“SUMMARY AND FUTURE PERSPECTIVES”
V. Summary and Future Perspectives

Uncontrolled hyperglycemia is associated with the development of complications in diabetes. One of the primary causes of diabetic complication is glycation, a non-enzymatic reaction between glucose and protein. Glycation is implicated in the pathogenesis of diabetes and is principally involved in aggravating diabetic complications. Serum albumin, the most abundant plasma protein undergoes glycation and albumin level is decreased in diabetes due to insulin deficiency. Therefore, this study was performed to understand the role of albumin in regulation of glycation i.e., whether decreased albumin levels are responsible for affecting the stoichiometry of plasma protein glycation. The study was performed in three systems (1) streptozotocin (STZ) induced diabetic mice plasma (2) diabetic clinical plasma (3) in vitro glycated plasma. Protein glycation was studied by using a combination of two dimensional electrophoresis (2DE), western blotting and LC-MS. In both mice and clinical experiments, increased plasma protein glycation was associated with low levels of albumin. Additionally, plasma albumin levels were negatively correlated with HbA1c. In vitro glycated plasma experiments with differential depletion of albumin mechanistically showed that the low albumin levels were associated with increased plasma protein glycation. In this study, for the first time it was addressed that variation in the albumin levels influences glycation of plasma proteins and HbA1c. Further, it was also shown that albumin competitively inhibits the glycation of low abundant proteins in vitro by using insulin as a model protein. These studies have suggested that at least in the initial stages of diabetes, albumin may protect other proteins from glycation. Therefore, reduced albumin in diabetes is a risk factor for glycation induced complications.

In addition, the influence of protein molecular mass on glycation of proteins was studied by using MALDI MS analysis with model proteins including insulin, apomyoglobin, papain, BSA, HSA and IgG. The study revealed that high molecular weight proteins were more prone to get glycated than the low molecular weight proteins suggesting the role of high molecular weight proteins like IgG and HSA in protecting the glycation of low molecular weight proteins.
Elevated blood glucose levels in diabetes have resulted in glycoxidative modification of plasma proteins. Moreover, decreased insulin synthesis has known to affect gene expression of albumin and fibrinogen contributing to the differential protein expression. Differential protein expression in diabetic plasma sample was studied by a combination of proteomic and western blot approaches. Plasma samples were categorized depending on HbA1c levels as non diabetic (ND) with HbA1c >5.8%, controlled diabetic (CD) with HbA1c 7-8 % and poorly controlled diabetic (PCD) with HbA1c > 8%. Six proteins including alpha-1-antitrypsin, vitamin D binding protein, fibrinogen gamma chain, haptoglobin, transthyretin and apolipoprotein A1 were differentially expressed in diabetic plasma. Amongst six differentially expressed proteins in diabetes, the down-regulation of apolipoprotein A1 was more prominent in poorly controlled diabetes. The 2DE analysis along with validation with dot blot strongly suggested the down regulation of Apo A1 may serve as an early marker for diabetic complications unlike microalbuminuria which is known to be one of the late markers of diabetic complications.

Low albumin levels in diabetic plasma are associated with increased plasma protein glycation. In addition, apolipoprotein A1 was found to be downregulated and is also glycated in poorly controlled diabetes. However the study has to be extended to a larger population for considering them as diagnostic markers for early diabetic complications. Further, maintaining normal or near normal levels of albumin in diabetes may help in reducing the glycation associated complications. Intervention with medicine or nutraceuticals that help in maintaining albumin levels may delay the onset of diabetic complications.