ROUTINE MEASUREMENT OF HAEMOGLOBIN A₁ AT THE DIABETIC OUTPATIENT CLINIC

by

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FOLLOWING the introduction of insulin therapy in 1922 one of the major problems in the long-term management of insulin-dependent diabetes was the lack of a convenient, reliable and objective test of longer term glycaemic control. In the 1960's and early 1970's haemoglobin A₁ (HbA₁, glycosylated haemoglobin) was shown to be elevated, usually, in diabetic patients¹-³ and its biochemical structure was elucidated.⁴-⁶ The mid-1970's saw many reports confirming that measurement of HbA₁ provided an accurate and objective assessment of glycaemic control in the preceding 6 to 8 weeks, a period corresponding to the approximate half-life of the average red blood cell. The vast majority of these studies were either cross-sectional in design⁷-¹⁰ or, if longitudinal, carried out in the controlled atmosphere of the clinical research centre or hospital ward.¹¹,¹²

On the basis of this accumulated knowledge, measurement of HbA₁ has become commonplace in many diabetic outpatient clinics. The purpose of this communication is to report how accurate measurement of HbA₁ has been successfully introduced to a large diabetic outpatient clinic in such a way that the result is available at the time of the patient's routine clinic visit. In addition, we have tried to assess whether, in insulin-treated patients, the test yields information which is distinct from that already available from plasma glucose measurements.

ORGANISATION OF OUTPATIENT CLINIC AND BLOOD SAMPLING

The diabetes clinics of the Royal Victoria Hospital, Belfast, are held on Tuesday and Friday mornings of every week and, once a month, also on a Saturday morning. Medical staff comprises at least two consultant physicians assisted by two junior grade doctors at each clinic. In addition, a "blood sampling" clinic is held every Wednesday morning, where patients, due to attend the following Friday, Saturday or Tuesday, have their blood samples drawn. This suits many of the patients who live not more than two or three miles from the hospital. Those patients who live further away arrange to have blood samples taken on the Monday or Tuesday either at home or at their local health clinic or general practice, and post them to the hospital laboratory. The custom has been for some years that patients who are solely on dietary therapy have fasting samples taken, while those on oral hypoglycaemic agents or insulin have samples taken 2.5 hours after breakfast.

This organisational scheme was already in existence before we introduced routine measurement of HbA₁ in December 1980. We surmised that the drawing of an extra 5 ml of blood and labelling of an extra specimen bottle would not place an intolerable strain on the system.

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LABORATORY METHODS

Plasma glucose is measured by the neocuproine method. The blood samples are put into fluoride-containing bottles.

Samples for HbA₁ measurement are drawn into EDTA and stored at 4°C until Wednesday afternoon. Red cells are then incubated in normal saline at 37°C until Thursday morning, to remove any "unstable" glycosylated fraction. Haemolysates are then prepared and HbA₁ is measured by agar gel electrophoresis (Corning Medical Ltd, Halsted, Essex). The normal range in our laboratory is 3.7 to 7.2 percent. Coefficient of variation within assay is 1.6 per cent and between assays 6.4 per cent. Two standard haemolysates (high and normal HbA₁) are run each week to ensure that there is no "assay drift".

As we were concerned that postal handling or delay might affect HbA₁ results we obtained duplicate samples from 21 diabetic and 5 control subjects. These were assayed after being sent directly to the laboratory or through the post. Mean ± SEM levels were similar — 9.25 ± 0.36 per cent (direct) v. 9.39 ± 0.39 per cent (postal), and the correlation between the two sets of samples was extremely close, \( r = 0.94 \).

During the period under investigation (December 1980 to February 1982) the laboratory were able to process approximately 110 samples for HbA₁ estimation each week and have them available, with the results on computer printout sheets, for the appropriate clinics. The maximum number of samples presented to the laboratory in any one week was 210, and these were processed satisfactorily in the normal way.

ASSESSMENT OF HbA₁ AND COMPARISON WITH PLASMA GLUCOSE

We have retrospectively identified 302 insulin-treated patients who, from December 1st 1980 to February 28th 1981 (initial visit) had HbA₁ measured for the first time under the scheme outlined as part of the routine clinic visit. Table 1 gives details of the patient characteristics. These patients were reviewed either twice or three times more in the subsequent 9 to 12 months; 181 patients were reviewed at 2 to 4 months (first review), 194 at 5 to 7 months (second review) and 248 at 9 to 12 months (third review) after the initial visit. In every case there was a period of at least 2 months between successive reviews. HbA₁ and plasma glucose (2.5 hour post-breakfast) were recorded for each clinic attendance.

| TABLE 1 |
| --- |
| Characteristics of 302 insulin-treated patients |
| Male/Female | 147/155 |
| Duration of diabetes (years) — range | <1 to 48 |
| — median | 12 |
| Age (years) — range | 7 to 83 |
| — median | 49 |
To attempt to compare the information available from each of these parameters in individual patients changes from the values at the initial visit, to subsequent reviews, were arbitrarily defined as follows:

**HbA₁**
- increase of $\geq 1.5$ per cent = clinically important increase
- decrease of $\geq 1.5$ per cent = clinically important decrease
- increase or decrease of $< 1.5$ per cent = no change

**Plasma glucose**
- increase of $\geq 3$ mmol/l = clinically important increase
- decrease of $\geq 3$ mmol/l = clinically important decrease
- increase or decrease of $< 3$ mmol/l = no change

These arbitrary values were chosen to take into account the reproducibility of the assay methods and, in the case of plasma glucose, the likelihood of day-to-day fluctuation.

Analysis of HbA₁ and plasma glucose values showed that neither of these parameters was normally distributed within the population studied. Therefore, comparisons were made using the Wilcoxon signed rank test for matched pairs, and correlations using the Kendall rank correlation coefficient. The conventional level of significance ($p < 0.05$) has been used in all analyses.

**RESULTS**

Initial visit HbA₁ values ranged from 5.5 to 21.0 per cent, with a mean ± standard error of $10.7 \pm 0.13$ per cent. Plasma glucose ranged from 1.0 to 33.6 mmol/litre with a mean of $10.16 \pm 0.35$ mmol/litre. There was a weak but statistically significant correlation between HbA₁ and plasma glucose — Kendall correlation coefficient = 0.18, $p < 0.001$. Similar weak correlations between HbA₁ and glucose were found at the first, second and third reviews, with Kendall correlation coefficients of 0.18, 0.21 and 0.12 respectively.

**Table 2**

Comparison of HbA₁ and plasma glucose at the initial visit with values at reviews

|            | HbA₁ (per cent) | Glucose (mmol/l) |
|------------|-----------------|-----------------|
|            | Initial Review  | Initial Review  |
| First review | 10.83 ± 0.17   | 10.49 ± 0.46   |
| (n = 181)    |                 | 10.06 ± 0.50   |
| Second review | 10.70 ± 0.15   | 10.05 ± 0.42   |
| (n = 197)    |                 | 10.41 ± 0.46   |
| Third review  | 10.74 ± 0.14   | 10.43 ± 0.38   |
| (n = 248)    |                 | 10.55 ± 0.39   |

Results as mean ± SEM.

* $p < 0.001$, Wilcoxon signed rank test. All other differences are not statistically significant.
Comparisons of mean HbA₁ and plasma glucose at the review visits with the mean levels at the initial visit, on a paired basis, are shown in Table 2. The significant falls in mean HbA₁ seen in patients at the first and second reviews were not reflected by decreases in plasma glucose. At the third review neither of these parameters differed significantly from mean levels at the initial visit.

Table 3 indicates the number of patients who had an increase, decrease, or no change (as defined above) in HbA₁ and plasma glucose at the first review compared to the initial visit. Thus, of the 70 patients whose HbA₁ level decreased, plasma glucose decreased in only 25, was unchanged in 28, and increased in 17. It can be seen that the changes in the two parameters were concordant in only 74 of 181 patients (40.8 per cent).

**TABLE 3**

*Comparison of individual "clinical changes" in HbA₁ and plasma glucose, from the initial visit to first review*

| Plasma glucose | Decrease | No change | Increase |
|----------------|----------|-----------|----------|
| HbA₁           | Decrease | 25        | 28       | 17       |
|                | No change| 28        | 42       | 26       |
|                | Increase | 3         | 5        | 7        |

Similarly, when HbA₁ and plasma glucose at the second review were compared in this fashion to levels at the initial visit, the concordance between the tests was 84 out of 194 (43.3 per cent), and for the third review 98 out of 248 (39.5 per cent).

**DISCUSSION**

Apart from accurate laboratory measurement of blood or plasma glucose, assay of HbA₁ is the only truly objective index of glycaemic control available to the practising diabetologist. Other aids to assessing control, such as urine sugar testing and home monitoring of blood glucose, are heavily dependent upon the patients' subjective interpretation and co-operation and are, in any case, less accurate.

Saunders et al demonstrated, in 1980, that measurement of HbA₁ by agar gel electrophoresis could be performed while patients waited at the clinic. However, their HbA₁ measurements, without prior incubation of red cells in saline, would have included the "unstable" Schiff base fraction which may be influenced by short term fluctuations in blood glucose. Like them, we chose to measure HbA₁ by agar gel electrophoresis. This has proven to be an accurate, reproducible method, with many samples being processed easily by a single technician. The problem of "unstable" HbA₁ has been overcome by incubating samples overnight in saline. The apparent reliability of results from postal samples should enable our system to be adopted by many other clinics where an extra visit to the hospital for blood sampling is inconvenient or impractical. When, in December 1980, we started this scheme, we confined routine HbA₁ measurement to insulin-treated patients as we were unsure if the laboratory could cope with the workload. Approximately a year later we expanded to include all patients at the clinic and the laboratory now satisfactorily processes 180 to 200 samples each week.
In comparison to plasma glucose, measurement of HbA$_1$ is relatively expensive; consumable materials alone cost 20 to 30 times more per test. We felt it important, therefore, to try to assess whether these two objective tests of glycaemic control give distinct information, if HbA$_1$ is to be measured routinely in patients with a condition as common as diabetes. The retrospective analyses we have presented here show this to be the case.

First, reductions in mean HbA$_1$ at the first and second reviews after the introduction of the test were not reflected by changes in the mean plasma glucose (Table 2). Secondly, the data concerning the trend of glycaemic control in individual patients shows that, according to the criteria we laid down, conclusions drawn from the two parameters would differ in approximately 60 per cent of cases (Table 3).

These conclusions are perhaps not unexpected bearing in mind that we are comparing HbA$_1$ with single post-prandial plasma glucose measurements. We believe that similar conclusions would hold for fasting plasma glucose measurements in insulin-treated patients. No doubt a greater degree of agreement would be obtained from comparison of HbA$_1$ with the mean of several plasma glucose levels, but we feel that our clinic is probably not unique in relying, in the majority of patients, on a single blood sample approximately every two to four months.

Conclusions concerning the possible impact which the availability of HbA$_1$ measurement may have on glycaemic control should not be based on a retrospective analysis such as this. Nevertheless, speculation on why HbA$_1$ levels decreased at the first and second reviews, but (disappointingly) increased again after 9 to 12 months, seems reasonable. It might be argued that since the number of patients at the first and second reviews was considerably less than at the initial visit, too much should not be read into the decreases in HbA$_1$. However, analysis showed no difference in terms of age, duration of diabetes or HbA$_1$ and plasma glucose at initial visit, between attenders and non-attenders at the first and second reviews. In any case every patient was included in either the first or second review. It is probable, therefore, that there was indeed an initial improvement in glycaemic control in the cohort.

We would suggest two possible explanations for this initial but unsustained improvement. First, enthusiasm for a "new" test on the part of the medical personnel, with subsequent waning, could certainly account for the described phenomenon. Interestingly, similar unsustained improvements in diabetic control have been observed after the introduction of home blood sugar monitoring programmes.$^{20-22}$ A second possible explanation is that we were observing a seasonal variation in glycaemic control, quite unrelated to any specific therapeutic manoeuvre. Our study began in mid-to-late winter, with first and second reviews largely in spring and summer, and final reviews in autumn through winter. It is easy to believe that more intercurrent illnesses (e.g., viral) in winter and dietary excess over the festive season would contribute to poor control, whereas increased physical activity in the spring and summer would tend to have the opposite effect. Although a seasonal variation in the onset of clinical diabetes is recognised,$^{23}$ we are not aware of any previous reports of seasonal variations in glycaemic control in established diabetes.
It is rather disappointing that approximately one year after introducing HbA\textsubscript{1} measurement routinely at our clinic, we seem to have achieved no substantial improvement in glycaemic control in the majority of the 302 insulin-treated patients studied. However, we believe we have demonstrated that accurate HbA\textsubscript{1} measurement can be provided with little or no disruption to established clinic routine, and that this test yields clinically useful information not previously available.

SUMMARY

Measurement of glycosylated haemoglobin (HbA\textsubscript{1}) by agar gel electroendosmosis was introduced as a routine test at a large teaching hospital diabetic outpatient clinic. An initial reduction in HbA\textsubscript{1} levels, in 302 insulin-treated patients, was not accompanied by a corresponding decrease in post-prandial plasma glucose levels, and was not sustained after 9 to 12 months. Changes in individual HbA\textsubscript{1} and plasma glucose were concordant in only approximately 40 per cent of observations. The facility of HbA\textsubscript{1} measurement appears to give clinically distinct information, but has not in itself led to an improvement in glycaemic control.

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REFERENCES

1 Husiman THJ, Dozy AM. Studies on the heterogeneity of haemoglobin. V. Binding of hemoglobin with oxidized glutathione. J Lab Clin Med 1962; 60: 302-319.
2 Rahbar S, Blumenfeld O, Ranney HM. Studies on an unusual hemoglobin in patients with diabetes mellitus. Biochem Biophys Res Commun 1969; 36: 838-843.
3 Trivelli LA, Ranney HM, Lai H-T. Hemoglobin components in patients with diabetes mellitus. N Engl J Med 1971; 284: 353-357.
4 Holmquist WR, Schroeder WA. A new N-terminal blocking group involving a Schiff base in hemoglobin A\textsubscript{1c}. Biochemistry 1966; 5: 2489-2503.
5 Bookchin R, Gallop PM. Structure of hemoglobin A\textsubscript{1c}: Nature of the N-terminal chain blocking group. Biochem Biophys Res Commun 1968; 32: 86-93.
6 Bunn HF, Haney DN, Kamin S, Gabbay KH, Gallop PM. the bio-synthesis of human hemoglobin A\textsubscript{1c}: slow glycosylation of hemoglobin in vivo. J Clin Invest 1976; 57: 1652-1659.
7 Gabbay KH, Hasty K, Breslow JL, Ellison RC, Bunn HF, Gallop PM. Glycosylated hemoglobins and long term blood glucose control in diabetes mellitus. J Clin Endocr Metab 1977; 44: 859-864.
8 Dunn PJ, Cole RA, Soeldner JS et al. Temporal relationship of glycosylated haemoglobin concentrations to glucose control in diabetics. Diabetologia 1979; 17: 213-220.
9 Tze WJ, Thompson KH, Leichter J. HbA\textsubscript{1c} — an indicator of diabetic control. J Pediatr 1978; 93: 13-16.
10 Gonen B, Rubenstein AH, Rochman H, Tanega SF, Horiwitz DL. Haemoglobin A\textsubscript{1}: an indicator of the metabolic control of diabetic patients. Lancet 1977; 2: 734-737.
11 Koenig RJ, Peterson CM, Jones RL, Sandek C, Lehman M, Cerami A. Correlation of glucose regulation and hemoglobin A\textsubscript{1c} in diabetes mellitus. N Engl J Med 1976; 295: 417-420.
12 Ditzel J, Kjaegaard J. Haemoglobin A\textsubscript{1c} concentrations after initial insulin treatment for newly discovered diabetes. Br Med J 1978; 1: 741-742.
13 Karamonos B, Cristacopulos P, Zacharion N, Korkolis S. Rapid changes of haemoglobin A\textsubscript{1c} fraction following alterations of diabetic control. Diabetologia 1977; 13: 406.
14 Svendsen PA, Christiansen JS, Soegaard U, Welinder BS, Nerup J. Rapid changes in chromatographically determined HbA1c induced by short term changes in glucose concentration. Diabetologia 1980; 19: 130-136.

15 Widness JA, Rogler-Brown TL, McCormick KL, et al. Rapid fluctuations in glycohemoglobin (hemoglobin A1c) related to acute changes in glucose. J Clin Lab Med 1980; 95: 386-394.

16 Goldstein DE, Peth SB, England JD, Hess RL, De Costa J. Effect of acute changes in blood glucose on HbA1c. Diabetes 1980; 29: 623-628.

17 Ditzel J, Forsham P, Lorenzi M. Rapid fluctuations in glycosylated haemoglobin concentration as related to acute changes in blood glucose. Diabetologia 1980; 19: 403-404.

18 Menard L, Dempsey ME, Blankstein LA, et al. Quantitative determination of glycosylated haemoglobin A1c by agar gel electrophoresis. Clin Chem 1980; 26 (II): 1598-1602.

19 Saunders J, Baron MD, Shenonda FS, Sonksen PH. Measuring glycosylated haemoglobin concentrations in a diabetic clinic. Br Med J 1980; 2: 1394.

20 Kelly CA, Barret EJ, Nash J. Home monitoring and self-management of diabetes in a rural population. Ir Med J 1981; 74: 321-324.

21 Worth R, Home PD, Johnston DG, et al. Intensive attention improves glycaemic control in insulin-dependent diabetes without further advantage from home blood glucose monitoring: results of a controlled trial. Br Med J 1982; 2: 1233-40.

22 Bell PM, Walshe K. Impact of home monitoring on diabetic lifestyle and blood glucose control. Practitioner 1983 (in press).

23 Gamble DR, Taylor KW. Seasonal incidence of diabetes mellitus. Br Med J 1969; 2: 631-633.