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The Future of HbA1c in Risk Prediction, Prevention and Management of Cardiovascular Events in Diabetes Mellitus: What are the Likely New Treatment Targets? A Perspective

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Abstract:-

- ***Introduction:***

Haemoglobin A1c (HbA1c) offers a retrospective analysis of patient's glucose excursions and is used in determining cardiovascular disease risks in diabetes mellitus patients. The availability of drugs of the class, Sodium-Glucose Co Transporter-2 inhibitors (SGLT), and Glucagon-like –peptide -1 (GLP-1) receptor agonists offer a proactive option to the management of cardiovascular disease complications in diabetes mellitus patients, despite failure to achieve HbA1c levels.

- ***Area covered:***

The lowest level of HbA1c associated with increased cardiovascular risks varies between the young and the old, and between races, as exemplified in the risk of diabetic retinopathy. The Metabolic Memory has also introduced the need to control hyperglycaemia as early as in the pre-diabetes stage, and also to address the factors that maintain the vicious circle of metabolic dysfunction.

- ***Perspective:***

Pre-diabetes carries cardiovascular risk, achievement and maintenance of HbA1c goals among diabetes patients are poor, thus emphasising the cardiovascular disease risks. This is pertinent in regions where the burden of diabetes mellitus is high, and frequent HbA1c monitoring cannot be achieved. The different HbA1c indices have not been shown to prevent stroke, diabetic foot ulcer syndrome and diabetic retinopathy, which would soon become new targets as we achieve greater reduction in the other cardiovascular disease complications. We here offer a new perspective for reasons to start/initiate treatments with lifestyle modification, Metformin and GLP-1 or SGLT therapy.

Keywords:- *Diabetes Mellitus, HbA1c, HbA1c Variability, Cardiovascular Disease Risk, Metabolic Memory.*

ABREVIATIONS:
HbA1c---Glycosylated hemoglobin A1c
HbC--- Hemoglobin C variant
DETECT-2---Diabetes Cardiovascular Risk-Evaluation: Targets and Essential Data for Treatment
DCCT---Diabetes Control and Complication Trial
UKPDS---United Kingdom Prospective Diabetes Study
EDIC---Epidemiology of Diabetes Interventions and Complications
MBG---Mean Blood Glucose
HR---Hazard Ratio
CL---Confidence Level
P---Probability
EMPA-REG--- Empagliflozin Cardiovascular Outcome Event Trial

I. INTRODUCTION: OVERVIEW OF HbA1C

Glycosylated haemoglobin A1c (HbA1c) is a glycosylation product of red cell haemoglobin. Determinations of HbA1c values are important in diabetes mellitus patients, and in those with increased risk of cardiovascular disease. The use of HbA1c as a measure of a patient's average blood glucose level is driven by the relationship of hyperglycemias to chronic complications of diabetes (microvascular and macrovascular).

Glycosylated haemoglobin (HbA1c) was first discovered in 1955, but its elevated levels in diabetes mellitus patients were noticed in 1968. [1] HbA1c is a glycosylation product of red cell haemoglobin. This is a non-reversible covalent bonding that is present throughout the 120 days life cycle of a red blood cell. Its use in the management of hospital cases began in 1976. [2] The HbA1c represents a weighted blood glucose level over the previous three months, with 50% contribution from the preceding month.

HbA1c is falsely low in conditions with reduced red cell life span as in haemolysis, splenomegally, chronic kidney disease, liver cirrhosis, haemorrhage, blood transfusion, hemoglobinopathies (sickle cell disease and

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HbC), and use of erythropoiesis-stimulating agents. The HbA1c level is however falsely elevated in other hemoglobinopathies like, iron deficiency anaemia, vitamin B12 deficiency anaemia that have reduced red blood cell turnover. [3]

HbA1c was recommended for use in the diagnosis of diabetes mellitus following the DETECT-2 study. The level for diagnosis of diabetes mellitus of 6.5% (48mmol/mol) was chosen because it was found that at values above this level the incidence of Diabetic Retinopathy rose sharply. It is considered that this level of HbA1c, the mean blood glucose level equivalent was 141mg/dl, HbA1c level of 6.5% (48mmol/mol) is less sensitive diagnostic index. [4] It was first recommended for use in the diagnosis of diabetes mellitus in 2009 by the International Expert Committee. [5]

II. ALTERNATIVE TESTS TO HbA1c

2a. Fructoseamine level reflects glucose levels over the past 2-3 weeks. It is accepted for blood glucose monitoring in pregnancy where there is need for frequent monitoring of blood glucose that cannot be provided by HbA1c. Pregnancy affects the HbA1c level, yielding falsely low level. The Fructoseamine is also used when HbA1c level is unreliable..[6] The reference range of serum Fructoseamine is 175-280mmol/l. In diabetic patients with good control, the range is about 210-420mmol/l while for uncontrolled diabetes the range is from 268 - 870mmol/l (REF)

2b. Glycated albumin: This reflects the blood glucose level over the past 2-3 weeks too. It is tested with an affinity chromatography with a reference range of 0.6-3%, and by enzymatic assay having a reference range of 11-16%. A typical diabetic range is 2-5 times above normal. It is used as an alternative to Fructoseamine. [7] Glycated albumin reflects more strong impaired glucose intolerance than HbA1c providing earlier indication of glucose dysmetabolism and may predict the trend of HbA1c. [7] It also has an independent clinical relevance concerning risk factors for hyperglycemic micro complications. [8]

Glycated albumin has lower reagent cost and its analysis can be automated on many conventional laboratory instruments. Glycated albumin assays do not vary as fructoseamine which changes with fluctuations in other serum proteins levels. Glycated albumin can be influenced by albumin metabolism as in obesity. Glycated albumin also shows greater correlation to postprandial glucose levels when compared to HbA1c. More studies though are still needed to demonstrate that Glycated albumin can complement or replace HbA1c in conditions where Hba1c values are unreliable. [9,10]

2c. GlycoMarkR (1,5-AG) 1,5 anhydroglucitol. This uses an automated assay. Hyperglycaemia reduces the reabsorption of 1,5-AG thus, giving a low serum level in hyperglycaemia. It reflects glucose levels over the preceding 1-2 weeks. A normal 1,5-AG concentration in women is 6.8-29.3mcg/ml, and in men 10.7-32.0 mcg/ml. [11]

III. USE OF HBA1c IN THE MANAGEMENT OF DIABETES MELLITUS

Treatment of diabetes mellitus is guided by glycemic goals set by Associations' and Societies. However, the goal differs in each case, depending on co-morbidity, presence of gestational diabetes, and age at diagnosis as applied in the individualised care approach in diabetes mellitus patient care. The goals also differ between Associations and Society Guidelines.

The increased use of HbA1c as a measure of a patient's average blood glucose level is driven by the relationship of hyperglycaemia to chronic complications of diabetes (microvascular and macrovascular [12, 13]. The DCCT and the UKPDS had earlier shown that while a 1% reduction in HbA1c in the DCCT study showed a 37% fall in relative risk of microvascular complications in Type 1 diabetes mellitus patients, the UKPDS showed a much smaller 14% non-statistically significant fall in macrovascular complications in Type 2 diabetes mellitus patients. [14]The Epidemiology of Diabetes Interventions and Complications (EDIC) study found that the intensive therapy in the short period of the DCCT study, reduced the long-term risk of cardiovascular diseases by 42%. [14] Laiteerapong and group in the "Diabetes and Aging Study" found that in the first year following the diagnosis and treatment of diabetes mellitus, when those with HbA1c below 6.5% (48mmol/l) are compared to those with levels greater or equal to 6.5% (48mmol/l), there was associated decreased microvascular and macrovascular events. [15] As new studies show that blood glucose variability is more significant in the development of diabetes cardiovascular complications, the need to better understand this variability in blood glucose and HbA1c in comparative analyses led to the studies of:

a) - the average blood glucose levels achieved.
b) - the variability of blood sugar measurements (fasting, postmeal)
c) - glycosylated haemoglobin and the variability of glycosylated haemoglobin
d) - updated mean HbA1c
e) - variability independent of the mean
f) - average real variability

- As measures of pooled averages for use in determining good control. [16,17] The study by Lind, and others, found that using the updated mean HbA1c does not take into account the temporal relationship between HbA1c and diabetes complications. [18] They concluded that the practice of using baseline HbA1c can lead to underestimation of the importance of HbA1c as a risk factor, where only one value is used. [18] However, in the Kilpatrick study it was shown that mean blood glucose (MBG) when compared with the HbA1c was significantly predictive of cardiovascular events during the short study period of the DCCT. The HR for a cardiovascular event and MBG was 1.148 (95% CL=1.036-1.372, p=0.008. For HbA1c the HR was 0.904 (95% CL=0.717-1.138, p=0.389. Specifically for macrovascular events and MBG HR was 1.124 (95% CL=1.032-1.234,

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p=0.007, and for HbA1c HR was 0.874 (95% CL=0.715-1.068, p=0.188. [14]

IV. HbA1c AND DIABETES MELLITUS CHRONIC COMPLICATIONS

The knowledge of HbA1c is important in diabetes mellitus patients, and in those with increased risk of cardiovascular disease. For these patients, achieving low levels early in the natural history may be beneficial. However, HbA1c test is imperfect, with pitfalls both in accuracy and interpretation. [3] It was also found that a single HbA1c measurement in time, underestimates the effect of glycemic excursions. Patients often do not achieve HbA1c targets for a prolonged period, and test intervals are variable. [18] In a study of 568 type 2 diabetes mellitus patients, 102 were found to have Diabetic Peripheral Neuropathy. The patients with the neuropathy had higher HbA1c coefficient of variation and HbA1c, than those without the neuropathy. Among the patients with diabetic peripheral neuropathy, HbA1c variability was found to be more discriminatory than the HbA1c. [19] In another study; by Gu J et al., they showed that HbA1c variability in the long-term was independently and similarly predictive of death or combined endpoints in Heart failure with preserved ejection fraction, and reduced ejection, and heart failure with mid-range ejection fraction. Glycemic variability was used to describe measurements of short-term or long-term fluctuations in glucose level. Short-term refers to within a day or between days glycemic variation and was measured by continuous glucose monitoring. Long-term refers to variations over months to years and was measured by visit-to-visit variability in either HbA1c of Fasting blood glucose or averages. [20] The predictive value of constructed HbA1c variability differed considerably, compared to that of an Updated Mean HbA1c. This risk was found to increase by up to 100% per standard deviation using a constructed Updated Mean HbA1c. Thus, the use of an index HbA1c was found to under-estimates risk of diabetes mellitus complications when compared with time-dependent effect of HbA1c. [18] Garst *et al* in an evaluation of 87,641 patients with diabetes mellitus in 20 studies revealed that higher HbA1c variability in type 1 diabetes mellitus was associated with increase in - renal disease (risk ratio:1.56, 95% CL: 1.08-2.25), cardiovascular events (risk ratio: 1.98, 95% CL:1.39-2.82). and among type 2 diabetes mellitus patients – renal disease (risk ratio: 1.34, 95% CL:1.15-1.57), cardiovascular events (risk ratio: 1.27, 95% CL:1.15-1.40). [21]

V. MEAN BLOOD GLUCOSE, BIOLOGICAL VARIATION OF HbA1c AND HbA1c

The mean blood glucose (MBG) calculated with a 24-hour recording with a continuous glucose monitoring over 5 minutes for 3 months among diabetes mellitus patients and normal patients was found to have a mathematical relationship with the HbA1c [22] This standard can only be achieved using a continuous glucose monitoring that can record both day and night glucose excursions. This can be used to view the HbA1c as a clinical estimate of a patient's mean blood glucose. In 2008, Nathan, *et al*. reported on the relationship of average blood glucose with HbA1c. The goal was to report HbA1c derived averages in the same unit used in self glucose monitoring (mg/dl, mmol/l), this is called the estimated average glucose (eAG). This is hoped to be used in the same way as the estimated glomerular filtration rate (eGFR) in chronic renal failure. [23] Other population studies also showed that although HbA1c correlates highly with preceding mean blood glucose, [24,25] the relationship between HbA1c and mean blood glucose among individuals within a population , show that there is a considerable variation around the population linear regression line at any given mean blood glucose level. [26] It was found that some individuals at the same mean blood glucose value have consistently higher HbA1c and others consistently lower. This finding does not agree with the hypothesis that HbA1c is solely determined by mean blood glucose. This variation is called a Biological Variation of HbA1c. Therefore, there are other factors other than the average blood glucose as represented by the HbA1c that effect an individual patient's risk for cardiovascular disease. This biological variation in HbA1c is an important predictor for the development and progression of diabetes complications. [27]

VI. REDUCTION OF RISKS OF ADVERSE CARDIOVASCULAR EVENTS

HbA1c variability is strongly related to cardiovascular diseases in patients with diabetes mellitus. Monitoring this is very important among young diabetes mellitus patients with good HbA1c control. It was found that the impact of HbA1c variability on young diabetes mellitus patients with lower HbA1c and longer duration of diabetes was greater than in the older age group. The explanation given was that intermittent hyperglycaemia is more effective in producing reactive oxygen species than chronic hyperglycaemia. The recurrent impaired endothelial function causes changes in the epigenetics, and activates release of cytokines. [28] There is no current agreement on the optimal HbA1c level to reduce mortality in heart failure. This is explained by a paradoxical or J-shaped relationship between HbA1c and clinical outcomes. [20, 29] Another study showed a U-shaped relationship between HbA1c and mortality, with the lowest risk seen in patients with moderate glycemic control at HbA1c 7.1-8.0% (53 – 64 mmol/l). [30]

However, in (EMPA-REG) Empagliflozin Cardiovascular Outcome Event Trial, there was a significant reduction in total mortality, morbidity and risk of heart failure co-morbidities of diabetes mellitus, despite the achieved HbA1c which was 7.8% (<64mmol/l). This adds to the unresolved issue of optimal HbA1c level for heart failure. [31]

The newer anti-diabetic drugs – Glucagon-like-peptide-1(GLP-1) receptor agonists (GLP-1RAs), and Sodium-Glucose Co-Transporter -2 inhibitors (SGLT-2is) can stop or reverse these co-morbidities. This is not related to their abilities in reducing blood glucose levels. [31, 32] When there is suboptimal glycemic and cardiovascular risk control in patients with type 2 diabetes mellitus, novel therapies give an added advantage to improve glycemic

control and cardiovascular and renal outcomes. This is in line with the changing guidelines, where second line drugs target morbidities rather than glycemic control. [33]

VII. ANGIOTENSIN CONVERTING ENZYME-2 IN THE MANAGEMENT OF DIABETES CARDIOVASCULAR COMPLICATIONS

The use of angiotensin converting enzyme (ACE) inhibitors to block the vasoconstriction and hypertrophic actions of angiotensin-II only slows but does not prevent the progression of such complications among diabetes mellitus patients. The discovery of angiotensin converting enzyme 2 (ACE2) in the heart and kidneys that acts in a counter-regulatory manner to angiotensin converting enzyme (ACE1), may play a role in mitigating the pathophysiological changes in cardiac and renal disease. [34] Increased Renin Angiotensin System (RAS) over activity contributes towards impaired glycaemia, and its' blockade is shown to be beneficial in improving glycaemia. [35]

ACE2 is protective against the detrimental effects of angiotensin-II (Ang-II) by decreasing the level of Ang-II and increasing the level of angiotensin 1-7 (Ang 1-7). This is postulated to be capable of decreasing the effect of Ang-II on vasoconstriction, fibrosis, inflammation, and endoplasmic reticulum stress and β-cell death. [35] All these would lead to protection of β-cell mass and improved insulin production. Components of RAS have been identified in systemic circulation, eyes, brain, heart, pancreas and islets of Langerhans. Angiotensin-II is known to moderate disorders such as hypertension, and heart failure, stroke. Angiotensin-II is now shown to impair glucose tolerance by inducing insulin resistance and blunting insulin secretion from the islet in the face of hyperglycaemia. [35]

ACE2 is expressed in the kidney, heart, lungs, testis, and also in glucose-regulating tissues such as the pancreas, adipose tissue and liver. ACE2 belongs to the M2 zinc metalloproteinase family. ACE2 degrades Ang-I and Ang-II, to produce Ang (1-9) and Ang (1-7) respectively. Ang (1-7) is an endogenous ligand for the Mas receptor (MasR), a vasodilator peptide that opposes some effects of vasoconstrictor Ang-II. [36]

In an animal study, increased expression of ACE2 in rats with streptozotocin-induced diabetes protected them against retinal vascular dysfunction. [36 37]

VIII. METABOLIC MEMORY

Glycemic control in the initial stages (pre-diabetes and at diagnosis) of diabetes, decreases macrovascular outcome and microvascular changes such as diabetic - retinopathy, nephropathy and neuropathy. The memory of poor control early in the disease continues even after a return to normoglycaemia in hyperglycaemic diabetes mellitus patients. This memory is dependent on the hyperglycaemia-stimulated production of reactive oxygen species (ROS) in mitochondria. The memory of hyperglycaemia initiates a vicious cycle of ROS induced mutations in the mitochondrial DNA. [38] This envisages a new strategy of not only aggressive treatment of hyperglycaemia, but additional use of compounds active on Active Glycation End Product (AGE), and those capable of targeting mitochondrial reactive species. [39 40]

IX. DISCUSSION

It has been shown that in the presence of the feared co-morbidities, glycemic goals become secondary to modifiable cardiovascular disease risk factors. [coronary artery disease, heart failure, cerebrovascular accidents, chronic renal failure, diabetic retinopathy, diabetic peripheral neuropathy, intracranial arteriosclerosis]. [26] Thus, the target becomes the pathophysiological changes to chronic hyperglycaemia.

This is evidenced by the finding that the mean percentage time in suboptimal HbA1c control in the first 2 years following diagnosis was found to be 30%, 34% and 40% for HbA1c thresholds of 8% (64mmol/mo/), 7.5%, 7% (53mmol/mol) respectively. [41] A study among Korean diabetes patients aged 20-39 years with early-onset diabetes and prediabetes found increased cardiovascular risks and all-cause mortality after a 10-year follow-up. Furthermore, recovery to normal fasting blood glucose among the group diagnosed as prediabetes with impaired fasting blood glucose at base line was associated with only a reduction in the cardiovascular disease risks and all-cause mortality.[42] Another study also found that prediabetes was associated with more cardiovascular disease risks and all-cause mortality in the general population and among those with artherosclerotic cardiovascular disease, leading the authors to call for an increase in screening for, and the management of prediabetes state, towards a primary and secondary prevention of cardiovascular disease.[43] This proactive approach would aim at preventing risks, and early management of common micro and macro- vascular complications of diabetes mellitus..

There is also a new hypothesis on what drives pro-inflammatory cytokines in diabetes. This is suggested to be related to lipid and mitochondrial dysfunctions and not glycolysis through glucose in Diabetes Mellitus patients. This explains why people with tight glucose control can nonetheless have disease progression. The initial hyperinsulinemia in type 2 diabetes leads to dyslipidaemia and mitochondrial dysfunction. [44]

To compound these, the data on HbA1c vary greatly between races, the HbA1C having been suggested as "invalid" or "misleading" as a diagnostic test in African Americans. [45, 46] The HbA1c levels vary with their blood glucose equivalents for risk of chronic complications. In a HbA1c study of the mean baseline HbA1c for incidence of diabetic retinopathy, the mean for individuals with black ancestry was 7.9% and white ancestry was 7.5%; $p<0.001$, while for both the baseline glucose level was the same at 9.0mmol/l. [47] Using diabetic retinopathy that was used in determining diagnostic HbA1c level, a population study showed; that after accounting for disease duration and other important cofounders, people with type 2 diabetes mellitus

diagnosed in their youth and early adulthood (or with a younger current age) were inherently more susceptible to retinopathy. [48] This is against the held belief that chronic complications (micro and macro-vascular) are based on chronic exposure to hyperglycaemia. This calls for a search for yet unidentified contributing factors for risk of retinopathy. The HbA1c cannot clarify past or future hypoglycaemic events, recovery with or without hyperglycaemia, post prandial hyperglycaemia, or glucose fluctuations. This explains the lack of benefit of tight versus standard glucose control in type 2 diabetes mellitus patients with relatively high baseline cardiovascular risk. Fluctuations in blood glucose levels lead to increase in free radical and greater endothelial dysfunction, and these radicals have a more deleterious impact than those produced under conditions of stable high blood glucose. [49]

X. CONCLUSION

The question therefore is, if these new molecules can stop, and reverse these co-morbidities, independent of their blood glucose reduction capacity, what will be the next goal of blood glucose control? What will be the next duration of diabetes to predispose to risks, and to qualify for the use of the new molecules? What new risks would become predominant [acute complications, retinopathy, neuropathy, stroke, foetal complications in pregnancy, wound healing, acute medical situations/admissions]?

The availability of the new molecules will get to the developing countries including Africa a long time after the new guidelines on use of SGLT2 enzyme inhibitors and GLP-1 agonists. Since the new molecules target the complications, we suggest using available resources towards the provision of these in resource poor countries, than the use of frequent HbA1c tests for determining cardiovascular risks after the memory had been fixed.

XI. EXPERT COMMENTARY:

The place of HbA1c and its indices will continue in the monitoring of response to lifestyle and drugs. They would guide treatment intensification and prevent treatment apathy.

However, they will no longer be used to offer/determine hope of averting complications when a vicious circle of metabolic dysfunction had been set in motion.

XII. 5-YEAR VIEW:

The universal acceptance of a first line regimen would reduce the cost and availability of these drugs. The burden of diabetes would be reduced. New research in areas of diabetic retinopathy and diabetic foot ulcer syndrome prevention will increase.

Screening for Pre-diabetes would now become cost effective and good practice. Prevention of diabetes should commence in these groups, with early inclusion of drugs used in the treatment of diabetes mellitus when goals are not achieved. Research towards the determination of test intervals would also spring up.

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