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Postmortem Diagnosis of Diabetic Metabolic Derangement: Elevated α_1-Antitrypsin and Haptoglobin Glycosylation Levels as an Index of Antemortem Hyperglycemia

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ABSTRACT: Fatal diabetic metabolic derangement is difficult to diagnose postmortem because of the paucity of characteristic morphologic findings. Hyperglycemia is an indicator of diabetic derangement. Conventional biochemical parameters for postmortem diagnosis of antemortem hyperglycemic states are not sufficiently resistant to antemortem and postmortem non-diabetic influences or are suited only for long and medium-term assessment of diabetes control. In the search for other, more reliable, indices of immediately antemortem blood glucose levels, we investigated the value of glycosylation levels of serum proteins with very brief biologic half-lives: a) In vitro studies were performed on the glycosylation course of the short-lived serum proteins α_1-antitrypsin (α_1-AT) and haptoglobin (HP). b) Glycosylation levels were measured after purification of α_1-AT and HP from sera of living and deceased non-diabetics and diabetics. c) The resistance of α_1-AT and HP glycosylation levels to autolysis was investigated. Our studies revealed the following: 1) α_1-AT and HP glycosylate considerably more rapidly than either albumin or hemoglobin. This rapid glycosylation, combined with the rapid turnover of both proteins, facilitates detection of short-term changes in glycemia. 2) α_1-AT and HP glycosylation levels are autolysis-stable and can be assessed even after advanced hemolysis. 3) α_1-AT and HP glycosylation levels appear to allow reliable ante- and postmortem discrimination between normoglycemic and hyperglycemic metabolic states. As a tool in the postmortem diagnosis of antemortem hyperglycemic states, α_1-AT and HP glycosylation levels combine the advantages of a short-term parameter with resistance to non-diabetic influences.

KEYWORDS: forensic science, diabetes mellitus, postmortem diagnosis of antemortem hyperglycemic states, α_1-antitrypsin and haptoglobin glycosylation levels

Identification of diabetic derangement as the cause of death can be of utmost importance in forensic practice, for example, if medical malpractice is suspected. Retrospective reconstruction of the metabolic state immediately preceding death based on morphologic findings obtained at autopsy is imprecise at best (1–10). Even Armanni-Ebstein cells, purportedly diagnostic for hyperglycemic diabetic derangement, are not found in every case; their demonstration, moreover, is problematic in cases of advanced autolysis (3,11–13). Biochemical studies on body fluids allow for a more specific assessment (1,3–10,14–28).

Postmortem diagnosis of "fatal diabetic coma" has to consider highly complex causal metabolic dysregulations: Diabetic coma may be caused by ketoacidotic or hyperosmolar diabetic derangement. A differentiation may be achieved even postmortem by determination of ketones or acetone levels in body fluids (3,7,8,16,19–21,24). In any event, one indicator of severe diabetic dysregulation is *hyperglycemia.* In living patients, diagnosis of hyperglycemia is a simple matter of blood glucose determination. Blood glucose levels in cadaver blood, however, are unreliable because of rapid postmortem glycolysis (15,19,20,29).

Consequently, other biochemical parameters are used in the postmortem diagnosis of antemortem hyperglycemia, including glucose (and lactate) levels in spinal fluid and vitreous humor, glycated hemoglobin (HbA_1), and glycosylation of total serum protein or albumin[3] (1,3,4,7,9,14,17–22,24–26,28). The diagnostic utility of these parameters varies. Glucose levels in spinal fluid and vitreous humor may reflect antemortem blood glucose levels (5,15,16,23,27). However, postmortem glycolysis also occurs in spinal fluid and vitreous humor and is regarded as the cause of the postmortem glucose decrease and lactate increase in these mediums (6,9,15,19,21,24,26). Although spinal fluid and vitreous humor are less subject to terminal and postmortem changes than blood, postmortem glycolysis may be quite rapid in these body fluids as well (9,15,19,21). Summary glucose and lactate values in spinal fluid and vitreous humor were therefore introduced in the postmortem diagnosis of antemortem hyperglycemia and have been regarded as valuable diagnostic tools by a number of authors (3,7,9,19–21,24,26). Nonetheless, as is the case with glucose levels, summary values may also be subject to non-diabetic influences (especially by hyperlactatemias of diverse genesis) (3,4,7,9,-19,22,24,25). HbA_1 levels and glycosylation of total serum protein or albumin, while being less susceptible to non-diabetic influences and resistant to autolysis (4,14,17,18,21,22,33), only reflect integrated blood glucose levels from the 6–8 weeks (HbA_1) or 2–3 weeks (glycosylated total serum protein or albumin) before death (4,17,22,28,30,31,34–41). What these parameters cannot ascertain are the blood glucose levels during the crucial period immediately prior to death.

An ideal parameter for evaluation of blood glucose levels immediately preceding death would have to have the following characteristics:

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[3]Since glycosylation of albumin represents up to 90% of total glycosylation, total serum protein glycosylation levels have the same diagnostic value as albumin glycosylation levels (30–32).

a) It must be a short-term parameter that rapidly and specifically registers changes in blood glucose.
b) It must be stable under ante- and postmortem non-diabetic influences, particularly autolysis.

The postmortem stability of HbA_1 and total serum protein or albumin glycosylation is due to peculiarities in the adduction of glucose to protein.[4] HbA_1, levels and glycosylation of total serum protein or albumin, however, are only suitable for long and medium-term monitoring of diabetes mellitus because of the relatively long biologic half-lives of hemoglobin (approx. 45 days) and albumin (approx. 21 days). Serum proteins other than albumin are also known to glycosylate in relation to the blood glucose levels to which they are exposed during their life-spans (30,31,37,42–48). It is to be expected that the glucose bond in these proteins is also highly stable. Glycosylation of proteins with much shorter half-lives than albumin should reflect short-term metabolic fluctuations better than glycosylation of albumin, total serum protein or hemoglobin. In postmortem diagnosis of diabetic hyperglycemic derangement, glycosylation levels of serum proteins with very short half-lives would therefore combine the advantage of postmortem stability with the capacity for retrospective registration of short-term glucose fluctuations.

The aim of the present study was to purify selected serum proteins with half-lives much shorter than albumin and to explore the utility of their glycosylation levels in postmortem diagnosis of antemortem hyperglycemic states. For reasons of practicability (adequate serum levels, feasible technical conditions), the serum proteins α_1-*antitrypsin* and *haptoglobin* (α_1-*AT* and *HP*, with half-lives of 4 and 2–4 days (49,50), were investigated.

Materials and Methods

In Vitro Studies on the Course of α_1-Antitrypsin (α_1-AT), Haptoglobin (HP) and Albumin Glycosylation

The course of α_1-AT, HP and albumin glycosylation was investigated in vitro as follows: Three different protein solutions containing either 1 g/L α_1-AT, 1 g/L HP, or 50 g/L albumin in PBS buffer (pH 7.2) were produced under sterile conditions. Each solution was fractionated and adjusted to one of two different glucose levels: 1) 5 mmol/L glucose (corresponding to normoglycemia) or 2) 30 mmol/L glucose (corresponding to hyperglycemia). Glucose-free solutions were used as controls. All solutions were incubated at 37°C for a maximum of 7 days. Aliquots for determination of glycosylation levels were removed every 24 h for α_1-AT and albumin, every 48 h for HP. Extent of glycosylation was determined as the ratio of fructosamine (30,31,37,41) to protein. Fructosamine levels were measured by a photometric test (Test-Combination Fructosamine, Boehringer, Mannheim, FRG), protein levels by the biuret method (Merckotest, Merck, Darmstadt, FRG).

α_1-AT and HP Glycosylation Levels and Conventional Parameters for Diagnosis of Hyperglycemic States in Living Subjects and Cadavers

Samples—Analyses were performed on samples from the following subjects:

[4]In (non-enzymatic) glycosylation, glucose adducts to free amino groups of the protein via an aldimine linkage (Schiff base), which then undergoes Amadori rearrangement to produce a stable ketoamine product (fructosamine) (30,31,37,41).

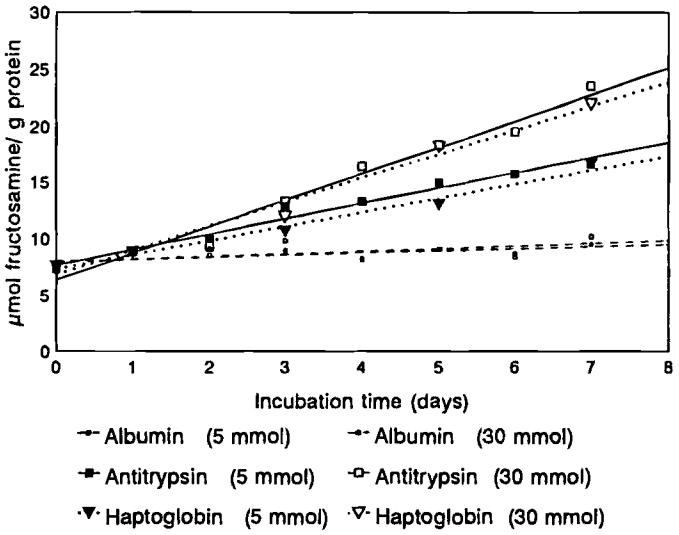

FIG. 1—*In vitro glycosylation of α_1-antitrypsin, haptoglobin and albumin during incubation at 37°C in 5 mmol/L and 30 mmol/L glucose solutions for up to 7 days.*

Living non-diabetics (n = 73; group "*ND am*" in Tables 1, 2A/B and Fig. 2): Blood samples (venous blood) from 73 healthy non-diabetic donors were analyzed.

Living diabetics (n = 26)—Residual blood (venous blood) from samples taken for clinical reasons from 26 inpatients was investigated. These living diabetics were divided into three groups according to their medical records:

Diabetics with normoglycemia—(n = 7; group "*Da am*" in Tables 1, 2A and Fig. 2):
Clinical diagnosis, "well-controlled diabetes mellitus";
* all fasting blood glucose levels (capillary blood) in the last 4 days before blood sampling <120 mg/dL;

Diabetics with hyperglycemia—(n = 17; group "*Db am*" in Tables 1, 2A and Fig. 2):
* Clinical diagnosis, "poorly-controlled diabetes mellitus";
* Fasting blood glucose levels (capillary blood) in the last 4 days before blood sampling frequently >120 mg/dL;

TABLE 1—*α_1-antitrypsin/haptoglobin glycosylation levels (µmol fructosamine/g protein) in living and deceased non-diabetics and diabetics* (am = *Living;* pm = *Postmortem;* ND = *Non-diabetics;* Da = *Diabetics, normoglycemia;* Db = *Diabetics, hyperglycemia;* Dc = *Diabetics, diagnosed or suspected fatal diabetic derangement:* Dc am = *Clinically diagnosed diabetic coma,* Dc pm = *Suspected fatal diabetic derangement;* MV ± SD = *Mean value ± standard deviation).*

Test group	Range	MV ± SD
ND am (n = 73)	8.19–15.17	12.51 ± 1.98
Da am (n = 7)	9.62–14.95	12.36 ± 1.98
Db am (n = 17)	14.97–20.51	18.19 ± 1.49
Dc am (n = 2)	24.14–25.62	24.88 ± 0.74
ND pm (n = 17)	7.50–15.06	11.01 ± 1.94
Da pm (n = 4)	11.89–15.05	13.52 ± 1.35
Db pm (n = 4)	17.17–19.86	18.49 ± 1.01
Dc pm (n = 16)	19.46–28.75	24.22 ± 2.77

TABLE 2A—*Conventional parameters for diagnosis of hyperglycemic states in living and deceased non-diabetics and diabetics* (am = *Living;* pm = *Postmortem;* ND = *Non-diabetics;* Da = *Diabetics, normoglycemia;* Db = *Diabetics, hyperglycemia;* Dc = *Diabetics, diagnosed or suspected fatal diabetic derangement:* Dc am = *Clinically diagnosed diabetic coma,* Dc pm = *Suspected fatal diabetic derangement;* MV ± SD = *mean value ± standard deviation).*

Parameter	Test group	Range	MV ± SD
Blood glucose concentration (venous blood) (mg/dL)	ND am (n = 73)	69–123	91 ± 12
	Da am (n = 6)	104–138	121 ± 18
	Db am (n = 15)	173–398	259 ± 56
	Dc am (n = 2)	401–482	441 ± 40
Glycosylation of total serum protein (μmol fructosamine/g protein)	ND am (n = 73)	2.35–4.91	3.44 ± 0.57
	Da am (n = 3)	3.98–5.12	4.53 ± 0.51
	Db am (n = 15)	4.13–6.24	5.42 ± 0.71
	Dc am (n = 2)	5.92–6.04	5.98 ± 0.06
HbA_1 (%)	Da am (n = 7)	7.3–12.1	10.1 ± 1.7
	Db am (n = 15)	9.1–14.3	12.1 ± 1.4
	Dc am (n = 2)	13.7–14.6	14.1 ± 0.4
	ND pm (n = 13)	4.4– 9.5	7.6 ± 1.7
	Da pm (n = 3)	9.7–14.1	11.9 ± 1.8
	Db pm (n = 3)	11.9–15.4	13.8 ± 1.4
	Dc pm (n = 12)	9.3–17.4	13.4 ± 2.4

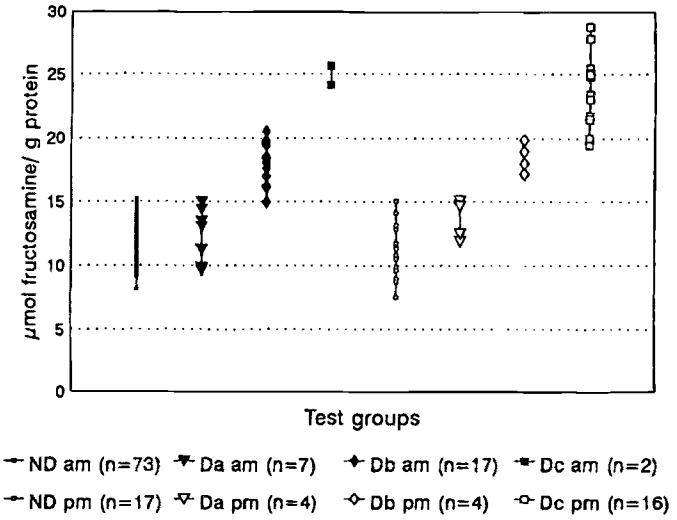

FIG. 2—*Distribution of α_1-antitrypsin/haptoglobin glycosylation levels in living and deceased non-diabetics and diabetics* (am = *Living;* pm = *Postmortem;* ND = *Non-diabetics;* Da = *Diabetics, normoglycemia;* Db = *Diabetics, hyperglycemia;* Dc = *Diabetics, diagnosed or suspected fatal diabetic derangement:* Dc am = *Clinically diagnosed diabetic coma,* Dc pm = *Suspected fatal diabetic derangement.*

TABLE 2B—*Conventional parameters for diagnosis of hyperglycemic states in deceased non-diabetics and diabetics* (**pm** = *Postmortem;* ***ND*** = *Non-diabetics;* ***Da*** = *Diabetics, normoglycemia;* ***Db*** = *Diabetics, hyperglycemia;* ***Dc*** = *Diabetics, suspected fatal diabetic derangement;* ***MV ± SD*** = *Mean value ± standard deviation).*

Parameter	Test group	Range	MV ± SD
Glucose levels in spinal fluid (mg/dL)	ND pm (n = 14)	32.2–94.8	68.4 ± 18.2
	Da pm (n = 4)	42.6–112.3	72.6 ± 16.4
	Db pm (n = 4)	102.5–166.4	138.8 ± 27.9
	Dc pm (n = 9)	184.9–426.8	292.8 ± 68.7
Glucose levels in vitreous humor (mg/dL)	ND pm (n = 14)	79.1–177.7	119.0 ± 28.2
	Da pm (n = 4)	94.9–170.6	128.7 ± 27.1
	Db pm (n = 4)	158.3–242.7	205.7 ± 31.0
	Dc pm (n = 11)	151.6–509.7	342.1 ± 95.7
Summary glucose and lactate values in spinal fluid (mg/dL)	ND pm (n = 14)	227.4–365.0	305.7 ± 35.8
	Da pm (n = 4)	295.8–427.0	359.3 ± 51.8
	Db pm (n = 4)	398.1–448.6	415.7 ± 19.5
	Dc pm (n = 9)	520.0–1026.0	696.2 ± 172.4
Summary glucose and lactate values in vitreous humor (mg/dL)	ND pm (n = 14)	208.7–419.6	301.4 ± 64.7
	Da pm (n = 4)	278.2–401.0	343.1 ± 44.4
	Db pm (n = 4)	387.7–460.8	420.3 ± 26.5
	Dc pm (n = 9)	421.3–902.8	640.5 ± 155.2

Diabetics with clinical diagnosis of "diabetic coma"—(n = 2; group "*Dc am*" in Tables 1, 2A and Fig. 2):

Table 3 includes the relevant clinical data on these two cases.

Cadavers of individuals with non-diabetic or diabetic metabolic antemortem state (n = 41)—Studies were performed on samples of blood (cardiac blood), spinal fluid and vitreous humor collected from 41 cadavers within 72 hours after death.

In 25 cases, samples were obtained at autopsy on patients who had died in hospital. A review of the medical records revealed that

* 17 of these patients were *normoglycemic non-diabetics* (group "*ND pm*" in Tables 1, 2A/B and Fig. 2),

* 4 were *diabetics with normoglycemia* (criteria see above; group "*Da pm*" in Tables 1, 2A/B and Fig. 2).

* and 4 were *diabetics with hyperglycemia* (criteria see above; group "*Db pm*" in Tables, 1, 2A/B and Fig. 2). None of these cases exhibited clinical signs of diabetic coma, all individuals died from other natural causes of death.

Specimens from a further 16 cadavers of subjects with a *diagnosis of suspected fatal diabetic derangement* (group "*Dc pm*" in Tables 1, 2A/B and Fig. 2) were provided by 5 institutes of forensic medicine.[5] In these cases, the diagnosis "suspected fatal diabetic derangement" was based on case history, autopsy findings and conventional parameters for diagnosis of diabetic derangements determined postmortem (cf. group "Dc pm" in Tables 2A/B; data for acetone not shown). Specific antemortem data were not available in these cases, since these individuals all died medically unattended.

Purification of α_1-AT and HP from Serum and Assessment of Glycosylation Levels—α_1-AT and HP were purified from sera of all blood samples. Due to their similar physicochemical properties, the two proteins were located after purification in the same fractions, which were then characterized by radial immunodiffusion, SDS-gel electrophoresis and nephelometric quantification of α_1-AT and HP.

Purification of α_1-AT and HP—Purification of α_1-AT and HP was done by affinity chromatography and subsequent preparative isoelectric focusing (IEF). For *affinity chromatography,* columns (5 mL bed volume) filled with Affi-Gel Blue (BioRad, Richmond, USA) were used. After equilibration of the columns with 0.03M monopotassium phosphate (KH_2PO_4, pH 7.1), 0.75 mL serum samples were applied. Stepwise elution was performed with 0.03 M KH_2PO_4 (pH 7.1) and 1.4M NaCl in 0.03M KH_2PO_4 (pH 7.1) at a flow rate of 1 mL/min; protein detection was performed at 280 nm. Eluted fractions were characterized as described as follows. Eluates containing α_1-AT and HP were desalted by dialysis against

[5]We thank the Institutes of Forensic Medicine of the Technical University of Aachen and the Universities of Frankfurt am Main, Hamburg, Heidelberg, and Ulm for kindly providing us with samples.

TABLE 3—*Case history, clinical data (upon admission) and α_1-AT/HP glycosylation levels in two living individuals with the clinical diagnosis "diabetic coma."*

	Case 1 (female, 74 years)	Case 2 (male, 53 years)
Case history	Diabetes mellitus, type 2; weakness, vomiting, polydipsia, polyuria for several days	Diabetes mellitus, type 2; polydipsia, polyuria, frequently blood glucose levels up to 800 mg/dL and disturbances of consciousness in the 7 days prior to admission
Clinical picture	Unconsciousness; feeble reflexes; exsiccosis, hypotension, tachycardia, hyperosmolarity; plasma ketones not elevated	Unconsciousness; exsiccosis, hypotension, tachycardia, oliguria, hyperosmolarity; plasma ketones not elevated
Glucose level (mg/dL)	922	1020
HbA1 (%)	13.7	14.6
Clinical diagnosis	Hyperosmolar, nonketotic diabetic coma	Hyperosmolar, nonketotic diabetic coma
α_1-AT/HP glycosylation level (μmol fructosamine/g protein)	24.14	25.62

water and further purified by *preparative IEF.* After addition of an ampholyte (Servalyt 3–6, Serva, Heidelberg, FRG) samples were focused in a Bio-Rad Rotofor Cell (Bio-Rad, Richmond, USA) and the pH of each fraction was determined. In accordance with the isoelectric points of α_1-AT and HP (4.0 and 4.1), fractions from the 4.0–4.1 pH range were pooled. After lyophilization of the pooled fractions, the samples were adjusted to the original volume of 0.75 mL with water, characterized, and the glycosylation levels of the purified serum proteins determined.

Characterization of the purified samples—Fractions derived from the affinity chromatography and preparative IEF procedures were characterized by radial immunodiffusion, SDS gel electrophoresis, and nephelometric quantification of α_1-AT and HP. Characterization with *radial immunodiffusion* was done using immunodiffusion plates (NOR-Partigen Albumin, α_1-AT and HP, Behring Diagnostika, Marburg, FRG). *One-dimensional SDS gel electrophoresis* for characterization of samples was performed as described by Lämmli (51) and protein detection was carried out using the silver staining method according to Merrill (52). In purified samples, α_1-AT and HP concentrations were assessed by means of *nephelometry* (Array 360-System, Beckmann, Munich, FRG).

Determination of α_1-AT and HP glycosylation levels—Glycosylation levels of α_1-AT and HP were assessed as the ratio of fructosamine (Test-Combination Fructosamine, Boehringer, Mannheim, FRG) to protein (Merckotest, Merck, Darmstadt, FRG) in the purified samples.

Assessment of Conventional Parameters for Diagnosis of Hyperglycemic States

Estimation of *blood glucose* levels was done enzymatically (hexokinase method; Gluc-Testpack, aca, Du Pont, Geneva, Switzerland). *Glycosylation of total serum protein* was calculated as the ratio of fructosamine (Test-Combination Fructosamine, Boehringer, Mannheim, FRG) to total serum protein (Merckotest, Merck, Darmstadt, FRG). *HBA_1 values* were measured using a microcolumn method (Test-Combination Hemoglobin A_1, Boehringer, Mannheim, FRG). In cadavers, *glucose and lactate concentrations in spinal fluid and vitreous humor* were determined enzymatically (hexokinase method and lactate dehydrogenase method; Gluc- and La-Testpack, aca, Du Pont, Geneva, Switzerland) and their summary values calculated.

Relation of α_1-AT and HP Glycosylation Levels to Conventional Parameters for Diagnosis of Hyperglycemic States

Linear regression analysis was used to evaluate the relation of α_1-AT and HP glycosylation levels to conventional diagnostic parameters for hyperglycemic states. Because of the low number of cases in some of the groups studied, further statistical analysis was not done.

Resistance of α_1-AT and HP Glycosylation Levels to Autolysis

Two fresh blood samples were stored at 25°C for a maximum of 12 and 14 days respectively. Rapidly progressing macroscopic hemolysis was detected within 3 days. An aliquot was taken from each sample every 24 h, centrifugated, and the supernatant stored at −20°C. After completion of the storage experiments, α_1-AT and HP were purified from the samples and their glycosylation levels determined as described.

Results

Courses of α_1-AT, HP and Albumin Glycosylation In Vitro

The courses of α_1-AT, HP and albumin glycosylation after incubation in 5 mmol/L and 30 mmol/L glucose solutions are shown in Fig. 1. Whereas albumin demonstrated no appreciable glycosylation, even after incubation in 30 mmol/L glucose solution for up to 7 days, both α_1-AT and HP underwent gradual glycosylation at roughly equal rates. Glycosylation of α_1-AT and HP progressed approximately twice as rapidly during incubation in 30 mmol/L as in 5 mmol/L glucose.

α_1-AT and HP Purification from Serum

Affinity chromatography enabled rapid removal of albumin from the serum samples. Following subsequent IEF, α_1-AT and HP were demonstrated by radial immunodiffusion in the pH 4.0–4.1 fractions. Besides α_1-AT and HP, SDS gel electrophoresis on these fractions detected only traces of other proteins, which could not be further characterized. Nephelometric quantification revealed that α_1-AT and HP were present in approximately equal concentrations. The fractions thus characterized were used to assess α_1-AT and HP glycosylation levels.[6]

[6]Since these fractions contained both α_1-AT and HP, they will be referred to in the following as "*α_1-AT/HP*".

α_1-AT/HP Glycosylation Levels in Living and Deceased Non-Diabetics and Diabetics

Table 1 shows the ranges, mean values, and standard deviations of the α_1-AT/HP glycosylation levels in the groups studied; Fig. 2 presents the distribution of values in the different groups. α_1-AT/HP glycosylation levels in non-diabetics and in diabetics with normoglycemia were all below 15.5 μmol/g (fructosamine/protein). Levels in diabetics with hyperglycemia were, by contrast, appreciably higher. The highest glycosylation values (up to 29 μmol/g) were registered in cases of clinically diagnosed diabetic coma or suspected fatal diabetic derangement. The area of overlap was small as far as could be determined from the material studied.

Conventional Parameters for Diagnosis of Hyperglycemic States in Living and Deceased Non-Diabetics and Diabetics

Tables 2A and 2B present the results for conventional clinical and postmortem parameters for diagnosis of hyperglycemic states (blood glucose concentrations, glycosylation of total serum protein, HbA_1, glucose levels and summary glucose and lactate levels in spinal fluid and vitreous humor).[7]

α_1-AT/HP Glycosylation Versus Conventional Parameters for Diagnosis of Hyperglycemic States

α_1-AT/HP glycosylation levels correlated closely with all conventional parameters for clinical and postmortem diagnosis of hyperglycemic states (for blood glucose concentration: $r = 0.89$; for glycosylation of total serum protein: $r = 0.85$; for HbA_1: $r = 0.75$; for glucose levels in spinal fluid: $r = 0.80$, and in vitreous humor: $r = 0.82$; for summary values in spinal fluid: $r = 0.85$, and in vitreous humor: $r = 0.84$).

Table 3 presents the clinical data (including the conventional parameters for diagnosis of hyperglycemic states) and α_1-AT/HP glycosylation levels of two living individuals with the clinical diagnosis of diabetic coma.

Resistance of α_1-AT/HP Glycosylation to Autolysis

Daily monitoring of blood samples stored for 14 days at 25°C revealed constant α_1-AT/HP glycosylation levels over the entire period. Even high-grade hemolysis did not disturb the determination of α_1-AT/HP glycosylation. The hemoglobin released during hemolysis was reliably separated from α_1-AT and HP by IEF.

Discussion

Features of protein glycosylation led to the hypothesis that assessment of glycosylation in short-lived serum proteins could be of value in postmortem diagnosis of antemortem hyperglycemic states.

Our findings in vitro experiments on the course of α_1-AT and HP glycosylation and the resistance to autolysis of α_1-AT and HP glycosylation support this hypothesis:

a) α_1-AT/HP glycosylation proved to be highly resistant to autolysis.

b) α_1-AT and HP glycosylated in vitro (in glucose solutions formulated to resemble physiologic and diabetic conditions in blood) considerably more rapidly than albumin.

Once Amadori rearrangement has occurred, glycosylation of serum proteins is irreversible (30,31,37). Hence, changes in blood glucose concentrations are reflected in serum protein glycosylation and registered for intervals the length of which depends on the half-life of the protein. The relatively short half-lives of α_1-AT and HP (4 and 2–4 days, respectively) preclude the registering of "old" blood glucose levels. Considering the rapid in vitro glycosylation of α_1-AT and HP, it can therefore be assumed that their glycosylation levels represent a rapid and specific index of short-term changes in blood glucose concentrations.

This was further investigated by analyzing blood samples from living non-diabetic and diabetic subjects as well as from cadavers of individuals with known metabolic states antemortem. α_1-AT and HP were purified from blood samples using affinity chromatography and IEF. After purification, both proteins were located in the same fractions, which contained only traces of other proteins. The purity attained was regarded as adequate since α_1-AT and HP have almost identical half-lives and since both proteins glycosylated in vitro at almost equal rates (Fig. 1). The determination of α_1-AT/HP glycosylation levels and conventional parameters for diagnosis of hyperglycemic states in living and deceased non-diabetics and diabetics revealed the following:

a) α_1-AT/HP glycosylation levels correlated closely with the metabolic state immediately preceding death or at the time of blood sampling (in living patients):

   A close correlation was found between α_1-AT/HP glycosylation and blood glucose in the same blood sample. This accords with the results of in vitro experiments showing that both α_1-AT and HP glycosylate rapidly when exposed to glucose (Fig. 1).

   Whereas non-diabetics and diabetics with normoglycemia had mean glycosylation levels of approximately 12 μmol/g (fructosamine/protein), corresponding levels in diabetics with hyperglycemia approached 18 μmol/g. The mean value in cases of clinically diagnosed or suspected fatal diabetic derangement was 24 μmol/g, twice as high as in non-diabetics (Table 1).

   Diabetic coma was diagnosed clinically in two living individuals. The α_1-AT/HP glycosylation levels in these cases (Table 1, group "Dc am," and Table 3) were much higher than those of diabetics with normoglycemia or hyperglycemia without signs of diabetic coma (Table 1 and Fig. 2, groups "Da" and "Db"). Comparably elevated values were seen in most of the cases of suspected fatal diabetic derangement analyzed postmortem (Fig. 2, group "Dc pm"). Antemortem data was not available in these cases. However, the conventional short-term parameters for diagnosis of hyperglycemia determined postmortem suggested a severe antemortem hyperglycemia (3,5,7, 9,15,16,19–21,23,24,26,27) in all cases with similarly elevated α_1-AT/HP glycosylation levels.

b) Compared with conventional parameters for the postmortem diagnosis of antemortem hyperglycemic states, α_1-AT/HP glycosylation determination may have valuable advantages:

   This parameter showed less inter-group overlap than either total serum protein glycosylation levels or HbA_1 values (Tables 1 and 2A). This was to be expected since α_1-AT and HP glycosylated considerably more rapidly in vitro than albumin and since their biologic half-lives are substantially shorter (4

[7]Since blood glucose levels in cadaver blood are of little diagnostic value due to the rapid postmortem onset of glycolysis, these were determined in living subjects only. Total serum protein glycosylation was also assessed in living subjects only, since analyses of cadaver blood may be disturbed by postmortem hemolysis.

and 2–4 days, respectively) than those of either albumin (approx. 21 days) or hemoglobin (approx. 45 days) (49,50). Changes in metabolism could therefore be reflected more rapidly and with greater reliability by α_1-AT/HP glycosylation than by either total serum protein glycosylation or HbA_1 values.

α_1-AT/HP glycosylation levels in cadaver blood correlated closely with glucose levels and summary glucose and lactate values in spinal fluid and vitreous humor. This supports the assumption that both α_1-AT/HP glycosylation levels and glucose levels, or summary values of glucose and lactate levels, are short-term parameters that reflect the metabolic state immediately preceding death. Glucose levels and summary values may, however, be subject to artefact, especially due to postmortem glycolysis, agonal hyperglycemia and hyperlactatemia of non-diabetic origin (3,4,6,7,9,15,19,21,22,24–26). By contrast, α_1-AT/HP glycosylation levels are unaffected by postmortem glycolysis or antemortem hyperlactatemia and are affected by short-term agonal fluctuations in blood glucose levels to a lesser extent than are glucose levels and summary values in spinal fluid and vitreous humor, since measurable changes in glycosylation are only attained after the proteins have undergone a sufficiently long incubation time.

Our findings allow the following conclusions regarding the suitability of α_1-AT/HP glycosylation determination as a tool in the postmortem diagnosis of fatal diabetic derangement:

1. α_1-AT/HP glycosylation levels closely reflect blood glucose concentrations at the time of blood sampling or shortly before death. They are therefore well suited as a short-term parameter for the diagnosis of hyperglycemic states.

2. α_1-AT/HP glycosylation levels are autolysis-resistant and can be determined even in hemolyzed blood samples.

3. The risk of spurious results is slight. Theoretically, long-lasting hyperglycemias of non-diabetic origin may falsify the results; such hyperglycemias may, however, also complicate diagnosis based on conventional parameters. Individual diagnosis must take into account possible underlying diseases. In cases of acute onset of hyperglycemia and a short interval between onset of hyperglycemia and death, α_1-AT/HP glycosylation levels may be artefactually low, since glycosylation requires a definite incubation period, and the half-lives of α_1-AT and HP are 4 and 2–4 days respectively. Cases of this type are, however, unusual. Fatal diabetic derangement is generally preceded by a longer period of hyperglycemia.

4. α_1-AT/HP glycosylation levels combine the advantages of a short-term parameter with resistance to autolysis as well as to non-diabetic influences.

On the basis of these attributes, α_1-AT/HP glycosylation determination may be a useful diagnostic tool in the postmortem diagnosis of hyperglycemic diabetic derangement. It may be of especial value when blood is the only available specimen or when influence of non-diabetic factors on conventional diagnostic parameters is suspected (3,4,6,7,9,15,19,21,22,24–26).

It should be added that the data presented here are preliminary. A sufficiently large number of cases of fatal diabetic coma with confirmed antemortem diagnosis will have to be investigated to determine definitively whether α_1-AT/HP glycosylation levels are superior to conventional parameters in the postmortem diagnosis of hyperglycemic diabetic derangement.

References

(1) Althoff H. Zur postmortalen diagnose diabetes mellitus—ein Beitrag zur HbA_{1c}-Bestimmung. Beiträge zur Gerichtlichen Medizin 1984;42:285–92.

(2) Hansen KH, Bonk U, Spaethe R. Zur methode der liquoruntersuchungen an der leiche. Verhandlungen der Deutschen Gesellschaft für Pathologie 1976;60:445.

(3) Kernbach G, Brinkmann B. Kombinierte befundanalyse zur diagnose der diabetischen stoffwechselentgleisung. Beiträge zur Gerichtlichen Medizin 1986;44:179–83.

(4) Kernbach G, Picht S, Brinkmann B, Püschel K. Erste ergebnisse zur postmortalen diagnose des diabetes mellitus durch HbA_1-Bestimmung. Zeitschrift für Rechtsmedizin 1983;90:303–8.

(5) Leopold D, Müller RK, Heilmann W. Zur postmortalen diabetes- und urämie-diagnostik am corpus vitreum. Deutsches Gesundheitswesen 1972;27(44):2098–101.

(6) Pfeifer K, Schneider W. Über das verhalten des liquorzuckers nach dem tode und seine diagnostische bedeutung. Virchows Archiv Pathologische Anatomie 1965;339:331–36.

(7) Püschel K, Beckmann ER, Kernbach G, Brinkmann B. Differentialdiagnose und beweis des coma diabeticum an der leiche. Beiträge zur Gerichtlichen Medizin 1984;42:315–8.

(8) Reh H. Die postmortale schnelldiagnose des coma diabeticum. Deutsche Zeitschrift für Gerichtliche Medizin 1966;57:183–9.

(9) Traub F. Methode zur erkennung von tödlichen Zuckerstoffwechselstörungen an der leiche (Diabetes mellitus und Hypoglykämie). Zentralblatt für Allgemeine Pathologie 1969;112(4):390–9.

(10) Zschoch H, Brüning EJ, Richter E. Die diagnose des diabetes mellitus an der leiche mit dem glukoteststreifen. Zentralblatt für Allgemeine Pathologie 1962;103(9):455–61.

(11) Beckmann ER, Püschel K, Picht S. Zur pathomorphologie des diabetes mellitus und des coma diabeticum. Beiträge zur Gerichtlichen Medizin 1984;42:307–13.

(12) Lasczkowski GE, Püschel K. Hyperglykämische stoffwechselentgleisung: Relation zwische glykogennephrose und postmortalen biochemischen parametern. Rechtsmedizin 1991;1:41–5.

(13) Püschel K, Kernbach G, Brinkmann B. Glykogennephrose und postmortale biochemische meßgrößen des glukosestoffwechsels. Verhandlungen der Deutschen Gesellschaft für Pathologie 1987;72:333–6.

(14) Chen C, Glagov S, Mako M, Rochman H, Rubenstein AH. Postmortem hemoglobin (HbA_{1c}): evidence for a history of diabetes mellitus. Ann Clin Lab Sci 1983;13(5):407–10.

(15) Coe JI. Postmortem chemistry of blood, cerebrospinal fluid and vitreous humor, In: Tedeschi, CG, Eckert WG, Tedeschi LG, editors. Forensic medicine, Vol. 2, Philadelphia: Saunders 1977:1030–60.

(16) DiMaio VJM, Sturner WQ, Coe JI. Sudden and unexpected deaths after the acute onset of diabetes mellitus. J Forensic Sci 1977;22:147–51.

(17) Gilg T, Liebhardt E, Kauert G. Untersuchungen der blutspiegel von glykosyliertem hämoglobin (HbA_1) an leichenbluten und asservierten blutproben und anwendungsmöglichkeiten in der rechts- und verkehrsmedizin. Beiträge zur Gerichtlichen Medizin 1984; 42:293–300.

(18) Hindle JH, Rostron GM, Gatt JA. The diagnostic value of glycated haemoglobin levels in post-mortem blood. Ann Clin Biochem 1985;22:144–7.

(19) Kernbach G, Brinkmann B. Postmortale pathochemie für die feststellung der todesursache coma diabeticum. Der Pathologe 1983;4:235–40.

(20) Kernbach G, Koops E, Brinkmann B. Biochemische parameter bei 31 tödlichen diabetischen stoffwechselentgleisungen. Beiträge zur Gerichtlichen Medizin, 1984;42:301–6.

(21) Kernbach G, Püschel K, Brinkmann B. Die biochemischen meßgrößen des glucosestoffwechsels in abhängigkeit von der todesart und postmortalen einflüssen. Zeitschrift für Rechtsmedizin 1986; 96:199–213.

(22) Kugler J, Oehmichen M. Untersuchungen zum glucosestoffwechsel an der leiche. Beiträge zur Gerichtlichen Medizin 1986;44:185–8.

(23) Leahy MS, Farber ER. Postmortem chemistry of human vitreous humour. J Forensic Sci 1967;12(2):214–22.

(24) Peclet C, Picotte P, Jobin F. The use of vitreous humor levels of glucose, lactic acid and blood levels of acetone to establish antemortem hyperglycemia in diabetics. Forensic Sci Int 1994;65:1–6.

(25) Ritz S, Kaatsch HJ. Postmortale diagnostik von tödlichen diabetischen stoffwechselentgleisungen: welchen stellenwert haben liquor- und glaskörperflüs-sigkeitssummenwerte sowie der HbA_1-wert? Der Pathologe 1990;11:158–65.
(26) Sippel H, Möttönen M. Combined glucose and lactate values in vitreous humour for postmortem diagnosis of diabetes mellitus. Forensic Sci Int 1982;19:217–22.
(27) Sturner WQ, Gantner GE. Postmortem vitreous glucose determinations. J Forensic Sci 1964;9(4):485–491.
(28) Valenzuela A. Postmortem diagnosis of diabetes mellitus. Quantitation of fructosamine and glycated hemoglobin. Forensic Sci Int 1988;38:203–8.
(29) Willner K, Schulz E, Schwerd W. Fermentative glucosebestimmung an leichenblut und-liquor. Deutsche Zeitschrift für Gerichtliche Medizin 1968;63:84–97.
(30) Kennedy L, Lyons TJ. Non-enzymatic glycosylation. Br Med Bull 1989;45(1):174–90.
(31) Mayer TK, Freedman ZR. Protein glycosylation in diabetes mellitus: a review of laboratory measurements and of their clinical utility. Clin Chim Acta 1983;127:147–84.
(32) Walker SW, Howie AF, Smith AF. The measurement of glycosylated albumin by reduction of alkaline nitro-blue tetrazolium. Clin Chim Acta 1986;156:197–206.
(33) Baker JR, Johnson RN, Scott DJ. Serum fructosamine concentrations in patients with type II (non-insulin-dependent) diabetes mellitus during changes in management. Br Med J 1984;288:1484–6.
(34) Baker JR, O'Connor JP, Metcalf, PA, Lawson MR, Johnson RN. Clinical usefulness of estimation of serum fructosamine concentration as a screening test for diabetes mellitus. Br Med J 1983; 287:863–7.
(35) Dolhofer R, Wieland OH. Increased glycosylation of serum albumin in diabetes mellitus. Diabetes 1980;29:417–22.
(36) Dolhofer R, Renner R, Wieland OH. Different behaviour of haemoglobin A_1 and glycosyl-albumin levels during recovery from diabetic ketoacidosis and non-acidotic coma. Diabetologia 1981; 21:211–5.
(37) Ledl F, Schleicher E. Die maillard-reaktion in lebensmitteln und im menschlichen Körper—neue ergebnisse zu chemie, biochemie und medizin. Angewandte Chemie 1990;102(6):597–626.
(38) Mashiba S, Uchida K, Okuda S, Tomita S. Measurement of glycated albumin by the nitroblue tetrazolium colorimetric method. Clin Chim Acta, 1992;212:3–15.
(39) Negoro H, Morley JE, Rosenthal MJ. Utility of serum fructosamine as a measure of glycemia in young and old diabetic and non-diabetic subjects. Am J Med 1988;85:360–4.
(40) Shima K, Ito N, Abe F, Hirota M, Yano M, Yamamoto Y, Uchida T, Nogucchi K. High-performance liquid chromatographic assay of serum glycated albumin. Diabetologia 1988;31:627–31.
(41) Wörner W, Pfleiderer S, Rietbrock N. Selective determination of nonenzymatic glycosylated serum albumin as a medium term index of diabetic control. Int J Clin Pharmacol Ther Toxicol 1993;31(5):218–22.
(42) Day JF, Thorpe SR, Baynes JW. Nonenzymatically glycosylated albumin. In vitro preparation and isolation from normal human serum. J Biol Chem 1979;254(3):595–7.
(43) Dolhofer R, Wieland OH. Glycosylation of serum albumin: elevated glycosyl-albumin in diabetic patients, FEBS Letters 1979; 103(2):282–6.
(44) Geiger M, Binder BR. Nonenzymatic glucosylation as a contributing factor to defective fibrinolysis in diabetes mellitus. *Haemostasis* 1986;16:439–46.
(45) Kordtlandt W, Benschop C, Erkelens DW, Thijssen JHH. A simple method for the measurement of total and glycated apolipoprotein B and its relevance to apolipoprotein-B metabolism in diabetes mellitus. Clin Chim Acta 1989;186:109–18.
(46) Schleicher E, Wieland OH. Specific quantitation by HPLC of protein (lysine) bound glucose in human serum albumin and other glycosylated proteins. J Clin Chem Clin Biochem 1981;19(2):81–7.
(47) Schleicher ED, Gerbitz KD, Dolhofer R, Reindl E, Wieland OH, Edelmann E, Haslbeck M, Kemmler W, Walter H, Mehnert H. Clinical utility of nonenzymatically glycosylated blood proteins as an index of glucose control, Diabetes Care 1984;7(6):489–565.
(48) Winter WE. Non-enzymatic glycosylation of low-density lipoprotein. Results of an affinity chromatography method. Diabetologia 1988;31:126–8.
(49) Gressner AM, Thomas L. Proteinstoffwechsel, In: Greiling H, Gressner, AM, editors. Lehrbuch der klinischen Chemie und Pathobiochemie, Schattauer, Stuttgart, 1987;135–85.
(50) Koj A. In: Allison AC, editor. Structure and Function of Plasma proteins, Vol. 1, Plenum Press, London, 1974, 73.
(51) Lämmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
(52) Merril C. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science 1981;211:1437–8.

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