

Assessment of Glycation Gap in Diabetes Mellitus Patients**R. Revathi¹, Rajini Samuel², Balaji Rajagopalan³**¹MSc, Ph.D., Assistant Professor of Biochemistry, Shri Sathya Sai Medical College and Research institute, Sri Balaji Vidyapeeth Deemed to be University, Guduvancherry-Thiruporur Main Road, Ammapettai, Chengalpet district: 603108²M.D, Professor of Biochemistry, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth Deemed to be University³M.D, Professor & HOD of Biochemistry, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth Deemed to be University

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Conflict of interest: Nil

Abstract:

Glycated hemoglobin (HbA1c) is a most preferred test for monitoring of diabetes mellitus patients but it is altered under certain conditions so fructosamine is used as an alternative for short term monitoring of blood glucose levels. HbA1c can be calculated from serum fructosamine. The difference between measured and calculated HbA1c is called glycation gap. This gap is altered under different glycation conditions which may serve as a simple tool to monitor the glycation levels. The aim of the present research study is to compare the serum fructosamine and HbA1c and then to calculate and assess the glycation gap in diabetic patients without complications. In our study, 72 diabetic patients without complications were included and based on serum fructosamine values they are divided into three groups. Serum fructosamine, HbA1c and Hemoglobin were measured. Calculated HbA1c values are obtained using calculated HbA1c-fructosamine regression equation. The difference between measured and calculated HbA1c termed the glycation gap was calculated for all the cases. The relationship between these parameters was graphically analyzed. Mean and standard deviation was calculated for all the 3 groups and one way statistical analysis test was done. The glycation gap was found to be negative in all these cases and statistically significant between the three groups.

Keywords: Glycation Gap, Fructosamine, Glycated Hemoglobin.

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Introduction

Haemoglobin (Hb)A1c results from non-enzymatic glycation in erythrocytes. Hence, HbA1c concentration reflects the concentration of glucose to which erythrocytes are exposed over their lifespan and so it was introduced into clinical practice as a marker of glycemic control. It is accepted that HbA1c correlates with average blood glucose levels over the preceding 2–3 months. [1-4] HbA1c is related to the risk of microvascular and macrovascular complications. The correlation between glycemia and HbA1c was influenced by various factors like age, hemoglobinopathies, drugs, renal failure and some diseases may affect HbA1c. HbA glycation in erythrocytes depends on glucose transport into erythrocytes and on intracellular glucose and protein metabolism. [5-7]

Fructosamine is a glycated serum protein (mainly albumin). It is the most widely used alternative to HbA1c. Some studies have attempted to interpret the parallel measurement of

fructosamine and HbA1c. [8-11] Fructosamine correlates rather well with HbA1c, The correlation is not observed in certain cases due to the difference in time duration during which HbA1c and fructosamine are exposed to mean glucose level and also individual variations between intracellular and extracellular protein glycation capacity. [1,2,8,9,10]

Fructosamine measuring extracellular glycated protein is not related to extracellular and intracellular glucose dynamics. The discordances between HbA1c and fructosamine is quantitated in the form of glycation gap (GG), and it correlates with the frequency of a major microvascular complication of diabetes. [3,5,6,11] Cohen et al defined the term glycation gap (GG) as the difference between the measured HbA1c and the HbA1c predicted from the measure of fructosamine, based on the HbA1c–fructosamine regression equation. [3,5] Glycation gap could denote the changes in HbA1c that may be a useful clinical tool for evaluating physiologic sources of

variation in diabetic complications beyond glycemic control. The observed discordance reflect presence of disease and diabetic complications. Glycation Gap can be either positive, negative or zero. Previous studies have observed positive glycation gap in diabetic complications and negative gap in diabetic without complications. GG predicts the progression of nephropathy in Type2DM. The aim of the present research study is to compare the serum fructosamine and HbA1c and then to calculate and assess the glycation gap in diabetic patients without complications.

Materials and Methods:

This study was conducted at Shri Sathya Sai Medical College and Research Institute for the period July 2023 to September 2023. Institute ethical clearance was obtained and IEC number is 2022/798. The sample size was 72 Diabetic patients without complications. Diabetic patients with complications were excluded for this study. The normal reference range for serum fructosamine is 205 to 285 micromole/Liter. Serum Fructosamine was estimated using BTS 350 semi-autoanalyzer and HbA1c was estimated using Mispa I2. The haemoglobin concentration was determined using Sysmex cell counter and noted for all the patients. The HbA1C(%) is calculated from fructosamine (micromol/litre) using the cohen et al regression

model. The difference between measured HbA1c and calculated HbA1c is called the glycation gap. [1,4,5]

Calculated HbA1c: $HbA1c = 0.017 \times \text{fructosamine} + 1.61$.

Glycation Gap (GG): $GG = \text{measured HbA1c} - \text{predicted HbA1c}$.

Results:

72 Patients were divided into 3 groups based on Serum fructosamine values (in micromole/litre). The values from 320 to 440 micromol/litre were included in group 1, values from 450 to 620 micromol/litre were included in group 2 and values from 630 to 1020 micromol/litre were included in group 3. The mean and standard deviation for serum fructosamine, Measured HbA1c, Calculated HbA1c, glycation gap and hemoglobin concentration was calculated for all the 3 groups and shown in table 1. Oneway ANOVA statistical test was used to compare the glycation gap and hemoglobin concentration in the 3 different groups and shown in the tables 2 and 3 respectively. The relationship between serum fructosamine, Measured HbA1c, Calculated HbA1c, glycation gap and hemoglobin concentration was graphically analyzed and shown in the graphs (1 to 8).

Table 1: Comparison of Glycation Gap in 3 different groups

Group 1: Serum Fructosamine values from 320 to 440 micromol/litre					
	Fructosamine micromol/litre	Calculated HbA1C(%)	Measured HbA1c(%)	Glycation Gap	Hemoglobin g/dl
Mean	384.17	8.14	6.10	-2.05	12.84
StdDev	39.44	0.67	0.76	0.73	1.95
Group 2: Serum Fructosamine values from 450 to 620 micromol/litre					
	Fructosamine micromol/litre	Calculated HbA1C(%)	Measured HbA1c(%)	Glycation Gap	Hemoglobin g/dl
Mean	530.83	10.63	6.83	-3.81	11.83
StdDev	58.67	1.00	1.16	1.31	1.33
Group 3: Serum Fructosamine values from 630 to 1020 micromol/litre					
	Fructosamine micromol/litre	Calculated HbA1C(%)	Measured HbA1c(%)	Glycation Gap	Hemoglobin g/dl
Mean	736.67	14.13	8.54	-5.59	13.44
StdDev	99.46	1.69	1.05	1.48	1.87

Table 2: One Way Anova Statistical Analysis for the Glycation Gap in 3 Groups

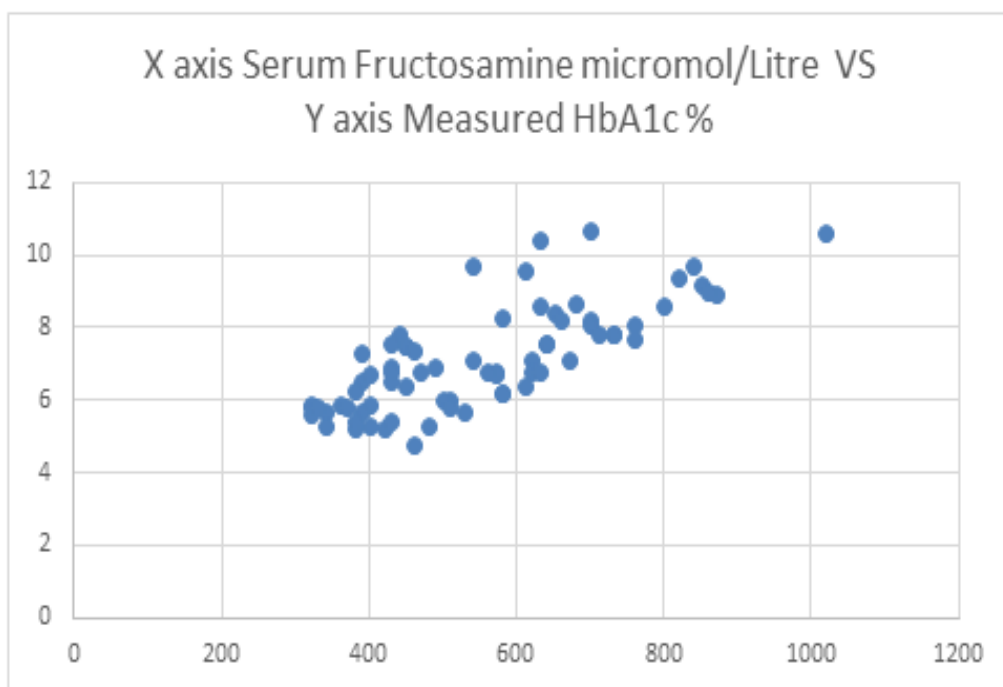
	Groups			Total
	1	2	3	
N	24	24	24	72
$\sum X$	-49.08	-91.42	-134.2	-274.7
Mean	-2.045	-3.8092	-5.5917	-3.815
$\sum X^2$	112.5754	387.966	800.6358	1301.1772
Std.Dev.	0.7285	1.3143	1.4779	1.8881

The f-ratio value is 50.96. The p-value is <0.00001. The result is significant at p.

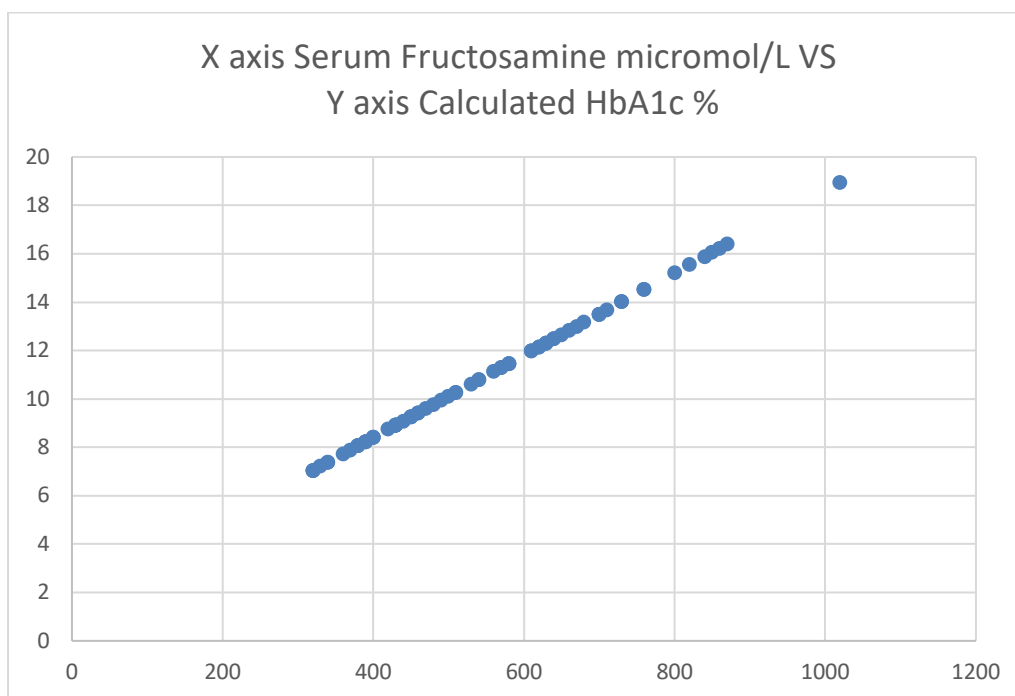
Table 3: One Way Anova Statistical Analysis for the Hemoglobin 3 Groups

	Groups			Total
	1	2	3	
N	24	24	24	72
$\sum X$	308.1	283.9	322.5	914.5
Mean	12.8375	11.8292	13.4375	12.701
$\sum X^2$	4042.35	3399.03	4414.29	11855.67
Std.Dev.	1.9462	1.3307	1.8731	1.8395

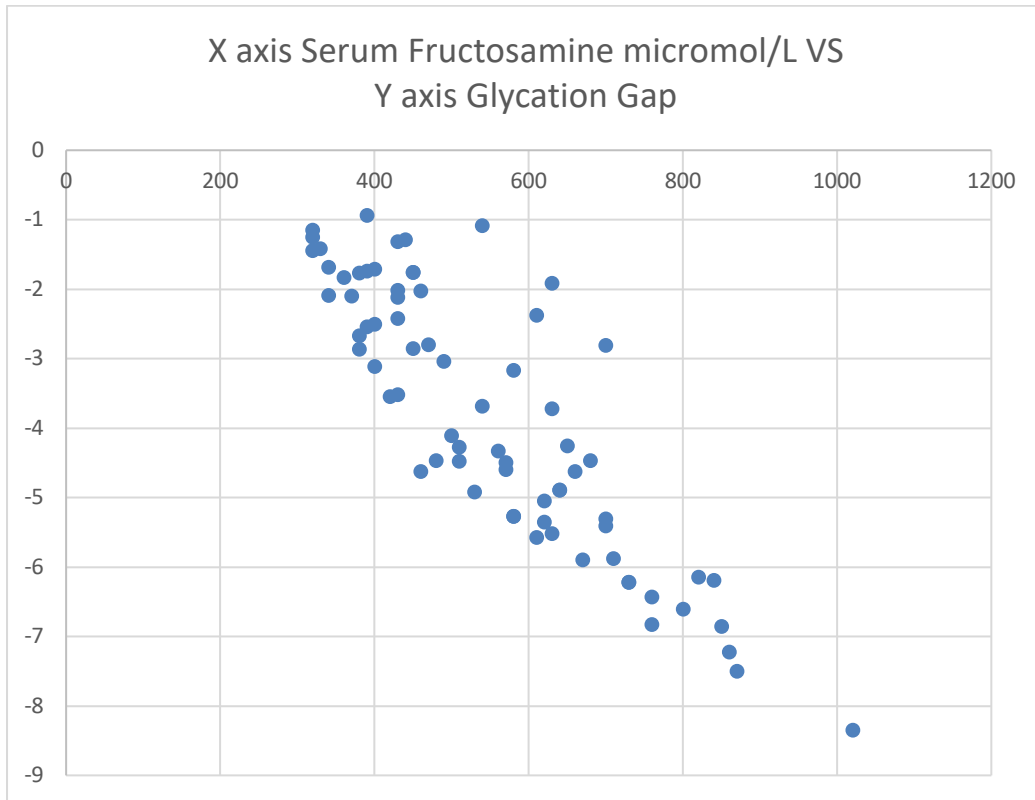
The f-ratio value is 5.24. The p-value is 0.0075. The result is significant at $p < 0.05$.



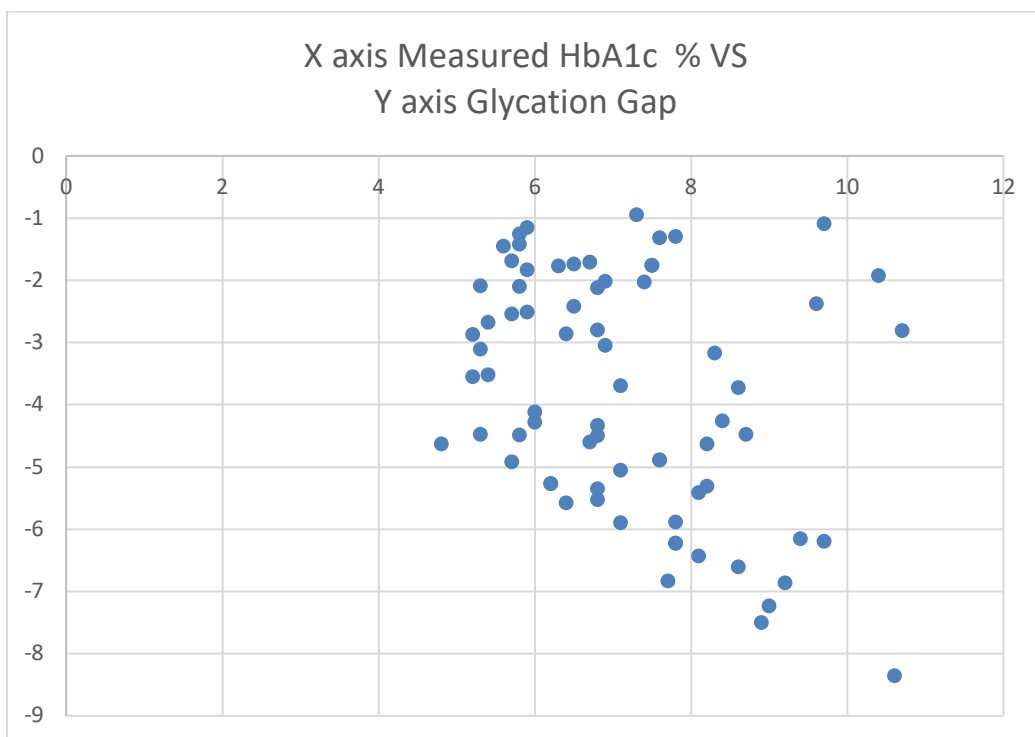
Graph 1: Serum Fructosamine VS Measured HbA1c



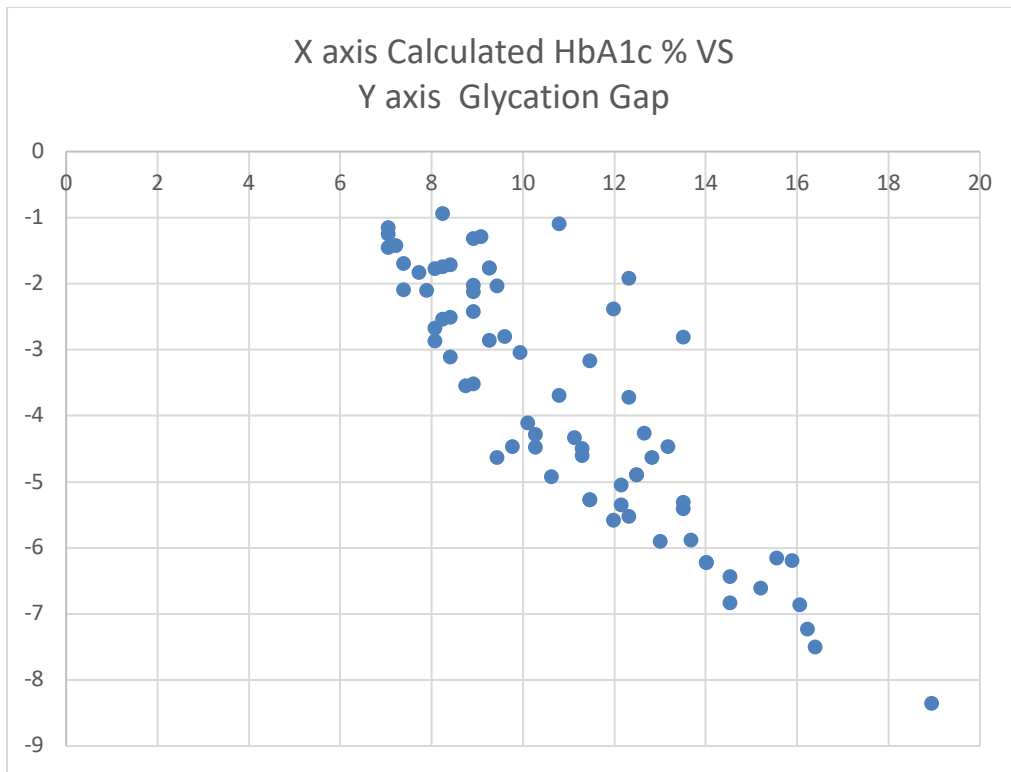
Graph 2: Serum Fructosamine VS Calculated HbA1c



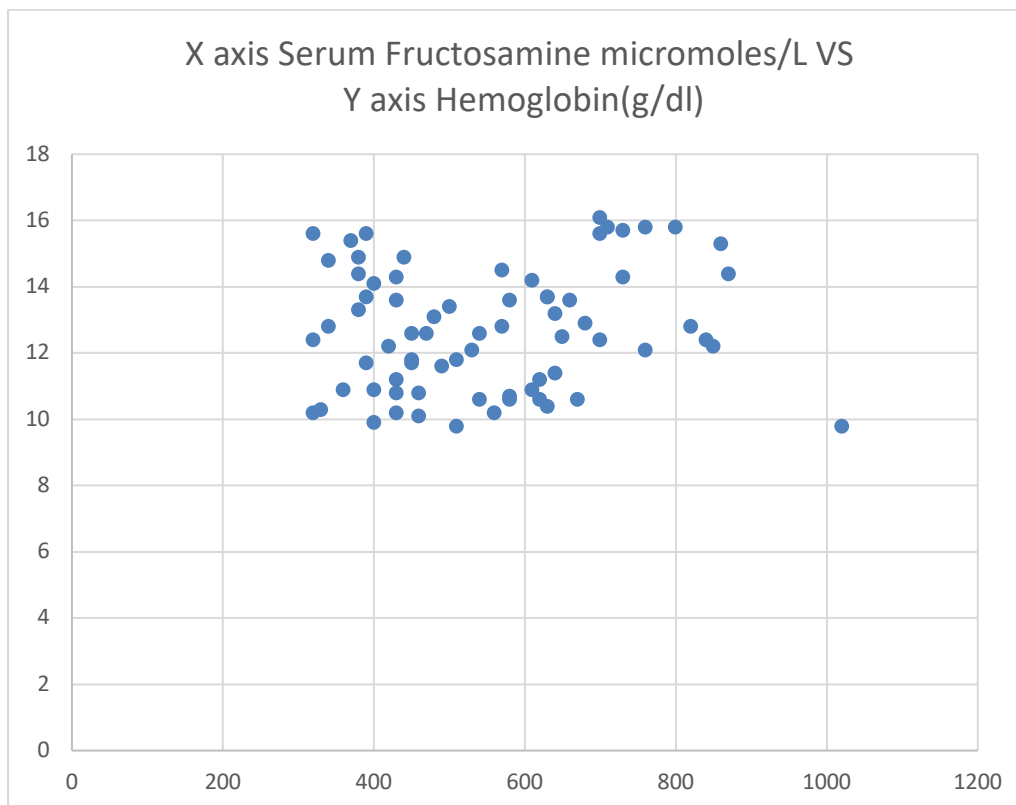
Graph3:Serum Fructosamine VS Glycation Gap



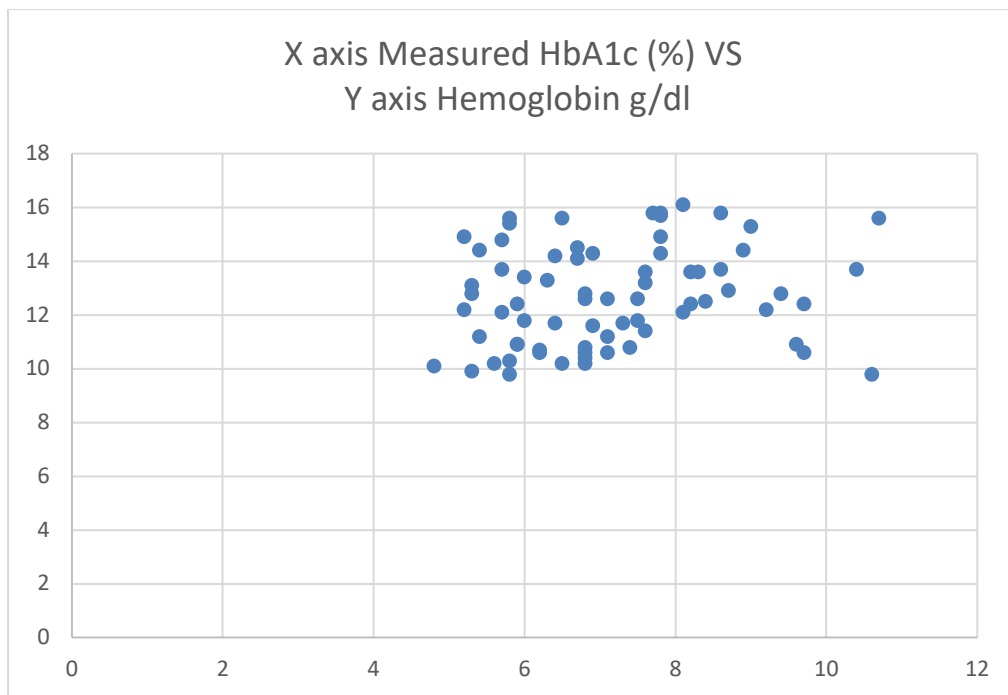
Graph4:Measured HbA1c VS Glycation Gap



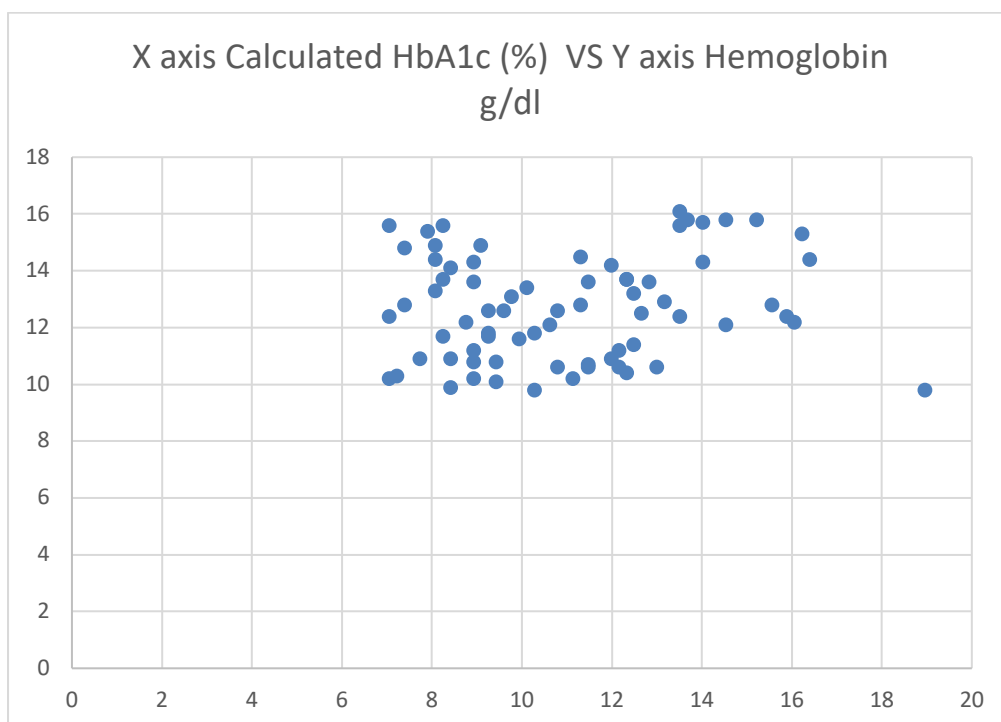
Graph 5: Calculated HbA1c VS Glycation



Graph 6: Serum Fructosamine VS Hemoglobin



Graph 7: Measured HbA1c VS Hemoglobin



Graph 8: Calculated HbA1c VS Hemoglobin

Discussion:

Diabetes mellitus is a major public health problem resulting in mortality and morbidity throughout the world. The glycation Process affects both microand microvasculature of the diabetes patients.[12,13] HbA1c and fructosamine plays a significant role in monitoring of long term and short term control of blood glucose respectively. The identification of the differences in the glycation process between these two parameters may serve as a useful marker to

monitor the glycemic status and its resulting complications in the patients.[5,9,14] The Glycation Gap (GG) is the difference between measured gly-cated hemoglobin (HbA1c) and HbA1c predicted from fructosamine.

Cohen et al defined glycation gap as the difference between the measured HbA1c and the HbA1c predicted from the measured fructosamine based on the HbA1c-fructosamine regression equation. It is a well known fact that diabetic complications are due

to prolong exposure to excess glucose. So long term control of blood glucose is required. The process of protein glycation serves as a marker for the progress of diabetes complication & underlying cause of most serious complication. The relation between fructosamine and Measured HbA1c is shown in the graph 1. The relation between fructosamine and Calculated HbA1c shown in the graph 2 is a straight line because it is directly calculated using the known derived relation. The relation between fructosamine, Measured HbA1c, Calculated HbA1c with glycation gap are shown in the graphs 3,4 and 5 respectively. The relation between fructosamine, Measured HbA1c, Calculated HbA1c with hemoglobin concentration was shown in the graphs 6, 7 and 8 respectively.

One way ANOVA statistical test was used. The f-ratio value is 50.96 and the p-value is <0.00001 for Comparison of Glycation Gap in 3 different groups so the p value is significant. The f-ratio value is 5.24 and the p-value is 0.0075 for Comparison of hemoglobin concentration in 3 different groups and so the result is significant at $p < 0.05$. The low F-value indicate that the group means are close together (low variability) relative to the variability within each group. The high F-value indicate that the variability of group means is large relative to the within group variability.

The mean and standard deviation of glycation gap in group 1(Serum Fructosamine values from 320 to 440 micromol/litre) is -2.05 ± 0.73 , group 2(Serum Fructosamine values from 450 to 620 micromol/litre) is -3.81 ± 1.31 and for group 3(Serum Fructosamine values from 630 to 1020 micromol/litre) is -5.59 ± 1.48 . The mean and standard deviation of hemoglobin concentration in group 1 is 12.84 ± 1.95 , group 2 is 11.83 ± 1.33 and for group 3 is 13.44 ± 1.87 . From our study, it is obviously seen that glycation gap was negative in all these diabetic patients without complications.

Conclusion

The glycation gap was negative in our study of diabetic patients without complications. The glycation gap may serve as a useful simple tool to assess the complications of diabetes mellitus with other routine parameters commonly used to monitor diabetes mellitus patients.

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