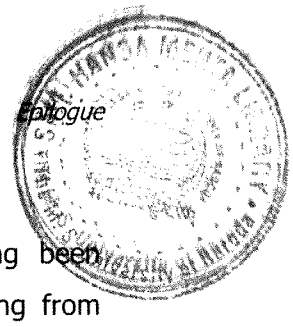
Epilogue



6.1 Summary

Neoplastic changes in cell surface and cell membrane have long been associated with profound alterations in protein modifications ranging from altered cellular expression, changes in specific activity and aberrant localization affecting cellular activity. The alterations in the oligosaccharide portion of glycoconjugates including glycoproteins on the cell surface are also involved in variety of cellular functions like cellular proliferation, cell-cell communications, cell adhesions and ECM interactions. The degradation of ECM and basement membranes is necessary for tumour invasion and metastasis. ECM is degraded by MMPs the major glycoproteins. The action of MMPs is counteracted by their natural tissue inhibitors (TIMPs). The balance of proteolysis and anti-proteolysis is disrupted in aggressive malignant tumors. This results in cancer transformation, tumour progression, angiogenesis, invasion and metastasis. The present study was designed to evaluate clinical significance of protein fractions, immunoglobulins, M-protein characterization, protein profiling, glycosylation changes, glycoprotein profiling, gelatinases (MMP-2 and MMP-9) and their natural tissue inhibitors (TIMP-1 and TIMP-2) in multiple myeloma and cervical cancer patients. The study was carried out using various bio-molecular techniques including spectrophotometry, radial immunodiffusion, ELISA, electrophoresis (agarose gel, Native-PAGE, SDS-PAGE), zymography, 2D-PAGE and necessary computational software. Statistical significance of the data was calculated using appropriate statistical methods. Noteworthy observations of the present study were as follows:

6.2 Serum protein profiling (Agar gel electrophoresis):

1. Serum total protein levels, alpha-2 and gamma globulins were significantly elevated and A:G ratio showed significantly decreased in MM patients as compared to the controls.
2. Serum total protein levels, gamma fractions as well as A:G ratio were significantly elevated in group-I MM patients as compared to group-II MM patients.

3. Serum total protein levels, alpha-2, beta and gamma fractions as well as A:G ratio could significantly discriminate between MM patients and controls. Serum total protein levels, gamma and A:G ratio could significantly discriminate between group-I and group-II MM patients.

6.3 Serum immunoglobulin profiling:

1. Serum IgG and IgM were significantly elevated in MM patients as compared to the controls. 79.4% of the group-I MM patients showed that M-protein were of IgG type.
2. The protein content of M-protein in MM patients were ≥3-<6 gm/dl in 45% and ≥6 gm/dl in 24% of MM patients.
3. M-protein was a glycosylated protein. TSA, fucose and hexoses content of M-protein were significantly higher.

6.4 Serum protein profiling (Native-PAGE and SDS-PAGE):

1. Higher levels of serum total protein levels, UnLMW, prealbumin, albumin, alpha and gamma globulins were observed in MM patients as compared to the controls. Serum prealbumin could significantly discriminate between controls and MM patients.
2. Serum total protein levels and ratio of UnLMW:gamma and prealbumin:gamma globulin were significantly higher in MM patients as compared to the controls. Ratio of UnLMW:gamma could significantly discriminate between controls and MM patients.
3. Serum total protein levels, albumin and gamma values were significantly higher in group-II MM as compared to the group-I MM patients.
4. Serum total protein levels, and ratio of UnLMW:gamma and prealbumin:gamma values were significantly elevated in group-I as compared to the group-II patients. Serum total protein levels, ratio of UnLMW:gamma and prealbumin:gamma could significantly discriminate between group-I and group-II MM patients.
5. Serum total protein levels, UnLMW, prealbumin, alpha and beta globulins were found to be significantly elevated in cervical cancer patients as

compared to the controls. Serum albumin could significantly discriminate between controls and cervical cancer patients

6. Multivariate analysis between protein profiles and clinicopathological parameters suggested that prealbumin and gamma globulin were significantly associated with early vs advanced stage and pathological tumor differentiation in cervical cancer patients, respectively.
7. Serum UnLMW:gamma, prealbumin:gamma, albumin:gamma, alpha:gamma and beta:gamma globulins ratios were significantly elevated in cervical cancer patients as compared to the controls.
8. Serum total protein levels, ratio of UnLMW:gamma, prealabumin:gamma, albumin:gamma, alpha:gamma and beta:gamma could significantly discriminate between controls and cervical cancer patients.
9. Multivariate analysis between total protein profiling ratio and clinicopathological parameters suggested that ratio of UnLMW:gamma, prealbumin:gamma, alpha:gamma and beta:gamma globulins were significantly associated with pathological tumor differentiation in cervical cancer patients. SDS-PAGE revealed 10-13 protein bands in the range of 14 kDa and 100 kDa in cervical cancer patients.

6.5 Glycosylation Changes:

1. Serum TSA, TSA/TP, fucose/TP, hexoses and MP levels were significantly higher in MM patients as compared to the controls and it could significantly discriminate between controls and MM patients. Serum TSA, fucose and hexoses showed significant positive correlation with TSA/TP, fucose/TP and MP in MM patients.
2. Serum TSA/TP and fucose/TP levels were significantly higher in group-II as compared to the group-I MM patients.
3. Serum TSA, TSA/TP, fucose/TP, and hexoses levels were significantly higher in cervical cancer patients as compared to the controls. And it could significantly discriminate between cervical cancer patients.

4. Serum TSA, fucose and hexoses showed significant positive correlation with serum TSA/TP, fucose/TP and MP in cervical cancer patients. Multivariate analysis among glycoconjugates, protein ratio of glycoconjugates and clinicopathological parameters suggested that hexoses and MP were significantly associated with early vs advanced stage of the disease.
5. Serum total glycoprotein values, prealbumin, albumin, alpha, beta and gamma glycoprotein fractions were significantly elevated in MM patients as compared to the controls. Serum total glycoprotein, prealbumin and albumin could significantly discriminate between MM patients.
6. Serum total glycoprotein values and ratio of prealbumin:gamma glycoprotein fraction were significantly higher in MM patients as compared to the controls. ROC curve analysis indicated good discriminatory efficacy of serum total glycoprotein, alpha:gamma and beta:gamma globulin between controls and MM patients.
7. Serum total glycoprotein values, alpha, beta and gamma glycoproteins were significantly elevated in group-II as compared to the group-I MM patients. Electrophoretograms revealed higher total glycoprotein values in group-II as compared to group-I MM patients.
8. Serum total glycoprotein values, prealbumin, albumin, alpha, beta and gamma glycoproteins were significantly higher in cervical cancer patients as compared to the controls and it could significantly discriminate between cervical cancer patients.
9. Multivariate analysis between total glycoprotein profiles and clinicopathological parameters suggested that total glycoprotein and gamma region glycoproteins were significantly associated with histopathlogy in cervical cancer patients.
10. Serum total glycoprotein and prealbumin:gamma were significantly elevated in cervical cancer patients as compared to the controls. Serum prealbumin:gamma, albumin:gamma, alpha:gamma and beta:gamma could significantly discriminate between controls and cervical cancer patients.

11. Multivariate analysis between ratio of glycoprotein electrophoretic patterns and clinicopathological parameters suggested that total glycoproteins, albumin:gamma and alpha:gamma were significantly associated with histopathology while beta:gamma was associated with early vs. advanced stage of the disease.

6.6 MMPs and TIMPs:

1. Total, pro- and active forms as well as activation ratio of MMP-2 were significantly higher in MM patients. Serum pro- and total forms as well as activation ratio of MMP-9 were significantly higher in MM patients as compared to controls. Serum proMMP-2 could significantly discriminate between controls and MM patients. Serum proMMP-2 showed significant negative correlation with active MMP-2 and pro MMP-9. Serum pro MMP-9 showed significant negative correlation with active MMP-2
2. Serum TIMP-1 levels were significantly higher in MM patients as compared to the controls, which could significantly discriminate between controls and MM patients. Serum total MMP-2 levels revealed significant positive association with serum TIMP-2 levels in MM patients.
3. The activation ratio of MMP-2 was significantly higher in group-I as compared to group-II MM patients. ProMMP-2 was positively and significantly correlated with serum active MMP-2 and pro MMP-9 in both the groups of MM patients.
4. Serum levels of active MMP-2, MMP-9, activation ratio of MMP-2 and MMP-9 were significantly elevated in cervical cancer patients as compared to the controls. Serum proMMP-2 was found to be significantly and negatively correlated with proMMP-9, whereas it was positively correlated with serum total MMP-9. Serum proMMP-9 levels were found to be significantly and positively correlated with serum proMMP-2 and total MMP-2 in cervical cancer patients. Serum proMMP-2, proMMP-9 and active MMP-9 could significantly discriminate between control and cervical cancer patients.

5. Serum proMMP-2 showed significant negative and significant positive correlation with serum active MMP-2 and serum total MMP-2, respectively. Serum proMMP-9 showed significant positive associated with total MMP-9. Serum proMMP-9 revealed significant negative association with active MMP-2 in cervical cancer patients. Serum active MMP-2 showed significant negative correlation with active MMP-9. Serum total MMP-2 was found to be significantly and positively associated with serum TIMP-2 cervical cancer patients.
6. Multivariate analysis between different forms of gelatinases and clinicopathological parameters suggested that activation ratio of MMP-2 was significantly associated with age and proMMP-9 was found to be significantly associated with pathological tumor differentiation in cervical cancer patients.
7. Total MMP-9 levels were significantly associated with early vs. advanced stage of the disease and pathological tumor differentiation in cervical cancer patients. Serum total MMP-9 levels could also significantly discriminate between controls and cervical cancer patients.
8. Multivariate analysis between TIMPs and clinicopathological parameters suggested that TIMP-1 was significantly associated with age in cervical cancer patients. Multivariate analysis of gelatinases and TIMPs complex with clinicopathological parameters suggested that TIMP-1 was significantly associated with age, while; MMP-2:TIMP-2 complex was significantly associated with early vs. advanced stage of the disease.

6.7 M-protein proteome analysis:

Isolation and separation of the M-protein on SDS-PAGE revealed seven distinct protein subunits with molecular weight of proteins ~11.48, ~17.78, ~24.54, ~34.67, ~45.70, ~69.18, ~100 kDa. M-protein proteome by 2D-PAGE approach revealed 7 distinct protein spots.

6.8 Conclusion:

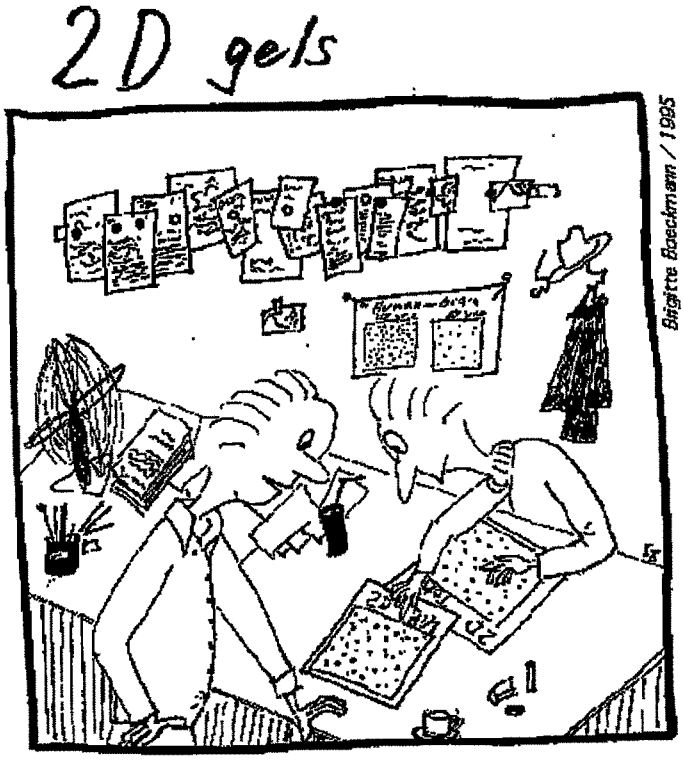
The data revealed that glycosylation changes play a major role in MM and cervical cancer. The alterations in glycoproteins as well as MMP-2, MMP-9, TIMP-1 and TIMP-2 have significant clinical usefulness for MM and cervical cancer patients. The gelatin zymography could be a cost effective, easy and simple alternative of kit based ELISA analysis. The electrophoretic patterns and M-proteome analysis revealed strong base for in-depth proteomics and glycomics approaches for MM and cervical cancer patients. The proteomics and glycomics approaches may be helpful to identify disease associated new protein markers to strengthen the armamentarium for diagnosis and prognosis as well as to select potential drug targets for molecular medicine.

6.9 Concluding remarks:

Cancer biology has found a new era from the holistic point of view that proteins coalesce into networks and circuits on command from external and internal stimuli. As stimuli fluctuate and response loops return information, newly formed protein networks rapidly break apart or are actively degraded. Carbohydrate chemistry helps to determine the 3D structures of proteins. Hence, identification of carbohydrate-protein interactions involving specific protein–linked oligosaccharides and their complementary binding proteins could be helpful. Such interactions takes place in the cells, on the cell surfaces, in the ECM or in the secretions. Understanding the aberrant glycosylation in cancer is a challenging task which requires deep introspection into this dynamic and fascinating field of clinical cancer research. Present approach revealed prolonged base to design extensive work-up for detailed proteome analysis as well as glycoprotein to identify a protein signatures in progression of MM and cervical cancer. In furtherance:

- ❖ It would be fascinating to assess these parameters in more number of patients with post-treatment studies to assess detailed clinical usefulness for MM and cervical cancer patients.
- ❖ In-depth study of degradome of matrix proteins (MMP-2 and MMP-9) fragments will establish their major role in MM and cervical cancer.

- ❖ A detailed proteomics, immunomics and glycomics approaches for M-protein may reveal better understanding to establish its role in clinical practice.
- ❖ A detailed study of serum proteomics and glycomics approach to may reveal newer biomarkers for cervical cancer patients.
- ❖ The present approach strongly support in-depth studies for carbohydrate-protein interactions based mimetic or inhibitors in targeted therapy directed against specific steps and stage of MM and cervical cancer progression.



"You've got one protein missing..."
"No, you've one extra protein!"