietary intake, water intake, etc. were also recorded for each subject. A 24 hr dietary recall questionnaire was used to find out the types of food stuffs consumed by each subject.
Materials and Methods

Picture demonstrating the get together of females during personal interviews

i) Marital status- Out of 248 rural females 79.8% were married, 19.8% were widow and 0.4% were separated whereas, out of 475 urban females 86.1% were married, 13.3% were widow, 0.4% were unmarried and 0.2% were separated (table-3.2).

Table-3.2: Marital status of urban and rural Bania females of District Panchkula

<table>
<thead>
<tr>
<th>Status</th>
<th>Rural Frequency</th>
<th>Rural Percent</th>
<th>Urban Frequency</th>
<th>Urban Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>198</td>
<td>79.8</td>
<td>409</td>
<td>86.1</td>
</tr>
<tr>
<td>Unmarried</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Widow</td>
<td>49</td>
<td>19.8</td>
<td>63</td>
<td>13.3</td>
</tr>
<tr>
<td>Separated</td>
<td>1</td>
<td>0.4</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>248</td>
<td>100.0</td>
<td>475</td>
<td>100.0</td>
</tr>
</tbody>
</table>

ii) Educational status- The percentage distribution of educational status of females, their spouses and children in the present study is demonstrated in table-3.3. Only 5.9% urban and 10.5% rural females were found to be illiterate whereas, among their spouses, only 0.2% urban and 2.0% rural were illiterate. 13.2% urban and 11.7% rural
children were illiterate. Maximum percentages of matriculates were found among rural females (34.7%) and their spouses (49.5%). Among urban females (30.9%) and their spouses (42.1%) maximum percentage was that of graduates.

**Table-3.3:** Percentage distribution of educational qualification among the present study

<table>
<thead>
<tr>
<th>Educational Qualification</th>
<th>Qualification of respondent</th>
<th>Husband’s Qualification</th>
<th>Qualification of children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urban</td>
<td>Rural</td>
<td>Urban</td>
</tr>
<tr>
<td>Illiterate</td>
<td>5.9</td>
<td>10.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Upto 5th Standard</td>
<td>0.8</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>5th-8th</td>
<td>12.4</td>
<td>19.4</td>
<td>5.1</td>
</tr>
<tr>
<td>8th-10th</td>
<td>21.3</td>
<td>34.7</td>
<td>24.4</td>
</tr>
<tr>
<td>10th-12th</td>
<td>9.9</td>
<td>14.5</td>
<td>14.8</td>
</tr>
<tr>
<td>Undergraduate</td>
<td>3.2</td>
<td>0.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Graduate</td>
<td>30.9</td>
<td>10.5</td>
<td>42.1</td>
</tr>
<tr>
<td>Above</td>
<td>15.6</td>
<td>7.3</td>
<td>11.0</td>
</tr>
</tbody>
</table>

**iii) Occupational status** The occupational status of females and their spouses in the present study is summarised in Table-3.4, which clearly shows that majority of females were house wives (R=83.9%, U=87.6%), while the working females were found to be higher among rural group (9.3%, 6.9%) in comparison with urban group (7.2%, 5.3%), respectively. Majority of spouses among rural (87.4%) as well urban (82.2%) were engaged in private jobs or were doing their own business.

**Table-3.4:** Percentage distribution of occupational status among the respondents and their spouses

<table>
<thead>
<tr>
<th>Occupational status</th>
<th>Occupation of respondent</th>
<th>Husband’s Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urban</td>
<td>Rural</td>
</tr>
<tr>
<td>House wife/Retired</td>
<td>87.6</td>
<td>83.9</td>
</tr>
<tr>
<td>Private job/ Business</td>
<td>7.2</td>
<td>9.3</td>
</tr>
<tr>
<td>Government job</td>
<td>5.3</td>
<td>6.9</td>
</tr>
</tbody>
</table>
iv) **Menarcheal Status:** The age at attainment of menarche among the subjects was determined using retrospective or recall method. Each subject was inquired about the age at which she began to menstruate. Although, this method is subjected to unknown degrees of bias and inaccuracy (Eveleth & Tanner, 1990), yet it is the only way to obtain information on an event which has not happened in the recent past.

v) **Menopausal Status:** The determination of age at menopause was done using status quo and retrospective methods. In status quo method, each subject was asked her precise age at the time of questioning (or her date of birth) and whether menopause has occurred or not on the day of investigation. This lead to record of percentage of affirmative answer at each successive age. Subsequently, a probit transformation was applied to that percentage distribution. This method was used for estimation of median and variance for the population. In retrospective or recall method, the mean age at menopause was calculated. It depended on the recall of age at menopause by the subjects. This method proves equally good if the phenomenon is of recent past.

vi) **Symptomatology of menopause:** The prevalence of menopausal symptoms (vasomotor, psychosocial and urogenital) among subjects was recorded based on a list of menopausal symptoms constituting 11 items, used by Jahanfar and associates (2006) for a study in Malaysia and subsequently recommended by Indian Menopausal Society (Singh, 2007). The symptoms which were experienced by any individual were further classified according to degree of severity as mild, moderate and severe.

For recording the symptoms of menopause the following list of symptoms as recommended by Indian menopausal society (Singh, 2007) were also included.

- (i) Hot flushes & Night sweats
- (ii) Heart discomfort
- (iii) Sleep problems
- (iv) Depression
- (v) Irritability
Materials and Methods

(vi) Anxiety
(vii) Weight changes
(viii) Physical & Mental exhaustion
(ix) Joint & Muscular discomfort
(x) Bladder problems
(xi) Dryness of vagina
(xii) Sexual problems
(xiii) Mood changes
(xiv) Fatigue & memory problems

B. Anthropometric Measurements: Each subject under investigation was measured using the standard techniques given by Weiner and Lourie (1981) for the under mentioned anthropometric measurements. The instruments used in anthropometric measurements included anthropometric rod, steel tape and body fat analyser. A body fat analyser (Omron HBF 302-Japanese Model) based on the bioelectrical impedance method was used to calculate total body weight and % body fat. All the instruments were checked for zero error before each measurement and the procedure for taking measurement was practised before hand to reduce personal error. Since all the measurements were taken by the researcher herself, there were no inter-observer technical errors of measurement (TEM). Random checks were made to find out technical error of measurements. In all such checks, it was found to be less than 1%. Intra-observer error for stature was 0.3–1 cm. For body weight, it was between 0 and 0.5 kg; for girth measurements, values ranged from 0.1 to 0.3 cm; for body fat error values ranged 0.1–0.2; for blood pressure values ranged from 1 to 5mmHg.

i) Height (cm): It is the vertical distance between the land mark ‘vertex’ and the ground with the subject standing erect, bare footed on a levelled ground and her face in Frankfurt Horizontal plane (F-H plane).

ii) Weight (kg): It is the gross body weight contained in the body when the subject stands erect with head present in F-H plane, wearing the minimum possible clothing.
iii) **Waist Circumference (cm)**: It measures the smallest circumference of the torso which is the level of the natural waist. It was measured midway between the umbilicus and xiphoid.

iv) **Hip Circumference (cm)**: It measures the maximum circumference of the hips at their widest portion, at a level in the middle of the two buttocks and at right angles to the axis of the body.

v) **Percent Body Weight (%):** Body fat percentage reflects total percentage of fat relative to body weight of an individual. It was measured with the help of body fat analyser.

vi) **Visceral fat (Kg):** Visceral fat is a type of abdominal fat that exists in the abdomen and surrounds the internal organs. It was measured with the help of body fat analyser. The value displayed by OMRON body fat analyser is the level indicating visceral fat area.

*Picture demonstrating the use of body fat analyser*
vii) Derived indices: The following indices were derived from the relevant anthropometric measurements recorded on the subjects.

a) **Body Mass index (BMI):** It is also known as Quetelet’s index, which expresses the relationship between the two most widely used parameters that monitor linear and ponderal growth, i.e., height and weight. Body mass index or BMI was computed as weight in kilograms divided by height (in m) squared.

\[
BMI = \frac{\text{Weight (Kg)}}{\text{Height}^2 \text{ (m)}}
\]

Body fatness is usually standardized for body weight and expressed as percent body fat [Fat mass (kg)/body weight (kg)], but an alternative is to express fat mass relative to height squared. This leads to the use of Fat Mass Index (FMI) \{fat mass/height squared\} and the Fat Free Mass Index (FFMI) \{fat free mass / height squared\} (Vantallie et al., 1990 & Wells, 2001). FMI + FFMI = BMI. The use of these indices allows one to easily assess whether BMI differences are due to fat or fat free mass (Freedman & Bettylou, 2009).

b) **Fat mass index (FMI):** Fat mass or body fat is calculated using percent body fat and overall body weight. It usually comprises the amount of overall adipose tissue contained in the body. The relation of fat mass with the height of an individual determines the fat mass index of the individual, determined as follows:

\[
FMI = \frac{\text{Fat mass (Kg)}}{\text{Height}^2 \text{ (m)}}
\]

c) **Fat free mass index (FFMI):** Fat free mass or the lean mass is the difference between the body weight and the fat mass of the body. The relation of fat free mass with the height of an individual determines the fat free mass index of the individual.

\[
FFMI = \frac{\text{Fat free mass (Kg)}}{\text{Height}^2 \text{ (m)}}
\]
d) Waist-hip Ratio (WHR): In the epidemiological study, it is the most common index of centralised fat and hence used as a measurement of obesity. It helps in determining the overall health risk. It measures the ratio of waist circumference to hip circumference.

\[
\text{WHR} = \frac{\text{Waist Circumference (cm)}}{\text{Hip Circumference (cm)}}
\]

e) Waist Height Index (WHI): Waist to height ratio is a simple measurement for assessment of lifestyle risk and overweight. It is an indicator of the body shape which in turn helps to assess the risk for obesity, Heart diseases, diabetes, stroke, and hypertension. It determines whether the body size is within frame or not. Waist to height ratio is a stronger predictor of cardio-metabolic risks than BMI. It is calculated by ratio of waist circumference to height.

\[
\text{WHtR/WHI} = \frac{\text{Waist Circumference (cm)}}{\text{Height (cm)}}
\]

f) Conicity Index (CI): It is an index of abdominal obesity. The conicity index evaluates waist circumference in relation to height and weight and therefore, it is determined through the measurements of weight, height and waist circumference (WC), by using the mathematical equation (Valdez, 1991):

\[
\text{CI} = \frac{\text{Waist Circumference (m)}}{0.109 \times \sqrt[3]{\text{Body Weight (kg)}/\text{Height (m)}}}
\]

g) A Body Shape Index (ABSI): Another index marking the abdominal fat deposits and determining the body shape using waist circumference and adjusted height and weight measures of an individual was proposed by Krakauer & Krakauer (2012). ABSI expresses the excess risk from high WC in a convenient form that is complementary to BMI and to other known risk factors. It is calculated as:

\[
\text{ABSI} = \frac{\text{Waist Circumference (m)}}{\text{BMI}^{2/3} \times \text{Height}^{1/2} (\text{m})}
\]
h) **Body Adiposity Index (BAI):** A new adiposity index was defined by Bergman *et al.* (2011) as an alternative parameter to reflect % body fat (PBF) for adult men and women of differing ethnicities without numerical correction. It is obtained using the hip circumference and height measurements of an individual without using the body weight.

\[
\text{BAI} = \frac{\text{Hip Circumference (m)} - 18}{\text{Height (m)} \times \sqrt{\text{Height (m)}}}
\]

C. **Physiological Parameters:** Each subject under study was also investigated for the following physiological parameters using the oscillometric method based digital OMRON Automatic Blood Pressure Monitor MX3 recommended by World Hypertension League. In order to obtain the normal readings, precaution was taken that the subject was completely relaxed and free from any stressful activities when her blood pressure and pulse rate were recorded.

a) **Blood Pressure (Systolic & Diastolic):** This measures the pressure exerted by the blood against the walls of the arteries, during the circulation of the blood to and fro from the heart. The measure is recorded as *Systolic Blood Pressure* during the contractile phase of the heart (maximum pressure exerted against arterial wall at the peak of ventricular contraction) while it is recorded as *Diastolic Blood Pressure* during the relaxing phase of the heart (minimum pressure exerted when the heart is at rest, just prior to the next heart beat).

b) **Pulse rate:** It has been as an index of metabolic activity or of energy expenditure. It was measured by palpation of radial artery at the wrist; the number of beats that occurred in half a minute were being counted and doubled to give the rate per minute.

D. **Bone Mineral Density:** Bone mineral density of each subject was assessed from the calcaneus using ultrasound based Bone densitometer McCue C.U.B.A. Clinical, a dry ultrasound sonometer which is designed to perform a subject’s measurement in less than 1 minute. It does not produce ionising radiations and operates on the voltage of 90-240V. It is
indeed very easy to operate and completely safe to use on a subject without any side effects. The following measurements were obtained on the densitometer based on the already existing normative data based on Caucasian population standards:

a) **Separation** which determines the width of the heel (in mm).

b) **BUA (broadband ultrasound attenuation)** that determines the complexity of bone structure.

c) **% expected** determines the comparative ultrasound analysis with respect to age matched normal mean.

d) **BUA Z-score** of individual gives the number of population standard deviations away from population mean for particular age.

e) **BUA T-score** of individual gives the deviation at peak bone mass (young normal aged 20).

The calcaneus is most commonly selected for bone ultrasound assessment because it is accessible to the transducer with minimal surrounding soft tissue.

**Picture demonstrating the set up of McCue CUBA Clinical bone densitometer**
E. ASSESSMENT OF OBESITY:

The prevalence of obesity among urban and rural females of District Panchkula was assessed with the help of Body Mass Index (BMI), Waist circumference (WC), Waist hip ratio (WHR), percent body fat (PBF) and Waist height ratio/index (WHI).

Body mass index is the most frequently used anthropometric index and has been recommended by the World Health Organization (WHO) to classify obesity (WHO, 1997). BMI has been used since the 1960s to assess obesity in adults (Keys et al. 1972; Garrow, 1981).

Table-3.5: The International Classification of adult underweight, overweight and obesity according to BMI

<table>
<thead>
<tr>
<th>Classification</th>
<th>Principal cut-off points</th>
<th>Additional cut-off points</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI(Kilogram/Meter²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>&lt; 18.50</td>
<td>&lt; 18.50</td>
</tr>
<tr>
<td>Severe thinness</td>
<td>&lt; 16.00</td>
<td>&lt; 16.00</td>
</tr>
<tr>
<td>Moderate thinness</td>
<td>16.00-16.99</td>
<td>16.00-16.99</td>
</tr>
<tr>
<td>Mild thinness</td>
<td>17.00-18.49</td>
<td>17.00-18.49</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.50-24.99</td>
<td>18.50-22.99</td>
</tr>
<tr>
<td>Overweight</td>
<td>≥ 25.00</td>
<td>≥ 25.00</td>
</tr>
<tr>
<td>Pre-obese</td>
<td>25.00-29.99</td>
<td>25.00-27.49</td>
</tr>
<tr>
<td>Obese</td>
<td>≥ 30.00</td>
<td>≥ 30.00</td>
</tr>
<tr>
<td>Obese class I</td>
<td>30.00-34.99</td>
<td>30.00-32.49</td>
</tr>
<tr>
<td>Obese class II</td>
<td>35.00-39.99</td>
<td>32.50-34.99</td>
</tr>
<tr>
<td>Obese class III</td>
<td>≥ 40.00</td>
<td>≥ 37.50</td>
</tr>
</tbody>
</table>


World Health Organization convened the Expert consultation on BMI in Asian populations. The experts reviewed scientific evidence that suggests that Asians population have different association between BMI, percentage of body fat, and health risks than do European populations. The consultation experts identified further potential public health action points (23.0, 27.5, 32.5 and 37.5 kg/m²) along the continuum of BMI, and proposed additional trigger points for public health action as 23 kg/m² or higher, representing increased risk, and 27.5 kg/m² or higher as
representing high risk. The suggested categories are as follows: less than 18.5 kg/m² (underweight); 18.5-23 kg/m² (increasing but acceptable risk); 23-27.5 kg/m² (increased risk); and 27.5 kg/m² or higher (high risk) (WHO Expert Consultation, 2004).

The special cut-off points for waist circumference (WC) and waist hip ratio (WHR) were recommended by WHO experts in an expert consultation meet at Geneva in December, 2008. The experts believed that waist circumference and waist–hip ratio (as measures of abdominal obesity) were correlated with BMI, but the level of association varied, suggesting that these measures may provide different information and thus may not be interchangeable. Practical considerations appeared to favour the use of waist circumference as an alternative to BMI (WHO, 2011).

Table 3.6: World Health Organization cut-off points and risk of metabolic complications

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Cut-off points</th>
<th>Risk of metabolic complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference</td>
<td>&gt;94 cm (M); &gt;80 cm (W)</td>
<td>Increased</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>94.1-101.9 (M); 80.1-87.9 (W)</td>
<td>Moderately increased</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>&gt;102 cm (M); &gt;88 cm (W)</td>
<td>Substantially increased</td>
</tr>
<tr>
<td>Waist–hip ratio</td>
<td>≥0.90 cm (M); ≥0.85 cm (W)</td>
<td>Substantially increased</td>
</tr>
</tbody>
</table>

Source: WHO, 2000, 2011; (M=men; W=women)

The cutoff used for assessing obesity based on percent body fat (PBF) and waist height index (WHI) was given by Dudeja et al. (2001) for PBF (≥ 30.0%) and by Ashwell et al. (1996) for WHI (≥0.5).

F. Screening for different categories of BMD:

The gold standard of BMD measurement i.e. hip measurement by DEXA (Dual energy X-ray absorptiometry) scan based values are universally accepted and used for screening individuals into different categories based on
BMD measures. The WHO proposed a consensus development statement and defined the strength of an individual’s bone relative to that seen in a young healthy population (T-score).

The World Health Organization has established the following definitions based on BMD measurement at the spine, hip or forearm by DXA devices (Kanis et al. 1994):

**Normal:**
BMD is within 1 SD of a “young normal” adult (T-score at -1.0 and above).

**Low bone mass (“osteopenia”):**
BMD is between 1.0 and 2.5 SD below that of a “young normal” adult (T-score between -1.0 and -2.5).

**Osteoporosis:**
BMD is 2.5 SD or more below that of a “young normal” adult (T-score at or below -2.5).

**Severe Osteoporosis (“established osteoporosis”):**
BMD 2.5 SD or more below the “young normal adult mean” (T-score at or below -2.5) in the presence of one or more fragility fractures.

*Source:* National Osteoporotic Foundation, 2010

However, the discrimination of osteoporotic patient against normal patients is -2.43SD for DXA BMD while it is only -2.02 SD for ultrasound. -2.0 SD ‘T’ score represents a BUA value of 53dB/MHz and is found to have discriminatory ability of 90% (Greenspan et al. 1997).

The classification of urban and rural Bania females of District Panchkula in the present study were classified into different categories of BMD relative to their BUA measurements by CUBA clinical, recommended as follows:

**Table-3.7:** T-score as recommended by CUBA clinical manual

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>Status</th>
<th>T-score (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Average</td>
<td>More than -1.0 SD</td>
</tr>
<tr>
<td>Osteopenic</td>
<td>Below average</td>
<td>Less than -1.0 SD but greater than -2.0 SD</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Risk of osteoporotic fracture</td>
<td>Less than -2.0 SD</td>
</tr>
</tbody>
</table>
Materials and Methods

Pictures demonstrating the setup and measurement of bone mineral density

G. Statistical Analysis:
Statistics is the science of collecting and analyzing of data. It is the science which deals with statistical methods and their application in an inquiry. Statistical method is technique to collect, classify, tabulate, analyze and interpret the quantitative data. The aim of statistical method is to present the data in a comprehensible and intelligent way. Thus, it serves to provide a shorthand description of large amount of data. These methods are very significant in anthropometric studies because they facilitate the
interpretation by giving numerical expression to the relationship and variation with respect to different aspects.

The data collected in the present study on rural and urban females was subjected to appropriate univariate, bivariate and multivariate statistical analysis which has been done using SPSS 16 and Window 7. The following statistical methods were used for analyzing the data:

1. Mean or Average.
2. Standard Deviation (S.D.)
3. t-test
4. Analysis of variance
5. Coefficient of correlation
6. Odds ratio
7. Confidence Interval
8. Regression
9. ROC curve Analysis

1. ARITHMETIC MEAN OR AVERAGE:

It measures the central value of distribution. The average values have been represented by arithmetic mean, which has been calculated by adding up all observations to be averaged and dividing the sum by the total number. The formula applied for calculation of Arithmetic mean is:

\[ \bar{X} = \frac{\sum X}{N} \]

Where,

\( \bar{X} \) = Arithmetic Mean

\( \sum \bar{X} \) = Sum of all the recorded observations.

\( N \) = Total number of observations.
2. **STANDARD DEVIATION:**

Standard Deviation is most widely used measure of dispersion of series. It gives the numerical expression to the extent of derivation of the observed data from, the arithmetic mean. Thus, it helps in studying dispersion. It can be obtained by taking the square roots of sum of the squares of deviations from mean. A high value of S.D. indicates a more dispersed or variable population and a lower value indicate a less variable population. S.D. is useful in comparing variability of groups with regard to some characters. The formula applied is:

\[
S.D. = \sqrt{\frac{\sum (X - \bar{X})^2}{N - 1}}
\]

Where,

- S.D. = Standard Deviation.
- \(\bar{X} - X\) = Deviation of values from the mean.
- \(N\) = Total number of individuals.

(To reduce errors due to small sample size, one is subtracted from \(N\)).

3. **t-TEST:**

Student’s t-test is a popular and frequently used statistical test to compare two different set of values. The ‘t’ is a statistics applied to assess the significance of differences between two populations or group of the data or two means \(X_1\) and \(X_2\) of two independent samples of size \(N_1\) and \(N_2\) and S.D. of \(S_1\) and \(S_2\). The main purpose of this is to test the hypothesis that the studied samples come from the same population.

i.e. \(\bar{X}_1 = \bar{X}_2\) or \(\bar{X}_1 - \bar{X}_2 = 0\)

It is calculated by using formula:

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{S_x \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}
\]

and

\[
S = \sqrt{S_1^2 N_1 + S_2^2 N_2 / N_1 + N_2 - 2}
\]
Where, $\bar{X}_1$ and $\bar{X}_2$ = means of two samples.
S1 and S2 = S.D. of two samples.
N1 and N2 = size of samples.
S = combined S.D.

4. **ANALYSIS OF VARIANCE (ANOVA):**

Analysis of variance is similar to an unpaired t-test but is used to test differences between two or more than two samples. It helps in drawing inferences regarding differences between several means, simultaneously. In one-way classification, there are two types of variations in data namely, variation between samples and variation within samples. If both variations are similar the sample is of same population.

\[
\text{Variance between samples} = (\text{MSB}) = \frac{SSB}{k-1}
\]
\[
\text{Variance within samples} = (\text{MSW}) = \frac{SSW}{N-k}
\]

Where:
- $SSB$ is sum of square of the deviations between samples.
- $SSW$ is sum of squares of the deviation within samples.

\[
F = \frac{\text{MSB}}{\text{MSW}}
\]

5. **THE COEFFICIENT OF CORRELATION:**

One of the most widely used statistics is the coefficient of correlation ‘$r$’ which measures the degree of association between the two values of related variables given in the data set. It takes values from + 1 to – 1. If two sets or data have $r = +1$, they are said to be perfectly correlated positively if $r = -1$ they are said to be perfectly correlated negatively; and if $r = 0$ they are uncorrelated.

The coefficient of correlation ‘$r$’ is given by the formula:

\[
r = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sqrt{\sum(x - x)^2 - \sum(y - y)^2}}
\]

Where:
Arithmetic mean of x and y is given by \( x \) and \( y \):

Deviations of the two series are squared and added up to get \( \sum x^2 \) and \( \sum y^2 \)

6. **ODDS RATIO:**

An odds ratio (OR) is a statistical measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure (Szumilas, 2010).

OR is used by epidemiologists in studies that involve the factors which may harm, it is in-turn a way of comparing patients who already have a certain condition (cases) with patients who do not (controls) – a “case–control study” (Harris & Taylor, 2003).

Odds ratios are used to compare the relative odds of the occurrence of the outcome of interest (e.g. disease or disorder), given exposure to the variable of interest (e.g. health characteristic, aspect of medical history). The odds ratio can also be used to determine whether a particular exposure is a risk factor for a particular outcome, and to compare the magnitude of various risk factors for that outcome.

Odds ratio is given as:

\[
OR = \frac{p \cdot q}{q \cdot p}
\]

Where, \( p \) is the probability for the first group, and \( q \) is the probability for the second.

- OR=1 Exposure does not affect odds of outcome
- OR>1 Exposure associated with higher odds of outcome
- OR<1 Exposure associated with lower odds of outcome

7. **CONFIDENCE INTERVAL:**

The confidence interval is one way of conveying our uncertainty about a parameter. Confidence intervals (CI) are typically used when, instead of simply wanting the mean value of a sample, we want a range that is likely to contain the true population
value. This “true value” is another tough concept – it is the mean value that we would get if we had data for the whole population. Statisticians can calculate a range (interval) in which we can be fairly sure (confident) that the “true value” lies (Harris & Taylor, 2003).

A confidence interval calculated for a measure of treatment effect shows the range within which the true treatment effect is likely to lie (subject to a number of assumptions). A confidence interval that embraces the value of no difference between treatments indicates that the treatment under investigation is not significantly different from the control. Confidence intervals aid interpretation of clinical trial data by putting upper and lower bounds on the likely size of any true effect (Davies & Crombie, 2009).

8. REGRESSION:

Regression analysis is a statistical tool for the investigation of relationships between the variables. Data regression analysis is a technique used for the modeling and analysis of numerical data consisting of values of dependent variable (response variable) and of one or more independent variables (explanatory variables). The dependent variable in the regression equation is modelled as a function of the individual variables.

8.1. LINEAR REGRESSION:

When one variable changes with other variable in some fixed ratio, this is called as linear regression. Such type of relationship is depicted on a graph by means of a straight line or a first degree equation.

Regression equation for a straight line is given by:

\[ y = a + bx \]

where, \( y \) = dependent variable

\( x \) = value of the independent variable

‘b’ = slope of the line

‘a’ = point where the line intercepts the vertical axis
The slope is called the regression coefficient. The difference between the observed value of the dependent variable \(y\) and its expected value by the regression model is called the residual. For the data points falling above the regression line then the residual is positive and for those falling below the line, then the residuals will be negative. The expression of the slope \(b\) and the intercept \(a\) are obtained as:

\[
b = \frac{\text{sum of } xy - \left(\text{sum } x \times \text{sum } y\right)}{\text{sum } x^2 - \left(\text{sum } x\right)^2}
\]

\[
a = \frac{1}{n} \left(\text{sum } y\right) - \frac{b \times \left(\text{sum } x\right)}{n}
\]

8.2 MULTIPLE REGRESSIONS:

When we study more than one variable (at least three variables) at a time, in which one is dependent variable and others are independent variables. In this analysis, the regression of dependent variables on a set of independent variables \((x_1, x_2 \ldots \ldots x_n)\) is studied. This analysis has helped in finding out whether the different anthropometric variables are related to systolic blood pressure, diastolic blood pressure and body mass index or not and if related then which all of them are significantly related.

\[
y = a_0 + a_1x_1 + a_2x_2 + \ldots + a_nx_n
\]

where, \(a_i\) - found approximation coefficients, \(x_i\) - i-th argument.

9. Receiver Operating Characteristic Curve Analysis

A receiver operating characteristic (ROC), or ROC curve, is a graphical plot that illustrates the performance of a binary classifier system as its discrimination threshold is varied. The curve is created by plotting the true positive rate against the false positive rate at various threshold settings. (The true-positive rate is also known as sensitivity in biomedical informatics, or recall in machine learning. The false-positive rate is also known as the fall-out and can be calculated as 1 -
specificity). The ROC curve is thus the sensitivity as a function of fall-out (Tape, 2001).

In general, if the probability distributions for both detection and false alarm are known, the ROC curve can be generated by plotting the cumulative distribution function (area under the probability distribution from $-\infty$ to $+\infty$) of the detection probability in the y-axis versus the cumulative distribution function of the false-alarm probability in x-axis.

ROC analysis provides tools to select possibly optimal models and to discard suboptimal ones independently from (and prior to specifying) the cost context or the class distribution. ROC analysis is related in a direct and natural way to cost/benefit analysis of diagnostic decision making.

An ROC curve demonstrates several things:

1. It shows the trade-off between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity).
2. The closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate is the test.
3. The closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test.
4. The slope of the tangent line at a cut-point gives the likelihood ratio (LR) for that value of the test.
5. The area under the curve is a measure of text accuracy.