MATERIAL AND METHOD
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The case material for the present study consisted of group A, 20 healthy young male subjects of age group 20-30 years and group B, 20 healthy (late) middle aged subjects of age group 40-60 years.

Young male volunteers were randomly selected among the medical students of this college. Some of them doing medical studies and others had completed their M.B.B.S. course doing resident or house jobs. Detailed history taking, thorough clinical examination and relevant investigations (See appendix) done in each case with special emphasise in xanthoma and xantholasma Blood sugar, blood urea, urinary proteins, TLC, D.L.C., E.S.R., Hb%, total urinary proteins and lipoaemia retinalis in fundus. Those case who did not revealed any abnormality in history examination and investigation were labelled as healthy. Middle aged volunteers were also selected randomly among the IIIrd and IVth class employees of the M.L.B. Medical College, Hospital, Jhansi and few patients attendant resident residing nearby the campus. They were also evaluated on the same way to declare healthy.

A detailed dietary history was elicited to assess the amount type of fat consumed weekly by these subjects in their usual diet. Specific consideration
was given to record weekly consumed amount and its type, milk and milk products, egg, non vegetarian diet, cooking media and manner of use food additives. His usual diet was noted and amount of fat and cholesterol in. It was calculated giving the consideration to the total amount of fat used in family. The number of family members and approximate percentage total food, the very individual was consuming. Any recent changes in diet, oral or parenteral medication 7 days before and during the study were noted. Most of the subjects were non alcoholic. Only few were consuming alcohol that too was occasional, non had consumed alcohol during study. Smokers were allowed to smoke as they were smoking previously, brand and number of smoke (Biri, Ciggerette) noted.

Informed consent was taken from each subjects to study on him.

Design of test

The fat load was given in the form of 25 g Amul butter with 2 eggs in the form of amlet (20 g vanaspati) with 250 ml sweet milk and four slices of bread plus 1000 mg crystalline cholesterol dissolved in milk plus a cup of tea. This high cholesterol fat
breakfast (HCFB) was given in place routine breakfast, consisted of 4 slices of bread, 1 egg amlet plus a cup of tea. No untoward effect to this high fat test meal was observed in any of the subjects.

The subjects were asked to have their dinner at 8 P.M. on previous night and not to take any thing after this except water. Next morning after at 8 A.M. after 12 hours fasting blood samples were taken in recumbent posture without producing venous stasis (Koerselmar et al. 1961).

1st (basal sample) : After 1st sample collection subjects were given HCFB continuously for 7 days in place of routine breakfast. On 8th test day morning 12 hours fasting blood samples were collected and same HCFB was given 8th and last day (IIInd or after HCFB). The subjects were again started their usual breakfast and on 15th test day 12 hours fasting samples were taken (IIIrd or HCFB withdrawal).
Table 1m: Showing the general characteristics of the subjects.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Variants</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Medical Student</td>
<td>6</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>b. Junior Doctor</td>
<td>13</td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td>c. Technician</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>d. Shopkeeper</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>e. Sweeper</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>f. Manual worker</td>
<td>8</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>g. Lab. attendant/ward boy</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>h. Chowkidar</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td>2.</td>
<td><strong>Dietary Habits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Vegetarian</td>
<td>23</td>
<td>57.5</td>
</tr>
<tr>
<td></td>
<td>- Non Vegetarian</td>
<td>17</td>
<td>42.5</td>
</tr>
<tr>
<td>3.</td>
<td><strong>Fat Consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Low fat consumption (less than 40 gm visible fat/day)</td>
<td>19</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>- High fat consumption (40 or more than 40 gm visible fat/day)</td>
<td>21</td>
<td>52.5</td>
</tr>
<tr>
<td>4.</td>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Smoker (Regular and occasional)</td>
<td>22</td>
<td>55.0</td>
</tr>
<tr>
<td></td>
<td>- Non smoker (Never smoked)</td>
<td>18</td>
<td>45.0</td>
</tr>
<tr>
<td>5.</td>
<td><strong>Alcohol consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Alcohol (including occasional and habitual)</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>- Non alcoholic (never consumed)</td>
<td>25</td>
<td>62.5</td>
</tr>
</tbody>
</table>
Serum was separated from blood within 4 hours by the centrifuging the supernatant of samples and following tests were performed.

1. **Cholesterol estimation**

   **Principle** : (Henley, 1957)

   A calorimetric method, colour develops when ferric chloride and cholesterol mixed in presence of sulphuric acid. This method was less accurate than enzymatic cholesterol estimation. Reaction during the colour development was known by the name of scientist Diberman Burchard Reaction.

   Optical density of test and standard was measured at 575 mU against blank to set zero.

   **Calculation**

   \[
   \text{Serum total cholesterol in mg/dl} = \frac{T}{S} \times 250
   \]

   \[T = \text{Optical density of test.}\]

   \[S = \text{Optical density of standard.}\]

   **Precautions**

   a. Calorimetric measurement should be done immediately (within 30 minutes) of colour development.

   b. Glassware should be free from moisture and detergent.
2. **Total lipid estimation** (Phospho Vanillin method)

   **Principle**

   The lipids on heating with concentrated sulphuric acid react with phosphovanillin reagent and produce pink coloured complex which is measured colorimetrically.

   Optical density (O.D.) of standard (S) and test (T) were measured with green filter.

   **Calculation**

   \[
   \text{Serum total lipid in mg/dl} = \frac{T}{S} \times 100
   \]

   Where \( T \) is Optical density of test.

   and \( S \) is Optical density of standard.

   **Precautions**

   If optic density of test exceeds \( S \), repeat the estimation after diluting the sample with saline

3. **Serum H.D.L. Cholesterol estimation**

   **Principle**

   HDL cholesterol is separated in serum from the other lipoproteins by precipitation using a precipitating reagent. Precipitate contains LDL, VLDL and chylomicron which are removed by ultra centrifugation.

   Supernatant contains HDL cholesterol which is estimated by HDL cholesterol colour reagent which gives a purple coloured complex that measured colorimetrically at 560mm.
Calculations

S.H.D.L. cholesterol concentration in mg/dl = \[ \frac{T}{S} \times 50 \]

T stands for optic density of test and
S stands for optic density of standard.

Precautions

a. The reagent with the specimen added should not be left at room temperature for longer than 1 minute after mixing since reaction also take place at room temperature.

b. Organish purple colour developed when reagent II was added in serum due to presence of serum proteins in absence of serum proteins, as in case of cholesterol standard purple colour complex is not formed. This difference in colour does not cause any variation in values of S.HDL cholesterol.

4. **S. TG estimation** (Serum Triglyceride)

**Principle**

Triglyceride + KOH → Glycerol + fatty acids.

Glycerol + periodate → Formaldehyde

Formaldehyde + NH₄⁺ + acetyl acetone → Diacetyldihydrobutidine.

Colour developed in the final step was be cause of diacetyldihydrobutidine.

Optical density (O.D.) of test and standard was measured at 415 nm against blank to set zero.
Calculation

\[ S_{TC} \text{ in mg/dl} = \frac{T}{S} \times 200 \]

\( T \) = Optical Density of test.

\( S \) = Optical Density of standard.

If the value of \( S_{TC} \) was more than 250 mg/dl, the test was repeated after diluting the plasma 1:1 (with normal saline) and the result so obtained was multiplied by two hundred.

Precautions

a. To avoid losses due to evaporation aluminium tests were applied on tubes before placing them in 50-60°C water bath.

b. Detergent were avoided to clean the glass ware.

5. \textit{S. Phospholipid estimation}

(Cormerty, Briggs. and Eaton, 1961)

Principle

Phosphates are separated with the help of Trichloracetic acid. Phosphorous was measured from phosphates by complex chemical reaction and colour development.

Optical density of test (T) and standard (S) was measured at 700 mu (red filter) against blank to set zero.
Calculation

Phospholipid in gm/dl = \( \frac{T}{S} \times 10 \times 25 \)
Phosphates in mg/dl = \( \frac{T}{S} \times 10 \)

Normal range of phosphate = 6-11 mg/dl
Normal range of phospholipids = 150-275 mg/dl

T = Optical density of test.
S = Optical density of standard.

Precautions

There should not be blood in serum otherwise value of S. Phospholipid will be higher because of higher cellular count.

6. S.LDL cholesterol estimation

Previously ultra centrifugation was necessary to obtain the isolated LDL fraction upon which to perform the assay. More recently establishment and validation of formula given by Friedwald and associates, 1972 has lead to the use of calculated LDL cholesterol. It is based on formula that VLDL cholesterol is represented by the total S. Triglyceride divided by five (it is valid till TG values are less than 400 mg%).

Calculation

S.LDL cholesterol = STC - (TG/5+HDL-C) mg%

by above mentioned formula we can calculate the cholesterol fraction of LDL which is almost equal to ultra centrifuge method.