Original Article

Comparison of Post-Mortem Vitreous Fluid and Blood Glucose Levels in Diabetes-Induced Rabbit Models

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ABSTRACT

Background: Diabetes is a common systemic disease in the world. Acute complications of diabetes may cause sudden unexpected deaths. Analysis done in vitreous fluid which is more protected and less contaminated by bacteria comparing to blood will produce more accurate results. **Aim:** Thus, we aimed to diagnose diabetes by comparing glucose levels of post mortem blood and vitreous fluid in death cases. **Materials and Methods:** A total of 17 New Zealand-type rabbits were divided into hyperglycemia (8), hypoglycemia (8), and control group (1). Rabbits were monitored for 5 days after experimental diabetes induction, and samples were taken at the point of death. Later rabbits were left in their environment, and samples were taken again at the post mortem first day. Mean blood glucose levels of hyperglycemia and hypoglycemia group were in diabetic range. **Results:** Blood glucose levels of hyperglycemic rabbits were measured as 512 ± 52,1 mg/dl, while vitreous glucose levels were 518,3 ± 76,8 mg/dl at the point of death. After one day, levels were measured as 433,9 ± 59,3 mg/dl and 329,8 ± 86,6 mg/dl. Blood glucose levels of hypoglycemic rabbits were measured as 39 ± 3,8 mg/dl, while vitreous glucose levels were 53,4 ± 13,9 mg/dl at the point of death. After one day, levels were measured as 36 ± 4,2 mg/dl and 1,6 ± 0,6 mg/dl. After analysis, there was a statistically significant difference between day 0 and 1 vitreous levels of hypoglycemia group. **Conclusion:** It can be clearly seen that vitreous fluid samples should be taken in judicial cases with sudden unexpected deaths like diabetes. This will contribute to identification cause of death.

KEYWORDS: *Alloxan, diabetes mellitus, post-mortem glucose, rabbit*

INTRODUCTION

Deaths in Forensic Science practices are split into two groups: natural and unnatural deaths. Sudden unexpected deaths are an important part of natural deaths.[1] Sudden deaths according to the definition made by the World Health Organization (WHO) are deaths that occur within 24 hours of the appearance of symptoms. Acute-serious complications of a systemic disease are one of the causes of sudden unexpected deaths.

Diabetes is known as a chronic metabolic disease that requires constant medical care because of the insulin deficiency or defects in the effect of insulin that the organism cannot adequately benefit from carbohydrates, fats, and proteins. It is stated that diabetes has acute and chronic complications, and these complications may cause mortality. Diabetic ketoacidosis and hyperosmolar, hyperglycemic status, hypoglycemia, and lactic acidosis, which occur as a result of decreasing or increasing insulin level, are among the acute complications of diabetes mellitus, and if these complications are not treated, they are reported to may cause deaths.[2]

In our country, according to the 2007 data of Turkey Statistical Institute (TSI), it is reported that 7.2% of the population has diabetes mellitus. It is reported that 32% of diabetics living in our country are not aware of their disease.[3] For this reason, especially in sudden death

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cases receiving a forensic case label, it is necessary to eliminate deaths due to diabetes mellitus or to diagnose those resulting from diabetes mellitus and its complications.[4]

Toxic chemicals are used in experimental animals to create diabetes. The most popular of these are alloxan and streptozotocin.[5-7] Alloxan is a uric acid derivative in the structure of alloxan monohydrate, and it easily dissolves in water and its solution should be stored below +4 degrees.[8-10] Alloxan selectively damages pancreatic beta cells and creates insulin-dependent diabetes.

This paper aims to compare the time-dependent levels of glucose concentrations in post-mortem blood and vitreous fluid in the diabetic rabbit models and to show the ratio of glucose level in vitreous fluid to blood glucose level at the time of death.

Methods

The ethics committee permission required for our study was obtained from the Ethics Committee of the Medical Sciences Experimental Research and Application Center of Çukurova University with 28 decision number and dated 28.04.2014.

In this study, 17 New Zealand-type rabbits with an average weight of 2.2–2.7 kg were used as experimental animals. These rabbits were divided into three groups as eight of them are hyperglycemia group, eight of them are hypoglycemia group, and one control group. The solutions that containing appropriate dose of active substance (alloxan) to create experimental diabetes determined by literature review were prepared. In the first and second groups, pancreatic injury was created with 100 mg/kg of alloxan. Rabbits were made to bleed from the ear vein for 5 days in the morning and afternoon, and glucose was monitored with a glucometer.

During the monitoring, the diets of the rabbits were arranged according to the blood glucose level. During blood glucose monitoring, in order not to enter the hyperosmolar coma of rabbits with high blood glucose; 1 IU/kg crystalline insulin was administered to those whose blood glucose was above 500 mg/dl, and 2 IU/kg crystalline regular insulin was administered to those with blood glucose above 600 mg/dl. Dextrose solutions were prepared according to blood glucose level and placed in their food.

At the end of the 5th day, 1 gr/kg ketamine substance was administered to the experimental animals, and they were decapitated. Blood and vitreous fluid samples were taken immediately after decapitation. Rabbits were left dead in their habitat for 1 day and on the post-mortem first day; blood and other eye vitreous fluid samples were taken and then placed in the refrigerator at -20 ^{0}C.

After the blood samples of hyperglycemia and hypoglycemia were completed, blood and vitreous fluid glucose measurements were made using the glucoxidase kit in the Çukurova University Faculty of Medicine Biochemistry Department.

Results

In our study, 100 mg/kg alloxan was given to rabbits to create experimental diabetes. In accordance with the literature, diabetes occurred after alloxan administration in all rabbits.

The blood glucose level averages of hyperglycemic rabbits were at diabetic level, and there was no obvious difference between days [Table 1].

Eight rabbits were injected 50 units of subcutaneous regular insulin from the abdomen. After insulin injection, hypoglycemia levels were evaluated by looking at the blood glucose levels of rabbits. Blood glucose levels of hypoglycemic rabbits were respectively determined as follows: 49, 46, 17, 26, 45, 46, 40, and 37 mg/dl.

Post-mortem blood and vitreous glucose levels of hyperglycemic rabbits were evaluated with the Glucoxidase kit [Table 2].

There was no significant difference between the blood and vitreous glucose levels of hyperglycemic rabbits

Table 1: Daily blood glucose level monitoring of hyperglycemic rabbits (mg/dl)

Rabbit no	Monday 15:30	Tuesday 09:00	Tuesday 15:30	Wednesday 09:00	Wednesday 15:30	Thursday 09:00	Thursday 15:30	Friday 09:00
1	412	75	137	329	533	469	510	549
2	69	113	209	334	412	421	540	433
3	314	74	305	449	541	553	159	High*
4	345	267	311	138	468	439	497	450
5	426	69	236	387	484	High	349	High
6	428	176	480	447	482	320	394	339
7	410	203	501	533	580	534	523	578
8	286	37	323	447	459	556	565	596

Note: * Glucometer device shows>600 mg/dl blood glucose level as high in the measurement

Table 2: Blood and vitreous glucose levels of the post-mortem hyperglycemic group

Rabbit no	Day 0 blood glucose level	Day 0 vitreous glucose level	Day 1 blood glucose level	Day 1 vitreous glucose level
1	312	576	600	471
2	329	171	179	5
3	650	670	416	191
4	620	629	680	537
5	685	650	622	45
6	339	244	348	17
7	578	367	378	174
8	583	839	260	621

Table 3: Post-mortem blood and vitreous glucose levels of hyperglycemic rabbits

	Day 0-blood	Day 0 vitreous	Day 1 blood	Day 1 vitreous
Mean	512,0	518,3	433,9	329,8
Standard deviation	147,2	217,2	167,8	244,9
Standard error	52,1	76,8	59,3	86,6
95% confidence interval	410-614	367,7-668,8	317,6-550,1	160-499,5

on post-mortem day 0 (P = 0.95). In addition, there was no statistically significant difference between blood and vitreous fluid values on post-mortem day 1 (P = 0.37) [Table 3].

Post-mortem blood and glucose levels of hypoglycemic rabbits were evaluated with the GOD kit [Table 4].

There was no significant difference between the blood and vitreous glucose levels of hypoglycemic rabbits on post-mortem day 0 (P = 0.37) [Table 5]. In addition, there was a statistically significant difference between blood and vitreous fluid values on post-mortem day 1 (P < 0,0001).

Conclusion

It is estimated that there are over 7 million diabetic patients in our country. Only 55% of all diabetic patients were diagnosed. Patients who cannot be diagnosed or diagnosed but whose treatment cannot be regulated may die due to acute and late complications of diabetes mellitus. Death cases, which are not diagnosed to be diabetic patients and occur due to acute complications of this disease, appear as sudden-suspicious deaths, and the cause of death is revealed by an autopsy.

In the studies carried out, it is stated that post-mortem vitreous fluid is more protective than other body fluids; therefore, it is less susceptible to bacterial contamination and shows little variability even in the awaited corpses.[11] In our study, blood and vitreous fluid glucose levels were compared in post-mortem day 0 and post-mortem day 1 in diabetic rabbits. It has been shown that the level of vitreous fluid glycemic helps to diagnose and shows less variation in post-mortem compared to blood.

In Vivero *et al*.'s study, post-mortem vitreous glucose levels were determined as 100.3 ± 116 mg/dl in the diabetic group (n = 111) and 21.7 ± 27.5 mg/dl in the non-diabetic group (n = 342). In our study, vitreous glucose level was found to be 518.3 ± 76.8 mg/dl and post-mortem 1st day vitreous glucose levels as 329.8 ± 86.6 mg/dl in the hyperglycemia group. The mean values in our study were higher than Vivero *et al*.'s study.[12] This was thought to be due to the fact that the experimental animals in our study were followed with higher blood sugar levels.

In a 500-case study performed by Palmiere *et al*., blood and vitreous fluid samples were collected in the post-mortem 3–51 hour intervals and diabetic ketoacidosis was attempted to be diagnosed. Sixteen of 500 cases were diagnosed with diabetic ketoacidosis, 13 cases were previously diagnosed with diabetes, and 3 cases were not diagnosed with diabetes. Diabetic ketoacidosis was diagnosed in patients with post-mortem vitreous glucose concentration above 10 mmol/L (104 mg/dl) and beta hydroxybutyrate level above 26 mg/dl. In a 3076-case study performed by Zilg *et al*., it is stated that the vitreous glucose rates taken in the early post-mortem remained stable and similar results were obtained in the second samples collected 1–3 days after autopsy. In this study, it is stated that the glucose concentration above 10 mmol/L has a good specificity in the diagnosis of diabetic coma. In Gürler *et al*.'s study, it is stated that the glucose value observed in the vitreous fluid post-mortem in the interval of 3–72 hours is over 13 mmol/L, indicating antemortem hyperglycemia. In Ali *et al*.'s study, it is stated that the post-mortem vitreous glucose concentration above 200 mg/dl indicates antemortem hyperglycemia. In the literature, it is seen that above 104 mg–200 mg/dl values show antermortem hyperglycemia.[11,13-15] In our study, it was found that the blood glucose level observed in the hyperglycemic group at the time of death was above 200 mg/dl in 7 of 8 cases, and the glucose level in the

Table 4: Blood and vitreous glucose levels of the post-mortem hypoglycemic group

Rabbit no	Day 0 blood glucose level	Day 0 vitreous glucose level	Day 1 blood glucose level	Day 1 vitreous glucose level
1	49	39	34	3
2	46	17	22	4
3	17	35	21	3
4	26	115	37	0
5	45	125	62	0
6	46	22	35	3
7	40	36	42	0
8	43	38	35	0

Table 5: Post-mortem blood and vitreous glucose levels of hypoglycemic rabbits

	Day 0 blood	Day 0 vitreous	Day 1 blood	Day 1 vitreous
Mean	39,0	53,4	36,0	1,6
Standard deviation	10,6	39,2	11,9	1,7
Standard error	3,8	13,9	4,2	0,6
95% confidence interval	31,6-46,4	26,2-80,6	27,7-44,3	0,5-2,8

vitreous fluid on post-mortem 1st day was above 200 mg/dl in 4 of 8 rabbits. However, in our study, it is seen that the glucose change observed in the hyperglycemic group is not statistically significant ($P = 0.14$).

In the study of Schöning and Strafuss, it is stated that vitreous glucose levels are not reliable in the diagnosis of post-mortem hypoglycemia and it reflects hyperglycemic conditions more accurately.[16] In our study, it was observed that the blood glucose values observed on the post-mortem day 0 and post-mortem day 1 were not statistically significant ($P < 0.62$) in the hypoglycemia group rabbits. In contrast, in the hypoglycemic group, there was a significant difference in post-mortem vitreous fluid glucose vary compared to hyperglycemia group ($P < 0.0036$).

In the study of Tumram *et al.*,[17] the relationship between the time of death and electrolyte and glucose concentrations, in vitreous fluid and synovial fluid, was investigated, and it was stated that glucose values decreased in vitreous fluid and synovial fluid. In our study, although no evaluation was made with the time of death, it was found that the glucose level in the vitreous fluid decreased depending on the time elapsed.

In the 453 case study of Vivero *et al.*,[12] it was stated that B-hydroxybutyrate values were higher in diabetic cases ($P < 000.1$), and it was a supportive marker in addition to glucose in diagnosing diabetic ketosidosis. Similarly, in our study, vitreous fluid samples were taken due to its sheltered structure and glucose level was examined here. However, unlike this study, beta hydroxybutyrate levels were not examined. As it is significant in diagnosing diabetic ketoacidosis, it will be useful to examine beta hydroxybutyrate levels in new studies.

In our study, post-mortem vitreous fluid and blood glucose values were examined, and it was found that post-mortem glucose measurement would help to detect undiagnosed diabetes cases, and it would be helpful to solve forensic events.

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Conflicts of interest

There are no conflicts of interest

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