Gaziantep Üniversitesi Tıp Fakültesi Dergisi 1998; 9: 52-57

FRUCTOSAMINE IS A MARKER FOR DIABETES MELLITUS

Necat YILMAZ, Binnur ERBAĞCI

Gaziantep Üniversitesi Tıp Fakültesi Biyokimya ve Klinik Biyokimya Anabilim Dalı

ÖZET

DİABETES MELLİTUSTA FRUCTOSAMINE

Diabetes mellitus evrensel bir sağlık problemidir; ve her yaşta görülebilir. Diabetli hastaların başlangıç tanısında biyokimyasal ölçümler yapılır: Bunlar, rastgele kan glikoz ve idrar glikoz ölçümleridir. Proteinin invitro glikolizasyonu 70 yıldan beri bilinmektedir. Bu reaksiyon geridönüşümsüzdür. Dolayısıyla glikolize protein oranı ile proteinin yarılanma ömrü ve hastanın glikoz konsantrasyonu arasında bir orantı vardır.

Anahtar Kelimeler : *Fruktozamin, diabetes mellitus, nitroblue tetrazolium (NBT).*

SUMMARY

Diabetes mellitus is a universal health problem and may occur at any age. The traditional biochemical measurements for initially detecting patients with diabetes mellitus are random estimations of blood and urine glucose concentrations. The occurence of protein glycation in vitro has been known for about 70 years. The reaction is irreversble so that the level of glycated serum proteins in a patient is dependent on serum glucose concentration and on half-life of the proteins.

Key Words : *Fructosamine, diabetes mellitus, nitroblue tetrazolium (NBT).*

FRUCTOSAMINE IS A MARKER FOR DIABETES MELLITUS

Diabetes mellitus - a condition affecting more than %5 of the popullation and considered to be the fifth leading cause of death in the U.S.- has been clinically defined as an absolute or relative deficiency of insulin that may progress to hyperglycemia and is often associated with specificmicro and macrovascular complications. Neuropathy, retinopathy, and nepropathy have been shown to be most prevalant in diabetic patients with poor glucose control and least prevalant in those patients with good glucose control. Three general classifications of diabetes have been described .Type I insulin dependent diabetes mellitus (IDDM), Type II non-insulin dependent diabetes mellitus (NIDDM) and Type III diabetes associated with other conditions. The aim of therapy in diabetes is to maintain a constant normal (or near normal) level of glucose in the blood. As blood glucose levels rise the increase in non-enzymatic glycosylation of proteins is proportional to both the level of glucose and life span of the protein in the circulation or tissues (1).

Diabetes mellitus is a universal health problem and may occur at any age. The traditional biochemical measurements for initially detecting patients with diabetes mellitus are random estimations of blood and urine glucose concentrations. Measurement of the glycosylated haemoglobin (HbA1c) concentration in random blood specimen is a more simple screening test for diabetes and has the advantage that it reflects blood glucose control under physiological condition (2). The occurence of protein glycation in vitro has been known for about 70 years. This non-enzymatic reaction was also found to occur under physiological conditions in the body and it was first dedected for

haemoglobin.The reaction is irreversible so that the level of glycated serum proteins in a patient is dependent on serum glucose concentration and on the half-life of the proteins (3). A rise in the glucose concentration in blood leads to chemical modificatication of circulating proteins by glycation. The initial product is labile glucose-protein adduct in the form of an aldimine. Unless the labile product reverts to its constituents the unstable double boand of the aldimine rearranges in a subsequent slower reaction in to stable single bond the glucose-protein complex is then in the ketoamine (fructosamine) form (4). Major product of this reaction in serum is glycated albumin and other proteins.

Monitoring glucose levels in blood and urine in hospitalized IDDM patients is insufficient and may be misleading. The daily level of plasma glucose is not stable and test must be performed several times daily to get a mean value of glucose concentration. The measurement of glycosylated haemoglobin has been widely used as an index of diabetic control. Because of the relatively long half-life of haemoglobin the concentration of glycosylated haemoglobin reflects the average level of control over a period of the preceeding 2-3 months (5). Howewer the techniques employed for it's determination are expensive, cumbersome, and difficult to standardize and therefore other alternative procedures have been developed. One of these is the measurement of serum fructosamine concentration which represents a value for the serum glycated proteins present during 1 to 3 weeks before the analysis (6). The measurements of glycated plasma proteins has been shown to be useful for the monitoring of medium term glycaemia in diabetic patients. Boronate affinity chromatography may be used to quantitate total glycated plasma proteins or glycated albumin specificalIy (major fraction). The fructosamine assay developed by Johnson et al (7).

The diabetic specialist's ideal picture is shown in the following Diagram 1.

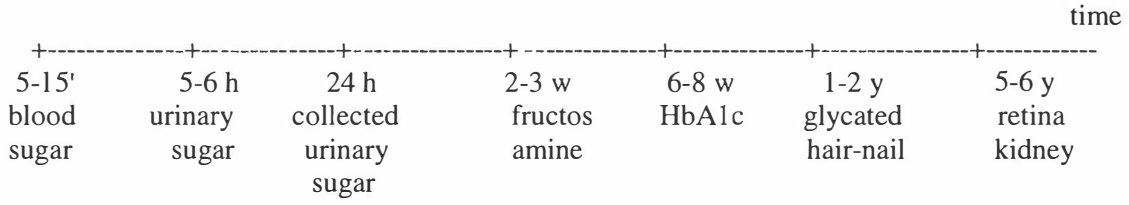

Diagram 1

A blood sugar determination is a snapshot that provides information spanning a period of a few minutes, self testing for urinary sugar at regular intervals of 5 to 6 hours yields an avarage over this collection period. A 24 hours urine samples again provides information about the behavior of the blood sugar during this period particulary whether it has exceeded the renal threshold and if so to what extent HbA1c provides information spanning a period of 6 to 8 weeks. Between these last two parameters lies the fructosamine value, which gives a picture covering 2 to 3 weeks. However it would also be useful to have parameters of diabetes control over a period longer than 6 to 8 weeks Glycated hair and glycated nails can provide information about the mean control of diabetes a period of 1 to 2 years and the situation over a period of 5 to 8 years can be read from eye and kidney findings (4). The glycation of structural proteins like that of circulating proteins leads to functional changes and ultimately to morphologically visible changes in tissue structures. The entire glycation sequence is currently at the center of views on the pathobiochemical development of micro and macro angiopathic concequences of long term diabetes. The substances that react with glucose include not only haemoglobin and transport proteins, structural proteins and enzymes but also nucleic acids.

Several methods for the determination of glycated serum proteins are described in the literature. In 1982 Johnson and Baker published a newsimple colorimetric method (Nitro-blue-tetrazolium reduction method) for other glycated serum proteins which were summarized under the name "fructosamines". Auto-mated techniques soon followed and the method was established as the “fructosamine test”. In 1991, Johnson and Baker published

modified fructosamine test plus including a detergent and urcase in the modified reagent by changing the concentrations of buffer and dye and by changing the approach to primary calibration (8). Johnson and Baker used 1-Deoxy-1-Morpholinofructose (DMF) for the calibration of their color test. This is a synthetic non-physiological seconder amine. The fructosamine values obtained by this method are therefore only relative values (4). Another primary standart useful for fructosamine tests is polylisine. The values obtained by these standardization methods show very good agreement (4). Kallener reported the use of polylisine as a calibrator was advantageus. Moshiba et al described a different method has been developed for the measurement of glycated albumin by the Nitroblue tetrazolium (NBT) colorimetrik method. According to method polyethylene glycol was added to serum and then the mixture was centrifuged to seperate globulin proteins from albumin proteins.

Several publications by Johnson and Baker have described a simple method, for fructosamine assays in clinical laboratories. The basis of this method is as follows: Proteins react with glucose to form ketoamine for which the term fructosamine has become accepted. In alkaline medium the fructosamine rearranges to the enaminol form which then reduces NBT to a dye, formazan. The rate of formation of this dye is used as a measure of the fructosamine concentration in the sample. Comparison of reagents (8) are shown in Table 1.

Protein + Glucose ----- Ketoamine (Fructosamine)---------- OH^-

Enaminol + NBT ---------Formazan(dye)

Diagram 2.

Reagent composition	**Fructosamine test**	**Modified Fructosamine test**
Nitroblue tetrazolium	0.25 mmol/L	0.48 mmol/L
Sodyum Carbonate buffer	100 mmol/L	200 mmol/L
	pH:10.35	pH:10.30
Detergent	----	22 g/L
Uricase	----	>2.5 kU/L
Assay settings	mmol/L	umol/L
Samp1e volume	20 ul	10 ul
Diluent volume	50 ul	30 ul
Reagent volume	200 ul	200 ul
Wawelength	530 nm	530 nm
Temperature	37 °C	37 °C

Table 1

This reagents fructosamine test, and modified fructosamine test are also available as a kit from L.A Roche, Boehringer Mannheim.

First Lylod and Marples have adapted this fructosamine assay reagent for use in a centrfugal analyzer and reported its performance, both analytically and as an index of hyperglicemia (Cobas Bio Centrifugal analyzer). Then Lim and Staley measured fructosamine for use in a centrifichem 400 centrifugal analyzer. Also six different automatic analyzer such as Ependorf, ACP, Epos, BM0/Hitachi (704, 705, 717, 737), were used for fructosamine assay (9,10). Yücel and Önder applicated fructosamine assay method to two diffrent analyzer Encore (Baker instrument) and Dacos (Coulter electronics) (11,12) and we applicated modified fructosamine and fructosamine assay method to Beckman CX-5 autoanalyzer (not pressed).

Glycated haemoglobin can be assayed chromatographically, electrophoretically or colorimetrically (13); glycated protein colorimetrically or chromatographically (14) (15). The colori-metric methods which are based on the thio-barbituric acid reaction and thus require an acid hydrolysis step reportedly are subject to interference from free glucose in the serum all of the methods require good technical skill and are not amenable to automation. Howewer fructosamine assay requires only simple colorimetry and is not expensive therefore particullary useful for a developing country.

Referance values for fructosamine test are plus 205-285 umol/L, unmodified fructosamine method 1.94-2.60 mmol/L (8) and normal range for children is 0.33-0.85 mmol/L (2). The fructosamine level is higher for girls aged 11-17 years in comparison with 11-17 year old boys and girls aged 3-11 years. There is strict corelation between the growth of the activity of glucuronidase and level of fructosamine (16,17).

The pregnant women reference ranges exhibit a significant decrease for the fructosamine concentration 0.13 mmol/L (6%), probably because of a shift of glucose to fetus (18,19). In well controlled diabetic pregnancy, both fructosamine and HbA1c reliably indicated ends in blood glucose but fructosamine estimated blood glucose levels more precisely because probably of increased erythropoesis in late pregnancy (20). That both HbA1c and fructosamine have very low sesitivity as predictors of gestational glucose intolerance. Chan et al reported indicating that albumin uria has an effect on the plasma fructosamine concentration which is independent of plasma albumin concentration (21,22,23). Effect of obesity on fructosamine is small (23). Interpretation HbX on subjects with a decreased erythrocyte half-life is difficult measurement of HbA1c fructosamine seems a suitable alternative. It has been shown that a significant correlation is present between blood pressure and plasma glucose concentrations among nondiabetic individuals, but no significant alteration is dedected in fructosamine values between normal blood pressure group and hypertensive group (24).

Criteria for screening diabetes mellitus using serum fructosamine level is 290 umol/L or hig-her (or fasting plasma glucose of 110 mg/dl or higher) agreed with the standart oral glucose tolerance test in identifiying patients with diabetes mellitus in %96.7 and the serum fruc-tosamine test simpler and less expensive (25).

In the fructosamine assay some interfering effect of triglyceride at 7.5 mmol/L and some minor interference is known. The effect of hyperlipidemia is reduced but not completely eliminated including a detergent in the reagent. Similarly, urate interference in the method had been eliminated by the addition of uricase to the reagent (8).

A comparison of fructosamine and bilirubin concentrations in specimens from nondiabetic patients confirmed in interferenal with icteric specimens (bilirubin>60 umol/L) (26), other minor interfering substance are heparin, EDTA, haemoglobin, penicillin, creatinine, ascorbate decreasing and glutathione, glucose, fructose, cholesterol increasing effect on serum fructosamine assay (4,27,28,29).

Fructosamine assay is not suitable for some situations especially serum total protein or albumin is low level such as nephrotic syndrome cirrhosis,of the liver or in any other clinical situation in which protein metabolism is altered (30-33).

The following conclusion can be said; Fructosamine falls more quickly than HbA1c; this can be explained by the shorter half-life of the glycated proteins. It is thus confirmed that fructosamine may be a parameter for assessment of a diabetic metabolic condition over a shorter period than HbA1c. Howewer fructosamine shows clear fluctations from day to day. Which can not be explained by fluctations in the blood sugar. They can be explained by variations of total serum protein concentration and these in turn are caused by differences in the degree of hydration. Fructosamine can be a good parameter for the assessment of diabetic metabolic condition over a short period.

REFERENCES

1. *Bio-Rad instruction manual p1, 1993*
2. *J Windeler, J Kooberling: The fructosamine assay in diagnosis and control of diabetes mellitus. J Clin Chem Biochem 129, 1990*
3. *Baker J, John O, Patricia. M et al: Clinical usefulness of estimation of serum fructosamine concentration as a sceening test for diabetes mellitus: British Med J 863, 1983*
4. *Bohringer Mannheim: Work shop report fructosamine 863, 1989*
5. *Baker J, Patrcia A, Robert S, et al: Fructosamine test-plus a modified fructosamine assay evaluated. Clin Chem 552, 1991*
6. *Costantic C, Simo J, Joven J: Serum fructosamine concentration in patients with cirrhosis of the liver the influence of hypoalbuminemia and hypergamma globunemia. Ann Clin Biochem 437, 1992*
7. *Sarrah J, Kerry W , Barry G: Biological variation in glycated proteins: Ann Clin Biochem 260, 1993*
8. *Mashiba S, Kazuo U, Shoji O: Measurement of glycated albumin by the nitroblue tetrazolium colorimetric method: Clinica Chimica Acta 3, 1992*
9. *Baker J, Patrioia A, Johson N et al: Use of protein-based standarts in automated colorimetric determinations of fructosamine in serum. Clin Chem 1550, 1985*
10. *David L, Marples J: Simple colorimetry of glycated serum protein in a centrifugal analyzer: Clin Chem 1686, 1984*
11. *Yong S, Michael S: Measurement of plasma fructosamine evaluated for monitoring diabetes. Clin Chemz 731, 1985*
12. *Önder F, Yücel D: Kolorimetrik fruktozamin yönteminin otomasyona uyarlanması. Biyokimya Dergisi 59, 1993*
13. *Miedami K, Casparie T: Glycosylated haemoglobins: Biochemical evaluation and clinical utility. Ann Clin Biochem 2, 1984*
14. *Yue DK, Morris K, Mellenom S: Glycosylation of plasma protein and its relation to glycosylated hemoglobin. Diabetes 296, 1980*
15. *Cole RA, Soelder JS, Dumm PJ: A Rapid method for the determination of glycosylated hemoglobins using high pressure liquid chromatography. Metabolism 289, 1978*
16. *Visser Van, Saleman S, Wersch Van J, et al: Glycosylated serum proteins and glycosylated haemoglobin in normal pregnancy. Ann Biochem 661, 1986*
17. *Parfitt VJ, Clark JD, Turner GM, et al: Use of fructosamine and glycated haemoglobin to verify self blood glucose monitoring data in diabetic pregnancy. Diabet Med 10:162, 1993*
18. *Gomo Z: Serum fructosamine a parameter for monitoring metabolic control in diabetes. Cen Afr J Med 362 , 1992*
19. *Chan JC, Yeuny VT, Cheung CK, et al: The inter relationships between albuminuria plasma albumin concentration and indices of glycemic control inview insulin dependent diabetes mellitus. Clin Chem Acta 179, 1992*
20. *Kurishitas M, Nakashima K, Kozu H: Glycated hemoglobin of fractionated erythrocytes, glycated albumin and plasma fructosamine during pregnancy . Am J Obst Gynecol 1372, 1992*
21. *Ciechanowski K, Ciechanowski A, Czekalski S: Value of fructosamine test in monitoring of diabetes complicated by proteinuria (abst). Pol Arch Med Wewn 180, 1993*
22. *Watts GF, Taub NA, Mazurkiewicz T: An Examination of the Covariability of subclinical albuminuria in insulin dependent diabetes mellitus, Implications for monitoring microalbuminuria. J Clin Endocrinol Metab 981, 1993*
23. *Woo J, Cockram C, Lau E, et al: Influence of obesity on plasma fructosa-*

mine concentration. Clin Chem, 2190, 1992

24. *Bilgin R, Donma S, Sağlıkery Y: Glucose glycated hemoglobin and fructosamine levels in essential hypertension. Biochem Mol Biol Int 1129, 1993*
25. *Kasezawa N, Kiyose H, Ito K, et al: Criteria for screening diabetes mellitus using serum fructosamine level and fasting plasma glucose level and fasting plasma glucose level. Methods Inf Med 237, 1993*
26. *Ewa T, Anna L: Fructosamine level in blood serum of patients with diabetes mellitus type I (IDDM) in different stages of the disease Materia Medica Polona 258, 1988*
27. *Anders K: Influeence of triglycerides and urate on methods for determination of fructosamine. Clinica Chimica Acta 99, 1992*
28. *Baker J, Zyzak D, Suzanne R, et al: Mechanism of fructosamine assayevidence aganist role of superoxide as intermediate in NBT reduction Clin Chem 2460, 1993*
29. *Baker J, Patriccia M, Tatmell M, et al: Quality assessment of determinations of serum fructosamine in 33 clinical chemistry laboratories. Clin Chem 2133, 1986*
30. *Lamb E, Venton T, Cattel R, et al: Serum glycated albumin and fructosamine in renal dialysis patients. Nephron 82, 1993 Toshikazu Y, Yasuo A, Takayoshi T, et al: Comparison of 1,5 anhydro glucitol, HbA1c, and fructosamine for detection of diabetes mellitus. Diabetes 52, 1991*
31. *Davie SJ, Whiting KL, Gould BJ: Biological variation in glycated proteins. Ann Clin Biochem 260, 1993*
32. *Lamb E, Venton TR, Cattel Wr, et al: Serum glycated albumin and fructosamine in renal dialysis patients. Nephron 82, 1993*