Glycosylated haemoglobin levels in children with diabetes mellitus

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SUMMARY Total glycosylated haemoglobin (HbA₁) levels were measured in 94 diabetic children aged between 3 and 19 years. The results were compared with traditional methods of assessing blood glucose control. HbA₁ levels correlated with home urine glucose testing ($P < 0.05$), with 24-hour urine glucose excretion ($P < 0.01$), and with height velocity ($P < 0.001$). Within the first two of these parameters there was a wide scatter of results, suggesting the inaccuracy of these methods for assessing control. The association of raised HbA₁ levels with height velocities < 10 th centile shows the effect of poor control on growth. HbA₁ may prove to be an objective method for assessing long-term blood glucose levels in diabetes, and thus it may be possible to determine the effect of good control in the prevention of the various diabetic complications.

The long-term complications of diabetes may have a multifactorial basis and it is still uncertain whether their development is related to blood glucose control. One of the reasons for the difficulty lies in our inability to assess accurately the degree of hyperglycaemia in individual patients. Recently it has been shown that an objective method of estimating control is the measurement of glycosylated haemoglobin (HbA₁) as this compound reflects the mean blood glucose concentration during the previous 2 or 3 months (Koenig *et al.*, 1976b; Gabbay *et al.*, 1977). We therefore examined the correlation between HbA₁ levels and those methods of assessment which are generally used in children with diabetes.

Patients and methods

All the children were attending the paediatric outpatient clinic regularly every 3 or 4 months. Their ages ranged between 3 and 19 years. They were being treated with Monocomponent insulins (Novo Laboratories), once-daily Monotard insulin in the younger children and twice-daily Actrapid and Semitard insulin in the older ones. Control value for HbA₁ levels were obtained in 14 nondiabetic children aged between 5 and 15 years. The controls were randomly selected from children attending the

outpatient department; none had any specific disease.

Four methods of assessment were used: HbA₁ levels, twice-daily home urine testing, 24-hour urine glucose output, and height velocity. The home urine tests were those recorded during the previous 3 months before the level of HbA₁ had been examined. The urine tested was the 2nd specimen passed before breakfast and before the evening meal. The results were classified as good (50% or more tests with no glycosuria and < 1 in 5 showing 2% glycosuria), fair ($< 50\%$ tests negative and < 1 in 5 showing 2% glycosuria), and poor ($< 25\%$ tests negative or > 1 in 5 tests showing 2% glycosuria). The 24-hour urine glucose output was measured in 82 children and expressed as a percentage of the planned carbohydrate intake. In the prepubertal children the height velocity during the previous year was expressed as a height velocity centile according to age.

Total glycosylated haemoglobins A_{1a}, A_{1b}, and A_{1c} (HbA₁) were measured in duplicate by column chromatography using the method of Welch and Boucher (1978). The results are expressed as a percentage contribution of HbA₁ to the total Hb concentration. The index of precision based on analysis of paired results was $\pm 0.63\%$ (Whitby *et al.*, 1967).

Results

HbA₁ levels in the control group of children were between 7 and 10% (mean 8.9%). The levels in the

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diabetic children ranged from 8 to 23% (mean 14.7%).

There was a significant difference between the mean HbA₁ levels of those assessed as having good control with home urine tests (mean 13.2%) and those assessed as being poorly controlled (mean 16.4%) ($P < 0.05$). There is however a wide scatter in all columns (Fig. 1). Nine of the 13 children assessed as well controlled but with HbA₁ levels above the mean were on once-daily insulin (Monotard), and in 7 of these 9 children the 24-hour urine glucose loss was between 20 and 70% of the planned carbohydrate intake (mean 44%).

A correlation ($r = 0.46$, $P < 0.01$) was found between HbA₁ levels and the 24-hour urine glucose output expressed as a percentage of the planned daily carbohydrate intake (Fig. 2).

Fig. 1 Levels of HbA₁ compared with assessment of control by twice-daily home urine tests.

In those children with height velocity < 10 th centile, the mean HbA₁ level was 16.2% compared with 12.4% in those with height velocities > 10 th centile ($P < 0.001$) (Fig. 3).

Fig. 2 Correlation of HbA₁ with 24-hour urine glucose output expressed as percentage of planned carbohydrate intake.

Fig. 3 Levels of HbA₁ in prepubertal children whose height velocity was less than or greater than the 10th centile.

Discussion

The glycosylated haemoglobins (A_{1a} , A_{1b} , A_{1c}) are structurally identical with HbA except for the presence of a glucose group linked to the terminal amino-acid of the β -chain. Glycosylation of HbA occurs by a slow nonenzymatic process in the circulating erythrocyte and forms a stable and relatively irreversible ketamine linkage (Bunn *et al.*, 1976). This results in the continuous formation and accumulation of glycosylated haemoglobins within the erythrocyte during the 120-day life span, and therefore reflects the degree of hyperglycaemia during this period. In health the glycosylated haemoglobins make up less than 10% of the total HbA. Of the 3 glycosylated haemoglobins, HbA_{1c} makes up the greatest fraction and appears to correspond most closely to the degree of hyperglycaemia (Koenig *et al.*, 1976b).

Several investigators have examined the relationship of HbA₁ with the more commonly used methods for assessing control in diabetes. They found a correlation of HbA₁ levels with physician's rating, urine testing, 24-hour urine glucose estimations, fasting plasma glucose levels, serum triglyceride levels, and response to treatment after diagnosis (Koenig *et al.*, 1976a; Gabbay *et al.*, 1977; Gonen *et al.*, 1977; Lanoe *et al.*, 1977; Peterson *et al.*, 1977; Koenig *et al.*, 1976b; Ditzel and Kjaergaard, 1978). However in nearly all these studies the scatter of results was wide, showing that present methods are inadequate for assessing control in diabetes. Indeed in some studies there was no correlation between the HbA₁ level and certain of the parameters used in assessment (Gonen *et al.*, 1977; Lanoe *et al.*, 1977). Overall, however, the evidence suggests that the measurement of HbA₁ may be the best method yet available for measuring blood glucose control (Koenig *et al.*, 1976a).

Although we found a correlation of HbA₁ levels with our present methods of assessing diabetic control, there is within each parameter a wide scatter of HbA₁ levels. This is not surprising as home urine tests are not an accurate method of assessment (Malone *et al.*, 1976), and the measurement of an occasional 24-hour urine glucose output cannot be regarded as an index of day-to-day diabetic control. In a review of studies evaluating the relationship of diabetic control with complications, Knowles (1964) concluded 'the most doubtful measurement of all is that of control'.

An objective parameter in assessing long-term control in children is height velocity. This study shows a clear difference in HbA₁ levels between those children who have grown poorly and those with satisfactory growth. Others have not found any

correlation between height velocity and traditional methods of assessing control (Jivani and Rayner, 1973; Craig, 1977) which may reflect the inadequacy of these methods, and suggests that HbA is a more accurate method of assessment.

Like others we found that a number of patients thought to be well controlled had raised HbA₁ levels. This was particularly apparent in a group of children who appeared well controlled as assessed by home urine tests. In some of these children inaccurate urine testing and book-keeping may account for the discrepancy. However, the majority were young children (<8 years) whose urine tests were generally both checked and recorded by the parents. These children were all on once-daily long-acting insulin injections. This type of insulin controls blood glucose levels between meals but does not prevent the postprandial blood glucose surge (Gokal *et al.*, 1977). Hence, urine tests before meals show little or no glycosuria but miss the marked hyperglycaemia which occurs after each meal. This was reflected in the children's high 24-hour urine glucose output and raised HbA₁ levels. It is now our policy to combine a quick-acting soluble insulin (Actrapid) with the once daily long-acting insulin (Monotard) to reduce the postbreakfast hyperglycaemia.

Our data confirm that the measurement of glycosylated haemoglobin may have an important role in evaluating diabetic control. They indicate the limitations of present methods of assessment and show how improbable it is that with these conventional methods we can hope to show a correlation between diabetic control and complications. Prospective longitudinal studies of HbA₁ in diabetes may not only provide an objective measurement of overall blood glucose control, but it may also more accurately relate this to the development of the long-term complications of diabetes.

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