MATERIAL AND METHOD
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Present study consisted of 32 healthy subjects their age ranged from 20-40 years with mean age of 29.62 ± 6.11 years. These all subject were selected from admitted patients in wards of MLB Medical college hospital Jhansi and few of them were patient’s attendant

Subjects were divided in two groups on the basis of age and sex. The age range were 22-40 year with Mean age 28.76 ± 5.85 years of 17th male subjects and 15th female age range 20-40 years with Mean age 30.6 ± 6.44 years.

Informed consent taken from each subject. A detailed history, thorough clinical Examination and relevant investigations e.g. Blood sugar, Bl urea, TLC, DLC, Hb% ESR and urine protien were done. All subjects were enquired about their dietary habit, socioeconomic status. Use of smoking, tobacco chewing and Alcohol Consumption were not allowed during whole period of study.

Detailed family history was enquired in every subjects regarding Diabetes Mellitus, Inschaemic heart desese : hypertension, obesity etc.

Design (Protocol) of Test

The subject were asked to take their dinner at 8. Pm. on Previous night and not to take anything except water after this, next morning fasting blood samples (2 ml) was taken serum was separated from blood within 4 hours by centrifugation & following test were performed
**Mode of estimation**

(1) Serum total cholesterol (STC) :-

STC estimation was done by kit: with the help of one step method supplied by ethnor India limited.

Procedure :-

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

<table>
<thead>
<tr>
<th></th>
<th>Test (T)</th>
<th>STD (S)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho cholesterol reagent</td>
<td>4 ml</td>
<td>4 ml</td>
<td>4 ml</td>
</tr>
<tr>
<td>Seum</td>
<td>29 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol standard (250 mg%)</td>
<td>-</td>
<td>20 ul</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>20 ul</td>
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</table>

Mix contents of each test tube simultaneously for 10 seconds and immediately place them in 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 calorimetric zero and calculation was done as
Cholesterol concentration of test samples (mg%) = \frac{\text{OD (T)}}{\text{OD (S)}} \times 250

(Cholesterol mg/dl or mg% / 38.7 = mmol/l).

Normal expected values of Cholesterol = < 200 mg/dl.