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Correlation between eAG Values Calculated from HbA1C Values and Fasting Plasma Glucose Levels for Diagnosis and Prognosis of Diabetes

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Abstract: Objective: The objective was to correlate eAG values calculated from HbA1c values obtained from Tosoh TLC-723 G8 based on cation exchange based HPLC and TBA 12 FR enzymatic fructosyl peptide oxidase (FPOX) method with fasting plasma glucose levels by hexokinase method for diagnosis and prognosis of diabetes.

Subjects and Methods: 300 randomly selected patient samples were analysed for fasting plasma glucose (FPG) by Hexokinase method and HbA1c values estimated by using Tosoh TLC-723 G8 analyser based on cation exchange HPLC and enzymatic fructosyl peptide oxidase (FPOX) (Manufacture by Seikisui medical co. ltd., Japan) programmed on TBA 12 FR biochemistry analyser. The estimated average glucose (eAG) values were calculated using Nanthan's regression equation for both HbA1c values. The correlation between FPG, HbA1c and eAG values has been done by Pearson correlation (r) and regression analysis was done by SPSS software.

Results: The correlation was good in entire data and poor in differentiated groups. Male data has shown good correlation between FPG and eAG as compared to female data with higher mean levels of FPG, eAG and HbA1c values. HPLC method has shown closer correlation between eAG values calculated from it and FPG values.

Conclusion: The overall correlation between eAG and FPG is good in entire data but mean value difference was found to be slightly high. So for diabetes diagnosis HbA1c values along with calculated eAG value along with FPG values is recommended as per ADA guidelines. As HPLC has shown good correlation between eAG and FPG values we recommend HbA1c values and eAG values calculated from it has to be used for diagnosis and prognosis of diabetes along with FPG values and other relevant clinical findings of patients. The use of HbA1c values alone for diagnosis and prognosis of diabetes still need multicentre studies with large sample size in different population.

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder marked by hyperglycemia with alteration in normal status of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion, insulin action or both. Diabetes mellitus is associated with retinopathy, nephropathy, peripheral neuropathy, cardiovascular disorder and other lethal complications of organ system. (Sarmah et al. 2012). According to International Diabetes Federation (IDF) 2013, prevalence of type 2 diabetes mellitus is increasing in epidemic proportions worldwide and developing countries like India has alarming situation for this disease. The number of people living with diabetes is expected to rise from 382 million in 2013 to 592 million by 2035. As per IDF report India alone has more than 65 million people living with diabetes and this number expected to reach 109 million by 2030.

An accurate measurement of blood glucose and evaluation of blood glucose control is helpful to assess the efficiency of particular therapy related to diabetes mellitus. Before 2010, all diabetes societies recommended blood glucose analysis as the exclusive method to diagnose diabetes. (Sacks, et al 2011) But it was year 2009, when an International Expert Committee that included representatives of the American Diabetes Association (ADA), the International Diabetes Federation (IDF), and the European Association for the Study of Diabetes (EASD) recommended the use of the A1C test to diagnose diabetes, with a threshold of $\geq 6.5\%$ (48 mmol/mol), and ADA adopted this criterion in 2010. The clinical significance of HbA1c was supported by Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS). (Sarmah et al. 2012) According to report published by ADA in 2010, criteria for diagnosis of diabetes are as follows:

- A. HbA1c $\geq 6.5\%$. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.* OR
- B. FPG ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.* OR
- C. 2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*OR
- D. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/l).

(*In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.)

HbA1c is now generally accepted as the single, most prominent and independent parameter of metabolic control, a risk factor for the development of diabetic complications and is widely used as treatment goal in disease management. (Farzana et al) The HbA1c value indicates a patient's average blood glucose level during the previous 60 – 90 days. As recommended by ADA, HbA1c test can be performed at least two times a year in patient with diabetes who are meeting treatment goals with stable glycemic control and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals.

Being a 'gold standard' test for diagnosis of diabetes, HbA1c value has prognostic potential also. HbA1c is useful biomarker for long-term glycemic control as well as a good predictor of lipid profile which eventually can predict the risk of cardiovascular complication in diabetes patients. A study done by Khan et al in 2007, on 1011 type 2 diabetic patients, HbA1c exhibited direct correlations with cholesterol, triglycerides, and low density lipoprotein cholesterol and inverse correlation with high-density lipoprotein cholesterol. There was a linear relationship between HbA1c and dyslipidemia as the levels of serum cholesterol and triglycerides were significantly higher and that of high-density lipoprotein cholesterol were significantly lower in patients with worse glycemic control as compared to patients with good glycemic control.

More than 20 different assay methods based on different physical, chemical or immunological characteristic of the Glycated hemoglobin and different analytical principles, such as immunoturbidimetry, cation exchange chromatography, and high-performance liquid chromatography (HPLC) have been used to measure HbA1c. Among all methods, measurement of HbA1c by HPLC became gold standard and has been appointed as the reference method for HbA1c assay by the National Glycohemoglobin Standardization Program (NGSP) in USA. (Sema et.al, 2012).

The HbA1c values are directly proportional to blood sugar value in diabetes mellitus. A diabetic patient with good glucose control has a HbA1c level either in the reference range or close to it. 6% of HbA1c value is equals to 135mg/dL of mean plasma glucose. For every increase in HbA1c of 1%, mean plasma glucose increases by 35 mg/dL. Many studies have shown that A1C is an index of mean plasma glucose over the preceding weeks to months. Many studies have proven that HbA1c is an index of mean plasma glucose over the preceding week to months. HbA1c does not reflect glycemic control over last three months as it claimed as well as HbA1c value as a sole tool for assessing the diabetic status cannot be used clinically. Since level of HbA1c may get affected due to alteration in red blood cell life span, and presence of different types of hemoglobinopathies, it may give rise to false results. (Sultanpur 2010)

However, combination of FPG, OGTT and HbA1c may be the useful battery of test in diagnosis of diabetes which is also recommended by WHO. The relationship between the mean blood glucose level and HbA1c value has been investigated in many studies with different equations. Nathan's regression equation which is recommended by ADA, convert HbA1c values into estimated Average Glucose (eAG) by formula 'eAG(mg/dl) = $28.7 \times \text{HbA1C} - 46.7$ ' (eAG(mmol/l) = $1.59 \times \text{HbA1C} - 2.59$).

In present study, eAG values were obtained from HbA1c values measured by two methods; Tosoh TLC-723 G8 analyser based on cation exchange based HPLC method and enzymatic fructosyl peptide oxidase (FPOX) (Manufacture by Seikisui medical co. Ltd., Japan) method programmed on TBA 12 FR biochemistry analyser. The correlation between these eAG and HbA1c values and fast plasma glucose has been investigated in this study. FPG values obtained by FPG kit based on Hexakinase method from Randox Laboratories (USA). The interchangeability of eAG and FPG values are also investigated for better diagnosis and prognosis of diabetes by measuring HbA1c values.

II. MATERIALS AND METHODS

This study was done at Pyramid Grouplab (PGL), New Panvel, Maharashtra. PGL receives clinical specimens from throughout the India from their logistics network in specially design specimen transport box and Test Requisition Form duly signature by patient.

The timeline of study is February 2011 to June 2013. In this study 300 samples were selected from daily work load at PRL, which came as suspected case of Diabetes (1 or 2) and for routine metabolic management of Diabetes. For these 300 samples we have chosen patients who has done fasting for overnight fasting (at least 8 hours) before collecting samples. Patients from all age group from 23 year to 79 years were included in study.

Venous blood specimens were collected in vacutainer containing EDTA (Ethylene Diamine Tetra acetic Acid) as an anticoagulant (BD Vacutainer with purple colour cap). After collection and prior to analysis, these specimens were mixed gently by inversion to ensure homogeneity. The specimens from different parts of country were collected and packed in specially designed, pre bar-coded specimen transport box containing cool pack for temperature control. Once the specimen reach to PGL, preliminary check was done for temperature and nature of sample. After preliminary checks sample information entered in Laboratory Management Software, sample is bar-coded and forward to different section of laboratory.

In our study, we collected approximately 2 mL of EDTA whole blood from each individual. Once process of patient data entry and bar coding was done, these samples were aliquoted equally in 2 tubes. The first aliquot was sent to satellite laboratory of PGL for FPG analysis. The second aliquot was processed in PGL. We have simultaneously processed sample for two techniques. First samples are processed on Tosoh automated Glycohemoglobin analyser HLC-723 G8. The BD vacutainer containing EDTA whole blood was directly processed by inserting sample tube in sample rack and push the start button. The optimum sample volume was 2mL and minimum was 50 μ L. For samples having less quantity as well as samples showing "area high" flag was diluted (e.g. 1:100, 1:200 as per requirement) manually with G8 variant Dilution solution for further analysis. The values are obtained as SA1c (Stable A1c) in % and were recorded on software as well as printout generated by instrument on thermal paper.

Once samples are processed on Tosoh automated Glycohemoglobin analyser HLC-723 G8 same samples are further processed for HbA1c detection by Enzymatic FPOX method from Norudia N HbA1c reagents (Manufacture by Seikisui medical co. Ltd., Japan) on fully automated TBA 120 FR instrument from Toshiba, Japan. EDTA whole blood sample was centrifuged for 5 minutes at 800g and after that from lower layer of blood cells, 25 μ L of Red blood cells are taken in 1.5mL vial. 500 μ L of Norudia N HbA1c pre-treatment solution is added in the vial and mixed well. This pre-treated sample was used for further analysis on fully automated TBA 120 FR instrument from Toshiba, Japan. Remaining samples stored at 2-8^oC for 14 days. The HbA1c results are expressed in % as well as μ mol/L as per NGSP guidelines. The results are recorded by TBA 120 FR software and printed on paper by dot matrix printer connected to instrument. In both the analytical technologies, erroneous results were repeated and if error remains consistent then patient history and fresh sample was requested.

Before analysing patient samples both methods were calibrated using calibrator level 1 and level 2 provided by manufacturer and the validity of calibration was checked by using control level 1 and level 2 provided by manufacturer. As per ADA guidelines for diagnosis of diabetes, FPG value ≥ 126 mg/dL considered to be diabetic range. In view with this guideline, we have categorised obtained values in three groups on the basis of their FPG values; one below 126 mg/dL, second 126-200 mg/dL and third above 200 mg/dL. As per ADA guideline first group should fall in non-diabetic range, second should fall in pre-diabetic range and third should be in diabetic individuals range. The correlation between FPG, HbA1c and eAG values has been done by Pearson correlation (r) and regression analysis was done by SPSS (version 22) software.

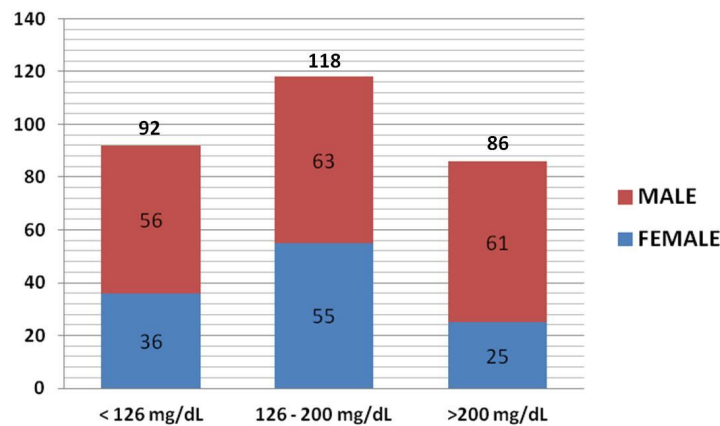
III. RESULTS

Total 300 patients included in the study, among which 4 samples were rejected due to gross hemolysis of sample. Hence, 296 samples were analysed for this study among which 116 (39%) patients were females and 180 (61%) patients were male. The mean age of patients included in the study was 51.31 ± 13.40 years (range 23 – 79)

With FPOX method, results were expressed in HbA1c percentage as well as mmol/L as per IFCC guidelines along with concentration of hemoglobin in μ mol/L and with HPLC methods, results are expressed as SA1c (Stable A1c) in % as well as mmol/L as per IFCC guidelines. In this study, calibration was done daily before assessing samples and controls. After calibration validity of calibration was checked by running control level 1 and level 2 for both methods. On the successful validation of calibration, samples were processed for HbA1c estimation. Also we have given standard guidelines to inculcate IFCC standard for outsourced samples to satellite laboratory for FPG estimation. The FPG results were expressed in mg/dL and mmol/L as recommended IFCC and ADA. The eAG values were calculated with HbA1c values obtained from FPOX method and HPLC method by Nathan's regression equation (i.e., 'eAG(mg/dl) = $28.7 \times \text{HbA1C} - 46.7$ ' (eAG(mmol/l) = $1.59 \times \text{HbA1C} - 2.59$)) which is recommended by ADA.

The data generated in this study is distributed in 3 groups on the basis of their FPG values; one < 126 mg/dL, second 126 - 200 mg/dL and third > 200 mg/dL. Maximum number of samples i.e., 118 samples have given values between 126 - 200 mg/dL,

followed by 92 and 86 number of samples has given values <126 mg/dL and >200 mg/dL respectively. Similar trend was observed in female and male samples involved in study. In female samples are 36, 55, and 25 number of samples have shown values of <126 mg/dL, 126 - 200 mg/dL and >200 mg/dL, respectively, where as in male samples 56, 63 and 61 number have shown values of <126 mg/dL, 126 - 200 mg/dL and >200 mg/dL, respectively. (Figure 1)



(Figure 1 : Number of samples distributed in defined groups)

In our study we have reported four correlation and regression analysis for each group. HbA1c values obtained from three techniques and eAG values calculated with Nanthan’s equation were correlated with FPG values. We have observed that entire data has good correlation values and significant different ($p < 0.05$) as compared to three differentiated groups. All four correlation analysis has shown poor correlation in normal individuals and individuals with higher values of FPG. The HPLC technique has shown good correlation in all the groups as compared to FPOX method. Male data has shown good correlation between FPG and HbA1c & corresponding eAG as compared to female data. We observed that mean levels of FPG, eAG, and HbA1c were higher in male than in females. Statistical difference was found significant in Male and Females eAG, HbA1c values obtained by FPOX method and HPLC method.

CORRELATION ANALYSIS	Entire data		< 126 mg/dL		126 - 200 mg/dL		>200 mg/dL	
	r	R square	r	R square	r	R square	r	R square
FPG Vs HbA1c % (Tosoh G8 by HPLC)	0.922	0.850	0.641	0.411	0.852	0.726	0.608	0.370
FPG Vs eAG (mg/dL) (Tosoh G8 by HPLC)	0.922	0.850	0.641	0.411	0.852	0.726	0.608	0.370
FPG Vs HbA1c % (Seikisui Norudia HbA1c by FPOX method)	0.852	0.726	0.432	0.187	0.565	0.319	0.504	0.254
FPG Vs eAG (Seikisui Norudia HbA1c by FPOX method)	0.852	0.726	0.432	0.187	0.565	0.319	0.504	0.254

(a)

CORRELATION ANALYSIS	Entire Female data		Entire Male data	
	r	R square	r	R square
FPG Vs HbA1c % (Tosoh G8 by HPLC)	0.896	0.803	0.932	0.868
FPG Vs eAG (mg/dL) (Tosoh G8 by HPLC)	0.896	0.803	0.932	0.868
FPG Vs HbA1c % (Seikisui Norudia HbA1c by FPOX method)	0.835	0.698	0.861	0.741
FPG Vs eAG (Seikisui Norudia HbA1c by FPOX method)	0.835	0.698	0.861	0.741

(b)

(Table 1 a & b: Pearson’s Correlation test data by SPSS software)

	Entire Data (N = 296)			Values < 126 mg/dL (N=92)		
	Mean (Min - Max)	Mean (± SEM)	SD	Mean (Min - Max)	Mean (± SEM)	SD
Age	51.31 (23-79)	51.31 (±0.78)	13.40	49.76 (23-74)	49.76 (±1.55)	14.86
FPG (mg/dL)	174.95 (71.31-440.12)	174.95 (±4.68)	80.57	117.98 (70.97-159.94)	117.98 (±2.24)	21.49
HbA1c % (Tosoh G8 by HPLC)	7.94 (4.1-15.8)	7.94 (±0.14)	2.43	6.12 (3.93-9.48)	6.12 (±0.1)	0.94
eAG (mg/dL) (Tosoh G8 by HPLC)	181.1 (70.97-406.76)	181.1 (±4.06)	69.79	128.87 (66.09-225.38)	128.87 (±2.8)	26.87
HbA1c % (Seikisui Norudia HbA1c by FPOX method)	8.28 (3.93-19.17)	8.28 (±0.15)	2.57	5.96 (4.2-7.1)	5.96 (±0.07)	0.65
eAG (Seikisui Norudia HbA1c by FPOX method)	190.93 (66.09-503.48)	190.93 (±4.28)	73.62	124.38 (73.84-157.07)	124.38 (±1.94)	18.63

(a)

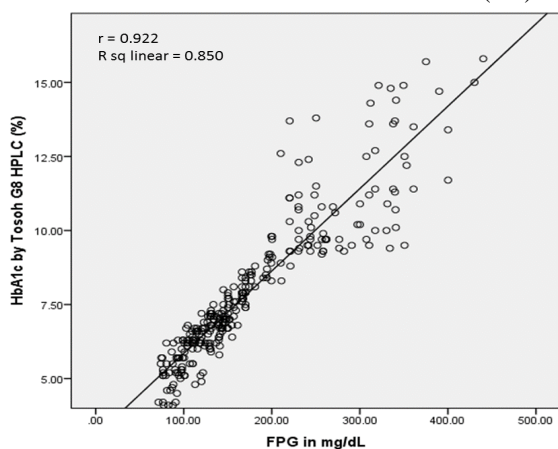
	Values 126 - 200 mg/dL (N= 118)			Values 200 mg/dL (N= 86)		
	Mean (Min - Max)	Mean (± SEM)	SD	Mean (Min - Max)	Mean (± SEM)	SD
Age	55.39 (29-77)	55.39 (±1.04)	11.28	47.36 (28-79)	47.36 (±1.41)	13.04
FPG (mg/dL)	165.14 (119.76 -234.56)	165.14 (±2.15)	23.39	270.5 (191.51 -406.76)	270.5 (±5.81)	53.89
HbA1c % (Tosoh G8 by HPLC)	7.77 (5.24 -10.61)	7.77 (±0.09)	0.93	11.29 (6.1 -19.17)	11.29 (±0.27)	2.47
eAG (mg/dL) (Tosoh G8 by HPLC)	176.29 (103.69 -257.81)	176.29 (±2.47)	26.78	277.42 (128.37 -503.48)	277.42 (±7.64)	70.84
HbA1c % (Seikisui Norudia HbA1c by FPOX method)	7.54 (6.1 -9.7)	7.54 (±0.07)	0.78	11.17 (8.5 -15)	11.17 (±0.2)	1.82
eAG (Seikisui Norudia HbA1c by FPOX method)	169.67 (128.37 -231.69)	169.67 (±2.05)	22.28	273.74 (197.25 -383.8)	273.74 (±5.65)	52.38

(b)

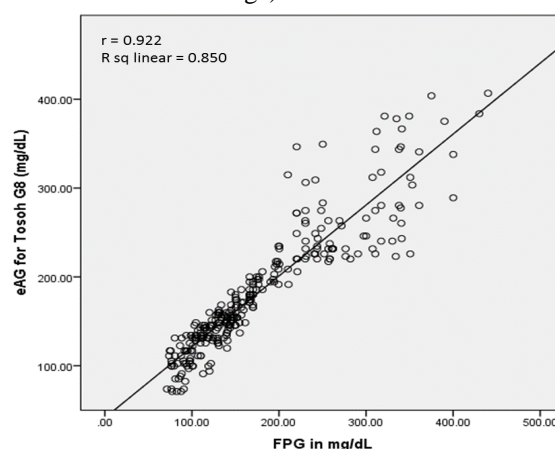
	Entire Female Data (N = 116)			Entire Male Data (N = 180)		
	Mean (Min - Max)	Mean (± SEM)	SD	Mean (Min - Max)	Mean (± SEM)	SD
Age	51.01 (25-77)	51.01 (±1.31)	14.13	51.5 (23-79)	51.5 (±0.96)	12.94
FPG (mg/dL)	164.02 (71.31 -360.97)	164.02 (±6.5)	69.97	181.99 (74.55 -440.12)	181.99 (±6.42)	86.18
HbA1c % (Tosoh G8 by HPLC)	7.62 (4.1 -14.4)	7.62 (±0.2)	2.15	8.14 (4.1 -15.8)	8.14 (±0.19)	2.58
eAG (mg/dL) (Tosoh G8 by HPLC)	171.87 (70.97 -366.58)	171.87 (±5.74)	61.81	187.05 (70.97 -406.76)	187.05 (±5.52)	74.04
HbA1c % (Seikisui Norudia HbA1c by FPOX method)	8.03 (3.93 -19.17)	8.03 (±0.23)	2.43	8.44 (4.46 -16.45)	8.44 (±0.2)	2.64
eAG (Seikisui Norudia HbA1c by FPOX method)	183.75 (66.09 -503.48)	183.75 (±6.47)	69.69	195.56 (81.3 -425.42)	195.56 (±5.66)	75.88

(c)

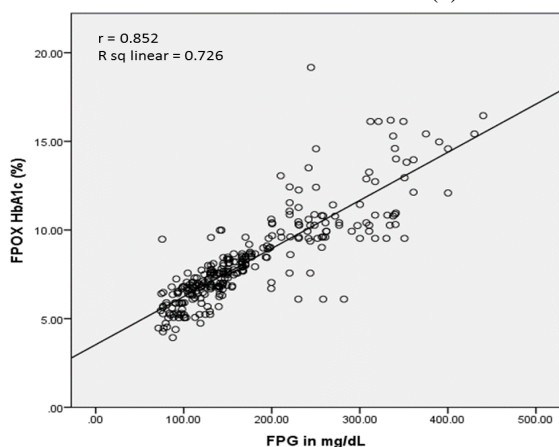
(Table 2 a, b & c: Statistical Comparison of glycemc parameter levels in differentiated groups with standard error mean (SEM), standard deviation (SD) and minimum – maximum range)



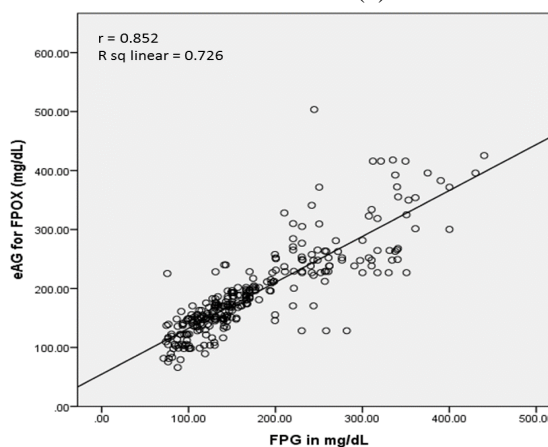
(a)



(b)

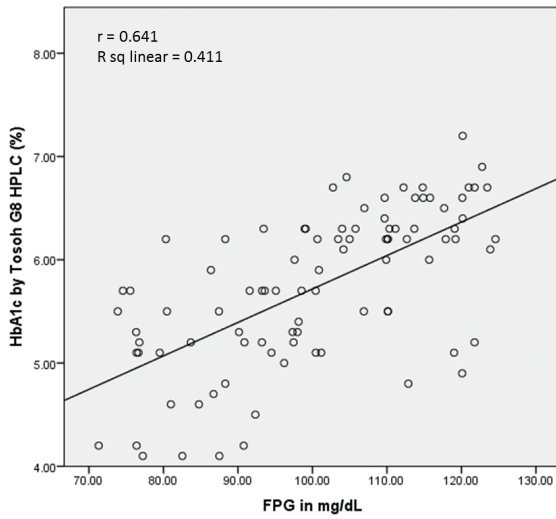


(c)

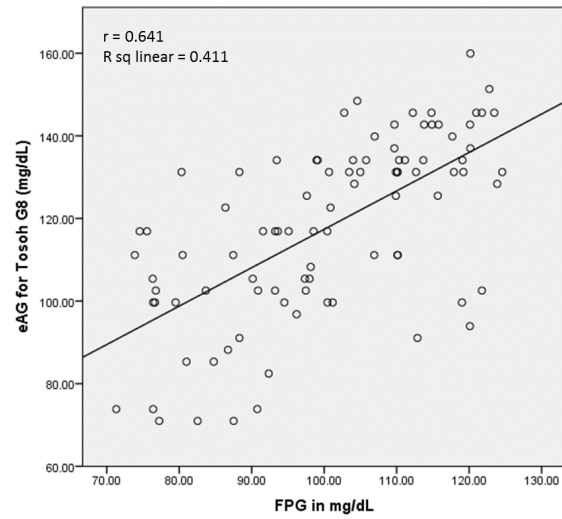


(d)

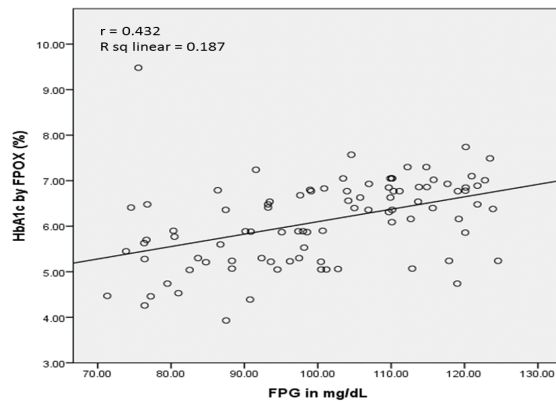
(Figure 2 a, b,c, & d: Correlation plot for “entire data” with r and r square values)



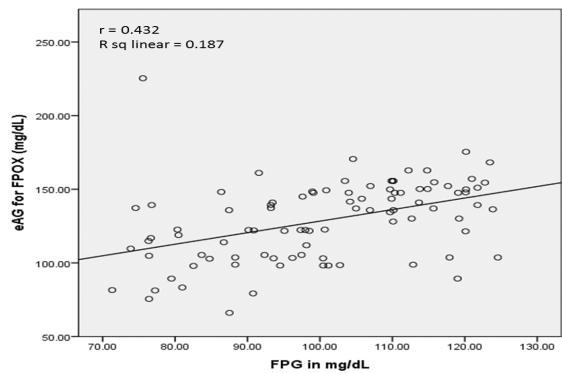
(a)



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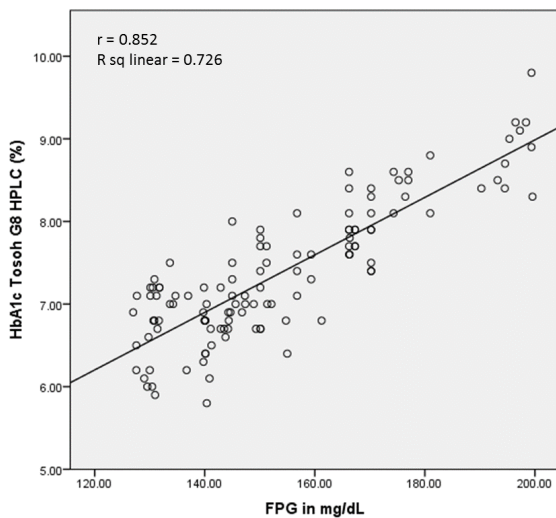


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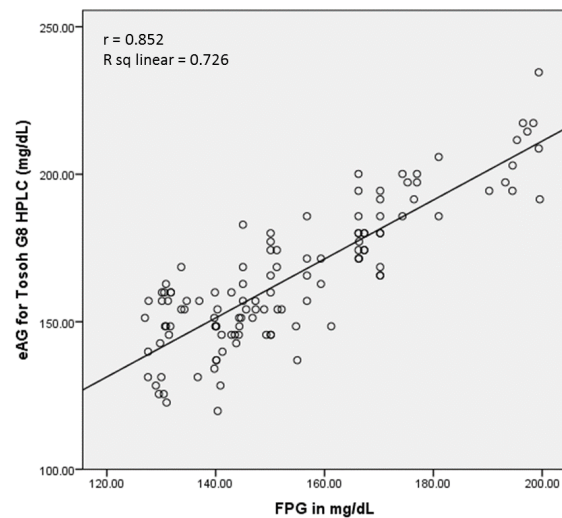


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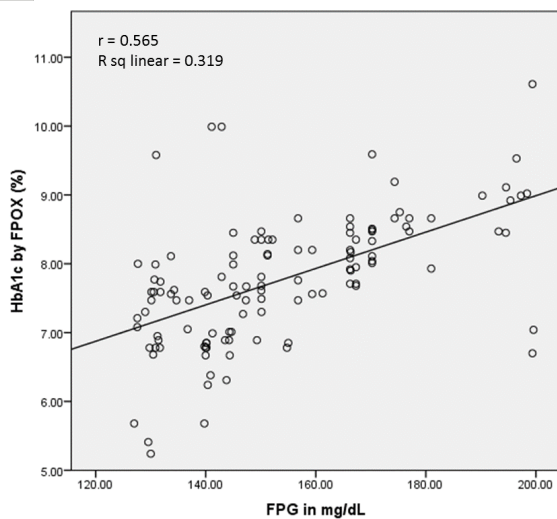
(Figure 3 a, b,c, & d: Correlation plot for samples having values “<126 mg/dL” with r and r square values)



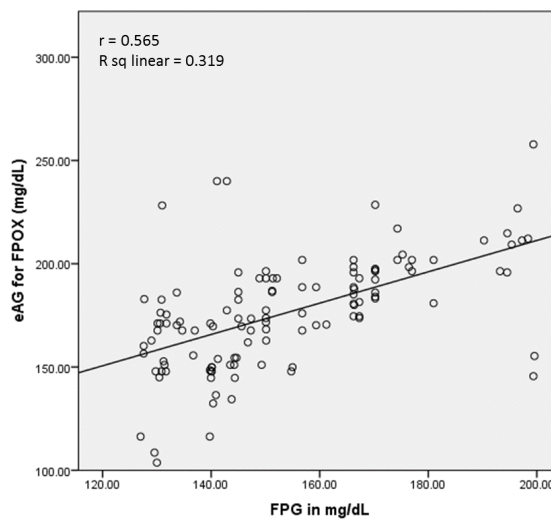
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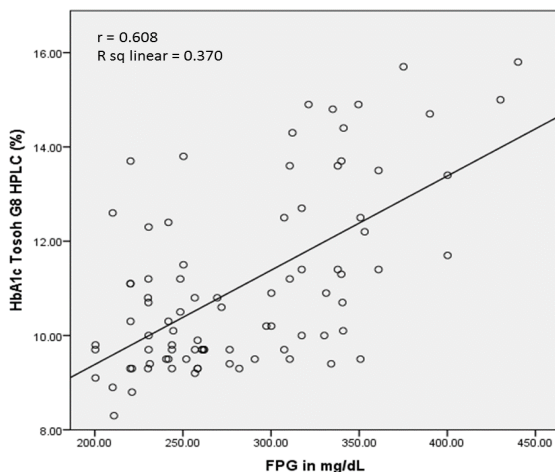


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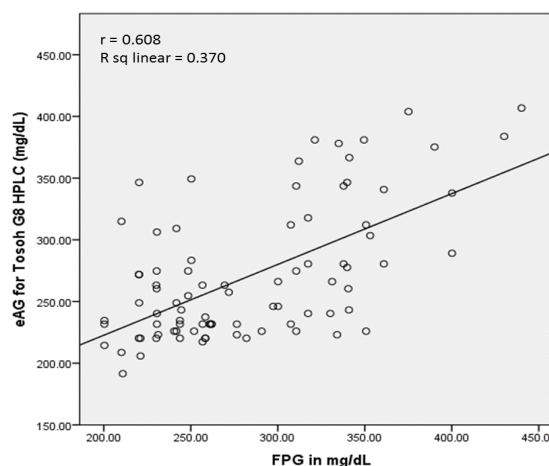


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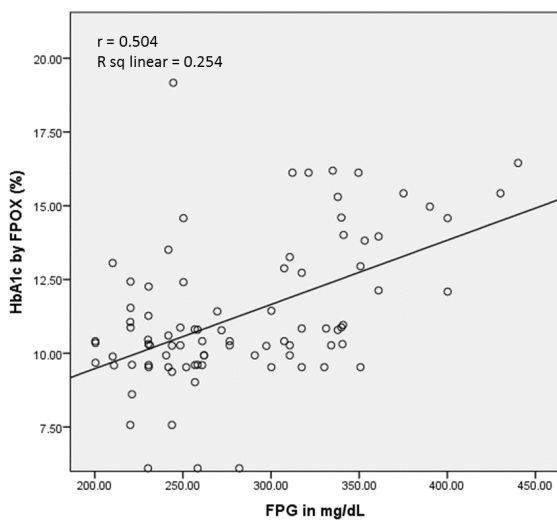
(Figure 4 a, b, c, & d: Correlation plot for samples having values between “126 - 200 mg/dL” with r and r square values)



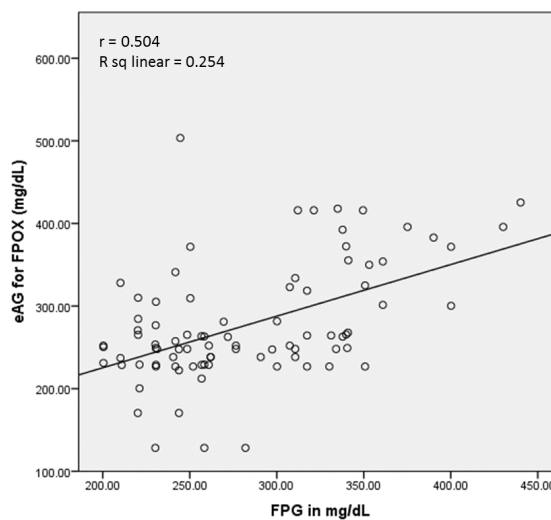
(a)



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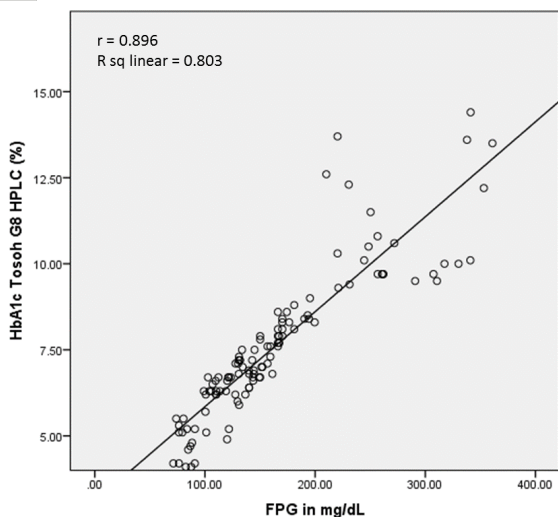


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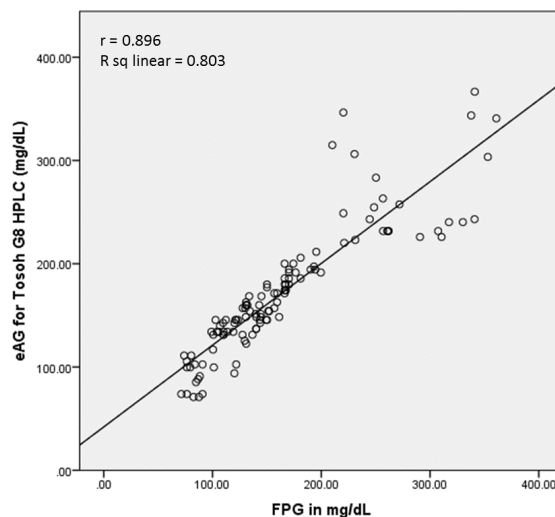


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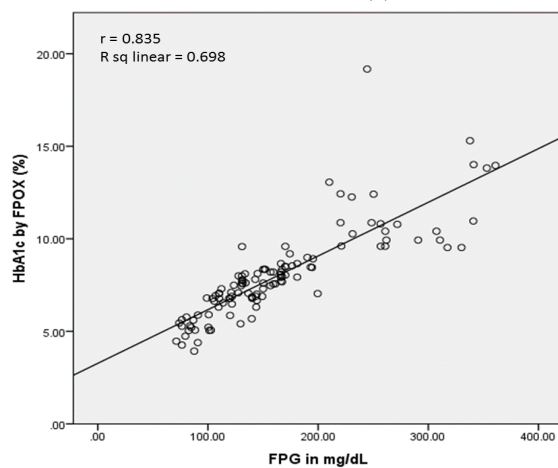
(Figure 5 a, b, c, & d: Correlation plot for samples having values “>200 mg/dL” with r and r square values)



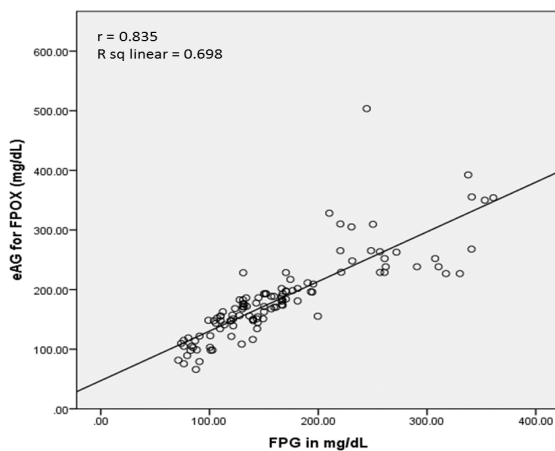
(a)



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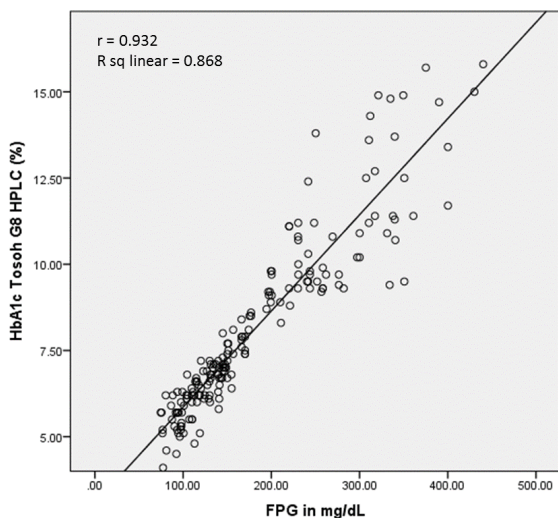


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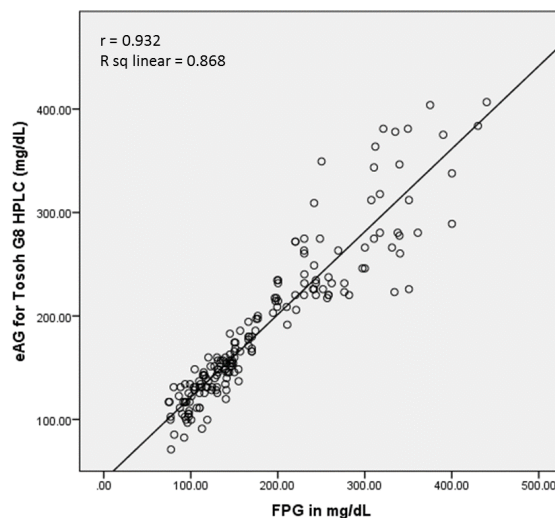


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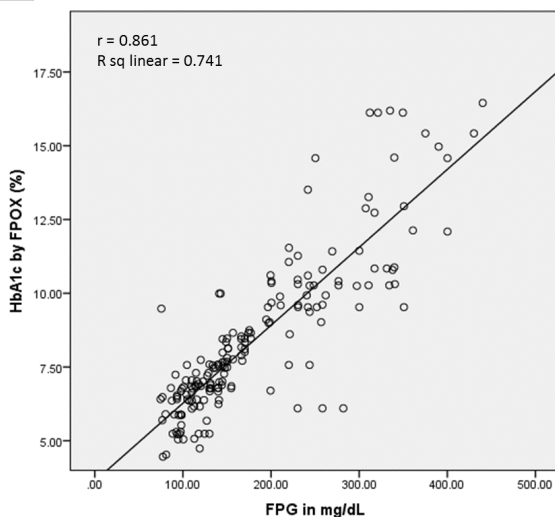
(Figure 6 a, b,c, & d: Correlation plot for “entire FEMALE data” with r and r square values)



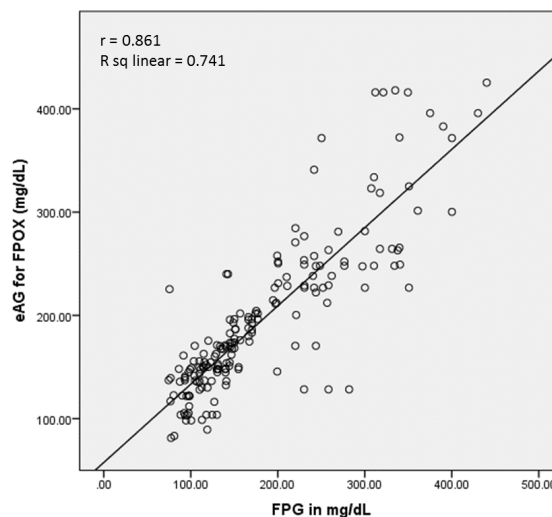
(a)



(b)



(c)



(d)

(Figure 7 a, b,c, & d: Correlation plot for “entire MALE data” with r and r square values)

IV. DISCUSSION

Still one of the remarkable issues with HbA1c measurement is risk of decreasing a patient’s glycemic control; when a patient’s HbA1c measurement is lower than those previous results, he or she might become confused and lead treatment to change diet which could negatively impact patient’s metabolic control. (Hanas 2002) The diabetes diagnostic guideline fixed by ADA which derived from reports of Diabetes Control and Complications Trial (DCCT) and the U.K. Prospective Diabetes Study (UKPDS) has shown acceptance worldwide. The Nanthan’s equation describing relationship between HbA1c and eAG; is used by health care provider and their patients in interpreting HbA1c values in units similar to those that the patients see regularly through self-monitoring worldwide. (Nathan et al 2008) In our study we used the same mathematical relationship equation we have found that eAG values obtained in entire data by HPLC method (i.e., $r = 0.922$ (Tosoh G8)) has shown closer correlation than FPOX methods (i.e., $r = 0.852$). The eAG values obtained from HbA1c values by HPLC method has shown closer mean levels with FPG values. The eAG values were slightly higher than FPG values in all group, and interchangeability of eAG values obtained from HPLC method and FPG will significant as per study. Still we recommend similar study has to be done with multicentre analysis and bigger sample number to make obtained data clinically meaningful. Overall, the data analysed in this study showed good correlation and significant difference between eAG and FPG. The correlation between eAG and FPG level in entire group disappeared when samples were differentiated in subgroup as per their FPG level. As patient’s level of glucose control worsened good correlation observed. In this study we also differentiated same data on the basis of gender. Both Female and male data has shown higher eAG values as compare to FPG values. Female has lower eAG and FPG levels than males.

In diabetes diagnosis and prognosis both values plays important role and interchangeability of these values still has to make a mark and clinical meaningful with strong studies. It is recommended strategy to use eAG level together with HbA1c values but clinical usefulness of eAG is not clear. (Lesile et al 2009) So for diabetes diagnosis HbA1c values along with calculated eAG value along with FPG values is recommended as per ADA guidelines. As HPLC has shown good correlation between eAG and FPG values we recommend HbA1c values and eAG values calculated from it has to be used for diagnosis and prognosis of diabetes along with FPG values and other relevant clinical findings of patients. The use of HbA1c values alone for diagnosis and prognosis of diabetes still need multicentric studies with large sample size in different population.

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