Glycosylated Haemoglobins in the Diagnosis of Diabetes Mellitus and for the Assessment of Chronic Hyperglycaemia

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Summary. Evidence is presented that two levels of glycosylated haemoglobin can be defined which when exceeded indicate respectively the presence of diabetes mellitus and a significant risk for the development of microvascular complications, as judged by current glucose tolerance test criteria for such risk.

Key words: Glycosylated haemoglobin, diabetes detection, glucose tolerance tests, microvascular complications (risk of)

Glycosylated haemoglobins (HbA₁) have been shown to be raised in chronic hyperglycaemia and to correlate closely with the average degree of hyperglycaemia over several weeks before their estimation [1–3]. Since glycosylation of protein by non-enzymatic binding [4] also appears to be related to the initiation of microvascular diabetic complications [5], there is good reason to aim at reduction of such glycosylation in diabetics. Similarly, since these complications develop with hyperglycaemia, whether overt or not, there is a need for a simple method for the detection of *clinically significant* hyperglycaemia. HbA₁ estimation appears to be such a method [6].

Evidence from long-term surveys suggests that hyperglycaemia becomes a risk factor for microvascular disease only once 2 h blood glucoses on oral glucose tolerance testing rise above 11.1 mmol/l (200 mg/100 ml) [7]. The present preliminary study was made to investigate the relationship of HbA₁ levels to oral glucose tolerance test (GTT) findings and to define HbA₁ levels above which chronic hyperglycaemia is likely to be a risk factor using the 2 h glucose levels quoted above.

Methods

Glycosylated haemoglobin HbA_{1(a+b+c)} was measured on aliquots of fasting venous blood samples from all hospital patients referred for routine oral 50 g GTTs over a 3-month period, with a rapid column chromatographic method [8]. The patients were aged from 20 to the late 70s, 65% were female, the excess being due to tests carried out during and after pregnancy. The GTTs were carried out fasting, sitting in a waiting-room, non-smoking, after 3 days adequate carbohydrate intake. The variation of the HbA₁ method was 4% between and 3% within assays. A normal range was established on 206 fit young adults aged 19–21 years over 2 years. There was no significant difference between the means of values in men and women (65 and 35% of subjects respectively). Plasma glucose levels were measured by a standard autoanalyser glucose oxidase method [9]. Correlation coefficients and regressions were calculated by standard methods [10, 11].

Results

The range for HbA₁ in 206 healthy young adults was 6.4 to 10.5% (mean 7.8% ± 0.095 SE). The ranges and means for the 27 newly diagnosed diabetics and the 58 non-diabetics are also shown in Table 1. In 27 newly diagnosed diabetics significant correlations were found between HbA₁ and the fasting, 30 min,

Table 1. Ranges and means ± SEM for HbA₁ in normal subjects, and in the subjects found to be non-diabetic or diabetic on oral glucose tolerance testing

Group	No.	HbA ₁		
		Range (%)	Mean (%)	± SEM
Normal subjects	206	6.4–10.5	7.8	0.095
Non-diabetic hospital patients	58	6.5–10.6	8.98	0.13
Newly diagnosed diabetics	27	5.7–22.1	15.68	0.53

2 h, and summed plasma glucose levels at these times (Table 2). Four of these subjects gave 2 h glucose values in the border-line range but each of these gave fully diabetic levels in the other samples. Thus no border-line group was defined for study. In 58 non-diabetic GTTs there were significant positive correlations between HbA₁ values and with fasting plasma glucose levels but not with the 30 min summed or 2 h levels (Table 2). Five grossly discrepant values of HbA₁ and oral GTT were found to be due either to recent delivery of gestational diabetics (three patients), to acute onset insulin-requiring diabetes (one patient), and to untreated pernicious anaemia with haemolysis (one patient).

Table 2. Correlation coefficients for HbA₁ levels with normal and diabetic blood glucose levels

	Non-diabetics		Newly-diagnosed diabetics	
	<i>n</i> = 58		<i>n</i> = 27	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
HbA ₁ versus fasting plasma glucose	0.28	0.02	0.86	<0.001
HbA ₁ versus 30 min plasma glucose	0.13	NS	0.69	<0.001
HbA ₁ versus 2 h plasma glucose	0.10	NS	0.68	<0.001
HbA ₁ versus summed plasma glucose	0.24	NS	0.79	<0.001

The regression of HbA₁ levels on fasting and 2 h plasma glucose levels were calculated (Figs. 1 and 2). The regressions indicated HbA₁ levels of $10.6 \pm 1.5\%$ SEM for fasting plasma glucose values of 7.0 mmol/l and levels of $12.6 \pm 1.4\%$ SEM for 2 h plasma glucose values of 11.1 mmol/l. Using the EASD definition of diabetes [7] and considering HbA₁ levels of above our normal range of up to 10.5% to be diabetic, we would appear to be diagnosing 2% diabetic false positives on HbA₁ levels without any false negative if a 2 h plasma glucose of 11.1 mmol/l or above were the criterion for diagnosis of diabetes. Since, however, the four subjects with lower 2 h glucose levels were clearly diabetic on fasting and 30 min samples, even with the 50 g load used, it appears that an HbA₁ of 10.6% does satisfactorily divide our non-diabetics from those currently diagnosed as diabetic.

Discussion

Oral GTTs are made difficult to interpret by their inherent variability [12] as well as by variability in their interpretation [13]. We have used current EASD definitions in this study. If, in addition, as Jarrett and Keen [14] and Sayegh and Jarrett [15] suggest, 2 h blood glucose levels of 11.1 mmol/l or above after carbohydrate loads may be a threshold above which there is a risk of microvascular disease, then there should be a corresponding value of HbA₁

Fig. 1. Showing percentage HbA₁ and fasting plasma glucose levels in the diabetics (○) and nondiabetics (●) found on oral GTTs. (Diabetics $r = 0.86$, $p \leq 0.001$; non-diabetics $r = 0.28$, $p \leq 0.02$)

Fig. 2. Showing percentage HbA₁ and 2 h plasma glucose levels for diabetics (○) and non-diabetics (●) identified on oral GTTs. (Diabetics $r = 0.68$, $p \leq 0.001$; non-diabetics $r = 0.1$ NS)

reflecting protein glycosylation above which this risk is present. Long-term studies will be needed to establish properly such thresholds for HbA₁ levels, measured by standardized methods, as a risk factor, but in the interim, values defined in the way that has been done in this study may be useful both in diagnosis and in the management of diabetes. Thus using the present method of HbA₁ estimation, levels of 10.6% and above indicate that diabetes mellitus is present. HbA₁ levels may make useful 'spot tests' for the detection of diabetes except in the presence of haemolysis, in diabetes of very recent onset, or where early diagnosis is vital as in pregnancy. The advantages of HbA₁ estimation are those of a simple procedure, lack of any need for dietary preparation or for fasting, elimination of the variability usually found on oral glucose tolerance tests as well as accurate reflections of overall chronic hyperglycaemia [3].

In attempts to assess glycaemia as a risk factor in diabetics it is necessary to avoid interference from short-term increased glycosylation. Storage of blood in sequestrene for 24 h pre-assay should allow removal of such short-term adducts [16]. Samples for long-term assessment should be taken when patients have apparently been fit and well, since values will be raised following acute failures of control (e.g. hyperglycaemic ketoacidosis or hyperosmolar coma). Within these constraints, levels of 12.6% and above in our laboratory are indications that hyperglycaemia is likely to be a significant risk for the development of microvascular complications. At present 60 and 40% of our current patients on treatment with or without insulin respectively fall into this category (unpublished data). Patients whose HbA₁ levels we find to lie between 10.5 and 12.6% can be considered to be borderline as regards the presence of hyperglycaemia as a significant risk factor. Standardization of methods, the establishment of normal ranges and studies versus oral GTT findings should add considerably to the usefulness of individual laboratories' HbA₁ levels for the diagnosis and long-term follow-up of diabetics.

Acknowledgements. The authors are grateful to Dr. Houghton and the staff in The Clinical Chemistry Department at The London Hospital for the preservation of basal blood samples from oral GTT and to Mr. Stephen Evans for guidance and practical help with the statistical analyses.

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Received: 11 November 1980

and in revised form: 30 December 1980

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