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Frequency of Autoantibodies Against 3-Deoxyglucosone H1 Protein in Type 2 Diabetes

Tip 2 Diabette 3-Deoksiglukazon-H1 Proteinine Karşı Otoantikorların Sıklığı

Jalaluddin M. ASHRAF[1], Shahnawaz REHMAN[2]

Abstract

Introduction: Advanced glycation end-products (AGEs) are contributing factors to diabetes complications. The antigenic nature of AGEs established the theory of incessant accumulation of AGEs can incite an autoimmune response in a diabetic patient. Glycating agents like 3-deoxyglucosone are increased in diabetic patients, leading to the high formation of AGEs aggravating the pathophysiological conditions in diabetes. We aimed to study the immunogenicity of glycated histone H1 protein and AGEs (N-carboxymethyl-lysine and pentosidine) as well as detection of autoantibodies in the sera of type 2 diabetic subjects.

Materials and Methods: Female rabbits were injected with native H1 and glycated-H1 to discern its immunogenicity. Diabetic subjects' sera were also scanned for the detection of autoantibodies against glycated-H1 and AGEs using immunochemical assay technique.

Results: Glycated-H1 was highly immunogenic, unlike the native analog. Diabetic sera showed 48% (72 of 150 samples) significantly strong binding with glycated-H1, further sera also showed 40% (60 of 150 samples), and 36% (54 of 150 samples) strong binding with CML, and pentosidine, respectively.

Conclusion: The findings support modified H1 as well AGEs are highly immunogenic in nature. The presence of autoantibodies against glycated-H1 and AGEs may be utilized in the assessment of diabetes or its complications.

Keywords: immunogenicity, 3-deoxyglucosone, advanced glycation end-products, diabetes

Öz

Giriş: İleri-glikasyonlanmış son ürün (İGS)'ler diabette komplikasyonların gelişimine katkıda bulunur. İGS'lerin antijenik özellikte olmaları, diabet olan hastalarda otoimmüniteyi tetikleyebileceklerini düşündürtmektedir. 3-deoksiglikazon gibi glikalleyici ajanlar, diabetik hastalarda daha fazla İGS birikimine ve böylece bazı patofizyolojik durumlara yol açıyor olabilir. Bu çalışmada glikallenmiş H1 histon proteini ve İGS'lerin (pentosidin ve N-karboksimetil-lizin) bağışıklığı uyarma özelliğini ve tip 2 diabeti olan hastalarda otoantikorların çalışılması planlanmıştır.

Gereçler ve Yöntemler: Dişi tavşanlara bağışıkl yanıtını uyarmak için doğal ve glikasyon olmuş H1 injekte edildi. Diabet olan deneklerin serumlarında immünohistokimyasal yöntem ile ve glikasyon olmuş H1'e İGS'ye karşı otoantikorlar araştırıldı.

Bulgular: Glikasyonlu-H1, doğal H1'in tersine bağışıklığı çok iyi uyarır bulundu. Dibetli deneklerden alınan serumların %48'inde (150 örneğin 72'sinde) glikasyonlu-H1'e karşı antikor bulunur iken, İGS'ye karşı %40 (150 örneğin 40'ında), pentosisidine karşı ise %36 hayvanda antikor saptandı.

Sonuçlar: Bulgulara göre, değiştirilmiş H1 ve İGS doğal olarak bağışıklığı oldukça kuvvetli bir şekilde uyarmaktadır. Glikasyonlu-H1 ve İGS'ye karşı oluşan otoantikorlar, diabet ya da komplikasyonlarının saptanmasında kullanılabilir.

Anahtar Sözcükler: Bağışıklanabilirlik, 3-deoksiglukozon, ileri glikasyon son-ürünleri, diabet

[1]Department of MLT, Applied Medical Sciences, Jazan university, Jazan, Saudi Arabia
[1]Medical Research Centre, Jazan University, Jizan, Saudi Arabia
[2]Integral University, Biosciences, Lucknow, India

Correspondence:
Jalaluddin M. ASHRAF
Department of MLT, Applied Medical Sciences, Jazan university, Jazan, Saudi Arabia

E-mail: jmashraf@gmail.com

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Introduction

A nonenzymatic chemical interaction involving reducing sugar and biomolecules containing amino group is known as Classical Maillard reaction.[1] The reaction starts with Schiff base formation and/or Amadori products that further undergo Advanced glycation end-product(AGE)s formation.[2] AGEs cause structural perturbation of proteins, affecting its functionality[3] and contribute to the development of diabetes complications.[4,5]

Previously reducing sugars were assumed as the key factors of AGEs formation but latter non-sugar molecules were also documented as the producer of AGEs. In consistent hyperglycaemia, endogenous glycating intermediates α-oxoaldehydes (3-deoxyglucosone (3DG), methylglyoxal and glyoxal) are increased manifold leading to high accumulation of AGEs in the body.[6,7] Apoptosis or necrosis or cell growth arrest has been detected due to the inactivation of some of the significant cellular proteins by the AGEs resulting from the glycation of 3-DG.[8,9]

Elastin collagen and lens crystalline are chiefly vulnerable to glycation because of its long life.[10] Nuclear histone proteins (H1, H2A, H2B, H3, and H4), indispensable parts of eukaryotic chromatin structure[11], have a long life that makes them prone to glycation.[12–14] Many studies have proved the glycation of histone proteins by reducing sugars and α-oxoaldehydes in vitro and in vivo.[15,16] Particularly, histone H1 is more glycation prone due to location on the periphery of nucleosomes[13,12,17], disturbing the structure and function of chromatin[18] that may compromise the integrity of DNA.

The disproportionate build-up of AGEs has been found to trigger cellular immune responses as well as humoral immunity, as the high-level antiAGEs antibodies have been reported in aging and pathological conditions.[19] Glycated histone H2A[20], HSA[21], IgG[22], have been reported highly immunogenic in animals than the native analogs. Further, glycated HSA from the sera of diabetic patients were found to be more immunogenic[22–24] than to the unmodified molecules. Likewise, Glycated rat skin collagen triggered the generation of anticollagen antibodies, which did not crossreact with native collagen. The antibodies were highly specific as 92% inhibition was observed against glycated lysine as an opponent. Finding suggests that glycated lysins on collagen are the utmost likely epitopes for anticollagen antibodies binding.[25]

The antigenic nature of AGEs established the theory of incessant accumulation of AGEs that can incite an autoimmune response in a diabetic body.[22,23,26] The previous finding has revealed AGEs and its immune complexes occurrence in numerous tissues including kidney of diabetes nephropathic subjects.[27] Furthermore, antigenic glycated-polylsine and autoantibodies were reported in animals and diabetic subjects, respectively.[28] Additionally, autoantibodies opposed to methylglyoxal glycated histones have been reported in type 1 diabetes subjects.[29] The H1 or "linker" histone proteins have a high content of lysine residues, signifying the status of autoantibodies against lysine polypeptide in diabetes subjects. Autoantibodies against AGEs may indicate the progression of complications. The presence of substantial amounts of AGEs and anti-AGE autoantibodies in diabetes reflects the role of glycation reaction in disease.

In the current work, the histone H1 protein was glycated by 3-DG. The degree of glycation, production of intermediates and AGEs (CML and pentosidine), and structure of glycated-H1 were analysed by physicochemical techniques.[12] Moreover, immunogenicity was checked by inducing antibodies in animals. The existence of autoantibodies in the sera of type 2 diabetes subjects as opposed to 3-DG glycated-H1 and AGEs (CML and pentosidine) was also investigated.

Materials and Methods

Random reared female New Zealand white rabbits with native and 3-DG-glycated-H1 histone were immunized as described before.[24] Rabbits (n=4; two each for native and glycated-H1) were injected with 100 μg of antigen emulsified with an equal volume of Freund's complete adjuvant intramuscularly at many sites. The booster dose of the same amount of antigen in Freund's incomplete adjuvant was injected into animals each week for 6 weeks. At every alternate booster dose antibody titer was estimated. Test bleeds executed 7 days post-boost substantial antibody titer was detected. For the inactivation of complement proteins, the animal's serum (preimmune and immune) was heated at 56°C for 30 min and stored at -20°C with 0.1% sodium azide.

Materials: Anti-rabbit IgG, anti-human IgG, 9.10-phenanthrenequinone, 2.4-dinitrophenyl hydrazine, *p*-nitro-phenyl phosphate, alkaline phosphatase conjugates, sodium dodecyl sulfate (SDS), Tween-20, Protein A-agarose (2.5 mL pre-pack column) and dialysis tubing, were from Sigma Chemical Company (USA). ELISA plates (96 wells) were from NUNC (Denmark). Triton X-100 was from Hi-Media (India). 3-DG-glycated-H1 was from our earlier study.[12]

Ethical Approval: Experiments on animals were carried out according to the local Ethical Committee laws and regulations. Animal ethical no: IU/IAEC/18/17, Date of approval: 28.02.2018.

Sera acquirement: Fasting blood samples of type 2 diabetes subjects were collected from IIMS&R, Integral University. The withdrawal of blood samples was performed after obtaining written consent from all subjects. All measures were in agreement with the Helsinki Declaration of 1975 as revised in 2008. All diabetic subjects were scanned for secondary complications (retinopathy, nephropathy, angiopathy, and neuropathy) and were under anti-diabetic treatment. Sera of the same sex and age of healthy subjects assigned as control. Blood samples were collected and allowed to coagulate for 30 min at temperature 37°C and were isolated by centrifuge method at 2000 *g* for 10 min Sera were heated at 56°C for 30 min and stored at –20°C with 0.1% sodium azide for the complement proteins inactivation. Table 1 contain the the clinical information about the subjects.

Consent Statement: The participants' rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration. The procedure was sanctioned by the Institutional Ethics Committee of IIMS&R. The clinical Institutional ethical number is-IEC, IIMS&R/2019/51, date of approval: 30.01.2019.

Table 1. Important clinical characteristics of normal and type II diabetic subjects

Variables	Healthy human subjects	Type II diabetic subjects
Sample size (n)	50	150
Disease duration (years)		13±5
Age (years)	matched	64±8
Sex (male: female)	30:20	90:60
HbA1 c (%)	4.7±0.1	8.2±0.6
BMI	22.1±3.9	29.2±4.8
Blood glucose (mg/dL) Fasting	89.0±6.8	210.0±21.3

Protein A-agarose Affinity Chromatography

Affinity chromatography (Protein A-agarose column) was employed purify the IgG from rabbit sera**,** the manufacturer's instruction and published literature were followed.[24] Briefly, 0.3 mL serum was diluted with an equal volume of PBS and employed on the column pre-equilibrated with the same buffer. The wash-through was recycled 2–3 times, and the detached substance was got rid of by thorough washing with PBS. The adhered IgG was rinsed with 0.58% acetic acid in 0.85% sodium chloride and accumulated in a tube having 1 mL of 1 M Tris-HCl (pH 8.5). 3 mL fractions were gathered, and absorbance was recorded at 278 nm. The separated IgG was dialyzed against PBS and put in at -20°C with 0.1% sodium azide.

Direct Binding Enzyme Linked Immunosorbent Assay (ELISA)

Direct binding ELISA was done on flat-bottom polystyrene plates as described previously.[24] Hundred micro liters of native or glycated-H1 or standard CML/pentosidine (10 µg/mL) in antigen coating buffer was coated in 96 wells Polystyrene PolySorp immune plates. After coating the antigens, the plates were incubated for 2 hours at 37°C and so kept overnight at 4°C. Each sample was analyzed in duplicate, and half of the plate was coated with antigens that served as control. The test plate was washed three times by washing buffer (TBS-T (20 mM Tris, 2.68 mM KCl, 150 mM NaCl, pH 7.4, containing 0.05% Tween-20), 150 µL of 2.5% fat-free skimmed milk in TBS (10 mM Tris, 150 mM NaCl, pH 7.4) was used to block unoccupied sites and incubated for 4–6 hours at 37°C. The plates were washed 5–6 times by TBS-T after incubation. Add serially diluted test serum in TBS-T in wells (100 µL/well) and incubated for 2 hours at 37°C and then kept overnight at 4°C. After that, the plates were washed three times with TBS-T, and then with coated anti-rabbit alkaline phosphatase conjugate in TBS and incubated for 2 hours. After 2 hours, again the plates were washed three times with TBS-T and twice with distilled water and then coated with *p*-nitrophenyl phosphate (substrate for alkaline phosphatase). An automatic microplate reader was used to monitor the absorbance (A) in each well at 410 nm. Results are calculated as a mean of (Atest – Acontrol).

All steps were the same for the detection of autoantibodies against glycated-H1 or N-carboxymethyl-lysine (CML) pentosidine in sera of diabetic patients apart from rabbit sera (1:100 dilution in TBS buffer).

Competition ELISA

The Competition ELISA was used to determine the antigenic specificity of the elicited antibodies in inoculated rabbits' sera and autoantibodies in diabetes subjects' sera.[24] Hundred microliter antigen (native or glycated-H1, 10 µg/mL) was coated on ELISA plates and incubated for 2 hours at 37°C later kept overnight at 4°C. A constant amount of affinity-purified IgG (40 µg/mL) was mixed with different amounts of inhibitors (0–20 µg/mL) and kept for 2 hours at room temperature and so overnight at

4°C. Subsequently, the procedure of direct binding ELISA is adopted. Percent inhibition was computed applying the formula:

$$\text{Inhibition (\%)} = (1 - A_{inhibited} / A_{uninhibited}) \times 100$$

Statistical Analysis

The data collected are demonstrated as mean ± standard deviation (SD). However, the Student's t-test, with probability <0.05 was performed for the statistical significance of data.

Results

Immunogenicity of native and 3-DG-glycated H1 histone

In the present study, the immunogenicity of 3-DG-glycated-H1 was estimated by inducing antibodies in the rabbits through direct binding ELISA. Animals were immunized with native and glycated-H1 and then sera of the animals were essayed on PolySorp wells coated with corresponding immunogens. The glycated-H1 was significantly strong immunogen, whereas native H1 was nonimmunogenic as no immune response was observed. 3-DG-glycated-H1 induced high titer antibodies (>1: 12,800), whereas native H1 showed negligible titer under identical experimental conditions. Direct binding ELISA also expressed high antibody titers (1 : 12,800) against standard CML and pentosidine (Figure 1). Preimmunized serum (negative control) exhibited negligible binding with corresponding immunogens. Furthermore, standard CML and pentosidine were also found to be highly immunogenic as high antibody titer was observed against it (p<0.001).

Characterization of anti-3-DG-glycated-H1 IgG antibodies IgG

Affinity chromatography (Protein A-agarose column) and SDS-PAGE (10% polyacrylamide gel) were applied to purify IgG from preimmune and immune rabbit antiserum and antibodies purity, respectively. Single band was detected in SDS-PAGE that confirms the purity of separated antibodies (Figure 2). The purified anti-glycated-H1 antibodies demonstrated stronger binding with its immunogen along with CML and pentosidine compared to that of native H1 (Figure 2).

Purified anti-glycated-H1 antibodies specificity was evaluated by competitive inhibition assay (p<0.001). In this assay, anti-glycated-H1 antibodies showed a maximum of 84.5% inhibition against glycated-H1 used as immunogen as an inhibitor at 20 µg/ml. The 2.1 µg/ml of glycated-H1 was needed to achieve 50% of inhibition. A maximum of 72.8 and 61.3 % inhibition were detected when 20 µg/ml CML and pentosidine was utilized as an inhibitor, respectively (Figure 3). The concentrations of

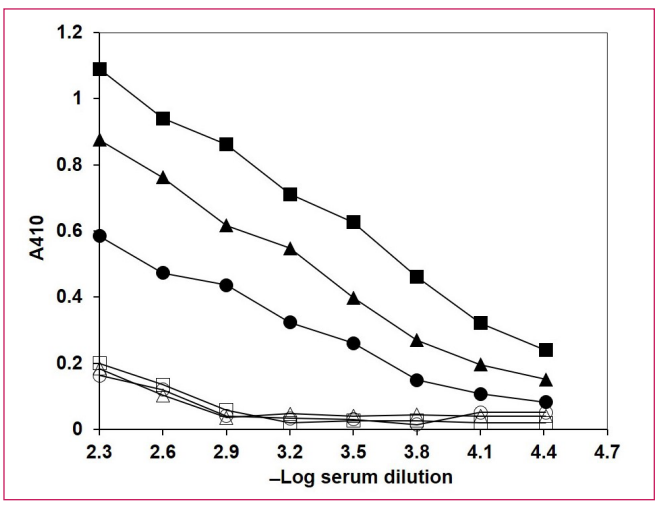

Figure 1. Level of antibodies against glycated H1, standard CML, and pentosidine antigens. Direct binding ELISA of glycated H1, CML, and pentosidine with preimmune (open square) and immune (closed square), preimmune (open triangle) and immune (closed triangle), and preimmune (open circle) and immune (closed circle) sera, respectively. The microtiter plates were coated with the respective antigens (10 µg/mL) (p<0.001).

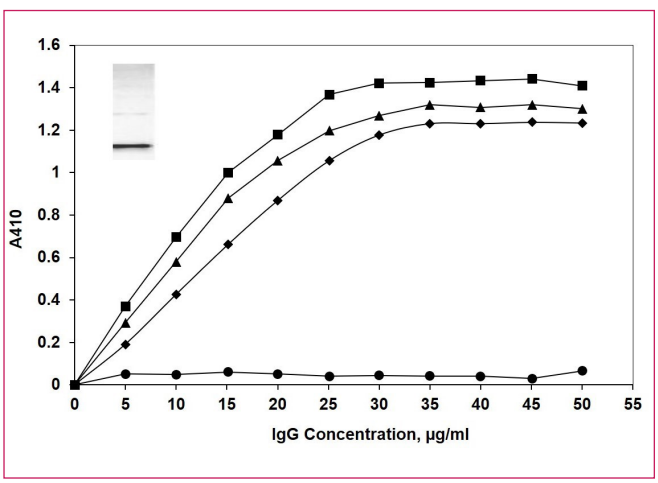

Figure 2. Direct-binding ELISA of affinity purified IgG (anti-glycated-H1 antibodies) with native H1 (closed circle), glycated H1 (closed square), CML (closed triangle), and pentosidine (closed rhombus). Microtiter plates were coated with the above-mentioned immunogens (100 µg/mL), and then increasing concentrations of purified IgG were applied (for details see "Materials and Methods") (p<0.001).

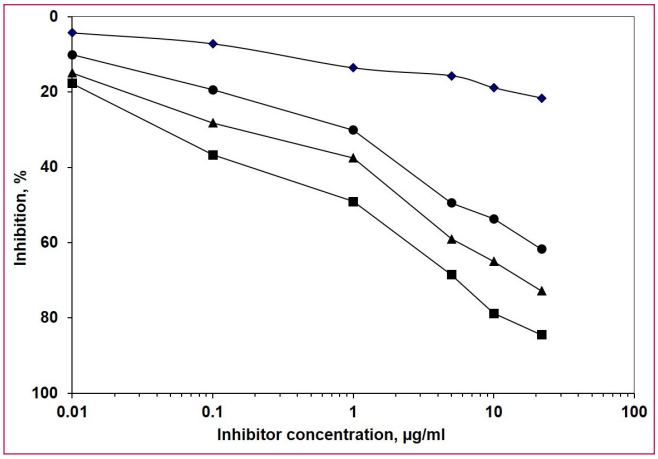

Figure 3. Characterization of antigenic specificity of the purified Anti-glycated-H1 antibodies by competitive inhibition assay. Inhibition of anti-glycated-H1 antibody binding by glycated H1 (closed square), standard CML (closed triangle), and pentosidine (closed circle) as well as inhibition of binding of preimmune antibodies (closed rhombus) by all three inhibitors (depicted as average of glycated H1, standard CML, and pentosidine) was determined. The microtiter plate was coated with glycated H1 (10 µg/mL) ($p<0.001$).

CML and pentosidine needed to achieve 50% inhibition were 3.9 and 4.4 µg/ml, respectively. Antibodies exhibited insignificant inhibition with glycated-H1, CML, and pentosidine used as inhibitors ($p<0.001$).

Use of anti-3-DG-glycated-H1 IgG antibodies to study epitope sharing by other glycated proteins or amino acids

Native H1, as inhibitor showed negligible inhibition against anti-glycated-H1 antibodies. The antibodies also identify the basic structure of H1 through modified epitopes or specificity. Table 2 represents the binding of anti-glycated-H1 IgG antibodies with a range of glycated proteins (or amino acids) and corresponding native equivalents. Findings show that glycated inhibitors are favorably bound by the experimentally induced antibodies against glycated-H1 compared to their corresponding native equivalents. In addition of other factors, the contribution of lysine and arginine in the generation of immunogenicity in glycated-H1 is apparent from the capacity of glycated-lysine and glycated-arginine in inhibiting the antibody binding to the degree of 64.3 and 53.7% (at a concentration of 20 µg/ml), respectively ($p<0.001$). Additionally, antibodies also exhibited cross-reactivity with CML, pentosidine, and other glycated-proteins or glycated-amino acids, a unique characteristic of polyspecific antibodies.

Table 2. Antigen binding specificity of anti-glycated-H1-IgG antibodies

Inhibitors	Maximum % inhibition at 20 µg/mL	Concentration for 50% inhibition (µg/mL)	% relative affinity
Native H1	21.6	-	-
Glycated-H	84.5	2.1	100
Pentosidine	61.3	4.4	56.9
CML	72.8	3.9	80.3
CEL	52.3	8.8	38.1
Arginine	31.4	-	-
Glycated arginine	48.6	-	-
Lysine	28.8	-	-
Glycated lysine	65.3	4.1	60.1
Phenylalanine	24.1		
Glycated phenylalanine	28.2	-	-
Tyrosine	20.3	-	-
Glycated tyrosine	40.4	-	-
Tryptophan	19.6		
Glycated tryptophan	34.3		
Native hemoglobin	21.7	-	-
Glycated hemoglobin	37.9	-	-
Native IgG	23.6	-	-
AGE-IgG	50.5	10.1	32.9
Native HSA	22.7	-	-
Glycated HSA	35.9	-	-

Antigenic specificity of the induced anti-glycated-H2A-IgG antibodies was investigated by competitive inhibition ELISA. Microtitre plates were coated with glycated-H1 (20 µg/mL). The induced antibodies exhibited wide range of heterogeneity in recognizing varied inhibitors. The notable feature of experimentally produced antibodies against glycated-H1 was the preferential recognition of glycated proteins or amino acids as compared to their native conformers.

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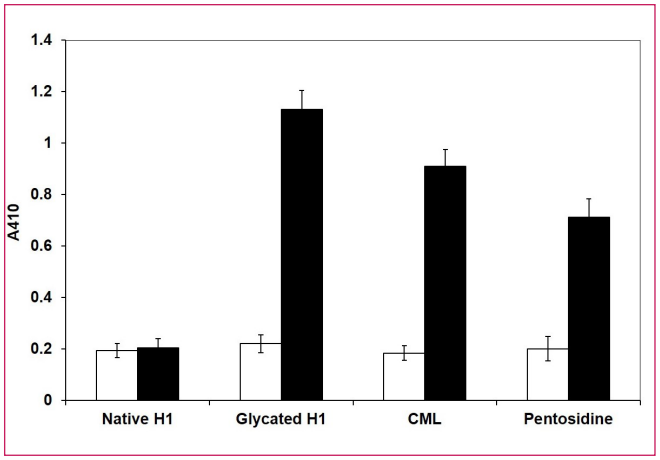

Figure 4. Direct-binding ELISA of native H1, glycated H1, standard CML, and pentosidine with autoantibodies of normal human (open square) and diabetic (closed square) sera. Normal human serum served as control (p<0.001).

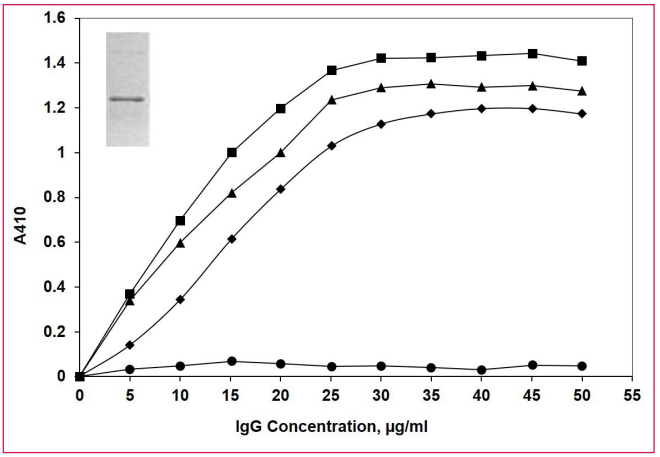

Figure 5. Direct-binding ELISA of affinity purified IgG from diabetic sera with native H1 (closed circle), glycated H1 (closed square), CML (closed triangle), and pentosidine (closed rhombus). Microtiter plates were coated with respective immunogens (100 µg/mL) and then increasing concentrations of purified IgG were used and the direct-binding ELISA procedure was followed as described in "Materials and Methods" (p<0.001).

Direct binding ELISA of diabetes sera with native H1, 3-DG-glycated, CML and pentosidine

In this part of the study, autoantibodies in diabetes sera opposed to native H1, glycated-H1, CML, and pentosidine was assayed by direct binding ELISA. The result shows that glycated-H1 exhibited statistically significantly higher (p<0.001) absorbance (1.131 ± 0.074) with diabetic sera as compared to that of healthy subjects (0.205±0.035). In case of CML and pentosidine, they also exhibited substantially higher (p<0.001) absorbance of 0.910±0.065 and 0.721±0.069, respectively as compared to that of healthy subjects (0.196±0.029 and 0.221±0.048). Native H1 showed negligible binding with healthy and diabetic subjects. The results are represented in Figure 4. diabetic sera showed 48% (72 of 150 samples) significantly higher binding with glycated-H1, further diabetic sera also showed 40% (60 of 150 samples), and 36% (54 of 150 samples) higher binding with CML, and pentosidine, respectively. Autoantibodies from normal sera showed similar binding with coated antigens.

Inhibition ELISA of purified diabetes IgG with native H1, 3-DG-glycated, CML and pentosidine

The purified IgG moved as a single band indicating IgG is highly pure (Figure 5). Moreover, purified IgG from diabetic sera displayed robust binding with all antigens (glycated-H1, CML, pentosidine) (Figure 5). The specificity of purified IgG with respective antigens was evaluated by inhibition ELISA (Figure 6). The mean inhibition of 70.76±4.84, 56.31±5.21, and 41.55±4.15%, in the binding of IgG of diabetic sera with glycated-H1,

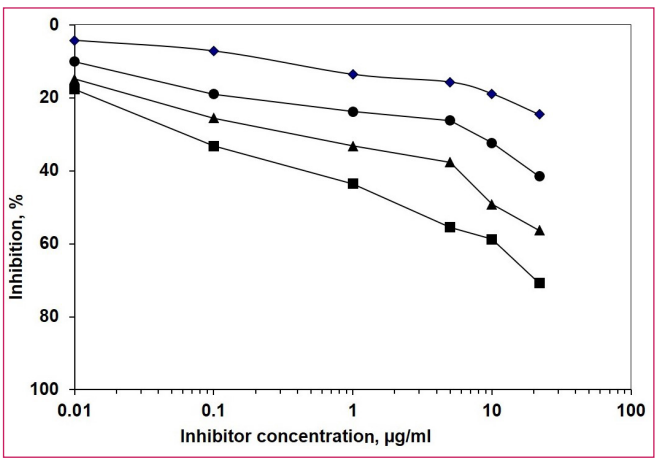

Figure 6. Characterization of antigenic specificity of the purified anti-glycated-H1 antibodies by competitive inhibition assay. Inhibition of anti-glycated-H1 antibody binding by glycated-H1 (closed square), standard CML (closed triangle), and pentosidine (closed circle) as well as inhibition of binding of normal human antibodies (closed rhombus) by all three inhibitors (depicted as average of glycated H1, standard CML, and pentosidine) was determined. The microtiter plate was coated with glycated H1 (10 µg/mL) (p<0.001).

CML, and pentosidine were seen, respectively (p<0.001). While native H1 exhibited insignificant inhibition of binding with the IgG (21.63±2.95%). All of these were statistically significant at p<0.001.

Discussion

Glycation reaction causes a structural perturbation in proteins that eventually affect the protein functionality. [12,30,31] It has been shown that structural perturbation of

protein by modifications is responsible for the change of protein immunogenicity.[32] It is well documented that autoantibodies against numerous proteins AGE structures have been detected in diabetic patients' sera that can trigger autoimmune response.[26, 27] Several studies have proved that these antibodies are accountable for the instigation and progression of many pathological disorders such as diabetic nephropathy and chronic renal failure.[29, 33]

In the present study, detection of high titer antibody against glycated-H1 is due to the formation of neoepitopes upon glycation by 3-DG that makes H1 highly immunogenic in nature. Moreover, the detection of antibodies opposed to standard CML and pentosidine confirm the CML and pentosidine formation in the previous study of glycation by 3-DG.[12] Glycation reaction causes structural changes in proteins.[12, 34, 35] that may promote the transition of native epitopes of H1 into powerful immunogenic neo-epitopes, indicating a straight relationship between glycation and immunogenicity.[12]

The specificity assay evidently confirms induced antibodies (anti-glycated-H1 antibodies) are extremely specific for the glycated-H1, CML, and pentosidine epitopes and points out antibodies are immunogen specific. Induced reaction with antibodies also exhibited cross-reactivity with CML and pentosidine, a typical characteristic of the polyspecific nature of antibodies. Therefore, the characteristic property of antibodies opposed to glycated-H1 was the favorable identification of CML and pentosidine. Our results are in agreement with earlier studies in which CML and pentosidine were detected as major antigens in the immune system serving as an immunological epitope.[36,37]

A notable feature of the induced antibodies opposed to glycated-H1 was the favorable identification of glycated-proteins or amino acids compared to their native equivalents. This characteristics supports that glycated-epitopes of numerous proteins and amino acids share common antigenic properties. Thus, glycated-lysine and glycated-arginine are preferred substrates for anti-glycated-H1 antibodies, which signify the role of lysine and arginine in neoepitopes generation on H1 protein upon glycation. Furthermore, antibodies also exhibited robust binding affinity towards CML and pentosidine, indicating CML and pentosidine are extremely immunogenic in nature. Hence, the immunological outcome establishes that glycation of H1 by 3-DG contributes to the structural modification besides the production of AGEs which are accountable for the neoepitopes production on H1, revealing it extremely immunogenic. The induced antibodies were found to be exceedingly specific as well as polyspecific in nature. The polyspecificity of antibodies may contribute to the aetiology of several autoimmune diseases. AGE-H1 might be one of the critical sources in the antigen-driven generation of autoantibodies, that might be considered as an indicator in the early diagnosis/prognosis of diabetic complications.

It has been reported in several studies that a high level of AGEs in the serum of diabetic subjects are considered as a pathological provocation for induction of anti-AGE antibodies which may be a causative factor in diabetic complications development.[38] Our study is in agreement with to earlier findings that were reported as the occurrence of anti-AGE antibodies in diabetic subjects accompanying various diabetic complications.[39, 40]

On the basis of previous findings, it has been proved that anti-AGE autoantibodies are generated in diabetes and its complications. But it has to be recognized the character of antibodies as well as which of the specific anti-AGE antibodies are applicable on the etiology of diabetic complications? Advancement in determining anti-AGE antibodies by reliable approaches would assist in establishing the role in the pathogenesis of many diseases, particularly diabetes and its complications. The specificity and binding pattern of the anti-AGE antibody indicates its contribution to diabetic complications. Consequently, it recommends anti-AGE antibodies may be utilized as biomarkers for the diagnosis and prognosis of diabetes and its complications.

Ethics Committee Approval: Experiments on animals were carried out according to the local Ethical Committee laws and regulations (Animal ethical no: IU/IAEC/18/17, Date of approval: 28.02.2018).

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare that there is no conflict of interests in the publication of this article.

Contribution of Authors: Concept: JMA; Design: JMA, SR; Data Collection or Processing: SR; Analysis or Interpretation: JMA, SR; Literature Search: SR; Writing: SR; Critical Review: JMA.

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