CHAPTER: 4

BIOCHEMISTRY
CHAPTER 4: BIOCHEMISTRY

4.1: INTRODUCTION

Carcinoma of the uterine cervix is the most important Cancer to affect the female genital tract. A variety of factors nutritional, demographic and biochemical appear to be associated with the disease. If these factors could be combined and their interrelationship taken into account, it may be possible to estimate more preciously the risk of future cancer. As there is little data available in India, on the biochemical profile of women suffering from cancer cervix, this study will provide the much needed baseline data encompassing nutritional and epidemiological status.
4.2: REVIEW OF LITERATURE

Research has shown studies on several biochemical and histochemical aspects of cervical carcinoma and explores their use in follow-up of patients undergoing radiotherapy. Material came from 19 patients with invasive cervical carcinoma admitted to Kenyatta National Hospital. A control group consisted of 20 women matched for age who attended clinics at the hospital but were not suffering from any malignant disease; control tissue for histological examination was obtained from 3 women who had undergone hysterectomy for uterine fibroids. Biochemical assays for alkaline and acid phosphatases in patients with cervical carcinoma show an increase in alkaline phosphatase in carcinomatous tissue (35.7 umoles/hr/mg) as opposed to normal tissue (7.2). When examined histochemically, increased alkaline phosphatase activity was observed in connective tissue, epithelium of the glands and blood capillaries of tumor tissue. 1 section containing normal tissue bordering carcinomatous tissue demonstrated normal alkaline phosphatase activity in the normal tissue and increased activity in the tumor tissue. Results demonstrate a clear difference in activities of these enzymes in carcinomatous tissue and normal tissue, which may be of value in follow-up care. Orinda et al., (1985).

The investigation interpretations of changes in serum enzymes in diseases like carcinoma is one of the most rapidly expanding filed in clinical biochemistry. Wroblewski et al., (1981), published his first paper on serum enzyme like SGOT, LDH and brought the possibilities of these enzymes assays in general notice. Thus, began the present efflorescence of clinical enzymology and large number of enzymes have been used for diagnosis and prognosis of various diseases including carcinoma cervix.
The body has a considerable factor of safety in renal as well as hepatic tissue. One healthy normal kidney can do the work of two, and if all the other organs are functionally properly less than a whole kidney can suffice.

Renal function begins with the appreciation of:

(a) **Patient history** of oliguria, polyuria, nocturia, ratio of frequency of urination in day and night time. Appearance of oedema is also important.

(b) **Physical examination** of urine in the side room for the presence/absence of albumin, microscopic examination of urinary deposits specially pus cells, RBCs and casts etc.

(c) **Biochemical parameters** of three nitrogenous constituents of blood is believed to reflect a deteriorating kidney function. Some authorities claim that serum uric acid normally rises first, followed by urea and finally increase in creatinine. However, other causes of uric acid rise should be kept in mind. *Chatterjee et al., 2007.*

Analysis of multiple proteins is thought to be essential for establishment of signature proteomic patterns that may distinguish cancer from non-cancer. Serum protein estimation along with surface-enhanced laser desorption/ionization (SELDI) is an affinity-based mass spectrometric method in which proteins of interest are selectively absorbed to a chemically modified surface on a biochip. This technology may provide protein profiling of a variety of biological specimens. In this study, it was explored whether the protein biochip SELDI approach could differentiate cervical cancer from non-cancer cohorts. Screening of protein profiles generated by SELDI in 62 cervical epithelial cell samples microdissected from 35 invasive cervical cancer and 27 age-
matched normal cervix tissue specimens, respectively. The cell lysates of pure populations of cervical cells were applied onto Ciphergen Protein Chip® WCX2 Arrays. Proteins bound to the chips were analyzed on a Protein Chip Reader Model PBS II. Derived proteomic patterns were converted to a simple proteomic scoring for distinguishing cancer from non-cancer cohorts. SELDI protein profiles of cell lysates from 20 cervical cancer and 15 normal cervix tissue specimens were used to train and develop a classification scoring system that used a seven-protein mass pattern. The training samples could be correctly discriminated. When a test set of 27 samples was used for evaluation of this scoring system to distinguish cervical cancer from non-cancer, a sensitivity of 87%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 86% for the test population were obtained. All seven proteins appeared to be down regulated in cervical cancer. The results from this study indicate that the proteomics approach of SELDI mass spectrometry, in combination with a simple scoring system, may distinguish cervical cancer from its normal counterpart. If this approach along with serum protein and the workable in the analysis of cervical exfoliated cell lysate, it might potentially be used in the early diagnosis of invasive cervical cancer.

In addition, the identification of these specific proteins in cervical cancer may also facilitate the discovery of new cervical tumour marker(s). Wonga et al., 2004.
4.3: MATERIALS & METHODS

One hundred patients were screened for cancer of the uterine cervix from the outpatient attending the gynaecology clinic at Silchar Medical College & Hospital, Silchar. Among these, fifty patients in different stages of the disease were selected. The entire study was conducted in patients prior to treatment.

To establish the normal parameters, twenty five healthy women in the age group 35-65 without any clinical evidence of cancer cervix, and who were not on oral contraceptive were used for the study. Ten age matched chronic cervicitis patients served as controls.

Staging was done according to an internationally accepted format of the International Federation of Gynaecology and Obstetrics (FIGO).

From the 50 patients selected for the study, 10, 15, 20 and 5 patients were in stages I, II, III, and IV respectively. Blood was collected in the fasting state without venous stasis. Serum free from hemolysis was used for the assay of various biochemical parameters under study. Erythrocytes were used for the assay of alanine and aspartate transaminases.

From the out patients attending the gynecologic clinic at Silchar Medical College and Hospital, Silchar, 50 patients with clinically and histopathologically confirmed diagnosis of carcinoma of the cervix (untreated) and were selected for the study. The age distribution of the patients was between 35-70 years and belonged to the lower class of social strata with 92 percent illiteracy. Staging of the disease was done by a gynaecologist according to the International Federation of Gynaecology and Obstetrics (FIGO). The majority of the cases (N=20) were in the
stage III, 10 were in stage I, 15 were in stage II and 5 were in stage IV of the disease.

To establish the normal parameters, twenty five healthy women in the age group of 35 to 70 years, without any clinical evidence of cancer cervix and who were not on oral contraceptives and non-smokers were taken as first set of controls/ (denoted as control I) Ten age matched chronic cervicitis patients served as second set of controls. (Denoted as control II).

Tests of liver function:

Because the functions of the liver are manifold, it is not surprising that tests of its efficiency are also numerous. These are of two main kinds, those which test the excretory powers of the liver and those which estimate the metabolic function.

Laboratory tests play an important role in the clinical assessment of hepatic dysfunction. First, they are of value in detecting abnormalities in the liver. This is particularly important in the non-jaundiced patient. Secondly, once an abnormality is demonstrated, the pattern of abnormal test results may confirm or suggest the nature of the underlying liver disorder in both jaundiced and non-jaundiced patients. Thirdly, same tests provide an index of the extent of hepatic disease and thus offer same prognostic information. Finally, the evaluation of test results serves as a valuable means of following the course of hepatic dysfunction and its management.

Many tests have been proposed for assessment of liver disease. The present discussion is oriented to an analysis of tests that we feel are of most value in the clinical appraisal of liver diseases. These include serum total protein, albumin, globulin, total bilirubin and enzymes-
alkaline phosphatase, alanine amino transferase, aspartate amino transferase, lactate dehydrogenase and gamma glutamyl transpeptidase.

**Tests of kidney function:**

There are a great many tests of renal function and many diagnostic procedures for determining the nature of the underlying renal disease, but in most patients, a few simple tests suffice. These include urea, creatinine and uric acid.

The entire exercise of kidney and liver function study has been done to eliminate from the study, those patients with impaired liver and renal involvement.

**Total protein (Kingsley, G.R. 1942):**

Protein gives a blue purple colour with alkaline copper sulphate solution.

**Reagents:**

**Stock biuret reagent:**
- Copper sulphate $\text{5 H}_2\text{O}$ : 0.06 moles $\text{l}^{-1}$
- Potassium tartarate : 0.21 moles $\text{l}^{-1}$
- Potassium iodide : 0.03 moles $\text{l}^{-1}$

Dissolved 45g of sodium potassium tartarate in 400 ml of 0.2 N sodium hydroxide. 15g of copper sulfate was weighed and dissolved separately. Then 5.0g of potassium iodide was added and made up to a litre with 0.2 N sodium hydroxide.

**Dilute biuret reagent:**

50 ml of stock biuret diluted to 500 ml with 0.2 N sodium hydroxide containing 5.0g of potassium iodide per litre.
Standard protein solution:
Weighed 6.0g of bovine serum albumin and made up to 100ml with distilled water.

Procedure:
To 5.0 ml of biuret reagent, 0.1ml of serum was added, incubated for 15 minutes at 37°C. Read at 540 nm against the reagent blank. Calculation was done against series of standard treated similarly. Albumin (Bartholomew, R.J., and Delony, A.M. 1966)

Bromocresol green in alkaline medium binds to albumin giving a green colour.

Reagents:
- Sodium citrate: 1 moles l\(^{-1}\)
- Dissolved 29.4 g of sodium citrate dehydrate in water and made up to 100ml with water.
- Citric acid: 1 moles l\(^{-1}\)
- Dissolved 21g of citric acid monohydrate and made up to 100 ml with water.
- Bromocresol green stock: 0.01 moles l\(^{-1}\)
- Added 0.698g of bromocresol green to 9.8 ml of 0.1 N sodium hydroxide and made up to 100ml with water. 17.3 ml of 1 M sodium citrate 32.7 ml of 1 M citric acid 6.0 ml of bromocresol green stock.
- Diluted to a litre adjusting the pH to 3.8 using citric acid or citrate.
Procedure:

To 5.0 ml of bromocresol green reagent, added 0.05 ml of serum. Incubated for 15 minutes at 37°C and read at 620 nm against the reagent blank. Calculation was done against series of standards treated similarly.

Globulin:

The differences between total protein and albumin has been taken as the globulin levels in g/dl.

Bilirubin (Jendrassik, L. 1938):

Bilirubin is coupled with diazotized sulfanilic acid in the presence of caffeine to give an azo dye.

Reagents:

Sulfanilic acid/Hydrochloride acid:

- Sulfanilic acid : 29 mM
- Hydrochloride acid : 0.17 N
- Sodium nitrite : 25 mM

Caffeine solution:

- Caffeine : 0.26 moles l⁻¹
- Sodium benzoate : 0.52 moles l⁻¹

Tartarate/sodium hydroxide:

- Tartarate : 0.98 moles l⁻¹
- Sodium hydroxide : 1.9 N
Procedure:

Into a series of test tubes, added 0.2 ml of sulfanilic acid in hydrochloric acid, one drop of sodium nitrite, 1.0 ml of caffeine-sodium benzoate solution and 0.2 ml of serum. Prepared corresponding sample blank for each test in the same manner, omitting sodium nitrite solution. Mixed and allowed to stand at 20°-25°C for 10 minutes. Then added 1.0 ml of tartarate in sodium hydroxide solution to the sample blank and the tests. Mixed and allowed to stand at 20°-25°C for 10 minutes and read the absorbance of the sample against the sample blank at 578 nm.

ALKALINE PHOSPHATASE (Bessey, O.A., Lowry, O.A., and Brock, M.J. 1946)

\[
\text{AP} \\
\text{R nitro phenyl phosphate + water} \rightarrow \text{phosphate + p nitro phenyl}
\]

Reagents:

Suffered substrate:

- Sodium p nitro phenyl phosphate : 5.5 mMl⁻¹
- Glycine buffer : 50 mM l⁻¹, pH 10.5

Procedure:

To 0.05 ml of serum, added 2.0 ml of buffered sodium p nitro phenyl phosphate. Mixed and incubated for 1 minute at 37°C and read through a flow cell at the interval of one minute for 3 minutes at 405 nm. Form the mean absorbance change per minute, the enzyme activity was calculated.
Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALAT) (Reitman, S., and Frankel, S. 1957)

Reagents:

- Phosphate buffer : 0.1 moles l\(^{-1}\), pH, 7.4
  
  11.3g of dry anhydrous disodium hydrogen phosphate and 2.7g of dry anhydrous potassium dihydrogen phosphate was dissolved and made up to a litre with distilled water. pH was adjusted to 7.4.

- DL Aspartic acid : 200 mM l\(^{-1}\)
- Ketoglutaric acid : 2 mM l\(^{-1}\)

  Dissolved 13.3g of DL aspartic acid in 90 ml of normal sodium hydroxide. Add 0.146 g of ketoglutaric acid and dissolved by adding sodium hydroxide, adjusted the pH to 7.4. Made up to 500 ml with phosphate buffer and stored at -20°C

Alanine substrate:

- Alanine : 200 mM l\(^{-1}\)
- Ketoglutaric acid : 2 mM l\(^{-1}\)

  Dissolved 9.0g of alanine in 90 ml of water with the addition of 2.5 ml normal sodium hydroxide to adjust the pH to 7.4. Added 0.146g of Ketoglutaric acid, adjusted the pH to 7.4 and made up to 500 ml with phosphate buffer and stored at -20°C.

- Sodium hydroxide : 0.4 N

  16g of sodium hydroxide was made up to 1 litre with distilled water.

2,4, Dinitrophenyl hydrazine (DNPH) : (1 mM l\(^{-1}\)).
Dissolved 19.8mg of DNPH in 10 ml of concentrated hydrochloric acid and made up to 100 ml with distilled water. Stored at room temperature.

Stock pyruvate standard (20 mM)
200mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer.

Working pyruvate
1 ml of stock standard was diluted to 5.0 ml with phosphate buffer and stored at -15°C.

Procedure:
To 0.1 ml of serum, added 0.5 ml of AST/ALAT substrates and incubated at 37°C for one hour and 30 minutes respectively. 0.5 ml of 2,4 dinitrophenyl hydrazine solution was added and allowed to stand for 20 minutes at room temperature. Added 5.0 ml of 0.4 N sodium hydroxide, mixed well and allowed to stand at room temperature for 20 minutes and read at 530 nm against the reagent blank. Values for the samples were obtained from the standard table.

Total Lactate Dehydrogenase (LDH) (Anon 1970):

\[
\text{Pyruvate + NADH + H}^+ \xrightleftharpoons{\text{LDH}} \text{L Lactate + NAD}
\]

Reagents:
Buffered substrate:
- Phosphate buffer : 53 mM l⁻¹, pH, 7.5
- Pyruvate : 0.63 mM l⁻¹
- NADH : 11 mM l⁻¹
To 3.0 ml of the buffered substrate, added 0.05 ml of NADH and 0.1 ml of serum, mixed well and read the initial absorbance after 30 seconds and repeated readings exactly at 1 minute intervals for 3 minutes at 340nm. From the mean absorbance change per minute, the enzyme activity was calculated.

_Urea_ (Wybenga, D.R., Glorgio, D.I., Jr., and Pileggi, 1971). Urea reacts with acidic diacetyl monoxime on heating to form a coloured complex. Thiosemicarbazide intensifies the colour of the reaction.

**Reagents:**

**Urea reagents:**
- Thiosemicarbazide : 0.5 mM l⁻¹
- Cadmium sulfate : 9.5 mM l⁻¹
- Orthophosphoric acid : 85%
- Concentrated sulfuric acid : 36 N

44 ml of concentrated sulfuric acid and 66 ml of 85% orthophosphoric acid was added to 100 ml of water, cooled to room temperature. 50mg of thiosemicarbazide and 2.0g of cadmium sulfate were dissolved separately and added to the above solution. Mixed and made up to 1 litre.

**Coloured reagent:**
- Diacetyl monoxime : 0.2 moles l⁻¹
  20g of diacetyl monoxime was dissolved in water and made up to a litre. Stored in an amber bottle.
- Stock urea standard : 8.32 mM l⁻¹
Weighed 500mg of urea and made up to 50 ml with distilled water.

**Working urea standard:**
Diluted 5.0 ml of stock urea to 50 ml with distilled water.

**Procedure:**
To a series of labeled test tubes, added 0.05 ml of serum, 5.0 ml of urea reagent and 0.5 ml of colour reagent. Mixed well and heated in a boiling water bath for 12 minutes. Cooled and read the absorbance at 540 nm against the reagent blank. Calculation was done against series of standards treated similarly.

**Creatinine (Taussky H.H.J, 1954):**
Creatinine reacts with alkaline picrate solution to give an orange red coloured creatinine picrate.

**Reagents:**
- Picric acid : $0.036 \text{ moles l}^{-1}$
- 9.16g of reagent grade picric acid was made up to a litre with distilled water.
- Sodium hydroxide : 2.4 N

**Tungstic acid:**
Dissolved 1.0g of polyvinyl alcohol in 100 ml of water by warming. Cooled and transferred to a litre volumetric flask, to which 11.1g of sodium tungstate (0.038 N) was added. Added 2.1 ml of
concentrated sulfuric acid in 300 ml of water in another container. This was added to the volumetric flask, mixed well and made up to a litre with distilled water.

**Creatinine standard:**

Creatinine 1.17 mM/hydrochloric acid (0.1 N).

**Procedure:**

To a series of labeled test tubes, added 4.0 ml of tungstic acid and 0.5 ml of serum. Mixed well, and centrifuged for ten minutes.

To 3.0 ml of the supernatant, added 1.0 ml of picric acid and 0.5 ml of 1.4 N Nabh. Allowed to stand at room temperature for 15 minutes and read at 500 nm against the reagent blank. Calculation was done against series of standards treated similarly.

Uric acid (Caraway, W.J. 1955)

Uric acid reacts with phosphotungstic acid in the presence of alkali to form a blue complex.

**Reagents:**

- Sodium carbonate : 10%

Diluted sodium tungstate :

To 50 ml of $\frac{2}{3}$ N sulfuric acid, added 50 ml of 10% sodium tungstate and made up to the volume to 800 ml with water.

**Stock uric acid standard:**

Weighed 60mg of lithium carbonate (8.1 mM/L) in about 15 ml of water. Heated to 60°C and poured this hot solution on to 100 mg of uric
acid (5.9 mM/L). 2.0 ml of 40% Formalin and 2.5 ml of concentrated sulfuric acid was added drop wise and diluted to 100 ml.

Diluted working standard : 59 mM/L

1.0 ml of the stock uric acid standard was diluted to 100 ml with distilled water.

Stock phosphotungstic acid

Dissolved 50 g of sodium tungstate (0.34 M) in 400 ml of water. 40 ml of 85% orthophosphoric acid was added. Refluxed for 2 hours. Cooled and made up to 500 ml with water.

Diluted phosphotungstic acid

Diluted 10 ml of Stock phosphotungstic acid to 100 ml with distilled water.

Procedure:

To a series of labeled test tubes, added 5.4 ml of diluted tungstic acid and 0.6 ml of serum. Mixed well and centrifuged after 10 minutes.

To 3.0 ml of the above supernatant, added 0.6 ml of sodium carbonate and 0.6 ml phosphotungstic acid. Kept at room temperature for 30 minutes. Read at 600 nm against a reagent blank. Calculation was done against series of standards treated similarly.
All statistical analysis was done by:

**T test for two population means with variance unknown but unequal**

**Hypothesis of the Test:**

1. Null Hypothesis: $H_0 : \mu_1 = \mu_2$
2. Alternative Hypothesis: $H_1 : \mu_1 \neq \mu_2$ or $H_1 : \mu_1 > \mu_2$ or $H_1 : \mu_1 < \mu_2$

**Test Statistic**

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)}} \sim t \text{ distribution with } n_1+n_2-2 \text{ df}$$

with $S_1^2 = \frac{1}{n_1-1} \sum_{i=1}^{n_1} (x_i - \bar{x})^2$ and $S_2^2 = \frac{1}{n_2-1} \sum_{i=1}^{n_2} (y_i - \bar{y})^2$

This test is also called as the Fisher’s t test. (*Wicox et al., 2010*)
4.4: RESULTS AND DISCUSSION

Table 4.1: Test of liver function:

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein g/dl Mean ± SD</th>
<th>Albumin g/dl Mean ± SD</th>
<th>Globulin g/dl Mean ± SD</th>
<th>Bilirubin (total) mg/dl Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Normal (N=25)</td>
<td>8.35 ± 0.61</td>
<td>5.29 ± 0.45</td>
<td>3.05 ± 0.20</td>
<td>0.80 ± 0.14</td>
</tr>
<tr>
<td>(ii) Chronic cervicitis (N=10)</td>
<td>7.50 ± 0.45</td>
<td>5.24 ± 0.33</td>
<td>3.01 ± 0.18</td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td>Cases:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stag I (N=10)</td>
<td>7.42 ± 0.50</td>
<td>4.22 ± 0.45</td>
<td>2.17 ± 0.45</td>
<td>0.50 ± 0.12</td>
</tr>
<tr>
<td>Stage II (N=15)</td>
<td>6.22 ± 0.52</td>
<td>4.13 ± 0.48</td>
<td>2.19 ± 0.37</td>
<td>0.45 ± 0.11</td>
</tr>
<tr>
<td>Stage III (N=20)</td>
<td>5.35 ± 0.51</td>
<td>3.09 ± 0.62</td>
<td>1.12 ± 0.33</td>
<td>0.38 ± 0.13</td>
</tr>
<tr>
<td>Stage IV (N=05)</td>
<td>4.49 ± 0.48</td>
<td>2.57 ± 0.32</td>
<td>1.01 ± 0.37</td>
<td>0.22 ± 0.20</td>
</tr>
</tbody>
</table>

4.4: DISCUSSION

Above table shows adverse effect of liver function in carcinoma of the cervix compare to normal healthy individual in our series. Wonga et al., 2004 also found similar result by proteomics approach of SELDI mass spectrometry.
4.5: CONCLUSION

The above table shows a significant difference (P value < 0.05) in all the parameters like albumin, globulin and bilirubin etc. between the control healthy individual and the second control with the difference stages of the carcinoma of the cervix.
Table 4.2: Serum levels of enzymes:

<table>
<thead>
<tr>
<th>Group</th>
<th>Alkaline phosphate U/L Mean ± SD</th>
<th>ALAT U/L Mean ± SD</th>
<th>AST U/L Mean ± SD</th>
<th>LDH U/L Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Normal (N=25)</td>
<td>36.09± 7.15</td>
<td>9.76±2.60</td>
<td>15.20±3.15</td>
<td>140.40±29.91</td>
</tr>
<tr>
<td>(ii) Chronic cervicitis (N=10)</td>
<td>39.90±3.80</td>
<td>20.60 ±2.63</td>
<td>29.75±2.25</td>
<td>165.00±20.30</td>
</tr>
<tr>
<td>Cases:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I (N=10)</td>
<td>40.60±6.07</td>
<td>38.77±3.20</td>
<td>38.65±3.80</td>
<td>177.40±20.37</td>
</tr>
<tr>
<td>Stage II (N=15)</td>
<td>42.50±7.60</td>
<td>55.93±3.37</td>
<td>40.15±3.90</td>
<td>182.03±20.07</td>
</tr>
<tr>
<td>Stage III (N=20)</td>
<td>47.85±6.34</td>
<td>65.69±3.57</td>
<td>48.60±3.82</td>
<td>197.47±21.50</td>
</tr>
<tr>
<td>Stage IV (N=05)</td>
<td>50.08±6.35</td>
<td>70.20±1.75</td>
<td>50.90±2.04</td>
<td>220.70±24.43</td>
</tr>
</tbody>
</table>

4.4: DISCUSSION

Orinda et al., (1985) also clearly mentioned about the increase of enzymatic activity with the gradual progress of the disease.

4.5: CONCLUSION

The above table clearly shows a significant difference of P value less than 0.05 by Fisher's Test indicating elevated enzymes levels with the progress of the disease which can be utilised as a prognostic factor before and after starting the treatment.
Table 4.3: Test of kidney function:

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mg/dl) Mean ± SD</th>
<th>Creatinine (mg/dl) Mean ± SD</th>
<th>Uric acid (mg/dl) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Normal</td>
<td>37.86 ± 5.62</td>
<td>1.20 ± 0.13</td>
<td>2.83 ± 0.72</td>
</tr>
<tr>
<td>(N=25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ii) Chronic cervicitis (N=10)</td>
<td>54.50 ± 4.65</td>
<td>2.25 ± 0.07</td>
<td>3.40 ± 0.53</td>
</tr>
<tr>
<td>Cases:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stag I (N=10)</td>
<td>62.75 ± 8.24</td>
<td>2.23 ± 0.27</td>
<td>4.73 ± 0.62</td>
</tr>
<tr>
<td>Stage II (N=15)</td>
<td>71.19 ± 7.25</td>
<td>3.28 ± 0.15</td>
<td>6.98 ± 0.72</td>
</tr>
<tr>
<td>Stage III (N=20)</td>
<td>82.65 ± 6.56</td>
<td>4.21 ± 0.13</td>
<td>8.97 ± 0.74</td>
</tr>
<tr>
<td>Stage IV (N=5)</td>
<td>100.30 ± 7.85</td>
<td>4.50 ± 0.12</td>
<td>10.83 ± 0.75</td>
</tr>
</tbody>
</table>

N: Number of samples.

4.4: DISCUSSION
The significant deterioration of kidney function between the control and progressing disease process is also reported by Chatterjee et al., 2007.

4.5: CONCLUSION
Statistical analysis shows the significant difference of the P value in all 3 parameters (urea, creatinine and uric acid) between the disease and healthy control groups. All are having P value less than 0.05 clearly indicating their role in evaluation of prognostic factor in carcinoma cervix.
4.6: BIBLIOGRAPHY