FRUCTOSAMINE AS AN INDEX OF GLYCAEMIA OF TYPE 2 DIABETES MELLITUS PATIENTS AT JOS UNIVERSITY TEACHING HOSPITAL.

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ABSTRACT

Introduction: With increasing prevalence of DM worldwide and imported lifestyle changes in our environment, there is a compelling need for adequate treatment and improved/novel monitoring tools. Fructosamine therefore may be useful as a complementary or substitute monitoring index given that is cheaper, technically easier to perform than HbA_{1c} and the assays have now been standardized and automated. This study aimed to determine the serum fructosamine concentration and the albumin adjusted fructosamine in type 2 diabetes mellitus patient as a measure of monitoring plasma glucose.

Methods: A cross-sectional study involving 180 study participants. A structured questionnaire was administered. The analysis was done using SPSS. Descriptive and inferential statistics were used with $p < 0.05$ considered statistically significant. Ethical clearance and informed consent were obtained before the commencement of the study. Participants were T2DM patients of JUTH aged 18-65 years while controls were healthy non-diabetic consenting staff of JUTH/clinical students of University of Jos. Samples collected were assayed for HbA1c, fasting glucose, Fructosamine and albumin.

Results: A hundred and twenty T2DM and 60 controls participated in the study. There were more females (87) than males (33) T2DM patients. The mean Fasting Blood Glucose (FBG), Fructosamine, HbA1c and Albumin amongst patients was 9.4±4.9 mmol/L, 392.5±137.1 µmol/L, 8.6±2.7% and 48.0±5.4 g/L compared to controls of 4.8±0.5 mmol/L, 258.7±21.0 µmol/L, 5.6±0.5 % and 50.9±2.6 g/L respectively. The differences in the mean FBG, fructosamine and HbA_{IC} levels among patients and controls was statistically significant ($P<0.001$). Adjusted fructosamine using three different formulae showed higher adjusted fructosamine in patients than in controls ($P<0.001$). Serum Albumin however was significantly higher in controls than patients with a p-value of less than 0.001.

Conclusion: *This study has shown that serum fructosamine correlate significantly with the FBG among T2DM patients and even revealed a slightly better correlation than HbA_{1c} and can therefore be used to monitor blood glucose level among T2DM patients requiring a shorter period of follow-up and with financial constraints.*

KEYWORDS: Fructosamine, Diabetes Mellitus, Glycated haemoglobin, Fasting glucose

INTRODUCTION

Diabetes Mellitus (DM) is a major public health problem worldwide. It was estimated by the International Diabetes Federation (IDF) to be 451 million people (aged 18-99 years) in 2017. These figures are expected to rise to 693 million by 2045.[1] An estimated 15.5 million adults aged 20-79 years were living with diabetes according to the IDF within the African region in 2017, representing a regional prevalence of 3.3%. Nigeria has witnessed a significant rise in the prevalence of DM over the past two decades. In 1992, the prevalence of DM was 2.2% as reported by the National Non-Communicable Disease (NNCD) survey with Lagos Mainland having the highest rate of 7.2%.[2] The IDF estimated a current overall prevalence of 5% DM in Nigeria.[3] The complications that are often associated with T2DM have a linear relationship with the average plasma glucose and the duration of such elevation. The management of diabetes in therefore hinged on adequate long-term monitoring of glycaemic control. The monitoring of glycaemic control in T2DM is often carried out by means of laboratory investigations such as fasting blood glucose (FBG), random blood glucose (RBG), post-prandial plasma glucose, glycated haemoglobin (HbA_{1c}) and sometimes by fructosamine and 1, 5-anhydroglucitol levels.[4] Fructosamine is a marker of glucose control reflecting the average serum glucose level over the preceding 2-3 weeks.[5] Consequently, it may be more appropriate for monitoring early response to treatment. This study is hopes to contribute to existing body of knowledge on Fructosamine and it seeks to evaluate the fructosamine levels in T2DM in relation to more established markers of blood glucose like plasma glucose and HbA_{1c} as well as the effect of albumin levels on these relationships.

MATERIALS AND METHODS

Jos university teaching hospital (JUTH) is a 600-bed tertiary health institution and serves as a referral centre for many neighboring states. This is a cross sectional study using specimen from consenting T2DM patients attending diabetic clinic in JUTH. The controls were healthy non-diabetic consenting individuals from staff of JUTH and some clinical student of University of Jos who have been instructed to fast overnight prior samples collection.

Inclusion Criteria

Patients aged 18-65 years who gave consent to participate in the study with confirmed T2DM. Controls were consenting adults within the same age bracket with FPG $\leq$ 5.6 mmol/L, not known diabetic nor on anti-diabetic medication(s).

Exclusion Criteria

Non-consenting patients, age outside 18-65 years or those having suspected T1DM. Controls with FPG > 5.6mmol/L or known Diabetics. Ethical approval was obtained from the Jos University Teaching Hospital ethical committee.

SAMPLE COLLECTION AND PROCESSING

The participants were instructed to fast for at least 8-12hours overnight. Sample collection was carried out between 8am and 10am with about 3-5mls each of venous blood collected from a peripheral vein (antecubital venipuncture) into an Ethylenediaminetetraacetic acid (EDTA), Sodium fluoride and plain vacutainer bottles for HbA_{1c}, glucose, fructosamine and albumin respectively. Specimen were centrifuged at 4000 revolutions per minute for five minutes. The serum from the plain bottle was separated promptly transferred to cryovials and stored at -20°C in a non-self-defrosting freezer for at most six weeks before analysis of fructosamine and albumin. Glucose was analyzed on the day of sample collection within an hour of sampling. The samples for HbA_{1c} collected in EDTA bottles were stored at -80°C until assayed within 60 days. The vacutainer needles were discarded into a biosafety box. The centrifuge (Universal 320 Hettich) and Cobas C311 chemistry autoanalyser (Roche® Diagnostic, Mannheim, Germany) were used during this process. The reagents and kits used for the measurement of Serum Glucose, Fructosamine, HbA_{1c} and Albumin

were procured from Roche® diagnostics (ISN).
To ensure adequate quality control samples were analyzed in batches (except for glucose) together with quality control sera from Roche® Diagnostics products. Analytical accuracy and precision was assured by simultaneous analyses of pooled serum and commercial quality control specimen at low and high control ranges. Glucose control was carried out per daily run. The general quality control measures was observed alongside instrument calibration before laboratory analysis

RESULTS

The study involves 180 participants comprising 120 T2DM Patients and 60 controls determined using the Fischer's formula and a prevalence rate of 8.4%[91, 92] in Nigeria. The mean age of the patients was slightly higher than the controls. There were more females than males among the study participants. The differences in the mean FBG, fructosamine and HbA_{IC} levels among patients and controls was statistically significant (P<0.001). Adjusted fructosamine using three different formulae showed higher adjusted fructosamine in patients than in controls (P<0.001). Serum Albumin however was significantly higher in controls than patients with a p-value of less than 0.001. (Table 1)

Table 1: clinical and biochemical characteristics of patients and controls

Characteristics	Patients Mean (SD)	Controls Mean (SD)	t-test	P-value
Age (years)	51.8±9.1	48.4±9.4	2.358	0.019
Sex				
Males (%)	33(27.5)	28(46.7)		
Females (%)	87(72.5)	32(53.3)	6.559	0.010
FBG (mmol/L)	9.4±4.9	4.8±0.5	7.505	<0.001
FRA (µmol/L)	392.5±137.1	258.7±21.0	3.870	<0.001
HbA_{1c} (%)	8.6±2.7	5.6±0.5	8.852	<0.001
Albumin (g/L)	48.0±5.4	50.9±2.6	8.592	<0.001

FRA= Fructosamine, Alb-FRA= Albumin Adjusted Fructosamine, FBG= Fasting blood glucose, BMI= Body mass index, HbA_{1c}= Glycated Haemoglobin
* Alb-FRA1=FRA X 40/ALB, ** Alb-FRA2= FRA + 0.3 (40-ALB), *** Alb-FRA3 =49 X FRA/ALB

The serum fructosamine value of the patients and controls ranged from 170-771 µmol/L and 214-310 µmol/L respectively. The mean fructosamine concentration of patients and controls were 392±137.1 µmol/L and 258.68±21.0 µmol/L respectively. The difference was statistically significant with a p-value of <0.001. The serum fructosamine of controls were Gaussian in distribution while those of patients were skewed to the right. (Figure 1 and 2).

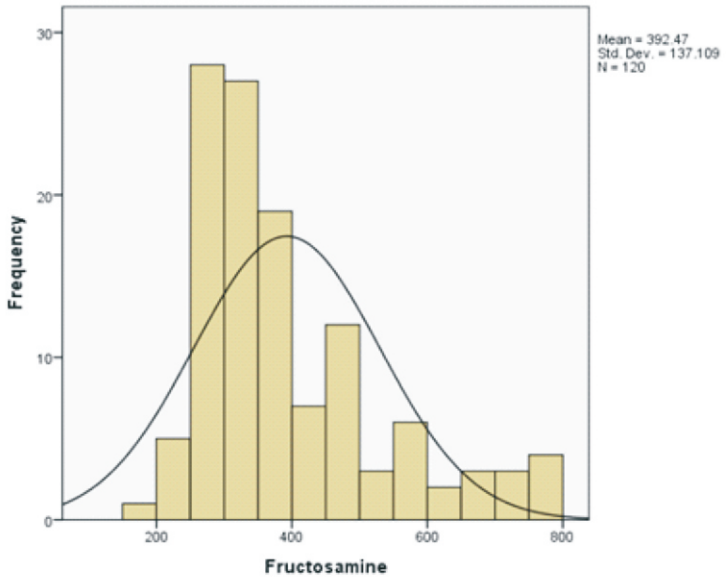

Figure 1: Distribution of Fructosamine in study patients

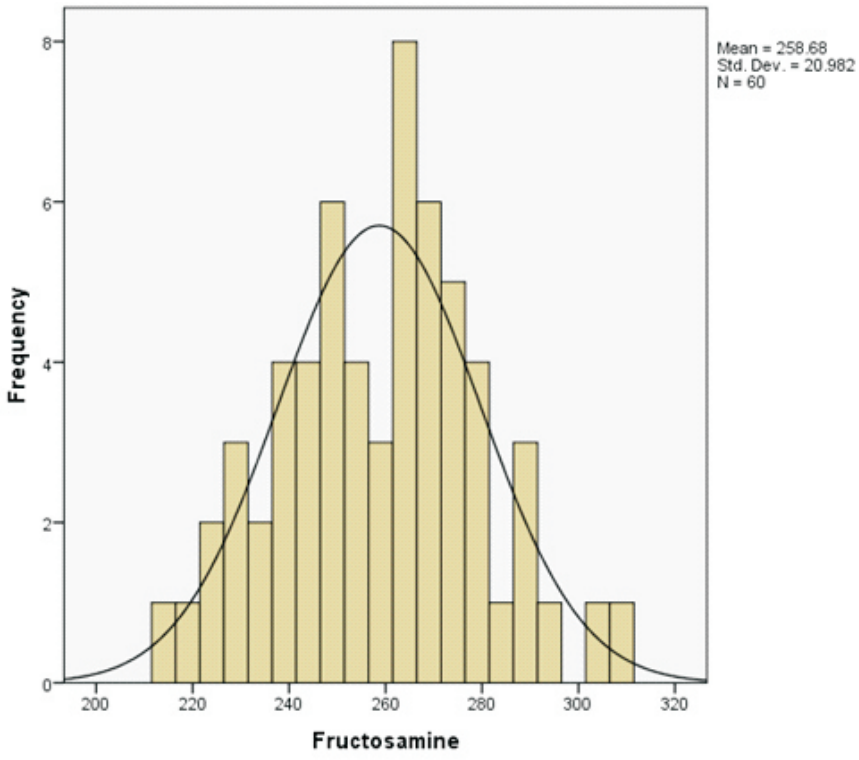

Figure 2: Distribution of Fructosamine in Controls

Table 2: median, range and inter-quartile range of biochemical parameters according to study groups

Parameter	Patients			Control			U-test *(p-value)*
	Median	**Inter-quartile range**	**Range**	**Median**	**Inter-quartile range**	**Range**	
FBG (mmol/L)	7.9	5.9-11.3	3.7-30.5	4.9	4.5-5.1	3.7-5.9	532.0 (<0.001)
FRA(µmol/L)	347.5	295.5-455.0	170-771	261.5	243.3-272.0	214-310	680.0 (<0.001)
Albumin (g/L)	49.0	46.0-51.0	12-59	50.0	49.0-53.0	47-56	2215.0 (<0.001)
Alb-FRA1 (µmol/L)	295.5	244.3-361.75	181-653	201.5	195.0-213.8	176-266	360.5 (<0.001)
Alb-FRA2 (µmol/L)	303.5	251.0-373.5	186-674	206.5	200-218.5	181-270	339.0 (<0.001)
Alb-FRA3 (µmol/L)	362.0	299.3-442.8	222-800	246.5	239.0-261.5	215-341	395.5 (<0.001)
HbA_{1C} (%)	8.0	6.0-11.0	5-15	6.0	5.0-6.0	5-6	814.5 (<0.001)

FBG= Fasting Blood Glucose, FRA= Fructosamine, Alb-FRA= Albumin Adjusted Fructosamine, HbA_{1c}= Glycated Haemoglobin, U = Mann-Whitney U test

* Alb-FRA1=FRA X 40/ALB, ** Alb-FRA2= FRA + 0.3 (40-ALB), *** Alb-FRA3 =49 X FRA/ALB

The median, inter-quartile range and range of biochemical parameters are shown in **Table 2.** This is also depicted using the Box-Whiskers Plot in **Figure 3**. The Box-Whiskers plot showed a better presentation of the spread and cluster of fructosamine among patients and controls. The plot showed a wider spread among the diabetic patients compared to controls. Mann-Whitney U test (non-parametric method) was used to compare the median fructosamine levels among cases and control. Except for Serum Albumin, the median levels of all biochemical parameters assessed were significantly higher among cases than controls (P< 0.001). The median albumin however was slightly higher among controls than cases. (p<0.001).

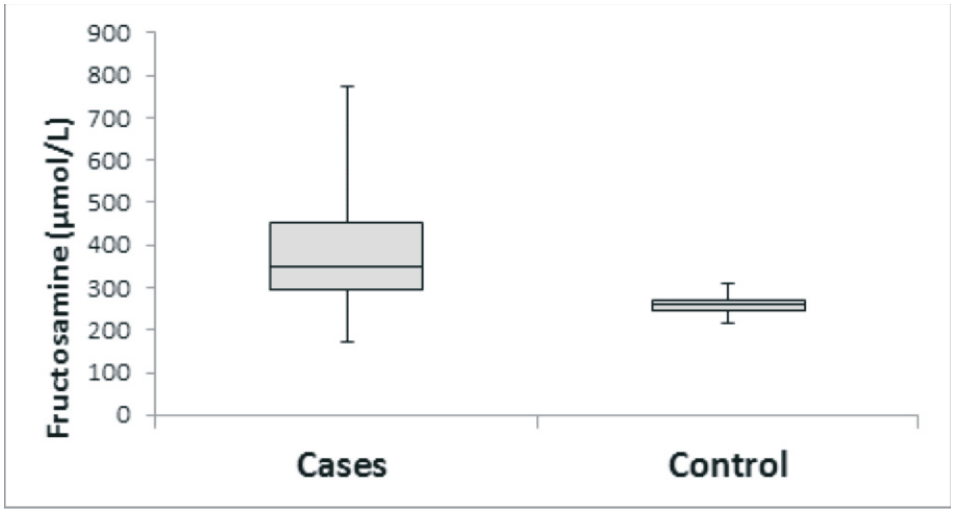

Figure 3-: Box-Whisker Plot showing the distribution of Fructosamine according to study groups

There was a positive correlation between fructosamine and all the parameters, ($p<0.05$). Fructosamine had a higher correlation with glucose (0.829) compared to HbA_{1c} (0.796) among the diabetic patients. The result among the control group was quite different when compared to that seen among patients. There was a negative correlation between fructosamine and HbA_{1c} while the correlation between fructosamine and glucose was 0.459.

The association between fructosamine (Adjusted and unadjusted) and FBG was significant (p-value is <0.001) as seen in **Table 3.** When 7.0 mmol/L was used as cut off value, fructosamine and FBG identified equal number of patients with glucose value of <7.0 mmol/L as 'normal'. However fructosamine classified only 42% of patients with **≥ 7.0 mmol/L as having elevated glycaemia. This may be explained by the different duration of glycaemia measured by both parameters. While FBG measures the spot glucose level, fructosamine measures the average of glucose over 2-3 weeks.**

Table 3: association between fructosamine and fbg of t2dm patients

Parameter	**FBG (<7.0mmol/L) n=49**	**FBG (≥ n=71**	Total **n=120**	Fishers p-value
Fructosamine				
Elevated	0(0.0)	30(42.3)	30(25.0)	
Normal	49(100.0)	41(57.7)	90(75.0)	<0.001
Adjusted Fructosamine 1				
Elevated	2(4.1)	28(39.4)	30(25.0)	
Normal	47(95.9)	43(60.6)	90(75.0)	<0.001
Adjusted Fructosamine2				
Elevated	2(4.1)	28(39.4)	30(25.0)	
Normal	47(95.9)	43(60.6)	90(75.0)	<0.001
Adjusted Fructosamine 3				
Elevated	2(4.1)	28(39.4)	30(25.0)	
Normal	47(95.9)	43(60.6)	90(75.0)	<0.001

Similarly, the association between fructosamine (Adjusted and unadjusted) and HbA_{1c} was equally significant as shown in Table 4. An HbA_{1c} value of 6.5% was used as a cut off. All patients with HbA_{1c} <6.5% equally had a normal fructosamine value, this constitute 28% of the patients. Out of the remaining 72% who had $HbA_{1c} \geq 6.5\%$, 34% of them equally had elevated fructosamine while the remaining patients in this category had a normal fructosamine values. The different time frame of glycaemia measured by these two parameters may also explain some of the statistical differences observed.

Table 4: Association between fructosamine and hba_{1c} of t2dm patients

Parameter	HbA_{1c} (<6.5%) n=33	HbA_{1c} (≥ n=87	Total n=120	Fishers p-value
Fructosamine				
Elevated	0(0.0)	30(34.5)	30(25.0)	
Normal	33(100.0)	57(65.5)	90(75.0)	<0.001
Adjusted Fructosamine1				
Elevated	0(0.0)	30(34.5)	30(25.0)	
Normal	33(100.0)	57(65.5)	90(75.0)	<0.001
Adjusted Fructosamine2				
Elevated	0(0.0)	30(34.5)	30(25.0)	
Normal	33(100.0)	57(65.5)	90(75.0)	<0.001
Adjusted Fructosamine3				
Elevated	0(0.0)	30(34.5)	30(25.0)	
Normal	33(100.0)	57(65.5)	90(75.0)	<0.001

Figure 4 shows the Receiver Operative Characteristic (ROC) curve of fructosamine and HbA_{1c} as predictors of glycaemic control.

The Area under curve (AUC) for unadjusted fructosamine was the highest (0.960) while the lowest was HbA_{1c} (0.878). At 285 μmol/L (upper reference limit used for this study), fructosamine has a sensitivity of 1.000 and a specificity of 0.449 for predicting good glycaemic control among all study participants.

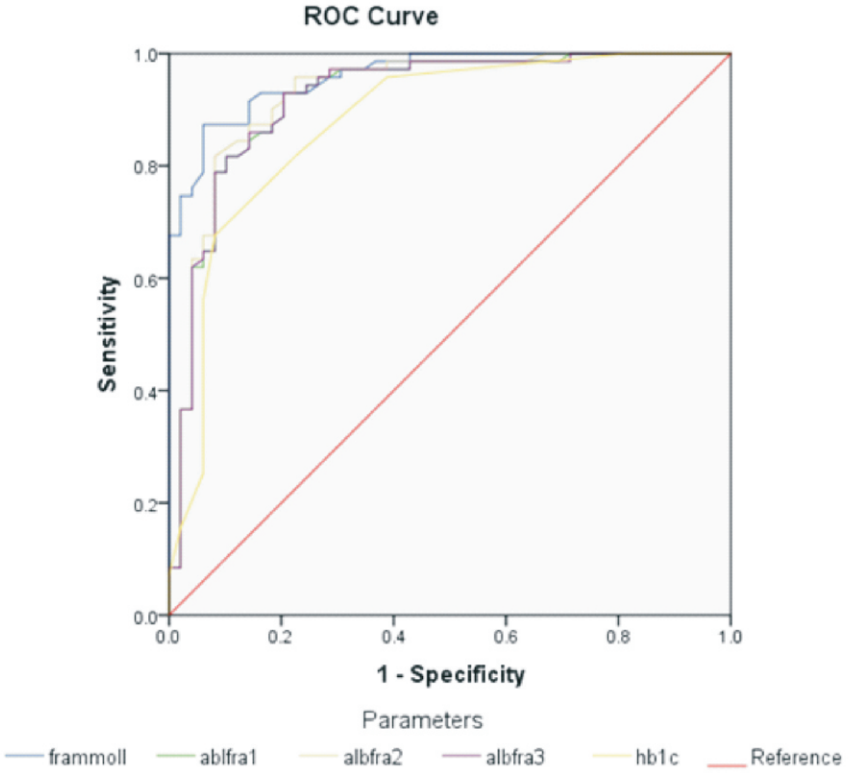

Figure 4: ROC Curve of fructosamine and HbA_{1c} (< 7mmol/L) as a Predictor of glycaemic control

DISCUSSION

The laboratory cut-off values for the diagnosis and management of the DM has constantly being changing over the years. For example, in 2011, WHO and ADA ratified the classification of HbA_{1c} as a diagnostic tool rather than just for monitoring of DM while fructosamine remains a monitoring tool[6, 7]. With the increasing prevalence of Diabetes mellitus and the forecasted increase due to modernization, adequate monitoring of short, intermediate and long-term blood glucose level becomes more important. While the use of FBG and HbA_{1c} for short and long-term monitoring of glycaemia among T2DM patients is relatively well established, the use of serum fructosamine as an intermediate-term glycaemic marker has not gained as much popularity as the earlier two especially in our environment.

The result from this study showed a significantly higher serum fructosamine values among T2DM patients compared to the controls as expected of an index of glycaemia. This is similar to the findings of the study carried out by Isah *et al* in Zaria in 1990 among diabetic and non-diabetic patients which also showed a higher fructosamine levels among T2DM patients when compared to non-diabetic individuals.[8] The study further revealed a significant positive correlation between the serum fructosamine values, FBG and HbA_{1c} among T2DM patients. This is similar to a study by Rosediani *et al* in Malaysia in 2006 which showed a significant correlation between FBG and both HbA_{1c} and fructosamine.[9] In the same study, HbA_{1c} correlated better with FBG than fructosamine. The disparity in the strength of correlation between the older study and this present study may be partly attributed to the relatively smaller sample size of 82 in the older study compared to 120 of the present one. The correlation of fructosamine with FBG and HbA_{1c} among controls was however different. While fructosamine had a weak positive correlation with FBG, it appeared to have no

correlation with HbA_{1c} among this group. This could mean that fructosamine may not adequately predict the glycaemic levels of non-diabetic individuals when compared with FBG and HbA1c according to the findings of this study. This may also limit the usefulness of fructosamine only to confirmed diabetic cases as pointed out also in an article by Justyna *et al*. [10]

Albumin was observed to be slightly higher among control than the diabetic patients. This could be explained by the fact that albumin being a negative acute-phase protein may be reduce in response to inflammatory conditions. These inflammatory conditions will likely be more prominent among the diabetic participants considering the pathophysiology of this ailment.

Fructosamine levels were higher in diabetics but had a positively skewed pattern of distribution when compared with controls with a normal distribution. The higher standard deviation of the cases is likely due to the wider spread of the individual values which is expected considering the different degrees of glucose control in this group of participants.

Our study showed little relationship between fructosamine, FBG and HbA_{1c} in normal participants. While HbA_{1c} had a positive correlation with FBG among the non-diabetic participants, fructosamine showed weak correlation to FBG and HbA_{1c} among this group of participants as against the correlation it had among the T2DM patients. It is therefore unlikely that fructosamine values will be helpful to dichotomize normal from diabetic patients in this population. This finding of a weak correlation of fructosamine with FGB among apparently normal participants was equally noted by Isah *et al* in Zaria Nigeria.[8] HbA_{1c} was not part of that study.

Diagnostic cut-point equivalents for fructosamine could be useful to identify T2DM patients with hyperglycaemia above desirable limits in settings where FBG or HbA_{1c} are not available or where the interpretation of these traditional measures is problematic, such as in settings with high prevalence of haemoglobinopathies. From the regression analysis of the data obtained from the T2DM patients of this study, a fructosamine levels of around 331 µmol/L which correspond to HbA_{1c} value of 7.0% could predict good glycaemic control in this group of patients according to this study. This would mean T2DM patients with fructosamine value of $\leq$ 331 µmol/L are in good glycaemic control while patients with fructosamine values >331 µmol/L would be regarded as having inadequate glycaemic control.

A positive correlation between fructosamine and albumin concentration was also observed in this study. This observation is in agreement with previous reports by Hindle *et al*.[11] It was also reported that serum fructosamine level may not be valid in hypoalbuminaemia, but in individuals with albumin level greater than 30 g/L. The least albumin value among the participants of this study was 38 g/L. Some other studies also found that serum fructosamine concentrations are valid and independent of albumin concentration.[12, 13] Albumin plays a significant role in fructosamine formation, being the most abundant protein in circulation and in view of its relatively long half-life (17-20 days) and possession of several lysine residues.[101] About 80% of the fructosamine in serum is said to be accounted for by albumin.[14]

The limitations of this study included the diagnosis of T2DM based on patient's history and clinical clues alone. Also the study assumed that every adult participant with Diabetes Mellitus had T2DM. Other laboratory tests were not done to properly classify patients as either T1DM or T2DM.

CONCLUSION

This study has shown that serum fructosamine correlate significantly with the FBG among T2DM patients and even revealed a slightly better correlation than HbA_{1c} and can therefore be used to monitor blood glucose level among T2DM patients especially when shorter follow-up and a cheaper test could benefit the patient. The study also revealed that serum fructosamine level of $\leq$ 331 µmol/L (corresponding to HbA_{1c} of $\leq$ 7.0%) could also predict adequate glycaemic control among

T2DM patients.

The study therefore recommends serum fructosamine assay as a complemetary glucose monitoring tool for shorter durations glucose monitoring alongside HbA_{1c} and FBG in the management of T2DM patients especially considering patients who access health care using out of pocket payments.

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