Section: Biochemistry

Original Research Article

ROLE OF 3-DEOXYGLUCOSONE-GLYCATED HUMAN SERUM ALBUMIN IN THE PATHOPHYSIOLOGY OF DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus complications are largely due to glycation, a non-enzymatic reaction between proteins and reducing sugars. 3-Deoxyglucosone (3-DG), a highly reactive dicarbonyl, is a major source of advanced glycation end products (AGEs) that alter biomolecule structure and function. Human serum albumin (HSA), the main plasma protein, is particularly affected. This study examined structural and biochemical changes in HSA glycated with 3-DG (20 µM, 28 days, 37 °C). Absorption spectroscopy showed increased hyperchromicity. Direct binding and inhibition ELISA revealed higher autoantibody levels against 3-DG-modified HSA in diabetics. Findings suggest glycation generates neo-epitopes on HSA, potentially triggering immune responses in diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia due to defects in insulin secretion, action, or both. The prevalence of DM in India is 9.3% and is increasing rapidly, with an estimated 640 million adults affected worldwide by 2040.[1] Chronic hyperglycemia promotes the formation of advanced glycation end products (AGEs) via non-enzymatic reactions between reducing sugars and proteins.[2] Highly reactive α-dicarbonyl compounds, particularly 3-deoxyglucosone (3-DG), are potent AGE precursors and are elevated in diabetes.[3,4] Albumin is especially prone to glycation, producing epitopes that can trigger autoantibody formation, potentially contributing to diabetic complications.[5,6] This study examines 3-DG–glycated serum albumin and associated autoantibodies in Type 2 DM patients.

Evidence from earlier studies supports the hypothesis that oxidative and glycated modifications of self-proteins can lead to the development of cross-reactive antibodies. Rasheed et al. demonstrated that hydroxyl radical-modified human serum albumin undergoes structural alterations that elicit the formation of cross-reactive antibodies, indicating the immunogenic potential of oxidatively damaged proteins.[7] Similarly, Khan et al. characterized hydroxyl radical-modified glutamic acid decarboxylase (GAD65) as a potential autoantigen in type 1 diabetes, suggesting that oxidative stress-induced modifications can unmask cryptic epitopes capable of triggering autoimmune responses.[8]

In diabetic patients, increased oxidative stress and elevated levels of reactive carbonyl species such as 3-DG act synergistically, promoting protein glycation and oxidation. Mustafa et al. reported that glycated human DNA serves as a preferred antigen for anti-DNA antibodies in diabetic individuals, further supporting the role of glycation-induced antigenicity in autoimmune manifestations associated with diabetes.[9] Additionally, Ashraf et al. observed a high prevalence of autoantibodies against 3-DG–glycated histone H2A in type 2 diabetes, reinforcing the link between carbonyl stress, protein modification, and immune activation.[10]

To the best of our knowledge, very little data is available to assess the role of glycated serum albumin in Type 2 DM patients. Glycated serum albumin and its resultant autoantibodies may have a role in the pathophysiology of Type 2 DM patients. Therefore, the present study has been undertaken to glycate serum albumin protein with 3-diabetes mellitus patients.

MATERIALS AND METHODS

A case control study was conducted in the Department of Biochemistry, New Delhi during a

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study period of six months from July 2024 to December 2024.

Study design: Case control

Study population: The study was conducted in a Tertiary care center in New Delhi. The study population included patients diagnosed with Type 2 diabetes mellitus according to latest American Diabetes Association (ADA) criteria including both males and females (aged 18-65 years).[11]

Sample size: The required sample size for this study was determined based on the desired 95 % confidence interval, an estimated prevalence of 9.3 % and a margin of error of 7 %. Using the standard formula for sample size calculation for population proportions:

$Z^2 \cdot p(1 - p)\, d2$

where Z = 1.96 for 95 % confidence interval, P = 0.093 for the estimated prevalence and d = 0.07 for the margin of error, the estimated sample size is approximately 65 participants with cases and 35 controls.

Inclusion criteria:

Type 2 diabetes mellitus patients having fasting plasma glucose (FPG) of ≥126 mg/dL (7.0 mmol/L) or 2-hour plasma glucose (PG) ≥200 mg/dL (11.1 mmol/L) or glycated haemoglobin ≥6.5% will be taken.

Sera from healthy individuals (n = 35) will serve as control. They will be matched for age, sex, gender and socio-economic conditions.

Exclusion Criteria:

Prediabetic patients having fasting plasma glucose (FPG) of 100 mg/dl (5.6 mmol/l) to 125 mg/dl (6.9 mmol/l) or 2-hour plasma glucose of 140 mg/dl (7.8 mmol/l) to 199 mg/dl (11.0 mmol/l) after ingestion of 75 g of oral glucose load (OGTT).

The pregnant females or subjects having anaemia, kidney, liver and thyroid disease or any coexisting autoimmune disorder will be excluded.

Patients who will not give consent will also be excluded from the study

Collection and processing of blood sample

Informed consent were obtained from all participants before taking blood samples for research purpose. Clinical history, physical examination, and relevant details of the patients were recorded. Ethical approval was se- cured prior to the study. A 5 ml blood sample was collected in plain vials, serum was separated, heat-inactivated at 56°C for 30 minutes to inactivate complement proteins, and stored at 20°C with appropriate labelling.

Modification of Human serum albumin by 3-Deoxyglucosone

20 µM Human serum albumin (HSA) using phosphate saline buffer (10 mM sodium phosphate, pH 7.4 with 150 mM NaCl) incubated with 3-Deoxyglucosone (3-DG) for 7–28 days at 37°C at final concentrations of 10 µM and 100 µM. maintaining alkaline ph between 10 and 11. Extensive dialysis was carried out at 4°C to eliminate unreacted 3-DG and other contaminants using sodium phosphate saline buffer (pH 7.4) with frequent buffer changes. Glycated HSA samples were aliquoted and kept at -80°C.

Hyperchromicity = $\frac{\text{Absmodified albumin} - \text{Absnative albumin}}{\text{Absmodified albumin}}$

Spectroscopic analysis of native and 3-Deoxyglucosone- modified-albumin

Structural changes in human serum albumin (HSA) due to 3-deoxyglucosone (3-DG) glycation were analyzed using UV–Vis absorption spectroscopy. Absorbance spectra of native (control) and 3-DG-modified HSA were recorded in a high-precision quartz cuvette (1 cm path length) over the 250–400 nm range, encompassing characteristic HSA peaks. Variations in absorbance intensity were used to calculate hyperchromicity, indicating structural alterations and chromophore modifications. These findings highlight protein aggregation and conformational changes associated with 3-DG-mediated glycation

Spectroscopic analysis of native and 3-Deoxyglucosone- modified-albumin: Structural changes in human serum albumin (HSA) due to 3-deoxyglucosone (3-DG) glycation were analyzed using UV–Vis absorption spectroscopy. Absorbance spectra of native (control) and 3-DG-modified HSA were recorded in a high-precision quartz cuvette (1 cm path length) over the 250–400 nm range, encompassing characteristic HSA peaks. Variations in absorbance intensity were used to calculate hyperchromicity, indicating structural alterations and chromophore modifications. These findings highlight protein aggregation and conformational changes associated with 3-DG-mediated glycation.

ELISA: Flat-bottom polysorp microtitre wells were coated with 100 µL of native or 3-deoxyglucosone (3-DG)–modified albumin (10 µg/mL in protein coating buffer) and incubated for 2 h at 37 °C followed by overnight at 4 °C. Duplicate samples were prepared, with control wells lacking antigen. After washing thrice with TBS-T, unoccupied sites were blocked with 150 µL of 2% non-fat dry milk (TBS, pH 7.4) for 4–5 h at 37 °C. In direct binding ELISA, diluted sera (1:100) or purified IgG were added and incubated under the same conditions, followed by washing. Anti-IgG alkaline phosphatase conjugate was added, incubated for 2 h at 37 °C, and washed. Para-nitrophenyl phosphate substrate was added, and absorbance was read at 410 nm. Results were expressed as the mean difference between test and control absorbance(Atest-Acontrol).[12]

Inhibition Elisa: The antigenic specificity of the antibodies was determined by inhibition ELISA varying amounts of inhibitors (0-20 µg/ml) were mixed with a constant amount of patient's sera/antisera or IgG. The mixture was incubated at room tempera- ture for 2 hr and overnight at 4°C. The immune complex thus formed was added in the wells instead of serum/IgG [13]. The remaining steps were same as in direct binding ELISA. Percent inhibition was calculated using the following formula;

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Percent inhibition = $\frac{1 - A\text{inhibited}}{A\text{uninhibited}} \times 100$ (1)

Statistical analysis: Data are presented as mean ± S.D. Statistical significance of control versus test was computed using student's t-test, A p value of <0.05 was considered statistically significant.

RESULTS

Absorption spectroscopy of native- and 3-deoxyglucosone- modified-Human serum albumin

Human serum albumin (HSA, 20 µM) was incubated at 37 °C with two concentrations of 3-deoxyglucosone (3-DG; 10 µM and 100 µM) to evaluate glycation-induced structural changes over time. Absorbance at 280 nm was measured on days 7, 14, 21, and 28 using a UV–Vis spectrophotometer. Both native (non-glycated) and 3-DG–modified HSA exhibited a gradual increase in absorbance, indicating progressive hyperchromicity [Figure 1, 2].

At 100 µM 3-DG, percentage increases in absorbance were 54.69%, 71.12%, 74.94%, and 76.27% on days 7, 14, 21, and 28, respectively [Table 1]. At 10 µM 3-DG, values were 48.05%, 65.03%, 71.27%, and 73.34% for the same time points [Table 2]. Glycation was slightly more pronounced at 100 µM, but changes after day 21 were minimal, suggesting near-complete modification by that time.

Table 1: Effect of time on absorbance and hyperchromicity of Human serum albumin incubated with 100µM 3-deoxyglucosone.

Incubation time	Absorbance at 280 nm	Hyperchromicity
0	0.214	0
7	0.532	54.69
14	0.741	71.12
21	0.854	74.94
28	0.902	76.27

Studies on the sera of Diabetes Mellitus patients

All 65 patients from whom the blood was withdrawn, after obtaining the con- sent, satisfied the criteria of the American Diabetes Association (ADA) for diagnosis of Diabetes Mellitus. Sera from 35 normal healthy subjects matched for age and sex who served as control.

Table 2: Effect of time on absorbance and hyperchromicity of Human serum albumin incubated with 10µM 3-deoxyglucosone.

Incubation time	Absorbance at 280 nm	Hyperchromicity
0	0.214	0
7	0.412	48.05
14	0.612	65.03
21	0.745	71.27
28	0.803	73.34

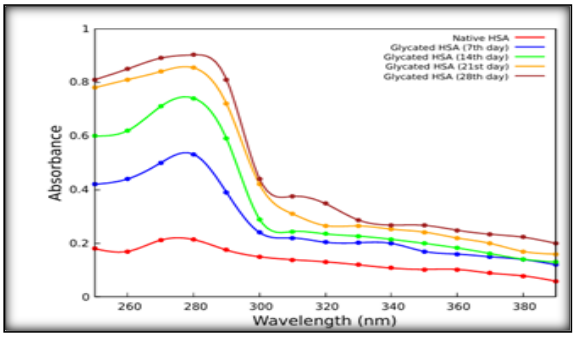

Figure 1: Profile of native human serum albumin incubated with 100µM for different time intervals.

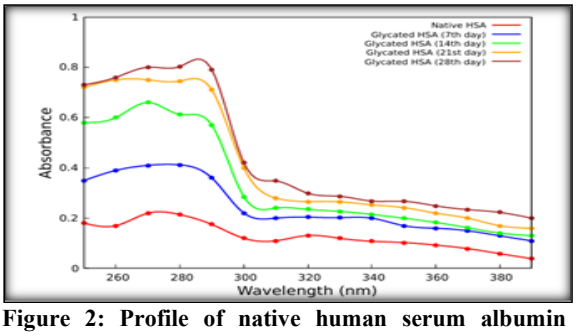

Figure 2: Profile of native human serum albumin incubated with 10µM for different time intervals

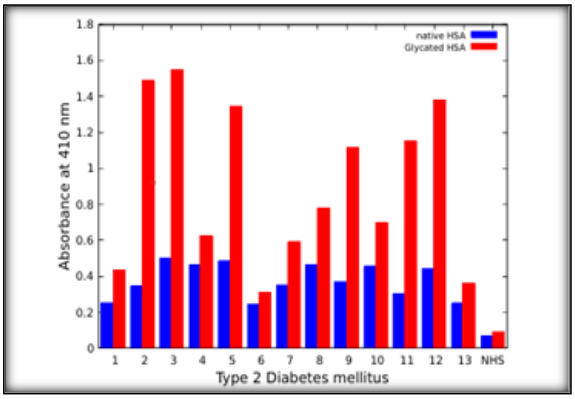

Figure 3: Direct binding ELISA of type 2 diabetes mellitus (1–13) with native HSA ( ) and Glycated-HSA ( ). Pooled normal human sera (NHS) served as control. microtiter plate were coated with respected antigens.

Enzyme immunoassay of diabetic mellitus sera with native HSA and 3-Deoxyglucosone-modified-HAS: A total of 65 serum samples were analyzed for their binding affinity to native and glycated human serum albumin (HSA). The sera were diluted at a 1:100 ratio and subjected to direct binding ELISA using microtiter wells, each coated with an equal amount of the respective antigen (Table 3). Serum

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samples from 35 apparently healthy individuals served as controls. The mean data suggested a notable presence of anti-glycated-HSA autoantibodies, while sera from healthy subjects exhibited minimal binding to the coated antigens shown in [Figure 3-7]. A total of 39 serum samples from individuals with diabetes mellitus demonstrated significantly higher binding to glycated HSA ($p<0.05$) and were selected for further investigation. The summarized data are presented in [Table 4].

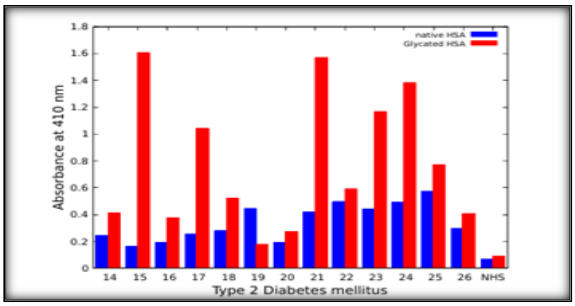

Figure 4: Direct binding elisa of type 2 diabetes mellitus patients (14–26) with native HSA ( ) and Glycated-HSA ( ). Pooled normal human sera (NHS) served as control. microtiter plate were coated with respected antigens.

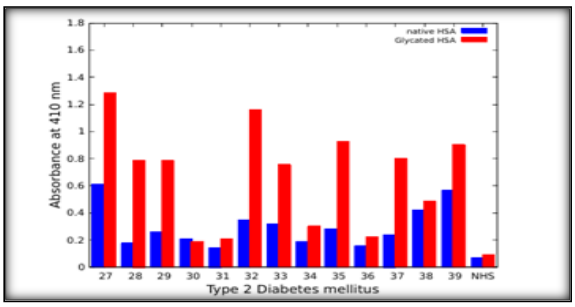

Figure 5: Direct binding ELISA of type 2 diabetes mellitus patients (27–39) with native HSA ( ) and Glycated-HSA ( ). Pooled normal human sera (NHS) served as control. microtiter plate were coated with respected antigens.

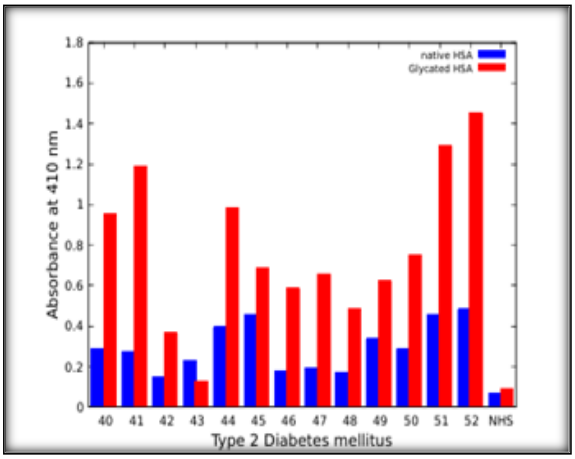

Figure 6: Direct binding ELISA of type 2 diabetes mellitus patients (40–52) with native HSA ( ) and Glycated-HSA ( ). Pooled normal human sera (NHS) served as control. microtiter plate were coated with respected antigens.

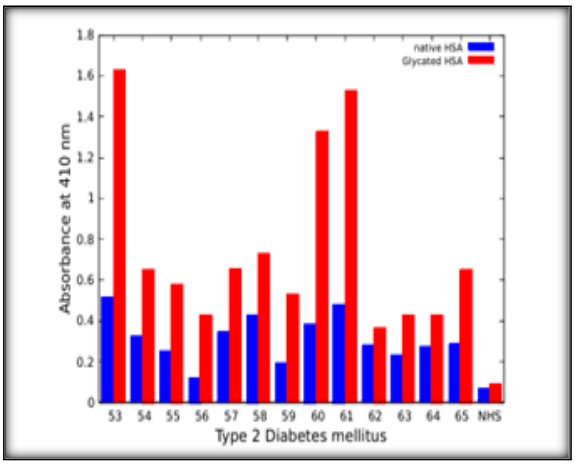

Figure 7: Direct binding ELISA of type 2 diabetes mellitus patients (53–65) with native HSA ( ) and Glycated-HSA ( ). Pooled normal human sera (NHS) served as control. microtiter plate were coated with respected antigens.

Table 3: Direct binding ELISA of Diabetes Mellitus patient sera with Native HSA and 3-Deoxyglucosone modified HSA.

Type 2 Diabetes Mellitus Sera	Native Albumin	3- Deoxyglucosone Modified HSA	Control
1	0.828	0.493	0.248
2	0.627	1.484	0.44
3	0.875	1.548	0.632
4	1.152	0.823	0.735
5	0.597	1.344	0.603
6	0.597	0.511	0.681
7	1.170	0.592	0.344
8	0.913	0.778	0.597
9	0.522	1.115	0.433
10	0.842	0.894	0.465
11	0.524	1.147	0.434
12	0.523	1.378	0.432
13	0.747	0.531	0.607
14	0.066	0.509	0.672
15	0.126	1.6	0.432
16	0.493	0.476	0.219
17	0.351	1.04	0.554
18	0.770	0.544	0.641
19	0.384	0.479	0.596
20	0.224	0.474	0.629
21	1.134	1.571	0.487

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22	0.599	0.993	0.472
23	0.692	1.163	0.532
Direct binding ELISA of Diabetes Mellitus patient sera with Native HSA and 3-Deoxyglucosone modified HSA. (Continued)			
Type 2 Diabetes Mellitus Sera	**Native Albumin**	**3- Deoxyglucosone Modified HSA**	**Control**
24	0.220	1.382	0.731
25	0.499	0.967	0.356
26	0.706	0.507	0.618
27	0.307	1.284	0.488
28	0.789	0.789	0.613
29	0.481	0.984	0.637
30	0.578	0.490	0.714
31	0.480	0.408	0.499
32	1.256	1.155	0.421
33	0.666	1.254	0.664
34	0.336	0.5	0.493
35	0.930	0.923	0.692
36	0.285	0.425	0.584
37	0.737	0.529	0.651
38	0.051	0.486	0.532
39	0.251	1.300	0.479
40	0.733	0.827	0.477
41	0.904	0.456	0.302
42	0.725	0.465	0.718
43	0.634	0.428	0.758
44	0.575	0.984	0.557
45	0.203	1.387	0.537
46	0.443	0.586	0.599
Direct binding ELISA of Diabetes Mellitus patient sera with Native HSA and 3-Deoxyglucosone modified HSA. (Continued)			
Type 2 Diabetes Mellitus Sera	**Native Albumin**	**3- Deoxyglucosone Modified HSA**	**Control**
47	0.525	0.457	0.461
48	1.005	0.484	0.528
49	0.779	0.921	0.649
50	0.114	0.952	0.671
51	0.773	1.26	0.559
52	0.549	0.82	0.625
53	0.457	1.628	0.646
54	0.864	0.653	0.501
55	0.996	0.98	0.488
56	0.965	0.427	0.603
57	0.405	0.654	0.251
58	0.573	0.931	0.487
59	0.775	1.452	0.573
60	0.979	0.529	0.511
61	0.519	0.531	0.375
62	0.612	0.467	0.612
63	0.321	0.518	0.542
64	0.293	0.431	0.517
65	0.927	0.452	0.365
Mean ± SD	0.615 ± 0.284	0.839 ± 0.378	0.538 ± 0.123

Table 4: Summary of inhibition ELISA of selected diabetes mellitus sera. Maximum percent inhibition at 20 µg/ml.

Type 2 Diabetes Mellitus Sera	Native Albumin	3-Deoxyglucosone Modified HSA
2	37.02	79.69
3	34.80	75.07
4	37.55	80.79
5	40.61	74.37
7	34.46	74.37
8	34.46	87.58
9	40.81	81.66
10	37.97	72.65
11	33.64	80.02
12	37.18	72.70
15	39.66	72.68
17	33.65	72.14
18	36.13	63.51
21	33.59	71.98
22	40.25	68.70
23	34.32	78.36
24	31.74	69.46

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25	36.38	65.79
27	39.11	86.75
28	30.34	74.43
29	40.41	76.56
32	34.49	65.70
33	35.52	72.11
35	30.30	76.88
39	42.38	67.69
40	35.67	78.81
44	31.25	71.70
45	36.60	73.95
46	33.18	71.69
49	34.26	89.56
50	33.18	75.97
51	41.77	68.37
52	35.24	82.06
53	31.58	67.18
54	38.16	77.59
55	33.01	61.80
57	37.01	66.40
58	28.42	87.17
59	33.63	77.51
Mean ± SD	35.63 ± 3.37	74.65 ± 6.72

Inhibition ELISA of selected type-2 Diabetes mellitus sera by native HSA and glycated-HSA

To assess the binding specificity of serum autoantibodies in type 2 diabetes mellitus (T2DM), inhibition ELISA was performed using native human serum albumin (HSA) and 3-deoxyglucosone (3-DG)–modified HSA as inhibitors (Fig. 8). Under identical conditions, native HSA produced an inhibition range of 28–36%, whereas 3-DG–modified HSA showed significantly higher inhibition of 65–82%. Mean percent inhibition was 34.63 ± 3.37% for native HSA and 74.65 ± 6.72% for glycated HSA (Table 4). Student's t-test revealed a highly significant difference ($p < 0.001$), confirming preferential autoantibody binding to glycated HSA and implicating protein glycation in immune dysregulation in T2DM

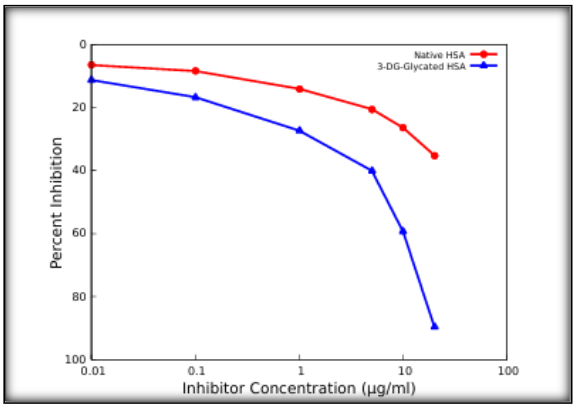

Figure 8: Inhibition of binding of Anti glycated antibodies of Diabetes mellitus serum (isolated from serum 49) by native-HSA ( ) and 3-DG-glycated-HSA ( ). Microtitre wells were coated with Native Albumin (2.5 µg/ml)

DISCUSSION

Chronic hyperglycaemia in diabetes mellitus promotes non-enzymatic glycation reactions between the amino groups of proteins or nucleic acids and the carbonyl groups of reducing sugars, initially forming Schiff bases and Amadori products that subsequently convert into advanced glycation end products (AGEs).[14–16] Among the AGE precursors, 3-deoxyglucosone (3-DG)—an α-dicarbonyl compound derived from glucose metabolism and fructosyl-lysine degradation—is a potent glycating agent.[17,18] It readily interacts with nucleophilic sites on proteins and nucleic acids, leading to structural and functional alterations. Human Serum Albumin (HSA), being the most abundant plasma protein, is particularly susceptible to such glycation-induced modifications.

In the present study, sera from Type 2 Diabetes Mellitus (T2DM) patients exhibited a significantly higher affinity toward 3-DG–modified HSA compared to native HSA, as demonstrated by direct binding ELISA. The elevated titres of anti-glycated HSA antibodies and the significant inhibition observed in competitive ELISA ($p < 0.001$) strongly indicate that circulating HSA-AGEs serve as potent immunogenic antigens capable of eliciting autoimmune responses.[19,20] These antibodies may form AGE–immune complexes, contributing to endothelial dysfunction and atherogenesis in diabetes.

Persistent hyperglycaemia also enhances free radical generation through glucose auto-oxidation and non-enzymatic protein glycation, augmenting oxidative stress and promoting polyol pathway activation.[21] Both experimental and clinical studies have established that reactive oxygen species (ROS) further exacerbate glycation-induced damage, altering the structure and function of HSA and other biomacromolecules. AGEs possess unique epitopes that can trigger autoimmune responses, resulting in the formation of autoantibodies against glycated proteins in diabetic sera.[22,23] These antibodies have been implicated in the etiopathogenesis of diabetic complications such as nephropathy and chronic renal

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failure.[24,25] It has also been suggested that glucose-derived adducts on autologous proteins can act as neoantigens, initiating immunogenic reactions. For instance, a hexitolamino derivative of collagen Amadori adduct has been shown to induce such immune responses.[26]

HSA, present at approximately 40 mg/mL in plasma, serves as a critical transport and antioxidant protein. It binds and transports free fatty acids, hormones, and various endogenous and exogenous molecules, maintaining osmotic balance between plasma and interstitial fluids.[27,28] Its versatile binding capacity allows HSA to sequester potentially toxic compounds, enhance their solubility, and protect against oxidative damage, thereby extending their plasma half-life.[29] Owing to these properties, HSA has been widely utilized in biomedical applications including drug delivery systems and diagnostic assays, and holds therapeutic relevance in disorders such as hypoalbuminemia, acute respiratory distress syndrome, liver disease, hemorrhage, and cardiopulmonary bypass.[30–33] Moreover, the pharmacokinetics and pharmacodynamics of several drugs are significantly influenced by their binding affinity to HSA, which affects their bioavailability, distribution, and toxicity profiles.[34–36]

Taken together, these findings suggest that hyperglycaemia-induced oxidative stress and glycation modify structural and functional properties of HSA, rendering it immunogenic. The resultant formation of anti-glycated albumin antibodies and AGE–immune complexes may contribute to the immunopathogenesis of diabetes-related complications.

CONCLUSION

This study evaluated autoantibodies against native human serum albumin (HSA) and 3-deoxyglucosone (3-DG)–modified HSA in type 2 diabetes mellitus (T2DM). Diabetic patients showed significantly higher autoantibody levels against both native and glycated HSA compared to controls. Direct binding ELISA demonstrated stronger binding to glycated HSA, indicating a high titre of anti-glycated HSA antibodies, which was confirmed by inhibition ELISA showing greater specificity for glycated HSA. 3-DG–mediated glycation alters HSA structure, reduces antioxidant capacity, and contributes to endothelial dysfunction, potentially worsening vascular complications, nephropathy, and retinopathy. These findings highlight 3-DG–glycated HSA as a potential biomarker for disease progression. Understanding these serological changes may guide targeted therapies aimed at reducing AGE formation and oxidative stress in T2DM.

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