MATERIAL AND METHODS
MATERIAL AND METHODS:

The present study was conducted on 22 subjects. All the subjects were young healthy medical students of M.L.B. Medical college Jhansi aged 19-28 yrs. Subjects were included in the study irrespective of their initial serum cholesterol level. Out of 22 subjects only 5 subjects were females. Detailed history was taken from all the subjects. No one was suffering from any chronic illness. Personal history revealed that all was doing moderate exercise. Only 3 subjects were smokers. All the subjects were taking their meals from hostel mess and were taking almost the same type of meals. Family history revealed a positive history of diabetes mellitus in 3, hypertension in 4 and IHD in 5 subjects where one or both the parents were suffering from the above mentioned disease.

An informed consent was taken from all the subjects included in the study. A thorough clinical check up and routine investigations done. Height, weight, and B.P. of all the subjects was recorded.

DESIGN OF TEST:

All the subjects were asked to take their dinner at around 7.00 PM on the day before study and then they were asked not to take anything in the night except water. No smoking was allowed during the test. In the morning (day 1) fasting sample was collected at about 9.00 A.M. Subjects were asked to take high cholesterol fat diet (2 boiled eggs and 250 ml of sweetened milk) in place of their usual breakfast. This provided approximately
650 mg of egg yolk cholesterol. Thereafter blood samples were collected at 1 hr. and 3 hr. interval.

The same protocol was followed and patients were given Semfibrozil (2 capsules of 600 mg.) along with high cholesterol fat diet after one week i.e. on 'day 7'. Fasting and postprandial samples were collected on day 7 also as were on day 1.

Serum was separated from the blood samples within 4 hr. of collection and following tests were performed.

1. SERUM TOTAL CHOLESTEROL (STC):

The estimation was done by one step method utilizing the kit provided by Ortho Diagnostic system, Ethnor Division.

PROCEDURE:

Three test tubes are taken and labelled as Test (T), Standard (S) and Blank (B) and then:

<table>
<thead>
<tr>
<th>Test (T)</th>
<th>Standard (S)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ortho cholesterol reagent</td>
<td>4 ml</td>
<td>4 ml</td>
</tr>
<tr>
<td>Serum</td>
<td>20 ul</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol standard (250 mg%)</td>
<td>-</td>
<td>20 ul</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix contents of each test tube simultaneously for 10 seconds and immediately place them to a boiling water bath for exactly 45 seconds followed by cooling with running tap water or cold water for 5 minutes. Dry the exterior of tube. mix their contents.

Measure optical density (OD) of each solution at 560 nm (range 560 to 600 nm). Set blank at colorimetric zero and calculation is done as:
Cholesterol concentration of test samples (mg%) = \( \frac{OD (I)}{PF (B)} \times 250 \) (Cholesterol mg/dl or mg%/38.7 = mmol/l).
(Range of normal expected values = 150-250 mg%).

2. SERUM TRIGLYCERIDES (STG):

It was estimated by using GPO-PAP method for quantitative determination (Enzymatic method) of STG.

PROCEDURE:

The reagents in kit are:
- 4 vial of reagent I (Lyophilised enzymes)
- 1 Vial reagent II (phenol solution)
- 1 Vial of triglyceride standard (300 mg%)

Reconstitute reagent I in 2.5 ml of distilled water, while reagent II and triglyceride standard are supplied ready to use.

<table>
<thead>
<tr>
<th></th>
<th>Test (I)</th>
<th>Standard (S)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent I</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Reagent II</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>

Mix well and add

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>20 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triglyceride standard (300 mg/dl)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>20 ul</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix and incubate in water bath at 37°C for 10 minutes.

Add distilled water & mix 2 ml 2 ml 2 ml
Take reading by measuring optical density (OD) of each solution at 500 nm (500-530 nm). Set blank at calorimetric zero. Calculation of STG is done as:

\[ \text{STG in test sample (mg%) = } \frac{\text{OD (T)}}{\text{OD (S)}} \times 300 \]

(for conversion m mol/l = mg/dl X 0.0114)

(Normal expected values = 30-150 mg%).

This test assay has validity for values upto 500 mg% only. If higher values expected then dilute serum suitably with distilled water and multiply value by dilution factor.

3. **SERUM HIGH DENSITY LIPOPROTEINS (HDL):**

It was estimated by kit provided by Ortho Diagnostic System, Ethnor Division.

**PROCEDURE:**

Two phases of test are involved:

**Phase I:** Lipogent TM agent (prepared solution for ready use) is used with serum to precipitate LDL and VLDL so that only HDL fraction remains.

**Phase II:** The supernatant of the above centrifuged solution is proceeded on with cholzyme-M kit for estimating HDL.

**PHASE I**

Mix 0.5 ml of lipogent TM with 0.5 ml of test serum. Keep at room temperature for 10 minutes and then centrifuge at 2000 rpm for 20 minutes to obtain a clear supernatant.
PHASE II

Dilute one part of cholzyme M standard (200 mg%) with seven parts of distilled water = Cholzyme TM working reagent.

<table>
<thead>
<tr>
<th>Test (T)</th>
<th>Standard (S)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholzyme TM working reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Supernatant</td>
<td>100 ul</td>
<td>-</td>
</tr>
<tr>
<td>Working standard</td>
<td>-</td>
<td>100 ul</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix and incubate in water bath at 37 °C for 15 minutes.

Add distilled water | 4 ml | 4 ml | 4 ml |

Mix and read optical density (OD) by 515 nm
(Range 500 - 530 nm) within 60 minutes.

Calculation is done as:

\[
\text{HDL concentration in test serum (mg%)} = \frac{\text{OD (T)}}{\text{OD (S)}} \times 50
\]

(Conversion into SI unit m mol/l = mg% / 38.76)
(Range of normal expected values = 30 - 60 mg%).

4. SERUM VERY LOW DENSITY LIPOPROTEINS (VLDL):

It was calculated by using formula given by Friedwald et al (1972):

\[
\text{VLDL (mg%)} = \frac{\text{STG}}{5} \quad \text{(This formula is valid only upto STG value / 600 mg%).}
\]

5. SERUM LOW DENSITY LIPOPROTEINS (LDL):

It was also calculated by the formula given by Fredrickson DS (1972):

\[
\text{LDL (mg%)} = \text{STC} - \left(\frac{\text{STG}}{5} + \text{HDL}\right)
\]

\[
= \text{STC} - (\text{VLDL} + \text{HDL})
\]