Combined Determination of Glucose and Fructosamine in Vitreous Humor as a Post-Mortem Tool to Identify Antemortem Hyperglycemia

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■ Abstract

In clinical practice, serum glucose levels are used to diagnose diabetes mellitus. In post-mortem diagnosis, however, biochemical markers in vitreous humor are more useful because of the difficulty involved in interpreting blood glucose levels and relatively non-specific pathological features. The aim of this study was to analyze the usefulness of post-mortem determination of glucose and fructosamine combined and to compare the results with those obtained for fructosamine and combined glucose and lactate levels in two diagnostic groups (one diabetic and the other non-diabetic). We studied 377 cadavers (mean age 57.6 yr, SD 20.4, range 15 to 98 yr) with a mean post-mortem interval of 14.9 h. (SD 6.0; range 2 to 24 h). The highest levels were obtained in cases where diabetes mellitus had previously been diagnosed. In relation to diagnostic performance, the most reliable values were those in which glucose and fructosamine were determined jointly. The findings provide information concerning the usefulness of measuring glucose and fructosamine levels as a post-mortem tool for identifying antemortem glycemic control in diabetics.

Keywords: diabetes · glucose · fructosamine · diagnosis · post-mortem · vitreous humor

Introduction

Uncontrolled diabetes mellitus is the cause of a variety of complications, which may on occasion lead to death. The incidence of diabetes mellitus in western countries is estimated to be 2-6% of the population, up to 50% of which goes undetected, meaning that this disease is under-reported on death certificates, both as an illness and cause of death [1-4]. Measuring serum glucose levels is of no value as a method of post-mortem diagnosis because of the glycolysis that occurs after death. Some authors have proposed that the combined determination of glucose and lactic acid in vitreous humor would be a useful indicator to confirm ante-mortem hyperglycemia as a probable cause of death [5-8]. In previous studies, we demonstrated the diagnostic usefulness of determining fructosamine levels in vitreous humor [9, 10]. Because serum proteins have a much shorter half-life than erythrocyte hemoglobin, the fructosamine test, which measures glycated proteins, reflects glycemic control during the preceding 1-3 weeks [11]. Fructosamine and glycated proteins are therefore directly correlated with ante-mortem glucose levels in serum [12, 13]. We

therefore consider the joint determination of free glucose and the glucose that participates in glycosylation and produces fructosamine, to be of diagnostic help in the presence of high ante-mortem glucose levels.

The purpose of the present study was to analyze the usefulness of the post-mortem determination of glucose and fructosamine combined and to compare the results with those obtained for fructosamine and glucose and lactate levels combined in two diagnostic groups (one diabetic and the other non-diabetic).

Materials and methods

We studied 377 cadavers (253 males and 124 females) with a mean age of 57.6 years (SD 20.4; range 15 to 98 years). The criteria for including cases were post-mortem interval, cause of death and circumstances of death. We excluded cases where individuals were already found dead. We selected cases with a post-mortem interval of below 24 hours, and, to control for post-mortem artifacts, the bodies were refrigerated. The average interval between death and refrigeration was 190 minutes and the mean post-mortem interval was 14.9 h. (SD 6.0; range 2 to 24 hours). The study was approved by the Ethics Committee of the Institute of Forensic Medicine and the Ethics Committee of the University of Murcia.

The criterion for selecting the group of diabetic subjects was a history of diabetes, as recorded by the general practitioner or contained in the patient's medical records. The groups were as follows: A. 96 cases with a previous diagnosis of diabetes mellitus, of which 55 were type l (insulin-dependent) and B. 281 cases without known history of diabetes mellitus which were used as control group to contrast the results obtained. Since the sample studied originated from forensic practice and most of the deaths had occurred outside hospital, the ante-mortem glucose levels shortly before death were not available. A puncture was made in each cadaver through the sclera in the outer canthus using a hypodermic syringe with a fine-gauge needle and 5 ml of vitreous humor from both eyes were collected before autopsy. The two samples were pooled and centrifuged at 1500 g for 15 min, stored at -80°C, analyzed in duplicate for glucose, lactate and fructosamine and tested in a HITACHI 917 autoanalyzer using Roche Diagnostic kits (Hoffmann La Roche, Ltd., Basel, Switzerland). The characteristics of the analytical methods have been previously described [9-13]. The detection limits for the different markers were as follows: 2 mg/dl (0.11 mmol/l) for glucose, 10 μmol/l for fructosamine and 2 mg/dl (0.22 mmol/l) for lactate. The validity and reliability of the different markers used in the post-mortem diagnosis have been demonstrated previously by our research group [9, 10].

For statistical analyses, the SPSS 15.0 Program was used. Descriptive, multivariate and discriminant analyses were performed. The Kruskal-Wallis test and Mann-Whitney U-test were used to compare groups. Also, for each of the variables, studied, a ROC (receiver operating characteristic) curve was drawn and the area under the curve was measured using a nonparametric method. Finally, to assess the diagnostic performance, we analyzed sensitivity and specificity, positive and negative predictive values, positive and negative likelihood ratio and Cohen's kappa concordance index.

Results

Lactate was the only marker among those studied to show a statistically significant correlation with the post-mortem interval (Pearson 0.220; $p < 0.001$). Table 1 shows the descriptive statistics obtained for the biochemical parameters in the two diagnostic groups. The levels of biochemical markers differed significantly ($p < 0.001$), while the highest vitreous humor levels were obtained in the group of diabetic subjects. We found statistically significant correlations between the diabetic group and levels of glucose ($p < 0.001$), fructosamine ($p < 0.001$), glucose+lactate ($p < 0.001$) and glucose+fructosamine ($p < 0.001$).

Table 1. Mean, standard deviation, median and range values for biochemical parameters in the diagnostic groups (diabetic and non-diabetic)

Parameter	Diabetic (n = 96)				Non-diabetic (n = 281)				p
	Mean	SD	Median	Range	Mean	SD	Median	Range	
Post-mortem interval (h)	15.4	5.7	15.5	3.0 - 24.0	14.7	6.1	14.0	2.0 - 24.0	
Glucose (mmol/l)	5.2	6.5	3.3	0.05 - 37.1	1.1	1.1	0.8	0.0 - 6.0	< 0.001
Fructosamine (mmol/l)	0.8	1.2	0.3	0.0 - 5.9	0.2	0.2	0.1	0.0 - 1.3	< 0.001
Lactate (mmol/l)	16.7	4.0	17.3	6.0 - 23.0	13.3	4.2	13.2	1.2 - 23.4	< 0.001
Glucose+lactate (mmol/l)	22.0	8.2	21.8	6.0 - 54.0	14.5	4.4	13.9	1.7 - 28.2	< 0.001
Glucose+fructosamine (mmol/l)	6.1	7.1	4.3	0.08 - 42.2	1.3	1.1	0.9	0.05 - 6.1	< 0.001

In the ROC analysis, all areas under the curve were significantly different from 0.5. The area corresponding to the summed values for glucose and lactate was 0.802, while that for the summed values for glucose and fructosamine was 0.783 (Figure 1).

In the discriminant analysis, biochemical marker levels were used with the diagnostic category 'diabetic' and 'non-diabetic' as the grouping variables. Correct classification was found in 80.9% of the cases (86.5% in the group of subjects with no known history of diabetes and 64.6% in the diabetic group). Table 2 summarizes the diagnostic performance for each of the biochemical markers studied.

posed to add the values for glucose and lactate when investigating deaths from diabetes [5, 6]. However, lactate levels are subject to interference produced by post-mortem autolysis, as confirmed in this study, since the levels of this marker are correlated with the post-mortem interval.

Glycated proteins, such as fructosamine in serum and vitreous humor, have been found to be very useful in diagnosing diabetes mellitus [9, 10, 12-15]. Because glycoprotein concentrations reflect mean serum glucose levels over a period of time, their determination provides a useful means of monitoring diabetic control [14, 16]. It may be worth determining glucose and fructosamine jointly since the concentration representing the glycosylation of proteins, as is the case for fructosamine, can be added to that for free glucose. Taking fructosamine into account in the determination of glucose helps dispel any doubts that may arise in the interpretation of results when glucose is determined on its own as result of post-mortem glycolysis.

The availability of ante-mortem glucose levels may be of diagnostic help and may also assist with a comparative study of post-mortem levels found in vitreous humor. However, the greatest difficulty in finding out the ante-mortem glucose levels in this study was the source of the cases used, as already mentioned.

The reliability of a diagnostic test is expressed in terms of sensitivity, specificity and positive and negative predictive values. In our case, the most reliable results were those in which glucose and fructosamine were determined jointly. The use of these statistics enabled us to confirm the statistical power of a test and to reduce the interferences introduced by the prevalence of an illness
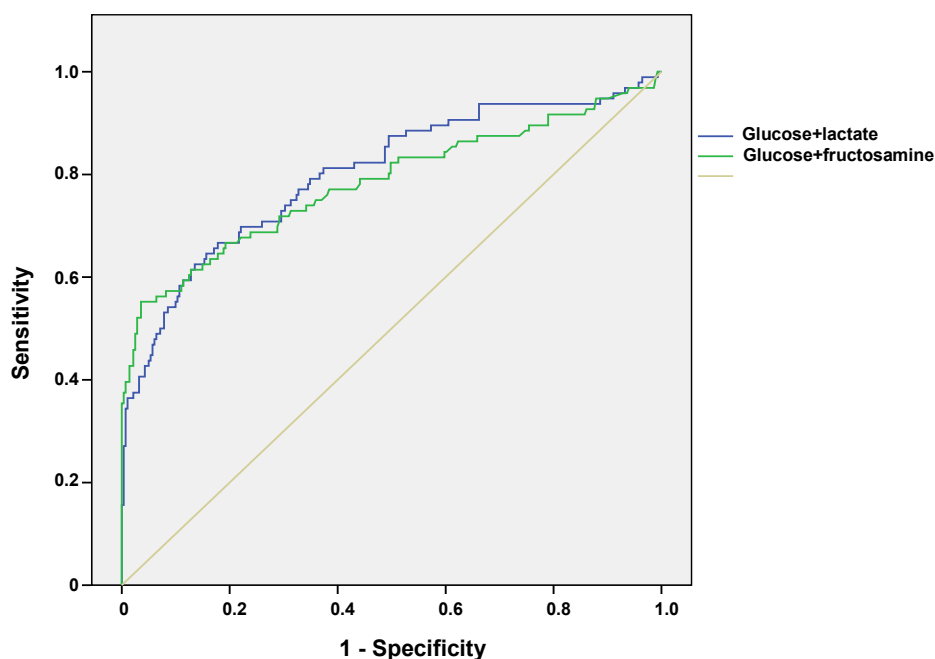

Figure 1. Receiver operating characteristic (ROC) curves for the summed values of glucose and lactate and for the summed values of glucose and fructosamine. The figure shows plots of the ROC curves of the true positive rate (sensitivity) against the false positive rate (1-specificity) for every possible cut-off value, and areas under the curves for the summed values of glucose and lactate and for the summed values of glucose and fructosamine. Accuracy is measured by the area under the ROC curves. The area corresponding to the summed values for glucose and lactate is 0.802, and for the summed values for glucose and fructosamine is 0.783.

Discussion

In the post-mortem diagnosis of ante-mortem hyperglycemia, glycolysis makes interpretation of serum glucose levels difficult. In the fluids of the cadaver, glucose is converted into lactate during the post-mortem period. Therefore, some authors have pro-

Table 2. Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratio, global value, kappa concordance positive and negative likelihood ratio, cut-off points values and areas below the ROC curves obtained for each of the biochemical marker

Statistics	Glucose	Lactate	Fructosa-mine	Glucose + lactate	Glucose + fructosa-mine
Sensitivity	58.3	76.0	60.4	62.5	55.2
Specificity	87.9	59.1	83.3	86.5	96.4
Positive predictive value	62.2	38.8	55.2	61.2	84.1
Negative predictive value	86.1	87.8	87.0	87.1	86.3
Global value	82.2	65.8	79.8	76.7	84.6
Positive likelihood ratio	4.8	1.8	3.6	4.6	15.2
Negative likelihood ratio	0.4	0.4	0.4	0.4	0.4
Cut-off point values	2.3	13.7	0.3	19.4	3.9
Area below the ROC curves	0.747	0.723	0.734	0.802	0.783

in a given population. For this purpose we used the Cohen's kappa concordance index, which provides the degree of coincidence in the classification of individuals. In the case of perfect concordance, the kappa value would be 1. If the agreement observed is as expected (i.e. fair), the value would be 0, and if the agreement observed was less than expected, the value would be less than 0. Landis and Koch [17] proposed limits to evaluate the degree of agreement as a function of the kappa index. In our case, the Cohen's kappa value was 0.9, so the degree of agreement was very high. As a general rule, tests with a (+) likelihood ratio greater than 10 and a (-) likelihood ratio less than 0.1 are useful for diagnostic purposes. In our study, the combined determination of glucose and fructosamine provided the highest (+) likelihood ratio of 15.2.

When the cut-off points were analyzed in the ROC curves, we found that for the joint determination of glucose and fructosamine the cut-off point was 5.9, with a sensitivity of 73.9 and specificity of 78.4. In contrast, for the combined determination of glucose and lactate the cut-off point was 168.8, with a sensitivity of 55.2 and specificity of 96.4. At first, bearing in mind the results obtained for the ROC curve, it might seem that the combined determination of glucose and lactate would be the most useful diagnostic marker. However, it is important to remember that lactate levels may increase as a consequence of states of glycolysis resulting from other metabolic processes or from intense suffering [18-20]. Indeed, in our study, lactate is the only marker to show a statistically significant correlation with the post-mortem interval. In this sense, it may be advisable to determine other free

markers of these interferences at short post-mortem intervals and to do so in fluids, including vitreous humor, which are more resistant to post-mortem autolytic processes [19, 21]. It should also be borne in mind that, unlike the extraction of other fluids, vitreous humor is easily extracted at the site of death, without having to wait for the autopsy.

In our study, significant differences were found for the combined concentrations of glucose and fructosamine between the diagnostic groups. In fact, they were five times higher in the group of diabetic subjects than in the control group. An additional reason for choosing the combined determination of glucose and fructosamine was to ascertain the likelihood ratio of the same prevalence. In this respect, the joint determination of glucose+fructosamine obtained the highest positive likelihood ratio (15.2) of all the markers analyzed. These results were corroborated in the discriminant analysis, where the percentage of correct classification was 80.9%, and as high as 86.5% in the control group. That is, only 38 of the 281 cases with no known history of diabetes were wrongly classified.

As we have pointed out, a high percentage of individuals with diabetes are undiagnosed. Although it might initially be thought that this would have some bearing on the interpretation of the results of the study, it should be remembered that in our study we included a group that we considered the problem group, since it consisted of subjects in whom the existence of diabetes mellitus was confirmed by medical records. No person for whom there was any doubt concerning the existence or not of diabetes was included in this group. We must also remember that some subjects included in the "non-diabetic" control group may have suffered from diabetes mellitus, since in forensic practice this information is frequently missing. This may be the reason for finding wrongly classified cases in this group, as we discovered from the discriminant analysis carried out.

In our opinion, these results are valuable and confirm the usefulness of combined determination of glucose and fructosamine in vitreous humor. The significance of the latter is due to its longer half-life and greater post-mortem stability.

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