Diagnostic Accuracy of Glycated Haemoglobin A1c and 1,5 Anhydroglucitol in Monitoring of Type 2 Diabetes Mellitus at University Teaching Hospital, Lusaka, Zambia

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Abstract

**Background:** Diagnosis and monitoring of diabetes has long been premised on fasting blood glucose and a hemoglobin-dependent biomarker, glycated hemoglobin A1c (HbA1c) which is a variant of hemoglobin A. However, adjunct to these markers, 1,5 anhydroglucitol (1,5AG), has since been introduced as a surrogate marker for diabetes. Because of limited capacity by routine blood and urine glucose tests in monitoring glycemic control over period of time, HbA1c and 1,5AG have the ability to predict diabetic complications and glycemic control over weeks and months. Our objective was to compare the monitoring of HbA1c and 1,5AG biomarkers in Type 2 Diabetes Mellitus (T2DM).

**Methods:** This cross-sectional study was conducted on 44 diabetic individuals and 42 non-diabetic individuals of age ranging from 20 to 80 years at University Teaching Hospital, Lusaka, Zambia. The threshold was set at various cutoff points for glycated hemoglobin A1c and 1,5AG, respectively. Data were coded and analyzed using multivariate methods for diagnostic efficiency, associations and compare of means.

**Results:** Mean serum 1,5AG mean was 61.6±10.1ng/dL in diabetics and 52±4.46ng/dL in non-diabetics, giving a non-statistically significant difference, p˂0.451. HbA1c was more specific (92%) and sensitive (95.9%) than 1,5AG, with sensitivity and specificity of 50.0 and 52.5, respectively. Serum HbA1c mean (7.75±0.34%) was significantly higher in diabetics than non-diabetics (4.36±1.6%), p˂0.001. Also, 1,5AG was poorly correlated with HbA1c in diabetics (r=0.123, p=0.298).

**Conclusion:** HbA1c was found to be robust and reliable in monitoring long-term glycemic control in T2DM than 1,5AG. Our study supports a possible cut-off point ≥5.5% of HbA1c in line with WHO recommendations below 7% threshold.

**Key words:** Diabetes mellitus, glycated haemoglobin, 1,5 anhydroglucitol, University Teaching Hospital, Zambia
I. INTRODUCTION

Background

Diabetes mellitus (DM) is a chronic metabolic condition affecting all age groups and gender but mostly associated with obesity and physical inactivity [1]. Traditional and non-traditional markers such as Glycated haemoglobin (HbA1c) and 1,5 Anhydroglucitol (1,5AG) have been suggested as glycemic metrics over long and short periods, respectively. Due to poor diagnosis and monitoring over periods of time, patients with type 2 diabetes mellitus are at higher risk of developing both microvascular and macrovascular complication [2]. Therefore, in 2009, an International Expert Committee that included representatives of the American Diabetes Association (ADA), the International Diabetes Federation and the European Association for the Study of Diabetes recommended the use of HbA1c test for monitoring glycemic control with a threshold of ≥6.5% and this criterion was adopted by ADA in 2010 [3]. However, another biochemical marker, 1,5AG, has been suggested as an adjunct to HbA1c. 1,5AG is a 1-deoxy form of glucose, haemoglobin independent, that has been measured and used clinically for monitoring of diabetes mellitus in Japan for decades [4,5].

Due to the long lifespan of erythrocytes [6], the percentage of HbA1c reflects the glycaemic control of a patient during the 8-10 week period before the blood sample is obtained [3]. However, another biochemical marker, 1,5AG, has been suggested as an adjunct to HbA1c. 1,5AG is a 1-deoxy form of glucose, haemoglobin independent, that has been measured and used clinically for monitoring of diabetes mellitus in Japan for decades [4,5].

According to Ishida, 1,5AG is more sensitive and robust than HbA1c or Fructosamine (FA) [8]. In addition, 1,5AG is a useful marker of short-term episodes of postprandial and acute hyperglycaemia, which might be missed, in standard used assays such as self-monitored blood glucose (SMBG) or HbA1c and Fructosamine [9,10]. Because of limited capacity by routine blood and urine glucose tests in monitoring glycemic control over period of time, HbA1c and 1,5 AG have been shown to predict the risk of developing diabetic complications and glycemic control over weeks and months. Hence, the study was undertaken to compare the monitoring efficiency of HbA1c and 1,5 AG as monitoring tools for diabetes mellitus.

Methods

All type 2 diabetic and non-diabetic individuals attending diabetic and medical clinics at University Teaching Hospital were included into the study. After review of medical records, individuals in the age group of 20-80 years of both sexes were included in the study. By using convenience sampling, a total of 86 participants were recruited in the study. While, individuals who gave a history of any cardiovascular, hepatic, renal, or anaemic disorders were excluded from the study.

Details pertaining to age and gender were captured using a structured questionnaire and Body Mass Index (BMI) calculated. Venous blood samples from all the subjects were collected after patient observed overnight fasting and analyzed for glycosylated hemoglobin (HbA1c) and, 1,5 Anhydroglucitol using the NeoBioLab® Enzyme Linked Immunosorbent Assay (ELISA), a quantitative competitive immunoassay for measurement of between fasting and post prandial glucose control [7].
human serum levels according to the manufacturer’s protocol.

The unpaired student t-test was used to compare mean values of HbA1c and 1,5 AG between the T2DM and non-T2DM groups. All statistical tests were performed at 5% significance level or 95% confidence interval with p-value of <0.05 to determine statistical significance. Area under the Receiver operator characteristics curve (AUROC) analysis was used to predict the accuracy of the biomarker and how well they discriminate diabetics from non-diabetics. The data was analyzed using SPSS v22 (IBM, Chicago, USA) and MATLAB 2016a (The Mathworks, Inc. Natick, Massachusetts). Verbal and written informed consent was obtained from each participant and the study approved (IRB00001131 of IORG0000774; Ref: 003-12-15) by the University of Zambia Biomedical Research Ethics Committee (UNZABREC).

Results
Our study population included 86 adult participants ranging from 20 to 80 years, 35 were males (41%) and 51 females (59%) with a mean age of 48.3±16.62 (mean±SD) years. The mean age for diabetics was 52.5±12.35 and 44.8±19.26 for non-diabetics who served as controls, respectively.

Demographics and Anthropometric Characteristics of the Study Population
Demographic and anthropometric characteristics are presented in Table 1 and Figure 1, respectively. T2DM was most prevalent between the ages of 41 to 70 years consistent with observations that T2DM affects the elderly more than the young. The mean BMI was higher among diabetics (27.18±4.45kg/m²) than non-diabetic individuals (25.25± 4.45kg/m²), Figure 1.

Interaction of T2DM and Gender on HbA1c and 1,5AG
A two-way ANOVA was conducted that examined the effect of T2DM and gender (two levels; male and female) and on serum HbA1c and 1,5AG levels, (Figures 2a and b).

Mean concentration for HBA1C and 1,5 Anhydroglucitol
T2DM participants, had a statistically significant higher mean HbA1c (7.75 ± 0.34%) compared to the non-diabetic group (4.36 ± 1.6%), t(59.1) = 9.01, p < 0.001. Serum mean 1,5AG, mean concentration was statistically different between diabetics (61.6±10.1 ng/dl) compared to non-diabetic group (52±4.64), t(80) =0.757, p<0.451(Figure 3).

Correlation analysis of HbA1c and 1,5AG
There was a weak correlation between HbA1c and 1,5 AG in T2DM participants without statistical significance (r = 0.123, p = 0.298) (Figure. 4).

Monitoring efficiency of HbA1c and 1,5AG in T2DM
Between HbA1c and 1,5 AG, HbA1c was the most specific (92.9%) and sensitive (95.9%) biomarker. The specificity (50.0%) and sensitivity (52.5%) for 1,5 AG was low with a poor overall monitoring of 51.2%. The AUROC and statistical significance is presented in Table 2 and figure 5.

Discussion
Our study demonstrated that diabetics were slightly older while females who participated were more in both diabetic and non-diabetic groups. These findings are in agreement with a World Health Organisations’ observation that T2DM is a condition mainly seen in adults (41-70yrs) [11]. The mean BMI is consistent with
study by Choi et al [12] and also in concordance with T2DM being largely associated with obesity and physical inactivity [11].

We demonstrated that HbAlc had a superior monitoring efficiency (94.0%) compared to 1,5 Anhydroglucitol (51.2%). These findings are in concordance to a study by Choi et al., which found HbAlc with an excellent specificity (91%) and sensitivity (68%), respectively [12]. Another study by Shimodaira et al. [13] found a sensitivity of 83.7%, and specificity 87.6% for optimal HbAlc cut-offs for monitoring diabetes also found similar results, sensitivity (86%) and specificity (86%) [11,13]. These studies confirm our finding and long-standing evidence that put HbAlc as the superior and gold standard biomarker for glycaemic control over time. However, a much lower sensitivity (<60%) that only improved to 78% when combine with glycated albumin and fructosamine has been reported Summer et al [14].

In contrast, specificity (50.0%) and sensitivity (52.5%) of 1,5 AG was low with a poor overall monitoring efficiency of 51.2%. A study by Pal et al. found a similar specificity of 42% in discriminating diabetes subtypes [15]. But these findings show a marked disparity with other studies that indicate 1,5-AG to reflect glycemic excursions, often in the postprandial state, more sensitive and robust than HbAlc [8,9,10]. This contrasting evidence in predicting diabetes is indicated by the remarkably lower positive and negative predictive values. This shift in the proven concept that, 1,5 AG is an inert metabolite and better predictor of hyperglycemia, should be investigated by conducting further research using larger sample sizes with a more stringent inclusion and exclusion criteria specific for 1,5-AG and a design that requires continuous monitoring of glucose concentrations over an extended period of time, for which 1,5AG will be measured. Nevertheless, HbAlc and 1,5AG were higher in diabetics consistent with several studies and observed trends indicative poor glycaemic control [16, 17].

In comparison between the diabetic and non-diabetic cohorts this study found that diabetics had a statistically significantly higher mean HbAlc compared to the non-diabetic group (Figure 3a) similar to the findings by Suzuki et al [18]. This value is above the American Diabetics Association recommended <7% upper limit; reference value 4.6 to 6.2 % for adults while the American Association Clinical Endocrinologists suggests levels less than 6.5% [18-22]. Our study demonstrated poor glycaemic control, occurrence that predisposes diabetics to the risk of developing both long-term macro and microvascular complications. There was no statistically significant mean in 1,5AG levels between the two groups. We are persuaded to postulate that this average monitoring accuracy obtained in this study could be due to diet, genetics and other epigenetic factors not covered in this study. This may further demonstrate that 1,5 AG was not a sensitive and specific marker for glycaemic control over time.

**Conclusion**

In our study, we found that HbAlc was a reliable and robust biomarker for monitoring hyperglycaemia overtime in individuals with T2DM than 1,5 AG. Mean serum levels of HbAlc were higher in diabetics than diabetics affecting mostly the middle, overweight and obese participants. Glycated haemoglobin could be a useful biomarker for diagnosing and monitoring glycaemic control. We propose a possible cut off point ≥5.5% in our Zambian population as opposed to World Health Organisation.
Figures and Tables

Table 1: Age distribution showing high prevalence of diabetes in the middle aged group (41-70 years old) among diabetics.

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Diabetics (n=44)</th>
<th>Non-Diabetics (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-40</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>31-40</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>41-50</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>51-60</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>61-70</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>71-80</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 1: Trend showing a proven concept that diabetes is most common among the overweight and obese. More women participated in the study and were more overweight and obese than men in both diabetic and non-diabetic groups.

Table 2: Monitoring of HbA1c and 1,5-AG

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>HbA1c (5.5%)</th>
<th>1,5-AG (42.6ng/dl)</th>
</tr>
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<tbody>
<tr>
<td>Specificity (%)</td>
<td>92.9</td>
<td>50.0</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>95.5</td>
<td>52.5</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>97.6</td>
<td>52.5</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>88.6</td>
<td>50.0</td>
</tr>
<tr>
<td>Overall efficiency (%)</td>
<td>94.0</td>
<td>51.2</td>
</tr>
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</table>

Monitoring efficiency was evaluated using logistic regression for each variable. Overall efficiency of HbA1c, Glucose, Self-Glucose were excellent, 1,5-AG displayed a poor overall efficiency. Cut-off points were calculated.

Figure 2a: Shows a high HbA1c concentration in both females and males among diabetics than non-diabetic participants. Figure 2b: Showing high levels of 1,5AG among the females in both diabetic and non-diabetic participants.

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Figure 3: Graph showing high levels of HbA1c (3a), 1,5AG (3b) among the diabetic than non-diabetic group indicating poor glycaemic control.

Figure 4: Showing a poor correlation between 1,5 AG and HbA1c in T2DM.

Figure 5: The ROC Curve for 1,5 AG and HbA1c in T2DM.
Acknowledgment
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Authors’ contributions
Makungu Peter and Trevor Kaile designed the study. Sinkala Musalula completed the laboratory analysis and statistical analysis. Timothy Kantenga, Choongo Kennedy, Angela Sinyani, Christopher Newton Phiri and Mildred Zulu contributed to Specimen processing and interpretation of Data. All authors contributed to writing, reading and approved the final manuscript.
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