4.1 Introduction

Occupational exposure to unburnt tobacco may occur during beedi making by women folk who are largely involved. Their ill health is due to the contact and inhalation of raw tobacco dust and volatile components. The health hazards which the women employees who are rolling beedis encounter are enormous. Biomonitoring of these women workers and health assessment is major hurdle in the improvement of their wellbeing. So far no epidemiological studies on tobacco related health risks in these workers have been carried out. Very few reports are available in literature based on the responses revealed by the volunteers. Tobacco related health hazards are mainly attributed to the various constituents such as nicotine cotinine and nitrosamines. These constituents may have diverse effects on the physiology of humans owing to its toxic nature. Contact and inhalation of raw tobacco dust may have systemic effect and lead to many disorders including hormone defects. According to Gupta (2001) smokeless tobacco poses a major risk to women and to the children born to them. The next phase of study aims to explore the health hazards experienced by women beedi rollers in Melapalyam based on the blood parameters and clinical findings of the volunteers. Since blood parameters serves as very good indices in assessment of health profile, steps were undertaken in the present study to evaluate the health status of women beedi rollers. Routine analysis of blood like TEC, TLC, Platelet count, haemoglobin count,
erythrocyte sedimentation rate and specific tests analyzing the trace elements were enumerated. Liver function tests which may be an indicator of the detoxification mechanism of toxic chemicals were also carried out. A special emphasis to the thyroid profile of the women beedi rollers were analysed in order to evaluate the possible thyroid dysfunction, prevalent in women subjects. Hence, this phase of work will deal with the health assessment of women beedi rollers apart from their occupational hazards which are symptomatic.

4.2 Materials and methods

4.2.1 Enumeration of total Total Erythrocyte Count

Materials

- Haemocytometer, Haeyem’s fluid, spirit, pin. Haemocytometer contain Naubauer’s counting chamber, TEC pipette and TLC pipette.

Composition of TEC fluid

1. Trisodium citrate - 3.13g
2. Commercial alchohol - 1 ml
3. Distilled water - 100 ml

Procedure

1. Blood is taken up to 0.5 mark, in the R.B.C.
2. Pipette and diluting fluid up to 101 mark.
3. It is mixed well and kept for 3 minutes.
4. 2-3 drops of fluid is discarded, from the pipette, which does not contain any cells.
5. The counting chamber is charged after 3 minutes. (to allow the cells to settle).

6. The RBC in the squire are ‘R’ in figure under high power. R.B.C. count is then calculated.

**Calculation**

\[
\text{TLC} = \frac{\text{NO. OF CELLS COUNTED} \times 10 \times 20}{4}
\]

**4.2.2 Enumeration of total count of Total Leucocyte Count**

**Materials**

Naubauer’s double counting chamber, TLC pipette, TLC diluting fluid, spirit, needle and microscope.

**Reagent Preparation**

TLC diluting fluid composition glacial acetic acid 2 cc gentian violet 1 cc, distilled water 100 cc.

**Procedure**

1. Clean the finger tip with sprit and make a deep prick. Such blood upto 0.5 marks. then such diluting fluid up to the mark 11.

2. Mix the blood thoroughly by rolling pipette horizontally in the palms.

3. Discard 2-3 drops, clean the counting chamber and fill the chamber with prepared solution.

4. Air bubbler should avoided, focus the field under low power, objective and count the TLC in all the corner squares.
5. So that the TLC in 64 squares are counted.

**Calculation**

\[
TLC = \frac{\text{NO. OF CELLS COUNTED} \times 10 \times 20}{4}
\]

**4.2.3 Estimation of Platelets and estimation of Erythrocyte Sedimentation Ratio**

**Method**

Westergrein’s method

**Procedure**

Blood is collected and mixed with anti coagulant in the ratio 1:4. The temperature should be 25-28°C. Blood is drawn up to the mark 0. The tube is kept exactly vertical for 1 hour. The height of plasma column after one hour is noticed.

**4.2.4 Estimation of Haemoglobin**

**Method**

Sahli’s method

**Materials**

By using pipette add 1ml in the haemoglobinometer up to the lowest marking. Drop blood up to 20ul in the sahli’s pipette. Adjust the blood column carefully without bubbles. Wipe excess of blood on the sides of the pipette by using a dry piece of cotton blow the blood into the acid solution in the graduated tube, rinse the pipette well. Mix the reaction mixtures and allow the mixture to stand at room temperature of 10 minutes.
Dilute the solution with distilled water by adding few drop of water carefully and mixing the reaction mixture until the color matches the color in the comparator. The lower meniscus of the fluid is noted and reading is noted.

### 4.2.5 Estimation of Packed Cell Volume

**Materials**

Wintrob’s haematocrit tube filter, oxalated venous blood. Wintrob’s haematocrit tube is a glass tube having 110 mm length and 3mm diameter, closed at one end. It is graduated 0-100cm at one side and 100-0 on another side.

**Procedure**

In Wintrob’s haematocrit tube, oxalated venous blood is taken without air bubbler. It is closed with cotton and kept in centrifuge, and rotated at 3000 rev/min for half an hour. Take out the test tube and not the blood celles packed at the bottom and buffy coat above it. Above the buffy coat, clean plasma is seen. The packed cell volume can be directly read out from the haematocrit tube normally buffy coat is of 0.5 – 1cm thickness. Wintrob’s haematocrit tube is also used for determination of ESR.

### 4.2.6 Determination of Serum Sodium and Potassium

**Method**

Flame photometry

**Specimen**

Serum or heparinized plasma.

**Requirements**

1. Test tubes (15 x 125 mm)
2. Dispenser or 10 ml volumetric pipette
3. 10 ml beakers or bulbs
4. 50 or 100 micro-liter push button pipette.
5. Flame photometer.

Mixed standards are prepared by using following two stock standards

1. Stock standard for sodium : 1000 mEq/l: It is prepared by dissolving 5.85 g of analar garde sodium chloride in glass distilled water and diluted to 100ml by using a volumetric flask.

2. Stock standard for potassium : 100 mEq/l: It is prepared by dissolving 0.740 g of potassium chloride (AR) in glass distilled water and diluted to 100ml by using a volumetric flask.

Mixed working standards are prepared as follows:

1. Sodium/potassium : 120/2.0 mEq/l: It contains 120mEq of sodium and 2.0 mEq of potassium per liter of distilled water. It is prepared by mixing 12ml of stock standard1 and 2.0ml of stock standard 2, in 86 ml of glass distilled water.

2. Sodium/potassium : 140/4.0 mEq/l: It is prepared by mixing 14 ml of stock standard I and 4.0 ml of stock standard. 2, in 82 ml of glass distilled water.

3. Sodium/potassium : 160/6.0 mEq/l: It is prepared by mixing 16 ml of stock standard 1 and 6.0 ml of stock standard 2, in 78ml of distilled water.
Procedure

Pipette in the tubes labeled as follows

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std :1</th>
<th>Std :2</th>
<th>Std :3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Glass distilled water, ml</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2. Serum or plasma, ml (heparinized)</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. Std : 120/2.0 ml</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. Std : 140/4.0 ml</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>5. Std : 160/60, ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Mix and transfer to beakers or bulbs for the flame photometric determination

Operation of a flame photometer

1. Put on the main switch.

2. Put on air compressor and adjust the required air pressure, by adjusting the knob meant for air.

3. Introduce glass distilled water, through automiser.

4. Put on gas and control the flame by adjusting the knob meant for gas. (It is adjusted till the flame is divided into five sharp cones)

5. Adjust the proper filters for the simultaneous determination of sodium and potassium (digital display)

6. Make zero adjustment by introducing distilled water.

7. Introduce the standard 120/2.0 and by using the knob meant for sodium the digits 120.0 and by using the knob meant for potassium the digits 2.0 are adjusted.
8. Introduce standard, 140/4.0 If the standards are accurately prepared the digital display will indicate exact concentration for both sodium and potassium.


10. Now introduce the test and record the readings for sodium and potassium.

4.2.7 Determination of Chlorides

Method

Schales and Schales.

Requirements

1. Test tubes: 15 x 125 mm
2. 0.2 ml and 2.0 ml graduated pipettes.

Reagents

1. Mercuric nitrate reagent: Dissolve 2.9-3.0 g of mercuric nitrate in about 800 ml of distilled water, add 20 ml of 2N nitric acid and make up to one liter. It is stable at room temperature in an amber colored bottle.

2. Diphenyl carbazole indicator: 100 mg/dl in 95% (v/v) ethanol. It is stable in an amber colored bottle at 2-8°C.

3. Chloride standard: 100 mEq/l: It is prepared by dissolving 5.85 g of analar grade sodium chloride in one liter of glass distilled water. It is stable at 2-8°C.
**Additional Reagents**

4) \(\frac{2}{3} \text{ N sulfuric acid}\)
5) \(10 \text{ g/dl, sodium tungstate.}\)

**Specimen**

Serum

**Procedure**

I. Prepare protein free filtrate of the serum sample as follows:

In a centrifuge tube, pipette

a) 4.0 ml distilled water
b) 0.5 ml serum
c) 0.25 ml \(\frac{2}{3} \text{ N H}_2\text{SO}_4\)
d) 0.25 ml 10 g/dl sodium tungstate

Mix thoroughly and centrifuge at 3000 R.P.M. for 10 minutes. Next procedure is as follows:

a) Pipette in a test tube, 2.0ml of protein free filtrate.

b) Add one drop of the indicator, (0.05ml)

c) Titrate against mercuric nitrate reagent. (End point: colorless to violet-blue color)

d) Note the titration reading : \(X\) ml.

e) Dilute standard 1:10 by using glass distilled water.

f) Pipette 2.0 ml of diluted standard in a test tube, and titrate it against mercuric nitrate reagent by using diphenylcarbazone indicator.

g) Note the titration reading : \(Y\) ml.
Calculation

\[
\text{Serum chlorides, mEq/L} = \frac{\text{Xml}}{\text{Yml}} \times 100
\]

4.2.8 Estimation of blood Sugar

Method

Trindie’s method

Reagent Composition

Reagent I – glucose

- Glucose oxidase (aspergillin) - 20,000 IU/l
- Peroxidase (horse radish) - 3,350 IU/l
- 4 amino antipyrine - 0.52 m mol/l
- 4 hydroxy benzoic acid - 10 m mol/l
- Phosphate buffer - 110 m mol/l

Also contains non reactive filter and stabilizers.

\[\text{pH} = 7 \pm 0.2 \text{ at } 25^\circ \text{C.}\]

Reagent II – glucose standard

Glucose standard – 100 mg/dl or 5.55 m mol/l

Reagent Constitution

Allow the vial to obtained room temperature. Dissolve the contents of each vial using glucose diluted with special with special lipid cleaning agent. Make up the volume to 200 ml or 500 ml transfer into a clear and dry amber colored bottle.
Procedure

Take approximately 1ml of blood mixed with anticoagulant and centrifuged it for 3 minutes. Thus we can separate plasma from blood. Take 3 test tube marked T,B,S (Test Blank and Standard) add 100ml working reagent to whole of them. Add 100 ml of distilled water to test tube marked as B and 100ml sample to test tube marked as T. Mix well and incubate for minutes at $37^\circ$ C. Read the absorbance of standard and each sample tube against reagent blank at 510 mm. (500-540) or 510/630 mm as brichromatic analyser.

4.2.9 Determination of total cholesterol

Reagent composition

Monoreagent PIPES 200 mmol/l pH 7.0 sodium cholate 1 mmol/l, cholesterol esterase > 250 U/l, cholesterol oxidase > 250 U/l, Peroxidase > 1 KU/l, 4-aminoantipyrine 0.33 mmol/l, phenol 4 mmol/l, non-ionic tensioactives 2 g/l (w/v). Biocides. Cholesterol standard. Cholesterol 200mg/dl (5.18mmol/L). Organic matrix based primary standard.

Reagent preparation

The Monoreagent and the Standard are ready-to-use.

Samples

Serum, EDTA or heparinized plasma free of hemolysis.

Cholesterol in serum or plasma is stable up to 5 days at 2-8$^\circ$C and for a few months at -20$^\circ$C.
Materials required

- Photometer or colorimeter capable of measuring absorbance at 500 ±10nm.
- Constant temperature incubator set at 37°C
- Pipettes to measure reagent and samples.

Procedure

1. Bring reagents and samples to room temperature.

2. Pipette into labelled tubes.

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Blank</th>
<th>Sample</th>
<th>Cal. Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1. Monoreagent</td>
<td>1.0 ML</td>
<td>1.0 ml</td>
<td>1.0ml</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>10 μl</td>
<td>-</td>
</tr>
<tr>
<td>Cal. Standard</td>
<td>-</td>
<td>-</td>
<td>10 μl</td>
</tr>
</tbody>
</table>

3. Mix and incubate the tubes 10 minutes at room temperature or 5 minutes at 37°C

4. Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

Calculations

\[
\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = \text{mg/dl total cholesterol}
\]

Samples with concentrations higher than 600 mg/dl should be diluted 1:2 with saline and assayed again. Multiply the results by .

If results are to be expressed as SI units apply:

\[ \text{mg/dl} \times 0.0259 = \text{mmol/l} \]
4.3 Determination of Urea

Name of the method

Diacetyl monoxime method.

Materials requirement

1. Test tubes : (15x125mm)
2. 10ml pipette, dispenser or burette.
3. Push button pipette or 0.1 ml serological pipette
4. Measuring cylinder of 100ml
5. Water-bath
6. Stopwatch
7. Photometer

Sample material

Serum.

Preparation & Stability of the reagents

1. Reagent 1 : (DMR) : It contains 0.2 g/dl, diacetyl monoxime in distilled water. The reagent is stable at room temperature ($25^\circ C \pm 5^\circ$) for one year.

2. Reagent 2 : (TSC) : It contains 40 mg/dl. thiosemicarbazide in distilled water. The reagent is stable at room temperature ($25^\circ C \pm 5^\circ$) for 6 months.

3. Reagent 3 : (Acid): It contains 60 ml of conc. sulfuric acid, 10 ml of orthophosphoric acid and 10ml of 1 gm/dl ferric chloride in orthophosphoric acid in one liter of the reagent prepared in distilled water. This reagent is stable at room temperature for one year.
4. Urea nitrogen standard: 20 mg/dl: It contains 42.8 mg of urea in 100ml of saturated benzoic acid. This standard is stable for one year when refrigerated.

**Preparation of working reagent**

It is prepared fresh by mixing one part of reagent 1, one part of reagent 2, and two parts of reagent 3. This reagent should be prepared fresh for each batch of the determination.

**Procedure**

Pipette in the tubes labeled as follows

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent, ml</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Serum/plasma, ml</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 20 mg/dl, ml</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water, ml</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mix the contents of the tubes thoroughly and place them in a boiling, water bath for exactly 15 minutes. Cool immediately by using tap water and after 5 minutes measure the intensities of the test and standard against blank at 520 nm (green filter).

**Calculation**

\[
\text{urea mg/dl} = \frac{\text{O.D. Test}}{\text{O.D. Std.}} \times 20
\]
4.3.1 Determination of Serum Creatinine

Method

Alkaline-picrate method

Reaction

Jaffe’ reaction

Specimen

Serum (or plasma)

Requirements

1. Test tubes : 15 x 125 mm
2. 5.0ml serological pipettes
3. 1.0 ml & 2.0 ml volumetric pipettes
4. Test-tube stand
5. Centrifuge tubes or test tubes, 100 x 10 mm
6. Centrifuge
7. Photometer

Preparation of the reagents

- Picric acid reagent : 0.91 gm/dl (0.04M)
- 10/dl, sodium hydroxide
- Working creatinine standards, 1 mg/dl, 5 mg/dl and 10 mg/dl.

These standards are prepared in 0.01N hydrochloric acid by using stock creatinine standard 100 mg/dl.
Stability of the reagent

Reagent 1 and 2 stable at room temperature (25°C ± 5°C). The working standards are stable at 2-8°C.

Preparation of the alkaline picrate reagent

It is prepared fresh by mixing 4 parts of reagent 1 & 1 part of reagent, 2. This working reagent is stable for one day.

Procedure

Pipette in the tubes labeled as follows:

<table>
<thead>
<tr>
<th></th>
<th>Test (in a centrifuge tube)</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water, ml</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Serum, ml</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Standard 1 mg/dl, ml</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>2/3N Sulfuric acid, ml</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>10 g/dl sodium tungstate ml</td>
<td>0.5</td>
<td>-</td>
</tr>
</tbody>
</table>

Centrifuge the contents in the test and get clear filtrate. Pipette in the tubes labeled as follows:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std : 1</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water, ml</td>
<td>3.0</td>
<td>3.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Filtrate. Ml</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diluted Std. 1 mg/dl ml.</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Alkaline picrate reagent. ml</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Mix and keep at room temperature ((25°C ± 5°C) for 20 minutes.

Read intensities of test and standard at 520 nm (green filter) by setting blank to 100%T.
Calculation

\[
\text{Serum creatinine, mg/dl} = \frac{\text{O.D. Test}}{\text{O.D. Std}} \times 1.0
\]

4.3.2 Estimation of Serum Bilirubin

Method

Malloy and Evelyn

Requirements

1. Test-tubes : 15 x 125 mm
2. 5.0, 0.2 ml serological pipettes
3. Stop watch
4. Test tube stand
5. Photometer

Preparation

1. Diazo ‘A’ : It is prepared by mixing 0.1 g of sulfanilic acid in 100 ml of 1.5% (v/v) hydrochloric acid.
2. Diazo ‘B’ : It is prepared by mixing 0.5 g of sodium nitrite in 100 ml of distilled water.
3. Diazo blank reagent : (1.5% hydrochloric acid) : It is prepared by adding 1.5 ml of concentrated hydrochloric acid to about 90 ml of distilled water in a 100 ml volumetric flask. Distilled water is added upto the mark.
4. Methanol
5. 10 mg/dl artificial bilirubin standard: It is prepared as follows

a) Stock standard: It is prepared by mixing 0.29 g of methyl red in 100 ml of glacial acetic acid.

b) Working standard: 0.1 ml of stock standard, 0.5 ml of glacial acetic acid and 1.44 g of sodium acetate are mixed in distilled water and diluted to 100 ml by adding distilled water.

**Stability of the reagents**

Reagent 1, 3, 4 & 5 are stable at room temperature \((25^\circ C \pm 5^\circ C)\) for one year.

Reagent 2 is stable at 2-8\(^\circ\)C in an amber colored bottle.

**Test Procedure**

Prepare fresh diazo mixture by mixing 5.0 ml of Diazo A and 0.15 ml of Diazo B. This mixture is stable only for a day.

Pipette in the tubes labeled as follows:

<table>
<thead>
<tr>
<th></th>
<th>Total Test</th>
<th>Total Blank</th>
<th>Direct Test</th>
<th>Direct Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water, ml</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Serum, ml</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Diazo mixture, ml</td>
<td>0.5</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Diazo blank reagent ml</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>Methanol, ml</td>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water, ml</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Keep in dark for 30 minutes. Read the intensities at 540 nm (green filter).

Read O.D. of the artificial bilirubin standard (undiluted) by transferring the standard solution in a dry cuvette at 540 nm (or green filter).
Calculations

\[
\text{O.D. of total Bilirubin} = \text{O.D. of total test} - \text{O.D. of total Blank.}
\]

\[
\text{O.D. of direct Bilirubin} = \text{O.D. of direct test} - \text{O.D. of direct blank.}
\]

\[
\text{Total bilirubin mg/dl} = \frac{\text{O.D. of total bilirubin}}{\text{O.D of std}} \times 10
\]

\[
\text{Direct bilirubin mg/dl} = \frac{\text{O.D. of total bilirubin}}{\text{O.D of std}} \times 10
\]

Indirect Bilirubin, mg/dl = Total Bilirubin, mg/dl – Direct Bilirubin, mg/dl.

4.3.3 Alkaline Phosphatase Determination

Method

Visible – Kinetic

Sample Material

Serum, heparinized plasma (Free from hemolysis).

Reagents

1. AMP buffer (pH : 10.3)
2. Magnesium chloride reagent : 30 mg/dl
3. P-nitrophenyl phosphate : (PNP) : Prepare fresh as follows :

Preparation and stability of the reagent : (PNP)

Dissolve 42.5 mg PNP in 0.5 ml of reagent No.2 This mixture is stable at 0-4°C for 4 weeks.
Procedure

Wavelength : 405 nm

Cuvette 1 cm : light path

Temperature : 30 or 37°C

Pipette into cuvette

<table>
<thead>
<tr>
<th>AMP Buffer, ml</th>
<th>2.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNP, ml</td>
<td>0.2</td>
</tr>
<tr>
<td>Serum (or plasma, ml)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Mix, read initial absorbance and start stop watch at the same time. Repeat readings after exactly 1, 2 and 3 minutes. Determine mean absorbance change per minute (ΔA/min).

Calculations

Serum alkaline phosphate :

IU (30°C, 37°C) = 1595 x ΔA 3 405/min.

4.3.4 Determination of Gamma G.T.

Method

End point reaction method.

Sample Material

Serum (Free from hemolysis)
Reagents

1. Substrate: It is prepared by mixing 250 mg of L-gamma-glutamyl–paranitroanilide, 872 mg of glycyl–glycine & 672 mg of magnesium chloride in 300 ml of AMP buffer, pH : 8.6 (0.05M)

2. Sodium hydroxide reagent : 0.0075 M.

3. P-nitroaniline standard : 12.4 mg/dl (0.9 mM/ml).

Stability of the reagents

Substrate is stable at 0-4°C for 3 months

Reagent 3 is stable at 2-8°C for several months.

Reagent 2 is stable at room temperature (25°C ± 5°C) for several months.

Procedure

Wavelength : 405 nm (violet filter)

Temperature : 37°C

Pipette into the tubes labeled as follows

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate, ml</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Serum, ml</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Incubate at 37°C for 45 min</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium hydroxide reagent, ml</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Serum, ml</td>
<td>-</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Read absorbances at 405 nm (Violet filter) against blank. Refer the standard graph for the calculations of enzyme units.
Standardization for the determination of gamma G.T.

Pipette in the tubes labeled as follows

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate, ml</td>
<td>0.9</td>
<td>0.7</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>P-nitroaniline std</td>
<td>0.1</td>
<td>0.3</td>
<td>0.5</td>
<td>0.8</td>
<td>0.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Sodium hydroxide R, ml</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>γ-GT, γU</td>
<td>10</td>
<td>30</td>
<td>50</td>
<td>80</td>
<td>90</td>
<td>00</td>
</tr>
</tbody>
</table>

Prepare a standard curve by plotting O.D. on Y-axis and IU on X-axis.

4.3.5 Determination of Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT)

Method

End point reaction

Name of the method

Reitman and frankel’s method.

Enzyme method

Karmen units

These are expressed as the unit of activity which produces change in O.D. of 0.001 per minute by enzyme present in 1.0 ml of serum.

Requirements

1. Test tubes: 15 x 125 mm
2. 5.0 ml serological pipettes
3. 0.1 ml serological pipettes
4. Constant temperature water bath
5. Stopwatch
6. Photometer.

**Preparation of the reagents**

1. **SGPT substrate**: It contains 1.78 g of alanine, 30 mg of alpha – keto – glutaric acid, 0.5 ml of 1.0 N sodium hydroxide in phosphate buffer pH 7.45 (M/15). The final quantity is adjusted to 100 ml by using the phosphate buffer. pH of this substrate should be 7.45.

2. **SGOT substrate**: It contains 2.66 g of aspartic acid, 30 mg of alpha – keto – glutaric acid, 20 ml of IN sodium hydroxide in the phosphate buffer, pH 7.45 (M/15). The final volume of the substrate is adjusted to 100 ml by using the phosphate buffer. pH of this substrate should be 7.45.

3. **DNPH reagent**: It contains 200 mg of dinitrophenyl hydrazine & 85 ml of conc. hydrochloric acid in distilled water; final volume should be adjusted to one liter by using distilled water.

4. 0.4 N sodium hydroxide

5. 22 mg/dl, sodium pyruvate standard.

**Stability of the reagents**

The reagents 1,2,3 and 5 are stable at 2-8°C. Reagent 4 is stable in a polyethylene container for several months at room temperature (25°C ± 5°C).
Sample material

Serum (Hemolysis interferes with the test). Use fresh serum.

Procedure

Wavelength : 546 nm (green filter, 530-550 nm)

Incubation temperature : 37°C

Incubation time

- SGPT 30 minutes.
- SGOT 60 minutes.

Procedure (SGPT Determination):

Pipette in the tubes labeled as follows:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate, ml</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Incubate at 37°C for 5 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum, ml</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Incubate at 37°C for 30 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNPH, ml</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Serum, ml</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>Mix thoroughly, keep at room temperature for (25°C ± 5°C) 20 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 N NaOH, ml</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Mix and keep at room temperature for 10 minutes. Afterwards, read intensity of test by setting blank at 100% T (540 nm: green filter)
1. **Procedure** (SGOT Determination):

   **Pipette in the tubes labeled as follows**

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate, ml</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Incubate at 37°C for 5 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum, ml</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Incubate at 37°C for 30 minutes (1 hr.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNPH, ml</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Serum, ml</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>Mix thoroughly, keep at room temperature for (25°C ± 5°C) 20 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 N NaOH, ml</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Mix and keep at room temperature for 10 minutes. Afterwards, read intensity of test by setting blank at 100% T (540 nm, green filter).

**4.3.6 Total Proteins determination**

**Name of the method**

Biuret method

**Requirements**

1. Test-tubes : 15 x 125 mm,
2. Serological pipette, 5ml
3. Test-tube stand
4. Push button pipette of 0.05 ml or serological pipette of 0.1ml
5. Photometer

**Specimen**

Serum
Preparation of the reagents

Stock Biuret reagent: Dissolve 45 g of Rochelle salt in about 400 ml of 0.2 N sodium hydroxide and add 15 g of copper sulfate by stirring continuously until the solution is complete. Add 5g of potassium iodide and make upto a liter with 0.2 N sodium hydroxide.

1. Protein reagent (ready to use): (working Biuret reagent). Dilute 200ml of stock reagent to a liter with 0.2 N sodium hydroxide which contains 5 g of potassium iodide per liter

2. Protein standard: 6.0 g/dl: 6g of bovine albumin dissolved in 100ml of normal saline, containing 0.1 g/dl, sodium azide.

Additional reagents

3. Sample blank reagent: 9.0 g of Rochelle salt & 5 g of potassium iodide dissolved in one liter of 0.2 N sodium hydroxide.

Stability of the reagents

Reagents 1 and 3 are stable at room temperature (25\textdegree{}C ± 50\textdegree{}C) for one year. Reagent 2 (Protein standard) is stable at 2-8\textdegree{}C for one year.

Procedure

Mono-step method
Pipette in three-tubes labeled as follows:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std.</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein in Reagent, ml</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Serum, ml</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein std 6 g/dl ml</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water, ml</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mix thoroughly and keep at room temperature \((25^0C \pm 5^0C)\) for exactly 10 minutes. Measure the intensities of the test and standard by setting blank at 100% T, by using 530nm (green filter).

### 4.3.7 Determination of Serum Albumin

#### Name of the method

Bromocresol green method.

#### Requirements

Test-tubes : 15 x 125 mm

1. Serological and graduated pipettes, 10ml, 5ml
2. Test-tube stand
3. Push-button pipette of 0.05 ml or serological pipette of 0.1ml.
4. Photometer
5. Serum

#### Preparation of the reagents

1. Albumin reagent (ready to use) : It is prepared by mixing following chemicals in 900 ml distilled water.
a) Succinic acid : 8.85 g.
b) Bromocresol green : 108 mg
c) Sodium azide : 100 mg
d) Brij-35 : 4.0 ml

pH of this solution is adjusted by using 1N sodium hydroxide to 4.1 Final volume is made to one liter by using distilled water.

1. Albumin std 4.0 g/dl : Bovine albumin 4.0 g in 100 liter of normal saline containing 0.1 g/dl sodium azide.

2. Sample blank reagent : It contains 0.885 g of succinic acid, 10 mg of sodium azide & 0.4 ml of Brij-35 pH of this solution is adjusted to 4.1

**Stability of the reagents**

Reagents 1 and 3 are stable at room temperature (25°C ± 5°C) for one year.
Reagent : 2 (albumin standard) is stable at 2-8°C for one year.

**Procedure**

Mono-step method

Pipette in three-tubes labeled as follows –

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin reagent, ml</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Serum, ml</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Albumin standard, ml</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water, ml</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Mix thoroughly and keep at room-temperature \((25^\circ C \pm 5^\circ C)\) for exactly 10 minutes. Measure the intensity of the test and standard by setting blank at 100% T, by using 640 nm (red filter).

**Calculations**

\[
\text{Serum albumin, g/dl} = \frac{\text{O.D. Test}}{\text{O.D. Std}} \times 4
\]

**4.3.8 Determination of Globulin**

Serum globulin = Total Protein - Albumin

**PLATE: 9**

Fig 47-52: Blood samples of beedi rollers are analysed for haematological parameters using auto analyser.
4.3.9 Triiodothyronine (T₃)

Method

Enzyme Linked Immunosorbent Assay

Sample

Serum

Materials provided with this kit

1. Anti-T₃ Antibody-Coated Microtiter Wells, 96 wells.
2. T₃ HRPO Conjugate Concentrate, 0.8 ml.
3. T₃ HRPO Conjugate Diluent, 15 ml
5. TMB Substrate, 12 ml
6. Stop Solution, 12 ml
7. Wash Buffer Concentrate (50X), 15 ml

Materials required

1. Distilled water.
2. Precision pipettes: 0.05 ~ 0.2 ml, 1.0 ml
3. Disposable pipette tips.
4. Microtiter well reader.
5. Vortex mixer or equivalent.
6. Absorbent paper.
7. Graph paper
Reagent preparation:

1. All reagents should be allowed to reach room temperature (18-22°C) before use.

2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

3. To prepare T3-HRPO Conjugate Reagent, add 0.1 ml of T3-HRPO Conjugate Concentrate to 2.0 ml of T3 Conjugate Diluent (1:20 dilution), and mix well. The amount of conjugate diluted is depend on your assay size. The Conjugate Reagent is stable at 4°C at least for two weeks.

Procedure

1. Secure the desired number of coated well in the holder. Make data sheet with sample identification.

2. Dispense 50 µl of standard, samples, and controls into appropriate wells.

3. Thoroughly mix for 10 seconds, then dispense 100 µl of Enzyme Conjugate Reagent into each well.

4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.

5. Incubate at room temperature for 60 minutes.

6. Remove the incubation mixture by flicking plate contents into a waste container.

7. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.

9. Dispense 100 µl TMB solution into each well. Gently mix for 5 seconds.

10. Incubate at room temperature in the dark for 20 minutes without shaking.

11. Stop the reaction by adding 100µl of Stop Solution to each well.

12. Gently mix for 15 seconds.

13. Read OD at 450 nm with a microtiter reader within 15 minutes.

**Calculation of results**

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of T\textsubscript{3} in ng/ml from the standard curve.

**4.4 Thyroxine(T\textsubscript{4})**

**Method**

Enzyme Linked Immunosorbent Assay

**Sample**

Serum
Materials provided with the kid:

- Antibody-coated microtiter wells. 96 wells per bag.
- Reference standard set, ready to use.
- T<sub>4</sub> HRPO Conjugate Diluent, 15 ml.
- T<sub>4</sub> HRPO Conjugate Concentrate, 0.8 ml
- TMB Substrate, 12 ml.
- Stop Solution, 12 ml.
- Wash Buffer Concentrate (50X), 15 ml

Materials required:

- Precision pipettes: 40µl-200µl and 1.0ml
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter well reader.

Reagent preparation:

1. All reagents should be brought to room temperature (18-22°C) before use.

2. To prepare T<sub>4</sub>-HRPO Conjugate Reagent, add 0.1 ml of T<sub>4</sub>-HRPO Conjugate Concentrate to 2.0 ml of T<sub>4</sub> Conjugate Diluent (1:20 dilution), and mix well. The amount of conjugate diluted is depend on your assay size. The Conjugate Reagent is stable at 4°C at least for two weeks.
The best temperature condition for this assay is from 19°C to 22°C. If, in the environmental assay condition, the temperature is higher than expected, we recommend increasing the T₄ conjugate dilution up to 1:40.

3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

Procedure

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.

2. Dispense 50µl of standard, specimens, and controls into appropriate wells.

3. Dispense 100µl of Enzyme Conjugate Reagent into each well.

4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this step.

5. Incubate at room temperature (18-22°C) for 60 minutes.

6. Remove the incubation mixture by flicking plate contents into a waste container.

7. Rinse and flick the microtiter wells 5 times with washing buffer (1X).

8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.

10. Incubate at room temperature for 20 minutes without shaking.
11. Stop the reaction by adding 100µl of stop solution to each well. Gently mix for 5 seconds

12. Read optical density at 450nm with a microtiter well reader.

**Calculation of results**

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µg/dl on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of T₄ in µg/dl from the standard curve.

**4.4.1 Thyroid Stimulating Hormone (TSH)**

**Method**

Enzyme Linked Immunosorbent Assay

**Sample**

Serum

**Materials provided with the kits**

- Anti-TSH antibody coated microtiter wells.
- Set of Reference Standards: 0, 0.1, 0.5, 2, 5, and 10µIU/ml - 1.0 mL/vial.
- Enzyme Conjugate Reagent, 12 ml.
- TMB Substrate, 12 ml.
Materials required

- Precision pipettes: 50µl, 100µl, 200µl, and 1.0ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

Reagent preparation

- All reagents should be brought to room temperature (18-22°C) before use.

- Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water.
  For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x).

Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 100µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of enzyme conjugate reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (22 2°C) for 120 minutes.

6. Remove the incubation mixture by flicking plate contents into a waste container.

7. Rinse and flick the microtiter wells 5 times with Washing Buffer (1X).

8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.

10. Incubate at room temperature for 20 minutes.

11. Stop the reaction by adding 100µl of stop solution to each well.

12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

13. Read absorbance at 450nm with a microtiter well reader within 30 minutes.

**Calculation of results**

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and patient samples.

2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in µIU/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.

3. Use the mean absorbance values for each specimen to determine the corresponding concentration of TSH in µIU/ml from the standard curve.
PLATE: 10

Fig 53-58: Blood samples of women beedi rollers are tested using thyroid kit (omega) to analyse thyroid profile in auto analyser.
7. PLATE - 10

Figure 53

Figure 54

Figure 55

Figure 56

Figure 57

Figure 58
4.4.2 Free Triido thyronine(FT$_3$)

Method

Competitive Enzyme Immunoassay – Analog Method for Free T$_3$

Sample

Serum

Reagents

A. Human Serum References -- 1ml/vial - Icons A-F

Six (6) vials of human serum based reference calibrators for free triiodothyronine at approximate concentrations of 0 (A), 1.0 (B), 3.0 (C), 5.0 (D), 8.0 (E) and 16.0 (F) pg/ml. Store at 2-8°C. A preservative has been added. Exact levels are given on the labels on a lot specific basis.

For SI units: 1pg/ml x 1.536 = pmoI/L

B. fT$_3$ –Enzyme Reagent - 13ml/vial - Icon –E

One (1) vial of triiodothyronine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T$_3$ Antibody Coated Plate 96 wells – Icon

One 96-well microplate coated with sheep anti-triiodothyronine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
D. Wash Solution 20ml - Icon

One (1) vial containing a surfactant in phosphate buffered saline. A preservative has been added. Store at 2-30ºC.

E. Substrate A – 7 ml/vial - Icon

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8ºC.

F. Substrate B – 7 .0ml/vial - Icon

One (1) bottle containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8ºC.

G. Stop Solution – 8ml/vial- Icon STOP

One (1) bottle containing a strong acid (1N HCl) Store at 2-30ºC

Materials required

1. Pipette capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials
Specimen collection and preparation

The specimens shall be blood serum in type and the usual precautions in the Collection of venipuncture samples should be observed. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow blood to clot. Centrifuge the specimen to separate the serum from the cells. Specimen may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) cannot be assayed within 48 hours, the sample(s) may be stored at temperatures of -20°C for up to 30 days. When assayed in duplicate, 0.100ml of the specimen is required.

Reagent preparation

1. Wash Buffer

Dilute contents of wash Concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.

2. Working Substrate Solution

Pour the contents of the amber vial labeled Solution A into the clear vial labeled Solution B. Place the yellow cap on the clear vial for easy identification. Mix and Label accordingly. Store at 2-8°C

Procedure

1. Format the microplate wells for each serum reference, control and specimen to be assayed in duplicate.

2. Pipette 0.050 ml (50 µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of fT₃-enzyme reagent solution to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two additional times for a total of three washes.

8. Add 0.100 ml (100µl) of working substrate solution to all wells.

9. Incubate for 15 minutes at room temperature.

10. Add 0.050ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within 30 minutes of adding the stop solution.

Calculation of results

A dose response curve is used to ascertain the concentration of free triiodothyronine in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader.

2. Plot the absorbance for each duplicate serum reference versus the corresponding fT₃ concentration in pg/ml on linear graph paper.
3. Draw the best fit curve through the plotted points.

4. To determine the concentration of FT$_3$ for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve and read the concentration in pg/ml from the horizontal axis of the graph.

4.4.3 Free Thyroxine (Free T$_4$)

Method

ELISA

Sample

Serum

Reagents:

A. Human Serum References -- 1 ml/vial - Icons A-F

Six (6) vials of human serum based reference calibrators for free thyroxine at approximate concentrations of 0 (A), 0.40 (B), 1.25 (C), 2.10 (D), 5.00 (E) and 7.40 (F) ng/dl. Store at 2-8°C. A preservative has been added. For SI units: ng/dL x 12.9 = pmol/L

B. fT$_4$- Enzyme Reagent -- 13 ml/vial

One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.
C. **Antibody Coated Microplate** -- 96 wells One 96-well microplate coated with anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. **Wash Solution Concentrate** -- 20ml One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

E. **Substrate A** – 7 ml/vial One (1) bottle containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

F. **Substrate B** – 7 ml/vial One (1) bottle containing hydrogen peroxide (H2O2) in acetate buffer. Store at 2-8°C.

G. **Stop Solution** – 8 ml/vial One (1) bottle containing a strong acid (1N HCl). Store at 2-8 °C.

**Materials required**

1. Pipette capable of delivering 50µl & 100µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
Specimen collection and preparation

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Specimen(s) may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) cannot be assayed within 48 hours, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml of the specimen is required.

Reagent preparation

1. Wash Buffer

   Dilute contents of Wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.

2. Working Substrate Solution

   Pour the contents of the amber vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly Store at 2-8 °C.

Procedure

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate.

2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of fT₄-Enzyme Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.

8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

**Calculation of results**

1. Record the absorbance obtained from the printout of the microplate reader.

2. Plot the absorbance for each duplicate serum reference versus the corresponding Cortisol concentration in µg/dl on linear graph paper.
3. Connect the points with a best–fit curve.

4. To determine the concentration of cortisol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/dl) from the horizontal axis of the graph.

4.4.4 AntiThyroglobin Antibody

Method

Enzyme Linked Immunosorbent Assay

Sample

Serum

Reagents

1. Thyroglobulin antigen coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)

2. Serum Diluent Type III: Ready to use. Contains buffer, BSA and Tween-20, and proclin (0.1%) as a preservative. (96T: one bottle, 30 ml)

3. High Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The High Positive Control is utilized to control the upper dynamic range of the assay. (96T: one vial, 0.4 ml)
4. **Calibrator**: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 ml)

5. **Negative Control**: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 ml)

6. **Low Positive Control**: Human serum or defibrinated plasma. Sodium azide(< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Low Positive Control is utilized to control the range near the cutoff of the assay. (96T: one vial, 0.4 ml)

7. **Horseradish-peroxidase (HRP) Conjugate**: Ready to use. Goat anti-human IgG, IgA, and IgM containing proclin (0.1%) and gentamicin as preservatives. (96T: one bottle, 15 mL)

8. **Wash Buffer Type II (20X concentrate)**: Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-80 and proclin (0.1%) as a preservative. (96T: one bottle, 50 mL)

9. **Chromogen/Substrate Solution Type I**: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If
allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 ml)

10. Stop Solution: Ready to use, contains a 1N H$_2$SO$_4$ solution. (96T: one bottle, 15 ml)

Materials

1. Wash bottle, automated or semi-automated microwell plate washing system.

2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µl volumes (less than 3% CV).

3. One liter graduated cylinder.


5. Test tube for serum dilution.

6. Reagent reservoirs for multichannel pipettes.

7. Pipette tips.

8. Distilled or deionized water (dH$_2$0), CAP (College of American Pathology) Type 1 or equivalent (17,18).

9. Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 minutes).

10. Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950 ml dH2O).

11. Single or dual wavelength microplate reader with 450 nm filter.
Procedure

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Calibrator determinations (one Negative Control, three Calibrators, one High Positive Control and one Low Positive Control) per run. A reagent blank (RB) should be run on each assay.

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. Mix Well.

3. To individual wells, add 100 µL of the appropriate diluted Calibrator, Controls and Experimental sera. Add 100 µL of Serum Diluent to reagent blank well.

4. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 1 minute.

5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

6. Add 100 µl Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.

7. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 1 minute.
8. Repeat wash as described in Step 5.

9. Add 100 µl Chromogen/ Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.

10. Incubate each well at room temperature (21° to 25° C) for 15 minutes +/- 1 minute.

11. Stop reaction by addition of 100 µL of Stop Solution (1N H₂SO₄) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.

12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm.

4.4.5 Anti Microsomal Antibody (AMA)

Method

Enzyme Linked Immunosorbent Assay

Sample

Serum

Reagents

1. Microsomal antigen coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
2. Serum Diluent Type III: Ready to use. Contains buffer, BSA and Tween-20, and proclin (0.1%) as preservative. (96T: one bottle, 30 mL)

3. High Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives.

4. Calibrator: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives.

5. Negative Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 ml)

6. Low Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added. The Low Positive Control is utilized to control the range near the cutoff of the assay. (96T: one vial, 0.4 ml)

7. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG, IgA, and IgM containing proclin (0.1%) and gentamicin as preservatives. (96T: one bottle, 15 ml)

8. Wash Buffer Type II (20X concentrate): Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-80 and proclin (0.1%) as a preservative. (96T: one bottle, 50 ml)

9. Chromogen/Substrate Solution Type I: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 ml)
10. Stop Solution: Ready to use, contains a 1N H₂SO₄ solution. (96T: one bottle, 15 ml)

Additional requirements

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µl volumes (less than 3% CV).
3. One liter graduated cylinder.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH₂0).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950 ml dH₂0).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm.

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Calibrator determinations (one Negative Control, three Calibrators, one High Positive Control and one Low Positive Control) per run. A reagent blank (RB) should be run on each assay.
2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µl + 200 µl) in Serum Diluent. Mix Well.

3. To individual wells, add 100 µl of the appropriate diluted Calibrator, Controls and patient sera. Add 100 µl of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.

4. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 1 minute.

5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

6. Add 100 µl Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.

7. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 1 minute.

8. Repeat wash as described in Step 5.

9. Add 100 µl Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.

10. Incubate each well at room temperature (21° to 25°C) for 15 minutes +/- 1 minute.
11. Stop reaction by addition of 100 µl of Stop Solution (1N H$_2$SO$_4$) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.

12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm.

4.5 Results

The health assessment of beedi rollers of various age groups through blood parameters has indicated a negative impact on the population.

4.5.1. Total Erythrocyte Count (TEC)

The total erythrocyte count in the control women population ranged from $4.05 \pm 0.452 \times 10^6$ mm$^3$ to $4.23 \pm 0.660 \times 10^6$ mm$^3$ in the age group of 20-30 to 40-50, which was within normal range ($4$ to $5 \times 10^6$ mm$^3$). But the beedi rollers at various age groups exhibited a decrease in TEC. In the age group of 20-30 the women beedi workers had a decrease in the TEC count ($3.84 \pm 0.583 \times 10^6$ mm$^3$) which was highly significant statistically. The same trend was observed in the age groups of 30-40 and 40-50 also (Fig: 59).

4.5.2. Total Leucocyte Count (TLC)

Total Leucocyte Count in the control population exhibited near the normal lower limit ($4.3$ to $10.0 \times 10^3$ mm$^3$). At the age group of 20-30, 30-40 and 40-50 TLC
was 3.19± 0.39, 3.97± 0.50, 3.77 ± 0.58 respectively. On the other hand, beedi rollers at the age group of 20-30, 30-40 and 40-50 showed TLC of a 3.44 ± 0.80, 4.27 ± 0.138, 4.57 ± 0.181 respectively. Significant decreased levels (P< 0.05) of TLC was determined in the women beedi rollers in all age groups compared to the non beedi rollers (Fig: 60).
4.5.3 Platelet Count

There was a decrease in the platelet counts among the beedi worker population. Sampling at the age group of 20 to 30 showed $1.65 \pm 0.64$ thousand cells/mm$^3$ when compared with normal individuals who have a count of $2.70 \pm 0.88$ thousand cells/mm$^3$. Similar observations were observed in the other age groups of beedi rollers. Even though platelet count was well within normal range of 1.5-4.0 thousand cells/mm$^3$ they showed a decreasing trend reflecting the health states of beedi workers (Fig: 61).

4.5.4 Haemoglobin content (Hb)

The normal range of haemoglobin content in females is 12.1 to 15.1 g/dl, 10.2 ± 1.15 and 10.5 ± 1.22 g/dl in the age group 20-30, 30-40, and 40-50 respectively. But the haemoglobin content of beedi workers population exhibited a decrease. It is $8.67 \pm 0.16$ g/dl in the age group of 20 to 30 and $8.89 \pm 0.18$ g/dl in 30-40 and $8.36 \pm 0.17$ g/dl in 40 to 50. Statistical analysis of the data showed highly significant values (P<0.001) in the age group of 20 to 30 and 40 to 50 and significant value (P<0.05) in the age group of 30 to 40 (Fig: 62).
4.5.5 Erythrocyte Sedimentation Rate (ESR)

ESR of the experimental subjects showed significantly high values, while the females have a value which lies within the normal range (0-20 mm/hr). The ESR of beedi workers at the age group 20-30 was $23 \pm 2.94$ mm/hr whereas that of control people in $16.8 \pm 1.38$ mm/hr. The same trend is observed in all beedi workers at the age group of 30-40 and 40-50(Fig: 63).
4.5.6 Packed Cell Volume (PCV)

Significant decrease (P<0.05) in PCV was observed in beedi rollers in all age groups. The control population exhibited a PCV within normal range (36-48%). At the age group of 20-30, 30-40 and 40-50 a PCV was 35.4 ± 2.14%, 35.2±3.48% and 35 ± 3.23% respectively. On the other hand, beedi rollers at the age group of 20-30, 30-40, 40-50 showed a PCV of 30.4 ± 2.91% 30.2±2.84% and 31.0 ±2.59% respectively(Fig: 64).
Trace Elements

Trace elements Sodium, Potassium and Chloride in the blood of beedi rollers were estimated and compared with normal population. Eventhough significant increase was noted they were within the normal range.

4.5.7 Sodium

The sodium level of the normal female is 135 to 145 mmol/l. Elevated levels of sodium was found in all age group of beedi rollers (128 ± 6.39 mmol/l to 129 ± 4.64 mmol/l) compared to non beedi rollers (126 ± 4.60 mmol/l to 128 ± 4.47 mmol/l) respectively(Table: 3a).

4.5.8 Potassium

A highly significant increase (P<0.001) in potassium level was observed in the beedi rollers among the age group of 40-50 (5.13 ± 0.72 mmol/l). This data slightly higher then the normal potassium level of normal healthy female (3.5 -5.0 mmol/l). In the age group of 20-30 and 30-40 the potassium level in beedi rollers was 3.18±0.18 and 3.20 ±0.27 mmol/l respectively. But in beedi rollers the age group of 20-30 and 30-40 exhibited the potassium level of 3.57 ±0.43 mmol/l and 4.03 ± 0.78 mmol/l respectively (Table: 3b).

4.5.9 Chloride

Eventhough highly significant increase (P<0.001) in the chloride levels among the beedi rollers irrespective of the age groups was identified, it was within range when compared to non beedi rollers. The chloride level in normal females ranges from 98-108 mmol/l. An observation of table indicates elevated level among the beedi rollers population compared to non beedi rollers (Table: 3c).
Table 3: Trace Elements in the beedi rollers population of various age groups.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>AGE</th>
<th>MEAN ± SD</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) SODIUM</td>
<td>20-30 N.B</td>
<td>127 ± 3.49</td>
<td>2.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30 B</td>
<td>129 ± 6.13</td>
<td>1.85</td>
<td>2.27**</td>
</tr>
<tr>
<td></td>
<td>30-40 N.B</td>
<td>128 ± 4.47</td>
<td>2.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-40 B</td>
<td>129 ± 4.64</td>
<td>1.85</td>
<td>2.30**</td>
</tr>
<tr>
<td></td>
<td>40-50 N.B</td>
<td>126 ± 4.60</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50 B</td>
<td>128 ± 6.39</td>
<td>1.15</td>
<td>1.82**</td>
</tr>
<tr>
<td>b) POTASSIUM</td>
<td>20-30 N.B</td>
<td>3.18 ± 0.18</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30 B</td>
<td>3.57 ± 0.43</td>
<td>.06</td>
<td>5.39**</td>
</tr>
<tr>
<td></td>
<td>30-40 N.B</td>
<td>3.20 ± 0.27</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-40 B</td>
<td>4.03 ± 0.78</td>
<td>.42</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>40-50 N.B</td>
<td>3.20 ± 0.22</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50 B</td>
<td>5.13 ± 0.72</td>
<td>.77</td>
<td>1.85**</td>
</tr>
<tr>
<td>c) CHLORIDE</td>
<td>20-30 N.B</td>
<td>93.5 ± 2.54</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30 B</td>
<td>95.4 ± 3.67</td>
<td>1.50</td>
<td>2.93**</td>
</tr>
<tr>
<td></td>
<td>30-40 N.B</td>
<td>93.8 ± 2.60</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-40 B</td>
<td>96.0 ± 4.46</td>
<td>0.50</td>
<td>3.19**</td>
</tr>
<tr>
<td></td>
<td>40-50 N.B</td>
<td>93.8 ± 2.73</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50 B</td>
<td>94.8 ± 3.08</td>
<td>0.75</td>
<td>1.24</td>
</tr>
</tbody>
</table>

** - highly significant. * Significant

4.6 Sugar

The random sugar estimated in the blood of non beedi rollers and beedi rollers showed a significant decrease (P<0.001). Non beedi rollers had an uniformly high sugar level of 175 ± 32.8 mg/dl to 176 ± 39.8 mg/dl against the normal sugar level of
80-120 mg/dl. On the otherhand, when compared to above range beedi rollers exhibited a significant decrease ranging from 132 ± 22.8 to 138 ± 19.7 mg/dl (Fig: 65).

4.6.1 Total Cholesterol

Total Cholesterol was found to be above the normal limit in beedi workers population irrespective of age it was 262 ± 33.7, 256 ± 38.6 and 251 ± 30.2 mg % in 20-30, 30-40 and 40-50 age group respectively (Fig: 66).
4.6.2 Urea

The possible level of urea in an adult female is 7-25 mg/dl. Analysis of blood urea in beedi rollers and non beedi rollers were within the normal range. However the beedi rollers exhibited a marginal increase in the blood urea level(Fig: 67).

4.6.3 Creatinine

Reports recording the creatinine level showed a highly significant increase (P< 0.001) in the beedi rollers population in the age group of 20-30 and 30-40 when compared to non beedi rollers (Fig: 68).
Liver Function Test

Liver is the vital organs in intermediary metabolism, indetoxification and in the elimination of toxic substance. Since the liver as considerable functional reserve, damage to the organ may not affect its activity. The results of the biochemical test provide a precise diagnostic and reflect the basic pathological process common to many condition. The liver function test is helps to scripe the beedi rollers exposed to potential heptotoxic materials if any in the industry. The routine liver function test includes determination of serum Total Bilurubin and enzymes test such as Alkaline Phosphate(ALP), Gamma Glutaminate Transferase(GGT), Serum Glutamic Pyruic Transaminase (SGOT), Serum Glutamite Oxaloacetic Transaminase (SGPT or AST) and Total Proteins, Albumin, Globulin.

4.6.4 Total Bilurubin

A measure of bile pigments and the bile salts in the blood can be determine by analyzing the total bilurubin. A highly significant increase in total bilurubin could be observed in beedi rollers in the age group of 20-30 (1.28 ± 0.385 mg/dl) In the age
group of 40-50 there is a significant decrease (P<0.05) in the total bilirubin level (1.20 ± 0.38 mg/dl) compared to non beedi rollers (1.36±0.395 mg/dl) (Table: 4a).

Table 4: Total Bilirubin, ALP, GGT of a Liver function test in the beedi rollers population of various age groups.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>AGE</th>
<th>MEAN ± SD</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) TOTAL BILIRUBIN (1.0 mg/dl)</td>
<td>20-30</td>
<td>N.B 1.20 ± 0.386</td>
<td>.052</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 1.28 ± 0.385</td>
<td>.056</td>
<td>.963**</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 1.37 ± 0.381</td>
<td>.053</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 1.29 ± 0.400</td>
<td>.042</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 1.36 ± 0.395</td>
<td>.054</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 1.20 ± 0.382</td>
<td>.081</td>
<td>1.52*</td>
</tr>
<tr>
<td>b) ALP (60-170 u/dl)</td>
<td>20-30</td>
<td>N.B 139 ± 3.60</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 132 ± 4.43</td>
<td>2.08</td>
<td>7.21**</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 120 ± 20.6</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 134 ± 4.83</td>
<td>2.61</td>
<td>35.8**</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 1.21 ± 19.8</td>
<td>2.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 134 ± 4.43</td>
<td>1.54</td>
<td>18.87**</td>
</tr>
<tr>
<td>c) GGT (15-45 u/dl)</td>
<td>20-30</td>
<td>N.B 39.14 ± 2.62</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 33.38 ± 5.09</td>
<td>1.01</td>
<td>6.64**</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 27.98 ± 7.50</td>
<td>2.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 33.65 ± 4.87</td>
<td>1.70</td>
<td>5.29**</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 27.01 ± 8.05</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 34.00 ± 5.69</td>
<td>2.54</td>
<td>3.47*</td>
</tr>
</tbody>
</table>

** - highly significant. * Significant
4.6.5 Alkaline Phosphate (ALP)

Changes in the enzyme Alkaline Phosphate determined in beedi rollers and non Beedi rollers did not deviate much from the normal level of 60-170 U/dl. However, the Alkaline Phosphate level decreased in beedi rollers in the age group of 20-30 and showed an increasing trend in other two age groups compared to non beedi rollers (Table: 4b).

4.6.6 Gamma Glutamitate Transaminase (GGT)

The enzyme GGT also exhibit a similar trend as that of ALP. Beedi rollers in the age group of exhibit a significant low level of GGT compared to non beedi rollers. But the GGT level in the age group 30-40 and 40-50 showed a significant increased 33.65 ±4.87 U/dl compared to 27.98 ±7.50 U/dl non beedi rollers and 34.0 ± 5.69 U/dl compared to 27.01±8.05 U/dl (Table: 4c).

4.6.7 Serum Glutamic Oxaloacetic Transaminase(SGOT)

Elevated levels of SGOT was determined in the women beedi rollers in all the age groups compared to the non beedi rollers. The non beedi rollers had a SGOT content of 39 ± 3.60 IU/l in 20-30 age group, 39.25 ± 3.07 IU/l in 30-40 age group and 38.35 ± 3.34 IU/l which was within the normal upper limit (5-40 IU/l). But in beedi rollers the SGOT range was 46.73 ±7.79 IU/l in 20-30 age group, 44.51 ±1.26 IU/l in 30-40 and 43.57 ± 6.12 IU/l which was higher than the normal value (Table: 5a).

4.6.8 Serum Glutamic Pyruvic Transaminase (SGPT)

The present observation of SGPT showed an increase in beedi rollers in the age group of 20 to 30 and 30-40. It was 47.80 ± 6.64 IU/l and 46.09 ± 8.80 IU/l
respectively, when compared to non beedi rollers who possessed 39.14±2.62 IU/l and 38.83 ± 2.78 IU/l in 20-30 and 30-40 age groups respectively. However in the age group of 40-50 the non beedi rollers had a high SGPT level than the beedi rollers (Table: 5b).

Table 5 : SGOT and SGPT levels of beedi rollers populations in different age groups.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>AGE</th>
<th>MEAN ± SD</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) SGOT (5-40 IU/l)</td>
<td>20-30 N.B</td>
<td>39.00 ± 3.60</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>46.73 ± 7.79</td>
<td>0.08</td>
<td>5.92**</td>
</tr>
<tr>
<td></td>
<td>30-40 N.B</td>
<td>39.25 ± 3.07</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>44.51 ± 1.26</td>
<td>1.42</td>
<td>2.98**</td>
</tr>
<tr>
<td></td>
<td>40-50 N.B</td>
<td>38.35 ± 3.34</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>43.57 ± 6.12</td>
<td>0.40</td>
<td>4.63**</td>
</tr>
<tr>
<td>b) SGPT(5-40 IU/l)</td>
<td>20-30 N.B</td>
<td>39.14 ± 2.62</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>47.80 ± 6.64</td>
<td>1.92</td>
<td>10.4**</td>
</tr>
<tr>
<td></td>
<td>30-40 N.B</td>
<td>38.83 ± 2.78</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>46.09 ± 8.80</td>
<td>1.99</td>
<td>10.2**</td>
</tr>
<tr>
<td></td>
<td>40-50 N.B</td>
<td>48.72 ± 2.68</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>47.89 ± 8.02</td>
<td>0.83</td>
<td>8.72**</td>
</tr>
</tbody>
</table>

** - highly significant. * Significant

4.6.9 Total Protein

The normal level of serum of total protein was 6-8 g/dl in non beedi rollers it amounts to 6.55 ± 0.291 g/dl to 6.75 ± 0.182 g/dl which was around the lower limit of the normal value. In beedi rollers there was a highly significant increase (P< 0.001) in
all age groups. It was $6.97 \pm 0.383$ g/dl, $6.87 \pm 0.310$ g/dl and $7.20 \pm 0.339$ g/dl in 20-30, 30-40 and 40-50 age groups respectively (Table: 6a).

**Table 6**: Total Protein, Albumin and Globulin Content in various age groups of beedi rollers.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>AGE</th>
<th>MEAN ± SD</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) T. PROTEIN</td>
<td>20-30 N.B</td>
<td>6.73 ± 0.875</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.97 ± 0.383</td>
<td>1.30</td>
<td>5.37**</td>
</tr>
<tr>
<td></td>
<td>30-40 N.B</td>
<td>6.75 ± 0.182</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.03 ± 0.310</td>
<td>1.53</td>
<td>7.21**</td>
</tr>
<tr>
<td></td>
<td>40-50 N.B</td>
<td>6.55 ± 0.291</td>
<td>2.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.20 ± 0.339</td>
<td>0.34</td>
<td>10.4**</td>
</tr>
<tr>
<td>b) ALBUMIN</td>
<td>20-30 N.B</td>
<td>5.28 ± 1.62</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.47 ± 1.22</td>
<td>1.16</td>
<td>-8.69**</td>
</tr>
<tr>
<td></td>
<td>30-40 N.B</td>
<td>5.46 ± 2.36</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.84 ± 1.16</td>
<td>1.13</td>
<td>-9.87**</td>
</tr>
<tr>
<td></td>
<td>40-50 N.B</td>
<td>5.42 ± 2.55</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.23 ± 2.03</td>
<td>1.46</td>
<td>-3.94**</td>
</tr>
<tr>
<td>c) GLOBULIN</td>
<td>20-30 N.B</td>
<td>1.45 ± 0.290</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.50 ± 0.390</td>
<td>1.54</td>
<td>8.20**</td>
</tr>
<tr>
<td></td>
<td>30-40 N.B</td>
<td>1.28 ± 0.904</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.02 ± 0.337</td>
<td>1.38</td>
<td>10.0**</td>
</tr>
<tr>
<td></td>
<td>40-50 N.B</td>
<td>1.13 ± 0.439</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.58 ± 0.391</td>
<td>0.89</td>
<td>11.3**</td>
</tr>
</tbody>
</table>

** - highly significant. * Significant

### 4.7 Albumin

Albumin decreased in the beedi rollers irrespective of the age. They possessed albumin level near the lower limit of the normal value (3.5-5.0 g/dl). The non beedi
rollers in the age group of 20-30 had an albumin level of 5.28 ± 1.62 g/dl whereas that of beedi rollers of the same age group showed a value of 3.47 ±1.22 g/dl. A similar decreasing in trend was observed in the other two age groups also (Table: 6b).

4.7.1 Globulin

Serum Globulin range from 1.5 to5.0 g/dl in normal women. While the non beedi rollers exhibited a lower limit of normal Globulin value, the beedi rollers showed a highly significant increase (P< 0.001) in all age categorise globulin level of beedi rollers was 3.50 ± 0.390 g/dl, 3.02 ± 0.337 g/dl and 3.5 ± 0.391 g/dl in 20-30, 30-40 and 40-50 age groups. This were very high values exceeding even the lower limit of the normal value (5.0 g/dl) (Table: 6c).

4.7.2 Thyroid profile

Analysis of Thyroid hormones such as  $T_3$, $T_4$, TSH, FT$_3$, FT$_4$, AMA and ATG in the blood of beedi rollers may help in the health assessment.

4.7.2 Total Triiodothyronine( $T_3$)

$T_3$ levels of a normal healthy women ranges from 60-200 ng/dl. In the present study the beedi rollers exhibited decreased levels of $T_3$ compared to non beedi rollers. In the age group of 20-30 the beedi rollers had a $T_3$ level of 68.80 ± 6.74 ng/dl compared to 95.23 ± 5.04 ng/dl among non beedi rollers. Similar observations was found among the age group of 30-40 and 40-50 which was much below than the normal lower limit. This decrease in $T_3$ level was highly significant (P < 0.001) in beedi rollers indicating hormone deficiency (Table: 7a).
Table 7: Thyroid Profile in beedi rollers showing T<sub>3</sub>, T<sub>4</sub> and TSH levels.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>AGE</th>
<th>MEAN ± SD</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N.B 95.23 ± 9.12</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>a) T&lt;sub&gt;3&lt;/sub&gt; (60-200 ng/dl)</td>
<td>20-30</td>
<td>B 68.80 ± 6.74</td>
<td>3.80</td>
<td>-5.97**</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 95.35 ± 5.04</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 62.20 ± 9.19</td>
<td>2.24</td>
<td>-10.5**</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 94.90 ± 8.16</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 57.52 ± 7.12</td>
<td>2.85</td>
<td>-12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N.B 95.23 ± 9.12</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>b) T&lt;sub&gt;4&lt;/sub&gt; (4.5-12 µg/dl)</td>
<td>20-30</td>
<td>B 4.43 ± 1.175</td>
<td>0.24</td>
<td>-11.5</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 8.66 ± 0.204</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 5.75 ± 0.247</td>
<td>0.61</td>
<td>-3.72**</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 8.94 ± 0.209</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 4.88 ± 0.189</td>
<td>0.43</td>
<td>-7.42*</td>
</tr>
<tr>
<td>c) TSH (0.3-5.5 µIU/ml)</td>
<td>20-30</td>
<td>N.B 3.09 ± 0.81</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 3.06 ± 0.89</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 4.39 ± 0.10</td>
<td>0.11</td>
<td>7.58</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 2.96 ± 0.95</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 4.55 ± 0.11</td>
<td>0.27</td>
<td>5.86**</td>
</tr>
</tbody>
</table>

** - highly significant. * Significant

4.7.3 Total Thyroxine (T<sub>4</sub>)

A decreasing trend in the T<sub>4</sub> content was observed in the beedi rollers. While non beedi rollers had a value within the normal limit of T<sub>4</sub> (4.5-12.5 µg/dl) beedi rollers possessed low level of T<sub>4</sub> data observed in 20-30, 30-40 and 40-50 age groups beedi rollers were 4.43 ± 0.175 µg/dl, 5.75 ± 0.547 µg/dl and 4.88 ± 0.189 µg/dl respectively.
whereas in non beedi rollers it was 8.86 ± 0.194 µg/dl, 8.66 ± 0.204 µg/dl and 8.94 ± 0.209 µg/dl (Table: 7b).

4.7.4 Thyroid Stimulating Hormone (TSH)

TSH level in beedi rollers and non beedi rollers were within the normal range (0.3-5 µIu/ml). However in beedi rollers the TSH level very near the upper normal limit in all age groups. Statistically highly significant results were observed in 20-30 age group where the beedi rollers showed 4.44 ± 0.95 µIu/ml of TSH compared to 3.09 ± 0.81 µIu/ml. In the age groups of 40-50 also the beedi rollers recorded 4.55 ± 0.11 µIU/ml of TSH against 2.96 ± 0.95 µIU/ml in non beedi rollers (Table: 7c).

4.7.5 Free Triiodo Thyronine (FT$_3$)

A decrease in the FT$_3$ level was observed in the beedi rollers which was synonymous with the T$_3$ level. Eventhough there was statistically highly significant decrease (P<0.001) all age groups of beedi rollers it remained within the normal limit of FT$_3$ level (Table: 8a).

4.7.6 Free Thyroxine (FT$_4$)

A corresponding decrease of FT$_4$ could be inferred from the data in the Beedi rollers. In the age group of 20-30 and 40-50 the FT$_4$ level went below the normal lower limit (0.7 ng/dl) and it was 0.69 ± 0.21 mg/dl and 0.66 ± 0.16 ng/dl respectively (Table: 8b).

4.7.7 Anti Microsomal Antibody (AMA)

The Antimicrosomal antibodies in beedi rollers showed statistically significant values (P<0.05) in the age group of 30-40 (48.66 ± 27.6 U/ml) and highly significant
Table 8: FT₃, FT₄, AMA and ATG levels of thyroid profile in beedi rollers population.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>AGE</th>
<th>MEAN ± SD</th>
<th>SE</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) FT₃ (1.7-4.2 pg/ml)</td>
<td>20-30</td>
<td>N.B 2.70 ± 0.76</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 1.42 ± 0.54</td>
<td>0.07</td>
<td>-9.51**</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 2.60 ± 0.83</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 1.48 ± 0.55</td>
<td>0.06</td>
<td>-9.27**</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 2.64 ± 0.70</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 1.33 ± 0.52</td>
<td>0.12</td>
<td>-7.39</td>
</tr>
<tr>
<td>b) FT₄ (.7-1.80 ng/dl)</td>
<td>20-30</td>
<td>N.B 1.20 ± 0.37</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 0.69 ± 0.21</td>
<td>0.02</td>
<td>-8.38**</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 1.51 ± 0.46</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 1.34 ± 0.53</td>
<td>0.59</td>
<td>-2.34*</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 1.21 ± 0.42</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 0.66 ± 0.16</td>
<td>0.36</td>
<td>-5.41**</td>
</tr>
<tr>
<td>c) AMA (36-49 U/ml)</td>
<td>20-30</td>
<td>N.B 38.52 ± 4.08</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 45.15 ± 6.95</td>
<td>2.96</td>
<td>5.45</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 38.57 ± 3.77</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 48.66 ± 27.6</td>
<td>2.11</td>
<td>2.65*</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 39.55 ± 3.80</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 45.47 ± 1.21</td>
<td>1.27</td>
<td>6.63**</td>
</tr>
<tr>
<td>d) ATG (225-325 IU/ml)</td>
<td>20-30</td>
<td>N.B 255 ± 14.8</td>
<td>4.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 287 ± 20.4</td>
<td>2.83</td>
<td>1.76*</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>N.B 268 ± 34.0</td>
<td>4.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 281 ± 15.4</td>
<td>1.73</td>
<td>2.95**</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>N.B 259 ± 28.6</td>
<td>3.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 277 ± 11.8</td>
<td>2.72</td>
<td>1.21</td>
</tr>
</tbody>
</table>

** - highly significant. * Significant

in the age group of 40-50 (45.47 ± 1.21 U/ml). While the non beedi rollers showed the AMA level near the normal lower limit (36 to 49 U/ml). The AMA level in the beedi worker population on the otherhand, were near the upper normal limit indicating elevated levels of AMA (Table: 8c).
4.7.8 Anti Thyro Globulin (ATG)

ATG in beedi rollers showed a marginal increase than the non beedi rollers and was found within the normal level (225-325 IU/ml). The increase in ATG was found to be statistically highly significant (p< 0.001) in 20-30 and 30-40 of beedi rollers population (Table: 8d).

4.8 Discussion

The present study dealing with the health assessment of women beedi rollers in Melapalayam has revealed that most of them suffer from pulmonary, gastrointestinal and cardiac disorders apart from various occupational hazards. Routine analysis of the blood parameters of 150 women beedi rollers from the age group of 20-50 and 150 non beedi rollers were carried out in Vimta Laboratory, Vannarpettai Tirunelveli. Total RBC (TEC) in beedi rollers were significantly lower in comparision to the control subjects. Yasmin et al., (2010) has reported that the TEC level of women beedi rollers of Patna in Bihar was significantly low than the control. In another comparable study, Karafakioglu et al.,(2009) found a significant fall in RBC count in women workers who harvest tobacco, after the harvest as compared to the count before the harvest. Metin et al.,(2004) and Rajesekhar et al.,(2007) found a reduction in RBC count in smokeless tobacco consumers. Adeniyi and Ghazal (2006) studied the effects of tobacco leaf extracts on Wister rats and found a significant reduction in RBC count. According to the doctors of Indian Medical Association Karim Nagar, majority of the women beedi rollers were suffering from anaemia and they have low count of RBC and WBC they added. They said that the beedi rolling was very high among the womenfolk of Sircilla and Jagtial revenue divisions such as Karimnagar town, Hushabad, Chigurumaidi Mandals (The Hindu, 2011). Total WBC count of beedi rollers was also significantly
lower as compared to control. These findings are in accordance with the earlier reports of Yasmin et al., (2010). Decrease in WBC count may be due to exposure to tobacco dust. Metin et al.,(2004) and Rajasekhar et al.,(2007) also found a reduction in WBC count in smokeless tobacco consumers. Valenca et al.,(2008) observed a reduction in leucocyte count in Wistar rats treated with oral nicotine. Rahman(2000) had also reported a drop in white blood cell count among beedi production workers.

In the present study haemoglobin levels were also lower among the beedi rollers. Rajesekhar et al.,(2007) also found a reduction in haemoglobin percentage in smokeless tobacco consumers. Adeniyi and Ghazal (2006) found a reduction in haemoglobin level of Wister rats in response to extract of tobacco leaves. Karafakioglu et al.,(2009) found a significant decrease in haemoglobin level in women workers who harvest tobacco, after the harvest as compared to the level before the harvest. Low haemoglobin levels with or without an absolute decrease of RBCs may lead to symptoms of anaemia. The platelet count of beedi rollers was also significantly lower as compared to the controls. Metin et al.,(2004) also found a reduction in platelet count in consumers of Maras powder (smokeless tobacco). Adeniyi and Ghazal (2006) found a significant reduction in platelet count in Wister rate as a result of exposure to tobacco leaf extracts. Increased ESR encountered in beedi rollers can be also be attributed to the inflammatory responses due to the inhalation of the toxic chemicals released by the raw tobacco dust Gupta et al., (2005). It is evident that inflammatory changes may be due to the proteins in the red blood cells causing them to bind one another clumps, making them denser than normal red blood cells. Thus the beedi rollers experienced inflammatory disorders causing stiffness and pain usually in the shoulder, neck, upper arms, hip and thigh (Sood,2002).
Other blood parameters such as trace elements also showed fluctuations in beedi rollers compared to the non beedi rollers. A decreased concentration of sodium reported as hyponatremia is prevalent among the beedi rollers. However, the level was not too little to be fatal. This condition occurs whenever there is a relative increase in amount of body water relative to sodium. This happens to occur with some diseases of liver and kidney and the patients with burn victims and numerous other conditions hyperkalemia (increased potassium level) has been observed in beedi rollers. Conditions of hyperkalemia may lead to irregular heart beats, since potassium is found to regulate the heart beat in the body. Chloride level shows an increasing trend in beedi roller which may lead to the condition of acitosis. There is no evidence of the status of blood urea and creatinine among the beedi rollers due to raw tobacco dust, eventhough increased creatinine level has been observed, which may be mainly due to the muscle degeneration caused by the ergonomic postures during beedi rolling.

The overall health status of a person can be assessed by infering results of the liver function test, since the liver plays a role in the removal of toxins. Differences in total bilirubin, ALP, GGT, SGOT, SGPT, and proteins may serve as indices of the health status. Bilirubin is the by product of the normal breakdown of red blood cells. Elevated bilirubin levels may be related to haemolytic anaemia among beedi rollers which may be correlated to a significant decrease in their total RBC content. ALP and GGT level are indicative of liver dysfunction. Beedi rollers exhibit fluctuation in these levels compared to non beedi rollers which may be caused due to nicotine toxicity (Cashman et al., 1992).

SGOT and SGPT are two important enzymes of the liver and other body tissues which are found to play a role in processing protein. Elevated levels in beedi rollers
indicate liver inflammation due to the inhalation of raw tobacco dust and its constituent nicotine. Similar observation was reported by Yasmin et al., (2010). It is suggested that nicotine may be absorbed through the skin of the fingers and palm and throughout the body. It is finally metabolized by the liver (Cashman et al., 1992; Snyder and Sheafor 1999; Neurath 1994). Karafakiooglu et al (2009) also found a significant rise in SGPT level of women workers who are involved in the harvest of tobacco.

Liver function test include measuring albumin and globulin, the major blood protein produced by the liver. Changes in the amount of albumin and globulin indicate abnormality of the plasma protein produced by the liver. In the present study the albumin level has decreased in the beedi rollers while globulin elicit a significant increase. Clinical significance of these findings prove that albumin decrease and globulin increase in advance liver disease (Godkar, 2007).

Thyroid diseases occur about five times more frequently women than in men (Srinivasulu, 1997). Smoke exposure may affect various metabolic and biological processes including hormone biosynthesis and secretion, interfere with thyroid hormone release, binding, transport, storage and clearance, associated with adverse effects on the thyroid resulting in changes in circulating hormone concentration. A comprehensive approach to assess the interrelationship between a raw tobacco dust and its constituents on the thyroid hormone levels revealed symptoms of physiological disorders in women beedi rollers. Behera et al. (1995) Yet published data are inconsistent to provide adequate evidence that unburnt tobacco smoke may affect beedi rollers. Free thyroid hormone (FT$_3$ and FT$_4$) represent a more useful index of thyroid status then total thyroid hormone because FT$_3$is influence by variations of thyroid hormone binding protein (Thyroid binding globulin TBG) (Bartalena et al., 1995). In the present
investigation detailed analysis of thyroid hormone such as TSH, T₃, T₄, FT₃, FT₄, AMA and ATG employing innovative modern hormone analysis techniques predict notable changes in the hormone levels which affects the beedi rollers when compared to normal subjects.

In the present study the T₃ and T₄ levels were significantly low in the beedi rollers, compared to non beedi rollers. Circulating TSH was found to be higher in beedi rollers. These results agree with the earlier findings of Behara et al.,(1995) who reported alternations in circulating T₃ and T₄ levels in Indian smokers. Sepkovic et al.,(1984) found reduced serum T₃ and T₄ levels in heavy smokers. Christensen et al.,(1984) and Nystrom et al.,(1993) have reported reduced T3 level in smokers. A danish study reported positive association between tobacco smoke and hypothyroidism. Vestergaard et al.,(2000) Fukata et al.,(1996) suggest that tobacco smoke may increase the risk of hypothyroidism in humans. However, high levels of T₃ and T₄ as well as decreased TSH levels. Melander (1981), Eden et al.,(1984), Hegedus et al.,(1985), Ericsson and Lindgarde et al.,(1991), Petersen et al., (1991), Fisher et al.,(1997) and Kundsen (2002), Vestergaard (2002) opined that association of tobacco smoke and hypothyroidism is controversial and previous results are not homogenous.

Pontikides and Krassas( 2002 ) in their review reports serum thyroxine level and serum T₃ level may remained unchanged or elevated due to tobacco dust. Hegetus et al.,(1985 and 1992) in two analogous studies found no different in Total T₃, T₄ and FT₃ levels in relation to tobacco smoke.

Exposure to passive smoke was accompanied by a statistically significant increased in T₃ and FT₄ levels. Whereas, TSH showed a non significant decrease. These findings are line with previous results (Greenspan et al.,1986, Fisher et al.,1997,
Ericsson *et al.*, 1991) and provide support to the notion that the increases in T$_3$ and T$_4$ seen in our study were not attributed to TSH secretion but to the probably short term effect of tobacco dust that cannot be identified with the current data.

Active and passive exposure to tobacco smoke is associated with mild inhibitory effect on the thyroid reflected in higher serum T$_4$ and T$_3$. Soldin *et al.*, (2009) recently documented mild inhibitory effect of secondhand smoke on serum T$_3$ and T$_4$ among women chronically exposed to it and further mean TSH level significantly low. Anti microsomal antibodies, Anti thyroglobulin estimated in beedi rollers also showed fluctuations indicating thyroid dysfunction. Thiocyanate in tobacco smoke may influence the thyroid gland positively or negatively and cause imbalance in the hormone levels. (Bertelsen and Hegedus, 1994).

Beedi rollers handle tobacco flakes and inhale tobacco dust as well as volatile components of tobacco dust as well as volatile components of tobacco which put them at a high risk of many health problems. Women beedi rollers face numerous health problems possibly due to direct inhalation of tobacco flakes and dust (Yasmin *et al.*, 2010). Nicotine get absorbed through the skin while rolling tobacco and has been demonstrated in the blood of beedi rollers who do not smoke (Ghosh, 2005). Nicotine is readily absorbed from the respiratory tract, buccal mucosa membrane and skin. While preparing beedi ingredients tobacco is likely to be absorbed through intact skin of the hands and inhaled as fine dust (Sumen *et al.*, 2007).

Scientists discovered large amount of nicotine a tobacco specific compound, found in its way into the blood stream of beedi rollers. The average level of cotinine a compound found when nicotine is broken down in the liver, found in the urine and
saliva of beedi rollers who did not consume tobacco, was eight times that found in passive smokers (Khanna, 1993).

The constituents of tobacco get absorbed into the body, get bioactivated and result in increased risk of developing ailments for which tobacco consumption is a major risk factor (Mittal et al., 2008). Investigations also show that these tobacco processors are exposed to extremely high levels of inspirable tobacco particulars. Considering the high content of Nicotine and other chemicals in beedi tobacco, these workers are at extremely high risk of developing systemic illness (Malson et al., 2001). From the above study it’s found that tobacco use is a critical health concern for women. “Protecting and promoting the health of women is crucial to health and development not only for the citizen of today but also for those of future generations”.
