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RESEARCH ARTICLE

THE CLINICAL USEFULNESS OF HBA1C AND FRUCTOSAMINE IN GLYACEMIC CONTROL OF PATIENTS WITH DIABETES MELLITUS

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ABSTRACT

Background: In recent years measurement of glycated haemoglobin Al (HbA1c) has been widely introduced as a measure of control of diabetes and is used by many diabetologists. However, it is subject to a number of disadvantages. It requires incubation for several hours to remove the unstable intermediate Schiff base, it is quite a time consuming assay to perform and, by comparison with the measurement of other ketoamines, relatively expensive. More recently, a method has been described for the measurement of glycated serum proteins, known as fructosamine, which is based on a colorimetric determination utilizing the reducing properties of fructosamine at high pH. It has the advantage that it is rapid, inexpensive, and can be automated, thus reducing the amount of laboratory time required for the assay. **Objectives:** The aim of the study is to evaluate the different methods for the glycemic control in diabetic patients. The primary objective is to compare the efficiency of NBT reduction method for estimation of serum fructosamine with HbA1c estimated by ion exchange resin method & secondary objective is an attempt to develop cost effective, manual, dye based method for estimation of serum fructosamine. **Method:** 57 patients with type 2 DM and 57 controls were enrolled for the study. EDTA blood sample were used for HbA1c by Ion exchange Resin Method. Serum samples were used to detect fructosamine, FBS, PPBS, urea, creatinine, total protein & albumin. For fructosamine NBT Reduction method was used. **Results:** The average value of HbA1c for cases was 7.82±1.12 and for controls 5.3±0.9. The average value of fructosamine for cases was 3.79±0.78 and for controls 1.99±0.28. There was significant correlation between HbA1c by Ion Exchange Resin Method and fructosamine by NBT Reduction Method. The correlation coefficient r for case was 0.633 and for controls r = 0.643 ($p< 0.05$). NBT Reduction method for fructosaminehas been standardized and linear graph was obtained. HbA1c and fructosamine showed significant correlation with FBS, PPBS values and duration of diabetes. Calculated HbA1c from fructosamine result show significant correlation with HbA1c by Ion Exchange Resin method. **Conclusion:** The significant correlation of fructosamine with HbA1c suggests that, fructosamine can be used as an early marker of glycemic control than HbA1c as the half life of serum albumin is only 14 – 21 days. NBT Reduction method is rapid, inexpensive and time saving technique can be used as a routine test.

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterised by hyperglycaemia, caused by deficiency in insulin release or action or both. The important metabolic disorder in diabetes mellitus is carbohydrate metabolism where glucose is underutilized, producing hyperglycaemia.

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The high costs of diabetes are attributable to care for both acute conditions (such as hypoglycaemia and ketoacidosis) and debilitating complications. The latter include both microvascular complications predominantly retinopathy, nephropathy, and neuropathy and macrovascular complications, particularly stroke and coronary artery disease (CAD). Together these make diabetes the seventh most common cause of death in the developed world (American Diabetes Association, 2009). Glycaemic markers are vital in routine practice as well as in research to guide therapy and to investigate the efficacy of medications on patients' glycaemic control. They include Haemoglobin A1c, Glycated serum proteins, Anhydroglucitol, Glycaemic variability indices, Mean

plasma glucose, Fasting plasma glucose and postprandial plasma glucose. These are excellent predictors of diabetes related complications. DCCT and UKPDS are the important landmark trials where they have shown that HbA1c is directly linked with development and progression of micro as well as microvascular complications (The Diabetes Control and Complications Trial Research Group, 1995; UK Prospective Diabetes Study, 1998). However, HbA1c gives false results in certain conditions such as haemolytic anaemia, blood transfusion, chronic renal or liver disease and drug treatment, pregnancy, etc (Koga, 2010; Mahajan, 2011). Searching for another more flexible parameter, which might permit an evaluation of the degree of blood glucose control within shorter periods. Analogous to haemoglobin, serum protein is also glycosylated non-enzymatically. This produce glycosylated protein or fructosamine, its levels are a function of blood glucose concentration. Such a relationship would suggest the possibility of using glycosyl protein as a tool for the semi long term control of diabetes, since the half-life of human serum albumin is much shorter (about 20 days) than that of HbA1c. Fructosamine is useful in short-term glycaemic control as in the case of gestational diabetes mellitus (Sherwani, 2016). Hence, we have conducted a study to evaluate the different methods for the glycaemic control in patients with diabetes.

MATERIALS AND METHODS

We conducted the cross section study in clinical biochemistry laboratory, Medical college hospital, Thiruvananthapuram and Biochemistry laboratory, department of MLT medical college, Thiruvananthapuram. We included the patients with T2DM, age between 35-55 years having Haemoglobin level between 12-16 g/dL with normal total protein and albumin level. The duration of the study was 6 months. Human Ethics Committee of Trivandrum Medical College has granted approval for conducting this study. We excluded the subjects with,anaemia, haemoglobin abnormalities, uraemia, Nephrotic syndrome, Hyperthyroidism, liver diseases, glucocorticoid administration and pregnancy, from the study. We employed Chi-square test to analyse the data by using the Statistical Package for Social Science (SPSS) software for windows version 17. The study population consists of 57 T2DM and 57 controls.

RESULTS

Majority of the cases (43.9%) were with age ranges from 46-55 years. There were 36.8% cases with age above 55 years. In case of controls, 42.1% were within the range 25-35 years. The average age. Out of 57 cases 33.3% are males and 66.7% are females. In case of controls, 38.6% are males and 61.4% are females (Table 2). There was a weak correlation of HbA1c with age in cases and controls. The duration of diabetes show a correlation coeffient of 0.322 with HbA1c values in 57 cases. There is no significant correlation between hemoglobin, urea, creatinine, total protein and albumin. Fasting blood glucose values shows a correlation with HbA1c in both cases and controls. (for cases r = .344, p = .009; for controls r = .346, p = .009). PPBS values are more correlated with HbA1c than FBS. (for cases r = .549, p = .000; for controls r = .401, p = .002). There is good correlation with HbA1c and fructosaminewas obtained in both cases and controls. (for cases r = .633, p = .000; for controls r = .643, p = .002). Mean blood glucose is a calculated value obtained from HbA1c results

Table 1. Percentage distribution of different age group included in the study

Age in years	Category				Total	
	Case		Control			
	N	%	N	%	N	%
25 – 35	3	5.3	24	42.1	27	23.7
36 – 45	8	14.0	15	26.3	23	20.2
46 – 55	25	43.9	12	21.0	37	32.4
> 55	21	36.8	6	10.6	27	23.7
Total	57	100	57	100	114	100

Table 2. Percentage distribution of gender included in the study

Sex	Category				Total	
	Case		Control			
	N	%	N	%	N	%
Male	19	33.3	22	38.6	41	36
Female	38	66.7	35	61.4	73	64
total	57	100	57	100	114	100

Table 3. Correlation of HbA1c

	Case (N=57)		Control (N=57)	
	r	p	r	p
Age in years	.215	.056	.210	.053
Duration of DM in years	.322	.010	.346	.009
FBS	.344	.009	.401	.002
PPBS	.549	.000	.232	.083
Hemoglobin	-.108	.426	.043	.753
Urea	-.307	.020	.041	.760
Creatinine	-.203	.129	.006	.963
Total protein	-.005	.969	.064	.638
Albumin	-.043	.751	.643	.000
Fructosamine	.633	.000	.687	.000
Calculated HbA1c	.898	.000	.789	.000
Mean blood glucose	.890	.000	.210	.053

Table 4. Correlation of fructosamine

	Case (N=57)		Control (N=57)	
	r	p	r	p
Age in years	.215	.056	.241	.071
Duration of DM (years)	.322	.010	.201	.110
FBS	.344	.009	.513**	.000
PPBS	.549	.000	.285*	.031
Hemoglobin	-.108	.426	-.138	.305
Urea	-.307	.020	-.064	.635
Creatinine	-.203	.129	-.074	.584
Total protein	-.005	.969	.080	.554
Albumin	-.043	.751	.643**	.000
HbA1c	.633	.000	.955**	.000
Calculated HbA1c	.898	.000	.751**	.000
Mean blood glucose	.890	.000	.241	.071

So it show good correlation with HbA1c. Calculated HbA1c from fructosamine result is also well correlated with HbA1c obtained directly (Table 3). There was a weak correlation of fructosamine with age in cases and controls. The duration of diabetes show a correlation coeffient of 0.314 with fructosamine values in 57 cases. There is no significant correlation between hemoglobin, urea, creatinine, total protein, albumin and fasting blood glucose values. PPBS values are correlated with fructosamine. (for cases r = .410, p = .002; for controls r = .513, p = .000). There is good correlation with HbA1c and fructosaminewas obtained in both cases and controls. (for cases r = .633, p = .000; for controls r = .643, p = .002). Mean blood glucose also show good correlation with fructosamine. Calculated HbA1c is obtained from fructosamine result is also well correlated.

DISCUSSION

Diabetes mellitus is associated with reduced life expectancy, significant morbidity due to the specific diabetes related micro vascular complications (retinopathy, nephropathy and neuropathy), and the increased risk of macro vascular complications (ischemic heart disease, stroke and peripheral vascular disease) (Kim *et al*., 2008). The development of these complications impacts on quality of life. T2DM results in premature death and irreversible long-term complications including myocardial infarction, stroke, retinopathy and blindness, renal disease requiring dialysis or transplantation, neuropathy, foot ulcer, amputation, and erectile dysfunction (Hoelzel *et al*., 2004). For the primary prevention and early intervention of type 2 diabetes, an identification of persons at high risk for developing future diabetes is important. For this purpose, many markers have been identified independently as a predictor or a risk factor and include the classic markers such as blood glucose profiles for the progression to T2DM (Pecoraro *et al*., 1979). Glycaemic biomarker levels such as plasma glucose at fasting (FPG) and postload, late insulin response at postload and HbA1c have been adopted as known biomarkers for predicting T2DM. The Diabetes Control and Complications Trial has now reported the impact of improved glycaemic control on long-term microvascular disease in diabetes. Using glycated haemoglobin as one of the major determinants of control, it found that improvements in glycaemic control brought about major reductions in the mean risks of developing nephropathy, neuropathy, and retinopathy (The Diabetes Control and Complications Trial Research Group, 1995). As the 'gold standard', albeit imperfect due to the various methods of analysis used, glycated haemoglobin analysis is likely to become increasingly important for monitoring the effectiveness of intensified diabetic management (Bunn *et al*., 1975). Glucose molecules are joined to protein molecules to form stable ketoamines, or fructosamines, through glycation, a nonenzymatic mechanism involving a labile Schiff base intermediate and the Amadori rearrangement. The concentration of fructosamine in serum thus reflects the degree of glycemic control attained by the diabetic patient and is useful in monitoring the effectiveness of therapy in diabetes over a period of several weeks, in a manner analogous to the determination of glycated haemoglobin (Armbruster, 1987). The present cross-sectional study was conducted for six months period in clinical biochemistry laboratory, Medical college hospital, Thiruvananthapuram and Biochemistry Laboratory, Department of MLT, Medical College, Thiruvananthapuram.

The study was mainly carried out in serum & EDTA blood samples which are collected from diabetic patients, attended in Diabetic clinic. Cases having anemia, hemoglobin abnormalities, uremia, Nephrotic syndrome, hyperthyroidism ,liver diseases , glucocorticoid administration and pregnancy were excluded from the study. Total of 57 cases and 57 controls were included in this study. Patients included in the study were having history of Diabetes mellitus and were undergoing treatment for Diabetes and were normotensive. Their age, gender, type of diet (veg or non-veg), duration of diabetes, treatment of diabetes, habits (smoking or alcoholic), other medications, other associated diseases were assessed. Biochemical parameters like FBS, PPBS, HbA1c, serum fructosamine, urea, creatinine, total protein and albumin were carried out. In this study, total number of cases was 57. According to HbA1c values, 8(14%) patients having diabetes were under good glycemic control (6.3-6.8%). For 21(36.8%), action suggested (6.9-7.6%). 27 (47.4%) undergoing poor glycemic control (>7.6%). According to serum fructosamine values, 10(17.5%) patients having diabetes were under good glycemic control (2.71-3.10mmol/L). For 16(28.1%), action suggested (3.11-3.5mmol/L). 30(52.6%) undergoing poor glycemic control (>3.5mmol/L). The average value of HbA1c for cases was 7.82±1.12 and for controls 5.3 ±0.9. The average value of fructosamine for cases was 3.79±0.78 and for controls 1.99±028. There was significant correlation between HbA1c by Ion Exchange Resin Method and fructosamine by NBT Reduction Method. The correlation coefficient r for case was 0.633 and for controls r = 0.643. (p value < 0.05). Which was same as that of Allgrove and B L Cockrillt study. They obtained a highly significant relation (p<0.001, r=0-69), the linear regression and 95% confidence limits. In J P H Shield etal study, the sensitivity and specificity for predicting poor control in the determination of glycated haemoglobin >12%, using a fructosamine concentration >3.5 mmol/l were respectively 88% and 68%. The positive predictive value of fructosamine was 54%. In a meta analysis by John R Baker, *et al*.,shown that there was a significant correlation both between fructosamine and glycosylated haemoglobin values (r=0-82). There were no significant (p >0.05) age- or sex-related differences in this population sample. The same result obtained in this study also. Fructosamine concentrations measured in these multiple blood specimens did not change significantly throughout the day (mean coefficient of variation 4.1%) despite wide variability of the respective plasma glucose concentrations (mean coefficient of variation 36.2%). It was observed that the plasma fructosamine values were 8-11% less than that of serum fructosamine (Baker *et al*., 1985). In this study, there was no significant correlation between HbA1c and parameters such as Hemoglobin, creatinine, urea, total protein and albumin. The same result obtained with serum fructosamine also.

A study by Saori Kashima and Kazuo Inoue in 2013, to assess the utility of mulitiple markers of diabetes such as non-glycaemic biomarkers (serum lipids, urea. Creatinine, albumin, total protein, liver enzymes, white-blood cell count, and uric acid) and non-blood biomarkers (age, blood pressure, and body mass index) may play some role in the pathogenesis of diabetes, these markers do not appear to add a practical precision to the diagnostic power of plasma glucose and HbA1c levels. A study by R. Clark Perry, Do1 R. Ravi Shankar (2001) suggested that HbA1c measurement improves the sensitivity of screening in high-risk individuals. A definable normal range (5.3±0.8%), any level that is above the normal range in any given assay should be considered abnormal (>6.1%) (Perry, 2001). In this study obtained the normal value 5.3±0.9%. FBS (r=0.344, p<0.05) and PPBS (r=0.549, p<0.05) values show good correlation with HbA1c. A review article by Iftikhar Ahmad Asim Syed (2011), with the introduction of the new term "estimated average glucose " (eAG) or Mean Blood Glucose, reported along with HbA1c results, make the result easy to interpret (Syed, 2011). In this study mean blood glucose also show good correlation with serum fructosamine, (r=0.75, p<0.001). The calculated HbA1c using fructosamine regression equation correlates well with HbA1c obtained by Ion Exchange method.(r=0.0726, p<0.001). CarlesZafon (2013) identified Glycation Gap, computed as the residual difference between the observed level of A1c and that predicted from its regression with fructosamine. GG strongly correlated with A1c. Qualitative

and quantitative variations in Hb might affect HbA1c levels. MCHC should be taken into account when interpreting HbA1c levels in type 2 diabetes patients (Zafon *et al*., 2013).

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Conclusion

- According to HbA1c values, 8(14%) patients having diabetes were under good glycemic control (6.3-6.8%). For 21(36.8%), action suggested (6.9-7.6%). 27 (47.4%) undergoing poor glycemic control (>7.6%).
- According to serum fructosamine values, 10(17.5%) patients having diabetes were under good glycemic control (2.71-3.10mmol/L). For 16(28.1%), action suggested (3.11-3.5mmol/L). 30(52.6%) undergoing poor glycemic control (>3.5mmol/L).
- The plasma fructosamine values were 8-11% less than that of serum fructosamine.
- There was significant correlation between HbA1c by Ion Exchange Resin Method and fructosamine by NBT Reduction Method. The correlation coefficient r for case was 0.633 and for controls r = 0.643. (p value < 0.05).
- There was no significant correlation between HbA1c and parameters such as Hemoglobin, creatinine, urea, total protein and albumin. The same result obtained with serum fructosamine also.
- FBS (r=0.344, p<0.05) and PPBS (r=0.549, p<0.05) values show good correlation with HbA1c. Butfructosamine show correlation with PPBS results only (r=0.410.p<0.05).
- The calculated HbA1c using fructosamine regression equation correlates well with HbA1c obtained by Ion Exchange method.(r=0.0726, p<0.001).

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