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ORIGINAL ARTICLE

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Vitreous humor fructosamine concentrations in the autopsy diagnosis of diabetes mellitus

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Abstract In clinical practice, biochemical markers, particularly serum glucose levels are used to diagnose diabetes mellitus. However, at autopsy this marker is of no value due to the substantial and capricious fluctuations in glucose levels after death. The aim of this study was to evaluate the usefulness of the postmortem determination of fructosamine in vitreous humor for confirming the presence of antemortem hyperglycemia. This was a study of 92 cadavers with a mean age of 60.05 years (SD 17.73) and a mean postmortem interval of 17.02 h (SD 9.76, range 2–58 h). Cases were assigned to two diagnostic groups according to the antemortem diagnosis of diabetes mellitus based on the patients' medical records. In vitreous humor statistically significant differences were found in glucose and fructosamine concentrations between the two diagnostic groups, the highest values being obtained in the group of subjects with a previous diagnosis of diabetes mellitus.

Key words Diabetes mellitus · Biochemistry · Fructosamine · Postmortem · Vitreous humor

Introduction

It would be very useful to specialists in forensic medicine to have a reliable method for the postmortem diagnosis of diabetes mellitus. Sudden, unexpected death due to the acute onset of diabetes is rare but is difficult to diagnose. Although an analysis of the biochemical constituents of the body is often useful in investigating deaths resulting from metabolic and biochemical disturbances (Knight 1996), biochemical diagnosis in the cadaver is often hindered by changes caused by autolysis, a factor which is closely linked to the postmortem interval. The interpretation of the results obtained with autopsy-derived body fluids is therefore at best quite fraught and complex.

In clinical practice, several biochemical markers, particularly serum glucose levels are used to diagnose diabetes mellitus. However, as a method of postmortem diagnosis of the disease, this marker is of no value due to the substantial fluctuations (Hill 1941; Coe 1977) brought about by the glycolytic phenomena that occur after death. Glycolysis continues spontaneously and the blood glucose concentration falls extremely rapidly (Coe 1993; Forrest 1993). Furthermore, death may be preceded by agonal processes and cardiopulmonary resuscitation which are often associated with the secretion or the administration of catecholamines, which result in further mobilization of liver glycogen and the release of glucose into the circulation (Lund 1964; Gormsen and Lund 1985) as a counterbalancing phenomenon.

Since glucose in the fluids of the cadaver is converted into lactate during the postmortem period, Traub (1969) proposed that hypoglycemia might be diagnosed by combining the values for glucose and lactic acid found in the cerebrospinal fluid. Also, several authors (Kernbach et al. 1984; Püschel et al. 1984) proposed the establishment of the sum values of glucose and lactate for the investigation of diabetic deaths and the metabolic disorders in chronic alcoholics (Brinkmann et al. 1998).

Because of the difficulty in interpreting postmortem serum glucose levels, other biochemical compounds such as glycated haemoglobin and glycated proteins (fructosamine) in serum have been studied (John et al. 1988; Valenzuela 1988). Protein glycosylation can occur as non-enzymatic post-translational modifications directly dependent on prevailing glucose concentrations (Bunn et al. 1978). Because glycoso-protein concentrations reflect mean serum glucose levels over a period of time, their determi-

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nation provides a useful means of monitoring diabetic control (Bunn et al. 1978; Kennedy and Merimee 1981).

Vitreous humor is very useful for postmortem diagnosis not least because of the ease with which this fluid can be obtained and the low risk of bacterial and other contamination, since it is anatomically well protected and usually the eye-globes are well preserved even in cases of serious trauma to the head (Coe 1974). It also resists the effects of putrefaction to a much greater and lengthier extent than other fluids (Coe 1974; Sturner et al. 1983) and is chemically stable (Coe 1969). It is a good postmortem indicator of diabetes mellitus because glucose levels in vitreous humor increase in direct correlation to the immediately antemortem serum levels with which they are in equilibrium (Coe 1973). Following the hypothesis of Traub (1969), some authors (Sippel and Mottönen 1982; Péclet et al. 1994) have proposed the combined determination of glucose and lactic acid in vitreous humor as useful indicators to confirm antemortem hyperglycemia as a probable cause of death.

The purpose of the present study was to evaluate the usefulness of the postmortem determination of fructosamine in vitreous humor for confirming the presence of antemortem hyperglycemia.

Material and methods

This study was conducted on 92 cadavers (61 males and 31 females) with a mean age of 60.05 years (SD 17.73; range 19–90 years). Of these, 35 deaths were due to violent causes (27 craniocerebral trauma and/or multiple trauma from motor vehicle collisions and 8 hangings) and 57 deaths from natural causes (54 cardiac deaths, 2 of pneumonia and pulmonary embolism and 1 of acute renal failure due to acute post-infectious glomerulonephritis). Cardiopulmonary resuscitation was used in 73 subjects. The mean postmortem interval was 17.02 h (SD 9.76, range 2–58 h).

On the basis of patients' medical records, cases were assigned to two diagnostic groups according to whether there was an antemortem diagnosis of diabetes mellitus. The groups were as follows: (A) 49 cases with previous diagnosis of diabetes mellitus (31 males and 18 females) with a mean age of 64.65 years (SD 13.72). The mean postmortem interval was 18.67 h (SD 9.73, range 3–58 h). In this group, 40 subjects had died as a result of cardiac disease, 7 from craniocerebral trauma, 1 from pulmonary embolism and 1 from hanging: (B) 43 cases (30 males and 13 females) with no known history of diabetes mellitus with a mean age of 54.69 (SD 20.37) and referred to as the control group. The mean postmortem interval was 15.13 h (SD 9.55, range 2–48 h). In group B there were 14 cardiac deaths, 20 of craniocerebral trauma, 1 pulmonary embolism, 1 acute renal failure and 7 hangings. Table 1 shows some of the variables studied in the two diagnostic groups.

The sclera was punctured by hypodermic syringe in the outer canthus with a fine-gauge needle and aspirate samples of vitreous humor were collected before autopsy. Samples were centrifuged at 1500 g for 15 min, stored at –70 °C and analysed in duplicate for fructosamine, glucose and microproteins. Glucose levels were tested in an autoanalyzer (Hitachi 747) using Boehringer Mannheim kits. Fructosamine was determined manually using the colorimetric determination method of QCA (Química Clínica Aplicada, SA). Finally, microprotein concentrations were determined in a Sinchron CX autoanalyzer using Beckman kits.

For statistical analyses of the data, the Mann-Whitney test, multivariate analysis and discriminant analysis were used.

Table 1 Description of the two diagnostic groups

	Diabetic	Non-diabetic
Number of cases	49	43
Mean age (years)	64.65	54.69
Postmortem interval	18.67	15.13
Sex		
males	31	30
females	18	13
Cause of death		
cardiac disease	40	14
craniocerebral trauma	7	20
pulmonary embolism	1	1
hanging	1	7
acute renal failure	–	1

Results

Table 2 shows the values (mean ± standard error of the mean, SD and range) obtained for glucose, fructosamine, microproteins and the fructosamine/microprotein ratio in the two diagnostic groups. The Mann-Whitney test was used to compare the mean values obtained. Statistically significant differences were found for glucose ($P = 0.000$) and fructosamine ($P = 0.000$) concentrations, and for the fructosamine/microprotein ratio ($P = 0.021$) between the two diagnostic groups (Table 3). The highest vitreous humor levels were obtained in the group of cases with a previous diagnosis of diabetes mellitus (Figs. 1 and 2). Table 4 shows the glucose, fructosamine and microprotein concentrations and the fructosamine/microprotein ratio in relation to the cause of death (natural or violent). The Mann-Whitney test was carried out to compare the mean values obtained and significant differences were found for glucose ($P = 0.002$) but not for fructosamine ($P = 0.343$)

Table 2 Mean (x), standard error of the mean (SE), standard deviation (SD), and range values for the biochemical parameters in the diagnostic groups (diabetic and non-diabetic)

	Diabetic ($n = 49$)				Non diabetic ($n = 43$)			
	mean	SE	SD	range	mean	SE	SD	range
Glucose (mg/dl)	148.0	19.3	135.3	2.0–674.0	24.8	2.7	17.9	0.0– 75.0
Fructosamine (mmol/l)	1.5	0.2	1.5	0.0– 5.9	0.5	0.1	0.7	0.0– 3.5
Microproteins (mg/dl)	130.2	20.5	143.8	24.0–530.0	64.9	7.6	50.1	13.0–270.0
Fructosamine/microprotein ratio	0.024	0.005	0.03	0.0– 0.20	0.012	0.002	0.017	0.0– 0.09

Table 3 Mann-Whitney test to compare mean values of biochemical markers for the two diagnostic groups (diabetics and non diabetics)

Variable	Mann- U Whitney	Wilcoxon W	Z	Probability
Glucose	326.0	1272.0	–5.694	0.000
Fructosamine	563.5	1509.5	–3.881	0.000
Microproteins	816.5	1762.0	–1.855	0.064
Fructosamine/microprotein ratio	762.0	1708.0	–2.307	0.021

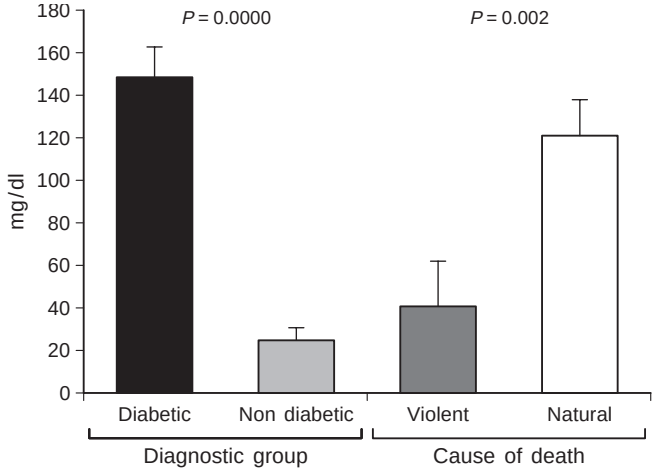

Fig. 1 Levels (mg/dl) of glucose (mean and standard error of the mean) in vitreous humor

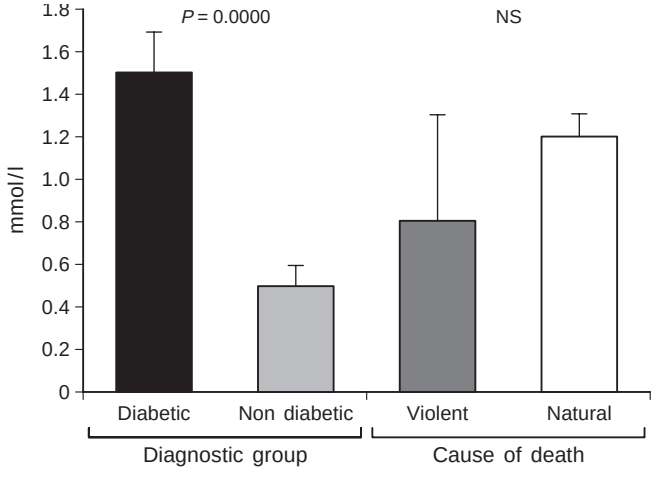

Fig. 2 Levels (mmol/l) of fructosamine (mean and standard error of the mean) in vitreous humor

or the fructosamine/microprotein ratio ($P = 0.925$) (Table 5). The highest vitreous humor levels of glucose were obtained in the group of subjects who died of natural causes (Fig. 1).

Table 6 shows the correlation matrix made for the biochemical markers and the variables postmortem interval, cardiopulmonary resuscitation, cause of death (violent or natural) and diagnostic group (diabetic or non-diabetic). No statistically significant correlations were obtained between the levels of the biochemical markers analysed in vitreous humor and postmortem interval or the use of cardiopulmonary resuscitation. However, we found statistically significant correlations between the diagnostic group and the levels of glucose ($P = 0.000$), fructosamine ($P = 0.000$) microproteins ($P = 0.005$) and fructosamine/microprotein ratio ($P = 0.041$). The correlation between the cause of death (natural or violent) and glucose levels ($P = 0.001$) was statistically significant. No significant correlations were found between the cause of death and fructosamine concentrations.

For discriminant analysis, fructosamine and glucose levels were used with the diagnostic category 'diabetic' and 'non-diabetic' as the grouping variable. Correct classification was found in 80.4% of the cases (93.0% in the group of subjects with no known history of diabetes and 69.4% in the diabetic group).

Discussion

Previous studies on vitreous humor have analysed postmortem glucose concentrations in subjects, however, there are no published studies concerning fructosamine levels in vitreous humor.

A high level of vitreous glucose can be taken as a reflection of high antemortem blood glucose levels. However the conditions frequently associated with marked terminal increases in blood glucose levels are not reflected

Table 4 Mean (x), standard error of the mean (SE), standard deviation (SD), and range values for the biochemical parameters in relation to the cause of death (violent or natural)

	Violent ($n = 35$)				Natural ($n = 57$)			
	mean	SE	SD	range	mean	SE	SD	range
Glucose (mg/dl)	40.4	25.0	42.4	0.0–185.0	121.2	18.0	136.1	0.0–674.0
Fructosamine (mmol/l)	0.8	0.5	1.1	0.0– 4.7	1.2	0.1	1.4	0.0– 5.9
Microproteins (mg/dl)	73.4	61.0	58.4	13.0–270.0	117.1	18.0	137.2	13.0–530.0
Fructosamine/microprotein ratio	0.017	0.004	0.025	0.0– 0.10	0.018	0.004	0.005	0.0– 0.20

Table 5 Mann-Whitney test to compare mean values of biochemical markers in relation to the cause of death (violent or natural)

Variable	Mann-Whitney U	Wilcoxon W	Z	Probability
Glucose	618.5	1248.5	–3.048	0.002
Fructosamine	881.0	1511.0	–0.948	0.343
Microproteins	896.5	1526.5	–0.812	0.417
Fructosamine/microprotein ratio	986.0	2639.0	–0.094	0.925

Table 6 Correlation matrix for the biochemical markers in vitreous humor and other variables

	Glucose	Fructosamine	Microproteins	Fructosamine/microprotein ratio
Postmortem interval	–0.010	0.153	–0.009	0.114
Cardiopulmonary resuscitation	–0.079	–0.034	–0.034	–0.027
Diagnostic group	–0.530**	–0.401**	–0.292**	–0.214*
Cause of death	–0.338**	–0.121	–0.186	–0.014

** $P < 0.01$
* $P < 0.05$

by similar increases in vitreous glucose (DiMaio et al. 1977). As the time between death and autopsy lengthens, the glucose level in the vitreous humor tends to fall, although this decrease will be gradual in the diabetic and high levels of glucose can still be shown for prolonged periods after death (Coe 1969, 1972, 1974, 1993; DiMaio et al. 1977).

In our study, the highest levels for glucose were obtained in diabetics. In routine postmortem analysis performed by Coe (1975) in 1000 consecutive natural deaths, peripheral glucose levels in excess of 500 mg/dl were found in 103 cases of non-diabetic individuals, while the vitreous humor level never exceeded 100 mg/dl. In our results, the group of non-diabetic subjects showed a mean glucose concentration of 24.83 mg/dl, the highest concentration in this group being 75 mg/dl.

Glucose levels substantially above 200 mg/dl should be considered as diagnostic of diabetes mellitus (DiMaio et al. 1977; Coe 1993). In our study the mean level of glucose in the group of diabetics was 148.08, with 11 cases showing values higher than 200 mg/dl (the highest being 674 mg/dl).

An assessment of fructosamine levels is a method for estimating glycated protein levels, which in turn provide an assessment of glycemic control during the preceding 1–3 weeks and which are therefore directly correlated with the antemortem glucose levels in serum (John et al. 1988; Valenzuela 1988). The Mann-Whitney test showed statistically significant differences for fructosamine in vitreous humor, the highest levels being seen in the diabetic group (mean 1.58). In our study, fructosamine levels were 3.09 times higher in diabetic than in non-diabetic subjects and the fructosamine/microprotein ratio was 2.16 times higher in diabetic than in non-diabetic subjects. Fructosamine concentrations in vitreous humor were significantly correlated with glucose levels and the diagnostic group. The absence of any statistical significant correlation between the postmortem interval and the biochemical markers analysed should be noted, since this implies no real interference from autolytic phenomena in the postmortem interval used in our study. Autolytic interference and, therefore, glycolysis were reduced to a minimum, a fact corroborated by the correlation analysis which found no statistically significant correlation with the postmortem interval. However, this absence of a statistically significant correlation does not necessarily mean that the glucose values did not change during the postmortem interval.

Certain causes of death are associated with postmortem increases in blood glucose levels. Such increases have been reported in asphyxial deaths, cerebral haemorrhage, congestive heart failure and electrocutions (Hill 1941; Coe 1976, 1977) and may be due to terminal stress associated with these conditions and the corresponding secretion of catecholamines. Such increases are not manifested in vitreous humor (DiMaio et al. 1977). Gormsen and Lund (1985) found that the only condition which undoubtedly induces high glucose levels in peripheral postmortem blood are attempts at resuscitation, including heart massage, although these authors felt that uncontrolled diabetes could still be diagnosed from properly performed blood glucose determinations on samples removed at autopsy. In our study, no correlation was found between biochemical concentrations in vitreous humor and the use of cardiopulmonary resuscitation. It must borne in mind that of the subjects who had received cardiopulmonary resuscitation, 40 had a known past history of diabetes mellitus and that 9 of the 19 subjects not receiving cardiopulmonary resuscitation had diabetes mellitus. This distribution of the different cases may explain why no statistically significant correlation was found between the use of cardiopulmonary resuscitation and the levels of the biochemical markers analysed.

However, we found statistically significant differences in the glucose levels in relation to the cause of death (natural or violent), the highest concentrations being observed in the group of subjects who died of natural causes. However, it must be noted that in this group 40 subjects had a case history of diabetes mellitus and had died of cardiac failure.

The results obtained in the discriminant analysis suggest that the combined determination of vitreous glucose and fructosamine concentrations are useful indicators for confirming the presence of antemortem hyperglycemia.

Combined elevated vitreous glucose and fructosamine values are indicative of diabetes.

Moreover the analysis of the biochemical markers assayed in this study is easy and cheap, for which reasons it can be recommended for use in the routine forensic diagnosis of diabetes mellitus after death.

References

Brinkmann B, Fechner G, Karger B, DuChesne A (1998) Ketoacidosis and lactic acidosis-frequent cause of death in chronic alcoholics. Int J Legal Med 111:115–119

Bunn HF, Gaffay KH, Gallop PM (1978) The glycosilation of hemoglobin: relevance of diabetes mellitus. Science 200:21–27

Coe JI (1969) Postmortem chemistries on human vitreous humor. Am J Clin Pathol 51:741–750

Coe JI (1972) Use of chemical determinations on vitreous humor in forensic pathology. J Forensic Sci 17:541–546

Coe JI (1973) Further thoughts and observations on postmortem chemistry. Forensic Sci Gaz 5:2–6

Coe JI (1974) Postmortem chemistries on blood: particular reference to urea nitrogen, electrolytes and bilirubin. J Forensic Sci 19:33–42

Coe JI (1975) Postmortem peripheral blood glucose and cardiopulmonary resuscitation. Forensic Sci Gaz 6:1–2

Coe JI (1976) Comparative postmortem chemistries of vitreous humor before and after embalming. J Forensic Sci 21:583–586

Coe JI (1977) Postmortem chemistry of blood, cerebrospinal fluid and vitreous humor. In: Tedeschi CG, Eckert WG, Tedeschi LG (eds) Forensic medicine, vol 2. Saunders, Philadelphia, pp 1030–1060

Coe JI (1993) Postmortem chemistry update. Emphasis on forensic application. Am J Forensic Med Pathol 14:91–117

DiMaio VJM, Sturner WQ, Coe JI (1977) Sudden and unexpected deaths after the acute onset of diabetes mellitus. J Forensic Sci 22:147–151

Forrest ARW (1993) Obtaining samples at postmortem examination for toxicological and biochemical analyses. J Clin Pathol 46:292–296

Gormsen H, Lund A (1985) The diagnostic value of postmortem blood glucose determinations in cases of diabetes mellitus. Forensic Sci Int 28:103–107

Hill E (1941) Significance of dextrose and nondextrose reducing substances in postmortem blood. Arch Pathol 32:452–473

John WG, Scott KWM, Hawkroft DM (1988) Glycated haemoglobin and glycated protein and glucose concentrations in necropsy blood samples. J Clin Pathol 41:415–418

Kennedy AL, Merimee TJ (1981) Glycosilated serum protein and hemoglobin A_1 levels to measure control of glycemia. Ann Intern Med 95:56–58

Kernbach G, Koops E, Brinkmann B (1984) Biochemische Parameter bei 31 tödlichen diabetischen Stoffwechselentgleisungen. Beitr Gerichtl Med 42:301–306

Knight B (ed) (1996) The pathophysiology of death. In: Forensic pathology. Arnold, London Sydney Auckland, pp 51–94

Lund A (1964) Adrenaline and nor-adrenaline in postmortem blood. Med Sci Law 4:194–197

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

Püschel K, Beckmann R, Kernbach G, Brinkmann B (1984) Differentialdiagnose und Beweis des Coma diabeticum an der Leiche. Beitr Gerichtl Med 42:315–318

Sippel H, Mottönen M (1982) Combined glucose and lactate values in vitreous humour for postmortem diagnosis of diabetes mellitus. Forensic Sci Int 19:217–222

Sturner WQ, Sullivan A, Suzuki K (1983) Lactic acid concentrations in vitreous humor: their use in asphyxial deaths in children. J Forensic Sci 28:222–230

Traub F (1969) Methode zur Erkennung von tödlichen Zuckerstoffwechselstörungen an der Leiche (Diabetes mellitus und Hypoglykämie). Zentrabl Allg Pathol 112:390–399

Valenzuela A (1988) Postmortem diagnosis of diabetes mellitus. Quantitation of fructosamine and glycated hemoglobin. Forensic Sci Int 38:203–208