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Glycated Hemoglobin Risk Stratification in New Onset Type 2 Diabetes Mellitus

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Abstract

Introduction: Glycated hemoglobin (HbA1c) is an internationally acceptable diagnostic test for diabetes mellitus (DM). This study aim to describe HbA1c levels in person(s) with new-onset type 2 DM.

Methods: This is a cross-sectional, case-controlled, multicenter study. A purposeful sampling technique was used to recruit 50 newly diagnosed type 2 DM subjects within 3 weeks of their diagnosis. Also, apparently healthy 50 non-diabetic with no significant medical history were recruited as controls.

Results: 100 participants comprising of 32 males,68 females (1:2) with age range 15 to 80 years were studied. HbA1c was from 3.19% to 14.0%. Estimated glucose average (eGA) correlated the most with HbA1c with a positive 0.997 value, at p-value of <0.0001. Random blood sugar (RBS) correlated well next at same p-value (p <0.0001). Scattered plot demonstrates a positive linear correlation for both.

Conclusion: HbA1c values varies in new-onset type 2 DM. The proposed "Glycated Hemoglobin Risk Stratification In Diabetes Mellitus" (GHRSIDM) categorizes HbA1c to classes 1 to 7 based on laboratory values. GHRSIDM can act as a lifelong chart for risk stratification at onset, used for monitoring, and prognostication among others.

Keywords: Glycated Hemoglobin; New Onset Type 2 Diabetes Mellitus; Glycated Hemoglobin Risk Stratification In Diabetes Mellitus Chart; Random Blood Sugar; Fasting Blood Sugar

Introduction

It took a long time for glycated hemoglobin (HbA1c) to be recognized internationally as an acceptable diagnostic test for diabetes [1,2]. Prior to this acceptance fasting blood sugar, (FBS), random blood sugar (RBS), and oral glucose tolerance test (OGTT) continue to be used for diagnosis of diabetes mellitus. Despite the use of FBS RBS and OGTT [3-6], the need for a long-term diagnostic and monitoring measure of glycemic control was obvious leading to research that culminated in the description of HbA1c as a diagnostic and therapeutic monitoring tool in the management of the patient with chronic hyperglycemia manifesting as type 2 diabetes mellitus, type 1 diabetes mellitus, or other forms

of diabetes. In the endocrine world today, HbA1c is the gold standard for the measurement of chronic hyperglycemia in people living with diabetes mellitus [1,2].

Studies done on glycated hemoglobin showed that HbA_{1c} values of ≥6.0% gave a reasonably high sensitivity and specificity for diagnosis using the WHO or ADA criteria [7]. Many large studies on HbA1c had demonstrated that the higher the level of HbA1c,the more the development and severity of complication, but these large studies did not go on to explicitly produce a tool or a model to categorize these high HbA1c values for use clinically and in the laboratory which can be used to address these findings. This creates a knowledge gap. Hence, there is an urgent need for risk stratification using HbA1c in newly diagnosed type 2 diabetes mellitus patient. The risk stratification is needed so as to place newly diagnosed type 2 diabetes mellitus patient in a stratum for the purpose of need of urgency of treatment, morbidity and mortality prediction at diagnosis, assessment of micro-damages that could have occurred to cells tissues, organs and systems, prediction of response to therapy, assessment of general status in the face of co-existing chronic or acute condition, consideration on management options at diagnosis of new onset type 2 diabetes mellitus , consideration of risk stratification based on gender line knowing female generally have lower hemoglobin level than male, general rehabilitative consideration/options, prognostification, and lifestyle modification appropriate for new onset type 2 diabetes mellitus based on HbA1c at diagnosis.

The risk factors for new onset type 2 diabetes mellitus includes obesity [8-10], central obesity [11-13], advancing age [14-16], sedentary lifestyle [17-19], macrosomia [20,21] and microsomia [22] at birth and a positive family history [23-26]. New onset type 2 diabetes mellitus occurs when these interplay results in biochemical and clinical manifestation of hyperglycemia which is the hallmark of type 2 diabetes mellitus.

The aim of this study is to describe glycated hemoglobin levels in new-onset type 2 diabetes mellitus.

Methodology

This is a cross sectional, case-controlled multicenter study. A non- probability technique, purposeful sampling technique was used to recruit 50 newly diagnosed type 2 diabetes mellitus subjects out of which 47 subjects had glycated hemoglobin of 6.5% and above while 3 newly diagnosed type 2 diabetes mellitus patient had HbA1c less than 6.5% but meeting fasting blood sugar criteria of 126mg/dl for diagnosis of new onset type 2 diabetes mellitus. All the 50 subjects were within their first three weeks of their initial diagnosis. Also,50 control participants who are apparently healthy with no significant medical history and not living with diabetes mellitus and has glycated hemoglobin less than 6.5% were recruited. This makes it a total of 100 participants in the study. It is a multicenter study on new-onset type 2 diabetes mellitus patients attending healthcare in tertiary and secondary hospitals; Lagos State University Teaching Hospital, Ikeja, Lagos, (LASUTH) and General Hospital, Gbagada. Lagos, Nigeria. A questionnaire was used to extract necessary information from the patients. The questionnaire was also used for data collection. The questionnaire is in three stages, section A collects demographic data including laboratory identifier number and hospital number, body weight, body height, waist circumference etc., excluding name. Section B collects laboratory parameters like value of blood sugar at diagnosis of new onset type 2 diabetes mellitus whether by point of care POC or laboratory analysis and also time testing was done was recorded. It also records, analytic value of HbA1c in percentages using gold standard of measurement of glycated hemoglobin, high performance liquid chromatography. Section C collects clinical information like type of diabetes, family history of type 2 diabetes mellitus, previous medical history and co-morbidity, type of glucose lowering agent patient is commenced on, if any, whether insulin, oral hypoglycemic agents or a combination of insulin and oral hypoglycemic agent and other relevant health history.

Glycated hemoglobin concentration estimation was done at Lagos State University Teaching hospital Laboratory. Point of care testing was through a brand-new glucometer that uses glucose oxidase as its active reagent in order to have a high sensitivity yield.

Research procedure includes collection through venipuncture of 5-7mls of venous whole blood in an ethylenediaminetetraacetic acid (EDTA) bottle and mixed with anticoagulant after which glycated hemoglobin estimation was done using high performance liquid chromatographic technique.

Inclusion and Exclusion Criteria

The inclusion criteria includes new onset type 2 diabetes mellitus on pharmacologic therapy or not within 3 weeks of diagnosis with glycated hemoglobin of 6.5% and above. In addition, three new onset type 2 diabetes mellitus patients that does not have HbA1c value up to 6.5% but meet the American diabetes Association fasting blood sugar level of 126mg/dl for diagnosis of new onset type 2 diabetes mellitus were recruited as subjects in this study to make up sample size of 50 subjects in the study that employs purposive sampling technique to recruit patient.

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Exclusion criteria includes new onset type 2 diabetes mellitus diagnosed by fasting blood sugar values without corresponding HbA1c equaling 6.5% or above (except the 3 patients mentioned above). New onset type 2 diabetes mellitus diagnosed by random blood sugar with values 200 mg/dl and above but with glycated hemoglobin less than 6.5% were also excluded. Recently diagnosed type 2 diabetes mellitus of more than 3 weeks were excluded. Other exclusion criteria were non-diabetes hyperglycemia, previously diagnosed type 2 diabetes mellitus, gestational diabetes mellitus, maturity onset diabetes of the young MODY, Latent Autoimmune Diabetes of Adulthood (LADA), unconscious new-onset type 2 diabetes mellitus patient with confounding cause of unconsciousness,

Sample Size

The sample size is 100 participants, 50 newly diagnosed type 2 diabetes mellitus and 50 apparently healthy non diabetics spread across the two centers.

Diagnosis of new-onset type 2 diabetes mellitus will be by fasting blood sugar of 126mg/dl or above, random blood sugar or by 2 hours post -prandial glucose measurement of 200 mg/dl and above or by glycated hemoglobin value of 6.5% or above according to American Diabetes Association diagnostic criteria.

Ethical approval was obtained from the ethical committees of the two centers. Lagos State University Teaching Hospital (LASUTH} with reference number LREC/06/26/3114 and General Hospital Gbagada Lagos with reference number GBGH/750/VOL.IV/782.

The World Medical Association (WMA) Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data was observed. A consent was duly obtained with signing of consent forms. The data was recorded in an excel sheet and stored in google drive file till sample size was completed. This data was later populated on SPSS version 26 for data analysis using descriptive statistics and pearson"s correlation test.

Results

Descriptive statistics showed a total of 100 participants were recruited into the study. 50 were newly diagnosed type 2 diabetes mellitus patient with HbA1c of 6.50% and above in 47 of them, while the fifty control had glycated hemoglobin of 6.40% or less and a random blood sugar or fasting blood sugar in less than diabetic range. Of the total population, 32 were male while 68 were female giving a male to female ratio of approximately 1:2. The age range is from 15 to 80 years with an age range of 65 years, a mean of 51.11years, a median of 52.50 years and a mode of 52 years with a variance of 220.42 and a standard deviation of 14.85. The age distribution is negatively skewed with a value of -0.31. The standard error of the mean is 1.49 and the standard error of skewness was 0.24.

The range of the glycated hemoglobin is 10.81%, from 3.19% to 14.00% in this study with a mean of 6.89% a median of 6.20% and a mode of 5.40% with a variance of 4.43 and a standard deviation of of 2.11. The glycated hemoglobin distribution is positively skewed among the studied cohort with a value of 1.17. The standard error of the mean is 0.21 while the standard error of skewness is 0.24.

Of the 100 participants. 72 had a random blood sugar recorded. The minimum was 66mg/dl while the maximum was 497mg/dl with a range of 431mg/dl. The mean of the random blood sugars was 150.83mg/dl, the median was 119mg/dl, mode was 100mg/dl,the variance was 6374.23 with a standard deviation of 79.84. The distribution of the random blood sugar was positively skewed with a value of 2.02. The standard error of the mean was 7.41 while the standard error of skewness was 0.28.

Of the 100 participants, 28 had a fasting blood sugar recorded after at least an 8 hours fasting overnight. The minimum value was 70mg/dl and the maximum was 519mg/dl with a range of 449mg/dl. The mean of the fasting blood sugars was 163.75 mg/dl, the median was 140mg/dl and the mode was 140 mg/dl, the variance was 7043.25 with a standard deviation of 83.92mg/dl. The distribution was positively skewed with a value of 3.18. The standard error of the mean was 15.86 while the standard error of skewness was 0.44.

Correlation between Hba1c and Estimated Glucose Average (Ega), Random Blood Sugar (RBS) and Fasting Blood Sugar (FBS)

Estimated glucose average correlated the most with glycated hemoglobin with a positive 0.997 value, almost equal to 1 at p-value of <0.0001 with a 2-tailed correlation statistic significant at p<0.01 (Table 1 and scattered plot diagram in Figure 1).

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		Glycated Hemoglobin in percentages (%)	Estimated Glucose Average (eGA) in mg/dl	Random Blood Sugar (RBS) in mg/dl	Fasting Blood Sugar (FBS) in mg/dl
Glycated Hemoglobin in percentages (%)	Pearson Correlation	1	.997**	.827**	0.326
	Sig. (2-tailed)			0	0
	N	100	100	72	28
Estimated Glucose Average (eGA) in mg/dl	Pearson Correlation	.997**	1	.828**	0.325
	Sig. (2-tailed)	0		0	0.092
	N	100	100	72	28
Random Blood Sugar (RBS) in mg/dl	Pearson Correlation	.827**	.828**	1	.b
	Sig. (2-tailed)	0	0		.
	N	72	72	72	0
Fasting Blood Sugar (FBS) in mg/dl	Pearson Correlation	0.326	0.325	.b	1
	Sig. (2-tailed)	0.09	0.092	.	
	N	28	28	0	28

Table 1: Correlations Between Glycated Hemoglobin (HbA1c), Estimated Glucose Average (eGA), Random Blood Sugar (RBS) and Fasting Blood Sugar (FBS)

**. Correlation is significant at the 0.01 level (2-tailed).

b. Cannot be computed because at least one of the variables is constant.

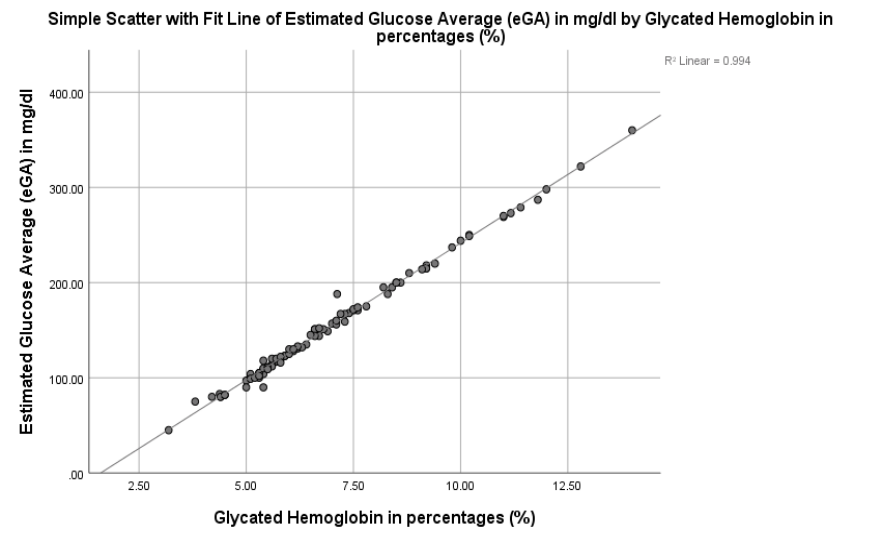


Figure 1: Estimated average glucose showed an almost perfect positive correlation with glycated hemoglobin (r = 0.997, p < 0.0001), indicating a highly significant linear association.

It showed a linear correlation on scatter plot diagram. Estimated glucose average also showed a strong positive correlation with random blood sugar at the same level of p-value of <0.0001 with a value of 0.83 with a 2-tailed correlation statistic. In this study, eGA showed a weak positive correlation with fasting blood sugar at a value of 0.33 but the p-value is 0.09, which is not significant.

Scatter plot Estimated Glucose Average (eGA) vs Glycated Hemoglobin (HbA1c)

The plot showed a near perfect positive linear correlation as both parameters increases concomitantly with virtually all the plots falling on the line of best fit Figure 1.

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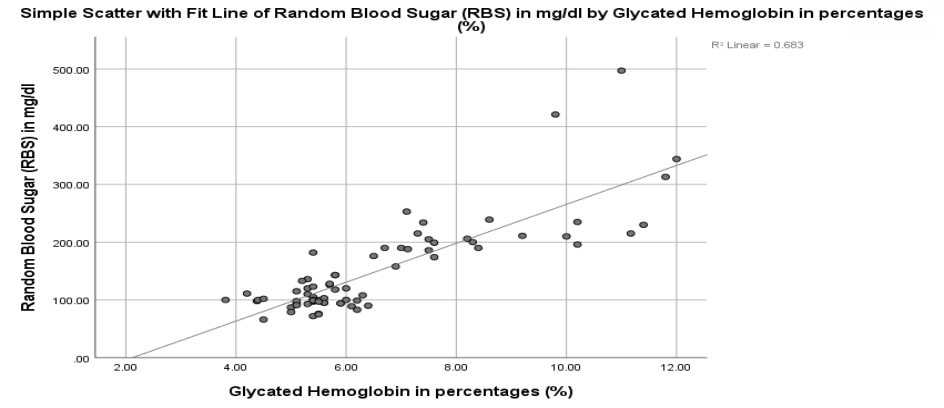


Figure 2: Near-perfect positive linear correlation between the two parameters, with both increasing concomitantly and nearly all observations closely aligned with the line of best fit.

Random blood sugar

Random blood sugar measurement correlated well next of the three parameters correlated with glycated hemoglobin. At a p-value of 0.0001 it correlated by a value of 0.83. This is also a very strong correlation to glycated hemoglobin. The correlation of random blood sugar to glycated hemoglobin and estimated glucose average is almost the same with that of HbA1c been 0.83 while that of eGA is 0.83 at the same level of significance of $p<0.0001$. Correlation between random blood sugar and fasting blood sugar was not calculated in this statistics.

Scatter Plot of Random Blood Sugar (RBS) Versus Glycated Hemoglobin (HbA1c)

This demonstrates a positive linear correlation as the values of random blood sugar increases as the value of HbA1c increases. The clustering around the line of best fit is more at lower HbA1c value than at higher HbA1c values though still in a linear fashion. In this plot, the distribution of the plots is approximately the same on both left and right side of the line of best fit Figure 2.

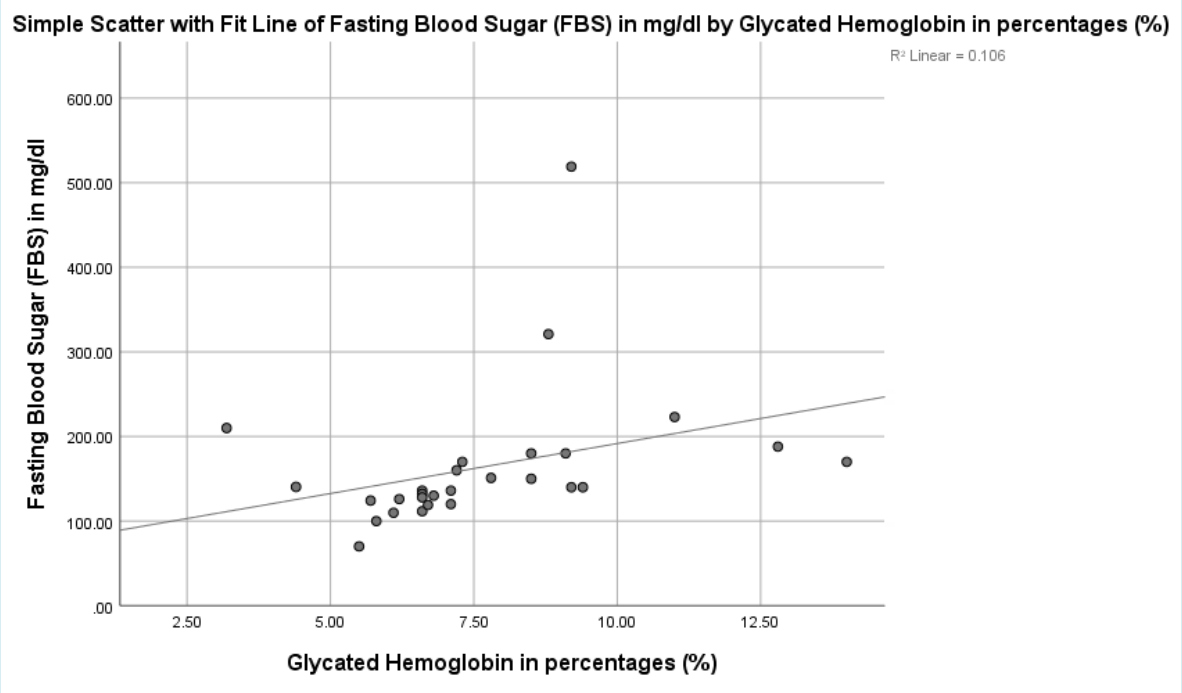


Figure 3: Random blood sugar showed a positive linear correlation with HbA1c, with greater clustering around the line of best fit at lower HbA1c values and an approximately even distribution of observations on either side of the regression line.

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Fasting blood sugar

Fasting blood sugar correlated the least with glycated hemoglobin in this study of the three parameters though it still demonstrate a weak positive correlation yet this was at a p-value of 0.09 for HbA1c and 0.09 for estimated glucose average (eGA) in mg/dl, both of which are not significant though the sample of fasting blood sugar correlated in this study is small,only 28.

Scatter Plot Fasting Blood Sugar and Glycated Hemoglobin

The scatter plot graph between fasting blood sugar and HbA1c also showed a linear correlation though with a greater variability compared to that shown by random blood sugar compared to glycated hemoglobin. (Figure 2 and 3).The diagram"s positive linear correlation is such that as an increase in fasting blood sugar results occur, there is also an increase in HbA1c. The correlation according to this study between fasting blood sugar and HbA1c is more at lower values of both parameters.

Discussion

In literature, the correlation between the parameters for diagnosing persons with new onset type 2 diabetes mellitus differs. The particular advantage of using HbA1c as a diagnostic tool for new onset diabetes mellitus measurement is that it avoid the use of clinical features which may have differential diagnosis as a factor in determining patient"s status. The fact that glycated hemoglobin is a predictor of average blood glucose level in the last 8 to 12 months [3,27,28] make it a very reliable laboratory parameter for diagnosing new onset type 2 diabetes mellitus without clinical or laboratory doubt. In this study too, some persons with newly diagnosed type 2 diabetes mellitus had a HbA1c more than twice the minimum required to diagnose them as new onset type 2 diabetes mellitus patient. This is dangerous as the risk these new onset type 2 diabetic patient will pose may be far higher than that of a newly diagnosed type 2 diabetes mellitus patient with 6.5% at diagnosis. In this study, the highest value of newly diagnosed type 2 diabetes mellitus patient was 14%. Doubtlessly, the duration of chronic hyperglycemia in the patient diagnosed with 6.5% at diagnosis may be shorter compared to the patient diagnosed with 14% as the transition from normal to prediabetes to new onset type 2 diabetes mellitus would have been a continuum until diagnosis with a lot of hyperglycemia memory mechanism [29,30] activated along the line. However, a patient with a HbA1c of 6.5% and 14% may develop new onset type 2 diabetes mellitus at different rates because there are patients who develop it more rapidly than others as factors like dietary, environmental and/or predisposition can affects this rate. It will be interesting to have a large-scale study that determine how soon patient develop microvascular and macrovascular complication of type 2 diabetes mellitus based on their glycated hemoglobin value at diagnosis. This will reveal how relevant value of HbA1c is relevant and related to development of type 2 diabetes mellitus complication among other confounding factors. No doubt risk stratification at diagnosis using HbA1c value is needed for better laboratory and/or clinical evaluation at onset and in monitoring the trajectory of the sequelae of clinical events following the level of HbA1c at diagnosis in newly diagnosed type 2 diabetes mellitus patient.

I hereby propose Glycated Hemoglobin Risk Stratification In Diabetes Mellitus (Table 2), the glycated hemoglobin risk stratification in diabetes mellitus (GHRSIDM) describes 7 classes of stratification with equivalent of the classes in standardized known eGA values since eGA correlated well with HbA1c as shown in this study. Under this glycated hemoglobin risk stratification in diabetes mellitus (GHRSIDM), class 1 carry the mildest risk while class 7 carry the worst risk with risk increasing as HbA1c level increase from class 1 to 7 before diagnosis. The lower the class of HbA1c at diagnosis of new onset type 2 diabetes mellitus, the better the prognosis for the patient and the lesser the cellular damage that is attributable to diabetes mellitus that would have occurred. Stratifying new onset type 2 diabetes mellitus based on their level of HbA1c at diagnosis like this gives more meaning to the clinician and laboratory scientist. The laboratory scientist will be able to advise on the class the level of HbA1c belong to at diagnosis and will be able to advise on relevant ancillary investigation needed for a better proper evaluation and management of the patient. The clinician, on the other hand will be guided on areas where particular clinical evaluation will be more relevant in the short and long term for the benefit of the patient.

Classes of Glycated Hemoglobin (HbA1c) values in Percentages (%)	Levels of Glycated Hemoglobin (HbA1c) in Percentages (%)
Class 1	6.5 to 8.4
Class 2	8.5 to 10.4
Class 3	10.5 to 12.4
Class 4	12.5 to 14.4
Class 5	14.5 to 16.4
Class 6	16.5 to 18.4
Class 7	18.5 and above.

N:B: HbA1c level between 5.7% to 6.4 % is prediabetes values and values of HbA1c less than 5.7 are normal HbA1c values.

Table 2: Glycated hemoglobin risk stratification in diabetes mellitus.

This proposed glycated hemoglobin risk stratification in diabetes mellitus (GHRSIDM) table or chart will also

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help to monitor chronic response to treatment better on a long run. This is a very useful clinical information which random blood sugar or fasting blood sugar may not easily give in the long run or may be cumbersome to give except if there is a continuous glucose monitoring reading of 2 to 3 consecutive months available for such purposes and which of course is more cumbersome not only for the clinician and laboratory scientist but also for the patient [31,32].This glycated hemoglobin risk stratification in diabetes mellitus table is also useful in persons with recently diagnosed type 2 diabetes mellitus and in persons with known type 2 diabetes mellitus irrespective of the duration of their type 2 diabetes mellitus management. The risk stratification table may not be relevant in gestational diabetes due to the instability of glycated hemoglobin in pregnancy because of anemia especially in the first trimester of pregnancy. The risk stratification table can be a lifelong evaluator in persons with a newly diagnosed type 2 diabetes mellitus for level of control purposes using HbA1c. The glycated hemoglobin risk stratification in diabetes mellitus table may be applicable to other type of diabetes, since non enzymatic glycation of hemoglobin is part of their pathogenesis.

On a practical note, l am going to use this glycated hemoglobin risk stratification in diabetes mellitus table to analyze the HbA1c of the 50 newly diagnosed persons with type 2 diabetes mellitus in this study for illustrative purposes of its application in real world day to day clinical and laboratory practice worldwide.In this study of persons with newly diagnosed type 2 diabetes mellitus, there are 50 glycated hemoglobin values. The distribution of the HbA1c according to the glycated hemoglobin risk stratification chart is as depicted in [Table 3].In this study, the glycated hemoglobin risk stratification in diabetes mellitus chart application showed that class 1 set of glycated hemoglobin reference values formed the commonest level of laboratory value presentation in person with new onset type 2 diabetes mellitus occurring beyond the first two interquartile range of total number of HbA1c values studied. That is over half of these persons with newly diagnosed type 2 diabetes mellitus present with HbA1c less than 8.5% at onset. Class 2 set of hemoglobin values forms the next commonest reference value of presentation in the laboratory with 26% of laboratory values studied falling in these range. Class 3 set of HbA1c values forms the third most common laboratory presentation in this cohort with 7 sets of values forming 14% of the total study population HbA1c that are in the newly diagnosed diabetic range in this study. Class 4 had only one person with new onset type 2 diabetes mellitus present with a HbA1c value of 14% and forms 2% of the newly diagnosed type 2 diabetes mellitus cohort. Classes 5,6 and 7 did not record any person with new onset type 2 diabetes mellitus in this study.

Classes of Glycated Hemoglobin. (HbA1c)	Number of Glycated Hemoglobin occurrences (n=50)	Percentages (%) of Glycated Hemoglobin that Occurred
Normal	2	4
Prediabetes	1	2
Class 1	26	52
Class 2	13	26
Class 3	7	14
Class 4	1	2
Class 5	None	
Class 6	None	-
Class 7	None	-
Total	50	100

N:B: One HbA1c with value of 6.2% (fasting blood sugar 126mg/dl) fell in the prediabetes range and two HbA1c fell in the normal HbA1c range 4.4% (FBS 140.40mg/dl) and 3.19% (FBS 210mg/dl) to complete the n=50 HbA1c. All these three had fasting blood sugar in diabetic range as shown in parentesis).

Table 3: Showing Distribution Of Glycated Hemoglobin At Presentation in New Onset Type 2 Diabetes Mellitus Using Glycated Hemoglobin Risk Stratification In Diabetes Mellitus (n=50)

Study strength and limitation

This is the first paper in literature that describes a chart, tool, instrument or model of risk stratification of new onset type 2 diabetes mellitus using glycated hemoglobin laboratory values as the parameter of prediction. Another strength of this study is that it is a multicenter study. Another strength of the study is that all participants whether control or subject had a glycated hemoglobin value that meets the study criteria for recruitment. this may not be possible with a probability study like randomized controlled study. Another strength is the fact that the proposed glycated hemoglobin risk stratification in diabetes mellitus is a model and a tool that can be used and reused in persons with newly diagnosed type 2 diabetes mellitus for a lifetime of living with diabetes mellitus allowing for objective monitoring and comparison. The user-friendly chart can be used by both physicians, healthcare workers, patients, caregivers, and patient"s relatives so that communication between healthcare provider and their patients/relative can be with mutual reference chart with ease of understanding. Another strength is that high performance liquid chromatography (HPLC), which is the gold standard for analysis of glycated hemoglobin among other methods was used in the study analysis. This study had limitations which includes the small sample size of new onset type 2 diabetes mellitus in the study. Another limitation is that though the 50 new onset type 2 diabetes mellitus patient in this study represent a multiracial

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group with two non-Nigerians (one male Egyptian and one female Guinea citizen), the overwhelming majority of 96% were Nigerians.Due to the small sample size involved in this study, a validation of this tool by a larger cohort study is proposed.

Conclusion

Glycated hemoglobin values varies in persons with new onset type 2 diabetes mellitus. The correlation between estimated glucose average, random blood sugar and fasting blood sugar is best with estimated glucose average, followed by with random blood sugar and then fasting blood sugar with a weak correlation. Hence, the eGA values of HbA1c can be used to interpret the classes of HbA1c proposed in this study. This description, proposal and calls for the use of "Glycated Hemoglobin Risk Stratification In Diabetes Mellitus" (GHRSIDM) in this study is timely and categorizes HbA1c to classes 1 to 7 based on laboratory values. This allows for risk stratification and assessment at onset, monitoring, prognostication among others where it can act as a life-long chart to guide therapy decision in people living with type 2 diabetes mellitus following their diagnosis at onset.

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Conflict of Interest

The author declare no conflict of interest.

Data availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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