The African Journal of Medicine and Medical Sciences

Volume 15
1986

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Palo Alto Melbourne

Afr. J. Med. med. Sci. (1986) **15**, 49–53.

Haemoglobin A_1 levels in Nigerian diabetic patients using microcolumn affinity chromatography

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Summary

Haemoglobin A_1 (HbA_1) levels were measured in forty-five diabetic Nigerians and thirteen non-diabetic controls using disposable micro-column kits based on affinity chromatography. The effect on HbA_1 results of storage of whole blood samples for 1 week at room temperature and in a refrigerator was also evaluated. The mean ± s.d. of HbA_1 levels in diabetic patients and controls were 12.1 ± 5.6% and 5.2 ± 0.90% respectively. The difference was highly significant, $P < 0.001$. Newly diagnosed diabetic patients who had not been on any treatment had significantly higher mean value of HbA_1, 14.4 ± 5.0%, than old patients already on treatment, 9.5 ± 2.9%, $P < 0.01$. In a group of nine patients, the mean ± s.d. of baseline HbA_1 value was 10.3 ± 4.2% and after 1 week storage of different portions of the samples at room temperature and in a refrigerator, the mean ± s.d. respectively were 9.8 ± 3.6% and 11.0 ± 4.2%. There was thus a slight decrease on storage at room temperature and a slight increase during refrigeration but the observed differences were not statistically significant. It is concluded that the glyc-affinity micro-column chromatography is a satisfactory and potentially useful method for measuring HbA_1 levels in tropical developing countries. The method was easy to use and the assays could be run under average ambient room temperature without recourse to use of an air-conditioned room or a special chromatography chamber.

Résumé

Les taux d'hémoglobine A_1 (HbA_1) furent mesurés chez 45 Nigérians diabétiques et 13 témoins non-diabétiques en utilisant des micro-colonnes en kits jetables, basées sur la chromatographie d'affinité. L'effet sur les résultats d'HbA_1 du stockage d'échantillons de sang total pendant une semaine à la température du local et dans un réfrigérateur fut aussi évalué. Les moyennes ± DS des taux d'HbA_1 chez les patients diabétiques et chez les témoins étaient respectivement 12.1 ± 5.6% et 5.2 ± 0.90%. La différence était hautement significative, $P < 0.001$. Des patients diabétique nouvellement diagnostiqués qui n'avaient pas été sous traitement avaient une valeur moyenne d'HbA_1, nettement plus elevée 14.4 ± 5.0% que les anciens patients déjà sous traitement, 9.5 ± 2.9%, $P < 0.01$. Dans un groupe de 9 patients, la moyenne ± DS de la valeur était 10.3 ± 4.2% et après une semaine de stockage de différentes portions d'échantillons à température du local et dans un réfrigérateur, les moyennes ± DS étaient respectivement 9.8 ± 3.6% et 11.0 ± 4.2%. Il y avait ainsi une légère diminution dans le cas de stockage à température du local et une légère augmentation dans le cas de réfrigération, mais les différences observées n'étaient pas statistiquement significatives. En conclusion, la chromatographie de la glyc-affinité par micro-colonne est une méthode satisfaisante et potentiellement utile pour mesurer les taux d'HbA_1 dans les pays tropicaux en développement. La méthode était facile à utiliser et les dosages pouvaient être effectués à température moyenne ambiante sans recourir à l'usage d'un local à air conditionné ou d'une chambre spéciale de chromotographie.

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Introduction

Glycosylated haemoglobin (HbA_1) estimation

has been established as a reliable method for assessing long-term metabolic control of diabetes mellitus because it represents the integrated blood sugar values for the preceding several (6–8) weeks (Koenig *et al.*, 1976; Gabbay, 1976; Gabbay *et al.*, 1977; Gonen *et al.*, 1977; Jovanonic & Peterson, 1981). Several techniques have been described for the measurement of glycohaemoglobins. These include the chromatographic methods of which the commercial disposable micro-columns are the most popular (Jones, Koler & Jones, 1978). High pressure liquid chromatography (Cole *et al.*, 1978) has the advantage that it can be automated and will therefore make possible the handling of a large number of samples. The electrophoretic techniques include isoelectric focusing on thin slabs of polyacrylamide gel (Spicer, Alloes & Buse, 1978) and also agar gel electrophoresis. Fluckiger and Winterhalter (1976) devised a colorimetric method which essentially involves direct chemical measurement of total glycosylation. In addition, a radioimmunoassay method has been described by Javid *et al.*, (1978). The relative efficacy and reliability of these various methods have been reviewed (Bunn, 1981; Gabbay & Fluckiger, 1981).

In a previous report, we noted the potential usefulness of HbA_1 determination in the management of diabetic patients in a developing country like Nigeria (Famuyiwa, Ogunmekan & Osotimehin, 1983). However, it was concluded in that study that the micro-column cation exchange chromatographic method (Isolab Fast Haemoglobin Kit) was not suitable for use in developing countries. The reasons were: the requirement for a highly regulated temperature of the environment for optimal performance of the test, hence the need for an air-conditioned room or a special chromatography chamber; the poor reproducibility of the assays in our hands; and interference by liable fractions of HbA_1c and Haemoglobin F.

Since that report, the manufacturers of the kit, Isolab Inc. (Akron, Ohio, U.S.A.) have developed another micro-column kit which is based on affinity chromatography instead of cation exchange. All the defects noted above which were present in the earlier kit were supposed to have been eliminated. In view of the relative simplicity of this micro-column method, the utilization of which is within the competence of any physician or laboratory technician, we have tested the new glyc-affinity chromatography kits in meauring HbA_1 levels in our diabetic patients. In addition, we have investigated the effects of sample storage under varying conditions: room temperature and refrigeration at 4°C, on the HbA_1 results.

Materials and methods

Patients

The patients were recruited among those attending the ambulatory Diabetic Clinic of the University College Hospital (UCH), Ibadan. Forty-five patients (twenty-three males and twenty-two females) were studied. Thirteen were newly diagnosed, thirty were old patients, and the duration of the disease was not accurately documented in two patients. Their ages ranged from 18 to 69 years with a mean of 48.9 years. Most of the patients had non-insulin dependent diabetes mellitus.

Controls

There were thirteen non-diabetic controls of whom nine were males and four were females. These were recruited among medical students and doctors in the hospital. Their ages ranged from 16 to 43 years with a mean of 31.1 years. One of the authors (OOF), who was one of the controls also served as a source of 'standard' sample to assess inter-assay variability. Blood was drawn from him weekly throughout the period that the assays were being carried out.

Haemoglobin A_1 (HbA_1) assay

Blood was drawn from the patients and controls into heparinized tubes and the whole blood specimens were initially kept in an ordinary refrigerator. The samples were usually analysed within 48 h of their being collected. In the study to determine the effects of a storage conditions on HbA_1 levels, blood samples from nine of the patients, as soon as they were drawn, were distributed into three separate heparinized tubes. One set of such samples was assayed immediately, another set was kept at room temperature and the third set was kept in a refrigerator. After a period of 1 week, these two sets of samples were analysed.

A 100-sample glyc-affin system kit with disposable microcolumns was provided by Isolab Inc., Akron, Ohio, U.S.A. The glyc-affin system is designed to separate haemolysates of human red blood cells into two fractions prior to spectrophotometric quantitation. A haemolysate of the patient's blood was prepared as per instructions in the package insert using reagents supplied with the kit. The glyc-affin system separates glycosylated haemoglobin (total HbA_1) from the other haemoglobins as the haemolysate passes through the pre-filled micro-columns. The resin in the column is an affinity medium composed of boronate groups bound to agarose. Glycohaemoglobin binds to these groups as the sample passes through. The non-glycoslyated haemoglobin will not bind and is eluted in the first fraction. Glycosylated haemoglobin is eluted from the column with a second buffer. Absorbances at 415 nm of both fractions are used to determine the percent total glycohaemoglobin (HbA_1). All the assays were performed under usual temperature conditions in the tropics in the dry season, which was about 30°C. A 'standard' sample obtained from one of the authors was run with each assay.

Statistical analysis

The *t*-test for paired and unpaired samples was used to assess for statistical differences. The level of significance was a P value < 0.05.

Results

The HbA_1 values in the patients and controls are shown in Table 1. The mean ± s.d. HbA_1 levels in the new patients, 14.4 ± 5.0; old patients, 9.5 ± 2.9; or all the patients combined, 12.1 ± 5.6 were significantly higher than the mean value for the controls, 5.2 ± 0.9, $P < 0.001$. The mean value for newly diagnosed patients who were just to be commenced on treatment was also significantly higher than the mean value for old patients who had been on some form of treatment from a few weeks to several years. Table 2 presents the data to show the effect of storage of whole blood samples for 1 week either at room temperature (mean temperature approximately 30°C) or in a refrigerator (mean temperature approximately 4°C). The results indicated that HbA_1 values tend to fall slightly on storage at room temperature while the opposite, a slight increase is observed during refrigeration at 4°C. The differences observed were however not statistically significant.

Samples were obtained from one of the authors every week and these were run with each weekly assay. In six such assays, the HbA_1 values for the 'standard' were as follows: 5.1,

Table 1. Haemoglobin A_1 values in diabetic patients and controls

	Number	HbA_1 (%) Mean ± s.d.
All patients	45	12.5 ± 5.6*
New patients	13	14.4 ± 5.0*†
Old patients	30	9.5 ± 2.9*
Controls	13	5.2 ± 0.9

*$P < 0.001$ compared to value in controls.
†$P < 0.01$ compared to value in old patients.

Table 2. Effects of sample storage under different conditions on HbA_1 levels

	Baseline	Storage at room temp for 1 week (30°C)	Storage in refrigerator for 1 week (40°C)
Number	9	7	9
HbA_1 % Mean ± s.d.	10.3 ± 4.2	9.8 ± 3.6*†	11.0 ± 4.2*

*$P > 0.2$ compared to baseline value
†$P > 0.1$ compared to post-refrigeration value.

5.3, 5.5, 5.1, 5.9 and 6.0. The mean ± s.d. was 5.48 ± 0.35 and the inter-assay coefficient of variation was 6.4%. The expected value was 5%.

Discussion

We had noted previously the desirability and potential usefulness of using HbA_1 measurements to monitor the metabolic control of diabetes among patients in developing countries given the lack of resources for frequent blood sugar determinations (Famuyiwa *et al.*, 1983). However, we found the micro-column cation exchange chromatography as most unsuitable because of the requirement for a highly regulated temperature of the environment (optimum 23°C), the poor reproducibility of the assays and interference by liable fractions of HbA_1c. Erasmus *et al.* (1983), have shown that the colorimetric method of Fluckiger and Winterhalter (1976) provides a highly precise and accurate method for measuring HbA_1 levels in Nigerian diabetic subjects. However, this method may be limited in its applicability in developing countries to tertiary care centres where the necessary support facilities and personnel will be available.

The simplicity of the micro-column method made it worthwhile to try the newly developed system which was based on affinity chromatography. The results showed a clear separation of HbA_1 values in diabetic patients from those in controls and diabetic patients previously on treatment had significantly lower mean values than newly diagnosed patients who were yet to be commenced on therapy. It was felt that the greatest advantage of the glyc-affin system was the fact that the columns could be run under average ambient temperature that obtain in a tropical country like Nigeria and satisfactory results still be obtained. The ability to use the kits without recourse to a refrigerated environment is a significant improvement over the cation-exchange method. This will make it possible for any physician in an urban or provincial setting in a developing country to use the kits.

Storage of patient samples at room temperature (30°C) or in a refrigerator (4°C) did not cause any significant change in the HbA_1 values. An important implication of this finding is that it may be possible to evolve an 'outreach programme' in developing countries whereby whole blood samples collected from diabetic patients who are being followed up in district and provincial hospitals or health centres may be collected on a scheduled basis and forwarded to a bigger facility for analysis. Many of these places are lacking in regular supply of electricity and they are therefore without reliable refrigerating facilities. The results from this study would suggest that non-refrigeration of samples for a period of 1 week does not significantly affect the results obtained. It should therefore be possible to make HbA_1 measurement available to a larger population of diabetic patients than would ordinarily have been the case.

However, the findings from this study should probably be considered only as preliminary. There is a need to investigate further the effect of longer storage of samples and also to test the feasibility of the type of 'outreach programme' referred to above. Efforts in these directions are currently underway.

Acknowledgments

We are grateful to Isolab Inc., Akron, Ohio, U.S.A. who supplied the kits and Mrs Akinpelu for typing the manuscript.

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(*Received* 2 *February* 1985; *accepted* 30 *October* 1985)