Family Medicine and Primary Care: Open Access
Sahu S, et al. J Family Med Prim Care Open Acc 6: 199.
www.doi.org/10.29011/2688-7460.100098
www.gavinpublishers.com
Research Article

Effect of Glycation on Urine Albumin Estimation by Immunoassay: An *in-vitro* Experiment

Suchanda Sahu[1*], Gopalaxmi Panda[2], Cecilia Xavier Jyothi[3], Lohithaksh Aavula[4]

[1]Additional Professor, Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

[2]Assistant Professor, Department of Biochemistry, Post Graduate Institute of Medical Education and Research, Capital Hospital, Bhubaneswar, Odisha, India

[3]Senior Resident, Department of Biochemistry, Lalithambigai Medical College and Hospital, Adalayamputtu, Chennai, India

[4]MBBS Student, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

[*]**Corresponding author:** Suchanda Sahu, Additional Professor, Department of Biochemistry, All India Institute of Medical Sciences, PO Dumduma, Bhubaneswar- 751019, Odisha, India

Citation: Sahu S, Panda G, Jyothi CX, Aavula L (2022) Effect of Glycation on Urine Albumin Estimation by Immunoassay: An *in-vitro* Experiment. J Family Med Prim Care Open Acc 6: 199. DOI: 10.29011/2688-7460.100099

Received Date: 24 August, 2022; **Accepted Date:** 05 September, 2022; **Published Date:** 09 September, 2022

Abstract

Background: This *in vitro* experiment was conducted to estimate the albumin by immunoassay method with and without incubation with high glucose and fructose to simulate the hyperglycemic condition as in diabetics. **Methods:** The pooled sera from 5 healthy (non-diabetic) individuals were incubated with fructose and glucose at concentrations of 250, 400, 500, and 600 mg%. Each set was again divided into aliquots for incubation at 4°, 25°, and 37° C for 1, 7, and 21 days. **Results:** There was a significant lowering of observed values when compared to the expected value ($p<0.001$) with a decreasing trend as the hexose concentrations were increased. At 250 and 400 mg% of fructose and glucose, the albumin levels were similar. However, at 500 and 600 mg%, the sera incubated with fructose had a lower level of albumin as compared to glucose. The ANOVA comparing the duration and the temperature of incubation was not statistically different within the groups. **Conclusion:** The extensive glycation at high levels of hexoses markedly decreases the albumin detected by the immunoturbidimetry assay. Hence, we suggest the development of polyclonal antibodies for glycated albumin rather than pure serum albumin for use in assay kits to be used for the detection of diabetic nephropathy.

Keywords: Glycated albumin; Immunoturbidimetry; Interference; Accuracy

Introduction

Diabetic Nephropathy (DN) is a grave complication of the type 1 and type 2 diabetes mellitus (DM). About 25% of the people with DM develop DN defined by the onset of persistent microalbuminuria, which when not detected and treated will lead to Chronic Kidney Disease (CKD) [1]. In elderly, the diabetic nephropathy today accounts for 46% of the chronic renal disease [2]. Although hyper filtration may persist, some patients experience a progressive decline in glomerular filtration rate (GFR). Incipient diabetic nephropathy usually develops after 10-15 years of type1 diabetes, but may already be present when type 2 Diabetes is diagnosed [1].

It has been consistently documented that in diabetic patients who do not have overt proteinuria (>300 mg/day), the presence of albumin in the urine is a powerful predictor of nephropathy, cardiovascular events, of progressive loss of GFR and of mortality. In the early days, the sensitivity of urine albumin tests was limited. Patients who did not have overt proteinuria, but who had albumin concentrations beyond the then-available detection threshold for albumin (approximately 30 mg/day), were given the diagnosis of "microalbuminuria" (30-300 mg/day). As urine albumin concentrations <30 mg/day predict renal and cardiovascular outcomes it is postulated to abandon the concept of "microalbuminuria" and to treat urinary albumin concentrations as a continuous variable [3].

Urinary albumin concentration is currently the most reliable predictor of diabetic nephropathy [1]. It is specifically measured

with nephelometric, turbidimetric, radioimmunoassay and enzyme immunoassay techniques, which is complicated because urinary albumin exists in many forms, most of which are not detected by immunoassays used in clinical laboratories [4,5]. The nature of this albumin which is immunochemically non-reactive is attributed to the fact that the epitope is altered by conformation change as a result of incomplete processing by the lysosomal pathway which is compromised in diabetes but not in healthy individuals [6,7], or to the attachment of ligands such as glucose or fatty acids, which are increased in diabetes. Because of these inconsistent modifications in urine albumin and the fact that antibodies in immunoassays for urine albumin are from serum albumin, there is a definite underestimation of urine albumin measurement [8]. From this it can be hypothesized that the epitopes of the albumin are blocked by the glucose, which are used in the immunoassay.

The present study is so planned as an *in vitro* experiment to estimate the albumin by immunoassay method with and without incubation with high glucose and fructose to simulate the hyperglycaemic condition as in diabetics.

Methods

This was an *in vitro* observational study performed as a controlled experimental trial after obtaining IEC approval. The pooled sera from 5 healthy (non-diabetic) individuals were divided into 8 parts and incubated with fructose and glucose at concentrations of 250, 400, 500 and 600 mg%. Each set was again divided into 6 aliquots for incubation at 4°, 25° and 37°C for 1, 7 and 21 days, considering the half-life of serum albumin.

This was done to assess both the short term and long-term effect of hyperglycemia. The pooled sera albumin was estimated by Bromocresol green (BCG) method. The sugar solutions of different concentrations were prepared in normal saline so that with 50% v/v dilution, the desired concentration was achieved. The albumin in the incubated samples were estimated by immunoturbidimetry method. All assays were run in triplicates in AU480 analyzer, Beckman Coulter, Brea, USA.

Statistical analysis

Mean values (from the triplicate run) of albumin were considered for statistical analysis. At the same concentrations of fructose and glucose, the albumin levels of both sets were compared by paired T test. The comparison between duration and temperature of incubation was done by ANOVA.

Results

The pooled sera had albumin levels of 3.6 gm%. This when incubated with equal volumes of sugar solutions, had albumin levels around 1.8 gm% as estimated by BCG method. Our expected albumin levels in the incubated samples were 1800 mg%. Table 1 shows the mean and standard deviation (SD) of the albumin estimated after *in vitro* glycation. There was a significant lowering of observed values when compared to expected value ($p<0.001$) with a decreasing trend as the hexose concentrations were increased (Figure 1). The mean values of albumin at various temperature and duration of incubation are shown in Table 2. At 250 and 400 mg% of fructose and glucose the albumin levels were similar. However, at 500 and 600 mg% the sera incubated with fructose had a lower level of albumin as compared to glucose. The ANOVA comparing the duration and the temperature of incubation were not statistically different within the groups (Table not shown in this paper).

Fructose and Glucose incubation Concentration (mg %)	Mean ± SD of albumin estimated in mg%	p[a]
250	1555 ± 169	<0.001
400	1237 ± 146	<0.001
500	913 ± 89	<0.001
600	727 ± 91	<0.001
[a]p value for One way T-Test done with expected albumin of 1800 mg%		

Table 1: Mean values of Albumin estimated after incubation with Fructose and Glucose.

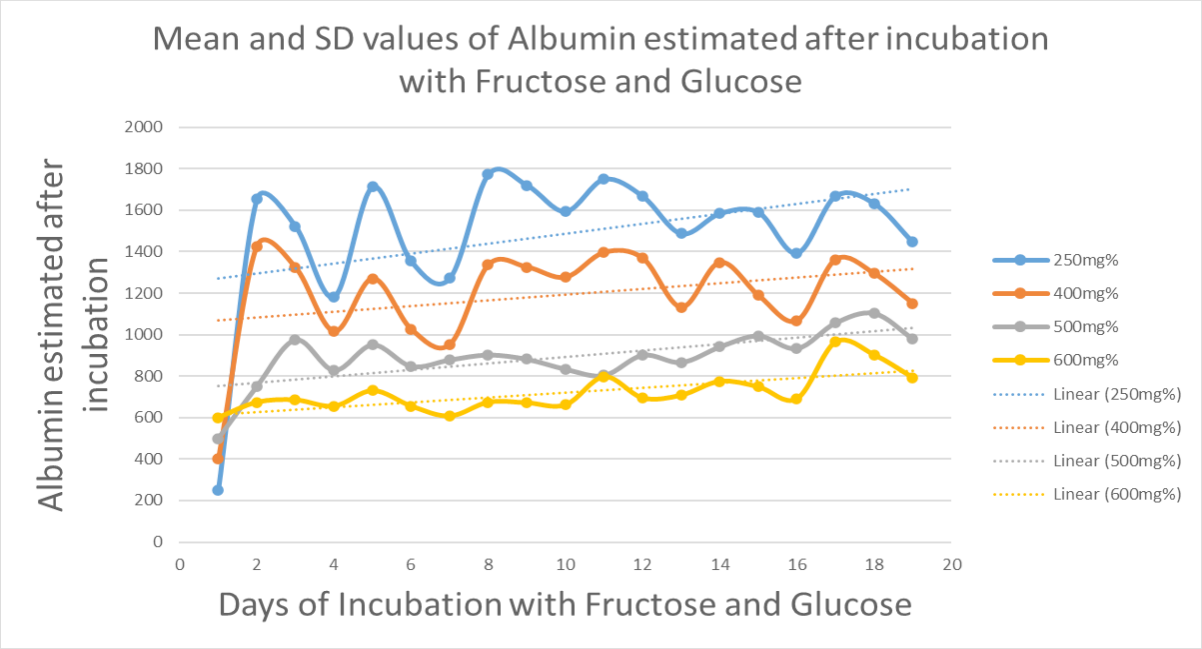

Figure 1: Albumin estimated after incubation with Fructose and Glucose.

Concentration (mg %) →				250		400		500		600	
				Fructose	Glucose	Fructose	Glucose	Fructose	Glucose	Fructose	Glucose
Temperature (°C) ↓	4	Duration of incubation(days) ↓	1	1653	1749	1426	1396	752	804	674	797
			7	1522	1667	1323	1368	976	902	685	694
			21	1183	1487	1016	1132	829	867	655	708
	25		1	1712	1584	1270	1348	952	942	730	775
			7	1354	1590	1026	1189	848	995	655	748
			21	1272	1393	953	1067	879	934	607	691
	37		1	1772	1668	1336	1362	903	1058	674	965
			7	1720	1632	1325	1294	882	1104	671	903
			21	1594	1446	1276	1151	834	981	661	793
			P[a]	0.417		0.225		0.031*		0.005*	

[a]p value for paired T test.

Table 2: Comparison of Albumin incubated at different concentrations of Fructose and Glucose for different days and temperature.

Discussion

From our experiment, it was suggestive that there is a marked decrease in albumin estimated by immunoassay after *in vitro* glycation with high concentrations of fructose and glucose. This can be explained by two reasons. One being the likelihood of increased glycation at lower concentrations of albumin [9]. In our experiment the serum albumin was about 1.8 gm% which was much below the normal serum levels. The extensive glycation causes change in conformation [10] of albumin which may alter epitope sites and decrease antibody binding even when polyclonal antibodies are used. Secondly, the natural milieu in serum contains antioxidants in the form of vitamins and minerals which diminish the rate of glycation [11]. No added antioxidants were present in the sugar solutions prepared

in our study. With poor diabetic control, glycation of albumin can be >90% [12] and the same albumin excreted in urine will be underestimated by immunoassays as the immunogenicity of the modified albumin is altered [13]. *In vivo*, in the hyperglycemic state in diabetics, apart from albumin, other membrane proteins and circulatory proteins are also glycated and later oxidized. These can lead to altered breakdown and excretion of albumin in urine [14]. Prolonged half-life of albumin in circulation may increase scope for its glycation. Though the diabetic kidney disease classification recommends albuminuria and eGFR measurement, it is not faultless [3]. The urine albumin method is derived with polyclonal antibodies of serum albumin, the reference methods are not standardized, though the reference material for calibration and standardization are validated [15]. The variability in commercially available kits for urine albumin can be as large as 40% [16] and that can lead to delayed diagnosis and misclassification of patients with DN [17]. There is a search for newer biomarkers for diabetic nephropathy which will help detect the disease in its early stages before the renal function deteriorates. Therefore, we recommend use of glycated albumin for generation of polyclonal antibodies for preparation of kits for detection of urine albumin though it may not be useful for other causes of albuminuria like hypertension or CKD.

Conclusion

The extensive glycation at high levels (≥400 mg%) of fructose and glucose markedly decreases the albumin detected by immunoturbidimetry assay. Hence, we suggest development of polyclonal antibodies for glycated albumin rather than pure serum albumin for use in assay kits to be used for detection of DN.

Author Contributions

Conceptualization: SS, GP, CXJ, LA: Methodology; SS, GP, CXJ, LA: Software; SS: Validation; SS, GP, CXJ, LA: Formal analysis; SS, GP, CXJ, LA: Investigation; SS, CXJ: Resources; SS: Data curation; SS, GP, CXJ, LA: Writing-Original Draft; SS: Writing-Review & Editing; SS, GP, CXJ, LA: Visualization; SS, GP, CXJ, LA: Supervision; SS, CXJ: Project administration; SS, GP, CXJ, LA: Funding acquisition.

Ethical Statement

This research was reviewed and approved by the Institutional Ethics Committee of All India Institute of Medical Sciences, Bhubaneswar, India had given approval for this study. It was registered as I/IM-NF/Biochem/20/128 dated 24.09.2020. Informed consent was obtained from all participants.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

Institutional Ethics Committee of All India Institute of Medical Sciences, Bhubaneswar, India had given approval for this study. It was registered as I/IM-NF/Biochem/20/128 dated 24.09.2020.

Consent to Participate: Written consent was obtained from the healthy volunteers.

References

1. Van JAD, Scholey JW, Konvalinka A (2017) Insights into Diabetic Kidney Disease Using Urinary Proteomics and Bioinformatics. J Am Soc Nephrol 28: 1050-1061.
2. Ritz E, Zeng X (2011) Diabetic nephropathy - Epidemiology in Asia and the current state of treatment. Indian J Nephrol 21: 75-84.
3. Persson F, Rossing P (2018) Diagnosis of diabetic kidney disease: state of the art and future perspective. Kidney Int Suppl 8: 2-7.
4. Greive K, Eppel G, Reeve S, Smith I, Jerums G, et al. (2001) Immuno –unreactive albumin excretion increases in streptozotoin diabetic rats. Am J Kidney Dis 38: 144-152.
5. Osicka T, Comper W (2004) Characterization of immunochemically nonreactive urinary albumin. Clin Chem 50: 2286-2291.
6. Burne M, Panagiotopoulus S, Jerums G, Comper W (1998) Alterations in the renal degradation of albumin in early experimental diabetes: a new factor in the mechanism of albuminuria. Clin Sci 95: 67-72.
7. Osicka T, Honlihas C, Chan J, Jerums G, Comper W (2000) Albuminuria in patients with type 1 diabetes is directly linked to changes in the lysosme mediated degradation of albumin during renal passage. Diabetes 49: 1579-1584.
8. Eppel GA, Nagy S, Jenkins MA, Tudball RN, Daskalakis M, et al. (2000) Variability of standard clinical protein assays in the analysis of a model urine solution of fragmented albumin. Clin Biochem 33: 487-494.
9. Schleicher ED, Olgemöller B, Wiedenmann E, Gerbitz KD (1993) Specific glycation of albumin depends on its half-life. Clin Chem 39: 625-628.
10. Rondeau P, Bourdon E (2011) The glycation of albumin: structural and functional impacts. Biochimie 93: 645-658.
11. Tarwadi KV, Agte VV (2011) Effect of micronutrients on methylglyoxal-mediated *in vitro* glycation of albumin. Biol Trace Elem Res 143: 717-725.
12. Kisugi R, Kouzuma T, Yamamoto T, Akizuki S, Miyamoto H, et al. (2007) Structural and glycation site changes of albumin in diabetic patient with very high glycated albumin. Clin Chim Acta 382: 59-64.
13. Neelofar K, Ahmad J (2017) An overview of *in vitro* and *in vivo* glycation of albumin: a potential disease marker in diabetes mellitus. Glycoconj J 34: 575-584.
14. Speeckaert MM, Speeckaert R, Van De Voorde L, Delanghe JR (2011) Immunochemically unreactive albumin in urine: fiction or reality? Crit Rev Clin Lab Sci 48: 87-96.
15. Miller G, Bachmann L (2019) National Institute of Diabetes and Digestive and Kidney Diseases National Kidney Disease Education Program Laboratory Working Group, Joint Meeting with the International Federation of Clinical Chemistry and Laboratory Medicine Working Group for Standardization of Albumin in Urine.
16. Seegmiller JC, Miller WG, Bachmann LM (2017) Moving Toward Standardization of Urine Albumin Measurements. EJIFCC 28: 258-267.
17. Boutten A, Delatour V, groupe de travail SFBC, SFNDT, SNP (2018) Urinary proteins: up-to-date reference methods for urinary protein analysis. Ann Biol Clin (Paris) 76: 638-642.