

COMPARISON OF GLYCOSYLATED ALBUMIN WITH OTHER GLYCOSYLATED SERUM PROTEINS

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ABSTRACT

This study was performed on 30 diabetic and healthy controls. Fasting blood glucose, glycosylated albumin, glycosylated hemoglobin, fructoseamine, total protein, globulin and albumin were assayed in both groups. Albumin was isolated from the serum by precipitation using 22.2% sodium sulphate and the glycosylated albumin level determined by the TBA method. Other parameters were determined by commercial assay procedures. A good correlation was found between glycosylated albumin and hemoglobin A1c and fructoseamine values. All of these levels were found to be significantly higher in the diabetic patients. However no significant difference was found in total protein, albumin, globulin and hemoglobin levels.

Key Words: Glycosylated albumin, glycosylated hemoglobin, diabet, fructoseamine, sodium sulphate precipitation.

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INTRODUCTION

After glycosylated hemoglobin was first reported by Allen et al (1) and its clinic importance understood, the investigation of nonenzymatic glycosylation of other blood and tissue proteins begun. The nonenzymatic glycosylation of many proteins has been found to increase in diabetic patients (2,5,6,7,8,9,10,11,12,13,14,34).

In diabetes mellitus, because of hyperglycemia, glucose binds nonenzymatically to protein free amino groups. First the unstable "Schiff base" an aldimine is formed which later by Amadori rearrangement converts to stable ketoamine product. This reaction is called nonenzymatic glycosylation. Its a first step of the Millard reaction. The second step is nonenzymatic browning in which advanced glycosylated products with high cross linked, brown yellow fluorescence, insoluble pigmented carbohydrate-protein polymers are formed. Since nonenzymatic glycosylation and then nonenzymatic browning cause the structural and functional changes in protein, it has been suggested that they may be factors underlying diabetic complications (4,6,7,15,16,17).

It has also been found that bilirubin and the drug binding capacity of albumin decreases due to nonenzymatic glycosylation in vitro, however the in vivo situation is still not clear. The reduced solubility of glycosylated albumin has been reported (18,19,20,21,22).

Glycosylated proteins are now used as the control parameters for diabetes mellitus. For long term glyceimic control glycosylated hemoglobin are now used in many laboratories for which many commercial kits are available. Its recomended that glycosylated serum proteins and albumin which reflect average blood glucose for one or two week, can be determined for short term glyceimic control (23,24,25,26,27).

In these study , glycosylated albumin , glycosylated hemoglobin, fructoseamine were determined in diabetics and healthy controls and checked for correlated for with one an other.

MATERIALS AND METHODS

Thirty diabetic and 30 healty controls (15 male and 15 female each) of age range 40 to 70 years who attending to İ.Pakize Tarzi Clinic, were included in this study. Diabetic type was not taken into consideration. Fasting blood glucose, glycosylated albumin, glycosylated hemoglobin, fructoseamine, total protein, globulin, albumin were assayed in both groups. These parameters were also evaluated with respect to sex.

Albumin was isolated from the serum by precipitation using 22.2 % sodium sulphate. Globulin precipitate was seperated by centrifugating at 1500 g for 10 min. The supernatant was taken and used for the determination of glycosylated albumin by the tiobarbuturic acid method (28,29). The purity of albumin was confirmed by cellulose acetate electrophoresis (Helena). The following kits were used to determine the following

parameters ; glycosylated hemoglobin , total protein and albumin; Eagles , serum fructoseamine by Roche, blood glucose: Biotrol and total hemoglobin: Boehringer.

The results were evaluated by using "t" test and regression analysis using the Microstat statistical computer package.

RESULTS

Glycosylated albumin , glycosylated hemoglobin and fructoseamine levels were found to be significantly higher in the diabetics. In contrast to glycosylated proteins, total protein , albumin , globulin and hemoglobin values between healthy donors and diabetics were not found significantly different (Table 1). Nor was there any significant correlation between these parameters and glycosylated proteins (Table 2). A good correlation was seen between glycosylated albumin, glycosylated hemoglobin and fructoseamine values. These parameters also correlated with fasting blood glucose (Table 2).

The findings with respect to sex are shown in Tables 3 and 4, are finding being that the glycosylated albumin levels in women were less significantly higher than the mens in the diabetic group, than in the control group. These tables also show that for both control and diabetic groups the serum albumin levels in women were significantly less than the mens.

Table 1: Mean values of all paratmeters in diabetics and controls and significense of differences (SD: Standard deviation).

	Control Group (n=30)		Diabetic Group (n=30)		P
	Mean	SD	Mean	SD	
Serum Glucose(%mg)	81.1	8.99	178.0	81.8	0.0001
Hemoglobin (%g)	13.4	1.03	13.4	0.77	0.45
Serum Albumin (%g)	4.72	0.57	4.88	0.57	0.15
Serum Globulin (%g)	2.58	0.59	2.62	0.52	0.374
Total Protein (%g)	7.29	0.35	7.50	0.36	0.01
Glycosylated Albumin (umolFructose/1g Alb)	9.06	1.83	13.9	4.22	0.0001
HbA1c (%)	6.78	0.67	10.80	1.94	0.0001
Fructoseamine(umol/L)	2.14	0.13	3.53	0.81	0.0001

Table 2: Regression analysis

Variables	Correlation coefficient (r)
Gly.Alb - Serum Glucose	0.8271
Gly.Alb - Fructoseamine	0.7571
Gly.Alb - Albumin	-0.2271
Gly.Alb - Total protein	0.1624
Gly.Alb - Hemoglobin	0.0218
Gly.Alb - Hba1c	0.6741
HbA1c - Fructoseamine	0.7374
HbA1c - Serum glucose	0.6676
Fructoseamine -. Serum glucose	0.8282

Gly.Alb: Glycosylated albumin

Table 3: Comparison of all parameters with respect to sex in control group (SD:Standard deviation)

	Women (n=15)		Men (n=15)		P
	Mean	SD	Mean	SD	
Serum Glucose (%mg)	83.33	10.21	78.80	7.22	0.0857
Hemoglobin (%g)	13.03	0.86	13.79	1.06	0.0203
Serum Albumin (%g)	4.63	0.69	4.81	0.42	0.1899
Serum Globulin (%g)	2.76	0.69	2.39	0.43	0.0397
Total Protein (%g)	7.39	0.38	7.20	0.29	0.0691
Glycosylated Albumin (umolFructose/1g Alb)	10.12	1.96	8.02	0.84	0.0001
HbA1c (%)	6.94	0.68	6.63	0.65	0.1045
Fructoseamine(umol/L)	2.19	0.14	2.03	0.09	0.0147

Table 4: Comparison of all parameters with respect to sex in diabetic group (SD:Standard deviation)

	Women (n=15)		Men (n=15)		P
	Mean	SD	Mean	SD	
Serum Glucose (%mg)	196.20	99.79	159.87	56.61	0.1151
Hemoglobin (%g)	13.03	0.53	13.84	0.78	0.0001
Serum Albumin (%g)	4.66	0.51	5.09	0.55	0.0171
Serum Globulin (%g)	2.82	0.53	2.49	0.45	0.0185
Total Protein (%g)	7.48	0.32	7.52	0.40	0.3825
Glycosylated Albumin (umolFructose/1g Alb)	15.19	4.96	12.66	2.97	0.0508
HbA1c(%)	10.45	1.81	11.17	2.05	0.1538
Fructoseamine (umol/L)	3.60	0.89	3.45	0.73	0.3291

DISCUSSION

For short term glycemic control determination of glycosylated albumin is one of the recommended methods (35). However there are few methods in literature for determining it. In affinity chromatography, glycosylated and nonglycosylated albumin are separated, then glycosylated albumin is determined specifically (26). In some studies glycosylated albumin is purified by DEAE cellulose chromatography and then assayed by the TBA method (28,30). In the Radio Immunassay (RIA) method, glycosylated albumin is determined by using monoclonal antibodies (31). In the present study albumin was isolated from serum by the simple and cheap method of precipitation with 22.2% sodium sulphate. Then purity of albumin was assayed by electrophoresis and spectrophotometer. The nonenzymatic glycosylation of albumin was determined by TBA method and found to 50% higher in diabetics than in controls.

In diabetic clinics glycosylated proteins determination is preferred to fasting blood glucose because the former better reflects average past blood glucose levels not being affected by daily blood glucose fluctuations. Blood samples can be taken from patients at any time. Fructoseamine and glycosylated hemoglobin are the factors mostly used for monitoring diabetes. These were determined in the present study and significantly increase in diabetics and positive correlation between one other and glycosylated albumin was

found as did others(23,24,25,26,27,32).

Garlic and Mazer investigated glucose binding site in human albumin.They found glucose residues on 534-525th amino acids and that this peptid e could not be cleaved by trypsin. Of normal serum albumin 10-12 percent has been found to be glycosylated (33).

Our results was consistant with those of Kemo et al who assayed glycosylated albumin in blood samples of type I diabetic children by affinity chromatography and found it correlated positively with glycosylated hemoglobin (22).

Dolhofer and Wieland purified serum or plasma albumin from type I and type II diabetic patients by DEAE cellulose chromatography determining glycosylated albumin by the TBA method and using hydroxymethylfurfuraldehyde (HMF) as a standard (ours:fructose) and finding an increase in diabetics (30).

Ohe et al determined glycosylated albumin levels in serum samples of diabetic patients and healthy controls by the RIA method, obtainig results were similar to ours (31).

Miyamoto et al measured higher glycosylated albumin levels in type I diabetic children than controls using high performance liquid chromatography (HPLC)(34), reporting glycosylated albumin not to change with age. They did find however an increase in fructoseamine levels with age.A better correlation between glycosylated albumin and blood glucose has been found than between fructoseamine and blood glucose (34).

Ryle et al investigated the effect of diet on glycosylated albumin and glycosylated hemoglobin. They formed two dietary groups: 1) Fibrous nutrients with less glucose 2) Less fibrous nutrients with more glucose. In neither group did they find change in glucose tolerance or glycosylated hemoglobin levels within the first two hours. After six weeks in first diet group a decrease in percent glycosylated albumin, an increase in it in the second diet group, was reported (35).

Ardawi et al determined glycosylated albumin, glycosylated hemoglobin and fructoseamine and subsequently recommended them for mid, long and short term glycemic control respectively (36).

Today there are many commercial kits for glycosylated hemoglobin and fructoseamine assaying. However for glycosylated albumin there are more suitable for routine clinical laboratories. Mainly chromatographic methods are used. It is therefore proposed that the simple and time saving method of isolating and determining glycosylated albumin utilized in this study be used for short term monitoring of diabetic patients.

REFERENCES

- 1 - Allen, D.W, Schroeder, W.A. and Balog, J.: Observations on the chromatographic heterogeneity of normal adult and fetal human hemoglobin. *J. Am. Chem. Soc.* 80:16:1628-1634, (1958).
- 2 - Brownlee, M., Viassara, H., Cerami, A.: Nonenzymatic glycosylation and pathogenesis of diabetic complication. *Ann Intern Med.* 4:527-37, (1984).
- 3 - Bunn, F.H.: Nonenzymatic glycosylation of protein: Its role in diabetes. In *diabetes Mellitus and Obesity*, Williams and Wilkins, Baltimore, London, s.137-145, (1982).
- 4 - Bauer, J.D., Ackerman, G.P., Toro, G., Brownlee, M., Vlassara, H., Cerami, A.: Nonenzymatic glycosylation on the pathogenesis of diabetic complications. *Ann. Inter. Med.* 101:527-537, (1984).
- 5 - Emekli, N.: *Clinical Biochemistry(I)*. M.Ü. Dişhekimliği Fakültesi Ders Notları, s.75-106, (1990).
- 6 - Kenedy, L., Mehl, Elder, E., Varghiese, M.: Non-enzymatic glycosylation of serum and plasma proteins. *Diabetes*, 31:52-56 (1982).
- 7 - Kennedy, L., Ragnes, W.: Non-enzymatic glycosylation and the chronic complications of diabetes. *Diabetologia* 26:93-98, (1984).
- 8 - Bakan, E., Bakan, N.: Glycosilation of nail in diabetes: Possible marker of long-term hyperglycemia. *Clin. Chim. Acta.* 147:1-5, (1985).

- 19- Yarat, A., Emekli, N.B.: The effect of non-enzymatically glycosylated collagen on normal human platelets. Clin. Chim. Acta. 183, 2203-206, (1989).
- 10- Yarat, A., Emekli, N.B.: NEG of collagen and its relation to glycosylated haemoglobin in acute short-term diabetes. J. Marmara Univ. Dental Faculty, 1, s. 12-15, (1990).
- 11- Yarat, A., Emekli, N.: Normal kişilerde TBA yöntemiyle tayin edilen HbA1c değerlerinin açlık kan şekeri ve hematokrit ile karşılaştırılması. M.Ü. Dişhekimliği Dergisi, 15: 45-51, (1987).
- 12- Yarat, A., Emekli, N., Noyan, Ü.: Nonenzymatic glycosylation of tail tendon collagen and gingival collagen from STZ induced diabetic rats. Pakistan Oral and Dental Journal, 10: 31-39, (1990).
- 13- Yarat, A., Emekli, N., İpbüker, A.: Serum IgE değerleri ile glikozillenmiş hemoglobin arasındaki ilişki. Year Book of Turkish Diabetology, Tur Matbaası, s. 97-112, (1990).
- 14- Goldman, Sajiman: Nonenzymatic glycosylation of insulin receptors results in decreased insulin binding. Diabetes 39: 308-311, (1990).
- 15- Andreassen, T.T., Oxlund, H.: Thermal stability of collagen in relation to nonenzymatic glycosylation and browning in vitro. Diabetologica 28: 687-91, (1985)
- 16- Monnier, V.M., Cerami, A.: Nonenzymatic Glycosylation and Browning in diabetes and Aging Studies on lens proteins. Diabetes 31: 57-63, (1982).

- 17- Saltmarch, M., Labuza, T. P.: Nonezymatic Browning via the millard reaction in foods. *Diabetes* 31:29-36, (1982).
- 18- Day, J. F., Thompson, R. W., Thorpe, S. R. and Baynes, J. W.: NEG of rat albumin. *J. Biol. Chem.* 254:9394-9400, (1979).
- 19- Day, J. F., Thorpe, S. R. ve Baynes, J. W.: Nonezymatically glycosilated albumin. *J. Biol. Chem.* 254:595-597, (1979).
- 20- Dolhofer, R., and Wieland, O. H.: Glycosilated of serum albumin elevated glycosyl albumin in diabetic patients. *FEBS letters.* 103:283-286, (1979).
- 21- Guthrow, C. E., Morris, M. A., Day, I. F.: Enhanced nonezymatic glycosylation of human of serum albumin in diabetes mellitus. *Proc. Natl. Acad. Sci.* 76:4258-4261, (1979).
- 22- Kemp, S. F., Czch, R. H. ve Horni, T. R.: Glycosylated albumin and transferin short-term markers of blood glucose control. *J. Peoliatr.* 105:394-398, (1984).
- 23- İpbüker, A., Bayer, H., Ersan, E., Aygözer, a., Barta, F., Çelyebi, A., Demiröz, T.: Diabetes Mellitus tanısında HbA1, Fruktosamin SGOTT ve glikoza insülinin cevabı. *Türk Diyabet Yıllığı*, Tur Ofset baskısı, s.141-147, (1989).
- 24- İpbüker, A., Ersan, E., Aygözer, M., İkten, Z., Bayer, H., Çelebi, A., Demiröz, T.: Diabetes Mellitus'un Tanı ve Kontrolunda Fructosaminler, *Türk Diyabet Yıllığı*, Tur Ofset baskısı, s.123-130, (1989).
- 25- Jones, I. R., Owens, D. R., Williams, S.: Glycosylated serum albumin: an intermediate index ay diabetic control. *Diabetes Care*, 6:501-503, (1983).

- 26- Johnson, R.N., Metcalf, P.A. ve Baker, J.R.: Fructoseamine: a new approach to the estimation of serum glycosylprotein: an index of diabetic control. *Clin. Chem. Acta.* 127:87-95, (1982)
- 27- Oresek, G., Seiffertof, U.B.: Fructosamin as a diagnostic parameter in the clinical routine. *Wien Klin Wechenschr* 74: 78-81, (1990).
- 28- Ney, K.A., Colley, K.J., Pizzo, S.V.: The standardization of the thiobarbituric acid assay for the nonenzymatic glycosylation of human serum albumin. *Anal Biochem.* 118:294-300, (1981).
- 29- Parker, K.M., England, J.D., Losta, J.D., Hessel, R., Goldstein P.E.: Improved colorimetric assay for glycosylated hemoglobin, *Clinical Chemistry*, 27/7, p.669-672, (1981).
- 30- Dolhofer, R., and Wieland, O.H.: Improvement of the thiobarbituric acid assay for serum glycosyl-protein determination. *Clin. Chim. Acta.* 112:197-204, (1981).
- 31- Ohe, Y., Matsuura, M., Nakajima, Y., Shin, S., Hashimoto, F., - Himoto, M. ve Shima, K.: Radioimmunoassay of glycated albumin with monoclonal antibody to glucitollysine. *Clin. Chim. Acta.* 169:229-238, (1987).
- 32- Hughes, R.C.: Membrane glycoproteins : A review of structure and function. Butterworths Ltd. Sti. London, p.182-228, (1976).
- 33- Garlick, R.L. and Mazer, J.S.: The principal site of nonenzymatic glycosylation of human serum albumin in vivo. *J. Biol. Chem.* 258:6142-6145, (1983).

- 34- Miyamoto, N., Shirakawa, N., Kuroda, Y., Abe, F., Shina, K.: Serum levels of glycated albumin in non-diabetic and insulin-dependent diabetic children. *Acta Paediatr. Jpn.* 32: 249-256, (1990).
- 35- Ryle, A. J., Davie, S., Gould, B. J., Yudkin, J. S.: Study of the effect of diet on glycosylated haemoglobin and albumin levels and glucose tolerance in normal subject. *Diabetic Med.* 7: 865-870, (1990).
- 36- Ardawi, M. S., Nasrat, M. N., Mira, S. A., Fatani, H. H.: Comparison of glycosylated fibrinogen, albumin, and haemoglobin as indices of blood glucose control in diabetic patients. *Diabetic Med.* 7: 819-824, (1990).