

## Evaluation of glycosylated hemoglobin and glycosylated albumin levels in forensic autopsies

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Received: 11.01.2009/ Accepted: 11.03.2010

**Abstract:** There may be difficulties in determining causes of sudden, unexpected deaths in medico-legal practice. It is known that sudden, unexpected deaths may result from diabetes mellitus and its complications. However, there is an ongoing debate on whether many parameters recommended to diagnose post-mortem diabetes mellitus are reliable. The aim of this study was to determine whether glycosylated haemoglobin (HbA<sub>1c</sub>) and glycosylated albumin (fructosamine) measures could be used to diagnose post-mortem diabetes mellitus in forensic autopsies and to contribute to limited data on the issue reported in the literature. Method: We obtained blood and vitreous humor specimens from 146 decedents aged over 18 years and exposed to forensic autopsies. Results: The mean age of the cases was  $46 \pm 16.39$  years (min 19yrs, max 85yrs) and 87.7% of the cases were male. HbA<sub>1c</sub> and fructosamine levels were higher than the standard values in 51 and 9 cases respectively. Of six cases with a history of diabetes mellitus, one had high fructosamine levels and five had high HbA<sub>1c</sub> levels. Fructosamine and HbA<sub>1c</sub> levels were high in three and 20 deaths from natural causes respectively and 12 of these deaths were caused by cardiovascular disorders. There was no significant relation between causes of deaths and HbA<sub>1c</sub> and fructosamine levels. Conclusion: The results of this study will contribute to the limited data on diabetes mellitus in sudden, unexpected deaths. It can be suggested that measurements of HbA<sub>1c</sub> and fructosamine levels should be included in routine autopsy protocols.

**Key words:** forensic autopsy, HbA<sub>1c</sub>, fructosamine

Sudden, unexpected deaths account for a large proportion of medico-legal deaths. It may be difficult to determine the mechanism of, causes of death, factors likely to play a role in death and to obtain evidence likely to shed light on how death has occurred in such cases [1, 2].

It is known that the most frequent natural cause of sudden, unexpected deaths is cardiovascular diseases and frequently appear in the middle aged and the elderly [3-5]. According to a report issued by Medical examiners commission in 2002, 32% of adult deaths were sudden, unexpected and sudden deaths from natural causes could be associated with diabetes mellitus (DM) [2].

It is known that microvascular, macrovascular and neuropathic complications of DM may play a role in sudden, unexpected deaths. It has been reported that cases of DM and glucose intolerance were at a high risk of cardiovascular and cerebrovascular mortality [6-8]. The prevalence of DM has been found to be 7,2% in Turkey [9, 10]. It has been reported that the prevalence of DM is 6,6% in the United States and that 190,000 people die of DM related conditions every year [11].

Many investigations have been described for post-mortem diagnosis of DM [12-16]. DM related morphological changes in the internal organs may not be detected at medicolegal autopsies. It

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has been reported that post-mortem biochemical analyses should be performed to determine DM reveal considerable changes in blood glucose levels and that there are difficulties in determining post-mortem blood glucose levels due to initiation of glycolysis. In addition, post-mortem glycolysis, agonal period, medical interventions such as cardiopulmonary resuscitation, intravenous administration of glucose and parenteral nutrition, conditions likely to affect pancreas cells such as acute pancreatitis and head traumas and use of post-mortem glucose by bacteria cause difficulties in evaluation of glucose levels in post-mortem blood specimens [12-14].

Apart from detection of post-mortem blood glucose levels, other biochemical measures such as blood pH, bicarbonate, lactate and potassium are recommended for the post-mortem diagnosis of DM [13]. Considering that glucose turns into lactate, glucose and lactate levels in post-mortem CSF and vitreous specimens can be used in combination to investigate DM associated deaths and metabolic disorders in alcoholics [14, 15]. However, prolonged agonal period, sudden deaths and acute infections affect the reliability of post-mortem extracellular lactate levels [14, 15].

It is known that detection of glucose, lactate, HbA<sub>1c</sub>, ketone compounds, insulin and other anti-diabetics and C-peptides in post-mortem vitreous, blood, CSF and urine specimens can be used to diagnose DM, terminal hyperglycaemia, hypoglycaemic coma and hunger-induced ketoacidosis [13, 14]. Acetone levels in post-mortem CSF and vitreous specimens can be high in cases of isopropyl alcohol intake, hypothermia, advanced liver disease and starvation. It has been suggested in many studies that lactate, fructosamine and microalbumine levels should be detected based on the fact that glucose metabolism occurs in vitreous fluid and CSF [13-16].

HbA<sub>1c</sub> and fructosamine are considered reliable and valuable parameters in the follow-up of antemortem blood sugar levels in clinical practice. There have been a limited number of studies on the use of HbA<sub>1c</sub> for the diagnosis of DM and it has been recommended in these studies that HbA<sub>1c</sub> levels can be determined for post-mortem evaluation of DM since HbA<sub>1c</sub> is stable, is not affected by post-mortem haemolysis and reflects glycaemia levels within post-mortem 6-8 weeks [13-19]. Based on the fact that acetone levels are increased in hunger-induced ketoacidosis, intoxications due to acetone-isopropanol, diabetic ketoacidosis and alcoholic ketoacidosis, it has been suggested that HbA<sub>1c</sub> can be useful in differentiation of alcoholic ketoacidosis and hunger-induced ketoacidosis from DM ketoacidosis in deaths, the cause of which is unknown [13].

The aim of this study was to determine whether post-mortem HbA<sub>1c</sub> and fructosamine levels could be used to diagnose DM in cases of medicolegal cases and to contribute to the limited data on the issue.

### **Material and methods**

This is a multi-centre and multidisciplinary study and was performed in Forensic Medicine Department of Dokuz Eylül University, Department of Biochemistry of Dokuz Eylül University, Endocrinology Department of İzmir Tepecik Education Hospital and Directorate of İzmir Forensic Medicine Institute, affiliating with the Ministry of Justice. Approval was obtained from the ethical committee.

#### **Selection of Cases**

The decedents aged over 18 years and autopsied in the Department of Forensic Medicine between May 2005 and February 2006 were included in the study irrespective of causes and manners of death. The corpses found to have any degrees of putrefaction (changes in skin colour, bullae, putrefactive venous network, epidermolysis) at the time of macroscopic examination were not included into the study.

Data about identity, history, information from judicial inquiry, findings from macroscopic examination and autopsy, results of toxicological analyses were recorded in the forms recommended in the international autopsy protocols. When history was missing in death investigation records, it was obtained from decedents' relatives.

#### **Collection and storage of specimens**

Blood and vitreous humor specimens were obtained from 146 decedents autopsied in the Morgue Department of the Directorate of İzmir Forensic Medicine Institute.

Blood specimens were drawn through 10cm-syringes from the intact and unputrefied vena cava inferior near the right atrium into sealed plastic tubes containing EDTA before the organs were removed.

The obtained specimens were kept at -60° C. Vitreous humor specimens were taken appropriately with sterile syringes (2 cc) and transferred into vacuumed plastic tubes. After the tubes were centrifuged at 2000g for 10min, obtained supernatant was transferred into Eppendorf tubes [17, 19].

Blood specimens could be obtained from 138 decedents and vitreous humor specimens from 114 decedents. Vitreous humor specimens from 32 decedents were traumatic (found to contain blood) and Blood specimens from 8 decedents were haemolysed. These specimens were not found appropriate for analysis and were excluded from the study.

### Biochemical Analysis

All specimens were analyzed in the central laboratory of Medicine Faculty of Dokuz Eylül University. The stored specimens were left to dissolve at room temperatures and homogenized and biochemical analyses of all the specimens were performed at a time.

For HbA<sub>1c</sub> measurements, blood specimens were added Hemolysis Reagent (Roche Diagnostics Inc, Cat. no: 20755699) as much as one second of the specimens and haemolysate was obtained. The haemolysate was analysed with immunoturbidimetric method and a commercial kit matching with haemolysate Integra 800 analyzer (Roche Diagnostics Inc.).

For fructosamine measurements, vitreous humor specimens were analyzed with a method based on nitro-blue tetrasolium reduction and a commercial kit matching with vitreous humor Modular Analytics P Module analyzer (Roche Diagnostics Inc.).

The specimens obtained for toxicological analyses were exposed to ethyl alcohol screening test and the obtained results were expressed in “+” or “-”.

Based on the reference values written in the manuals of the kits and in the literature, HbA<sub>1c</sub> and fructosamine levels were classified into low, normal and high values [16, 19].

### Statistical Analysis

Data were recorded in the SPSS for Windows 11.0 and Mann-Whitney U test was used to determine the relation between HbA<sub>1c</sub> and fructosamine levels and such variables as age, gender, manner of death and results of ethyl alcohol analyses.

## Results

The mean age of 146 medico-legal cases included in the study was  $46 \pm 16.39$  year (min 19, max 85), 87.7% of the cases were male and 12.3% were female. Figure 1 shows the distribution of male and female decedents by age groups. The

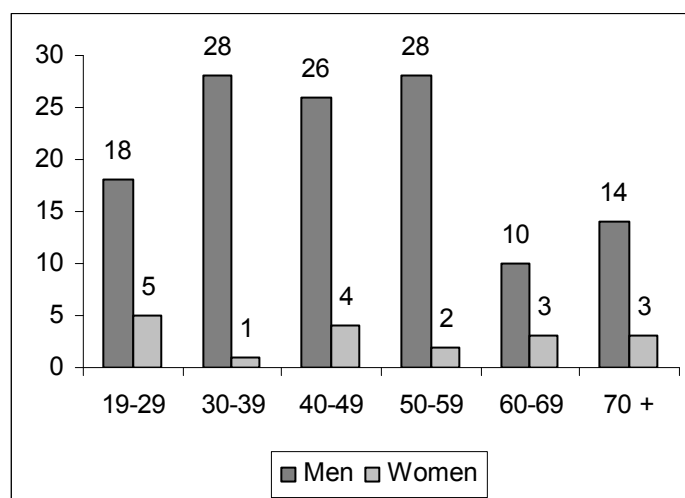


Fig. 1 Distribution of Male and Female Decedents by Age Groups

Table 1 Manner of Death

Manner of Death	Frequency (n)	Percent (%)
Homicide	38	26.0
Suicide	24	16.4
Accident	35	24
Natural	49	33.6
<b>Total</b>	<b>146</b>	<b>100.0</b>

Table 2 Causes of Death

Causes of death	Frequency (n)
<b>Traumatic deaths</b>	<b>97</b>
Gunshot	23
Blunt Force Injuries	23
Intoxication	17
Stab wounds	15
Mechanic asphyxia (hanging)	12
Drowning	4
Burns	2
Others	1
<b>Natural deaths</b>	<b>49</b>
Cardiovascular system	24
Respiratory system	17
Gastrointestinal system	3
Central nervous system	2
Others	1
Undetermined	2
<b>Total</b>	<b>146</b>

Table 3 Distribution of HbA<sub>1c</sub> and Fructosamine Levels\*

	HAB1c		Fructosamine	
	Frequency	Percent (%)	Frequency	Percent (%)
Normal	84	57.5	38	26
Low	3	2.1	67	45.9
High	51	34.9	9	6.2
Undetermined	8	5.5	32	21.9
<b>Total</b>	<b>146</b>	<b>100</b>	<b>146</b>	<b>100</b>

\* HbA<sub>1c</sub> and fructosamine levels were classified according to the definition in the commercial kit used for biochemical examinations.

distribution of decedents by manner of death is presented in Table 1. The most frequent cause of death was gunshot wounds (15.7%). The distribution of decedents by causes of death is shown in Table 2.

The minimum and maximum HbA<sub>1c</sub> levels were 3.64% and 9.66%

respectively (SD: 0.83) and minimum and maximum fructosamine levels were 7.00  $\mu\text{mol/L}$  and 445.00  $\mu\text{mol/L}$  respectively (SD: 81.57). Table 3 shows HbA<sub>1c</sub> and fructosamine levels.

Six decedents (4.1%) were found to have DM based on the information obtained from their relatives. Out of six decedents with a history of DM, one had high fructosamine levels, five had high HbA<sub>1c</sub> levels and two were found to die of natural causes.

The distribution of HbA<sub>1c</sub> and fructosamine levels by causes of death is shown in Table 4.

Out of 49 deaths from natural causes, three decedents had high fructosamine levels, 20 had high HbA<sub>1c</sub> levels and 12 were found to die from cardiovascular conditions. There was no significant relation between causes of death and HbA<sub>1c</sub> and fructosamine levels. Half of the deaths from traumas had high HbA<sub>1c</sub> levels.

There was no significant difference in HbA<sub>1c</sub> and fructosamine levels between deaths from violent acts and behaviour and deaths from natural causes (for HbA<sub>1c</sub>  $p = 0,441$ ; for fructosamine  $p = 0,784$ ).

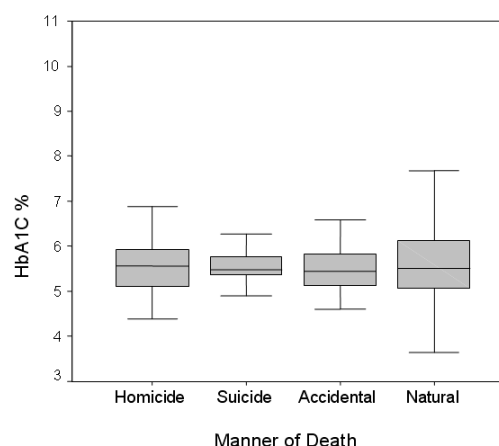
There was no significant difference in HbA<sub>1c</sub> and fructosamine levels between genders (Mann-Whitney U test for HbA<sub>1c</sub>  $p = 0,610$ ; for fructosamine  $p = 0,701$ ). The distributions of HbA<sub>1c</sub> and fructosamine levels by manner of death are shown in Figures 2 and 3.

There was no significant difference between the distributions of HbA<sub>1c</sub> and fructosamine levels by manner of death. The mean Post-mortem interval was  $0.97 \pm 0.361$  day ranging between 6 hours and 3 days. Post-mortem interval shorter or longer than 12 hours did not significantly affect HbA<sub>1c</sub> and fructosamine levels (Mann-Whitney U test for HbA<sub>1c</sub>  $p = 0,841$ ; for fructosamine  $p = 0,254$ ).

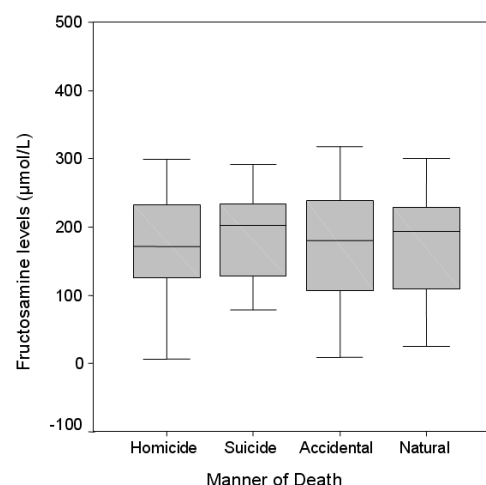
Out of 26 decedents, 22 had ethyl alcohol and 4 did not have ethyl alcohol. Presence or absence of ethyl alcohol did not significantly affect HbA<sub>1c</sub> and fructosamine levels (Mann-Whitney U test for HbA<sub>1c</sub>  $p = 0,80$ ; for fructosamine  $p = 0,519$ ).

**Table 4** The Relation between HbA<sub>1c</sub> and Fructosamine Levels and Causes of Death

Cause of death	HbA <sub>1c</sub> levels			Fructosamine levels		
	Normal	Low	High	Normal	Low	High
<b>Natural deaths (N=49)</b>	<b>24</b>	<b>1</b>	<b>20</b>	<b>16</b>	<b>24</b>	<b>3</b>
Cardiovascular system	10	-	12	7	13	2
Respiratory system	8	-	7	8	7	-
Gastrointestinal system	2	1	-	1	1	-
Central nervous system	1	-	1	-	1	1
Others	1	-	-	-	1	-
Undetermined	2	-	-	-	1	-
<b>Traumatic deaths (N=97)</b>	<b>60</b>	<b>2</b>	<b>31</b>	<b>22</b>	<b>43</b>	<b>6</b>
Gunshot	14	2	7	3	14	1
Blunt Force Injuries	12	-	9	8	13	-
Intoxication	13	-	4	3	7	-
Stab wounds	9	-	5	4	4	3
Mechanic asphyxia (hanging)	9	-	3	4	2	1
Drowning	1	-	2	-	2	-
Burns	1	-	1	-	1	-
Others	1	-	-	-	-	1
<b>Total (N=146)</b>	<b>84</b>	<b>3</b>	<b>51</b>	<b>38</b>	<b>67</b>	<b>9</b>



**Fig. 2** The distribution of HbA<sub>1c</sub> levels by manner of death



**Fig. 3** The distribution of fructosamine levels by manner of death

## Discussion

It has been reported that 44-45% of sudden, unexpected deaths exposed to medico-legal investigations are due to natural causes [2, 20]. However, difficulties in determining causes of sudden, unexpected deaths may arise.

Causes of death in order of frequency in Turkey were cardiac conditions, accidents and diseases other than cardiac conditions and cancers as in the world [21]. Studies on deaths exposed to medicolegal investigations have revealed that the rate of deaths from natural causes range from one thirds to one sevenths of autopsy series [4, 22]. DM causes sudden, unexpected deaths since it is not treated and followed adequately, the disease is difficult to diagnose in the post-mortem period and the rate of undiagnosed DM cases are high. Therefore, it is of great importance to distinguish DM from other causes and manners of sudden, unexpected deaths [23, 24].

It has been reported that DM and glucose intolerance increase the risk of mortality from cardiovascular diseases and in turn lead to a higher risk of sudden, unexpected death [6-8]. There have been few studies reported in the literature on sudden, unexpected deaths from DM and to our knowledge there have not been any studies on the issue in Turkey. We think that DM, a frequent systemic disease which may cause sudden, unexpected deaths, should be included in autopsy protocols and that this will contribute to both post-mortem studies and clinical data. Comparative studies on decedents known to have DM versus controls have demonstrated that as HbA<sub>1c</sub> levels increase so does the frequency of DM in advanced age groups [14, 15, 24].

In the present study, there was no significant relation between HbA<sub>1c</sub> and fructosamine levels and age and gender. This might have been due to lack of a control group, small sample size and problems with taking history of DM.

In this study, HbA<sub>1c</sub> and fructosamine levels were not affected by post-mortem interval. This supports the idea that HbA<sub>1c</sub> and fructosamine are stabile parameters, least affected by post-mortem biochemical changes [14, 17, 19]. It is known that where specimens are obtained is just as important as the parameters tested. Further studies including decedents with longer post-mortem intervals and using specimens from various parts of the body are needed to confirm the findings concerning the relation between HbA<sub>1c</sub> and fructosamine levels and post-mortem interval [15, 17, 19].

Osuna et al. showed that post-mortem glucose levels were affected by many factors including causes and manners of death [19]. Antemortem medical interventions and cardiopulmonary resuscitation are two other factors which affect post-mortem glucose and other biochemical parameters. However, we found no significant difference in HbA<sub>1c</sub> and fructosamine levels between deaths from trauma and deaths from natural causes, consistent with several other studies in the literature [14, 24, 25].

It has been reported that ethyl alcohol has no impact on HbA<sub>1c</sub> and fructosamine levels in the acute stage. Comparable with the literature, this study showed no significant difference in HbA<sub>1c</sub> and fructosamine levels between decedents found to have alcohol and those found not to have alcohol [15, 17].

Winecker et al. reported that HbA<sub>1c</sub> and fructosamine levels were reliable indicators of post-mortem DM since these parameters showed glicemia which appeared a few weeks or a few months before death and did not fluctuate greatly compared to glucose [24]. Likewise, this study revealed that HbA<sub>1c</sub> and fructosamine could be used for the post-mortem diagnosis of diabetes mellitus. We think that the results of this study will make a valuable contribution to the limited relevant data in the literature.

## Recommendations

We believe that HbA<sub>1c</sub> and fructosamine levels in post-mortem blood and vitreous specimens should be determined when medico-legal aspects of deaths especially sudden, unexpected deaths are investigated.

We can also suggest that HbA<sub>1c</sub> and fructosamine measures should be incorporated into autopsy protocols in that they are considered more reliable than post-mortem glucose levels and lactate levels and provide guidance in the diagnosis of chronic DM.

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