Proteins modifications in diabetes may lead to early glycation products (EGPs or Amadori product) as well as advanced glycation end products (AGEs). Early glycation involves non-enzymatic covalent attachment of glucose with N-terminal and lysyl side chain ε-amino groups to form unstable Schiff base adduct that rapidly progresses to a stable ketoamine derivative, the Amadori product. After Amadori, the reactions become more varied and complicated leading to the formation of AGEs. Modifications of structural as well as circulating proteins by glycation have drawn much attention because of their potential role in the etiopathogenesis of diabetes. Although Amadori product is the principal form of glycation mediated modification on proteins, most studies so far have demonstrated the formation and role of AGEs in various diseases including diabetes, whereas the possible effects of EGPs are poorly defined. However, recent investigations by several scientists have shown that elevated concentrations of Amadori products (such as glycated albumin) have a substantive role in diabetes related complications.

Glycation of plasma proteins occur during hyperglycemic stages such as those preceding diabetes. Protein functions can be impaired by glycation and in conditions such as diabetes, the extent of glycation and the degree of damage increases in parallel. Early glycation products can have a damaging effect on nonstructural proteins such as enzymes. Large-scale clinical studies have established that hyperglycemia, the defining metabolic abnormality in diabetes, increases the risk for diabetic complications via accelerated formation of Amadori product. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes related complications. Furthermore, Amadori-albumin generates free radicals and augments oxidative stress in diabetes. Moreover, deciphering the mechanism of protein damage caused by glycation is fundamental in understanding
the biochemical basis of diabetes related complications. This may help in the
development of new therapeutic strategies to minimize the hazards of diabetes.
Compounds that can block the attachment of sugars with proteins may prove
beneficial in preventing, delaying or ameliorating the complications of diabetes.

In the present study, commercially available human serum albumin (HSA)
was incubated with varying concentration of glucose for one week to generate
Amadori-rich HSA. The Amadori adduct was assayed by NBT and authenticated by
boronate affinity chromatography. The structural perturbations in the Amadori-
albumin was analyzed by UV, fluorescence, CD, thermal denaturation studies, FT-IR,
HPLC, LC-MS and ESI-MS techniques. The studies revealed remarkable changes in
HSA upon glycation. Furthermore, antibodies were induced in rabbits against native
and Amadori-albumin. The induced antibodies were characterized by direct binding
and inhibition ELISA. Antigen-antibody interactions were further confirmed by band
shift assay. In order to assess the possible role of Amadori-albumin in the etiology of
diabetic complications, sera from type 1 diabetes patients with and without secondary
complications were analyzed for their binding to native and Amadori-albumin.
Furthermore, albumin was also purified from the plasma of diabetic patients and
changes in the isolated HSA were analyzed with experimentally produced antibodies
against glycated-HSA.

UV, fluorescence, CD, thermal denaturation and FT-IR results suggest
structural changes in HSA modified by glucose. NBT assay showed Amadori product
formation which was authenticated by retention on boronate affinity matrix.
Furthermore, generation of furosine (acid hydrolysed product of Amadori adduct;
fructosyl lysine) was detected by HPLC and LC-MS. Formation of the Amadori

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product was further confirmed by ESI-MS. Antiglycation studies using aminoguanidine (AG) and thymoquinone (TQ) showed an inhibition in glucose mediated HSA glycation. Fructosamine yield in glycated-HSA significantly decreased in presence of AG and TQ. Free radical generation during glycation was confirmed by quantitation of superoxide radicals in presence and absence of AG, TQ and superoxide dismutase (SOD). The results indicate that early glycation generates free radicals. Anti-glycation and anti-oxidant role of AG and TQ was also studied in diabetic rabbits. Experimental diabetes in rabbits was induced by alloxan and glucose, insulin, glycated hemoglobin (HbA1c) and fructosamine were measured in control, diabetic and AG and TQ treated diabetic rabbits. Effect of chronic hyperglycemia on oxidative stress parameters like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), glutathione (GSH), nitric oxide, lipid peroxidation and protein carbonyl level was studied. Compared to control rabbits, diabetic animals showed decreased level of SOD, CAT, Gpx and GSH. Nitric oxide (NO) level was significantly higher in diabetic animals. AG and TQ treatment significantly restored the level of the above biochemicals.

Immunogenicity of native and glycated-HSA was probed in experimental animals. Glycated-HSA was found to be a highly immunogenic and induced high titre antibodies compared to native HSA. The result suggest generation of glucose mediated neo-epitopes on HSA (Amadori product) rendering it highly immunogenic. In inhibition ELISA, the anti-glycated-HSA antibodies showed preferential binding with glycated-HSA. In band shift assay retardation in antigen-antibody complex was observed. The anti-glycated-HSA antibodies also showed binding with glycated proteins rich in lysine residues. This suggests common epitopes on glycated-HSA and other glycated proteins.
Fifty sera of type 1 diabetes patients (with and without secondary complications) were tested for the autoantibodies reactive with native, glycated and NaBH₄ reduced glycated-HSA. Seventy three percent (16 of 22) of type 1 diabetes sera with secondary complications showed enhanced binding with glycated-HSA as compared to native and NaBH₄ reduced glycated-HSA. Whereas, only sixty four percent (18 of 28) of type 1 sera without secondary complications showed enhanced binding with glycated-HSA. Normal human sera did not show appreciable binding with coated antigens. The higher recognition of glycated-HSA by autoantibodies of diabetic patients with secondary complications, compared to those without secondary complication, point towards the possible involvement of Amadori-HSA (and its autoantibodies) in diabetes related complications. Inhibition ELISA reiterated the true interaction of autoantibodies with glycated-HSA. Affinity purified IgG of diabetic patients showed appreciably high binding with glycated-HSA. The results suggest that autoantibodies seen in the sera of type 1 diabetic patients with secondary complications might be the consequence of accelerated immunological activity of neo-epitopes that have appeared on HSA by glucose modification.

Albumin purified from the plasma of type 1 diabetic subjects with and without complications were subjected to inhibition ELISA with experimentally generated antibodies against glycated-HSA. The results demonstrated that albumin purified from patients’ plasma were glycated because they inhibited anti-glycated-HSA antibody binding. However, the degree of albumin glycation varied in the patients as the inhibition results were different. Albumin purified from normal healthy individuals did not show appreciable binding with the anti-glycated HSA antibodies. Moreover, higher inhibition in antibody binding was observed with the HSA isolated from diabetic patients with secondary complications. This is in conformity with earlier
studies that diabetic state promotes Amadori-modification of many circulating proteins.

Results of this study conclusively point out that the Amadori-albumin is highly immunogenic and the major contribution is by the immunogenic epitopes of glycated lysine residues. The prevalence of autoantibodies against Amadori-albumin in type 1 diabetic patients may be attributed to the powerful immunogenicity of endogenously formed Amadori-albumin. Antiglycation properties exhibited by AG and TQ has prompted us to think and work in the direction of preventing the formation of Amadori proteins in diabetic state. This may prove effective in reducing or ameliorating the diabetes related vascular complications.