

Glycated haemoglobin and glycated protein and glucose concentrations in necropsy blood samples

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SUMMARY Glycated haemoglobin and glycated protein (fructosamine) and blood glucose concentrations were measured in blood samples collected from 75 patients at necropsy. Estimation of blood glucose was a poor indicator of glycaemia before death. Measurement of glycated haemoglobin by affinity chromatography distinguished non-diabetic patients from diabetic patients. The distinction was not as clear cut when HbA_{1c} was estimated using electroendosmosis. Seven patients, who at necropsy had no known history of diabetes, had glycated haemoglobin concentrations in the diabetic range. Two of these patients were found to be diabetic, and diabetes had been suspected at some time in another three patients.

It is concluded that measurement of glycated haemoglobin or HbA_{1c} in necropsy specimens is a valuable tool for assessing glycaemic control in known diabetic patients, and may be useful in diagnosing previously unsuspected diabetes.

Biochemical examination of necropsy material may be of value for determining the metabolic abnormalities responsible for death: results may establish a cause of death when necropsy fails to show clinically important anatomical disease, or may help evaluate the physiological effects of recognisable anatomic lesions. Hill suggested that blood glucose estimation after death was of no value.¹ He showed that blood glucose concentrations decreased by about 0.7 mmol/hour after death due to continued cell metabolism and bacterial degradation. Based on these findings a normal blood glucose concentration in life would be expected to have fallen to concentrations approaching zero in specimens collected six to 10 hours after death. In contrast, Tonge and Wannan showed that true glucose was detectable up to 60 hours after death.²

Glycated haemoglobin provides an assessment of long term carbohydrate control in diabetic patients.³ Interest in this began with the observation by Rahbar in 1968 that diabetic patients had raised concentrations of "fast-moving" haemoglobin (HbA_{1c}) when analysed by electrophoresis.⁴ Subsequent work in several laboratories led to the identification of HbA_{1c} as glycated haemoglobin⁵⁻⁶ with the Amadori product of glucose binding preferentially to the N-amino terminal valine of the β -chain. Glycation at other sites on the haemoglobin molecule does not result in an electrophoretically distinct fraction.

The methods available for the estimation of

glycated haemoglobin either measure total HbA_{1c} (electroendosmosis)⁷ or the main stable glycated fraction HbA_{1c} after isolation from the other fractions (isoelectric focusing).⁸ More recently methods using affinity chromatography separation have been described.⁹ This separates haemoglobins to which is bound a glucose molecule and therefore measures total glycated haemoglobin.

Several workers have investigated the possibility of using glycated haemoglobin as an indicator of glucose intolerance with methods based on charge separation.¹⁰⁻¹² They reported poor sensitivity with only patients with pronounced glucose intolerance showing high results. Workers using affinity chromatography have recently described more favourable sensitivity.¹³⁻¹⁴

In this study we used affinity chromatography and electroendosmosis to estimate total glycated haemoglobin and HbA_{1c} (respectively) in samples collected from 75 cadavers coming to necropsy. Glycated protein was estimated as fructosamine. The results were then compared with the known clinical state of the patients before death.

Patients and methods

A series of unselected patients who came to necropsy over one year were included in the study. Of the 75 patients studied, 42 were men (seven known diabetics)

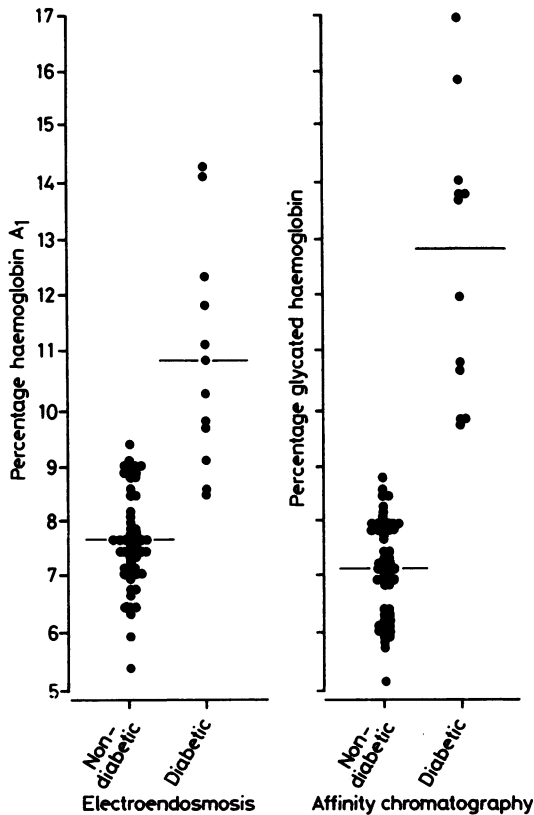


Figure 2 Glycated haemoglobin (measured by affinity chromatography) and HbA_{1c} (measured by electroendosmosis) found in necropsy blood samples of known diabetics and patients with no previous history of diabetes.

and 33 women (five known diabetics). The mean time from death to necropsy was 51.3 (range 12–144) hours, 16.5 (range four to 36) hours of which were at room temperature and 40.0 (range five to 120) at 4°C. The mean age of the non-diabetic patients was 69.2 (range 23–90) years. The mean age in the diabetic group was 69.2 (range 61–82) years.

Blood was collected at necropsy from the femoral vein and added to tubes containing fluoride oxalate as an anticoagulant. Twenty patients also had blood collected from the right side of the heart: five also had blood collected from the opposite femoral vein and from axillary veins. Three patients had blood samples collected from the right and left femoral veins, the aorta, the superior vena cava, the right ventricle, the inferior vena cava and the hepatic vein.

Blood glucose concentration was measured the day of collection using a glucose oxidase/4-aminophenazone method on a YSI analyser. Glycated haemoglobin was estimated by affinity chromatography as previously described⁹ and HbA_{1c} was estimated using the Corning Glytrac electroendosmosis

procedure (Corning Medical and Scientific). The latter two measurements were performed within five days of collection, the haemolysates being stored at 4°C. Estimation of fructosamine value was performed by a modification of the method described by Johnson *et al*¹⁵ on a Technicon RA 1000. Specimens were stored at –20°C for up to one month before estimation.

Results

BLOOD GLUCOSE

In all but one case the glucose concentration in the blood taken from the femoral vein was decreased—in most cases to less than 2.0 mmol/l. One diabetic patient showed a high glucose concentration of 22.8 mmol/l. Twenty patients with no history of diabetes also had glucose estimated on blood collected from the right side of the heart. The mean blood glucose concentration in these specimens was 15.7 (range 0.2–33.2) mmol/l and was significantly higher ($p < 0.001$) than the mean blood glucose concentration of 1.7 (range 0–6.4) mmol/l found in the femoral veins of these patients. The increased blood glucose concentration in the right side of the heart was not related to the length of time after death. Three of these patients had further blood samples collected, and the mean blood glucose concentrations in these specimens were: 0 (right femoral vein), 0.2 (left femoral vein), 0 (aorta), 17.5 (superior vena cava), 16.7 (right ventricle), 28.5 (inferior vena cava) and 48.2 (hepatic vein) mmol/l.

GLYCATED HAEMOGLOBIN AND HAEMOGLOBIN A_{1c}
Glycated haemoglobin and HbA_{1c} were estimated on specimens collected from the right and left femoral veins, the right and left axillary veins, and the right side of the heart of five patients. In all patients the variation in the glycated haemoglobin results was within the precision of the method. In one, the variation of HbA_{1c} results was greater than the precision of the method.

The mean glycated haemoglobin and HbA_{1c} values in the patients with no known history of diabetes were found to be 7.7% (range 5.2–15.2%) and 8.0% (range 5.3–12.3%), respectively. The diabetic patients showed a mean glycated haemoglobin result of 12.8% (range 9.9–17.2%) and a mean HbA_{1c} of 10.9% (range 8.5–14.4%). Using Smirnov's test,¹⁶ the glycated haemoglobin and HbA_{1c} results found in the diabetic population were significantly different from those in the non-diabetic population (at the 95% confidence level).

Seven patients, who at necropsy had no known history of diabetes, produced results that in life would be regarded as abnormal.^{9,14} The medical histories of these seven patients were further investigated. The results (table) show that two of the seven were probably diabetic and another had had the blood glucose artificially raised with a dextrose infusion. These cases were excluded from the non-diabetic

Table Percentage of patients showing increased glycated haemoglobin at necropsy

HbA _{1c}	Glycated haemoglobin	Known clinical state	Necropsy findings	Subsequent information
10.2	10.0		Ischaemic heart disease	6 years ago glucose 6 mmol/l
9.0	10.0	Strokes, amputation, renal and cardiac failure	Ischaemic heart disease	No mention of diabetes mellitus
10.9	13.0		Ischaemic heart disease	BM glucose stick 9 mmol/l
10.0	11.4	Rheumatoid ischaemic heart disease, fracture of femur	Cholecystitis, ischaemic heart disease, rheumatoid arthritis	Numerous blood glucose estimations of 7-7.5 mmol/l
12.3	15.2	Depression	Suicide by electrocution	Normal OGTT
10.0	10.2	Sudden death, profound hypoglycaemia	Infarcted bowel, pulmonary emboli, ? nodule in pancreas	7 years ago On dextrose for two days before death; (glucose 25 mmol/l)
10.7	12.5	Haematemesis, abdominal pain	Perforated duodenal ulcer	Glucose 10.9 mmol/l before death

group. The other four patients had no recorded history of diabetes. Using Healy's method for the removal of outliers, the range of results (95% confidence limits) in the non-diabetic group was 5.9-8.6% for glycated haemoglobin and 6.4-9.4% for HbA_{1c}. With the exception of these seven patients, the distribution of results for both groups is shown in the figure.

FRUCTOSAMINE

Most blood specimens were haemolysed when collected and were therefore considered to be unsuitable for analysis. Other specimens had very little plasma associated with the red cells. Most samples had a high total protein content, with a mean concentration of 78.8 (range 44-120) g/l. The albumin concentration was low with a mean result of 19.2 (range <5-39) g/l, resulting in a protein : albumin ratio of 3.8. The mean fructosamine value measured in 26 non-diabetics was 1.06 (range 0.33-1.84) mmol/l, which, when corrected for protein concentration, gave a mean of 14.3 (range 3.2-26) μ mol/g protein. The mean fructosamine value in three diabetics was 2.09 (1.73, 1.83 and 2.70) mmol/l, which, when corrected for protein concentration, was 31.1 (25.1, 40.7 and 27.6) μ mol/g protein.

Discussion

Despite the introduction of insulin in the treatment of diabetes over 60 years ago, diabetes continues to devastate the health of a considerable proportion of people around the world. It is the seventh leading cause of death in the United States, accounting for about 150000 deaths annually.¹⁷ In the United Kingdom diabetes is reported to be the primary cause in 0.7% of male and 0.95% of female deaths. Diabetes as a secondary cause may be a contributory factor in many more deaths, with the suspected incidence of diabetes being between 2% and 4% in the general population.

Attempted assessment of glycaemic state before death is normally based on the estimation of glucose in blood samples collected at necropsy. In this study we have shown that glucose concentrations in necropsy peripheral blood samples were significantly decreased when compared with non-fasted blood glucose concentrations in a healthy population (3.6-7.0 mmol/l). Similar results have been reported by other workers.¹ The high glucose concentrations in blood collected from the right side of the heart have also been reported,¹ although the finding seems to be largely ignored by pathologists. The high blood glucose concentration in the right heart is probably due to glycogen breakdown occurring in the liver; this would account for the finding that the highest glucose concentrations are found in blood samples collected in the hepatic vein. Due to the large variation in blood glucose concentrations no normal range for necropsy could be established.

Unlike glucose, in most cases glycated haemoglobin and HbA_{1c} concentrations did not differ in blood samples collected from various sites in the body. The range (after removal of outliers) of glycated haemoglobin found in the patients with no known history of diabetes was similar to that previously reported in a healthy population (5.0-8.5%),⁹ but the slight increase may be due to this population being older than that previously investigated. The range of HbA_{1c} in this group was higher than that reported by Menard *et al*⁷ in a healthy population. This may be due to necropsy adducts (other than glucose) adding to haemoglobin and thereby affecting its charge characteristic. This would affect the electroendosmosis method but not the affinity method. In the diabetic patients the glycated haemoglobin results tended to be higher than those for HbA_{1c}, confirming previously reported findings that in the diabetic ranges affinity chromatography produces higher results.⁹

The distinct results found in the diabetic and non-

diabetic groups using affinity chromatography were similar to those reported in life by investigators who compared glycated haemoglobin results (measured by affinity chromatography) with those of an oral glucose tolerance test (OGTT), although in this study we did not consider the group with impaired glucose tolerance. There was an overlap in the HbA_{1c} results obtained in both groups, with three diabetic patients displaying results in the non-diabetic range. Other workers who used electroendosmosis to estimate HbA_{1c} and compared the results with the outcome of an OGTT reported poor sensitivity for HbA_{1c} in diagnosing diabetes. The difference in the diagnostic sensitivity of the two methods may be due to the better precision of the affinity method, or that electroendosmosis may be affected by several factors that do not interfere with the affinity method. When glycated haemoglobin was used to assess glycaemic control in the known diabetics, three patients showed good, three moderate, and six poor controls.

Seven patients, who at necropsy had no known history of diabetes, displayed results (glycated haemoglobin or HbA_{1c}, or both) that in life would be regarded as abnormal. All seven patients displayed an increased glycated haemoglobin result; HbA_{1c} was raised in only six patients. The case histories of these seven patients were further investigated. It was found that before death three had been suspected at some time of being diabetic, although diabetes was not confirmed. Two patients had had a glucose estimate that in itself was diagnostic of diabetes.

Assessment of fructosamine value is a recently described method for estimating glycated protein, which is reported to reflect glycaemic control over the preceding one to three weeks. The estimate is performed on serum or plasma. Unfortunately, specimens collected at necropsy tend to be haemolysed or very haemoconcentrated, or both, and are therefore unsuitable for analysis. Fructosamine estimation was only possible in 26 non-diabetic and three diabetic patients. None of the seven non-diabetic patients who displayed an increased glycated haemoglobin estimation had a fructosamine estimate. There was reasonably clear distinction between the diabetic and non-diabetic groups, especially after the results were corrected for protein content. Interpretation of the fructosamine results was difficult in these patients as the protein content (albumin : protein ratio) differed considerably from that found in life.

We conclude that measurement of glycated haemoglobin or HbA_{1c} in specimens collected at necropsy provides a valuable tool for assessing glycaemic control of known diabetic patients just before death, and may be useful for the diagnosis at necropsy of previously unsuspected diabetes. Estimation of blood glucose concentration is of no value in

assessing glycaemic control at necropsy, although pathologists should be aware of the differences found at various sites in the body.

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