MATERIAL AND METHODS
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The case material for study consisted of healthy children of both sex of different age groups and cord blood of newborn. Informed consent will be taken from the parents of each and every subjects. Detailed history and meticulous clinical examination and investigation will be done to find out any pathological condition. Subjects having pathological condition like pregnancy induced hypertension, Eclampsia, Diabetes mellitus, liver and renal diseases in mother and any systemic illness in children will be excluded from the study. The umbilical cord blood sample is collected from the neonates preferably in Obstetrics & Gynaecological department, M.L.B. Medical College, Jhansi. 4-5 ml of blood will be collected from children of different age groups preferably 8-10 hours fasting. The lipoprotein estimation was done at Lipid Research Laboratory, attached to the Department of Medicine. The following tests will be performed by Standard kits:

1. STC (Serum Total Cholesterol),
2. STG (Serum Triglycerides),
3. HDL-C (High Density Lipoproteins).

The serum total cholesterol, serum triglyceride and high-density lipoprotein were estimated by standard method, but very low-density lipoprotein (VLDL) and low density
lipoprotein (LDL-C) were calculated by the following formula given by Friedwald et al (1972) and Friedrickson D.S. (1972) respectively.

\[ VLDL \text{ (mg/dl)} = \frac{STG}{5} \text{ (valid till STC value are below 400 mg/dl)} \]

\[ LDL-C \text{ (mg/dl)} = STC - (STG/5 + HDL) \]

\[ = STC - (VLDL + HDL) \]

Statistical analysis was done by using Student 't'-test.

The children of different ages are grouped into five groups and umbilical cord blood of newborns also. They are -

- **Group A** (umbilical cord blood of newborn),
- **Group B** (0-1 year age),
- **Group C** (2-5 year age),
- **Group D** (6-9 year age),
- **Group E** (9-12 year age),
- **Group F** (13-16 year age).

These groups are further divided into sub-groups, according to gender and socio-economic status.

- **M** = Male,
- **F** = Female,
- **LSES** = Low socio-economic status,
- **MSES** = Middle socio-economic status.

The classification of socio-economic status was given by many authors like - Kuppuswamy, S.N. Prasad etc.
Now-a-days, modified S.N. Prasad Classification is used. It is also called R.N. Srivastava Classification. The classification was based on income per month (in Rupees).

<table>
<thead>
<tr>
<th>Social Class</th>
<th>Income per month (in rupees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>600 and above</td>
</tr>
<tr>
<td>II</td>
<td>300 - 599</td>
</tr>
<tr>
<td>III</td>
<td>140 - 299</td>
</tr>
<tr>
<td>IV</td>
<td>60 - 139</td>
</tr>
<tr>
<td>V</td>
<td>60 and below</td>
</tr>
</tbody>
</table>

So, I & II class included in Upper socio-economic status and III class being to middle socio-economic status and IV & V class belong to low socio-economic status.

**Principle and Procedure of Cholesterol estimation**

**Principle**

\[ \text{Cholesterol Esters} \xrightarrow{\text{Esterase}} \text{Cholesterol} + \text{Fatty acids}, \]

\[ \text{Cholesterol} + \text{O}_2 \xrightarrow{\text{oxidase}} \text{Cholesterol-3-one} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4-\text{amino-antipyrine} + \text{pHES} \xrightarrow{\text{Peroxidase}} \]

\[ \text{Quinoneimine (Red dye)} + 2\text{H}_2\text{O}. \]

The intensity of red colour produced is directly proportional to the total serum cholesterol is the same when read at 520 nm.
**Procedure**

- Prepare reagent according to instructions.
- Label tubes: Blank (B), Standard (S), Test (T).
- Pipette - 1.0 ml of reagent of all tubes and pre-warm at 37\(^{\circ}\)C for at least five minutes.
- Add 0.01 ml (10 ul) of sample to respective tube, mix and return to 37\(^{\circ}\)C.
- Incubate all the tube at 37\(^{\circ}\)C for five minutes.
- Zero spectrophotometer with the reagent blank at 520 nm.
- Read and record absorbance of all vial.

**Calculation** - Total cholesterol (mg/dl) = \( \frac{\text{Test}}{\text{Standard}} \times 200 \)

**Principle and Procedure of Triglyceride estimation**:

**Principle** -

Serum triglycerides are hydrolysed to glycerol and free fatty acids by lipase. In presence of ATP and glycerokinase, the glycerol is converted to glycerol-3-Phosphate oxidase to yield hydrogen peroxide. Hydrogen peroxide reacts in the presence of peroxidase with ESPAS (N-ethyl-N-Sulfopropyl-m-anisidine) and 4-amino antipyrine to form a coloured complex. The intensity of the colour developed is proportional to triglyceride concentration and is measured photometrically at 546 (530 to 570 nm) or with green filter.
Triglyceride + H₂O \( \xrightarrow{\text{Lipoprotein lipase}} \) \( \text{Glycerol + fatty acids.} \)

Glycerol + ATP \( \xrightarrow{\text{Glycerokinase}} \) \( \text{Glycerol-3-Phosphate + ATP.} \)

Glycerol-3-Phosphate + O₂ \( \xrightarrow{\text{Oxidase}} \) \( \text{Glycerol-3-Phosphate + H₂O₂} \)
\( \xrightarrow{\text{Dihydroxyacetate Phosphatase.}} \)

H₂O₂ + Amino-antipyrine + ESPAS \( \xrightarrow{\text{Peroxidase}} \) \( \text{Oinocidine + H₂O.} \)

Procedure -

There are three methods. Only one which is done in our lipid research laboratory by colorimeter and spectrophotometers.

<table>
<thead>
<tr>
<th></th>
<th>Blank (B)</th>
<th>Standard (S)</th>
<th>Test (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogen Reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.02 ml</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>0.02 ml</td>
</tr>
</tbody>
</table>

Mix well and incubate at 37°C for 10 min. or at room temperature to 30°C for 20 min. After that add 2 ml of distilled water into each tube (B), (S), (T).

Calculation - Triglyceride concentration (mg/dl)

\[ \text{Calculation} = \frac{\text{AT} - \text{AB}}{\text{AB} - \text{AB}} \times 200 \]
Principle and Procedure of HDL-C Estimation:

Principle -

Chylomicron, VLDL (very low-density lipoprotein) and LDL-C (low density lipoprotein) of serum are precipitated using buffered polyethylene glycol (PEG 6000). After centrifugation, high density lipoprotein (HDL) remain in supernatant. The cholesterol in HDL fraction is then estimated by an enzymetric method using cholesterol esterase, cholesterol oxidase, peroxidase, 4-amino-antipyrine and phenol.

Procedure -

Step I : HDL separation -

Add 0.3 ml (sample), one precipitating reagent (0.3 ml) then mix, keep at room temperature and then centrifuge at 4000 rpm for 10 min. or 2000 rpm for 20 min. to obtain a clear supernatant.

Step II : HDL-C Estimation -

<table>
<thead>
<tr>
<th></th>
<th>Blank (B)</th>
<th>Standard (S)</th>
<th>Test (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol working Reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>1.0 ml</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>
Mix and incubated at 37°C for 10 min. or at room
temperature (25°C ± 2°C) for 20 min. Mix and read AT, AS
and AB at 500 nm (490 – 550 nm) or with Green filter.

**Calculation** -

$$\text{HDL-C (mg/dl)} = \frac{\text{AT} - \text{AB}}{\text{AS} - \text{AB}} \times 50 \times 2 \text{ (dilution factor of sample)}$$

To convert mg/dl into m mol/L, there are certain
formula given as -

$$\text{HDL-C (mg/dl) is converted by m mol/L}
= \text{mg/dl} \times 0.02586$$

**LDL-C and serum total cholesterol (mg/dl)
is converted by m mol/L = mg/dl \times 0.02526**

**Serum Triglyceride (mg/dl) is converted by m mol/L = mg/dl \times 0.01129.**