CHAPTER – 3

METHODOLOGY

3.1 INTRODUCTORY STATEMENT

There is no alternative to truth and therefore to research. The purpose of any research work is to discover the answer to the question through the application of a scientific procedure, the main aim of the research is to find out the truth which is hidden and which has not been discovered yet. The research is one way to penetrate deep into the fact leading to knowledge. Thus, in order to organize the facts collected, proper scientific methodology must be used.

According to P.V. Young:- "We may define social research as the systematic method of discovering new facts, of verifying old facts, their sequences interrelationship, causal explanations and the natural laws which govern them."

According to Clifford Woody:- “Research comprises defining and redefining problems, formulating hypothesis or suggested solution; collecting, organizing and evaluating data; making deduction and reaching conclusion; and at last carefully testing the conclusions to determine whether they fit the formulating hypothesis.”

3.2 HYPOTHESIS

In scientific research we have to make new discoveries, but we cannot proceed in complete ignorance. We must have some ideas as to new aspects that are likely to be discovered. Then of course, we proceed to find out whether the ideas conceived are true. Those may be totally correct, as only partially correct or may be altogether false, but they do help us to get going and guide us in our study.
Methodology

According to G.A Lundberg, "A hypothesis is a tentative generalization, the validity of which remains to be tested in its most elementary stage. The hypothesis may be any hunch, guess imaginative ideas, which may become the basis for action or investigation."

In the present study data have been collected and conclusion has drawn under the light of following **Hypothesis:**

- There shall be no significant difference in nutritional status, health status and life style pattern of post menopausal women.
- There shall be no significant association between some factors of nutritional status, health status and life style pattern with stress level of post menopausal women.

### 3.3 WORKING DEFINITIONS

(I) **Age at Menopause:** - Postmenopausal women whose menses is seized for at least one & half years.

(II) **Food Frequency:** - food group intake was assessed in terms of frequency consumption of food on weekly, monthly and daily basis.

(III) **Dietary nutrient intake:** - Calculated for some nutrients like - Energy, Carbohydrate, Protein, Fat, Calcium & Iron consumed in a day.

Some factors of nutritional status in relation to stress level of post menopausal women. We have assessed Age, Weight and Energy, keeping in view that if energy intake increases, weight is directly affected. Beside this in the present study health status and life style pattern with stress level of postmenopausal women has been assessed.

(IV) **Physical Activity:** - In present study three category of physical activity has been considered-

- Sedentary worker: Teacher, Executives and Housewife etc.
- Moderate worker: Servant maid, Basket maker and Weaver etc.
- Heavy worker: Stone Cutter (We have not found post menopausal women in this category).

(V) **Exercise:** - This study includes light exercise, walk & Yoga etc.
3.4 RESEARCH DESIGN

Research design is a plan according to which observations are made and data assembled. It provides the empirical and logical basis for drawing conclusions and gaining knowledge. In present study, the research design has been developed systematically in following step:-

Sample Design:- According to P.V Young “sample is a miniature picture of the universe.” When a small group is taken, it is called sample study. The whole group from which sample is drawn is technically known as universe or population and the group actually selected for the study is known as sample. When the sample is representative of a population and certain things can be predicated about the population from which it was drawn, and then it is known as statistical inference. Taking this in consideration, following sample design has been propagated systematically

Sample technique

The present study was carried out on 200 Post menopausal women aged 40-70 years. Samples were selected by purposive sampling method.

Table 3.1
Sample at a glance

<table>
<thead>
<tr>
<th>Demographic Variable</th>
<th>Population Particulars</th>
<th>Frequency (N=200)</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td>Illiterate</td>
<td>42</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>89</td>
<td>44.5</td>
</tr>
<tr>
<td></td>
<td>Higher Secondary</td>
<td>50</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Graduate</td>
<td>19</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>Post-Graduate and Above</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>200</td>
<td>100.0</td>
</tr>
<tr>
<td>Occupation</td>
<td>Working</td>
<td>39</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>Non-working</td>
<td>161</td>
<td>80.5</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>200</td>
<td>100.0</td>
</tr>
</tbody>
</table>
### Demographic Variable

<table>
<thead>
<tr>
<th>Population Particulars</th>
<th>Frequency (N=200)</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>177</td>
<td>88.5</td>
</tr>
<tr>
<td>Spinster</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Widow</td>
<td>23</td>
<td>11.5</td>
</tr>
<tr>
<td>Divorsee</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>200</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

### Category

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency (N=200)</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>145</td>
<td>72.5</td>
</tr>
<tr>
<td>OBC</td>
<td>37</td>
<td>18.5</td>
</tr>
<tr>
<td>SC</td>
<td>18</td>
<td>9.0</td>
</tr>
<tr>
<td>ST</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>200</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

### Types of Family

<table>
<thead>
<tr>
<th>Type</th>
<th>Frequency (N=200)</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear</td>
<td>104</td>
<td>52.0</td>
</tr>
<tr>
<td>Joint</td>
<td>96</td>
<td>48.0</td>
</tr>
<tr>
<td>Extended</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>200</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

### Monthly Income

<table>
<thead>
<tr>
<th>Income Category</th>
<th>Frequency (N=200)</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIG 8001/- to 20000/-</td>
<td>29</td>
<td>14.5</td>
</tr>
<tr>
<td>MIG 20000/- to 40000/-</td>
<td>116</td>
<td>58.0</td>
</tr>
<tr>
<td>HIG 40001/- and above</td>
<td>55</td>
<td>27.5</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>200</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Source: currentnewsandinformation.blogspot.com/.../mhada-housing-lottery

**Table 3.2**

Classification of sample as per locale

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Locale</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sri Aurobindo Institute of Medical Sciences, Indore</td>
<td>70</td>
</tr>
<tr>
<td>2.</td>
<td>Bhandari Hospital &amp; Research Center, Vijay Nagar, Indore</td>
<td>45</td>
</tr>
<tr>
<td>3.</td>
<td>Medicare Hospital, Ravindra Nagar, Indore</td>
<td>40</td>
</tr>
<tr>
<td>4.</td>
<td>Choithram Hospital &amp; Research Center, Manik Bagh Road, Indore</td>
<td>31</td>
</tr>
<tr>
<td>5.</td>
<td>Dr. Aruna Tiwari’s Clinic, Sadar Bazar Main Road, Indore</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>200</strong></td>
</tr>
</tbody>
</table>
3.5 TOOLS AND TECHNIQUES

Adopting proper tools and techniques for the collection of data is a foremost important step of the research design. The collection of data refers to a purposive gathering of the information relevant to the subject matter of the study from the samples under investigation. The method of collection of the data depends upon the nature, purpose and the scope of the study on one hand and the availability of resources and time on the other.

In present study the collection of data was done mainly by following methods.

**Questionnaire method :-** In this study, a structured questionnaire was used regarding demographic trends (marital status, education, income status, occupation and caste) life style pattern (Physical activity, Food Pattern, Exercise) Health status ( Age at menopause, health problems like Blood pressure, arthritis, joint pain, diabetes, Obesity Anemia)

**Diet survey:-** For the collection of data regarding consumption of different food items, a 24-hour dietary recall method has been conducted through questionnaire method. The food list was used to collect information about the quantity of various food consumed by respondents, during the period of survey (one day). For this a questionnaire containing a list of foods was prepared .The quantities consumed by the samples were entered carefully on the sheet. Some points were particularly considered while taking information regarding the dietary intakes which were as follows:

- Sizes and volumes of some foods like size of chapatti, minimum amount of dal and katori size, consistency of dal, amount of sugar in tea etc.
- Information regarding use of oil or fat in vegetables.
- Food items consumed outside were recorded.

The nutrients were calculated by especially designed computer software based on nutritive value of Indian foods by C.Gopalan (1996) and consumed nutrients were checked against recommended dietary allowances (ICMR 1989) for the assessment of nutrient intake status.
Methodology

Anthropometric measurements

Nutritional anthropometry is measurement of human body at various ages and levels of nutritional status. It is based on the concept that an appropriate measurement should reflect any morphologically variation occurring due to significant functional physiological changes.

For example, a significant reduction in fat fold measurement reflects a shift in the individual’s energy balance.”

“The pattern of growth and the physical state of the body through genetically determined are profoundly influenced by diet and nutrition hence anthropometric measurements are useful criteria for assessing nutritional status” (Swaminathan - 1998)

Following procedures were adopted for it:

Age :- The age of the subjects was taken up by asking date of birth. Those who were not knowing their date of birth, then the age of the subject has been assessed by school records, season, festival and important incident if any took place at that time of the birth.

Weight :- The weight of the subjects was measured in kg. on a spring weight machine. The subject was wearing minimum clothing and no shoes.

Height :- The measurement of height was done by asking the person to stand the women on a plain surface and rating her back on fixed support. The height was measured using non stretchable tailoring tape. The subjects were not wearing shoes. The measurement of Height and Weight was done by taking full precaution, which are as following:

- The zero error of the weighing scale was checked before taking the weight and was corrected as and when required.
- In the measurement of height, the subject was allowed to stand erect, looking straight and leveled surface, without shoes, with heels together and toes apart.
- In the measurement of weight. The person was wearing minimum clothing and was without shoes.
Methodology

BMI :- After the cessation of linear growth around 21 years weight for height indicates muscles fat mass in the adult body the ratio of weight (in kg) height (m$^2$) is referred to as body mass index (BMI). It provides a reasonable indication of the nutritional status of adults. The BMI has good correlation with fitness. It may also be used as an indicator of health risk. Body mass index of subject was determined by using formula -

\[ \text{BMI} = \frac{\text{Weight in kg}}{\text{height in m}^2} \]

Waist to Hip Ratio:- The predominant distribution of fat in an obese person, whether in upper part or the lower part of the body may determine the disease pattern, but with upper body obesity the ratio is 0.85 in women and greater than 1.0 in males. Abdominal obesity does not always go hand with overweight or obesity.

\[ \text{The normal of ratio} \quad \frac{\text{Waist}}{\text{Hip}} = 0.7 \]

Clinical assessment

Clinical examination is the most essential part of all nutritional surveys, since the ultimate objective is to assess levels of health of individuals and population groups as influenced by the diet they consume. The assessments of clinical manifestations were done as per ICMR Clinical Examination Performa. (Appendix 6)

Biochemical Method

Blood Hemoglobin level, serum calcium and Lipid Profile were estimated biochemically by following procedures:

Estimation of Hemoglobin

   Sahli’s Method

Apparatus

   It consists of a square tube with markings of Hb% on one side and percentage on the other side; 100%=14.5gm%Comparator has a flat surface
Methodology

of a standard brown colour. Hb pipette has mark of 0.02ml (20cumm). A dropper is used to put N/10 HCl and distilled water into the square tube. Hb meter with square tube flat comparator surface is preferable to round tube.

Principle

Blood is added to N/10 HCl, which converts Hb into acid Hematin. Brown colour of acid hematin is matched against the brown colour of the comparator.

Technique

- Add N/10 HCl with a dropper into the Hb meter tube up to the mark 3gm%.
- Fill the Hb pipette with 0.02ml of blood and wipe off the excess blood at nozzle of the pipette with moistened cotton. Blood may be taken from an EDTA vial or from a finger prick.
- Blow off the blood in the pipette into the Hb meter tube. Rinse the pipette by drawing in and discharging the blood-acid solution at least twice.
- Withdraw the pipette from the solution and rinse it with 2-3 drops of HCl so that Hb acid solution sticking to the pipette goes into the tube. Withdraw the pipette. Allow the acid to act on the RBC’s for 10 minutes to eyes the red cells and convert Hb to acid hematin.
- Mix acid hematin with a glass rod provided with the Hb meter set.
- Match colour of the solution with that of the comparator in the natural light. If it is draker, add distilled water with a dropper and stir the solution with a glass rod. This process is continued till the colour of the solution matches the comparator colour.

Take out the stirrer from the solution but keep it in the upper inside of the tube and take the reading from the upper meniscus in gm%.
Methodology

Blood Hemoglobin

Requirements

1. **Drabkin's reagent**
   - It contains in 1000 ml of distilled water.
     (a) potassium ferricyanide : 400 mg
     (b) potassium dihydrogen phosphate : 250 mg
     (c) Postassium cyanide : 100 mg
     (d) Nonidet : 1 ml
   - This reagent is stable in polythene contains at 2.80 c.

2. Cyanmet hemoglobin (HICN) standard (Hb standard) is commercially available. This standard is directly pipette in a curette and optical density is measured at 540 nm (green filter): The reading obtained, correspondence to 15 g/dl. Hemoglobin (the international committee for standardization in hematology has defined specification on the basis of a molecular weight of 64458 and a millmolar coefficient extinction of 44.0 solution of HICN contain 550, 850 mg. Hb per liter and the exact concentrations indicated on the label.

3. Hb – pipette. (20µl calibrated)
4. Test tube (15 x 125 mm)
5. Photometer or spectrophotometer

Procedure

Pipette in the tubes labeled as follows:-

**Table 3.3**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Drabkin's reagent, ml</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>2. Blood, ml</td>
<td>0.02</td>
<td>--</td>
</tr>
</tbody>
</table>
Methodology

Mix the contents in the tube labeled as test thoroughly and wait for 5 minutes. (1) Read absorbance of test by setting blank to 100% T at 540 nm (green filter). If reading of blank is equal to distilled water, it is not necessary to keep a blank. (2) Read a absorbance of standard (15 g/dl) by pipetting it directly in a cuvette.

Calculations

\[
\text{Hemoglobin (g/dl)} = \frac{\text{O.D. TEST} \times 15}{\text{O.D. STD}}
\]

Requirements

1. Drabnkin's reagent
2. Cyanmet hemoglobin (Hb standard) standard : 15g/dl (O.D. of this standard at 540 nm (green filter) corresponds to 15g/dl. hemoglobin.

Pipette in the tubes labeled as follows:-

<table>
<thead>
<tr>
<th>Table 3.4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagent contents of blank and standard solution</strong></td>
</tr>
<tr>
<td><strong>Reagents</strong></td>
</tr>
<tr>
<td>1. Drabnkin's reagent, ml</td>
</tr>
<tr>
<td>2. Hb Standard ,ml</td>
</tr>
</tbody>
</table>

Mix well and read intensities of these standards by setting blank to 100% T at 540 nm (green filter). Prepare a green by plotting O.D. readings on Y-axis and concentrations of hemoglobin standars,i.e.5.0g,10.0g and 15.0g on X-axis.

Precautions

1. The reagent is poisonous, handle it carefully.
2. Mix anticoagulant blood by swirling properly before pipetting Adjust carefully the blood column up to the graduations mark and use dry cotton to wipe excess blood on the pipette.
Methodology

3. Do not discard Drabkin’s reagent in the sink. Poisonous cyanide (HCN) gas is released if the sink has an acidic solution. Flush the sink with water and then discard the Drabkin’s reagent and continue to flush water for some time.

4. If capillary bloods is used, keep the Drabkin’s reagent ready in a test tube. Collect the free flowing blood in to Hb-pipette (Sahli pipette) wipe the excess blood and dispense in the reagent. Mix immediately to avoid clotting of the blood.

5. Turbidity may develop due to HbS or HbC or due to lipaemic blood. In that cases if it is due to abnormal hemoglobin add 0.1 g of potassium carbonate, centrifuge and read absorbance of the supernatant solution. In the case of a lipaemic blood specimen use 20 µl of serum or plasma, mix with 5ml of Drabkin’s reagent and use it as a blank. Read test reading against this blank.

ESTIMATION OF SERUM CALCIUM

Principle

Calcium ions present in sample reacts with ortho corsolphaltein complex one in alkaline medium in presence of 8-hydroxy quino line and forms a violet coloured complex, the intensity of which is measured at 578 nm (530-590 nm)

Reaction

\[ \text{OCPC} + \text{Ca} \xrightarrow{\text{Alkaline medium}} \text{Violet colour complex} \]

Reagent

Reagent 1: Calcium Reagent
Reagent 2: DEA Buffer
Reagent 3: Calcium Standard, 10 mg/dl
Methodology

Material Required

Clean 2 dry glassware laboratory glass pipettes or micropipettes 2 compatible micro tips.

Bio-chemistry analyzer.

Procedure

1. Bring all the assay reagents to room temperature.

2. Working Assay

<table>
<thead>
<tr>
<th>Table 3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagent contents of blank and standard solution</strong></td>
</tr>
<tr>
<td><strong>Reagent</strong></td>
</tr>
<tr>
<td>Working reagent</td>
</tr>
<tr>
<td>Standard</td>
</tr>
<tr>
<td>Sample</td>
</tr>
</tbody>
</table>

Mix well and incubate for 5 minute at room temperature.

Measure the absorbance of the standard and sample against the reagent blank at 578 nm (530-590 nm) within 15 minutes.

The final colour is stable for at least 15 minute.

Calculation

\[
\text{Calcium conc. (mg/dl) = } \frac{OD (sample)}{OD (standard)} \times 10
\]

Limitation and Precautions

1. Storage condition mentioned on the kit must be adhere.

2. Do not freeze or expose the reagent to higher temperature as it may affect the performance of the kit.

3. Before assay bring all the reagents to room temperature.

4. Use clean glassware free from dust or debries.

5. If the reagent is hazy or cloudy do not use it.
**Methodology**

**ESTIMATION OF SERUM CHOLESTEROL**

Serum Cholesterol was estimated by the CHOD-PAP method.

**Principle**

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent oxidation by cholesterol oxidase $\text{H}_2\text{O}_2$ is liberated. The colorimetric indicator quinoneimine is generated from 4-aminoantipyrine and phenol by $\text{H}_2\text{O}_2$ under the catalytic action of peroxidase.

\[
\text{CHE} \\
\text{Cholesterol ester} + \text{H}_2\text{O} \rightarrow \text{cholesterol} + \text{Fatty acid}
\]

\[
\text{CHO} \\
\text{Cholesterol} + \text{O}_2 \rightarrow \text{Cholesterol-3-one} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \rightarrow \text{Quinoneimine} + 4\text{H}_2\text{O}
\]

**Sample**

Serum, or EDTA plasma

The stability in serum or plasma is 7 days at 2-8°C.

**Assay Parameters**

- Analysis Mode: mono.endpoint
- Reading: bichromatic
- Sample volume (µl): 3
- Reagent volume (µl): 300
- Delay time (Reading 1): ----
- Kinetic interval (Reading 2): 300 sec
- Wavelength 1 (nm): 505 nm
- Wavelength 2 (nm): 670 nm
- Linearity limit: 1000 mg/dl
**Methodology**

**Procedure**

<table>
<thead>
<tr>
<th>Table 3.6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Reagents Serum Cholesterol</strong></td>
</tr>
<tr>
<td>Sample or standard</td>
</tr>
<tr>
<td>Mono reagent</td>
</tr>
</tbody>
</table>

Mix, incubate for 15 sec. at 37º C and read absorbance A1 exactly after 300 sec.

**Calculations**

\[
\text{Cholesterol (mg/dl)} = \frac{A \text{ of Sample}}{A \text{ of Standard}} \times \text{conc. of standard (mg/dl)}
\]

**ESTIMATION OF SERUM TRIGLYCERIDES**

Serum triglycerides were estimated according to the GPO – POD method.

**Principle**

Determination of triglycerides involves enzymatic splitting with lipoprotein lipase; indicator is quinoneimine, which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxides.

\[
\text{Lipase} \\
\text{Triglycerides} + H_2O_2 \rightarrow \text{Glycerol + fatty acids}
\]

\[
\text{GK} \\
\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol 3-phosphate}
\]

\[
\text{GPO} \\
\text{Glycerol 3-phosphate} + \text{O}_2 \rightarrow \text{Dihydroxyacetone phosphate + H}_2\text{O}_2
\]

\[
\text{POD} \\
2 \text{H}_2\text{O}_2 + \text{Aminoantipyrine + 4 Chlorophenol} \rightarrow \text{Quinoneimine} + 4\text{H}_2\text{O}
\]
Methodology

Sample
Serum or EDTA plasma
The stability in serum or plasma is 7 days at 2-8°C.

Assay Parameters

- Analysis Mode: endpoint
- Reading: bichromatic
- Sample volume (µl): 3
- Reagent volume (µl): 300
- Delay time (Reading 1): 300 sec
- Kinetic interval (Reading 2): ----
- Wavelength 1 (nm): 505nm
- Wavelength 2 (nm): 670nm
- Linearity limit: 600 mg/dl

Procedure

Table 3.7
Standard Reagents Serum Triglycerides

<table>
<thead>
<tr>
<th>Sample or standard</th>
<th>3µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono reagent</td>
<td>300 µl</td>
</tr>
</tbody>
</table>

Mix, incubate for 300 sec. at 37°C and read absorbance A.

Calculations

\[
\text{Triglycerides (mg/dl)} = \frac{A \text{ of Sample}}{A \text{ of Standard}} \times \text{conc. of standard (mg/dl)}
\]

ESTIMATION OF HDL CHOLESTEROL
Direct. Enzymatic – Liquid
**Methodology**

**Principle**

The method depends on the properties of a detergent which solubilizes only the HDL, so that the HDL-C is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoproteins LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to absorption of the detergents on their surfaces.

The intensity of the colour formed is proportional to the HDL-C concentration in the sample.

**CHE**

\[ \text{Cholesterol ester} + \text{H}_2\text{O} \rightarrow \text{cholesterol} + \text{Fatty acid} \]

**CHO**

\[ \text{Cholesterol} + \text{O}_2 \rightarrow \text{cholesterol-3-one} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{DSBmT} \rightarrow \text{Quinoneimine} + 4\text{H}_2\text{O} \]

**Sample**

Serum or EDTA plasma

The stability in serum or plasma is 7 days at 2-8°C.

**Assay Parameters**

- Analysis Mode: mono.fixed-time
- Reading: monochromatic
- Sample volume (µl): 7
- Reagent 1 volume (µl): 750
- Delay time (Reading 1): 5 min
- Reagent 2 volume (µl): 250
- Kinetic interval (Reading 2): 5 min
- Wavelength 1 (nm): 600
- Wavelength 2 (nm): ----
- Linearity limit (mg/dl): 200
Methodology

Procedure

Table 3.8
Standard Reagents HDL

<table>
<thead>
<tr>
<th>Sample or standard</th>
<th>7µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>750µl</td>
</tr>
<tr>
<td>Mix, incubate for 5 min at 37º C and read absorbance A1</td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>250 µl</td>
</tr>
</tbody>
</table>

Mix, incubate for 5 min at 37º C and read absorbance A₂. \( \Delta A = A₂ - A₁ \)

Calculation

Cholesterol (mg/dl) = \( \frac{\Delta A \text{ of Sample}}{\Delta A \text{ of Standard}} \times \text{conc. of standard (mg/dl)} \)

Calculation of LDL and VLDL By Friedwald Equation

VLDL (mg/dl) = Triglycerides / 5
LDL Cholesterol (mg/dl) = Total Cholesterol – (VLDL + HDL)

Specimen use 20µl of serum or plasma mix with 5ml of drabnkin’s reagent and use it as a blank. Read test reading against this blank.

Source: www.randox.com

STRESS TEST

In this age of industrialization and technological development, where the individual’s life is becoming complex and he or she has to face day to day problems, the concept of anxiety is considered to be an important factor in psychology. Psychologists are diverted their interest to measure this phenomenon.

In India, S.D. Kapoor has adapted Cattell’s I P A T Anxiety Scale Questionnaire in Hindi in Indian situations. In 1966, Durganand Sinha has
constructed and standardized a W-A Sinha Anxiety Scale to measure anxiety, one aspect of personality. It includes 100 items which manifest the individual’s anxiety in ten different areas. This scale was based on Taylor’s Manifest Anxiety scale and Cattell’s I P A T Anxiety Scale Questionnaire. A Hindi adaptation of Taylor’s Anxiety scale was developed in 1967 by B.N. Singh and R.C. Thukur. The adapted scale is standardized on 706 college student’s aging 16 to 19 years. In all the scales mentioned as above it is found that they are almost based on foreign tests or they are Indian adaptations. In the anxiety field, no original scale is developed in our country. Keeping this in view, the present authors took up the work of constructing the test.

The disagreement and confusion centering around the concept of anxiety during the past three decades led the person authors to develop a comprehensive test of anxiety covering a variety of anxiety indices proposed by different investigators from time to time. This scale has also found to be diagnostic of general neuroticism, besides of anxiety status.

To assess the stress level of the respondent (post menopausal women) sinha’s comprehensive anxiety test and stress inventory test (Dr. Hari’s Chandran) were used to determine, the stress level of post menopausal women. Some questions were taken from Hari’s stress inventory for purpose of this study. Analysis and scoring was done according to Sinha’s comprehensive anxiety test manual SCAT (Sinha’s comprehensive anxiety test) along with Dr. Hari’s Chandran stress inventory.

**Instruction and Administration**

1. It is a self administering inventory. It is administered in group as well as individual. The instructions printed on the test form should be read by test administrator and the testee.

2. No time limit is fixed for completing the test. However, usually an individual takes 15 to 20 minutes in completing the test form.
3. It should be emphasized that there is no right and wrong response to the statements. They are designed to study individual’s reactions to different situation.

4. It should be emphasized that each item has to be respond in either positive or negative i.e., Yes or No and that no statement is to be left out.

5. It is undesirable to tell the testee the aim for which the test is used.

**Scoring :-** The inventory can be scored accurately by hand and no scoring key or stencil is provided. For any response indicated as ‘Yes’ should be awarded the score of one and zero for ‘No’ response. The sum of all the positive or yes responses would be the total anxiety score of the individual.

<table>
<thead>
<tr>
<th>Percentiles</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>Extremely High Anxiety</td>
</tr>
<tr>
<td>95</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>High Anxiety</td>
</tr>
<tr>
<td>70</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Normal Anxiety Level</td>
</tr>
<tr>
<td>40</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Low Anxiety Level</td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Extremely Low Anxiety</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>
3.6 STATISTICAL ANALYSIS

The Data was entered into the computer database. The responses of frequencies were calculated and analyzed by using statistical software SPSS version 11.0. Prevalence of an outcome variable along with 95% confidence interval was calculated. The Hari’s stress inventory (2009) and Sinha’s comprehensive anxiety test (SCAT) was analyzed by categorization of the three levels (Low Anxiety, Normal Anxiety and High Anxiety) of stress as presence of stress. The descriptive statistics like mean and standard deviation for different study variables were calculated. Significance of difference in frequency distribution of studied sample have been found out using Chi square and difference in mean has been found out using ‘t’ test.