Glycated Haemoglobin A_{1c} Measurement in Stored Whole Blood Sample is Reliable for Clinical Use

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ABSTRACT

Glycated haemoglobin A_{1c} (HbA_{1c}) gives an integrated plasma glycaemia for the previous 2–3 months and its measurement is central in the management of diabetic patients. However, in many developing countries because kits/regents or expertise for HbA_{1c} measurement are not always available and the test must be conducted on fresh whole blood samples, HbA_{1c} tests are not routinely performed. Thus, this study aimed to determine if the degradation products from whole blood sample storage are significant enough to compromise the diagnostic value of HbA_{1c} measurements. Two hundred and thirty-one fresh whole blood samples with pre-determined HbA_{1c} values were stored at between 2–8°C and using boronate affinity immunoassay technique, HbA_{1c} values were then measured in the same whole blood samples after 20 days of storage. The results showed that there were no significant differences in the mean values of the initial HbA_{1c} measurement and the values obtained after storage (7.5 ± 2.0 vs. 7.5 ± 2.1, $p > 0.05$) and this was irrespective of gender.

Furthermore, irrespective of gender, there were significant correlations between the HbA_{1c} values measured in fresh whole blood samples and values obtained after storage ($r = 0.83$, $p < 0.01$). Therefore, based on these findings and other previous reports, the effect of storage degradation product was not significant enough to compromise the clinical or research use of HbA_{1c} test results from stored whole blood samples. However, we recommend that diagnostic laboratories should evaluate their HbA_{1c} measurement techniques for HbA_{1c} determination in stored whole blood samples. Any persistent upward or downward bias in stored whole blood samples should be reported to guide the physician in interpreting HbA_{1c} results from stored whole blood samples from that laboratory and/or technique.

La Medición de la Hemoglobina Glicada en Muestras de Sangre Entera Almacenadas es Confiable para el uso Clínico

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RESUMEN

La hemoglobina glicada o glicosilada A_{1c} (HbA_{1c}) produce una glicemia plasmática integrada en los últimos 2–3 meses y su medición es fundamental para el tratamiento de pacientes diabéticos. Sin embargo, en muchos países en vías de desarrollo – debido a que no siempre hay kits/reactivos o conocimiento experto para la medición de HbA_{1c}, y la prueba tiene que realizarse con muestras de sangre entera fresca – no se realizan tests de HbA_{1c} de forma rutinaria. Así, este estudio apuntó a determinar si los productos de degradación del almacenamiento de la muestra de sangre entera son suficientemente significativos como para comprometer el valor del diagnóstico de las mediciones de las dimensiones de HbA_{1c}. Doscientos treinta y una muestras de sangre entera fresca con valores HbA_{1c} pre-determinados, fueron almacenadas entre 2–8°C y usando la técnica de inmunoensayo de afinidad al boronato, los valores de HbA_{1c} fueron entonces medidos en las mismas muestras de sangre entera después de 20 días de almacenamiento. Los resultados mostraron que no había ninguna diferencia significativa en los valores promedios de la medición inicial de HbA_{1c} y los valores obtenidos después del almacenamiento (7.5 ± 2.0 vs. 7.5 ± 2.1, $p > 0.05$), independientemente del género. Además, con independencia del género, hubo correlaciones significativas entre los valores de HbA_{1c} medidos en las

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muestras de sangre entera fresca y los valores obtenidos después del almacenamiento (r = 0.83, p < *0.01). Por lo tanto, basado en estos hallazgos y otros informes anteriores, el efecto del producto de la degradación por almacenamiento no fue suficientemente significativo como para comprometer el empleo clínico o investigativo de los resultados de la prueba de HbA_{1c} a partir de muestras de sangre entera almacenada. Sin embargo, recomendamos que los laboratorios de diagnóstico evalúen sus técnicas de medición para la determinación de HbA_{1c} en las muestras de sangre entera almacenada. Cualquier tendencia persistente ascendente o descendente en las muestras de sangre entera almacenada debe ser reportada a fin de orientar al médico en la interpretación de los resultados de HbA_{1c} de las muestras de sangre entera almacenadas por el laboratorio y/o para la técnica.*

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INTRODUCTION

The International Diabetes Federation (IDF) has projected that by the year 2025, 11.8% of the population of Trinidad and Tobago will be diagnosed with Type 2 diabetes mellitus, representing one of the highest prevalence rates in the North American region (1). Previous research studies in Trinidad have shown increased prevalence of cardiovascular disease risk factors which were associated with poor glycaemic control in Type 2 diabetic patients (2–4). Indeed, poor glycaemic control is linked to macrovascular complications in Type 2 diabetes (5). Although it is still controversial whether it is poor fasting or postprandial glycaemia that contributes more to macrovascular complications in diabetes (6), it is well known that Type 2 diabetic patients are two to four times more likely to develop coronary heart disease (CHD) than patients without diabetes (7). Thus, regular assessment of long-term plasma glycaemia in Type 2 diabetic patients is important in monitoring the risk of cardiovascular disease. Glycated haemoglobin A_{1c} (HbA_{1c}) is well accepted as the biochemical parameter for assessing long-term glycaemic control in diabetic patients (8). Indeed, the United Kingdom Prospective Diabetes Study (UKPDS) coronary heart disease (CHD) risk tool, UKPDS Risk Engine, incorporated HbA_{1c} as one of the important parameters in CHD risk prediction (9). HbA_{1c} is usually measured on fresh whole blood samples but current clinical chemistry guidelines state that whole blood samples stored at -70°C remained stable for measurement of HbA_{1c} using High Pressure Liquid Chromotography (HPLC) technique (8). Indeed, other workers, using HPLC technique have found strong correlations between fresh whole blood HbA_{1c} values and values obtained from the same whole blood samples stored for over a decade (10). In our research laboratory and the allied hospital, there is no research report on HbA_{1c} measurement from stored blood samples hence HbA_{1c} is typically conducted on fresh whole blood samples. Delays in procurement of assay kits *eg* due to their temporarily being out of stock often result in cancellation of HbA_{1c} tests for patients or even for research activities. Thus, this study was aimed at determining if the degradation products from blood storage are significant enough to compromise the diagnostic value of HbA_{1c} measurements. It is envisaged that the result of the study would assist laboratory management staff to take decisions when there is shortage of reagents or when the procurement of assay kits is delayed.

MATERIALS AND METHODS

Two hundred and thirty-one fresh whole blood samples submitted to the diagnostic laboratory of the Eric Williams Medical Sciences Complex (EWMSC) for HbA_{1c} measurements were collected and stored at between 2–8°C for 20 days after determining the HbA_{1c} levels. The initial HbA_{1c} levels were determined using boronate affinity immunoassay technique (Axis-Shield PoC AS, N-0504 Oslo, Norway) before storage. The manufacturer's instruction (Axis-Shield PoC AS, N-0504 Oslo, Norway) indicated that blood samples stored at 2–8°C for up to 10 days before analysis could still yield reliable results. Based on our estimation that delays in procuring assay kits could take up to 21 days to arrive, we decided to store the samples for twice times the manufacturer's recommendation to determine if longer storage could significantly affect diagnostic values of the results. Thus, the initial HbA_{1c} values and date of measurements were recorded in the computer spread sheet. After 20 days of the initial test, the HbA_{1c} measurements were repeated on the stored whole blood samples using the same boronate affinity immunoassay technique and the values recorded. The two hundred and thirty-one blood samples were determined in 14 batches based on the dates blood samples were drawn. The repeat laboratory tests for HbA_{1c} in the stored whole blood samples were performed by the same technician that performed the initial analysis.

The results are expressed as mean ± SD. The Statistical Package for the Social Sciences (SPSS Inc, Chicago, USA) software was used in all analyses. The difference between the initial HbA_{1c} values and the values after storage were determined using paired student's t-tests while the correlation between the initial HbA_{1c} values and the values after storage were determined by Pearson correlation technique. A p-value < 0.05 was considered statistically significant on two-tailed testing for all analysis.

RESULTS

The assay has a measuring range of 3–18% HbA_{1c} while the measuring interval is 0.1% HbA_{1c}. The repeat tests for the 231 blood samples (14 batches) had overall inter-assay coef-

ficient of variation of 3.9% and 7.3% for the normal and the abnormal quality control samples respectively. Table 1 shows the initial mean ± SD values of the HbA_{1c} obtained from fresh whole blood samples and values obtained after 20 days of storage at 2–8°C. There was no significant difference in the mean value of the initial HbA_{1c} measurement and the value obtained after 20 days of storage irrespective of gender ($p > 0.05$). Although the mean of the differences range from -0.04% to 0.02% in female and male subjects respectively, the overall mean of the differences and the standard deviation (measure of upward or downward bias) obtained were -0.01% and 1.2% (Table 1). Figures 1 and 2 show the correlations between the HbA_{1c} values obtained from fresh whole blood samples and the values obtained after storage. There were significant correlations between the HbA_{1c} values measured in fresh whole blood samples and values obtained after storage (r = 0.83, $p < 0.01$) and the correlations were also strong in male and female patients (Fig. 2, all $p < 0.01$).

Table 1: Assessment of the differences in HA_{1c} values in fresh whole blood samples and in stored whole blood samples

Characteristic of samples	Subjects All	Males	Females
Fresh blood sample mean (±SD) (%)	7.5 (2.0)	7.3 (2.1)	7.5 (2.0)
Stored blood sample mean (±SD) (%)	7.5 (1.6)	7.4 (1.8)	7.5 (2.1)
Mean of the difference (stored – fresh sample) %	-0.01	0.02	-0.04
±SD of the mean of the differences (measure of upward or downward bias) %	1.2	1.1	1.3

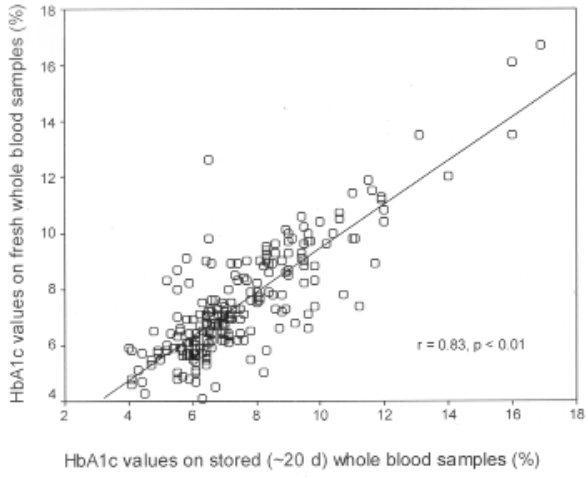

Fig. 1: A scatter plot showing a strong correlation between glycated haemoglobin A_{1c} values on fresh whole blood and stored (2–8)°C for. 20 days) whole blood samples.

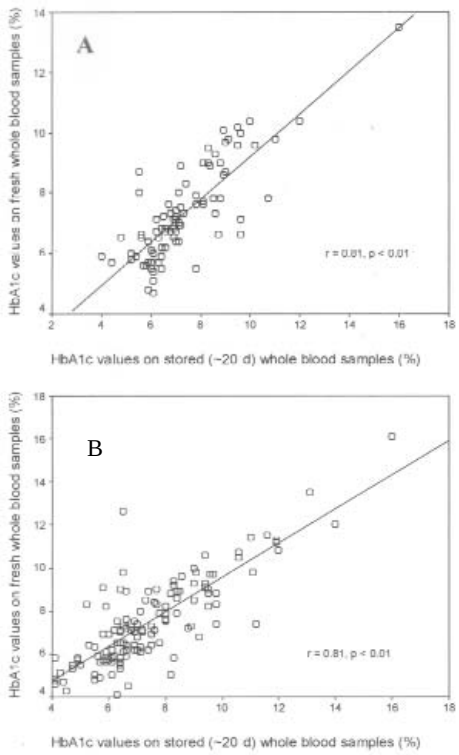

Fig. 2: A scatter plot showing a strong correlation between glycated haemoglobin A_{1c} values on fresh whole blood and stored (2–8°C for 20 days) whole blood samples of male (A) and female (B) patients.

DISCUSSION

The present study attempted to determine the reproducibility of HbA_{1c} measurements in stored whole blood samples and the results have shown that reliable HbA_{1c} results could still be obtained from whole blood samples stored for up to twice the kit manufacturer's storage recommendation. This is consistent with other reports on frozen whole blood samples stored for even longer periods (10). This has important implications in the management and treatment of diabetic patients and for diabetes research.

The present observation is potentially useful for physicians and researchers in developing countries where HbA_{1c} measurement is typically conducted on fresh blood samples and yet the reagents/kits for HbA_{1c} measurements are often in short supply. The apparent limitations (resources, expertise *etc*) in measuring HbA_{1c} on a routine and/or regular basis in many developing countries might have contributed, in part, to several research reports of poor glycaemic control in many developing countries (11–14). In Trinidad and Tobago, reports of poor glycaemic control is particularly worse at the primary care setting where we have previously reported on the cardiovascular risk implications of

poor glycaemic control (14, 15). Of concern is the fact that in most primary care clinics in Trinidad and Tobago, the mean HbA_{1c} value for each clinic exceeded the 7.0% cut-off point recommended from the landmark UKPDS report (5). In our experience, laboratory requests for HbA_{1c} tests for diabetic patients are not routine and regular given that physicians, especially at the primary care settings, rely on the routine fasting blood glucose measurement in the clinic for the review of the patient's medical prescription. We are of the view that HbA_{1c} measurement should be routine and regular to guide prescription review especially as diabetes health educators and dieticians are in short supply in many developing countries (16). Furthermore, measurement and use of fasting blood glucose results, as a guide in determining the patient's plasma glycaemia, is not technically reliable given that most patients do not always conform to the requirements for determining fasting blood glucose concentration as stipulated in the new diagnostic criteria (17).

Therefore, for effective management of diabetic patients, it is important that the physician know the true integrated long-term glycaemic level of his/her patient as estimated by HbA_{1c} tests. In many developing countries, this is largely dependent on the availability of the HbA_{1c} test kits given that non-availability of the test kit/reagents or even technical and laboratory expertise, as is often the case at primary care settings, means that the physician would have missed this important laboratory information that would better guide prescription review. This is essentially the general uptake in many primary care settings in the developing countries and since there is no research documentation to suggest or recommend blood sample storage for HbA_{1c} measurement, HbA_{1c} tests are often omitted when the reagents/kits are not readily available. For instance, in the present study, the leaflet in the kit used (Axis Shield PoC AS, N-0504, Oslo, Norway) recommended storage duration of less than 10 days. Therefore, the results of the present study, which found a strong correlation between the fresh HbA_{1c} values and storage values, should encourage researchers and laboratory managers currently using the above manufacturers' HbA_{1c} kits to carry out tests on whole blood samples stored longer than the manufacturer's recommendation. Indeed, the present study has demonstrated that the effect of storage degradation product was not significant enough to compromise the clinical or research use of HbA_{1c} test result from stored whole blood samples. The finding of 1.2% bias in this study for stored samples is further strengthened by a previous report that showed only 0.35% upward bias in frozen whole blood samples stored for over a decade (10). Therefore, based on the present findings and other previous reports (10), researchers and clinical diagnostic laboratories should evaluate their HbA_{1c} measurement techniques for HbA_{1c} determination in stored whole blood samples. Any persistent upward or downward bias in stored whole blood samples should be reported to guide physicians in interpreting HbA_{1c} results from stored whole blood samples from that particular laboratory and/or technique.

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