4.0 RESULTS

4.0 RESULTS

4.1 LEVELS OF THE GLYCOPROTEIN CONSTITUENTS IN CONTROLS, PATHOLOGICAL CONTROLS AND UNTREATED CANCER PATIENTS

Breast cancer study:

Mean values of the glycoprotein constituents in healthy individuals, patients with benign breast diseases (BBD) and untreated breast cancer patients are shown in **tables 1-2.** The levels of biomarkers were found elevated in patients with BBD and untreated breast cancer patients as compared to the healthy individuals (controls). As documented in the tables, levels of the markers were higher in untreated cancer patients also as compared to the patients with BBD. The levels of free, protein bound and total sialic acid, ratio of protein bound sialic acid to total protein, ratio of total sialic acid to total protein, fucose, mucoid proteins, hexoses, ratio of fucose to total protein, ratio of mucoid proteins to total protein and ratio of hexoses to total protein were significantly higher (p=0.000, 0.000, 0.000, 0.005, 0.011, 0.000, 0.000, 0.000, 0.000, 0.000 and 0.000, respectively) in untreated breast cancer patients as compared to the controls. The total sialic acid values were significantly higher (p=0.000) in patients with BBD as compared to the healthy females. Levels of free sialic acid, protein bound sialic acid, ratio of protein bound sialic acid to total protein, ratio of total sialic acid to total protein, fucose, mucoid proteins and hexoses were elevated in patients with BBD as compared to controls but the difference was not statistically significant. However, when fucose and mucoid proteins were normalised by total protein levels, it showed significant difference (p=0.000) between the two groups. In comparison between patients with BBD and untreated breast cancer patients, the levels of free sialic acid, protein bound sialic acid, total sialic acid, ratio of protein bound sialic acid to total protein, ratio of total sialic acid to total protein, fucose, mucoid proteins, hexoses, ratio of fucose to total protein, ratio of mucoid proteins to total protein and ratio of hexoses to total protein were significantly raised (p=0.024, 0.006, 0.000, 0.098, 0.022,

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0.000, 0.000, 0.026, 0.003, and 0.000, respectively) in untreated breast cancer patients.

Oral cavity cancer study:

Comparison of serum glycoprotein constituent levels between healthy individuals, patients with oral precancerous conditions (OPC) and untreated oral cancer patients are detailed in **tables 3-4.** The mean values of all the glycoprotein constituents were significantly higher in untreated oral cavity cancer patients as compared to the controls ($p < 0.000$). Mean values of the markers were higher in patients with OPC when compared with controls. Elevations in marker levels in patients with OPC was not statistically significant in case of protein bound sialic acid, ratio of protein bound sialic acid to total protein, fucose, ratio of fucose to total protein and ratio of hexoses to total protein as compared to the controls. The oral cavity cancer patients showed higher mean values of all the markers as compared to the patients with OPC. The alterations in free sialic acid levels were not statistically significant. As detailed in **tables 3-4,** Oral cavity cancer patients showed significant elevation in serum levels of free sialic acid, protein bound sialic acid, total sialic acid, ratio of protein bound sialic acid to total protein, ratio of total sialic acid to total protein, fucose, mucoid proteins, hexoses, ratio of fucose to total protein, ratio of mucoid proteins to total protein and ratio of hexoses to total protein (p=0.005, 0.030, 0.000, 0.001, 0.000, 0.000, 0.000, 0.000,0.000, 0.000 and 0.000 respectively) as compared to the patients with OPC.

In both the groups, Untreated cancer patients showed significant elevations in serum glycoprotein constituents levels when compared with controls as well as pathological controls. Further pathological controls showed elevations in the levels of marker when compared with respective controls.

Table-1

COMPARISON OF SERUM SIALICACID LEVELS BETWEEN CONTROLS, PATIENTS WITH BBD AND UNTREATED BREAST CANCER PATIENTS

	Group	Mean	S.E	"t"Value	"p"Value
FSA	Controls	0.93	0.118	1.432^a	0.157^a
(μg/dl)	Pts. with BBD	1.11	0.043	2.274^b	0.024^b
	Untreated BC pts.	1.35	0.047	3.310^c	0.000^c
PSA	Controls	10.55	1.880	0.542	0.621
(μg/dl)	Pts. with BBD	11.09	0.469	2.845	0.006
	Untreated BC pts.	12.86	0.409	2.381	0.009
TSA	Controls	47.61	1.460	3.802	0.000
(μg/dl)	Pts. with BBD	53.89	0.770	5.431	0.000
	Untreated BC pts.	62.55	1.135	5.227	0.000
PSA/TP	Controls	1.628	0.030	1.500	0.370
(μg/gm)	Pts. with BBD	1.740	0.075	1.614	0.098
	Untreated BC pts.	1.894	0.059	4.019	0.005
TSA/TP	Controls	7.30	0.280	0.833	0.408
(μg/gm)	Pts. with BBD	7.53	0.171	2.308	0.022
	Untreated BC pts.	8.23	0.231	2.582	0.011

a   - Controls versus patients with BBD

b   - Patients with BBD versus untreated BC patients

BBD   - Benign Breast Disease

BC   - Breast Cancer

c   - Controls versus untreated breast cancer patients

FSA   - Free Sialic acid

PSA   - Protein Bound Sialic Acid

Pts. - Patients

TP   - Total Protein

TSA   - Total Sialic Acid

Table-2

COMPARISION OF SERUM FUCOSE AND SEROMUCOID FRACTION BETWEEN CONTROLS, PATIENTS WITH BBD AND UNTREATED BREAST CANCER PATIENTS

	Group	Mean	S.E.	"t" Value	"p" Value
Fucose	Controls	11.46	0.764	1.499[a]	0.135[a]
(μg/dl)	Pts. with BBD	13.60	0.666	3.733[b]	0.000[b]
	Untreated BC pts.	18.61	0.406	7.056[c]	0.000[c]
MP	Controls	117.90	7.010	1.887	0.062
(μg/dl)	Pts. with BBD	134.26	5.105	5.796	0.000
	Untreated BC pts.	184.41	5.095	6.721	0.000
Hexoses	Controls	12.64	0.460	0.370	0.712
(μg/dl)	Pts. with BBD	13.05	1.033	4.978	0.026
	Untreated BC pts.	22.51	0.849	9.415	0.000
Fucose/TP	Controls	1.58	0.138	4.452	0.000
(μg/mg)	Pts. with BBD	2.25	0.061	3.042	0.003
	Untreated BC pts.	2.62	0.105	6.010	0.000
MP/TP	Controls	13.95	0.600	5.143	0.000
(μg/gm)	Pts. with BBD	18.41	0.632	4.886	0.000
	Untreated BC pts.	24.42	0.696	9.263	0.000
Hexoses/TP	Controls	1.704	0.090	0.813	0.418
(μg/gm)	Pts. with BBD	1.827	0.121	6.069	0.000
	Untreated BC pts.	2.830	0.122	6.210	0.000

a   - Controls versus patients with BBD

b   - Patients with BBD versus untreated BC patients

BBD - Benign Breast Disease

BC   - Breast Cancer

c   - Controls versus untreated breast cancer patients

MP   - Mucoid proteins

Pts. - Patients

TP   - Total Protein

Table-3
COMPARISON OF SERUM SIALIC ACID LEVELS BETWEEN CONTROLS, PATIENTS WITH OPC AND UNTREATED ORAL CAVITY CANCER PATIENTS

	Group	Mean	S.E.	"t" Value	"p" Value
FSA	Controls	0.71	0.112	3.561[a]	0.004[a]
(μg/dl)	Pts. with OPC	1.05	0.083	2.872[b]	0.005[b]
	Untreated OC Pts.	1.27	0.051	3.474[c]	0.001[c]
PSA	Controls	7.23	0.564	1.290	0.113
(μg/dl)	Pts. with OPC	10.54	0.493	2.273	0.030
	Untreated OC Pts.	12.06	0.541	5.174	0.000
TSA	Controls	44.62	0.442	4.501	0.000
(μg/dl)	Pts. with OPC	52.28	1.184	9.742	0.000
	Untreated OC Pts.	63.20	0.833	7.924	0.000
PSA/TP	Controls	1.34	0.090	1.664	0.099
(μg/gm)	Pts. with OPC	1.64	0.081	3.263	0.001
	Untreated OC Pts.	1.78	0.094	3.262	0.001
TSA/TP	Controls	6.37	0.142	5.463	0.000
(μg/gm)	Pts. with OPC	7.67	0.290	6.500	0.000
	Untreated OC Pts.	9.69	0.144	4.161	0.000

a - Controls versus patients with OPC

b - Patients with OPC versus oral cavity cancer

c - Controls versus untreated oral cavity cancer

FSA - Free Sialic acid

OC - Oral Cavity Cancer

OPC - Oral Precancerous Conditions

PSA - Protein Bound Sialic Acid

Pts. - Patients

TP - Total Protein

TSA - Total Sialic Acid

Table – 4

COMPARISON OF SERUM FUCOSE AND SEROMUCOID FRACTION BETWEEN CONTROLS, PATIENTS WITH OPC AND UNTREATED ORAL CAVITY CANCER PATIENTS

	Group	Mean	S.E.	"t" Value	"p" Value
Fucose	Controls	10.46	0.332	1.529[a]	0.127[a]
µg/dl)	Pts. with OPC	11.91	0.579	7.606[b]	0.000[b]
	Untreated OC pts.	18.55	0.621	6.325[c]	0.000[c]
MP	Controls	105.38	2.136	3.699	0.000
(µg/dl)	Pts. with OPC	130.98	3.947	8.145	0.000
	Untreated OC pts.	169.04	4.383	12.237	0.000
Hexoses	Controls	11.88	0.190	2.328	0.018
(µg/dl)	Pts. with OPC	13.72	0.670	5.797	0.000
	Untreated OC pts.	18.92	0.598	5.971	0.000
Fucose/TP	Controls	1.09	0.054	1.484	0.159
(µg/gm)	Pts. with OPC	2.21	0.093	0.516	0.000
	Untreated OC pts.	2.83	0.102	6.131	0.000
MP/TP	Controls	13.86	0.452	4.969	0.000
(µg/gm)	Pts. with OPC	18.83	0.898	7.289	0.000
	Untreated OC pts.	26.12	0.678	8.762	0.000
Hexoses/TP	Controls	1.40	0.090	1.533	0.179
(µg/gm)	Pts. with OPC	1.70	0.121	5.301	0.000
	Untreated OC pts.	3.01	0.122	9.981	0.000

a   - Controls versus patients with OPC

b   - Patients with OPC versus oral cavity cancer patients

c   - Controls versus untreated oral cavity cancer patients

MP   - Mucoid proteins

OC   - Oral Cancer

OPC   - Oral Precancerous Conditions

Pts.   - Patients

TP   - Total Protein

4.2 CORRELATION OF THE GLYCOPROTEIN CONSTITUENT LEVELS WITH STAGE OF MALIGNANT DISESASE (Spearman's correlation curves):

Spearman's correlation curves were constructed to evaluate association of alterations in serum levels of glycoprotein constituents with stagewise disease activity in untreated breast cancer patients and oral cavity cancer patients. Levels of significance were calculated using ANOVA.
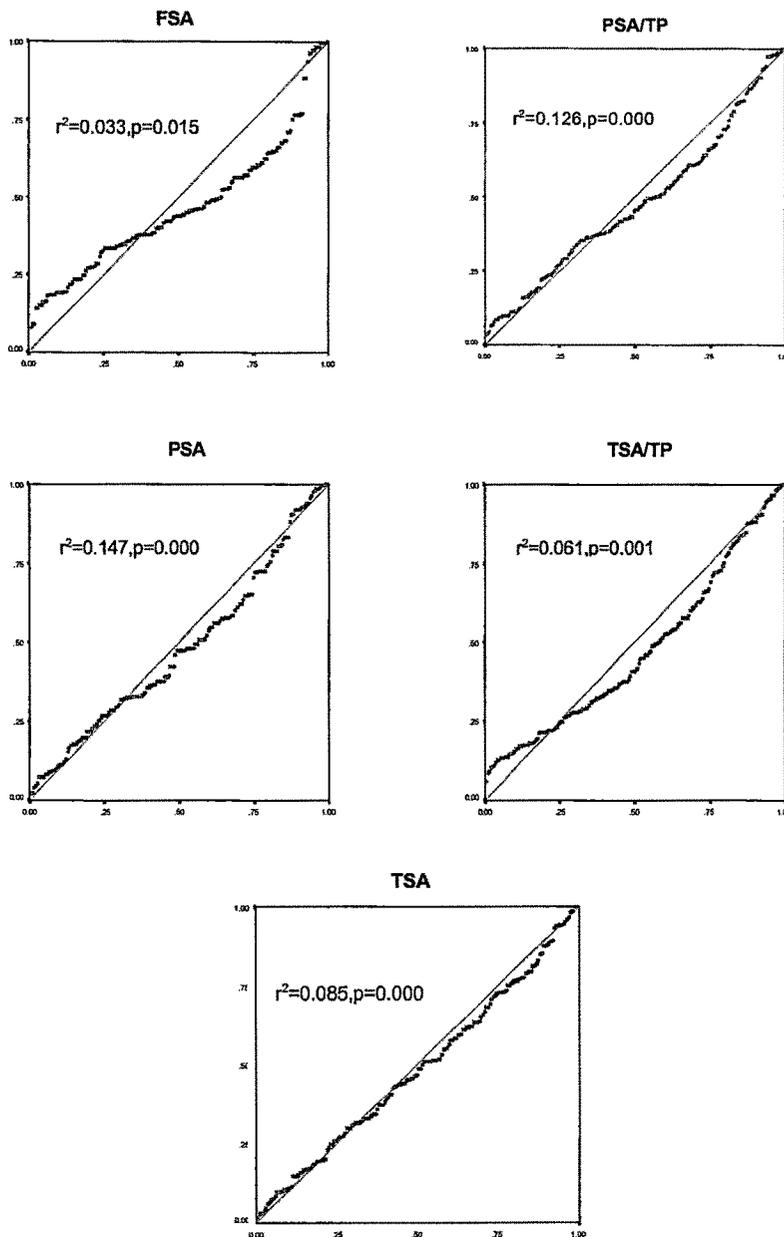
Breast Cancer Study:

The correlation of variations in the marker levels with stagewise disease activity in breast cancer patients is depicted in **figures 1-2.** The sialic acid forms as well as fucose and seromucoid fraction showed positive correlation with the stage of malignant disease of breast. The r^2 ranged between 0.033 - 0.147. The "p" values indicated that the correlation was statistically also significant between serum levels of free sialic acid, ratio fucose, mucoidproteins, hexoses, ratio of fucose to total protein, ratio of mucoidproteins to total protein and ratio of hexoses to total protein with pvalues 0.033, 0.004, 0.01, 0.000, 0.01, 0.01, and 0.0001 respectively. Serum levels of protein bound sialic acid and total sialic acid, did not show statistically significant association with stage of the disease. However their ratio to total protein revealed statistically significant (p=0.000) associaftion with stage of the disease.
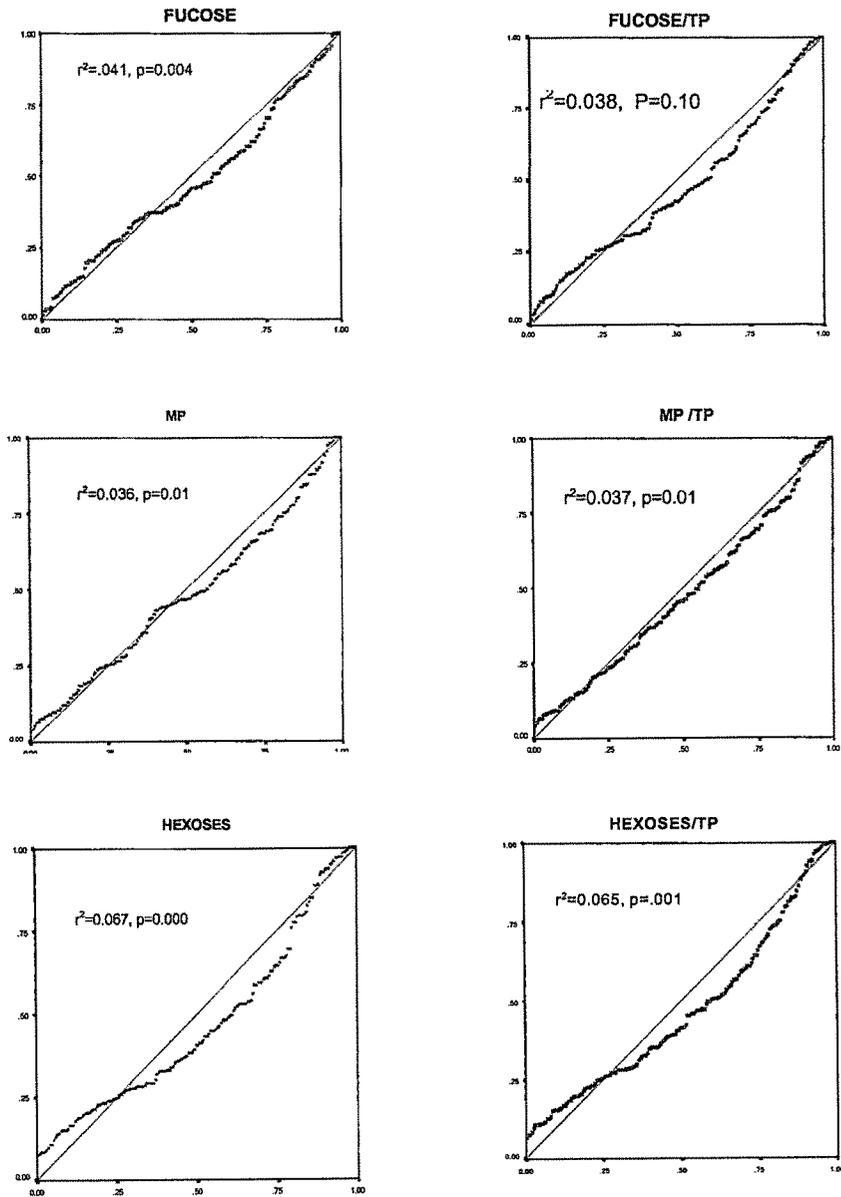
Oral Cavity Cancer Study:

Figures 3-4 exhibit association of the markers with stagewise disease activity in oral cavity cancer patients. The levels of markers elevated progressively with increase in stage of the disease. A strong correlation was observed between extent of the diseases and serum levels of protein bound sialic acid, total sialic acid, hexoses and ratio of hexoses to total protein (p=0.030, 0.034, 0.029 and 0.05, respectively). The remaining markers did not show statistically significant correlation with disease progression where the values of r^2 ranged between 0.00 - 0.012.

Figure-1
CORRELATION OF SERUM SIALIC ACID LEVELS WITH STAGEWISE DISEASE ACTIVITY IN BREAST CANCER PATIENTS

FSA

$r^2=0.033, p=0.015$

PSA/TP

$r^2=0.126, p=0.000$

PSA

$r^2=0.147, p=0.000$

TSA/TP

$r^2=0.061, p=0.001$

TSA

$r^2=0.085, p=0.000$

X axis - Observed cumlative probability
Y axis - Expected cumlative probability
FSA   - Free sialic acid
PSA   - Protein bound sialic acid
TSA   - Total sialic acid
TP   - Total protein

Figure-2
CORRELATION OF SERUM FUCOSE AND SEROMUCOID FRACTION LEVELS
WITH STAGE WISE DISEASE ACTIVITY IN BREAST CANCER PATIENTS

FUCOSE — $r^2=.041, p=0.004$

FUCOSE/TP — $r^2=0.038, P=0.10$

MP — $r^2=0.036, p=0.01$

MP /TP — $r^2=0.037, p=0.01$

HEXOSES — $r^2=0.067, p=0.000$

HEXOSES/TP — $r^2=0.065, p=.001$

MP   - Mucoid protein
TP   - Total protein
X axis - Observed cumlative probability
Y axis - Expected cumlative probability

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Figure-3
**CORRELATION OF SERUM SIALIC ACID LEVELS WITH STAGE WISE
DISEASE ACTIVITY IN ORAL CAVITY CANCER PATIENTS**
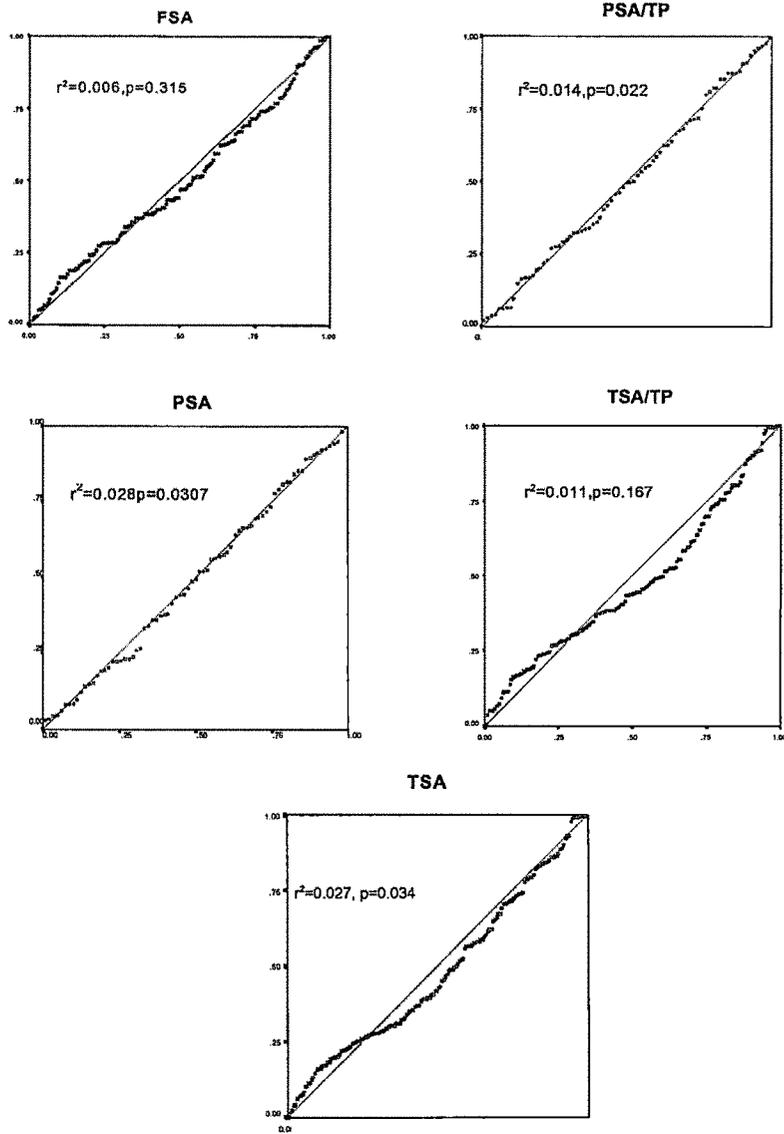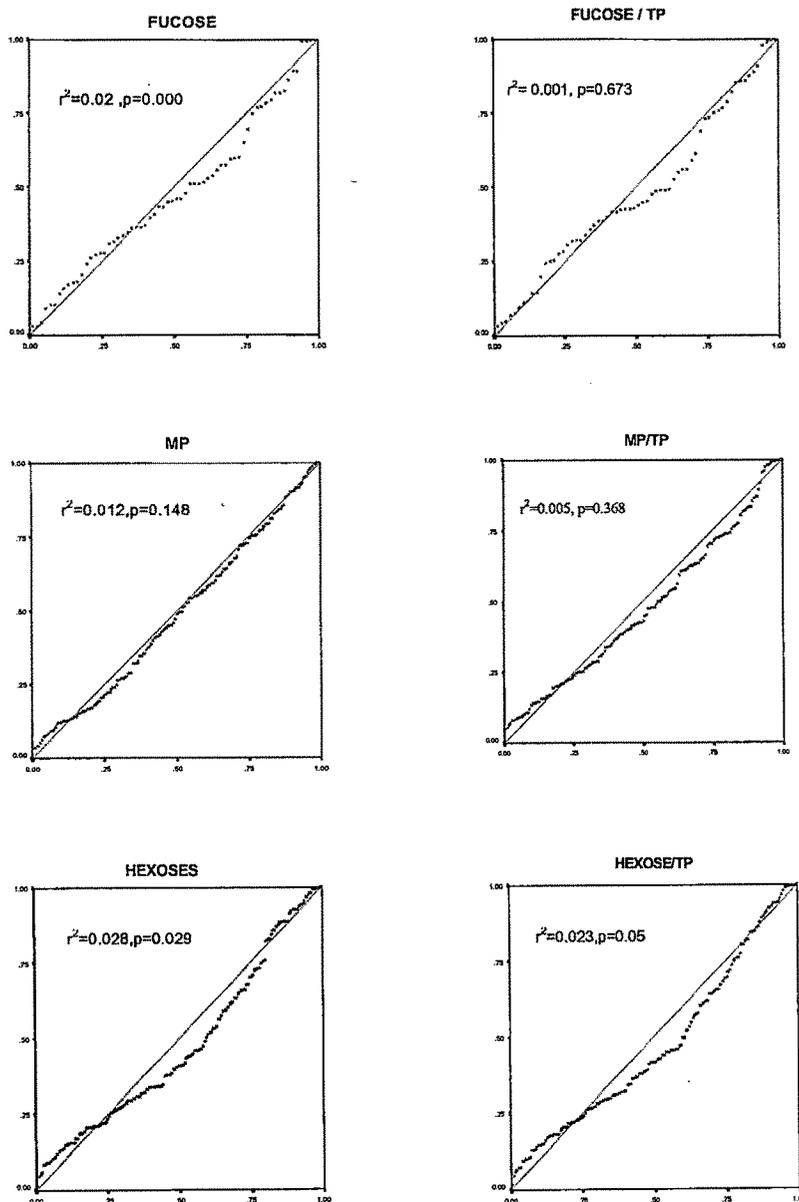

X axis - Observed cumulative probability
Y axis - Expected cumulative probability
FSA   - Free sialic acid
PSA   - Protein bound sialic acid
TSA   - Total sialic acid
TP   - Total protein

Figure-4
CORRELATION OF SERUM FUCOSE AND SEROMUCOID FRACTION LEVELS
WITH STAGE WISE DISEASE ACTIVITY IN ORAL CAVITY CANCER PATIENTS

FUCOSE $r^2=0.02$, $p=0.000$

FUCOSE / TP $r^2= 0.001$, $p=0.673$

MP $r^2=0.012$, $p=0.148$

MP/TP $r^2=0.005$, $p=0.368$

HEXOSES $r^2=0.028$, $p=0.029$

HEXOSE/TP $r^2=0.023$, $p=0.05$

Xaxis - Observed cumulative probability
Yaxis - Expected cumulative probability
MP   - Mucoid protein
TP   - Total protein

50
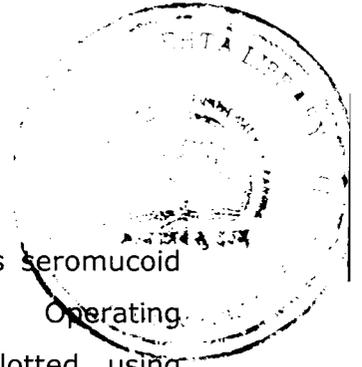
4.3 ROC CURVE ANALYSIS OF THE MARKERS

Diagnostic values of sialic acid forms and fucose as well as seromucoid fraction were evaluated by constructing Receiver's Operating Characteristic (**ROC**) curves. The ROC curves were plotted using sensitivity and specificity at various cut-off levels. ROC curves between 45^0 diagonal line and 90^0 line denotes acceptable diagnostic values of the markers. Further closeness of curve to upper left corner indicates better diagnostic efficacy of the markers. 45^0 diagonal line represents ROC curve of an useless marker.

Breast Cancer Study:

As documented in **figure-5**, total sialic acid and ratio of total sialic acid to total protein showed better diagnostic values as compared to protein bound sialic acid, ratio of protein bound sialic acid to total protein and free sialic acid. The variations in protein bound sialic acid and ratio of protein bound sialic acid to total protein levels were unable to discriminate between controls and untreated breast cancer patients at 30% or less sensitivity levels. **Figure–6** depicts ROC curves of fucose and seromucoid fractions in breast cancer patients. As evident from the ROC curves, fucose and seromucoid fraction levels were within acceptable range of diagnostic value. The fucose levels showed ROC curves with maximum discriminatory values. The ROC curves for fucose and seromucoid fraction indicated better diagnostic values as compared to that for sialic acid levels.

Oral cavity cancer study

Figures 7 – 8 exhibit ROC curves of glycoprotein constituents in untreated oral cavity cancer patients. As illustrated, different forms of sialic acid, fucose and seromucoid fraction revealed ROC curves in acceptable range. It is clear from the figure that serum glycoprotein constituents have ability to discriminate between healthy individuals and untreated cancer patients. Among all the markers, protein bound sialic acid, total sialic acid, ratio of total sialic acid to total protein fucose, and ratio of fucose to total protein were found to be more sensitive indicators of oral cavity cancer.

Figure-5
ROC CURVES OF SIALIC ACID FORMS IN BREASTCANCER PATIENTS

Figure-6
ROC CURVES OF FUCOSE AND SEROMUCOID FRACTIONS IN BREASTCANCER PATIENTS

TP-Totalprotein   MP-Mucoid protein   FUC- Fucose   HEX-hexoses

Figure-7

ROC SURVES OF SIALIC ACIDSIN PATIENTS WITH ORAL CAVITY CANCER PATIENTS

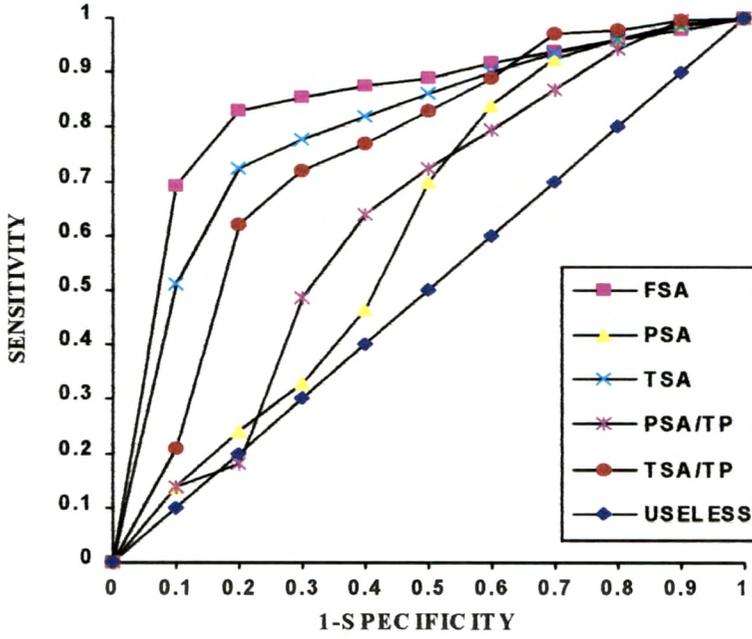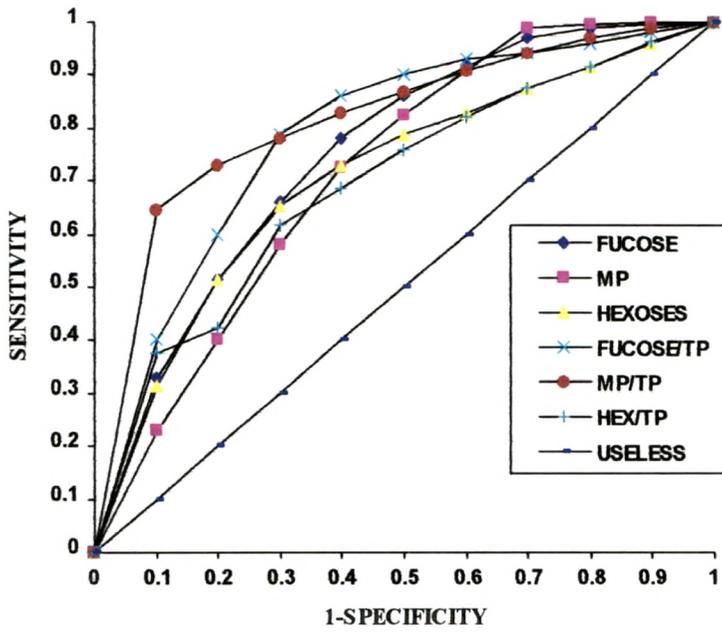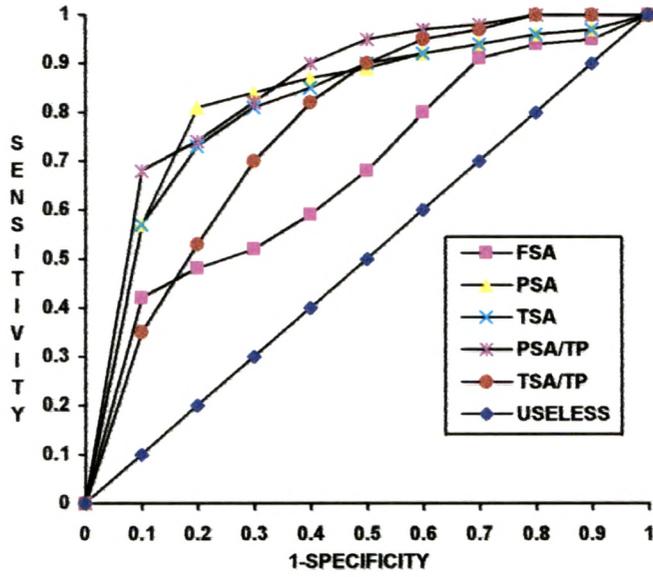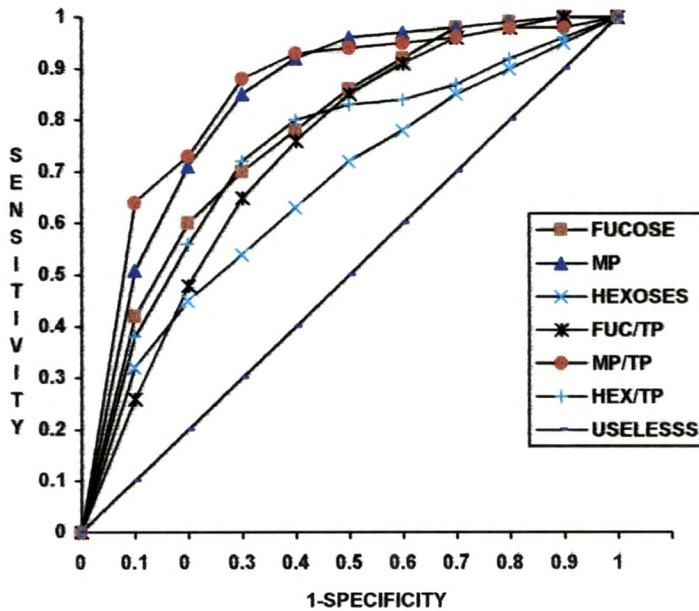**Figure-8**

ROC CURVES OF FUCOSE AND SEROMUCOID FRACTION IN ORAL CAVITY CANCER PATIENTS

TP-Total protein   MP-Mucoid protein   FUC-Fucose   HEX-Hexoses

## 4.4: SURVIVAL CURVES OF THE MARKERS (Kaplan-Meire model)
Breast cancer study:

To evaluate prognostic value of the markers, survival curves (log survival) were plotted for breast cancer and oral cavity cancer patients. Mean pretreatment values of the markers were used as cut off levels. Survival of patients having levels of biomarkers above cut off values was compared with survival of the patients whose pretreatment marker levels were below cut off. **Figures 9-10** indicate overall survival curves for sialic acid levels in breast cancer patients. Total sialic acid, ratio of protein bound sialic acid to total protein, ratio of total sialic acid to total protein, fucose, mucoid proteins, hexoses, ratio of fucose to total protein, ratio of mucoid proteins to total protein, and ratio of hexoses to total protein demonstrated significant difference indicating a poor prognosis of the group of the patients whose marker values were above cut off levels. There was no significant correlation between overall survival and serum levels of free sialic acid and protein bound sialic acid. The patients having the marker levels below mean values had better overall survival. The results documented that high pretreatment levels of glycoprotein constituents were indicators of poor prognosis.

Oral cavity cancer study:

Survival curves for the glycoprotein markers for oral cavity cancer patients are shown in **Figures 11-12**. The survival curves indicated significantly better survival in patient whose levels of protein bound sialic acid, ratio of protein bound sialic acid to total protein, ratio of total sialic acid to total protein, fucose, mucoid proteins, ratio of fucose to total protein and ratio of mucoid proteins to total protein were below mean values of pretreatment as compared to their counter parts. Pretreatment levels of free sialic acid, total sialic acid hexoses and ratio of hexoses to total protein did not show any significant association with survival in oral cavity cancer patients.

Figure-9
SURVIVAL CURVES (LOG SURVIVAL) OF FSA, PSA AND TSA IN BREAST CANCER PATIENTS ACCORDING TO THE MEAN VALUES AS CUTOFF.
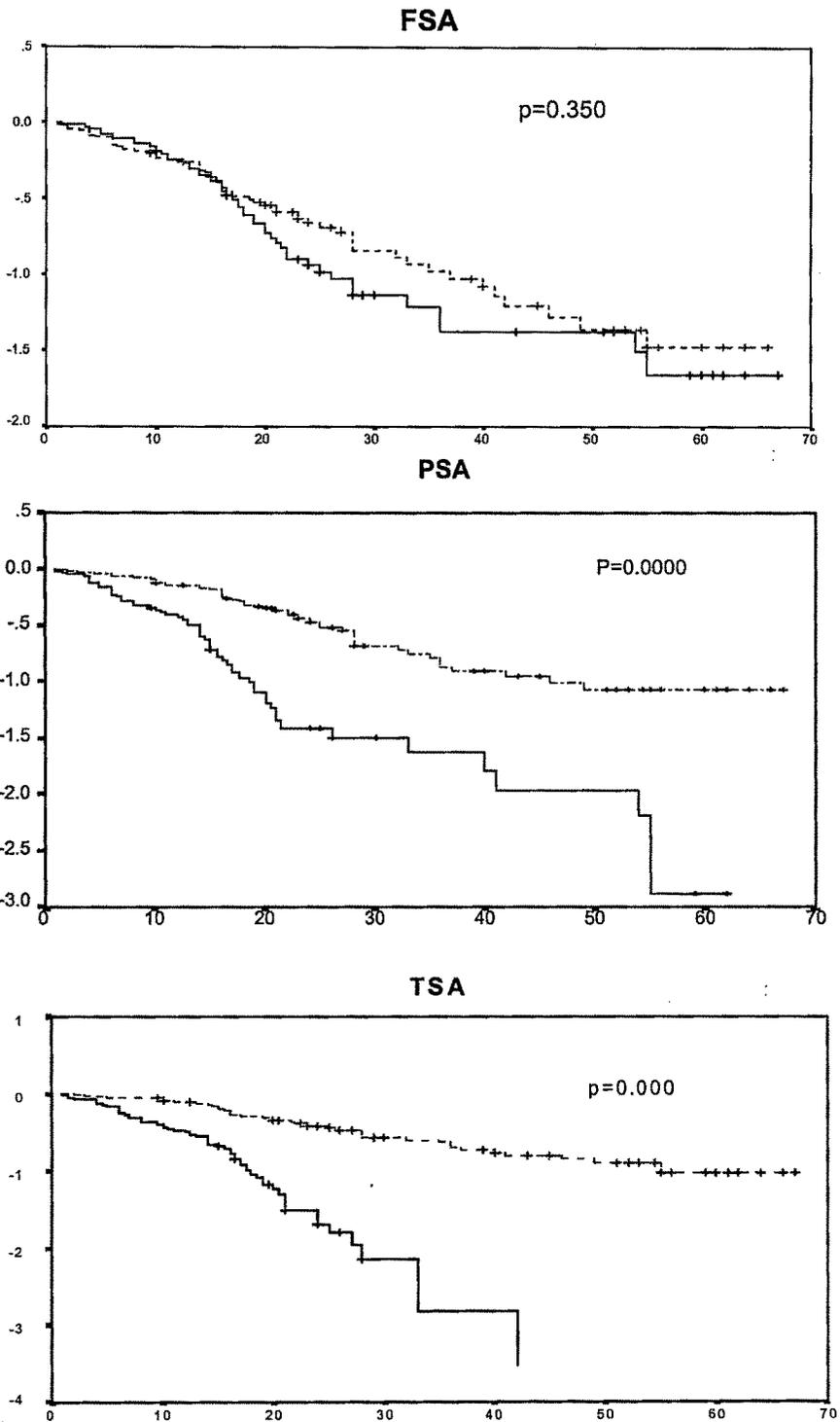
FSA

p=0.350

PSA

P=0.0000

TSA

p=0.000

————— - Above mean value ― ― ― - - Below mean value

Xaxis - Duration in months   Y axis - Log survival

PSA   - Protein bound sialic acid   TSA   - Total sialic acid   TP   - Total protein

FSA   - Free sialic acid

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Figure- 9(cont.)
SURVIVAL CURVES (LOG SURVIVAL) OF PSA/TP AND TSA/TP IN BREAST CANCER
PATIENTS ACCORDING TO THE MEAN VALUES AS CUTOFF.
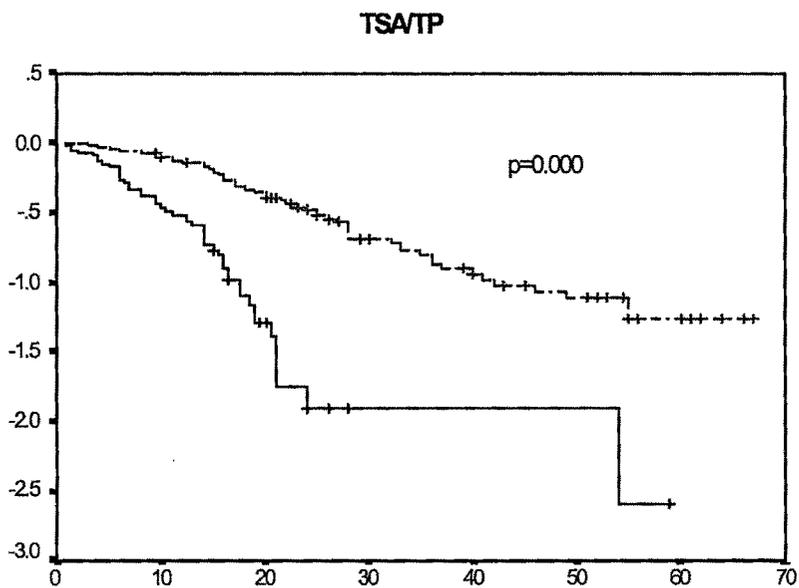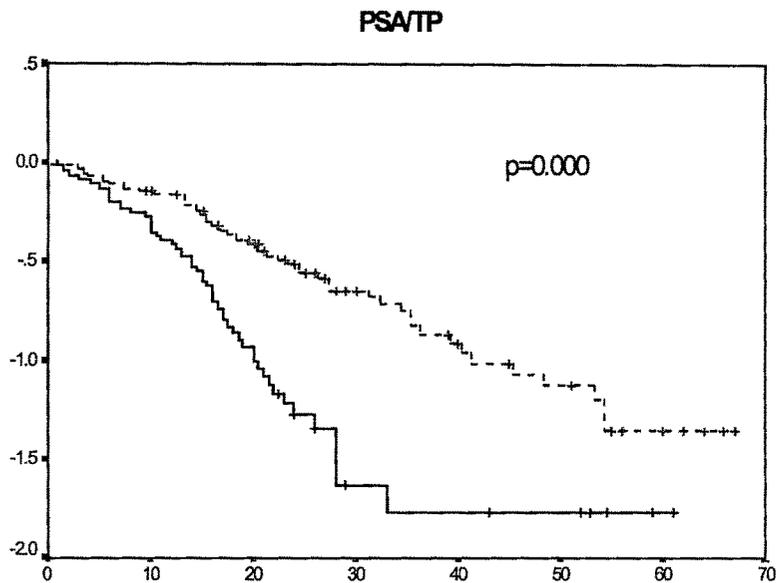
PSA/TP

TSA/TP

————— - Above mean value
— · — · — - Below mean value

Xaxis - Duration in months
Y axis - Log survival
PSA   - Protein bound sialic acid
TSA   - Total sialic acid
TP   - Total protein

Figure-10

SURVIVAL CURVES (LOG SURVIVAL) OF FUCOSE, MUCOID PROTEIN AND HEXOSES IN
BREAST CANCER PATIENTS ACCORDING TO THE MEAN VALUES AS CUTOFF.

FUCOSE

MUCOID PROTEIN

HEXOSES

—————— - Above mean value
— · — · - Below mean value

Xaxis   - Duration in months
Y axis - Log survival

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Figure-10 (Cont.)
SURVIVAL CURVES (LOG SURVIVAL) OF FUCOSE/TP, MUCOID PROTEIN/TP AND
HEXOSES/TP IN BREAST CANCER PATIENTS ACCORDING TO THE MEAN VALUES AS
CUTOFF.

FUCOSE/TP

p=0.000

MUCOID PROTEIN/TP

p=0.000

HEXOSES/TP

P=0.0000

——————— - Above mean

-·-·-·-·-· - Below mean

X axis - Duration in months
Y axis – Log survival

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Figure-11

SURVIVAL CURVES(LOG SURVIVAL) OF FSA, PSA AND TSA IN ORAL CAVITY CANCER PATIENTS ACCORDING TO MEAN VALUE AS CUTOFF

Xaxis - Duration in months
Yaxis - Log survival
FSA   - Free sialic acid
PSA   - Protein bound sialic acid
TSA   - Total sialic acid

Figure-11(Cont.)
SURVIVAL CURVES (LOG SURVIVAL) OF PSA/TP AND TSA/TP IN ORAL CAVITY CANCER
PATIENTS ACCORDING TO MEAN VALUES AS CUTOFF

PSA/TP

TSA/TP

———— - Above mean value

-------- - Below mean value

Xaxis   - Duration in months
Y axis - Log survival
PSA   - Protein bound sialic acid
TSA   - Total sialic acid
TP   - Total protein

Figure-12
SURVIVAL CURVES (LOG SURVIVAL) OF FUCOSE MUCOID PROTEIN AND HEXOSES IN ORAL CAVITY CANCER PATIENTS USING MEAN VALUES AS CUTOFF

FUCOSE — p=0.000

MUCOID PROTEIN — p=0.0291

HEXOSES — p=0.8730

——————— - Above mean value
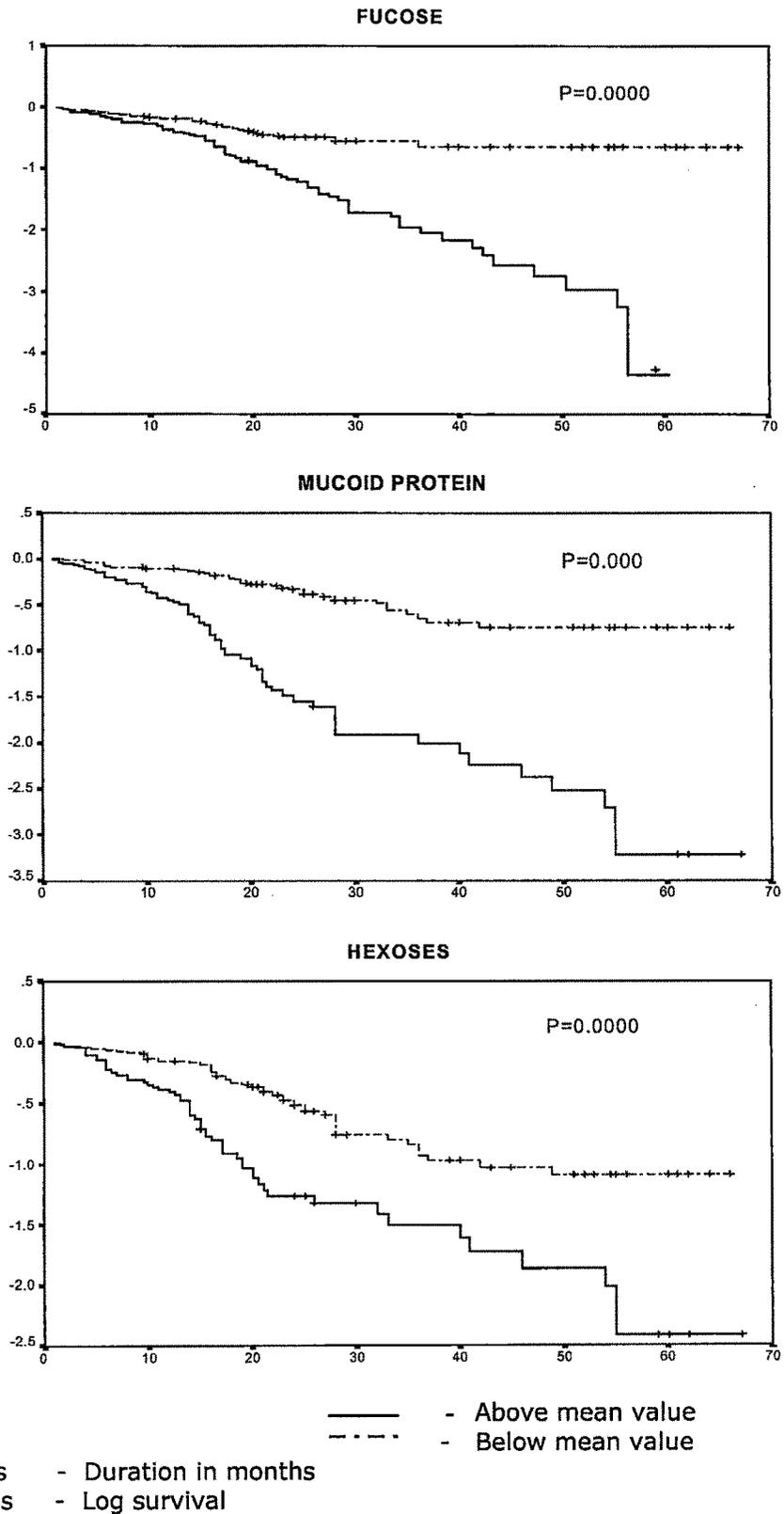— - — - - - Below mean value

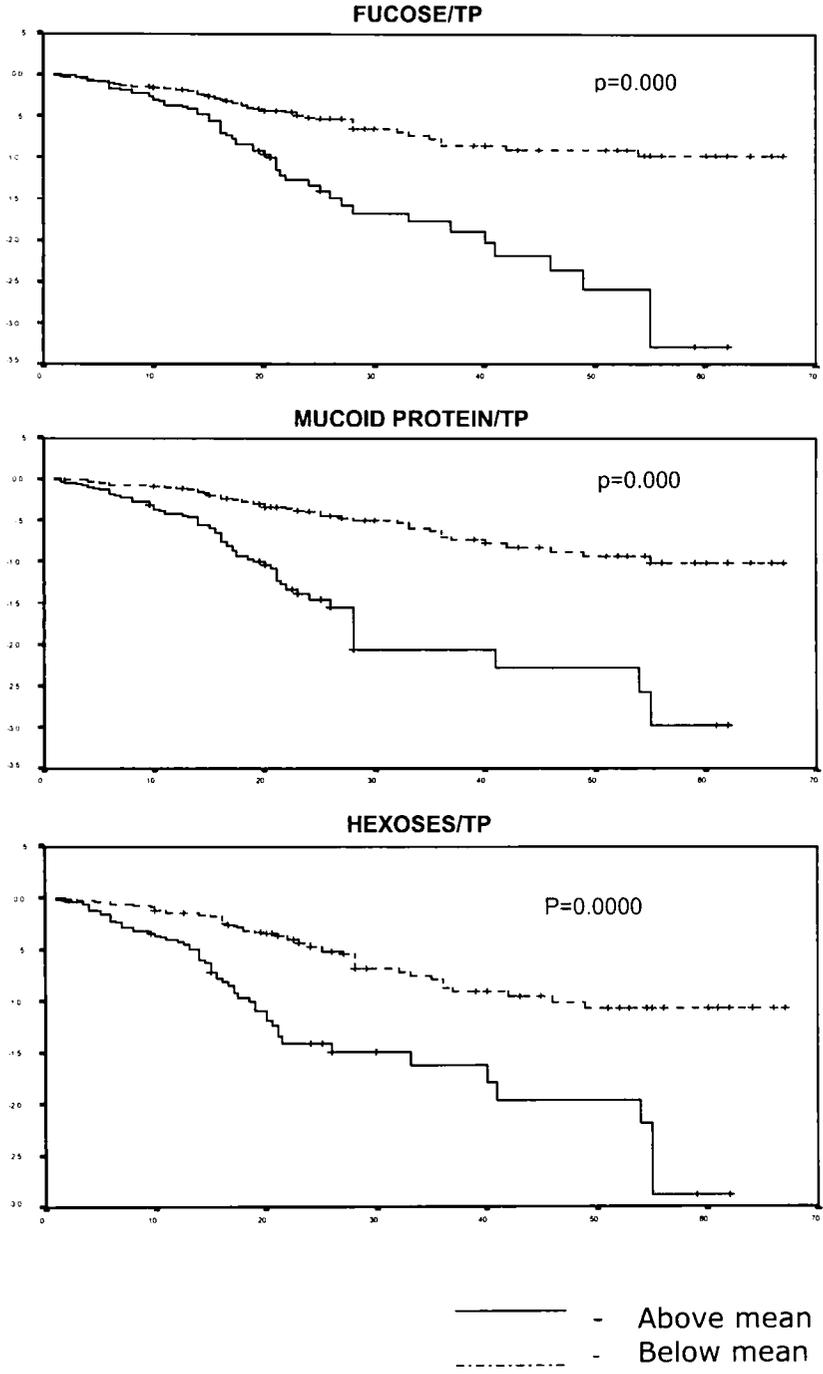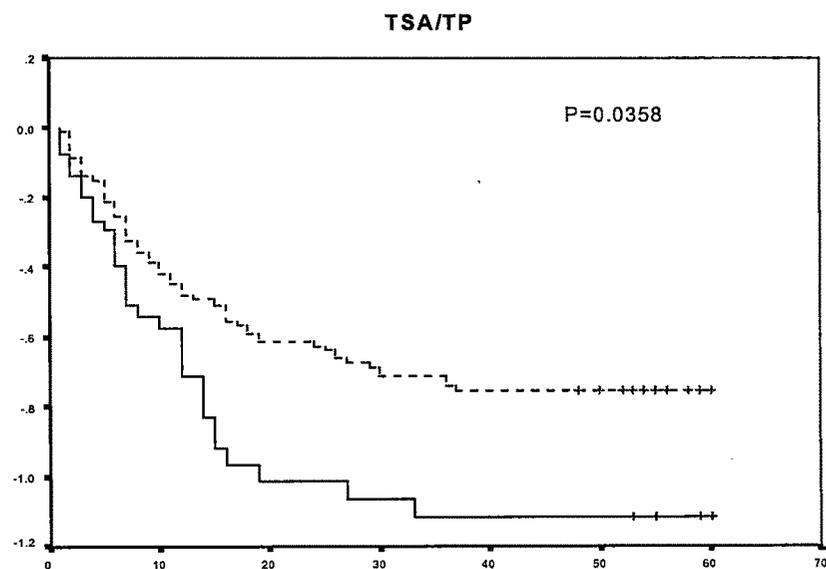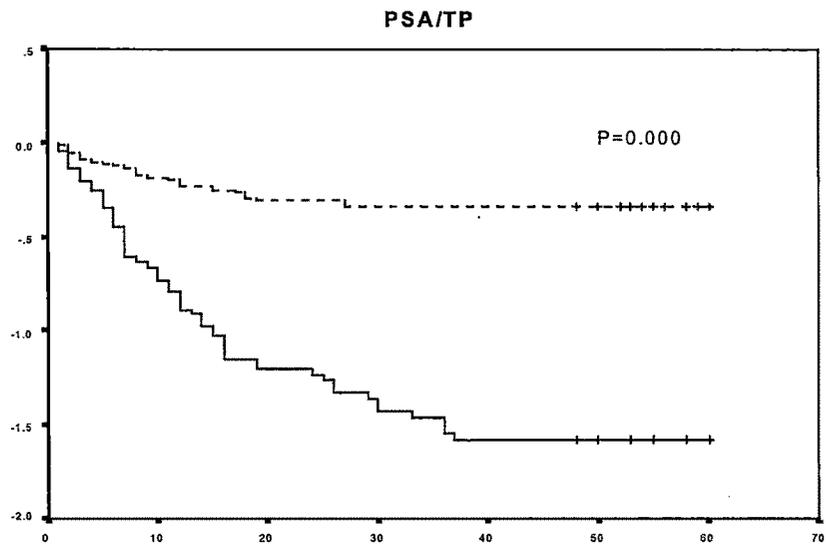X axis   - Duration in months
Y axis   - Log survival

61
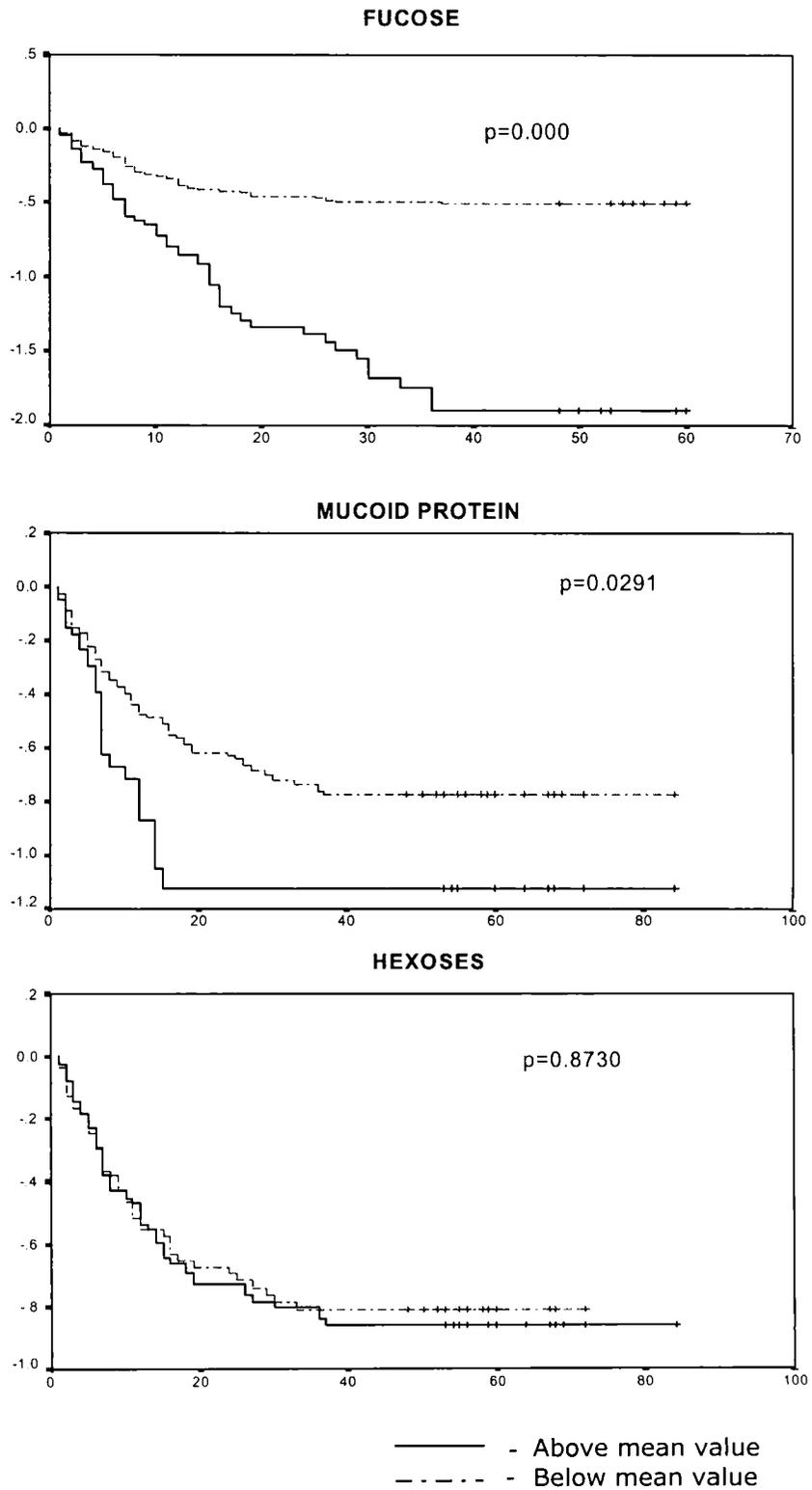
Figure-12 (Cont.)
SURVIVAL CURVES (LOG SURVIVAL) OF FUCOSE/TP, MUCOID PROTEINS/TP AND
HEXOSES/TP IN ORAL CAVITY CANCER PATIENTS USING MEAN VALUES AS CUTOFF

FUCOSE/TP

MUCOID PROTEIN / TP

HEXOSE/TP

——— - Above mean value
— · — · - Below mean value
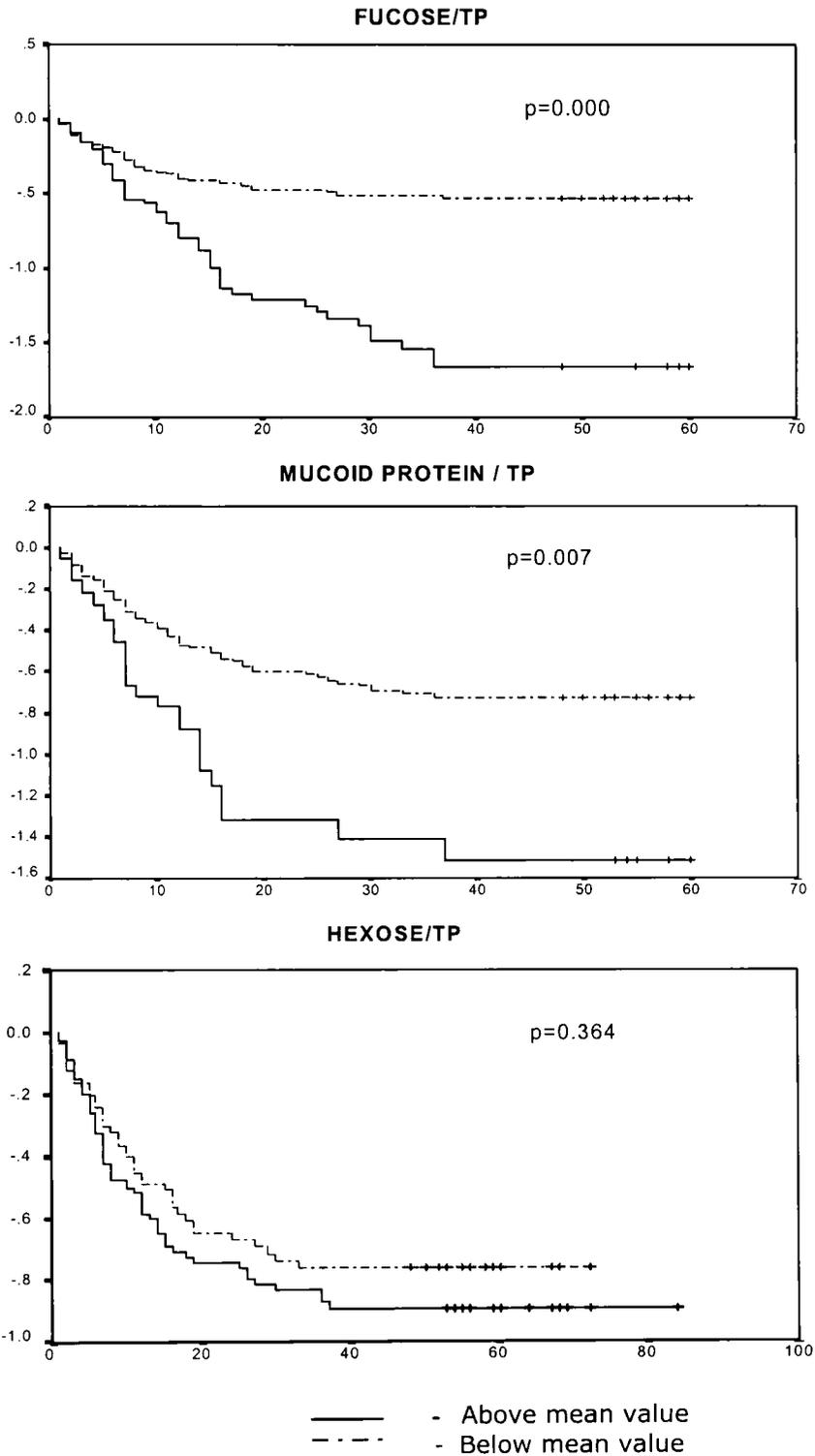
Xaxis   - Duration in months
Y axis   - Log survival
TP   - Total protein

4.5 COMPARISON BETWEEN PRETREATMENT AND POST-TREATMENT MARKER LEVELS

Levels of the biomarkers at diagnosis were compared with their marker values found during follow-ups. The follow-up samples were grouped into complete responders and non-responders as detailed earlier. Levels of biomarkers were also compared between reponders and non-responders.

Breast Cancer Study:

Figure 13 and **Table 5** exhibit pretreatment and post-treatment mean values of the glycoprotein constituents in breast cancer patients. As can be seen from the illustrations and table that serum levels of free sialic acid, protein bound sialic acid, total sialic acid, ratio of protein bound sialic acid to total protein and ratio of total sialic acid to total protein were significantly lower (p=0.045, 0.004, 0.003, 0.000 and 0.000 respectively) in reponders as compared to their levels at the time of diagnosis. As evident from **table-5** responders showed significantly lower levels of mucoid proteins, hexoses, ratio of mucoid proteins to total protein and ratio of hexoses to total protein (p=0.000, 0.030, 0.000 and 0.000 respectively) as compared to the pretreatment levels. Serum levels of fucose and its ratio to total protein were lower in responder when compared to pretreatment levels however, the difference was not statistically significant. Serum levels of sialic acid, fucose and seromucoid fractions as well as their ratio to total protein kept on circulating either at same levels or were elevated in nonresponders as compared to their levels at diagnosis. In comparison between complete responders and non-responders, the complete responders showed significantly lower levels of the glycoprotein constituents.

Oral cancer study

Tables 6-7 provide comparison of the glycoprotein constituents between untreated oral cavity cancer patients, complete responders and non-responders. The complete responders showed lower concentrations of the markers as compared to both, poor/no response groups (nonresponders) as well as untreated oral cavity cancer patients. Statistical analysis revealed a significant fall in the levels of free sialic acid, total sialic acid, ratio of total sialic acid to total protein, mucoid proteins, hexoses, ratio of mucoid proteins to total protein and ratio of hexoses to total protein (p=0.025, 0.000, 0.000,0.000, 0.040, 0.000 and 0.000, respectively) in complete responders as compared to untreated oral cavity cancer patients. Levels of the biomarkers were significantly increased in nonresponders as compared to the complete responders. The mean values of ratio of total sialic acid to total protein, mucoid proteins, hexoses, ratio of mucoid proteins to total protein and hexoses to total protein were significantly higher in nonresponders (p=0.013, 0.006, 0.001, 0.04 and 0.093, respectively) as compared to that in untreated oral cavity cancer patients. The alterations in levels of free sialic acid, protein bound sialic acid, total sialic acid, ratio of total sialic acid to total protein, fucose, ratio of fucose to total protein and ratio of mucoid proteins to total protein were comparable between untreated oral cavity cancer patients and nonresponders. Serum levels of fucose and seromucoid fractions were significantly higher in nonresponders when compared with responders.

In both group of the patients (Breast cancer and oral cavity cancer), patients with favourable treatment out come showed a declined in circulating levels of glycoprotein constituents as compared to their levels in both untreated cancer patients as well as non-responders.

Figure-13

COMPARISION OF SERUM SIALIC ACID LEVELS (MEAN±S.E.) IN UNTREATED BREASTCANCER PATIENTS, COMPLETE RESPONDER AND NONRESPONDER.
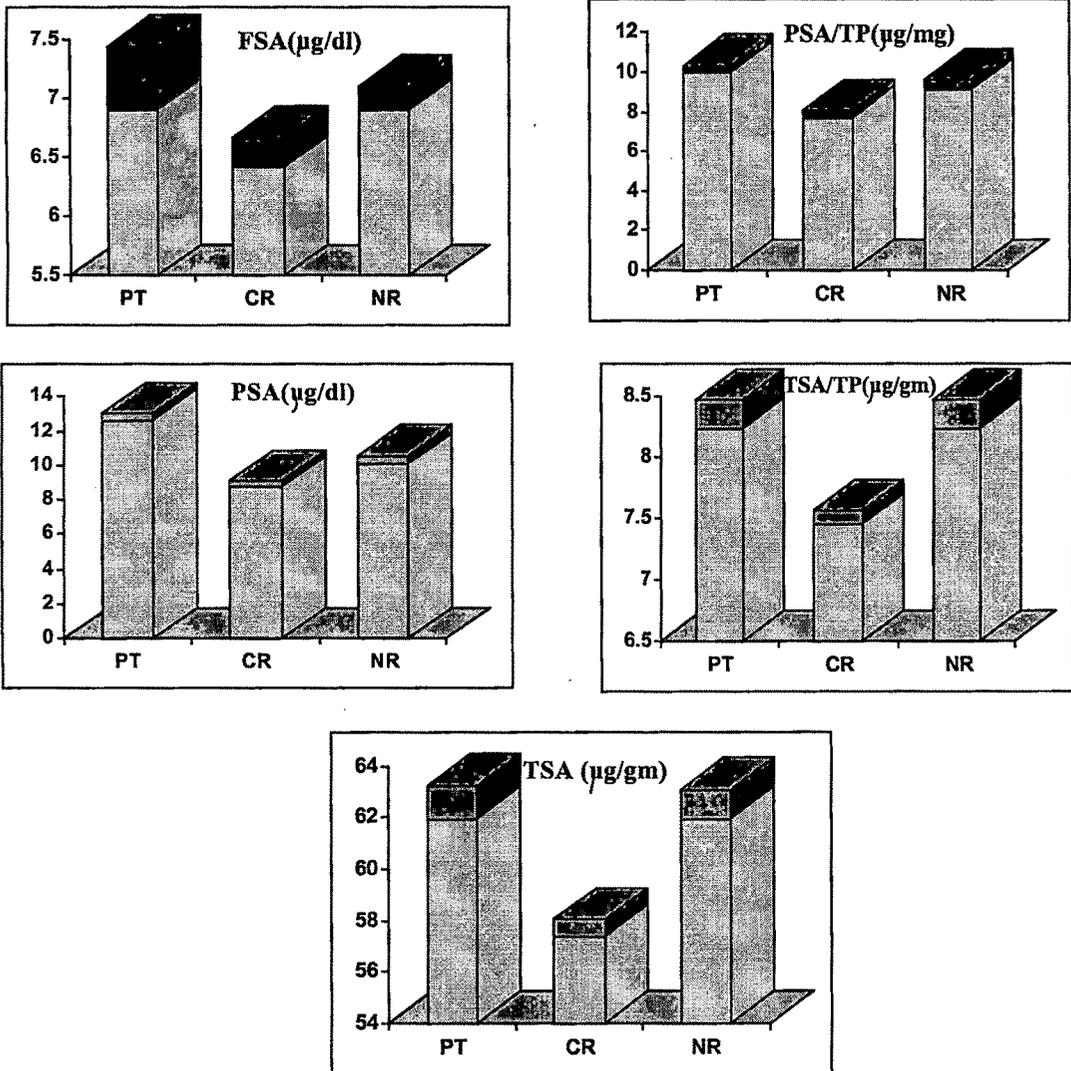
	FSA	PSA	TSA	PSA/TP	TSA/TP
Untreated BC pts.Vs CR "t"	4.053	8.394	8.803	0.101	16.93
"p"	0.045	0.004	0.003	0.751	0.000
Untreated BC pts.Vs CR "t"	1.007	0.011	0.385	2.966	0.236
"p"	0.045	0.004	0.003	0.751	0.000
CR.Vs NR "t"	1.582	5.970	5.367	3.780	14.84
"p"	0.098	0.015	0.021	0.053	0.000

65

Table-5

COMPARISON OF SERUM FUCOSE AND SEROMUCOID FRACTION LEVELS IN UNTREATED BREAST CANCER PATIENTS, COMPLETE RESPONDERS AND NONRESPONDERS

		Mean	S.E.	"t" Value	"p" Value
Fucose	PT	18.61	0.666	1.343[a]	0.180[a]
(μg/dl)	CR	16.62	0.294	1.284[b]	0.200[b]
	NR	17.23	0.052	0.482[c]	0.958[c]
MP	PT	180.66	6.156	4.554	0.000
(μg/dl)	CR	149.69	3.519	1.174	0.241
	NR	142.47	4.124	5.263	0.000
Hexoses	PT	22.51	0.849	2.172	0.030
(μg/dl)	CR	18.34	1.549	0.368	0.718
	NR	21.49	0.968	1.930	0.045
FUCOSE/TP	PT	2.82	0.0616	1.866	0.063
(μg/gm)	CR	2.12	0.0362	0.053	0.958
	NR	2.62	0.0681	2.612	0.009
MP/TP	PT	24.42	0.696	4.956	0.000
(μg/gm)	CR	19.25	0.436	0.395	0.690
	NR	19.52	0.595	4.001	0.000
Hexoses/TP	PT	2.83	0.122	4.745	0.000
(μg/gm)	CR	2.35	0.173	0.156	0.876
	NR	2.71	0.111	2.653	0.008

a - Untreated breast cancer patients versus complete responders

b - Untreated breast cancer patients versus nonresponder

c - Complete responders versus nonresponders

CR - Complete responders

NR - Nonresponders

PT - Pretreatment

TP - Total Protein

Table - 6

COMPARISON OF SERUM SIALIC ACID LEVELS BETWEEN UNTREATED ORAL CAVITY CANCER PATIENTS, COMPLETE RESPONDERS AND NON RESPONDERS

		Mean	S.E.	"t" Value	"P" Value
FSA	PT	1.27	0.051	2.132	0.025^a
(µg/dl)	CR	1.19	0.081	0.492	0.124^b
	NR	1.55	0.093	3.000	0.002^c
PSA	PT	12.06	0.541	1.844	0.033
(µg/dl)	CR	9.81	0.552	0.690	0.070
	NR	12.85	0.714	2.271	0.001
TSA	PT	63.20	0.833	3.80	0.000
(µg/dl)	CR	58.98	0.740	0.67	0.535
	NR	68.00	0.953	7.55	0.000
PSA/TP	PT	1.78	0.090	3.35	0.007
(µg/gm)	CR	1.53	0.101	1.50	0.155
	NR	2.00	0.123	3.04	0.003
TSA/TP	PT	9.69	0.142	4.57	0.000
(µg/gm)	CR	8.84	0.132	1.68	0.013
	NR	10.25	0.174	6.64	0.000

a - Untreated oral cavity cancer patients versus complete responders

b - Untreated oral cavity cancer patients versus nonresponders

c - Complete responders versus nonresponders

CR - Complete responders

NR - Nonresponders

PT - Pretreatment

TP - Total Protein

TABLE - 7

COMPARISON OF SERUM FUCOSE AND SEROMUCOID FRACTION LEVELS BETWEEN UNTREATED ORAL CAVITY CANCER PATIENTS, COMPLETE RESPONDERS AND NONRESPONDERS

		Mean	S.E.	"t" Value	"p" Value
Fucose	PT	18.55	0.621	2.451[a]	0.016[a]
(μg/dl)	CR	13.90	0.569	1.493[b]	0.139[b]
	NR	19.65	1.104	4.630[c]	0.000[c]
MP	PT	169.04	4.382	4.991	0.000
(μg/dl)	CR	135.25	3.078	2.784	0.006
	NR	174.60	4.369	7.524	0.000
Hexoses	PT	18.92	0.398	5.002	0.000
(μg/dl)	CR	10.62	0.281	3.294	0.001
	NR	17.51	0.419	8.564	0.000
Fucose/TP	PT	2.88	0.102	1.901	0.060
(μg/gm)	CR	2.48	0.091	0.370	0.714
	NR	2.94	0.170	2.083	0.040
MP/TP	PT	26.12	0.678	5.294	0.000
(μg/dl)	CR	20.37	0.513	2.062	0.040
	NR	26.46	0.748	6.881	0.000
hexoses/TP	PT	3.01	0.122	5.373	0.000
(μg/gm)	CR	1.59	0.044	2.803	0.093
	NR	2.26	0.068	8.312	0.000

a - Untreated oral cavity cancer patients versus complete responders

b - Untreated oral cavity cancer patients versus nonresponders

c - complete responders versus nonresponders

CR - Complete responders

MP – Mucoid proteins

NR - Nonresponders

PT - Pretreatment

TP - Total Protein

4.6 PAIRED "t" ANALYSIS

To evaluate association of the alterations in biomarkers with response to anticancer therapy, each follow-up marker value were paired with the individual's pretreatment marker levels. The statistical significance of the alterations with treatment outcome in responders and nonresponders was calculated by paired "t" analysis.

Breast cancer study

Tables 8-11 shows paired "t" analysis of glycoprotein constituents between untreated cancer patients and their follow-ups. As detailed in tables 8 and 10, the group of breast cancer patients with favourable treatment outcome showed significantly lower levels of all the markers as compared to their levels at the time of diagnosis. As can be observed in table-8, the protein bound sialic acid, total sialic acid and ratio of total sialic acid to total protein showed significantly decreased values (p=0.006, 0.016 and 0.018, respectively) in complete responders as compared to their values at the time of diagnosis. As shown in table-10 fucose, mucoid proteins, hexoses, ratio of mucoid proteins to total protein and ratio of hexoses to total protein values were also significantly lower (p=0.014,0.000,0.001, 0.000 and 0.002, respectively) in complete responders as compared to their pretreatment levels. Circulating levels of the markers during follow-up period in nonresponders were comparable or significantly increased than their pre treatment values (tables 9 and 11). The levels of protein bound sialic acid (p=0.000), ratio of protein bound sialic acid to total protein (p=0.000), mucoid protein (p=0.032), hexoses (p=0.000) ratio of mucoid proteins to total protein (p=0.000) and hexoses to total protein ratio (0.000) showed statistically significant elevation in nonresponders as compared to untreated breast cancer patients. Serum levels of fucose and its ratio to total protein showed comparable values between nonresponders and untreated breast cancer patients.

Oral cavity cancer study:

The mean values of differences in the marker levels in complete responders and nonresponders are shown in **tables 12-15**. Levels of glycoprotein constituents were lower in complete responders when compared with their levels at diagnosis. The decline in levels of protein bound sialic acid, total sialic acid, ratio of protein bound sialic acid to total protein, ratio of total sialic acid to total protein, mucoid proteins, hexoses, ratio of fucose to total protein, ratio of mucoid protein to total protein and ratio of hexoses to total protein in complete responders were statistically significant (p=0.033, 0.000, 0.001, 0.000, 0.000, 0.000, 0.043, 0.000 and 0.000, respectively). However, the difference in levels of free sialic acid, was not statistically significant. The levels of sialic acid forms and seromucoid fraction$ levels in nonresponders were either higher or comparable with their pretreatment levels. Serum levels of free sialic acid, protein bound sialic acid, total sialic acid, ratio of protein bound sialic acid to total protein, ratio of total sialic acid to total protein, fucose, mucoid protein, ratio of mucoid proteins to total protein and ratio of hexoses to total protein showed statistically significant elevations(p=0.042, 0.000, 0.000, 0.000, 0.000, 0.001, 0.020, 0.035, 0.000 and 0.000 respectively) in nonresponder in comparison with their levels at diagnosis. However, serum level of hexoses and ratio of fucose to total protein did not show significant alterations among nonresponders as compared to their pretreatment levels.

Table-8

PAIRED "t" TEST FOR SIALIC ACID LEVELS IN COMPLETE RESPONDERS (PAIRED WITH PRETREATMENT VALUES OF BREAST CANCER PATIENTS)

	Mean difference	S.E	"t" Value	"p" Value
FSA (μg/dl)	0.122	0.067	-1.831	0.072
PSA (μg/dl)	1.292	0.464	-2.736	0.006
TSA (μg/dl)	3.855	1.591	2.423	0.016
PSA/TP(μg/gm)	0.152	0.077	-1.980	0.054
TSA/TP(μg/gm)	0.597	0.25	2.395	0.018

Table –9

PAIRED "t" TEST FOR SIALIC ACID LEVELS IN NON RESPONDERS (PAIRED WITH PRETREATMENT VALUES OF BREAST CANCER PATIENTS)

	Mean difference	S.E	"t" Value	"p" Value
FSA(μg/dl)	0.080	0.055	1.464	0.146
PSA(μg/dl)	-3.050	0.374	-8.173	0.000
TSA(μg/dl)	0.236	0.738	0.322	0.749
PSA/TP(μg/gm)	-0.400	0.056	-7.222	0.000
TSA/TP(μg/gm)	-0.004	0.112	2.385	0.965

FSA – Free Sialic Acid
PSA – Protein bound Sialic Acid
TP – Total Protein
TSA – Total Sialic Acid

Table-10

PAIRED "t" TEST FOR FUCOSE AND SEROMUCOID FRACTION IN COMPLETE RESPONDERS (PAIRED WITH PRETREATMENT VALUES OF BREAST CANCER PATIENTS)

	Mean difference	S.E.	"t" Value	"p" Value
Fucose (μg/dl)	0.888	0.363	2.473	0.014
MP (μg/dl)	-37.93	6.072	-6.253	0.000
Hexoses (μg/dl)	5.248	1.592	3.304	0.001
Fucose/TP (μg/gm)	0.093	0.049	1.915	0.056
MP/TP (μg/gm)	6.984	0.636	10.991	0.000
Hexoses/TP(μg/gm)	0.657	0.184	3.582	0.002

Table-11

PAIRED "t" TEST FOR FUCOSE AND SEROMUCOID FRACTION IN NONRESPONDERS (PAIRED WITH PRETREATMENT VALUES OF BREAST CANCER PATIENTS)

	Mean difference	S.E.	"t" Value	"p" Value
Fucose (μg/dl)	0.4874	0.507	0.962	0.337
MP (μg/dl)	-18.630	3.498	3.580	0.032
Hexoses (μg/dl)	-3.276	0.657	-4.991	0.000
Fucose/TP (μg/gm)	-0.072	0.069	1.202	0.230
MP/TP (μg/gm)	-5.260	0.919	-5.731	0.000
Hexoses/TP(μg/gm)	-0.4311	0.094	-4.591	0.000

MP – Mucoid Proteins
TP – Total Protein

72

Table – 12

PAIRED "t" TEST FOR SIALIC ACID LEVELS IN COMPLETE RESPONDERS (PAIRED WITH PRETREATMENT VALUES OF ORAL CAVITY CANCER PATIENTS)

	Mean difference	S.E.	"t" Value	"p" Value
FSA(μg/dl)	0.106	0.067	-1.580	0.116
PSA(μg/dl)	1.878	0.871	-2.164	0.033
TSA(μg/dl)	4.203	0.818	5.140	0.000
PSA/TP(μg/gm)	2.307	0.657	-3.510	0.001
TSA/TP(μg/gm)	0.795	0.153	5.210	0.000

Table – 13

PAIRED "t" TEST FOR SIALIC ACID LEVELS IN NON RESPONDERS (PAIRED WITH PRETREATMENT VALUES OF ORAL CAVITY CANCER PATIENTS)

	Mean difference	S.E.	"t" Value	"p" Value
FSA(μg/dl)	0.2731	0.133	2.053	0.042
PSA(μg/dl)	-2.597	0.653	3.984	0.000
TSA(μg/dl)	5.2863	1.000	5.280	0.000
PSA/TP(μg/gm)	0.4259	0.111	3.836	0.000
TSA/TP(μg/gm)	0.5879	0.183	-3.216	0.001

FSA – Free Sialic Acid
PSA – Protein bound Sialic Acid
TP – Total Protein
TSA – Total Sialic Acid

Table – 14

PAIRED "t" TEST FOR FUCOSE AND SEROMUCOID FRACTION IN COMPLETE RESPONDERS (PAIRED WITH PRETREATMENT VALUES OF ORAL CAVITY CANCER PATIENTS)

	Mean difference	S.E.	"t"Value	"p"Value
Fucose (μg/dl)	0.9951	0.347	1.433	0.155
MP (μg/dl)	46.275	5.420	8.543	0.000
Hexoses (μg/dl)	14.835	2.277	-6.515	0.000
Fucose/TP(μg/gm)	0.0966	0.068	2.415	0.043
MP/TP (μg/gm)	2.0056	0.746	1.352	0.178
Hexoses/TP(μg/gm)	0.5348	0.064	8.402	0.000

Table – 15

PAIRED "t" TEST FOR FUCOSE AND SEROMUCOID FRACTIONS IN NONRESPONDERS (PAIRED WITH PRETREATMENT VALUES OF ORAL CAVITY CANCER PATIENTS)

	Mean difference	S.E.	"t"Value	"P"Value
Fucose(μg/dl)	0.9203	0.391	2.352	0.020
MP (μg/dl)	23.400	4.399	3.374	0.035
Hexoses (μg/dl)	0.7987	0.462	1.734	0.085
Fucose/TP (μg/gm)	0.1196	0.065	1.835	0.070
MP/TP (μg/gm)	6.114	0.908	6.735	0.000
Hexoses/TP (gm/dl)	0.1813	0.074	2.466	0.014

MP – Mucoid Proteins
TP – Total Protein

4.7 REPRESENTATIVE PATTERNS SHOWING POST-TREATMENT VARIATIONS IN LEVELS OF THE GLYCOPROTEIN CONSTITUENTS IN CANCER PATIENTS.

To evaluate efficacy of the biomarkers in treatment monitoring of cancer patients, levels of the biomarkers were analysed serially after initiation of anticancer therapy. Representative patterns of the markers during follow-up with different treatment out come are shown in **figures 14 - 15. Figures 14A** and **15A,** shows post-treatment variations of markers in 70 years old female with breast cancer ($T_4N_0M_0$). The patient was treated with surgery followed by Tamoxifen. She had disease free survival after surgery. Levels of the boimarkers during follow up were calculated as percentage of pretreatment levels. The pretreatment values were considered 100%. As shown in the figures, serum levels of all the markers remained below pretreatment level throughout follow up.

The alterations in circulating levels of markers in the group of patients with no response to therapy are represented in **figures 14B** and **15B.** Figures show post- treatment variations in marker levels in a 45 years old female with invasive ductal carcinoma of breast ($T_4N_2M_0$). Subsequent to surgical resection of the tumour, the patient was treated with radiotherapy and chemotherapy. She did not respond to any of the therapies and developed metastasis at 14 months after initiation of treatment. As clear from the figures, marker levels remained above pretreatment level throughout 24 months of follow up duration.

Figures 14C and **15C** depict alterations in marker level in a 60 years old female diagnosed as having breast cancer ($T_3N_1M_0$). The patient showed positive response to surgery and had disease free survival for 20 months. Later on she developed metastasis. As clear from the figures, the marker levels remained below pretreatment values initially but then started rising. Importantly, the rise in the marker levels was observed 3-4 months prior to the clinical detection of the metastasis.

Figure 16A and **17A** illustrate individual levels of the biomarkers at diagnosis and during/after anti cancer treatment in oral cavity cancer patients. **Figure 16A** and **17A** show the biomarkers values in a patient bearing epidermoid carcinoma of lower gum with Stage III ($T_3N_1M_0$) disease. There was no evidence of disease after surgical resection of the tumour. As depicted in the figures there was a sharp fall in marker levels during follow-up and they remained below pretreatment value throughout the whole follow-up duration. The pattern suggests that disease free survival was associated with decline in the levels of glycoprotein constituents.

Figure 16B and **17B** depict a case of squmous cell carcinoma of tongue ($T_4N_0M_0$) who did not respond to anticancer therapy (radiotherapy and chemotherapy) and had persistently progressive malignant disease during follow-up. As clear from the figure, levels of the markers were raised progressively during follow-up period. Throughout the follow up period marker levels either remained near pretreatment levels or were elevated.

Figures 16C and **17C** represent alterations in serum levels of glycoprotein constituents in a patient with epidermoid carcinoma of tongue ($T_2N_0M_0$), who had disease free survival for a period of 20 months after surgery followed by Radiotherapy. Subsequently, the patient developed recurrent disease. As shown in the figure, concentrations of the markers were declined when patient was clinically found to be in remission. However, recurrence of the disease was detected after 22 months. It was noteworthy that the marker levels began rising prior to clinical detection of the recurrence.

Figures 14-17, representatively show post-treatment variations in breast cancer patients and oral cavity cancer patients. The post-treatment changes in all cancer patients exhibited almost similar trend as represented in Figures14-17.

Figure-14
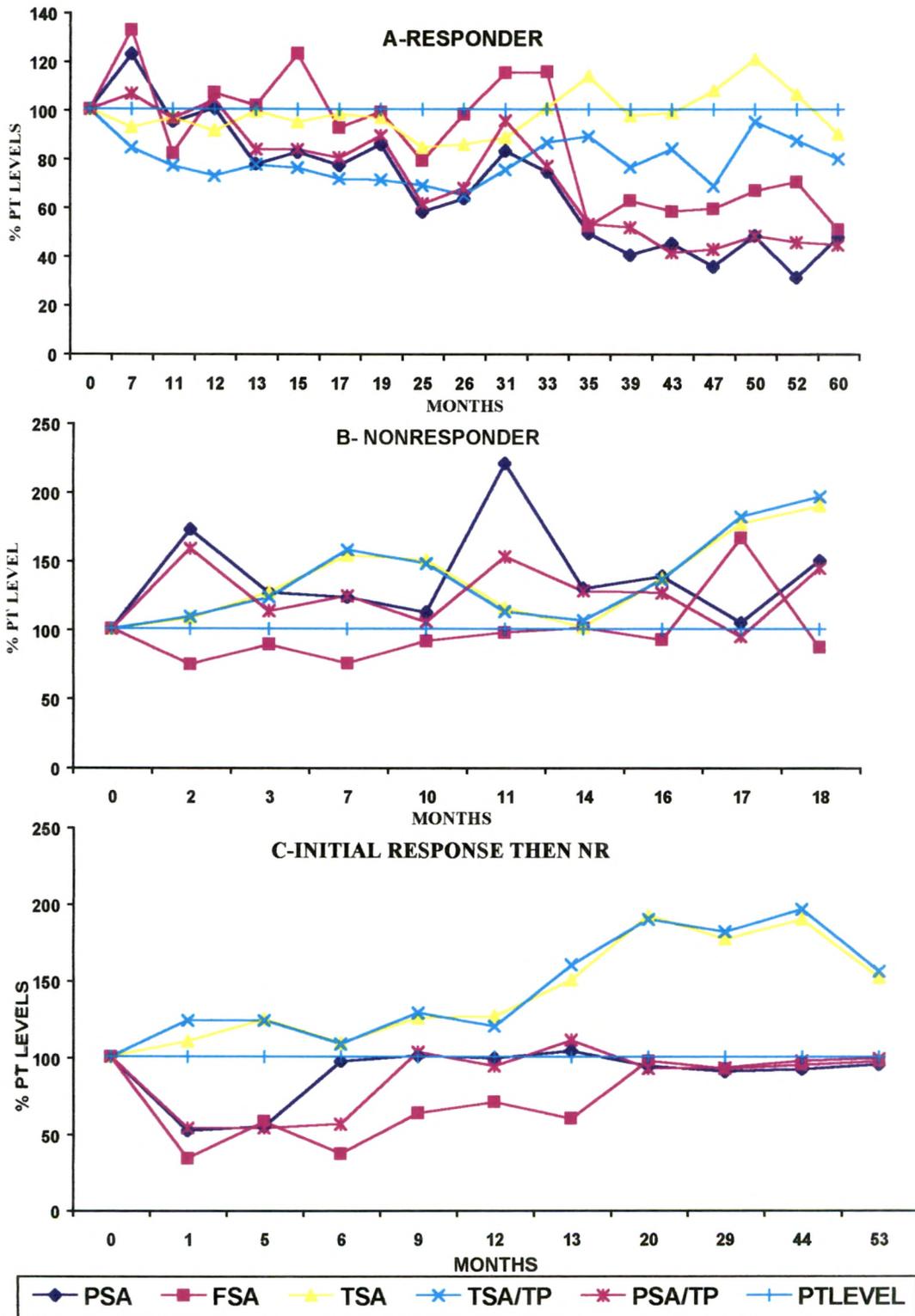## POST-TREATMENT VARIATIONS IN SERUM SIALIC ACID LEVELS IN BREAST CANCER PATIENTS

Figure - 15
POST-TREATMENT VARIATIONS IN FUCOSE AND SEROMUCOID
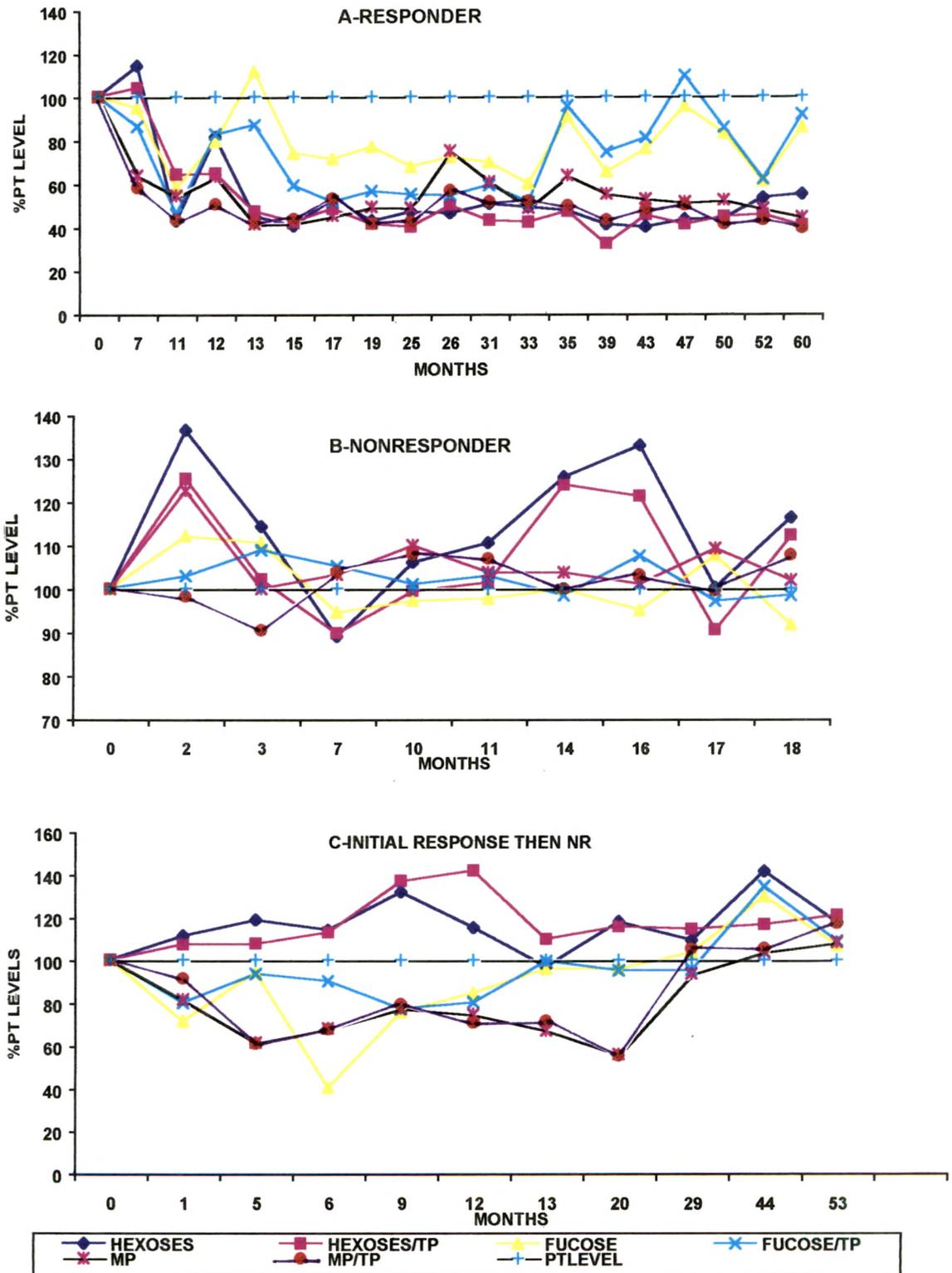FRACTION LEVELS IN BREAST CANCER PATIENTS

Figure-16
POST-TREATMENT VARIATIONS IN SIALIC ACID LEVELS IN ORAL CAVITY CANCER PATIENTS
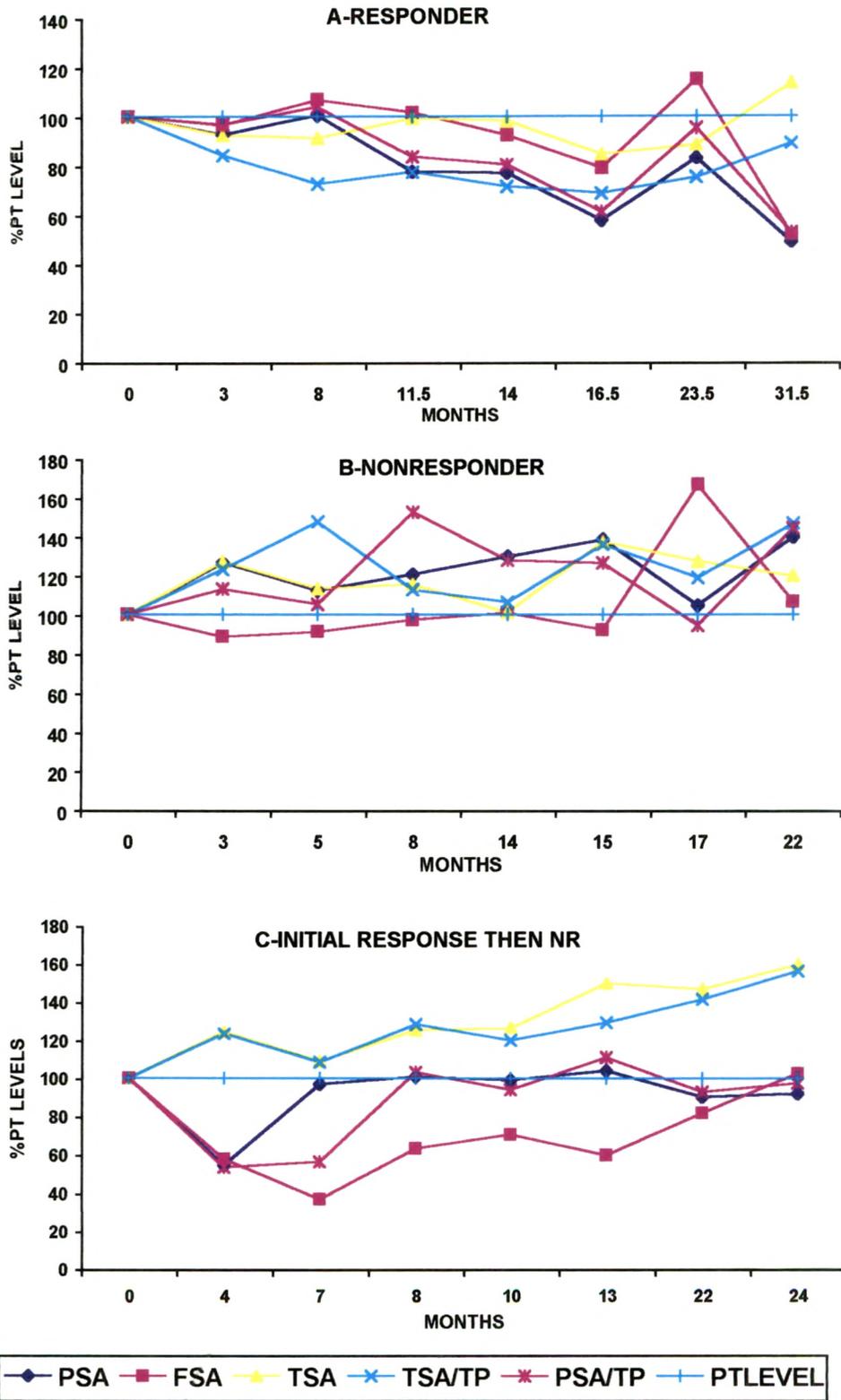
Figure - 17
POST-TREATMENT VARIATIONS IN FUCOSE AND SEROMUCOID FRACTION LEVELS IN ORALCAVITY CANCER PATIENTS
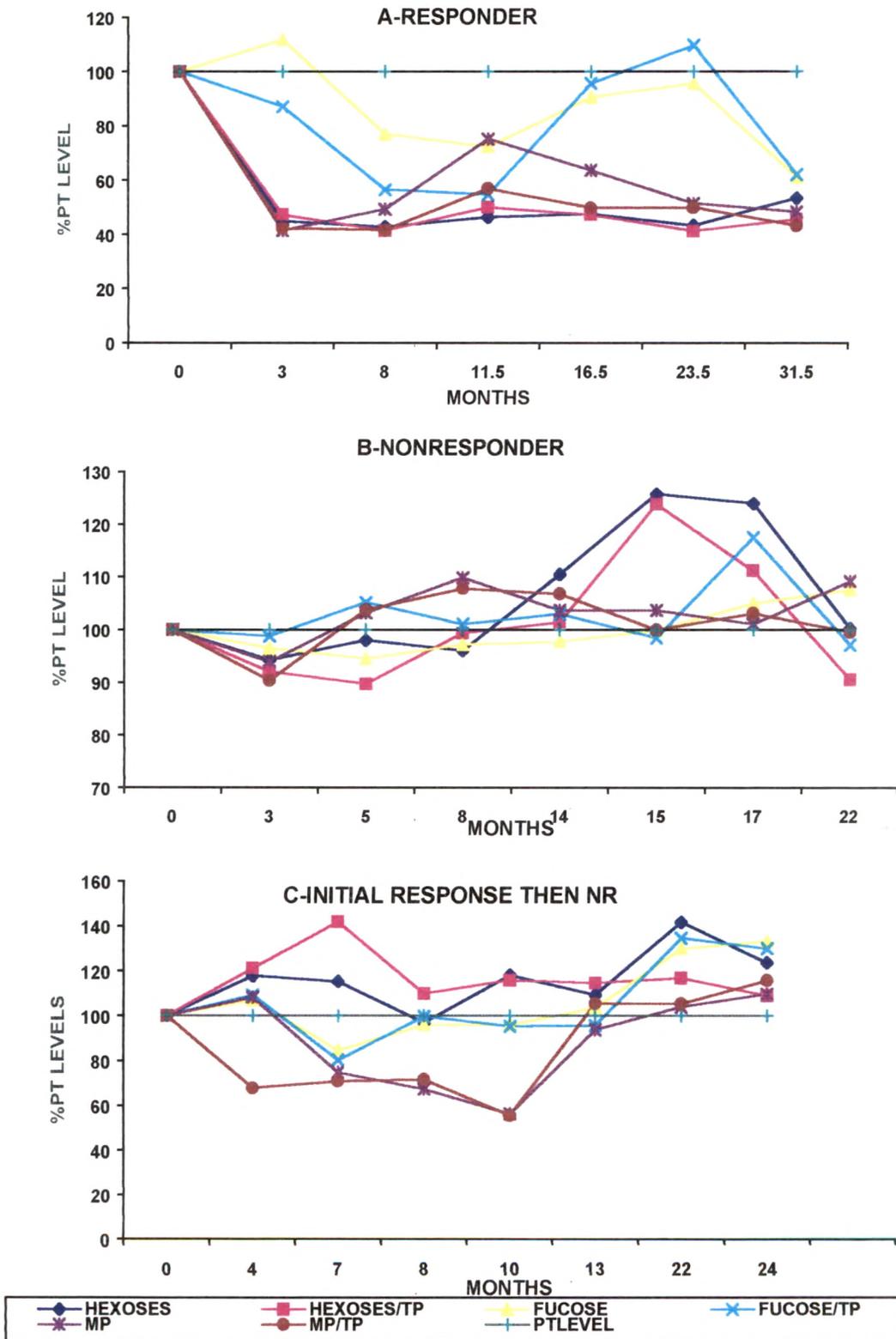
Figure-18

Lane **a** - Serum glycoproteins stained by periodic acid schiff's (PAS)

Lane **b** - Serum proteins stained by coomassie brilliant blue (CBB)

Lane **c** - Molecular weight markers

E - Extra protein band

i - Albumin region glycoproteins

ii - Alpha region glycoproteins

iii - Beta region glycoproteins

iv - Gamma region glycoproteins

4.8 ALTERATIONS IN GLYCOPROTEIN ELECTROPHORETIC PATTERNS:

Figure-18 shows separation of serum proteins by polyacrylamide gel electrophoresis. The proteins were stained using PAS (Periodic acid schiff) staining for detection of glycoproteins. As illustrated in the figures, serum glycoproteins were grouped into: **(i)** Albumin **(ii)** Alpha, **(iii)** Beta and **(iv)** Gamma region glycoproteins depending on the simultaneous run for total proteins. The molecular weight marker was also electrophoresd simultaneously. The glycoprotein electrophoresis was carried out in blood samples obtained from controls, pathological controls and cancer patients at diagnosis as well as during post-treatment follow-ups. During analysis of the electrophoretic patterns, an extra band was observed in post-beta region in some of the samples. The band was termed as "E "band

Figure-18
SEPARATION OF SERUM PROTEINS / GLYCOPROTEINS BY POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)
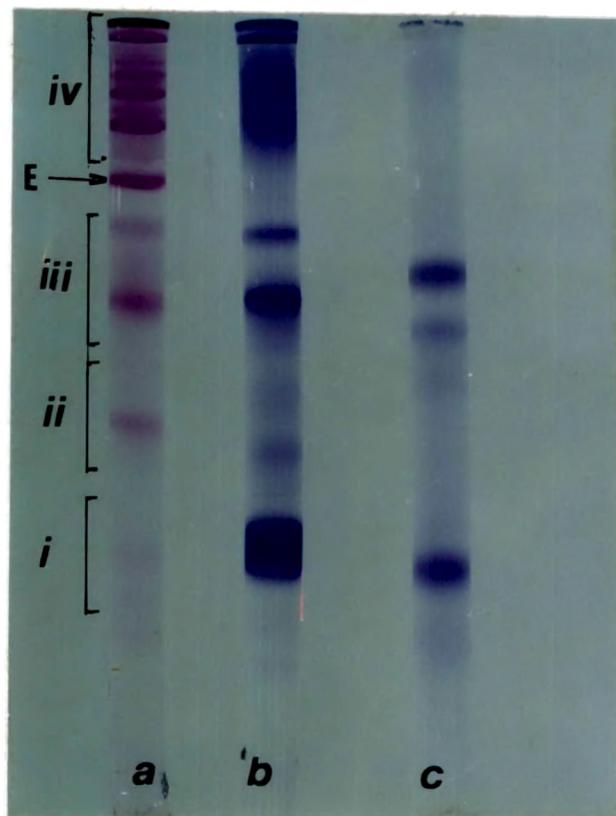
Breast Cancer Study

Mean values of various fractions of glycoproteins in healthy females, patients with BBD and untreated breast cancer patients are shown in **table 16**. The levels of various region glycoproteins were comparable between controls, pathological controls and untreated breast cancer patients. However, when percentage of individual fractions were compared, albumin to total glycoprotein ratio showed significant decline in patients with BBD and untreated breast cancer patients as compared to controls (p=0.000). The patients with BBD showed lower levels of percent albumin as compared to that in untreated breast cancer patients. However, the difference was not stastically significant. The percentages of alpha and beta region glycoproteins were comparable between all the three groups. However, the ratio of gamma region to total glycoprotein showed significantly lower levels (p=0.05) in healthy individuals as compared to untreated breast cancer patients. When the ratio of albumin to gamma region glycoproteins was compared among three groups untreated breast cancer patients showed significantly lower (p=0.047) values as compared to the controls. However, the patients with BBD showed comparable values of albumin/gamma region glycoprotein with controls as well as untreated breast cancer patients.

Paired "t" analyses were performed to study the association of various region glycoproteins with treatment response. As depicted in **table-17**, complete responders showed significantly higher values of albumin, alpha, and beta region glycoproteins (p=0.001). The gamma region glycoproteins were significantly lower (p=0.000) in complete responders as compared to their pretreatment levels. When percentage of each fractions were compared between complete responders and untreated cancer patients, it revealed significantly declined gamma/total glycoprotein (p=0.000) levels and significant elevations (p=0.000) in albumin region glycoproteins during follow up. Whereas, total glycoprotein levels were significantly lower (p=0.000) in patients with positive treatment outcome as compared

to their initial levels. The ratio of albumin to gamma region glycoproteins showed significant elevations (p=0.000) in complete responders. In comparison between untreated breast cancer patients and nonresponders, the ratio of albumin to gamma region glycoproteins showed significant difference (p=0.000), in the levels of total glycoprotein as well as various glycoprotein fractions were comparable at diagnosis and during follow-up.

As the alterations in glycoprotein fractions were found to be associated treatment outcome, the follow-up samples were grouped into: **(i)** glycoprotein fractions below their pretreatment levels and **(ii)** glycoprotein levels above their pretreatment levels. **Figure-19** shows number of individuals during follow-up with values of the glycoprotein fractions above and below pretreatment level. A more number of patients with favorable treatment response (complete responders) had albumin region glycoprotein above pretreatment levels. Further, a higher number of nonresponders had gamma glycoproteins above pretreatment levels as compared to the complete responders.

Oral Cavity Cancer Study

Mean values of serum glycoproteins in controls, patients with OPC and untreated oral cavity cancer patients are shown in **table 19**. Among various fractions of glycoproteins, albumin and gamma region glycoproteins showed noteworthy alterations. As compared to the healthy individuals, the patients with OPC and untreated oral cavity cancer patients showed significantly lower (p=0.001) levels of albumin/total glycoprotein ratio. The gamma region glycoproteins were significantly higher (p=0.000) in patients with OPC as well as untreated oral cavity cancer patients as compared to the controls. The ratio of albumin/gamma region glycoproteins revealed significantly lower (p=0.001) levels in OPC and oral cavity cancer patients as compared to the healthy individuals.

A representative pattern of alterations in untreated oral cavity cancer patients, complete responders and nonresponders is shown in **figure-19**. The disappearance of gamma region glycoproteins was significantly associated with successful treatment outcome. The data in the tables shows that the quantitative analysis also revealed significant changes in complete responders as compared to their values at diagnosis. The glycoprotein electrophoretic patterns in untreated oral cavity cancer patients and nonresponders were almost similar.

The detailed comparison of the levels of glycoprotein fractions at diagnosis of oral cavity cancer and during their follow-up is shown in **table 20.** The mean values of albumin, alpha, beta and gamma region glycoproteins were compared between untreated oral cavity cancer patients, complete responders and nonresponders. The levels of albumin region glycoproteins were decreased in complete responders as compared to both untreated oral cavity cancer patients and nonresponders ($p=0.007$ and 0.002, respectively). The statistical analysis indicated positive correlation between response to therapy and serum level of albumin region glycoproteins. Whereas, the gamma region glycoproteins showed reverse association i.e. gamma region glycoproteins showed decreased values in complete responders as compared to untreated oral cavity cancer patients and nonresponders ($p = 0.001$ and $p = 0.000$, respectively), When the levels of glycoprotein fractions were compared between untreated oral cavity cancer patients and nonresponders. However, alpha and beta region glycoproteins did not show significant alterations between the levels at diagnosis and during follow-ups with respect to the treatment outcome. Ratio of albumin to gamma region glycoproteins was significantly higher in complete responders as compared to untreated oral cavity cancer patients and nonresponders ($p = 0.000$ and 0.001, respectively).

MEAN VALUES OF GLYCLOPROTEIN FRACTIONS IN CONTROLS, PATIENTS WITH BBD AND UNTREATED BREAST CANCER PATIENTS

		Controls	Pts. With BBD	Untreated BC pts.	"t" Value	"p" Value
Albumin	Mean	69.827	67.305	52.516	0.700[a]	NS
	S.E.	14.004	6.317	10.375	0.993[b]	NS
					1.464[c]	NS
Alpha	Mean	67.500	67.586	63.506	0.352	NS
	S.E.	10.333	8.297	4.616	0.148	NS
					0.309	NS
Beta	Mean	164.770	165.87	163.45	0.061	NS
	S.E.	15.415	14.697	8.509	0.022	NS
					0.142	NS
Gamma	Mean	329.751	332.27	347.586	0.076	NS
	S.E.	28.906	15.575	26.798	0.125	NS
					0.494	NS
Albumin/TGP	Mean	11.342	9.748	8.4154	3.650	0.000
(%)	S.E.	2.223	0.801	0.0143	4.708	0.000
					1.664	0.050
Alpha/TGP	Mean	10.310	10.3667	9.748	0.563	NS
(%)	S.E.	0.012	0.986	0.7882	0.623	NS
					0.470	NS
Beta/TGP	Mean	27.290	25.819	24.693	1.787	0.048
(%)	S.E.	0.014	0.8229	1.743	1.490	0.097
					0.584	NS
Gamma/TGP	Mean	50.9751	53.596	54.509	1.171	NS
(%)	S.E	0.728	2.1173	1.603	2.00	0.047
					0.343	NS
Alb/Gamma	Mean	0.2117	0.2025	0.1510	0.143	NS
(%)	S.E.	0.0150	0.0624	0.0201	2.428	0.03
					0.787	NS
TGP/TP	Mean	68.542	71.77	73.95	0.182	NS
(%)	S.E.	3.026	4.011	4.30	1.998	0.045
					1.215	0.064

a   - Controls versus patients with BBD
b   - Controls versus untreated breast cancer patients
BBD   - Benign Breast Disease
BC   - Breast Cancer
c   - Patients with BBD versus untreated breast cancer pts.
NS   - Not Significant   TGP   - Total glycoprotein   TP   - Total Protein

Table – 17

PAIRED "t" ANALYSIS OF GLYCOPROTEIN FRACTIONS: COMPARISON BETWEEN UNTREATED BREAST CANCER PATIENTS AND COMPLETE RESPONDERS

	Mean Difference	"t" Value	"p" Value
Albumin	-14.60	3.01	0.000
Alpha	-15.20	3.54	0.000
Beta	-31.20	3.84	0.000
Gamma	43.80	-3.15	0.000
Albumin/TGP (%)	-3.90	5.61	0.000
Alpha/TGP (%)	-1.78	2.22	0.030
Beta/TGP (%)	-4.65	5.62	0.000
Gamma/TGP (%)	10.60	-6.51	0.000
Albumin/Gamma(%)	-21.93	9.65	0.000
TGP	32.10	-3.46	0.000

Table – 18

PAIRED "t" ANALYSIS OF GLYCOPROTEIN FRACTIONS: COMPARISON BETWEEN UNTREATED BREAST CANCER PATIENTS AND NONRESPONDERS

Parameter	Mean difference	"t" Value	"p" Value
Albumin	17.1	1.95	0.06
Alpha	8.02	1.19	0.24
Beta	8.44	0.72	0.47
Gamma	11.4	0.58	0.57
Albumin/TGP(%)	1.84	1.88	0.07
Alpha/TGP(%)	0.60	0.86	0.40
Beta/TGP(%)	-0.40	-0.4	0.69
Gamma/TGP(%)	-3.50	-1.8	0.07
Albumin/Gamma(%)	20.58	7.91	0.00
TGP	-50.00	-1.3	0.20

TGP = Total glycoprotein

Figure-19

PERCENTAGES OF RESPONDERS AND NON RESPONDERS WITH LEVEL OF GLYCOPROTEIN FRACTIONS ABOVE / BELOW PRETREATMENT(PT) VALUE IN BREAST CANCER PATIENTS
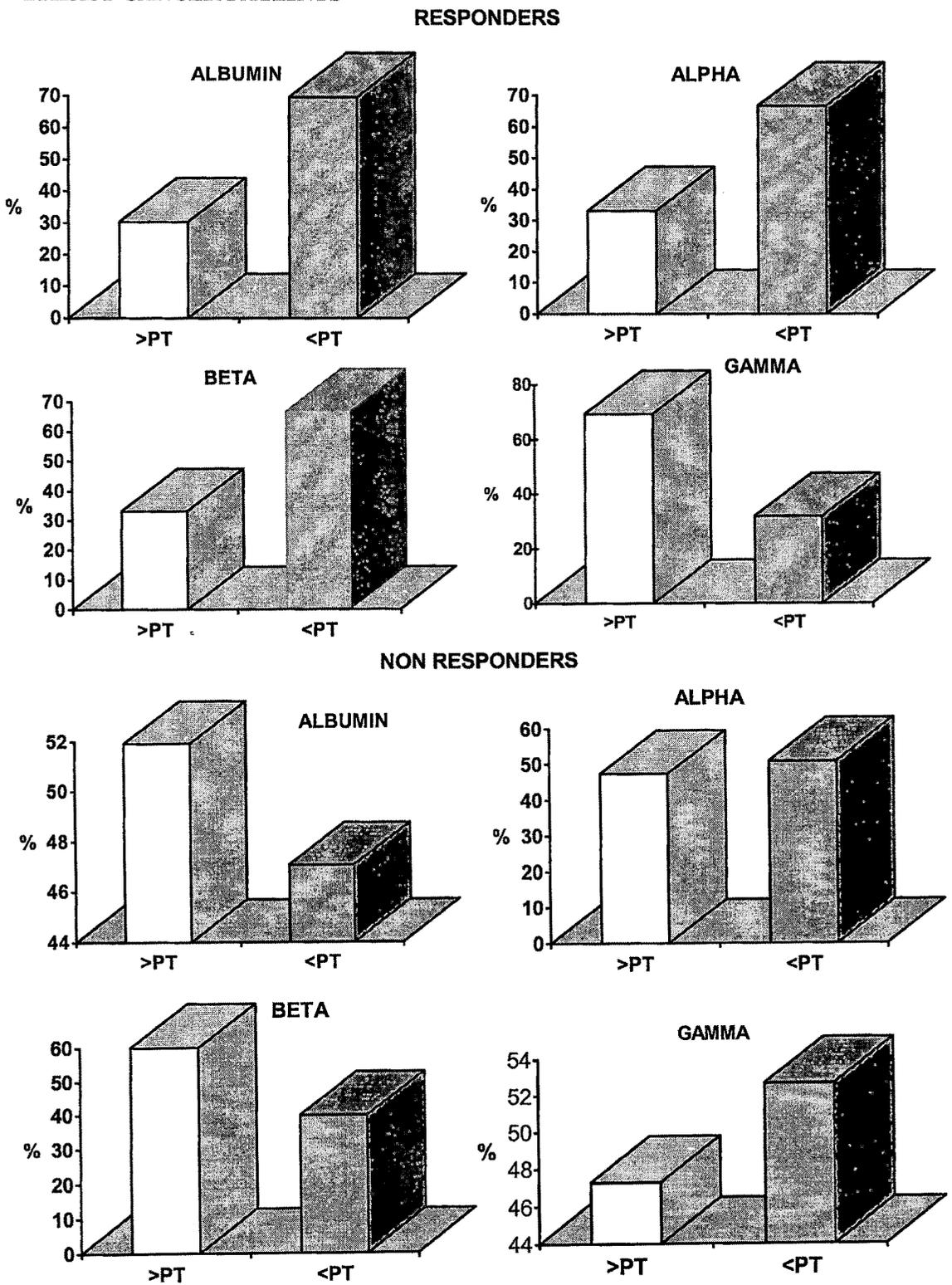
RESPONDERS

NON RESPONDERS

Table – 19
**MEAN VALUES OF GLYCLOPROTEIN FRACTIONS IN CONTROLS, PATIENTS
WITH OPC AND UNTREATED ORAL CAVITY CANCER PATIENTS**

		Controls	Pts. With OPC	Untreated OC patients	"t" Value	"p" Value
Albumin	Mean	68.798	68.0704	51.758	1.888[a] 0.157[b] 1.598[c]	0.097[a] 0.887[b] 0.157[c]
	S.E.	7.1076	7.9234	4.8813		
Alpha	Mean	66.428	68.7037	59.078	0.383 0.832 0.142	0.438 0.237 0.884
	S.E.	7.0163	4.1120	5.3528		
Beta	Mean	162.520	163.342	177.503	0.585 1.164 1.190	0.653 0.189 0.178
	S.E.	10.505	9.2687	7.4623		
Gamma	Mean	321.480	331.27	339.892	0.502 1.255 0.440	0.629 0.101 0.669
	S.E.	20.308	16.5614	10.4263		
Albumin/TGP (%)	Mean	13.4537	8.197	7.068	5.993 5.108 1.251	0.000 0.000 0.170
	S.E.	0.872	0.100	0.897		
Alpha/TGP (%)	Mean	10.406	10.5422	9.949	0.135 0.448 0.863	0.851 0.669 0.417
	S.E.	0.887	0.472	0.501		
Beta/TGP (%)	Mean	27.9834	25.3166	24.893	2.410 2.156 0.351	0.055 0.078 0.463
	S.E.	0.928	0.819	0.885		
Gamma/TGP (%)	Mean	50.175	51.652	54.742	0.924 3.089 1.984	0.217 0.001 0.085
	S.E.	1.076	1.182	1.015		
Alb/Gamma (%)	Mean	0.3115	0.179	0.144	3.742 4.917 1.045	0.001 0.000 0.158
	S.E.	0.026	0.0243	0.022		
TGP/TP (%)	Mean	71.176	73.0911	78.565	0.653 2.492 1.807	0.715 0.042 0.092
	S.E.	2.024	2.1187	2.166		

a   -Controls versus patients with OPC
b   -Controls versus untreated oral cavity cancer patients
c   -Patients with OPC versus untreated oral cavity cancer pts.
NS   -Not Significant
TGP   -Total glycoproteins   Alb-albumin   TP-Total Protein

Table - 20

GLYCOPROTEIN FRACTIONS IN UNTREATED ORAL CAVITY CANCER PATIENTS, COMPLETE RESPONDERS AND NONRESPONDERS

	Group	Mean	S.E.	"t"Value	"p" Value
Albumin/TGP	PT	7.0683	0.897	3.976[a]	0.001a
(%)	CR	12.0412	0.872	0.856[b]	0.321b
	NR	5.5113	1.582	3.612[c]	0.002c
Alpha/TGP	PT	9.9498	0.501	2.80	0.007
(%)	CR	11.8881	0.472	1.51	0.058
	NR	8.0637	1.138	3.10	0.007
Beta/TGP	PT	25.8931	0.728	0.48	0.635
(%)	CR	25.3166	0.720	0.72	0.475
	NR	23.7988	0.90	0.90	0.377
Gamma/TGP	PT	54.3421	1.015	3.74	0.000
(%)	CR	50.1767	1.267	0.47	0.641
	NR	62.6262	3.041	3.78	0.001
Alb/Gamma	PT	0.1448	0.022	4.80	0.000
(%)	CR	0.2762	0.026	0.80	0.398
	NR	0.1068	0.034	3.59	0.001

a - Untreated oral cavity cancer patients versus complete responders

b - Untreated oral cavity cancer patients versus complete esponders

c - Complete responders versus nonresponders

CR- Complete responders

NR- Nonresponders

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As mentioned earlier (figure-18), an extra band (E-band) was observed in controls, pathological controls, untreated cancer patients, complete responder and non-responder. To check the association of presence of E band with malignancy, frequency of E band were calculated in healthy individuals, patients with BBD/OPC and untreated cancer patients. The frequency of appearance of the E-band in various groups is shown in table-21 and figures-20. When the frequency of this band were compared in various groups more number of untreated cancer patients showed presence of this band. However, there was no association between presence of E band and treatment outcome. Figures-20 (A, B and C) shows slab gel protein electrophoretic pattern among controls, patients with BBD, and untreated breast cancer patients respectively. As clear from photograph, 2 out of 9 healthy individuals showed presence of E band. However, from pathological controls 3 out of 9 showed presence of E band. Among cancer patients 5 out of 10 subjects showed presence of E band. Further, more number of breast cancer showed presence of E band when compared with its presence in oral cavity cancer patients.

Table - 21
FRQUENCY OF "E" BAND

Group	Frequency
Controls	31.0% (31/100)
Patients with BBD	35.0% (35/100)
Untreated breast cancer patients	56.0% (126/225)
Controls	28.0% (28/100)
Patients with OPC	48.0% (48/100)
Untreated Oral cavity cancer patients	53.5% (107/200)

Figure-20
**SERUM PROTEIN ELECTROPHORETIC PATTERNS SHOWING PRESENCE OF
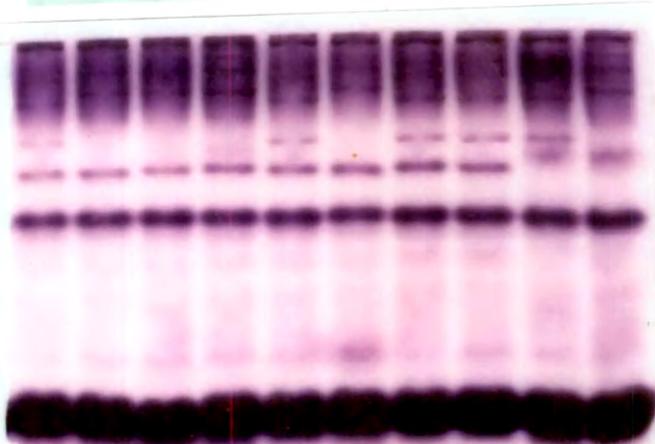E BAND IN DIFFERENT GROUPS**

CONTROLS
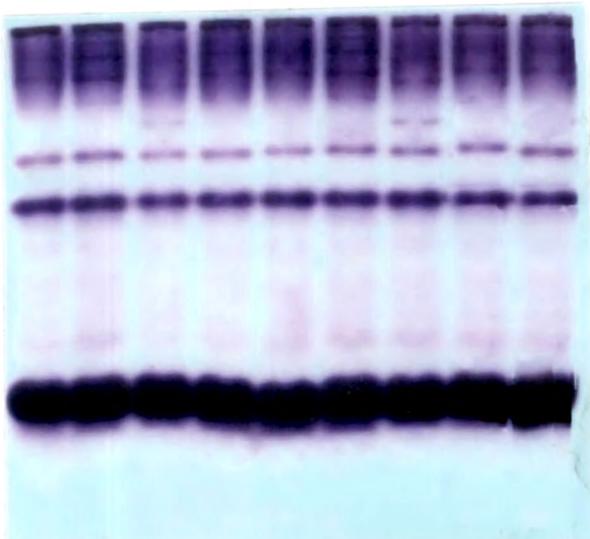
PATHOLOGICAL
CONTROLS
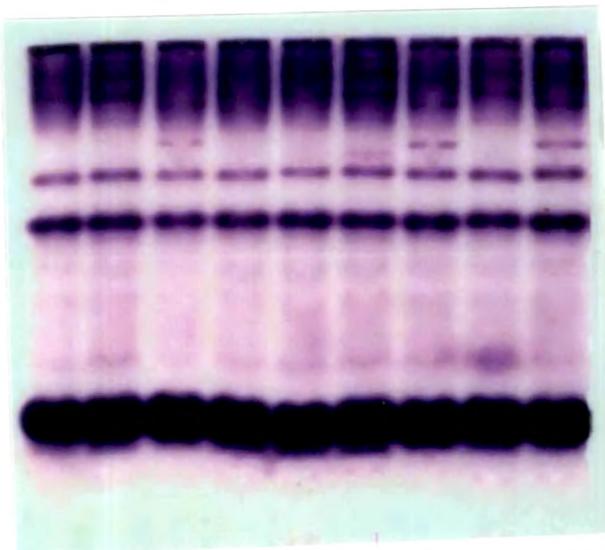
CANCER
PATIENTS

4.9 REPRESENTATIVE PATTERNS SHOWING POST-TREATMENT CHANGES IN GLYCOPROTEIN ELECTROPHORETIC PATTERNS IN CANCER PATIENTS

Sensitivity of serum glycoproteins to anticancer treatment was studied by performing glycoprotein electrophoresis from the serum of cancer patients at diagnosis as well as at different intervals during/after anticancer treatment.. **Figure 21(A and B)** depicts glycoprotein electrophoretic pattern in breast cancer patients ($T_2N_1M_0$ and $T_4N_0M_0$ respectively). **Figure 22(A)** shows glycoprotein separation by polyacrylamid gel electrophoresis(PAGE) in patients with cancer of cheek mucosa **Figure 22(B)** shows glycoprotein separation by polyacrylamid gel electrophoresis(PAGE) from sera of patients with cancer of lower gum. All patients showed favorable treatment outcome. As clear from the photograph patients with successful treatment outcome showed less number of bands in post-beta region throughout the follow-up period **[lane. b to d, lane. b to p, lane. b to f, and lane b to g respectively]**.

Figure 23 represents serum glycoprotein electrophoretic patterns in a patient with breast cancer($T_3N_0M_0$ and $T_3N_0M_x$ respectively) and **figure 24 (A and B)** represents patient with cancer of lower gum and cancer of tongue ($T_2N_0M_0$ and T_4N_0) who did not responded to anticancer therapy. As can be seen from the figure, in all four patients serum glycoprotein electrophoretic patterns were simillar at diagnosis and during follow-up duration.

Figure 25A and 25B represents serum glycoprotein electrophoretic changes in a breast cancer patients with $T_4N_2M_0$ and $T_2N_1M_0$ who initially responded to anticancer therapy and later on developed recurrence. **Figure 25A and 25B** shows glycoprotein electrophoretic pattern in serial followup of oral cavity cancer patients (cancer of tongue $T_4N_2M_0$ and cancer of floor of mouth $T_2N_1M_0$ respectively). As clear from the plates, presence of more number of glycoprotein bands in post-beta region were associated with poor treatment outcome. However, disappearnce of these bands were

associated with favourable treatment out come.It is clear from the photographs that there was a disappearance of various glycoprotein band in post beta region during initial followup duration when patients had disease free survival. However these band were reappeared lateron with reccurence of the disease.

Above results were obtained on tube gel electrophoresis. The tube gel electrophoretic analysis has its own advantages. However, single run on single tube might subject to scarce reproducibility and errors. But, runs on slab gel where more number of samples can be loaded on uniformly polymerized matrix are more creditable and can be compared with less valuation errors. To confirm reproducibility and accuracy of results on tube gel, the glycoproteins were also separated using slab gel from the subjects in all the groups. Glycoprotein electrophoretic patterns on slab gel in controls, pathological controls and cancer patients at diagnosis as well as during/after treatment are shown in **figures 27**. **Figures 27A** shows glycoprotein electrophoretic patterns in controls, pathological controls, cancer patients at diagnosis and during followup with favourable treatment outcome. **Figure 27B** shows glycoprotein electrophoretic patterns in controls, pathological controls, cancer patients who initially responded to the therapy but latter on deyloped metastasis. As can be seen in the photographs more number of glycoprotein bands are present in untreated cancer ptients as compared to both controls as well as pathological controls. As shown in **Figure 27A (lane-g)** and **Figure 27B (lane-e)** glycoprotein bands of post gamma region were disappeared with succesful treatment outcome.These bands were reappeared with devlopment of recurrence(**Figure 27B lane f, g and h**). As clear from the figures, glycoprotein electrophoretic patterns on slab gel were simillar to that obtained from tube gel electrophoresis.

Figure 21

A:
Cancer of Lt. Breast with $T_2N_1M_o$, Age-46 years, HPE- ductal cell carcinoma

Lane	Duration	Treatment	Comments
a	00 months	pretreatment	
b	05 months	surgery+2 CT	diseasefree survival
c	14 months	surgery+3 CT+TMX	disease free survival
d	25 months	surgery+2 CT+TMX	disease free survival

B:
Cancer of Lt. Breast with $T_4N_1M_o$, Age-46 years, HPE- ductal cell carcinoma

Lane	Duration	Treatment	Comments
a	0 months	pretreatment	
b	1.5 months	surgery	disease free survival
c	2.5 months	surgery+3 CT	disease free survival
d	4.5 months	surgery+4 CT+ 6CT *	disease free survival
e	7.0 months	surgery+4 CT+ 6CT	disease free survival
f	9.0 months	surgery+4 CT+ 6CT	disease free survival
g	11.0 months	surgery+4 CT+ 6CT	disease free survival
h	13.0 months	surgery+4 CT+ 6CT	disease free survival
i	16.0 months	surgery+4 CT+ 6CT	disease free survival
j	18.0 months	surgery+4 CT+ 6CT	disease free survival
k	20.0 months	surgery+4 CT+ 6CT	disease free survival
l	22.0 months	surgery+4 CT+ 6CT	disease free survival
m	24.0 months	surgery+4 CT+ 6CT	disease free survival
n	26.0 months	surgery+4 CT+ 6CT	disease free survival
o	29.0 months	surgery+4 CT+ 6CT	disease free survival
p	36.0 months	surgery+4 CT+ 6CT	disease free survival

CT - Chemo therapy
TMX-Tamoxifen

Figure-21

REPRESENTATIVE PATTERN OF SERUM GLYCOPROTEIN ELECTROPHORESIS IN RESPONDERS (BREAST CANCER PATIENTS)
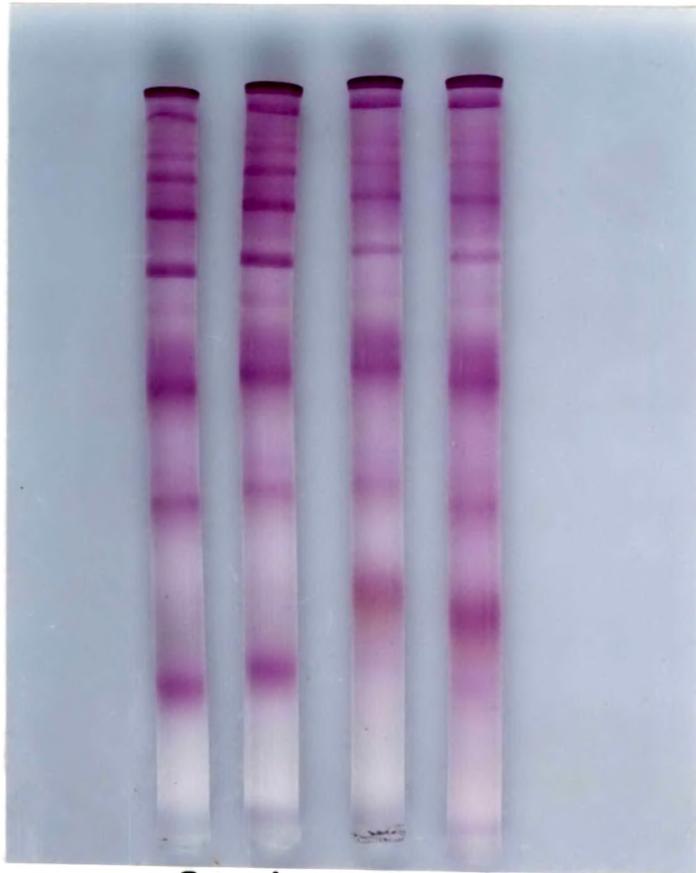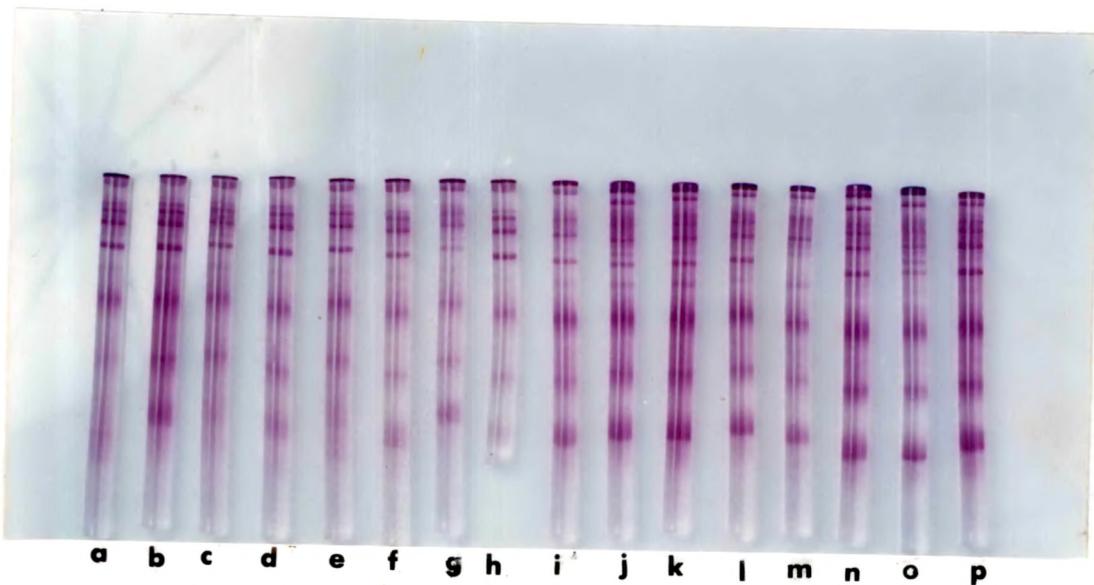
Figure 22

A:

Cancer of Lower gum with $T_2N_0M_o$, Age-42 years, HPE- epidermoid Carcinoma

Lane	Duration	Treatment	Comment
a	0 0 months	pretreatment	
b	3.0 months	surgery+RT	NED
c	6.0 months	surgery+RT	NED
d	11.5 months	surgery+RT	NED
e	16.5 months	surgery+RT	NED
f	31.5 months	surgery+RT	NED

B:

Cancer of cheek mucosa with $T_4N_0M_x$, Age-53 years, HPE- epidermoid Carcinoma

Lane	duration	Treatment	Comments
a	0.0 months	pretreatment	
b	2.0 months	surgery+RT	NED(Bx-Negative)
c	5.0 months	surgery+RT	NED
d	8.5 months	surgery+RT	NED
e	15.0 months	surgery+RT	NED
f	21.0 months	surgery+RT	NED
g	25.0 months	surgery+RT	NED

Bx   - Biopsy
NED - No evidence of disease
RT   -Radiotherapy

Figure-22

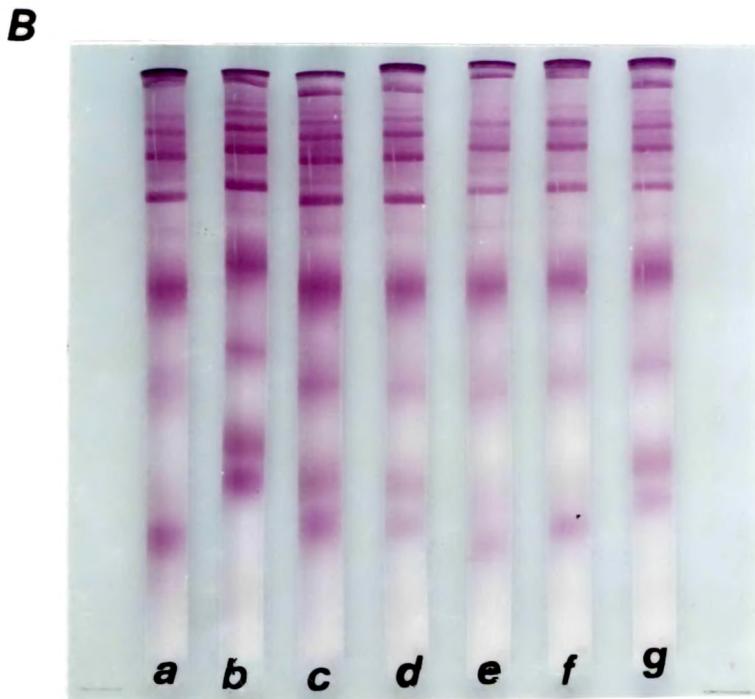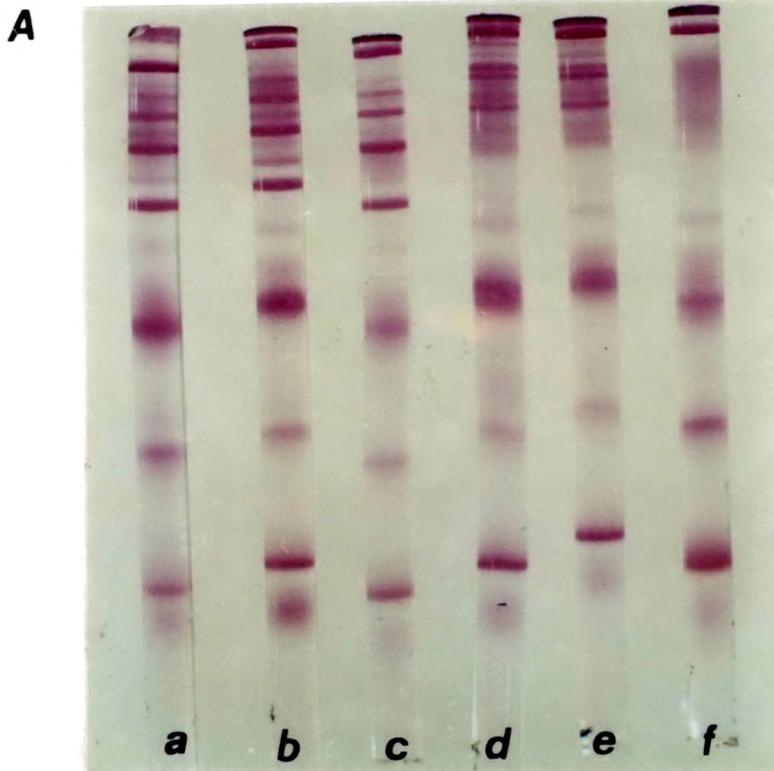**REPRESENTATIVE PATTERN OF SERUM GLYCOPROTEIN ELECTROPHORESIS IN RESPONDERS (ORAL CAVITY CANCER PATIENTS)**

Figure 23

A:

Cancer of Rt. Breast with $T_3N_0M_o$, Age-43 years, HPE- ductal cell carcinoma

Lane	Duration	Treatment	Comment
a	0 months	pretreatment	
b	5.5 months	surgery+3 CT+RT	persistant disease
c	10.0 months	surgery+3 CT+RT+TMX	advance disease
d	14.0 months	surgery+3 CT+RT+TMX	advance disease

B:

Cancer of Lt. Breast with $T_3N_0M_x$, Age-35 years, HPE- Invasive lobular Carcinoma

Lane	Duration	Treatment	Comments
a	0 months	pretreatment	
b	11.5 months	surgery+6 CT	secondary in Rt. Breast
c	19.5 months	surgery+6 CT+ 7CT	secondary in Rt. Breast
d	21.0 months	surgery+6 CT+ 7CT+RT	secondary in Rt. Breast

After 21 month of follow-up patient was on palliative treatment. Patient expired after 5 months of last followup

CT - Chemo therapy
RT - Radio therapy
TMX -Tamoxifen

Figure-23

REPRESENTATIVE PATTERN OF SERUM GLYCOPROTEIN ELECTROPHORESIS IN NONRESPONDERS (BREAST CANCER PATIENTS)
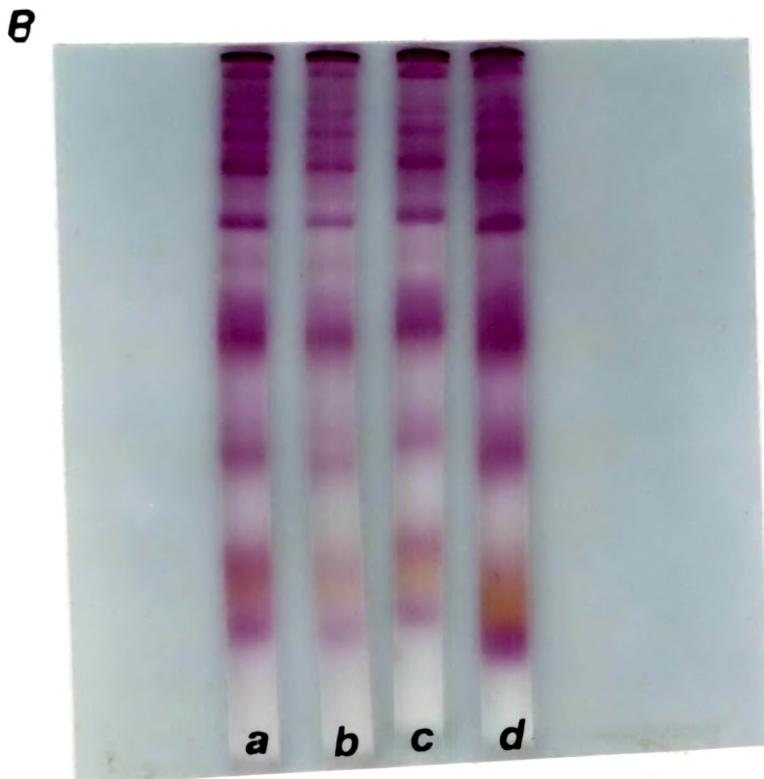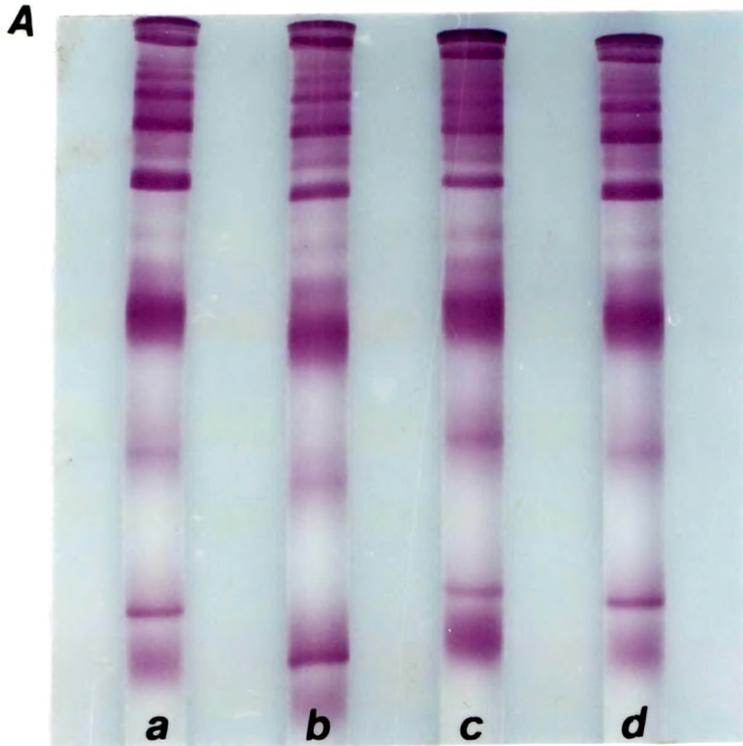
Figure 24

A:
Cancer of Lower Alveolus with $T_4N_2M_o$, Age-38 years, HPE- epidermoid carcinoma

Lane	Duration	Treatment	Comment
a	0.0 months	pretreatment	
b	2.0 months	palliative RT	Secondary on opposite side
c	8.0 months	palliative RT+ palliative care	Advance disease

patient was lost to follow-up after 9 months

B:
Cancer of tongue with $T_4N_0M_x$, Age-56 years, HPE- epidermoid carcinoma

Lane	Duration	Treatment	Comments
a	0 months	pretreatment	stageIII
b	4 months	palliative RT	advance disease
c	5 months	palliative RT	advance disease
d	6 months	palliative RT	advance disease
e	8 months	palliative RT +pain care	advance disease
f	11 months	palliative RT +pain care	advance disease
g	14 months	palliative RT +pain care	advance disease
h	18 months	palliative RT +pain care	advance disease

Patient expired after 15 days of last follow-up collection

RT- Radio therapy

Figure-24
REPRESENTATIVE PATTERN OF SERUM GLYCOPROTEIN
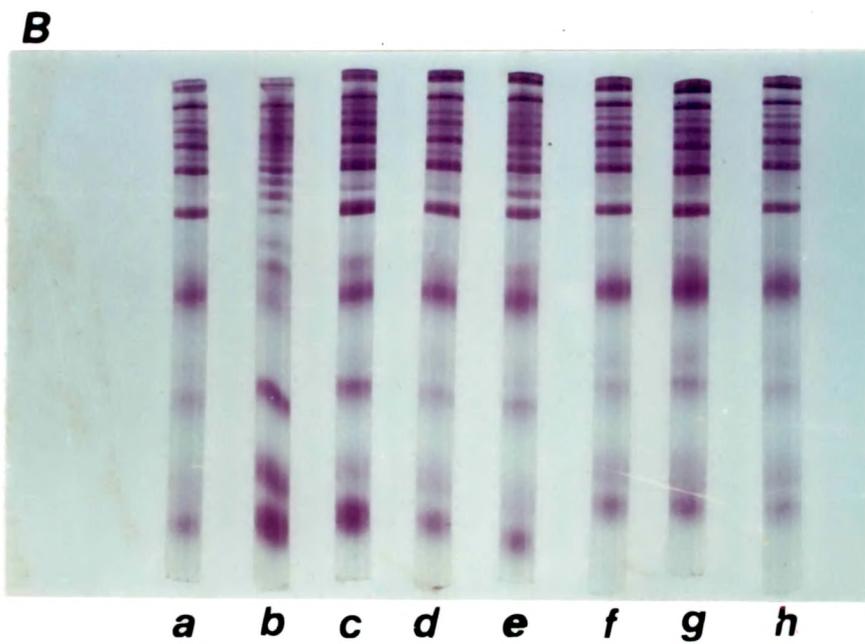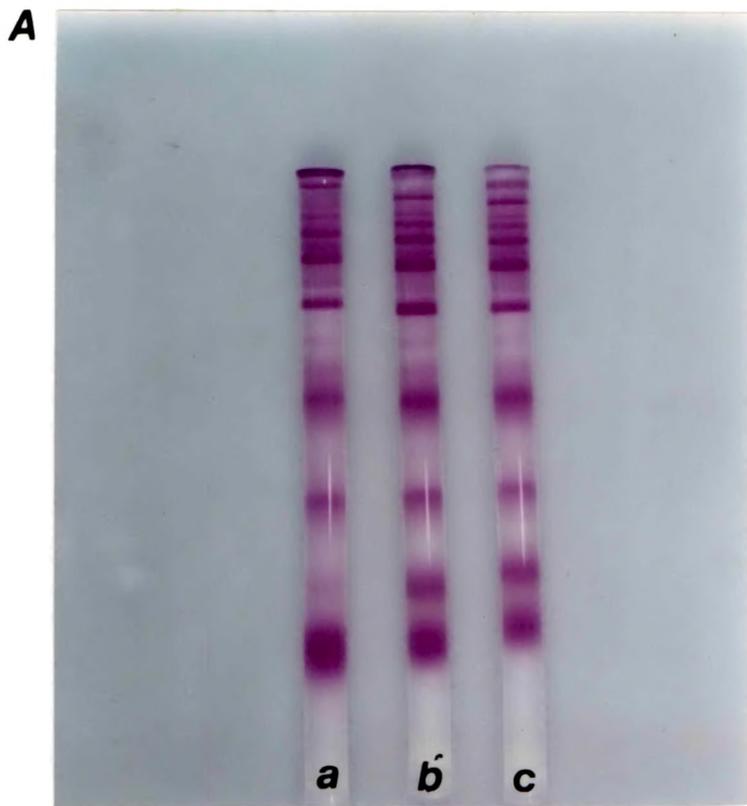## ELECTROPHORESIS IN NONRESPONDERS
(ORAL CAVITY CANCER PATIENTS)

Figure 25

A:

Cancer of Lt. Breast with $T_4N_2M_o$, Age-42 years, HPE- ductal cell carcinoma

Lane	Duration	Treatment	Comment
a	0 months	pretreatment	
b	2.0 months	surgery	diseasefree survival
c	3.5 months	surgery+3 CT+during RT	disease free survival
d	4.5 months	surgery+3 CT+RT+2CT	disease free survival
e	10.0 months	surgery+3 CT+RT+3CT	disease free survival
f	14.0 months	surgery+3 CT+RT+3CT+TMX	disease free survival

patient devloped recurrence at 17.5 months

g	19.0 months	surgery+3 CT+RT+3CT+TMX+CT	recurrent disease
h	20.5 months	surgery+3 CT+RT+3CT+TMX+CT	persistant disease

B:

Cancer of Rt. Breast with $T_2N_1M_o$, Age-30 years, HPE- ductal cell carcinoma

Lane	Duration	Treatment	Comment
a	0 months	pretreatment	
b	1.5 months	surgery+1 CT	disease free survival
c	5.0 months	surgery+6 CT+TMX	disease free survival
d	10.5 months	surgery+6 CT+TMX	disease free survival

secodary disese was detected on Lt breast after 15 month

CT   - Chemotherapy
RT   - Radiotherapy
TMX -Tamoxifen

Figure-25

REPRESENTATIVE PATTERN OF SERUM GLYCOPROTEIN ELECTROPHORESIS IN BREAST CANCER PATIENTS WITH INITIAL RESPONSE FOLLOWED BY NO RESPONSE TO THERAPY
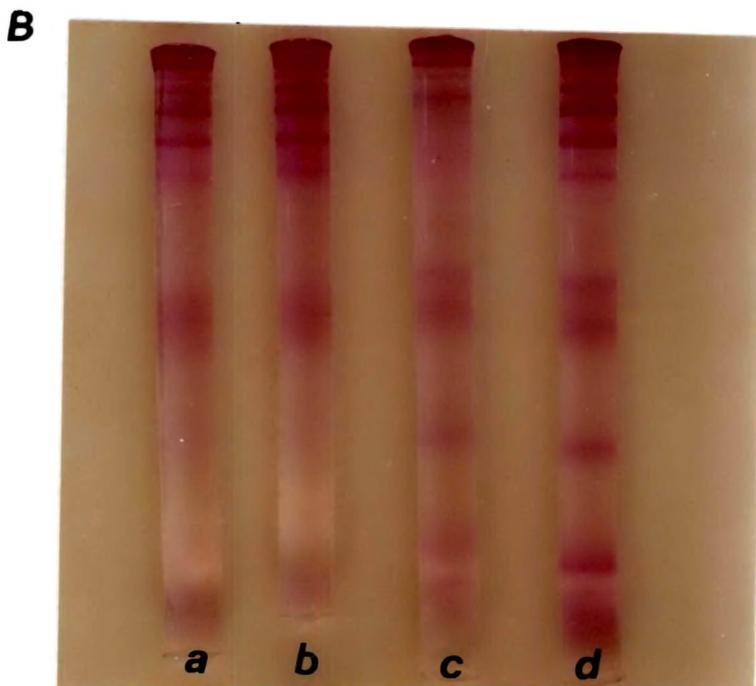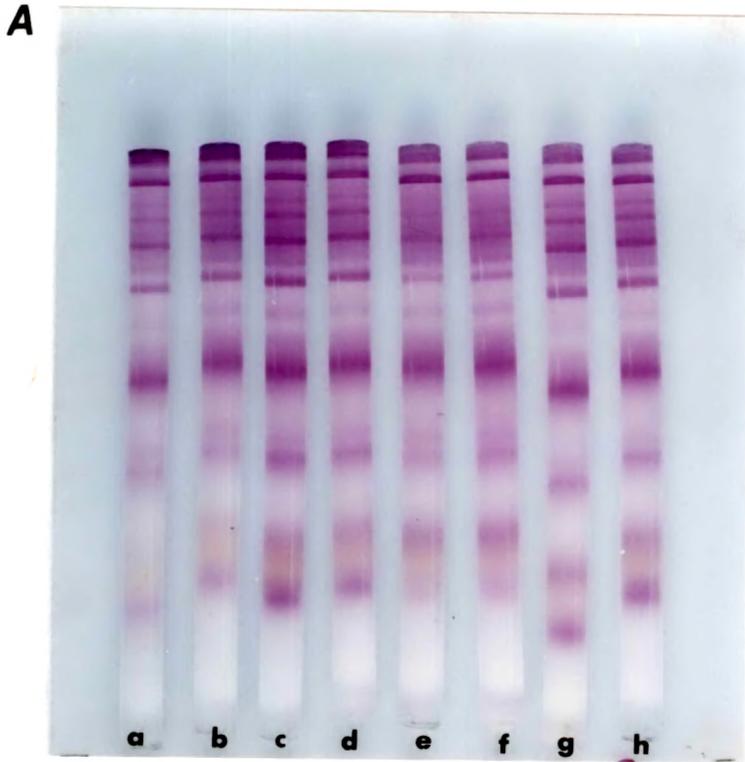
Figure 26

A:

Cancer of floor of mouth with $T_4N_1M_0$ Age-39 years, HPE- Epidermoid carcinoma

Lane	Duration		Treatment	Comments
a	0	months	pretreatment	
b	1.0	months	surgery	disease free survival
c	3.0	months	surgery	disease free survival
d	6.0	months	surgery	disease free survival
e	8.0	months	surgery	disease free survival
f	10.0	months	surgery	disease free survival
g	11.0	months	surgery	disease free survival
h	15.0	months	surgery+RT	metastasis

patient not come for followup after last followup.

B:

Cancer of tongue with $T_4N_3M_0$, Age-55 years, HPE- epidermoid carcinoma

Lane	Duration		Treatment	Comments
a	0	months	pretreatment	
b	1.5	months	surgery +RT	disease free survival
c	2.5	months	surgery+RT	disease free survival
d	5.5	months	surgery+RT	disease free survival
e	7.0	months	surgery+RT	disease free survival
f	9.0	months	surgery+RT+ 2CT	recurrent disease
g	11.0	months	surgery+RT+ 2CT	recurrent disease
h	16.0	months	surgery+RT+ 2CT	recurrent disease

CT- Chemotherapy
RT- Radiotherapy

Figure-26

REPRESENTATIVE PATTERN OF SERUM GLYCOPROTEIN ELECTROPHORESIS IN ORAL CAVITY CANCER PATIENTS WITH INITIAL RESPONSE FOLLOWED BY NO RESPONSE TO THERAPY
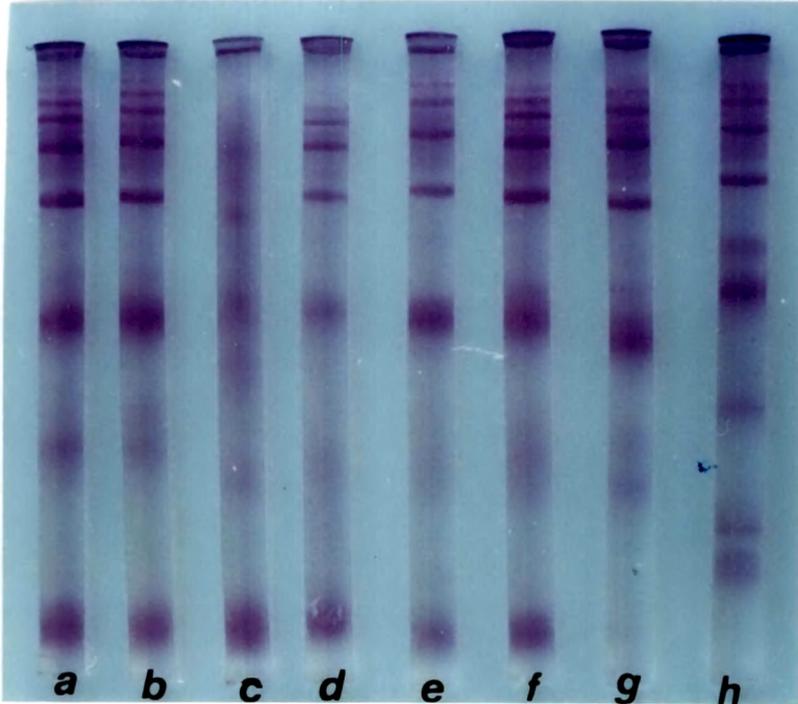
Figure 27

A:

Cancer of Lt. Breast with $T_2N_1M_o$, Age-46 years, HPE- ductal cell carcinoma

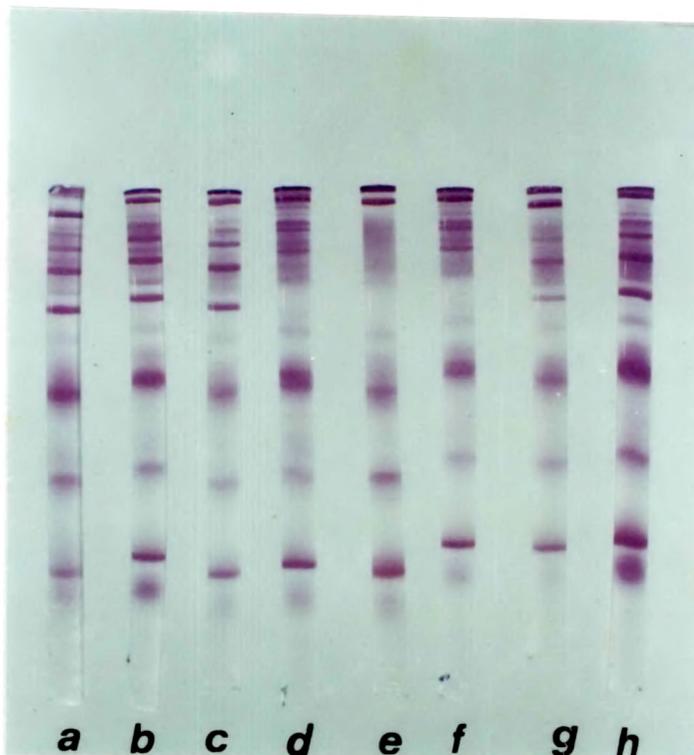| Lane	Duration	Treatment	Comments
d	0 months	pretreatment	
e	5 months	surgery+2 CT	disease free survival
f	14 months	surgery+3 CT+TMX	disease free survival
g	25 months	surgery+2 CT+TMX	disease free survival

B:

Cancer of Lt. Breast with $T_4N_1M_o$, Age-46 years, HPE- ductal cell carcinoma

| Lane	Duration	Treatment	Comments
d	0 months	pretreatment	
e	1.5 months	surgery	disease free survival
f	2.5 months	surgery+3 CT	disease free survival
g	4.5 months	surgery+3 CT+6 CT	recurrence of disease
h	7.0 months	surgery+3CT+6 CT	persistant disease

Lane a   **- controls**
Lane b and c   **– pathological controls**

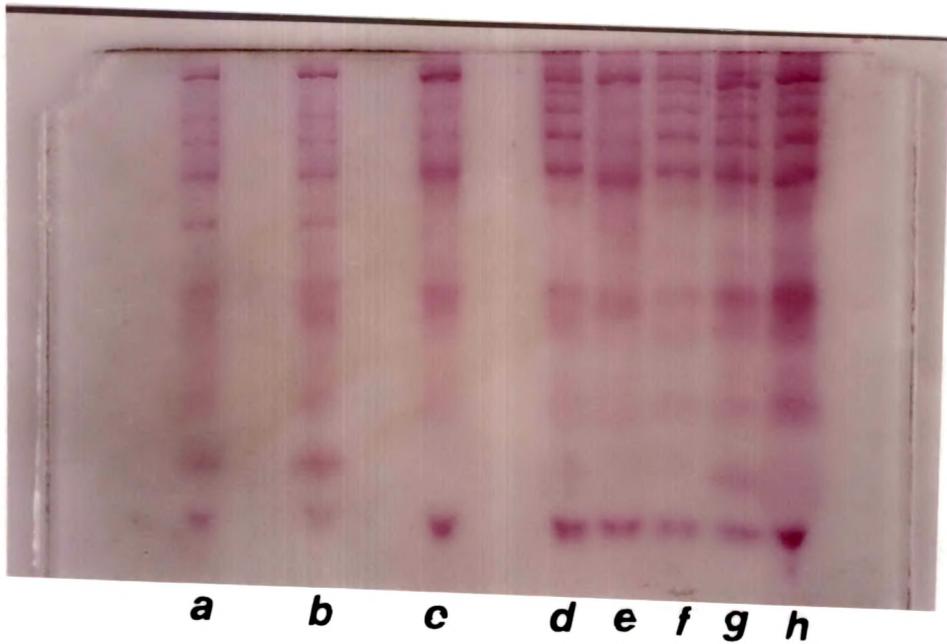CT- Chemotherapy
RT- Radiotherapy

Figure-27

REPRESENTATIVE PATTERN OF SERUM GLYCOPROTEIN ELECTROPHORESIS SEPRATED ON SLAB GEL
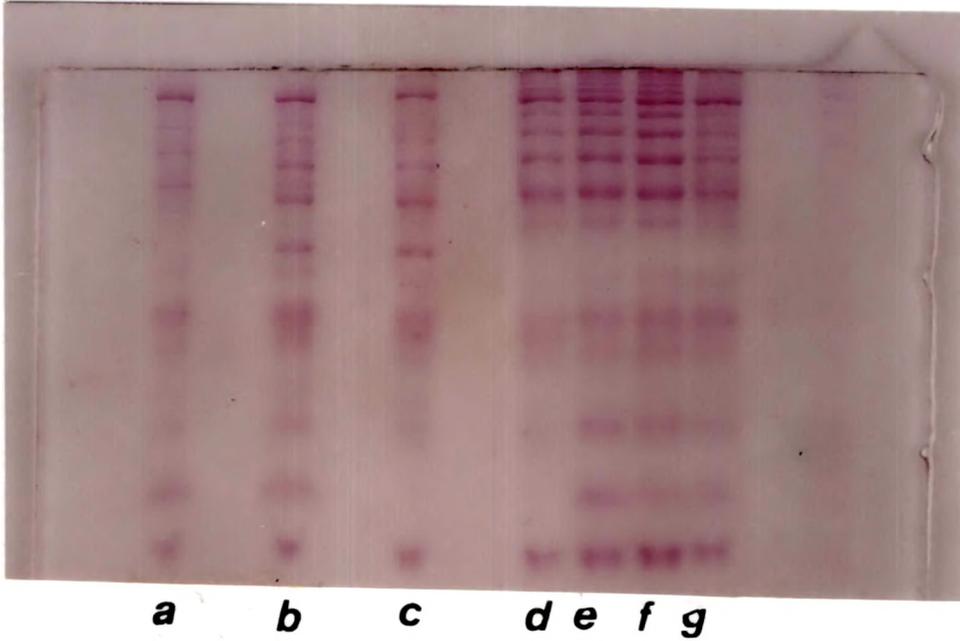
4.10 LECTIN AFFINITY CHROMATOGRAPHY FOR EVALUATION OF SIALOPROTEINS AND FUCOPROTEINS

Proteins with terminal sialic acid or fucose residues were separated using sialic acid specific lectins (Sambucus Nigra) and fucose specific (Lotus tetragonolobus) lectins. The lectins were coupled with CNBr activated sepharose prior to incubation with serum. Various control elutes were run to confirm isolation of specifically desired proteins. **Figure-28** shows separation of proteins by SDS-PAGE of various elutes which were stained by silver staining. Lane **a** represents elute lectin incubated with sepharose, lane **b** represents elute of CNBr activated sepharose in absence of serum and lectin, lane **c** represents elute of lectin incubated with serum, Lane **d** represents elute of only lectin, lane **e** represents elute of serum incubated with CNBr activated sepharose. It is clear from the photograph that lectin coupled with sepharose were able to bind with more number of proteins as compared to lectin alone. Further, the elutes of only sepharose, only lectin and serum with sepharose showed only one band which indicate that resultant separated proteins were only lectin binding protein without impurities of other proteins. This also documentes that lectin-eluted fractions were specifically only alpha2, 6 sialylated or fucosylated proteins of serum.

Figure-29(A) shows separation of serum protein by SDS-PAGE stained by silver staining. Sera of the subjects as well as lotus tetragonolobus lectin elute of the same sera were loaded on a single gel. As is clear from the figure, less amount of the proteins and less number of the bands were observed in the lectin eluted fractions which indicates that lectin-eluted fractions specifically showed only fucoproteins.

Figure 29A shows electrophoretogram of serum fucoprotein and total protein of a healthy individual and breast cancer patient at diagnosis as well

as during follow-up. lane **a** represents serum protein of the cancer patient at diagnosis however, Lane **b** represents cancer patients during followup, Lane **c** represents controls and lane **d** represents another cancer patient at the time of diagnosis. Lane **f to i** represents protein pattern of lectin eluted fraction from sera of the same control, and breast cancer patient. Lane **e** shows molecular weight markers. As clear from the figure, more number of bands were present in elute of sera from untreated breast cancer patient as compared to that of control and pathological control. Similar patterns of fucoprotein and total protein were observed in the entire subject studied. It indicates that more number of fucoproteins were present in sera of cancer patients at the time of diagnosis and with poor treatment outcome during followup. However, serum total proteins revealed similar pattern in control, patient with BBD and untreated cancer patients as well as in nonresponder. It is clear that there was no difference in electrogram of serum total protein between controls and cancer patients. However, cancer patients showed elevation in serum fucoproteins.

Figure 29B shows separation of serum fucoprotein extracted from serum of oral cavity cancer patient at the time of diagnosis and during followup. Lane **a** shows fucoprotein of serum from untreated cancer patient. While lanes **b to f** exhibit serum fucoprotein of cancer patient during after anti cancer treatment who has responded to anticancer therapy. As is clear from the figure, less amount of protein and less number of bands were observed in the lectin eluted fraction of cancer patient during folowup as compared to their levels at the time of diagnosis.

Figure30A Compares electrophoresis pattern of sialoprotein isolated using lectin sambucus nigra from controls and breast cancer patient. Lane a to d shows sialoproteins of healthy individual, breast cancer patients at the time of diagnosis, and two responder follow-ups. As is clear from the figure, more number of sialoproteins were observed in cancer patients at diagnosis. More number of sialoproteins was observed in the sera of patients obtained at the time of diagnosis as compared to that obtained during

Figure-28

Lane		elute of
a	-	Lectin coupled with CNBr activated sepharose
b	-	Only sepharose
c	-	Serum incubated with lectin
d	-	Only lectin
e	-	Serum incubated with CNBr activated sepharpose

follow-up in patient with favourable treatment outcome.

Figure-30B shows 1D electrophoretic pattern of lectin (Sambucus Nigra) extracts of serum from a oral cavity cancer patient who did not responded to anticancer therapy. Lane **a** shows sialoprotein of serum from untreated cancer patient. While lanes **b to f** exhibit serum sialoproteins of cancer patient during /after anticancer treatment. As exhibited in figure serum sialoprotein pattern were almost similar at the time of diagnosis and during followup in cancer patient.

Figure-28

SDS-PAGE OF SERUM PROTEIN SHOWING SPECIFIC AFFINITY TO THE LECTINS

a   b   c   d   e

Figure- 29 (A)
SDS-PAGE OF SERUM PROTEIN AND SERUM FUCOPROTEIN USING LECTIN LOTUS TETRAGONOLOBUS

Lane a to d   - serum protein   Lane f to i – serum fucoprptein
Lane e   - Molecular weight marker
Lane a and f - Untreated Breast cancer patients ($T_3N_1M_0$)
Lane b and g - 8 month follow-up of above patient with no response
Lane c and h - Healthy female
Lane d and i - Untreated Breast cancer patient ($T_4N_2M_0$)

Figure- 29 (B)
SDS PAGE OF SERUM FUCOPROTEIN IN BREAST CANCER PATIENTS

a - Molecular weight marker   b - Pretreatment breast cancer patient
c - 3.0   month follow-up (DFS)   d - 8.0   month follow-up (DFS)
e - 11.0 month follow-up (DFS)   DFS- Disease free survival

Figure – 30

SERUM SIALOPROTEIN ELETROPHORECTIC PATTERN IN CANCER PATIENTS
(LECTIN SAMBUCUS NIGRA)

A

B

Lane a – control
Lane b –untreated breast cancer patient
Lane c – 6. 0 month follow up
Lane d – 10.5 month follow up
Lane e – molecular weight marker

Lane a – untreated cancer patient
Lane b – 3.5   month follow up
Lane c – 5.5   month follow up
Lane d – 7.0   month follow up
Lane e – 12.0 month follow up

4.11 ALTERATIONS IN LEVELS OF THE ENZYMES OF GLYCOPROTEIN METABOLISM:

Variations in the levels of important enzymes (viz. Fucosidase, Sialyltransferase and Fucosyl transferase) were evaluated from the sera of the subjects.

Variations in fucosidase levels

Breast cancer study

Mean values of fucosidase in the controls and patients are shown in **table 22.** The fucosidase levels were higher in healthy individuals as compared to pathological controls as well as untreated breast cancer patients. The enzyme levels were significantly decreased (p=0.026 and 0.020, respectively) in patients with BBD and untreated breast cancer patients as compared to the controls.

To examine association of the alterations in fucosidase levels with treatment outcome the enzyme activities were also measured from blood samples obtained from cancer patients during/after treatment. Mean fucosidase levels were found higher in complete responders as compared to untreated breast cancer patients as well as nonresponders. The increase in fucosidase values in complete responders was statistically significant as compared to untreated breast cancer patients and nonresponders (p=0.02 and 0.05, respectively). Fucosidase levels were comparable between untreated breast cancer patients and nonresponders.

Figure 31A shows regression analysis comparing relationship of disease status with the enzyme activity. The extent of disease (TNM stages) showed negative correlation with serum fucosidase levels in untreated breast cancer patients. Correlation was statistically significant with r^2 0.028 and p=0.026.

Figure 31B shows survival curves of fucosidase in untreated breast cancer patients. The patients were divided into two groups: **(i)** patients with enzyme activity below mean pretreatment levels and **(ii)** patients with enzyme activity above mean pretreatment levels. As illustrated in the figure, breast cancer patients with higher activity of fucosidase had better survival as compared those with lower levels of the enzyme.

Oral Cavity Cancer Study:

The changes in fucosidase activities in patients with OPC and untreated oral cavity cancer patients with respect to healthy individuals are documented in **table-23**. As clear from the table untreated oral cavity cancer patients showed significant decrease in the fucosidase activity when compared with patients with OPC (p=0.001) and healthy individuals (p=0.010). In comparison between controls and patients with OPC, the enzyme concentrations were found to be significantly lower (p=0.001). The oral cavity cancer patients with favorable response showed significantly higher levels of fucosidase as compared to untreated oral cavity cancer patients (p=0.02) and nonresponders (p=0.001). The nonresponders showed elevated fucosidase levels as compared to untreated oral cavity cancer patients; however, the difference was not statistically significant.

Correlation of the stage of malignant disease with levels of serum fucosidase is shown in **figure 32A.** Serum fucosidase concentration showed negative correlation with stage of the disease in untreated oral cavity cancer patients.
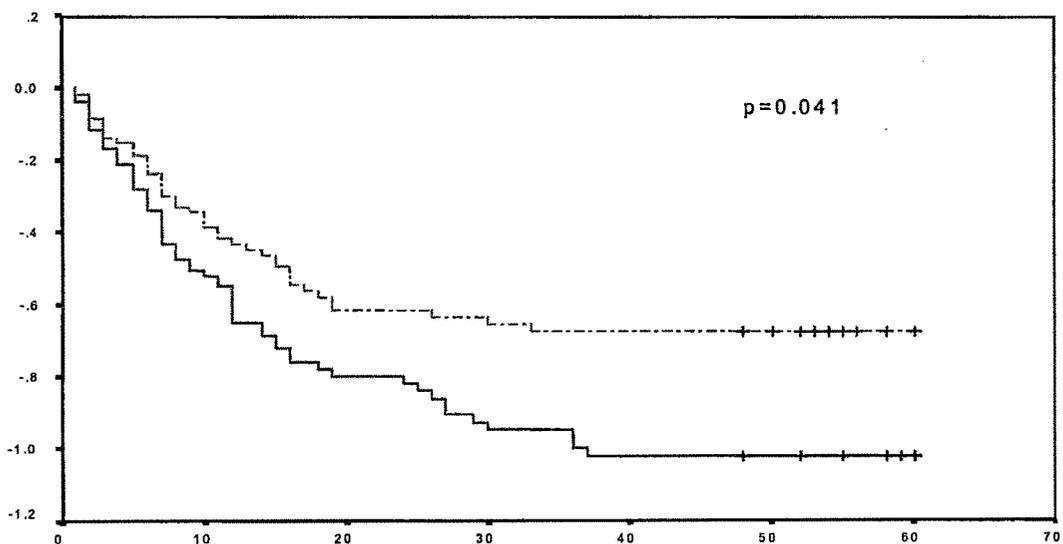
Correlation of serum of fucosidase levels at diagnosis of oral cancer with overall survival of the patients was studied by constructing overall survival curves. As indicated in **Figure- 32B** patients with lower enzyme levels (below mean of pretreatment) had poor overall survival than their counter part.

Figure-31

A: CORRELATION OF SERUM FUCOSIDASE LEVELS WITH STAGE WISE DISEASE ACTIVITY IN BREAST CANCER PATIENTS

$r^2=0.026$,p=0.028
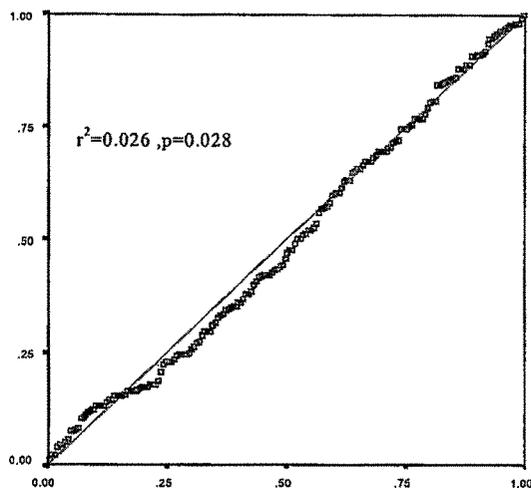
X axis - Observed cumlative probability
Y axis - Expected cumlative probability

B: SURVIVAL (LOG SURVIVAL) CURVE OF SERUM FUCOSIDASE IN BREAST CANCER PATIENTS

p=0.041

——————— - Below mean
--·--·--- - Above mean

Xaxis- Duration in months
Yaxis- Log survival

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Figure-32
**CORRELATION OF SERUM FUCOSIDASE LEVELS WITH STAGE WISE
DISEASE ACTIVITY IN ORAL CAVITY CANCER PATIENTS**

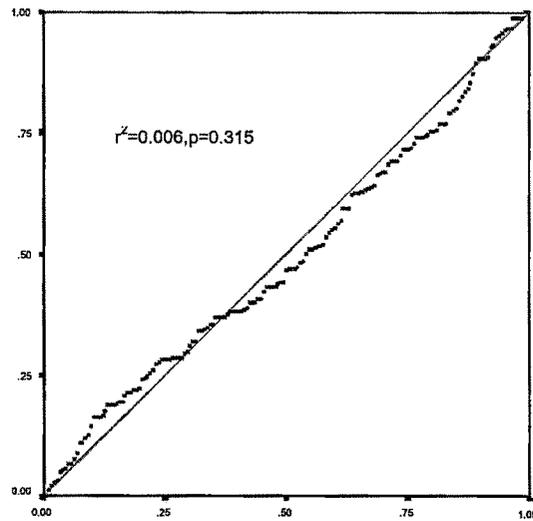X axis- Observed cumulative probability
Y axis- Expected cumulative probability

**B: SURVIVAL CURVE (LOG SURVIVAL) OF SERUM FUCOSIDASE IN ORAL
CAVITY CANCER PATIENTS**

--------- - Above mean
————— - Below mean
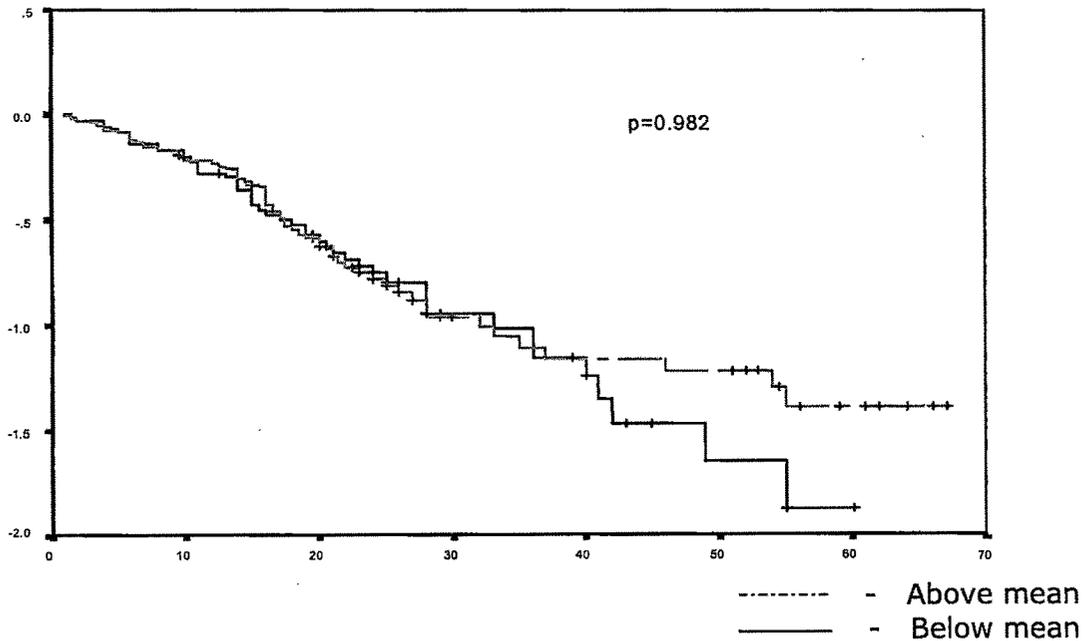
X axis- Duration in months
Y axis- Log survival

Table - 22

MEAN VALUES OF FUCOSIDASE IN CONTROLS, PATIENTS WITH BBD AND UNTREATED BREAST CANCER PATIENTS

	Mean	S.E.	Groups Compared	"t" Value	"p" Value
Controls	14.23	1.043	Control vs.BBD	1.171	0.280
BBD	7.18	0.240	Control vs. BC	5.094	0.026
BC	6.83	0.203	BBD vs. BC	5.359	0.020
CR	7.62	0.421	BC vs. CR	5.506	0.020
NR	8.17	0.362	BC vs. NR	1.385	0.241
			CR vs. NR	2.266	0.133

Table – 23

MEAN VALUES OF FUCOSIDASE IN CONTROLS, PATIENTS WITH OPC AND UNTREATED ORAL CAVITY CANCER PATIENTS

	Mean	S.E.	Groups compared	"t" Value	"p" Value
Controls	14.65	1.332	Controls vs. OPC	2.633	0.010
OPC	10.32	0.966	Control vs. OC	6.196	0.001
OC	4.77	0.532	OPC vs. OC	3.294	0.001
CR	7.52	0.266	OC vs. CR	2.942	0.020
NR	7.02	0.400	OC vs. NR	1.870	0.100
			CR vs. NR	0.0271	NS

BBD - Patients with BBD
BC - Untreated breast cancer patients
CR - Complete responders
NR - Nonresponders
NS - Not significant
OC - Untreated oral cavity cancer patients
OPC - Patients with OPC
Vs - Versus

VARIATIONS IN FUCOSYLTRANSFERASE LEVELS

Breast Cancer Study

As documented in **table-24,** untreated breast cancer patients showed significantly elevated level of fucosyltransferase as compared to the controls (p=0.001) and the pathological controls (p=0.01). The patients with BBD showed higher levels of fucosyltransferase as compared to the controls. The enzyme activities were also compared between untreated breast cancer patients, complete responders and nonresponders. It is clear from the table that fucosyltransferase activities were lower in complete responder as compared to both untreated breast cancer patients (p= 0.01) as well as nonresponders (p=0.05). The enzyme levels were comparable between untreated breast cancer patients and nonresponders.

Oral Cavity Cancer Study

Table-25 show comparison of fucosyltransferase levels between controls, patients with OPC, untreated oral cavity cancer patients, complete responders and nonresponders. It is clear from the table that in comparison with all the remaining groups, untreated oral cavity cancer patients had higher fucosyltransferase levels. The enzyme levels were significantly lower (p=0.05) in controls, patients with OPC and complete responder as compared to untreated oral cavity cancer patients. However, the comparison between nonresponders and untreated oral cavity cancer patients revealed non-significant difference. The patients with OPC patients showed significantly higher (p=0.05) levels of fucosyltransferase as compared to the controls. The fucosyltransferase levels were significantly higher in nonresponders (p=0.05) as compared to the complete responders.

Table - 24

MEAN VALUES OF FUCOSYLTRANSFERASE IN CONTROLS, PATIENTS WITH BBD AND UNTREATED BREAST CANCER PATIENTS

	Mean	S.E.	Groups compared	"t"Values	"p"Values
Controls	6676.4	1278.5	Control vs.BBD	1.875	NS
BBD	10838.6	1759.9	Control vs. BC	4.045	0.001
BC	18001.7	3291.3	BBD vs. BC	2.364	0.010
CR	11434.4	2798.7	BC vs. CR	2.579	0.010
NR	16839.1	4805.8	BC vs. NR	0.6554	NS
			CR vs. NR	2.878	0.050

Table - 25

MEAN VALUES OF FUCOSYLTRANSFERASE IN CONTROLS, PATIENTS WITH OPC AND UNTREATED ORAL CAVITY CANCER PATIENTS

	Mean	S.E.	Groups compared	"t"Values	"p"Values
Controls	6886.4	537.44	Controls vs.OPC	1.3058	NS
OPC	21697.0	3439.51	Controls vs.OC	2.7452	0.05
OC	26577.1	3080.47	OPC vs. OC	2.3908	0.05
CR	16731.8	2406.65	OC vs. CR	2.660	0.05
NR	24271.2	3362.53	OC vs. NR	0.0453	NS
			CR vs. NR	2.5567	0.05

BBD - Patients with BBD
BC - Untreated breast cancer patients
CR - Complete responders
NR - Nonresponders
NS - Not significant
OC - Untreated oral cavity cancer patients
OPC - Patients with OPC

VARIATIONS IN SIALYLTRANSFERASE LEVELS:

Breast Cancer Study:

Mean values of sialyltransferase in the controls, patients with BBD and breast cancer patients are shown in **Table-26.** Untreated breast cancer patients revealed elevated levels of sialyltransferase as compared to the controls and patients with BBD (p=0.001 and 0.01, respectively). The patients with BBD showed higher concentrations of sialyltransferase as compared to the controls. When the level of sialyltransferase were compared between untreated breast cancer patients, complete responders and nonresponders, the complete responders exhibited significantly lower levels of sialyltransferase as compared to untreated breast cancer patients (p=0.05) and nonresponders (p=0.02). The enzyme concentrations were comparable between untreated breast cancer patients and nonresponders.

Oral Cavity Cancer Study:

The levels of sialyltransferase in controls, patients with OPC, untreated oral cavity cancer patients, complete responders and nonresponders are shown in **table-27.** The levels of sialyltransferase were significantly higher in untreated oral cavity cancer patients as compared to the controls (p=0.001). The patients with OPC showed significantly lower sialyltransferase levels as compared to untreated oral cavity cancer. In comparison between the controls and patients with OPC, the later group revealed higher concentrations of the enzyme. However, the difference was not statistically significant. The table also represents changes in sialyltransferase values in the oral cavity cancer patients during follow-up. As indicated in table, complete responders showed significantly lower sialyltransferase levels as compared to untreated oral cavity cancer patients (p=0.05) and nonresponders (0.05). The difference in sialyltransferase levels was not significant in comparison between untreated oral cavity cancer patients and nonresponders.

Table – 26

MEAN VALUES OF SIALYLTRANSFERASE IN CONTROLS, PATIENTS WITH BBD AND UNTREATED BREAST CANCER PATIENTS.

	Mean	S.E.	Groups compared	"t"Values	"p"Values
Controls	2419.9	369.12	Controls vs. BBD	1.913	0.10
BBD	3380.6	355.41	Controls vs. BC	3.207	0.001
BC	4680.4	419.52	BBD vs. BC	2.918	0.01
CR	3219.7	380.37	BC vs. CR	2.519	0.05
NR	4289.5	423.89	BC vs. NR	0.199	NS
			CR vs. NR	2.930	0.05

Table – 27

MEAN VALUES OF SIALYLTRANSFERASE IN CONTROLS, PATIENTS WITH OPC AND UNTREATED OC PATIENTS.

	Mean	S.E	Groups compared	"t"values	"p"values
Controls	2380.05	399.48	Control Vs OPC	1.825	0.10
OPC	3200.22	484.66	Control Vs OC	3.086	0.001
OC	3402.56	329.22	OPC Vs OC	2.698	0.05
CR	2489.6	440.26	OC Vs CR	2.762	0.05
NR	3380.25	365.38	OC Vs NR	0.479	NS
			CR Vs NR	2.360	0.05

BBD - Patients with BBD
BC - Untreated breast cancer patients
CR - Complete responders
NR - Nonresponders
NS - Not significant
OC - Untreated oral cavity cancer patients
OPC - Patients with OPC

4.12 MULTIVARIATE ANALYSIS OF THE CLINICO-PATHOLOGICAL PARAMETERS AND BIOLOGICAL MARKERS WITH TREATMENT OUTCOME IN CANCER PATIENTS.

Cox's regression model was used to evaluate correlation between established clinicopathological parameters and variations in serum tumor markers in predicting the prognosis.

Breast cancer study

Table-28 shows multivariate analysis of the clinicopathological and biological parameters with treatment outcome in breast cancer patients. TNM stage, lymphnode involvement, histopathological examination and site of the disease were clinicopathological parameters included. TNM classification, lymphnode involvement, total sialic acid, ratio of mucoid proteins to total protein, fucose, ratio of total sialic acid to total protein, and mucoid proteins showed significant association with treatment outcome. However, presence of extra band, histopathological examination, site of the disease, protein bound sialic acid and its ratio to total protein and free sialic acid levels did not show any relation with treatment response. In this model, prognostic significance of TNM stage was followed by ratio of mucoid proteins to total protein, total sialic acid, ratio of fucose to total protein, hexoses, ratio of total sialic acid to total protein, fucose, mucoid protein and lymphnode involvement.

Oral Cavity Cancer Study

The multivariate analysis of the clinicopathological and biological parameters with treatment outcome in oral cavity cancer patients is documented in table-29. In addition to TNM stage, protein bound sialic acid, ratio of protein bound sialic acid to total protein, fucose and ratio of fucose to total protein levels showed significant correlation with treatment response in oral cavity cancer patients. The prognostic significance of TNM stage in oral cavity cancer patients was followed by protein bound sialic acid, ratio of protein

bound sialic acid to total protein, fucose and ratio of fucose to total protein. Total sialic acid levels did not show association with response to therapy, however, its ratio to total protein showed strong association with treatment response. Free sialic acid, hexoses, mucoid protein, ratio of mucoid protein to total protein and ratio of hexoses to total protein did not show association with treatment outcome.

Table -28
ASSOCIATION OF TREATMENT RESPONSE WITH BIOMARKERS AND CLINICOPATHOLOGICAL PARAMETERS IN BREAST CANCER PATIENTS

Variable	Significance
Histology	0.4555
Stage	0.0000
Lymphnode involvement	0.0118
Site	0.8838
E-band	0.5778
Free sialic acid	0.5784
Protein bound sialic acid	0.1788
Total sialic acid	0.0010
Protein bound sialic acid/ Total protein	0.8040
Total sialic acid/Total protein	0.0020
Fucose	0.0027
Hexoses	0.0014
Mucoid proteins	0.0078
Fucose/Total protein	0.0013
Mucoid protein/Total protein	0.0004
Hexoses/Total Protein	0.0016

Table – 29

ASSOCIATION OF RESPONSE WITH BIOMARKERS AND CLINICO-PATHOLOGICAL PARAMETERS IN ORAL CAVITY CANCER PATIENTS

Variable	Significance
Stage	0.0000
Free sialic acid	0.8522
Protein bound sialic acid	0.0000
Total sialic acid	0.4430
Protein bound sialic acid/ Total protein	0.0000
Total sialic acid/Total protein	0.0565
Fucose	0.0000
Hexoses	0.9854
Mucoid proteins	0.5943
Fucose/Total protein	0.0000
Mucoid proteins/Total protein	0.1559
Hexose/Total proten	0.5524

4.13 COMPARISION OF GLYCOPROTEIN CONSTITUENTS AND ENZYME LEVELS BETWEEN NONMALIGNANT AND MALIGNANT TISSUES

The levels of glycoprotein constituents and enzymes were analysed from malignant and surrounding normal tissues to check the association of circulatory levels with the presence of tumour. As is clear from **table 30**, the glycoprotein constituents and enzyme levels were higher in tumour tissues as compared to the surrounding normal tissues. The elevations in free sialic acid, hexoses, mucoid proteins and sialyl transferase were statistically significant (p=0.097, 0.024, 0.027 and 0.047, respectively). The increased values of the glycoprotein constituents and enzymes in tumour tissues confirmed that the elevations in the markers in circulation were due to the presence of malignant tumours.

Table - 30

COMPARISION OF GLYCOPROTEIN CONSTITUENTS AND ENZYME LEVELS BETWEEN NONMALIGNANT AND MALIGNANT TISSUES

Parameters	Group	Mean	S.E.	"t" Value	"p" Value
FSA (μg/mgprotein)	NM MT	1.429 1.606	0.185 0.364	2.921	0.097
Fucose (μg/mgprotein)	NM MT	0.505 0.247	0.142 0.069	1.600	0.123
Hexoses (μg/mgprotein)	NM MT	1.600 5.516	0.4218 1.839	5.883	0.024
MP (μg/mgprotein)	NM MT	3.3518 4.3576	0.4986 1.011	5.369	0.027
Hexoses/TP (μg/mgprotein)	NM MT	0.0478 0.1458	0.0099 0.0619	15.11	0.001
MP/TP (μg/mgprotein)	NM MT	0.1174 0.1788	0.0162 0.0637	4.987	0.033
Fucosidase (units/mgprotein)	NM MT	0.2534 0.4182	0.0655 0.1211	2.588	0.117
SiTase (CPM/mgprotein)	NM MT	268.99 1058.78	65.34 508.87	4.262	0.047

FSA   - Free sialic acid   MP   - Mucoid Protein
MT   - Malignant tumour tissue   NM   - Normal Tissue
SiTase- Sialyl transferase   TP   - Total Protein