Original Article

Glycation Induced 3D Structural Changes of Human Serum Albumin in Diabetes Mellitus

Structural Changes of Proteins in Diabetics

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ABSTRACT

Objective: The present study was planned to find out the structural changes of proteins due to glycation in diabetic and non-diabetic patients.

Study Design: Experimental Study.

Place and Duration of Study: This study was conducted at the Civil Hospital Faisalabad and Bioinformatics Lab GC University Faisalabad from January 2018 to June 2019.

Materials and Methods: A total 60 subjects, 30 without diabetes and 30 with diabetes were selected. From each subject 0.5 ml was drawn from antecubital vein using plastic disposable syringe. Serum was separated after centrifugation of clotted blood. Serum glucose, total proteins and albumin was immediately analyzed and a part of serum was stored in storage cup for future analysis of glycated albumin. In silico prediction models was used to predict change in glycated proteins, for this purpose different bioinformatics tools were used in present research work. These tools helped to construct three dimensional (3D) models of proteins before glycation and after glycation as well as their stability after glycation.

Results: The study revealed that structural and functional features of glycated HSA, isolated from diabetic patients were significantly different from the HSA isolated from non-diabetic subjects. These findings suggest that active sites of HSA may not be available under extensive glycation, leading to the impairment of its important functions. Results amino acid residue at 114 position was glycated, the normal functioning of human serum albumin was stopped. Ramachandran plot was constructed for glycated and non-glycated human serum albumin, hence showed UN functionality of protein with red color. Thus, glycated HSA may be involved in the pathogenesis of diabetes and its complications.

Conclusion: The overall glycation rate, the thermodynamics of this process, and the modification rate in certain regions were considered in such studies. The role of glycation conditions in the types of modifications formed was also examined.

Key Words: Glycation, Human Serum Albumin, Diabetes, amino acid

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INTRODUCTION

Glycation is a non-enzymatic unconstrained procedure in proteins which has astounding effect on its physical and utilitarian angles. The procedure of glycation has important impact on structures and elements of proteins. Three-dimensional auxiliary difference in HSA is so unfaltering and stable that it replaces HbA1C as infection marker for diabetes[1].

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Alongside auxiliary changes the cancer prevention agent movement of albumin additionally diminished with non-enzymatic Glycation[2]. Also, advance discoveries demonstrated that after NEG of albumin may prompt hindrance of dynamic site and engaged with diabetic intricacies like interminable kidney infection[3].

Albumin is one of the major and biggest plasma proteins. The typical grouping of albumin in plasma contributes 35-50g/l which empowers it most plenteous plasma protein having various physiological capacities. Human albumin additionally adds to half sound people plasma proteins.[3]. The protein has three areas I,II,III that further subdivided into two subdomains, A and B that empowers it to into practical conformational structure nearness of 17 disulphide extensions makes it impervious to the adjustment in the pH and other modifying condition. The typical physiological capacity of albumin is to keep up osmotic weight in the plasma: this interesting property emerges because of its low sub-atomic weight contrasted with other plasma proteins [(4)].

The structure of albumin alters with enzymatic and non-enzymatic Glycation [5]. The glycation of albumin has huge impact on albumin activity and cell capacities [6]. These auxiliary and useful changes because of Glycation in vivo has been found in vitro in commercial Bovine Serum Albumin (BSA). Bovine serum albumin has proof to indicate 76% arrangement homology to human serum albumin (HSA) and the two has comparable ellipsoidal shape having three spaces I, II, III connected together through helical expansions. Because of glycation, the albumin structure is changed and it diminished the medication liking of albumin [7].

Non - enzymic glycosylation begins with the buildup of a free amino gathering of an amino acid or protein and the carbonyl gathering of a diminishing sugar in its open chain structure bringing about the development of a temperamental adduct, named an aldimine or Schiff base which, quickly experiences cyclisation to the relating n-substituted aldosylamine (Aglycosylamine). The Schiff base quickly and balance is come to in matter of hours. The consistent state level of Schiff base mirrors the encompassing glucose focus (over a predefined timeframe). The procedure of glycation has vital impact on structures and elements of proteins. The investigation of proteins has consistently been a significant piece of therapeutic research and sub-atomic analysis. Proteins coursing in human blood are promptly available and can break down legitimately to deliver symptomatic data on infection status in patients.[8] The Glycation procedure in hyperglycemic condition for the most part chooses coursing proteins like albumin, lipoproteins, hemoglobin, and so on non-enzymatic glycation (Millard response) is a mixed drink of arrangement of responses that incorporates buildups, adjustments of practical gatherings, and oxidative alterations. Further oxidation of the early Glycation items that framed by the arrangement of Schiff's base and Amadori produces progressed glycated finished results (AGE) which is non reversibly aggregate and structure further cross connecting with one another. Arrangement of the late propelled Glycation final results are viewed as solid marker of smaller scale just as large scale vascular complexities of diabetes mellitus. The present investigation intended to discover the degree of Glycation in diabetic and non-diabetic and to examine the basic changes in protein because of Glycation. The main objectives were to study glycation status and identification of three-dimensional structural modification of albumin in diabetic and non-diabetic patients**.**

MATERIALS AND METHODS

Purpose of the study was to access Glycation status in diabetic and non- diabetic patients and 3-D structural and functional changes in albumin serum. A total 60 subjects, 30 without diabetes and 30 with diabetes were selected. From each subject 0.5 ml was drawn from antecubital vein using plastic disposable syringe. Serum was separated after centrifugation of clotted blood. Serum glucose, total proteins and albumin was immediately analyzed and a part of serum was stored in storage cup for future analysis of glycated albumin. Glycation was measured using the Thiobarbituric Acid Colorimetric reaction. In silico stability assay for albumin prediction models were constructed through bioinformatics tools which was used to predict albumin structure in normal and diabetic patients. Following tools of bioinformatics were used in the research work. 3D model building of albumin Template search with Blast was performed against SWISS-MODEL template library (SMTL). The target sequence was searched with BLAST (9)against the primary amino acid sequence contained in the SMTL. Model was built based on the target-template using UCSF Chimera. Superimposition of 3D Model Albumin. The change in albumin structure after diabetes was predicted by superimposition of normal albumin and albumin of diabetic patients. Ramachandran Plot and the Stability of Albumin. Ramachandran plot was used to visualize the back bone of polypeptide chain, to calculate the phi and psi angles and for structural validation. Ramachandran plot was introduced by an Indian Physicist G. N. Ramachandran. Ramachandran analysis was done to check whether the mutated amino acids fall in allowed region or not (10).

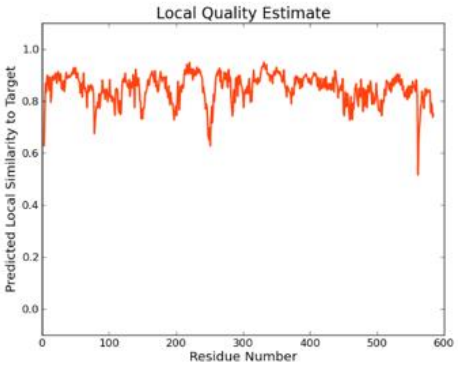
RESULTS

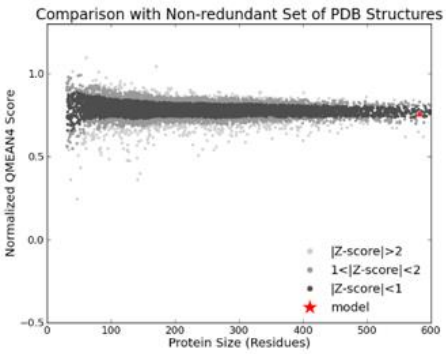
Our findings suggest, the glycation level ranged between 0.024 mole/mole of protein to 0.30 mole / mole of protein in normal individuals. The value of glycation level in diabetes patients ranged from 0.032 mole/mole to 1.96 mole/mole of protein.

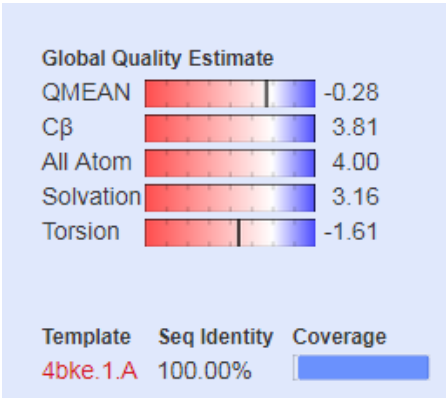
The change in albumin structure after diabetes was predicted by glycation of normal albumin and albumin of diabetic patients in the form of carbonyl group. The comparison of both models has no structural changes but due to glycation three-dimension protein is inactive.

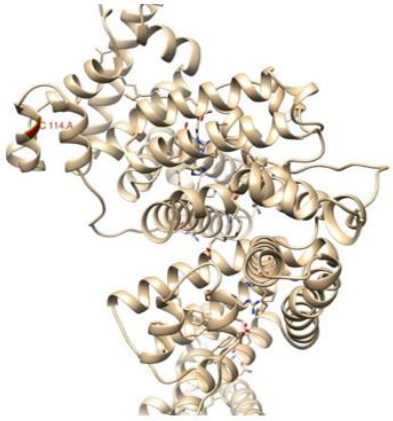
In the structures below first is three-dimension structure of human serum albumin and it is completely functional but after glycation the green lines showed the glycation of protein and in this form, it is not able to perform its normal functions. Below the pictures explained single amino acid glycation consequences. When amino acid residue at 114 position was glycated the normal functioning of human serum albumin is stopped.

When the Ramachandran plot was constructed for normal human serum albumin all amino acids fall in allowed region and protein is fully functional. When the Ramachandran plot was constructed for glycated human serum albumin all amino acids fall in allowed region but the protein is un functional. This is due to glycation, all amino acids lost their functionality. Their red color showed dead proteins.

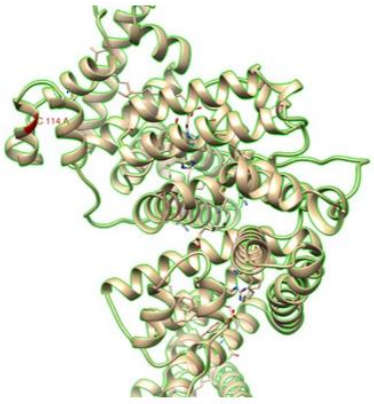




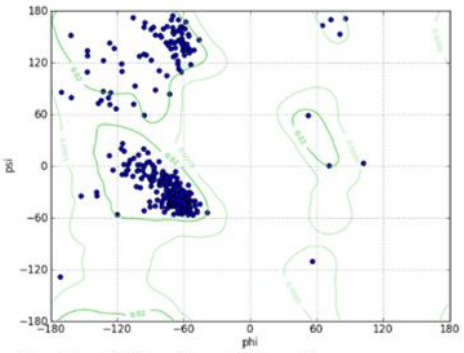




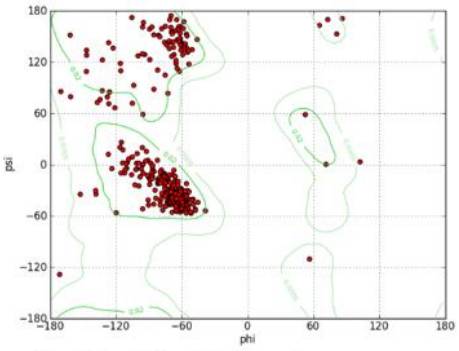
Residue 114 without Glycation



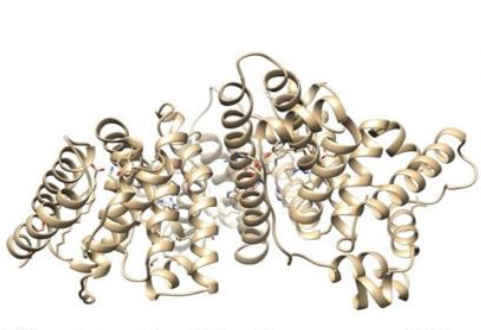
Residue 114 with Glycation



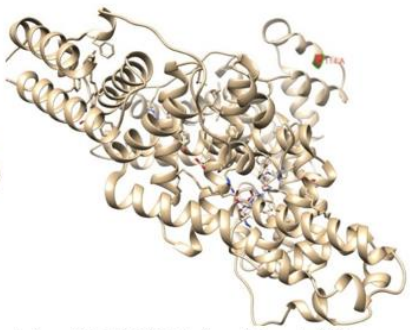
Residue 114 without Glycation



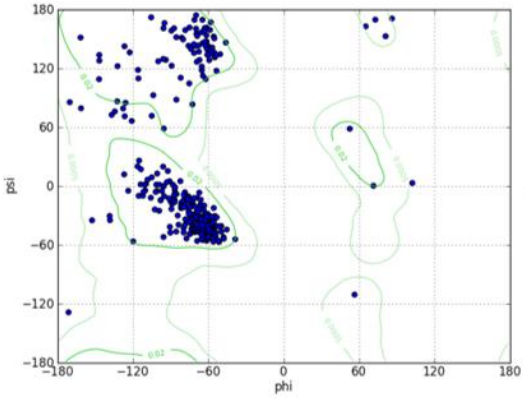
Residue 114 with Glycation



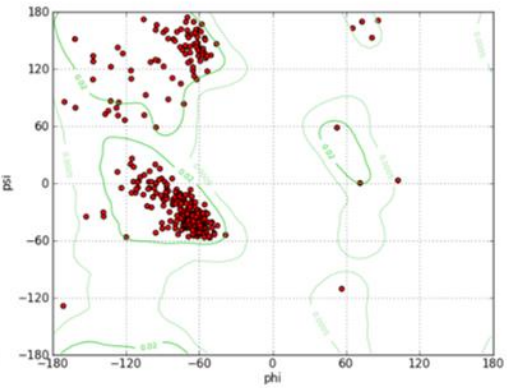
HSA protein without Glycation



HSA protein with highlighted residue at 114



Ramachandran HSA protein with all residues



Ramachandran HSA protein with Glycation

DISCUSSION

Glycation-induced changes in the structure and function of proteins are of particular interest since numerous studies in vivo have reported the strong involvement of glycated albumin in the development and progression of chronic diabetes complications[6,11]. Various beneficial functions linked to Albumin, including regulating oncotic pressure, the transport and binding capacities of metabolites and therapeutic agents[12]. The most striking property of HSA is its antioxidant activity, which has been shown to be impaired due to glycation[13].The change in albumin structure after diabetes was predicted by glycation of normal albumin and albumin of diabetic patients in the form of carbonyl group. The comparison of both models showed that due to glycation three-dimension protein is inactive.

Our results can be compared with the work of Monacelli who found that the structure of albumin modifies with enzymatic and non-enzymatic glycation[5]. The glycation of albumin has significant effect on albumin action and cell functions[6]. These structural and functional changes due to glycation in vivo has been found in vitro in commercial bovine serum albumin (BSA). Due to glycation, the albumin structure is changed and it reduced the drug affinity of albumin[14]. Our results showed lost functionality of protein in (Fig 1, 2) where green lines displayed glycation.

CONCLUSION

The glycation of HSA has been an issue of interest for several decades and especially in the past ten years. Part of this interest relates to the clinical use of glycated HSA as a biomarker for the control of blood sugar over short to medium periods. There is also increasing interest in the effects of glycation on the structure of HSA. A variety of methods have been used to examine the number, location, and type of modifications that can occur with glycated HSA. Many possible modification sites for both early-stage glycation products and AGEs have been identified on this protein, but a smaller subset of these sites appear to be involved in most of these reactions. Some information about the similarities or differences between in vivo and in vitro glycated HSA has been obtained from such work. The overall glycation rate, the thermodynamics of this process, and the modification rate in certain regions were considered in such studies. The role of glycation conditions in the types of modifications formed was also examined.

Author's Contribution:

Concept & Design of Study:	Sajjad Ghani
Drafting:	Fariha Niaz, Saira Mushtaq
Data Analysis:	Attiya Anwar
Revisiting Critically:	Sajjad Ghani, Fariha Niaz
Final Approval of version:	Sajjad Ghani

Conflict of Interest: The study has no conflict of interest to declare by any author.

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