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Original Article

Biochemistry Section

Evaluation of Plasma Glucose Estimations as Reliable Economical Surrogate for HbA1c in Monitoring Glycaemic Status of T2DM Patients: A Retrospective Study

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ABSTRACT

Introduction: In Low and Middle Income Countries (LMIC) like India, either Fasting Plasma Glucose or Postprandial Plasma Glucose (FPG/PPG) estimations were adopted as surrogate alternative to Glycated Haemoglobin (HbA1c) in Type 2 Diabetes Mellitus (T2DM). However, the reliability of this correlation remains ambiguous due to lack of consensus among the previous studies.

Aim: To determine the correlation of FPG and PPG as well as their calculated mean with HbA1c in T2DM subjects for monitoring glycaemic status.

Materials and Methods: A single centre, retrospective, cross-sectional data survey was carried out for a sampling frame of 13 months (August 2017 to August 2018) encompassing 1268 T2DM subjects. The data was collected during September 2018 to March 2019 and subsequently analysed during April 2019 to August 2019. The analysis was carried out in two approaches. In the first approach: the data was segregated into two major groups and six subgroups to understand relative concordance and discordance percentage; sensitivity, specificity and accuracy; and absolute and percentage difference recruiting relevant statistical tools. In the second approach, Area Under Curve (AUC) of Receiver Operating Characteristic (ROC) curves were employed to understand changes in FPG/PPG/calculated mean with increasing severity of T2DM.

Results: With increasing severity of T2DM (HbA1c), not only gradual exacerbation of underestimation in FPG and overestimation in PPG but also declination of sensitivity in either of them was apparent. Though calculated mean of FPG and PPG measurements appended with intermittent features yet mimics PPG. AUC of ROC analysis revealed relatively high PPG levels at lower HbA1c levels and its replacement with FPG with increasing HbA1c levels.

Conclusion: An integrated utility of both FPG and PPG as tuning tools of treatment modalities to achieve desired HbA1c levels in T2DM could be a promising approach.

Keywords: Estimated average glucose, Fasting plasma glucose, Glycated Haemoglobin, Postprandial plasma glucose, Type 2 diabetes mellitus

INTRODUCTION

In LMIC like India, T2DM is recognised as a major public health hazard especially not only because of alarming rise but also due to the rapid shift of its onset in individuals below 50 years of age [1-3]. The devastating aspect of T2DM is chronic hyperglycaemia resulting in significant morbidity and premature mortality [4,5]. Hence, the management of good glycaemic control is the cornerstone of diabetes care. Several groups consolidated the existence of a direct relationship between the diabetic complications and the mean plasma glycaemic value [6-10].

Owing to the inherent attributes, HbA1c established as the Standard Of Care (SOC) for testing and monitoring mean glycaemic status in T2DM [8,9]. HbA1c is well-known to reflect the retrospective mean glucose values as well as the impact of lifestyle and medication on glycaemic control over the past three months [6-10]. Especially in LMIC like India where the majority of accessible laboratories are equipped with resource-poor settings, it is either unavailable or unaffordable [11]. In order to provide an economical and feasible alternative, the existence of a correlation between plasma glucose estimations and HbA1c was explored [12-28]. Various methodological approaches were adopted to explore whether the FPG or PPG is the best surrogate for HbA1c [12-28]. As per these studies, a weak-to-moderate range of correlation coefficient of plasma glucose estimations (FPG: 0.28-0.84 & PPG: 0.20-0.86) with HbA1c was reported [12-28]. The documented cut-off (mg/dL) range for FPG and PPG at HbA1c ≤7% was 110-130 mg/dL and 126-180 mg/dL, respectively [12-28]. The literature survey evidences equivocal reports wherein one of the plasma glucose estimations i.e., either FPG or PPG had relatively high correlation with HbA1c [12-28]. Though correlation coefficient analysis, adopted as traditional analytical tool in earlier studies, explores the linear association of plasma glucose estimations and HbA1c yet from the analytical point of view quantifying the extent of difference between them in terms of overestimation and underestimation as well as percentage difference will also be more eloquent [12-28].

In view of the above facts, the present retrospective study was undertaken with an objective to evaluate the correlation of FPG, PPG, and their mean with HbA1c in terms of whether these plasma glucose measurements (FPG, PPG, and their mean) overestimates or underestimates and if so, the extent of percentage difference between them and its possible implications in clinical intervention in T2DM management. As mean plasma glucose of HbA1c is basically presumed to be the retrospective reflection of integrated fasting and postprandial glycaemic states [16,17], mean of FPG and PPG was included to comprehend its relation with HbA1c in the present study. This is the first study on Indian population exploring the correlation of mean of FPG and PPG with HbA1c.

MATERIALS AND METHODS

The sampling frame duration for the present single-centred, retrospective, cross-sectional data survey was 13 months encompassing August 2017 to August 2018 in the tertiary care hospital "Kovai Medical Centre and Hospital (KMCH), Coimbatore".

The data was procured during September 2018 to March 2019 and subsequently analysed during April 2019 to Aug 2019. The Institutional Human Ethical Committee (REF: EC/AP/634/09/2018; Dated: 02/10/2018) clearance was obtained for this study. Owing to the retrospective nature of the study, IHEC has waived the requirement of informed consent.

Inclusion criteria: Only the medical records of those adults (above 18 years) with a history of T2DM irrespective of its severity and on anti-diabetic therapy were included. The records of the subjects with HbA1c, FPG, and PPG quantitative estimation carried out on the same day during the follow-up visits were only included in this study.

Exclusion criteria: The patient records apparent with anaemia, haemoglobin abnormalities and blood disorders (polycythaemia, leukaemia etc.,), recent blood transfusion, use of drugs that stimulate erythropoiesis, end stage renal disease and pregnancy were excluded from the study.

Sample size calculation: A minimum sample size of 328 was derived, after incorporating the local population T2DM prevalence of 22.6% as well as sensitivity (FPG: 74%; PPG: 79%) and specificity (FPG: 84%; PPG:74%) based on previous analogous study on Indian population and assuming 95% confidence interval with 10% precision [25,29-31]. Even as a rule-of-thumb, a minimum sample size of 300 is recommended as sufficiently large for evaluating both sensitivity and specificity of most screening and diagnostic tests [32,33]. However, in the present study, a total of 1268 subjects medical record data of both in-patients and out-patients was acquired from Medical Records Department (MRD), KMCH, Coimbatore.

Study Procedure

Demographic features, anthropometric measurements, clinical (duration as well as severity of T2DM and details of anti-diabetic treatment protocols) and laboratory data (HbA1c and plasma glucose measurements i.e., FPG and PPG) of each subject were extracted from their respective medical records.

Data segregation: In the present study, in order to validate the agreement of plasma glucose measurements with increasing levels of HbA1c, the data is segregated into two major groups i.e., Group I (HbA1c ≤7%; n=267) and Group II (HbA1c >7%; n=1001) based on the current treatment guidelines [8]. Subsequently group II is further segregated into six unit interval subgroups: i) (HbA1c >7 to ≤8%; n=318), ii) (HbA1c>8 to ≤9%; n=281), iii) (HbA1c>9 to ≤10%; n=177), iv) (HbA1c>10 to ≤11%; n=108), v) (HbA1c>11 to ≤12%; n=54) & vi) (HbA1c>12%; n=63). As a final step, in order to comprehend the relation of plasma glucose measurements with increasing HbA1c based on the AUC of ROC curves, the entire pooled data is reorganised into six groups i.e., A (≤7 vs >7 to ≤8%), B (≤8 vs >8 to ≤9%), C (≤9 vs >9 to ≤10%), D (≤10 vs >10 to ≤11%), E (≤11 vs >11 to ≤12%), and F (≤12 vs >12).

Biochemical examination: The entire process of sample collection, processing, and analysis were strictly carried out under aseptic conditions as per standard laboratory protocols. Both HbA1c and plasma glucose quantification i.e., FPG and PPG estimations were carried out on Cobas Integra 400 Plus Chemistry Analyser (Roche Diagnostics Ltd., Switzerland) using System Packs. The quality control products for HbA1c were also provided by the same company. The HbA1c estimation was based on the "Turbidimetric Inhibition Immunoassay" (TINIA). The measuring range was 4.2-20.1%. The Coefficient of Variation (CV) of repeatability and intermediate precision were within the manufacturer's computations. The plasma glucose estimation is based on the "Hexokinase method" popular as the reference method. The measuring range is 2-720 mg/dL. The Coefficient of variation (CV) of repeatability and intermediate precision were in concurrence with the manufacturer's measurements. Robust routine "Internal Quality Assurance Program" (Bio-Rad Laboratories Pvt., Ltd., India) and "External Quality Assurance Scheme" (CMC-EQAS, Under Aegis of ACBI, Christian Medical College, Vellore, India) were exercised not only to meet and sustain NABH accreditation requirements but also to provide clinically relevant accurate and precise measurements. The mean of FPG and PPG was computed. The estimated Average Glucose (eAG) of HbA1c (%) was derived using the formula "eAG (mg/dL)= 28.7×HbA1c-46.7" [9]. Henceforth, in order to minimise the reprise of "mean of FPG and PPG" in the subsequent sections, it would be presented as "Mean". Similarly, "eAG (mg/dL) of HbA1c" as "eAG".

STATISTICAL ANALYSIS

In the present study, Statistical Package for Social Sciences (SPSS) version 24.0 software was employed for data analysis. The normal distribution of all data was examined with Shapiro-Wilk (SW) test. Continuous variables were presented as mean±standard deviation (SD) or median (interquartile range, IQR) according to the distribution state. Categorical variables were analysed using Chi-squared test (χ^2) and presented as percentages. Spearman correlation was applied to find out the existence of a linear association between fasting/postprandial/mean plasma glucose and the HbA1c (%) as well as its eAG (mg/dL) in type 2 diabetics. Cross tabulations were generated for concordance in classification between eAG and plasma glucose (fasting, postprandial and mean). Post-hoc Chi-squared test (χ^2) with Bonferroni adjustment was used for understanding the concordance percentage difference in multiple pairwise comparisons. In order to understand the difference between plasma glucose (fasting, postprandial, and mean) and eAG, an absolute difference was computed. Absolute difference was presented as median with an interpercentile range encompassing 5th and 95th percentile. Percentage difference between eAG and plasma glucose (fasting, postprandial and mean) was computed as "{(plasma glucose-eAG)/eAG}×100". Sensitivity, specificity, and Positive Predictive Value (PPV) with cut-off value of two major groups were extracted from the AUC of ROC curves whereas for the subgroups, cross-tabulations were used. For the final step, ROC curves of plasma glucose measurements against HbA1c across increasing intervals were constructed and AUC were extracted. The relative distribution of the AUC of plasma glucose measurements (FPG/PPG/Mean) were plotted against the six groups of HbA1c. A two-sided $p<0.05$ was considered significant for all analyses. All the assumptions of the statistical tests were respected.

RESULTS

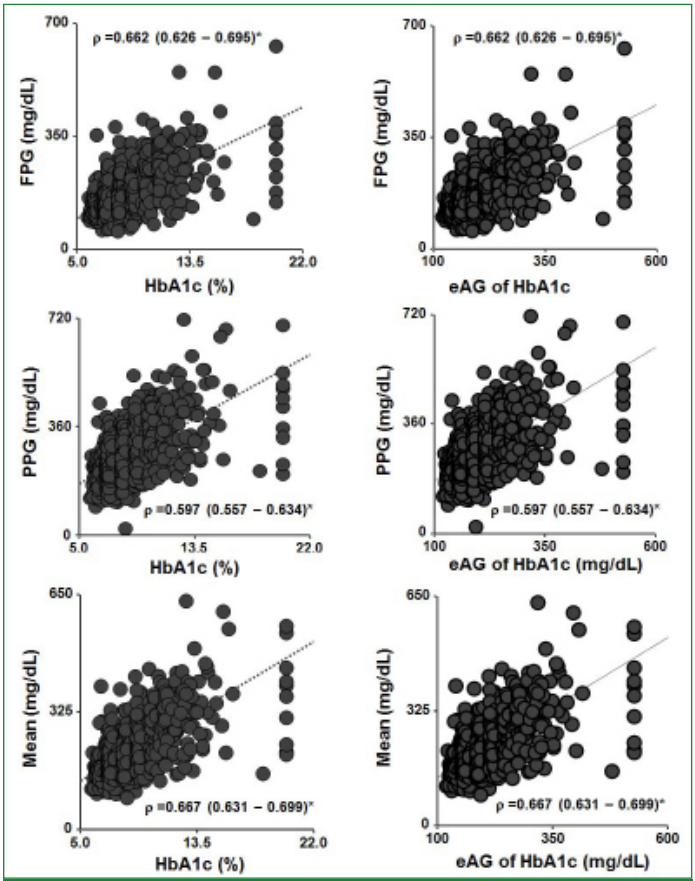
The baseline characteristics of the subjects are discussed in [Table/Fig-1]. The age and BMI of the subjects recruited in the present study were 56 years (49-64 years) and 25 kg/m^2 (23-28 kg/m^2), respectively. Out of 1268 medical records, males constituted 743 (58.6%) and females comprised the remaining 525 (41.4%). The entire pooled data, group I and group II of HbA1c were inherent with median of 8.2% (7.2-9.5%), 6.7% (6.4-6.8%), and 8.7% (7.8-9.9%), respectively. As anticipated, the subgroups i-vi showed gradual escalation of their median HbA1c. Similarly, even the mean plasma glucose (200 mg/dL) had intermittent median between FPG (154 mg/dL) and PPG (248 mg/dL). The duration of T2DM among the subjects recruited in the present study was five years (3-9 years).

A moderate positive correlation coefficient was apparent between plasma glucose measurements (FPG, PPG and Mean) and HbA1c in the entire pooled data analysis [Table/Fig-2]. Only group II has sustained significant moderate positive correlation whereas in group I significant correlation has declined to a very weak level [Table/Fig-3a]. Even the group II couldn't sustain its moderate correlation when the analysis was stretched to the subgroup (i-vi) level [Table/Fig-3b]. Even among the subgroups, only subgroup i–iii sustained significant but weak correlation. There was no correlation in the remaining three subgroups (Subgroup: iii-vi). Therefore, gradual

declination and disappearance of the linear association of plasma glucose measurements with increasing HbA1c was obvious. In the present study, the correlation of FPG/PPG/Mean (mg/dL) against either HbA1c (%) (or) eAG (mg/dL) yielded same results, as evident in the form a typical representation [Table/Fig-2].

Variables	Total (1268)	p-value (Test)
Age, years	56 (49-64)	0.001 (S-W test)
Gender, n(%)		
Male	743 (58.6)	0.0001 (χ^2 test)
Female	525 (41.4)	
Body Mass Index (BMI) in kg/m^2	25 (23-28)	0.001 (S-W test)
HbA1c (%)	8.2 (7.2-9.5)	0.001 (S-W test)
Group I (HbA1c ≤7.0%)	6.7 (6.4-6.8)	0.001 (S-W test)
Group II (HbA1c >7.0%)	8.7 (7.8-9.9)	0.001 (S-W test)
Subgroup i (HbA1c >7 to ≤8)	7.5 (7.3-7.8)	0.001 (S-W test)
Subgroup ii (HbA1c >8 to ≤9)	8.5 (8.2-8.8)	0.001 (S-W test)
Subgroup iii (HbA1c >9 to ≤10)	9.5 (9.3-9.8)	0.001 (S-W test)
Subgroup iv (HbA1c >10 to ≤11)	10.5 (10.3-10.7)	0.001 (S-W test)
Subgroup v (HbA1c >11 to ≤12)	11.5 (11.2-11.9)	0.001 (S-W test)
Subgroup vi (HbA1c >12)	13.5 (12.7-15.3)	0.001 (S-W test)
Fasting Plasma Glucose (FPG) in mg/dL	154 (126-199)	0.001 (S-W test)
Postprandial Plasma Glucose (PPG) in mg/dL	248 (204-307)	0.001 (S-W test)
Mean of FPG and PPG in mg/dL	200 (168-249)	0.001 (S-W test)
Duration of T2DM, years	5 (3-9)	0.001 (S-W test)

[Table/Fig-1]: Baseline characteristics of the subjects.
Not normally distributed data is expressed as median (interquartile range) and categorical variables as number (percent). (χ^2: Chi-squared test; and S-W test: Shapiro-Wilk test)



[Table/Fig-2]: Spearman correlation coefficient (ρ) analysis of blood glucose measurements against HbA1c data. (FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; mean: mean of FPG and PPG; eAG: estimated average glucose of HbA1c; &*: p<0.05).

In order to quantify the agreement of plasma glucose measurements with increasing HbA1c levels, concordance and discordance percentages were computed. The concordance percentage

Sl. No.	HbA1c Interval (%)	Spearman correlation (ρ) with 95% confidence intervals (CI)					
		FPG (mg/dL) vs HbA1c (%)		PPG (mg/dL) vs HbA1c (%)		Mean (mg/dL) vs HbA1c (%)	
		ρ (95% CI)	p-value	ρ (95% CI)	p-value	ρ (95% CI)	p-value
A. Correlation in the major groups							
1.	Group I	0.216 (0.097-0.329)	0.0004	0.186 (0.066-0.301)	0.0022	0.199 (0.079-0.312)	0.0011
2.	Group II	0.602 (0.558-0.644)	0.0001	0527 (0.477-0.573)	0.0000	0.600 (0.555-0.641)	0.0001
B. Correlation analysis in the group I and subgroup (i-vi) of group II							
1.	Group I	0.216 (0.097-0.329)	0.0004	0.186 (0.066-0.301)	0.0022	0.199 (0.079-0.312)	0.0011
2.	Subgroup i	0.187 (0.078-0.292)	0.0008	0.174 (0.064-0.279)	0.0019	0.206 (0.097-0.310)	0.0002
3.	Subgroup ii	0.187 (0.070-0.298)	0.0017	0.138 (0.020-0.251)	0.0210	0.167 (0.050-0.279)	0.0050
4.	Subgroup iii	0.140 (-0.008-0.282)	0.0630	0.119 (-0.029-0.263)	0.1134	0.151 (0.003-0.293)	0.0437
5.	Subgroup iv	0.099 (-0.092-0.283)	0.3066	0.091 (-0.100-0.275)	0.3495	0.113 (-0.077-0.296)	0.2419
6.	Subgroup v	-0.186 (-0.434-0.088)	0.1773	0.029 (-0.240-0.295)	0.8328	-0.073 (-0.335-0.198)	0.5968
7.	Subgroup vi	0.165 (-0.088-0.398)	0.1969	0.145 (-0.108-0.380)	0.2559	0.161 (-0.091-0.394)	0.2065

[Table/Fig-3]: Spearman correlation analysis: Blood glucose measurements against estimated average glucose (eAG) of HbA1c in mg/dL.

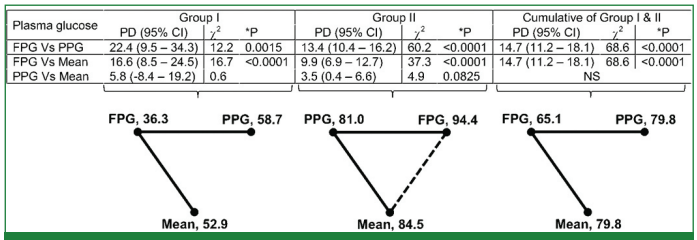
variations of group I (PPG>Mean>FPG) was contrary to group II (FPG>Mean>PPG) whereas their cumulative concordance percentage exhibited PPG≈Mean>FPG [Table/Fig-4a]. Hence, PPG at group I, FPG at group II, and both PPG and mean on a cumulative basis outstood with relatively high concordance percentages. Overall, mean was in concurrence with respective dominant parameters without any significant difference [Table/Fig-5]. On subgroup analysis, further worsening of even weak concordance percentages with an increase in HbA1c unit intervals (except at subgroup vi) was eminent [Table/Fig-4b]. The first two subgroup unit intervals i and ii shared overall common concordance percentage gradation of mean>FPG>PPG whereas the remaining unit intervals (subgroup iii-vi) exhibited concordance percentage gradation of FPG>mean>PPG [Table/Fig-6]. At each and every subgroup of group II, PPG showed significant difference with relatively dominant parameter whereas mean reserved such significant difference at iii, iv, and vi subgroups [Table/Fig-6]. On the other hand, as anticipated, the overall discordant percentage of PPG>Mean>FPG [Table/Fig-7]. Within the discordant percentage, relatively dominant underestimation in FPG and overestimation in PPG were apparent. Although mean expressed intermittent discordant percentage yet mimics PPG with features of overestimation.

In lines with above observations, contrary presentation of group I and group II was apparent with reasonable specificity and accuracy but suffered with weaker sensitivity [Table/Fig-8a]. Even in the subgroup analysis, all the three plasma glucose measurements were apparent with weaker and compromised sensitivity (barring subgroup vi) [Table/Fig-8b]. Overall, the plasma glucose measurements revealed exacerbation of weaker sensitivity with increasing intervals of HbA1c.

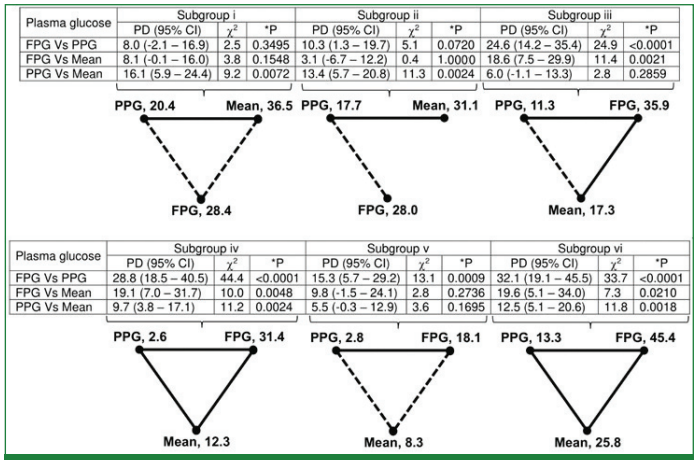
In order to quantify the differences and their pattern of variations between plasma glucose measurements and eAG with increasing levels of HbA1c, absolute difference analysis was performed [Table/

HbA1c %	FPG C/T	FPG % (95% CI)	PPG C/T	PPG % (95% CI)	Mean C/T	Mean % (95% CI)
A. Concordance analysis in the major groups						
Group I	232/639	36.3 (32.6-40.0)	37/63	58.7 (46.6-70.9)	100/189	52.9 (45.5-60.2)
Group II	594/629	94.4 (92.3-96.1)	975/1205	81.0 (78.6-83.1)	912/1079	84.5 (82.2-86.6)
Total	826/1268	65.1 (62.4-67.7)	1012/1268	79.8 (77.5-82.1)	1012/1268	79.8 (77.5-82.1)
B. Concordance analysis in the group I and subgroup (i-vi) of group II						
Group I	232/639	36.3 (32.6-40.0)	37/63	58.7 (46.6-70.9)	100/189	52.9 (45.5-60.2)
Subgroup i	67/236	28.4 (22.6-34.1)	22/108	20.4 (13.8-28.9)	101/277	36.5 (30.8-42.5)
Subgroup ii	37/132	28.0 (0.20-0.35)	38/214	17.7 (12.6-22.9)	82/264	31.1 (25.6-37.1)
Subgroup iii	33/92	35.9 (26.0-45.7)	23/204	11.3 (6.9-15.6)	30/173	17.3 (12.0-23.8)
Subgroup iv	22/70	31.4 (20.5-42.3)	4/184	2.6 (0-4.2)	14/114	12.3 (6.9-19.8)
Subgroup v	8/44	18.1 (6.8-29.6)	4/143	2.8 (0-5.4)	8/96	8.3 (3.6-15.7)
Subgroup vi	25/55	45.4 (32.3-58.6)	47/352	13.3 (9.8-16.9)	40/155	25.8 (19.1-33.4)
Total	424/1268	33.4 (30.8-36.3)	175/1268	13.8 (11.9-15.7)	375/1268	29.6 (27.1-32.2)

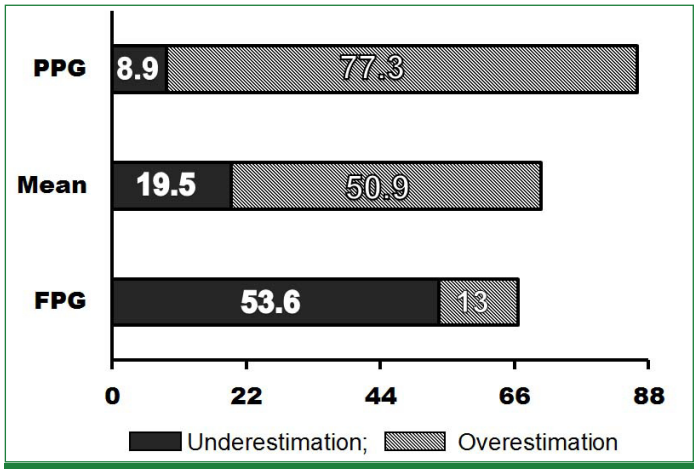
[Table/Fig-4]: Concordance of plasma glucose measurements with eAG of HbA1c.
FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; Mean (FPG & PPG): Mean of FPG & PPG; C/T: Concordant number/Total number; CI: Confidence interval; Concordance was in accordance to estimated average glucose of HbA1c



[Table/Fig-5]: Post-hoc Chi-square test among fasting plasma glucose (FPG in mg/dL), postprandial plasma glucose (PPG in mg/dL) and Mean (mean of FPG & PPG in mg/dL) with estimated average glucose (eAG in mg/dL) of HbA1c of Group I, Group II and their cumulative % with respective diagrams.
[Each node in Post-hoc test: concordant percentage; dark interconnecting line: significant Bonferroni corrected p-value; dotted line: not significant Bonferroni corrected p-value; NS: Not Significant; and without interconnecting line: p>1.000]



[Table/Fig-6]: Post hoc Chi-square test among Fasting plasma glucose (FPG in mg/dL), Postprandial plasma glucose (PPG in mg/dL) and mean (mean of FPG and PPG in mg/dL) with estimated Average glucose (eAG in mg/dL) of HbA1c at various subgroups with respective diagrams.
(Each node in Post-hoc test: concordant percentage; dark interconnecting line: significant Bonferroni corrected p-value; dotted line: not significant Bonferroni corrected p-value; and without interconnecting line: p>1.000)



[Table/Fig-7]: Overall discordant percentage of Fasting plasma glucose (FPG), Mean (Mean of FPG and PPG) and Postprandial plasma glucose (PPG). Discordance percentage was derived in comparison with estimated average glucose (eAG) of HbA1c.

Fig-9,10]. Even the absolute difference interpretations were in lines of observations inferred from discordance percentage computations. The gradual widening of interpercentile range with increasing HbA1c intervals is the most common and generalisable feature.

In addition to absolute difference analysis, the differences between the plasma glucose parameters were further quantified in terms of percentage differences. As apparent from; overall, only 14% of FPG, 17% of mean and 7% of PPG were within 0±5% percentage differences [Table/Fig-11]. Approximately, 80% of FPG and mean outstood with 0±30% percentage differences. Even at a 0±30% percentage difference, PPG accounted only for 46% of samples. However, among the FPG and mean, mean accounted marginally high percentage of samples at various range. These observations were apparent not only at major groups of HbA1c but also mostly even at the subgroups of group II (subgroup i-vi) [Table/Fig-12].

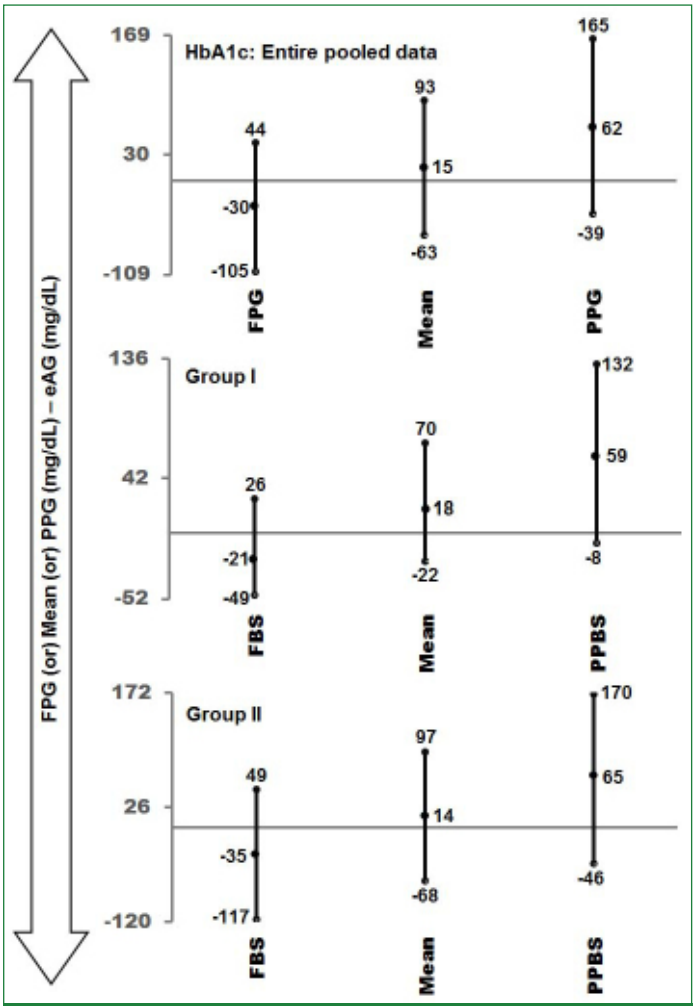
In a final step, the cut-off for plasma glucose measurements for good glycaemic control were extracted from ROC curve analysis. The cut-off (sensitivity, specificity and PPV) for FPG, PPG and mean were 140 mg/dL (78.2%, 71.1% and 41.9%), 220 mg/dL (70.4%, 74.1% and 42.0%) and 180 mg/dL (73.7%, 75.3% and 44.3%), respectively. In an additional approach, in order to explore the relationship between plasma glucose measurements with increasing HbA1c, scattered plots of the AUC of ROC curve analysis at various intervals of HbA1c were computed. In the same perspective, as apparent, PPG at a lower interval of HbA1c (group A) showed relatively the highest AUC whereas thereafter FPG replaced the PPG [Table/Fig-13].

DISCUSSION

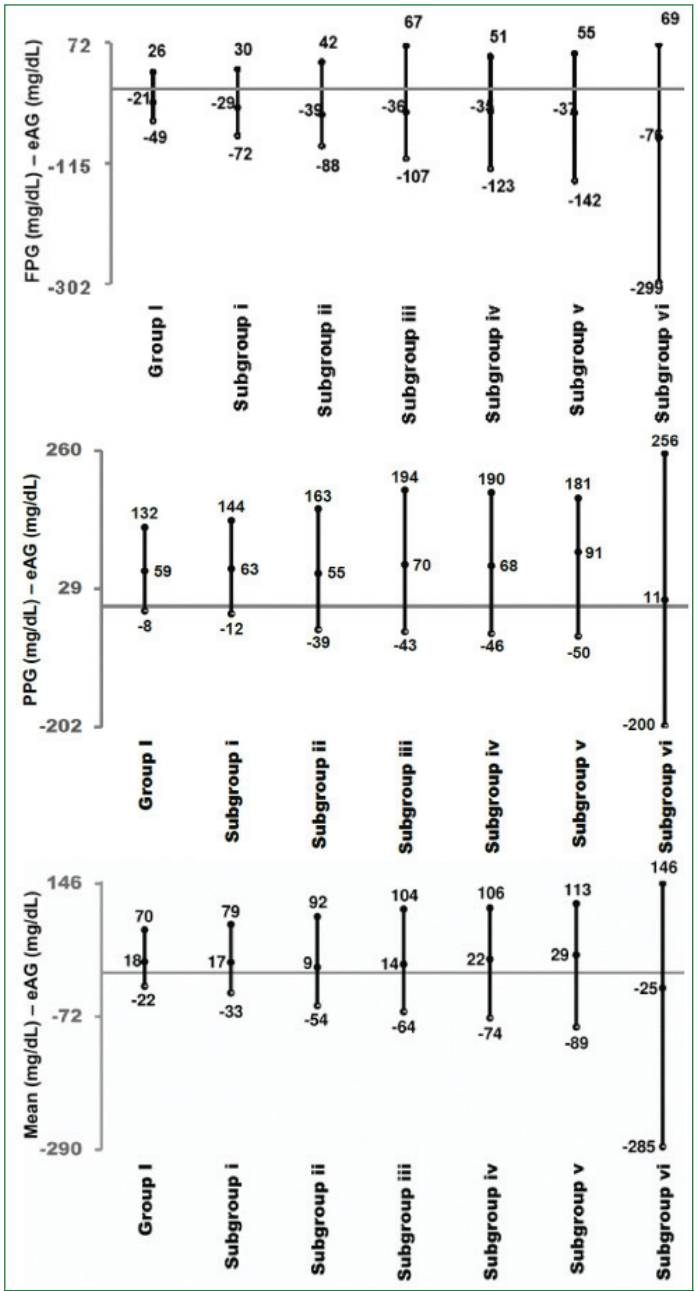
Measurement of HbA1c is an effective approach in monitoring long-term glycaemic patterns in T2DM. The merits of HbA1c comprises no special preparation of the patient, requirement of non fasting (random) sample, robust stability in sample material/ room temperature, minimal intraindividual variability (CV <1%) and insusceptibility to acute factors (stress/exercise) [6-9]. The plasma glucose estimations were vulnerable to stress factors accounting to erratic fluctuations. However, FPG and PPG estimations were routinely adopted as reliable economical surrogate providing snapshot measure of glycaemia with a targeting treatment goal of 80-130 mg/dL and <180 mg/dL, respectively [8]. Lack of consensus among previous studies raised ambiguity over the reliability of either FPG or PPG estimations as an economical surrogate for HbA1c in monitoring Type 2 Diabetes [12-28].

HbA1c	FPG (mg/dL)			PPG (mg/dL)			Mean (mg/dL)		
	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy
A. Major groups									
Group I vs group II	86.9%	59.3%	65.1%	13.9%	97.4%	79.8%	37.5%	91.1%	79.8%
Group II vs group I	59.3%	86.9%	65.1%	97.4%	13.9%	79.8%	91.1%	37.5%	79.8%
B. Group I and Subgroup (i-vi) of Group II									
Subgroup i vs remaining	21.1%	82.2%	66.9%	6.9%	90.9%	69.9%	31.8%	81.5%	69.0%
Subgroup ii vs remaining	13.2%	90.4%	73.3%	13.5%	82.2%	67.0%	29.2%	81.6%	70.0%
Subgroup iii vs remaining	18.6%	94.6%	84.0%	13.0%	83.4%	73.6%	16.9%	86.9%	77.1%
Subgroup iv vs remaining	20.4%	95.9%	89.4%	3.7%	84.5%	77.6&	13.0%	91.4%	84.7%
Subgroup v vs remaining	14.8%	97.0%	93.5%	7.4%	88.6%	85.1%	14.8%	92.8%	89.4%
Subgroup vi vs remaining	39.7%	97.5%	94.6%	74.6%	74.7%	74.7%	63.5%	90.5%	97.0%

[Table/Fig-8]: Impact on sensitivity and specificity of Fasting plasma glucose (FPG), Postprandial plasma glucose (PPG) and mean (mean of FPG and PPG) at various intervals of HbA1c



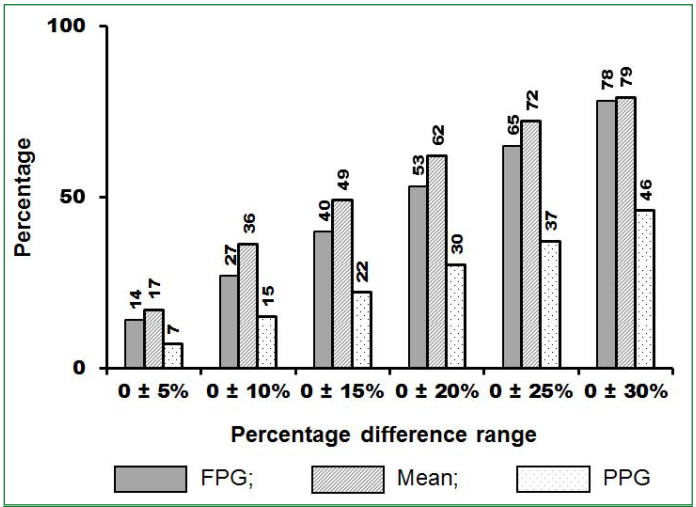
[Table/Fig-9]: Absolute difference of FPG (mg/dL), PPG (mg/dL) and mean (mg/dL) from estimated Average glucose (eAG, mg/dL) of HbA1c. (Absolute difference plot: median with 5th and 95th interpercentile range; FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; Mean: mean of FPG and PPG).



[Table/Fig-10]: Absolute difference between plasma glucose measurements (mg/dL) in comparison to estimated Average glucose (eAG, mg/dL) of HbA1c in group I and Subgroup i-vi. (Absolute difference plot: median with 5th and 95th interpercentile range; FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; and, Mean: Mean of FPG and PPG).

The correlation coefficient analysis of previous studies reported negligibly to high rankings as apparent in [Table/Fig-14]. In the present study, both plasma glucose measurements with a moderate correlation coefficient exhibited FPG>PPG. These correlation coefficient findings were not only in consensus with earlier studies but also in dissensus with some studies [13,14,17,20-27]. However, major group and subgroup analysis unraveled the gradual declination and disappearance linearity association of plasma glucose estimates with increasing HbA1c.

The cut-off for good glycaemic control (FPG: 140 mg/dL and PPG: 240 mg/dL) observed in the present study was relatively higher to previous studies [Table/Fig-14] [13,14,17,20-27]. Though sensitivity and specificity were considered during in near approximation with previous studies yet suffered with weak PPV (FPG: 41.9% and PPG: 42%). In the previous studies on Indian population, the reported cut-off (PPV) for FPG was 110 mg/dL (89%) and 130 mg/dL (87%)



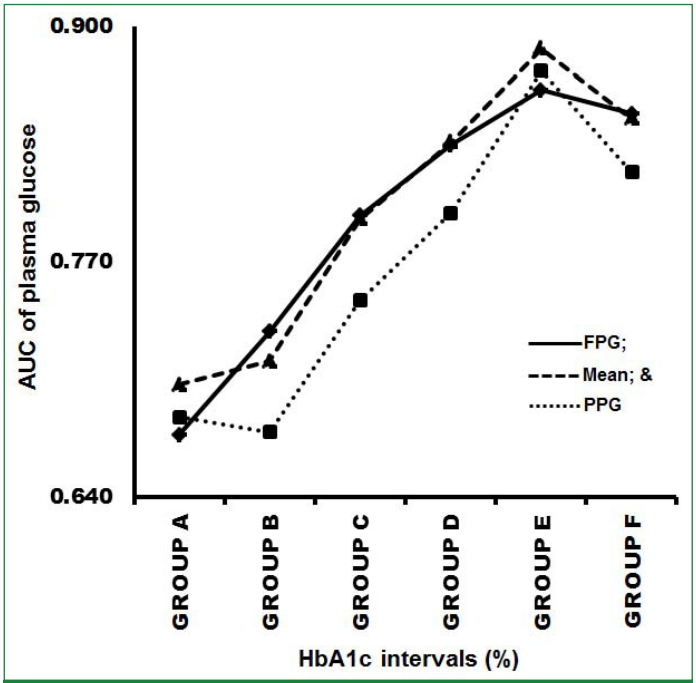
[Table/Fig-11]: Proportions of percentage difference of FPG (mg/dL), PPG (mg/dL) and Mean (mg/dL) with respect to eAG of HbA1c (mg/dL). (FPG: Fasting plasma glucose; mean: mean of FPG and PPG; PPG: Postprandial plasma glucose; and eAG: estimated average glucose of HbA1c).

Only the present study analysis unraveled the inherent feature of exacerbating of either underestimation in FPG or overestimation in PPG with diabetes worsening. Moreover, any change of about ~30 mg/dL in plasma glucose level is associated with a 1% change in HbA1C while any change in HbA1c value by at least 0.5% is considered as both statistically and clinically significant [11]. But the present study explored existence of only 14% FPG and 7% PPG with 0±5% difference. Hence, the direct correlation of plasma glucose values with HbA1c could be erroneous. Hence the provision of mean was exploited not only to minimise the erratic fluctuations of FPG and PPG estimations but also to explore its correlation with HbA1c. Even though mean exhibited intermittent features between FPG and PPG yet the analytical attributes implicate inclination towards PPG features. Relative to plasma glucose estimations mean showed modest improvement in proportion of samples at each percentage difference range.

Only a couple of studies demonstrated the impact of FPG and PPG on the overall glycaemic indicator, HbA1c. In one of those study, based on the degree of glycaemic control, the relative predominant contribution of PPG in moderate diabetics whereas FPG with diabetes

PD range	Percentage difference (%)																							
	Group						Subgroup																	
	I			II			i			ii			iii			iv			v			vi		
	FPG	Mean	PPG	FPG	Mean	PPG	FPG	Mean	PPG	FPG	Mean	PPG	FPG	Mean	PPG	FPG	Mean	PPG	FPG	Mean	PPG	FPG	Mean	PPG
0±5%	12	14	10	14	20	7	14	23	4	10	20	7	18	15	12	20	13	5	17	6	9	10	14	11
0±10%	29	35	16	27	37	15	27	39	8	20	38	18	29	33	23	34	29	14	35	35	19	27	27	21
0±15%	44	51	22	39	49	22	39	53	15	35	50	26	40	44	27	45	44	27	46	46	24	35	37	25
0±20%	62	60	27	51	63	31	52	66	24	47	67	34	50	60	37	56	57	33	65	56	26	43	43	38
0±25%	77	69	31	62	73	39	67	76	31	56	74	43	60	69	42	67	73	43	76	70	39	52	57	46
0±30%	89	76	37	75	80	48	80	83	41	72	80	50	72	79	50	78	80	56	81	78	48	56	67	40

[Table/Fig-12]: Proportions of percentage difference between FPG (mg/dL), PPG (mg/dL) and mean (mg/dL) with respect to estimated Average Glucose (eAG, mg/dL) of HbA1c at various intervals. (FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; mean: mean of FPG and PPG).



[Table/Fig-13]: Changes in the AUC of FPG, PPG and Mean (mg/dL) with gradual increase in HbA1c intervals [FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; Mean: Mean of FPG and PPG; AUC: Area under the curve derived from Receiver operating characteristic (ROC) curve; group A: ≤7% Vs >7% to ≤8%; group B: ≤8% vs >8% to ≤9%; group C: ≤9% vs >9% to ≤10%; group D: ≤10% vs >10% to ≤11%; group E: ≤11% vs >11% to ≤12%; and group F: ≤12% vs >12%).

whereas for PPG it was 126 mg/dL (95%) and 180 mg/dL (80%) [25-27]. But at the cut-off values of previous studies [25-27], plasma measurements of the present study exhibited compromised PPV with a sensitivity of approximately ≤50%.

worsening was demonstrated [16]. Probably this understanding could be extrapolated as the reason behind the observations with respect to concordance percentages in both major and subgroups as well as changes in the AUC of FPG and PPG with increasing HbA1c intervals in the present study. In another study, it had been demonstrated that basal insulin therapy primarily reduces FPG but subsequent treatment with oral antidiabetic drugs, especially in patients with uncontrolled hyperglycaemia, PPG accounted for the majority of residual hyperglycaemia [28]. In the present study, inclination of analytical attributes of mean towards PPG features probably suggests the relevance towards the existence of residual hyperglycaemia due to PPG. Of note, PPG is also acknowledged as an independent risk factor for cardiovascular deaths [34,35]. Hence, most of the recent treatment guidelines comprised not only specific FPG targets but also PPG and A1c targets.

Accumulating body of evidence also demonstrates the broad-spectrum application of HbA1c even in diagnosis of diabetes mellitus, as a predictor of lipid profile and; elevated levels implicating significant risk for cardiovascular diseases and stroke in individuals with diabetes [36]. Indubitably, it's elevated levels was also known to alarm the individual's susceptibility to and macrovascular diabetic complications. On the other side, further insistence was on improving glycaemic control in T2DM patients rather than treating dyslipidemia for the prevention of diabetic complications [37]. Even on a comprehensive note and in lines of basic understanding of biomolecular integration of glucose metabolism in pathophysiological conditions, chronic hyperglycaemia is the main felon for diabetic complications and comorbidities [38,39]. Recent cohort study reports association of hyperglycaemia with hospital mortality in non diabetic COVID-19 patients [40]. Hence, maintenance of glycaemic status should be adage of diabetic treatment. However, as snapshots of plasma glucose estimation (FPG/PPG) are susceptible

A) Tabulated cut-off values with sensitivity, specificity and PPV

Studies	Cut-off		Sensitivity (%)		Specificity (%)		PPV (%)	
	FPG	PPG	FPG	PPG	FPG	PPG	FPG	PPG
Avignon A et al., 1997 [24]	120	140	69	73	85	92	62	76
Rosediani M et al., 2006 [17]	110	145	81	75	58.3	80.6	70.6	82.5
Datta S et al., 2014 [26]	110	126	85	92	81	90	89	95
Swetha NK 2014 [25]	130	180	74	79	84	74	87	80
Current study	140	220	78.2	70.4	71.1	74.1	41.9	42

B) Tabulated cut-off values and correlation analysis report

Studies	Cut-off		Correlation analysis	
	FPG	PPG	HbA1c Vs FPG	HbA1c Vs PPG
Avignon A et al., 1997 [24]	120	140	0.62	0.81
Bonora E et al., 2001 [14]	120	160	0.48	0.48
Rosediani M et al., 2006 [17]	110	145	0.58	0.60
Saeed MK [20]	120	160	0.60	0.20
Haddadinezhad S and Ghazaleh N, 2010 [23]	120	160	0.32	0.43
Azim W et al., 2011 [13]	126	200	0.28	0.44
Gupta S et al., 2011 [21]	120	140	0.68	0.62
Saiedullah M et al., 2013 [22]	-	-	0.81	0.77
Shreshta L et al., 2013 [27]	120	200	0.45	0.63
Datta S et al., 2014 [26]	110	126	0.84	0.86
Swetha NK 2014 [25]	130	180	0.74	0.76
Current study	140	220	0.66	0.59

[Table/Fig-14]: Tabulations of cut-off for good glycaemic control (mg/dL), sensitivity, specificity and PPV as well as correlation analysis between HbA1c and plasma glucose measurements of previous and current study [13,14,17,20-27].

to erratic fluctuations, clinical decisions drawn solely on such glucose-based estimations (FPG/PPG) could be perilous. Eventually, even clinical decisions cannot be moulded based on only HbA1c measurement. Therefore, tuning the treatment approaches using glucose-based estimations at regular short intervals and evaluating whether or not success using the HbA1c target could be meritorious. In order to improve the accessibility of HbA1c tests to every diabetic patient in India, the facility can be standardised, centralised, and subsidised. The samples can be pooled, mobilised, analysed and the reports can be released within stipulated turn-around time.

Limitation(s)

The limitations of the present study comprise the non involvement of the independent population which was more general. Hence, these observations may not be generalisable to the overall population due to the existence of baseline differences between the subjects recruited in the present study and the general population. Moreover, the present study was also unable to establish the relative contribution of glucose-based measurement at various intervals of HbA1c. However, the present study provides insight into risks associated with adopting glucose-based estimations as an alternative to HbA1c estimation and on the feasible merits of combined application of both in achieving desired levels of glycaemic control in T2DM. Further, rigorous validation studies are warranted in the Indian population in order to establish the cut-off as treatment targets for FPG and PPG in order to achieve HbA1c ≤7%. Owing to the analytical attributes in the present study, the provision of involving mean can also be further evaluated.

CONCLUSION(S)

On direct comparison to eAG: FPG with narrower interpercentile range underestimated whereas both PPG and mean overestimated but only mean had relatively narrow interpercentile range in the major as well as subgroup analysis. Weak to moderate sensitivity encompassing only poor sample size with 0±5% percentage difference was inherent in plasma glucose measurements. Hence, neither from a diagnostic laboratory nor clinical point of view, the output of the in-depth analysis of the present study warrants the utility of the glucose-based measurements as an economical alternative to HbA1c. Rather close monitoring of glucose-based measurements and accordingly tuning the treatment modalities in order to achieve clinically desired glycaemic control; the success of which can be evaluated using HbA1c measurement could be meritorious.

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