Chapter 7

Insulin in Forensic Medicine and Toxicology

Rafał Skowronek

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76691

Abstract

Today insulin is used not only in medicine in the treatment of diabetes but also in sport as a doping agent and for criminal purposes. Suicides and homicides with insulin maybe are not so common, but are seen in the routine medicolegal and toxicological-clinical practice. Despite the often quite clear circumstances of death and a well-established mechanism of action of insulin and its analogs, it is difficult to analytically confirm its excessive exogenous administration in postmortem biological material. There are no uniform international standards of conduct in such cases, both at the stage of the material sampling during autopsy and forensic laboratory analysis and the final interpretation of the obtained results. The aim of the study is to present the current state of basic knowledge about nonmedical use of insulin, with particular emphasis on the possibility of postmortem diagnosis. The study also highlighted the little known clinical problem of insulin abuse for recreational purposes.

Keywords: overdose, homicide-suicide, postmortem diagnostics, thanatobiochemistry, medicolegal autopsy, forensic histopathology

1. Introduction

Insulin is a potent anabolic polypeptide hormone which stimulates the uptake and storage of carbohydrates, fatty acids, and amino acids into glycogen, fat, and protein, respectively [1]. The typical physiological effect of its action is hypoglycemia (reduction in blood glucose). The result of a casual or intentional overdose of insulin is a hypoglycemic coma and in extreme cases even death of the user. Today it is used not only in medicine in the treatment of diabetic patients but also in competitive sport as a common doping agent for body building [2, 3] and for different criminal purposes [4–8]. Suicides and homicides with insulin maybe are not so common, but are seen in the routine medicolegal and toxicological-clinical practice. The first documented case of murder by insulin is dated to the year 1957 (Kenneth Barlow case) [9]. Vincent Marks in his review has analyzed case histories of 66 people alleged or proven to have been poisoned by insulin (murders, manslaughters, attempted murders, Munchausen-by-proxy cases) [7].

Of course, some insulin overdoses are accidental and associated with incorrect dosage of the drug by the patient [10, 11]. Most of these cases are not clinically serious. It seems, however, that the risk of intentional (suicidal) insulin overdose in patients with diabetes of both types (1 and 2) is underestimated. The population-based study of suicide victims in Northern Finland performed by Löfman et al. revealed that 3.1% of all suicide victims had diabetes (34.6% type 1 and 65.4% type 2) [12]. In victims with type 1 diabetes, insulin as a suicide method covered half of the self-poisoning cases, while the proportion in type 2 diabetes was 13%. It is known that the risk of depression and attempted suicide is higher in patients with chronic diseases, including diabetes, so physicians who treat diabetic patients should evaluate co-occurring depression and substance abuse, both of which are major risk factors of suicide [13, 14].

The aim of the author is to present the current state of basic knowledge about the nonmedical use of insulin, with particular emphasis on the possibility of postmortem diagnosis. The study also highlighted the little known, rare clinical problem of insulin abuse for recreational purposes.

2. Case reports

In order to illustrate the abovementioned problems, I present two typical cases from routine medicolegal practice of the Department of Forensic Medicine and Forensic Toxicology in Katowice, School of Medicine in Katowice, Medical University of Silesia, Poland [15, 16].

2.1. Suicide

A 44-year-old nondiabetic man was found dead lying on the bed in his flat. Near the body, an ampoule and almost empty syringe were found and taken for further analysis. Two days earlier, the man had called his wife and said that he is going to commit suicide. The forensic autopsy did not reveal the cause of death. The initial stage of putrefaction, blood fluidity, acute blood stagnation (hyperemia) in the internal organs, and two supravital point wounds on the right thigh, which might have been injection sites, were found. Histopathological findings in the main internal organs were the following: brain, hyperemia with numerous petechiae, and edema; heart, adipositas, medium grade of atherosclerosis of the coronary arteries, and local fragmentation of muscle fibers; and lungs, hyperemia with local hemorrhages into alveoli and edema. Additional histochemical staining (Periodic Acid-Schiff, PAS) disclosed low amounts of glycogen in the liver (**Figure 1**). The standard toxicological analysis disclosed no evidence of drug abuse or alcohol, so due to the suspicion of suicide by insulin injection, a directed analysis with immunoradiometric assay (IRMA Kit Immunotech), routinely used for the in vitro determination of insulin in human serum and plasma, was conducted. It revealed a high insulin concentration level—24.42 μIU/ml in the vitreous humor (measuring range, 0.5–300 μIU/ml; the norm for serum, <22 μIU/ml)—and the presence of insulin in the material

http://dx.doi.org/10.5772/intechopen.76691

secured at the crime scene (in the syringe, 1853.91 μIU/ml). All these results (information from the prosecutor about the crime scene, results of medicolegal autopsy, results of histopathological and toxicological studies) clinched the thesis of insulin overdose.

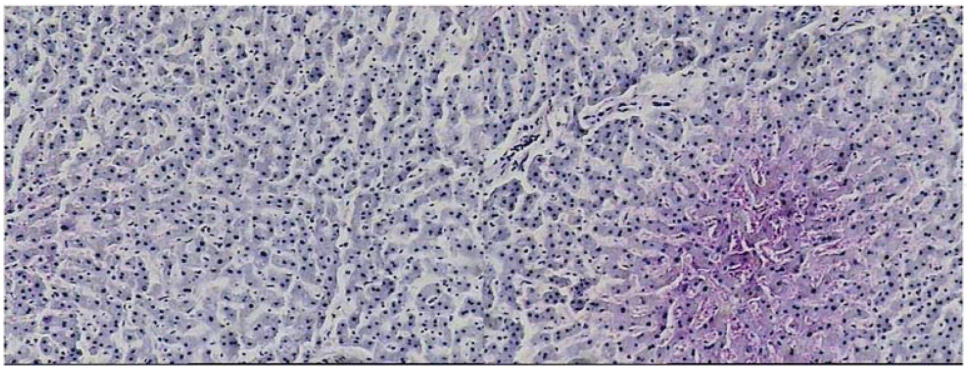

Figure 1. Low amounts of glycogen in the liver—50% of control sections taken during autopsy of sudden traumatic death victims.

Figure 2. Empty packages after insulin, NovoMix 30 Penfill (a mixture of fast and long-acting insulin analogue), revealed in the apartment of victim. Needles can be analyzed by forensic geneticists for the presence of DNA mixture of the victim and murderer in the case of homicide-suicide death.

2.2. Homicide-suicide

According to the information of the Prosecutor's Office, a 63-year-old man was supposed to kill his wife and dog and then commit suicide. Such situation in forensic medicine is called homicide-suicide or dyadic death. In the apartment a farewell letter and empty packages after insulin, NovoMix 30 Penfill (a mixture of fast and long-acting insulin analogue), were revealed (**Figure 2**). External medicolegal examination and forensic autopsies carried out at the Department of Forensic Medicine and Forensic Toxicology of the Medical University of Silesia in Katowice did not explain the cause of death. However, potential injection sites on the thighs and the shoulder of woman were revealed (**Figure 3**). Different biological materials for additional tests—biochemical, chemical-toxicological, and histopathological and for forensic genetics—were taken. Due to the inability to quickly determine insulin level in body fluids and the site of injection using the reference chromatographic methods [17–20], the determination of this hormone was ordered to two clinical diagnostic laboratories (by chemiluminometric and immunoradiometric methods). In addition, C-peptide (short 31-amino-acid

Figure 3. Numerous supravital point wounds and surrounding bruises on the thighs—potential insulin injection sites.

http://dx.doi.org/10.5772/intechopen.76691

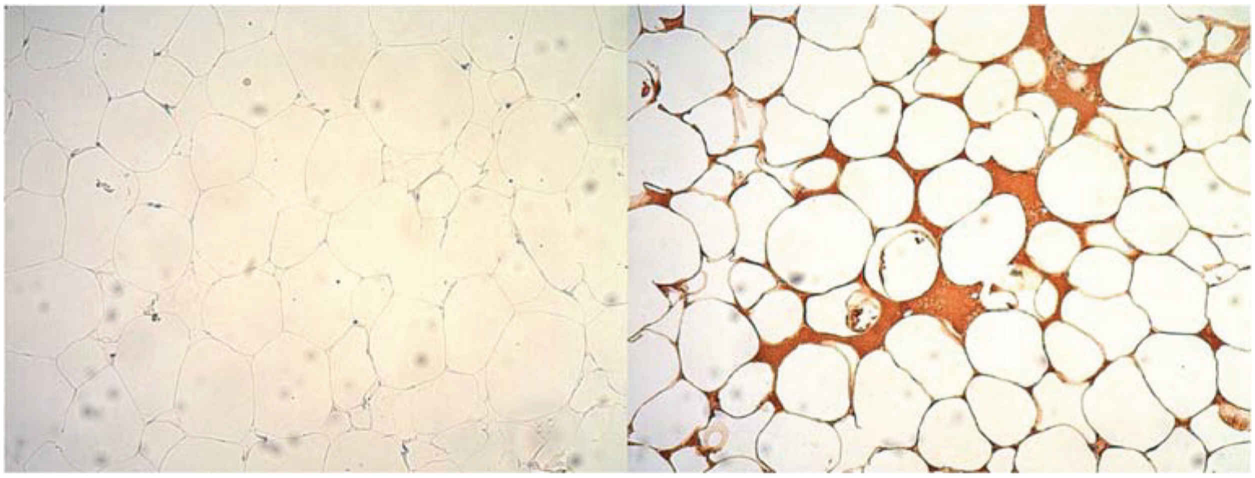

Figure 4. Positive immunohistochemical (IHC) detection of insulin in the subcutaneous tissue around needle tracts between adipocytes (right) and control section from distant area of the skin with no reaction (left).

polypeptide that connects insulin's A-chain to its B-chain in the endogenous proinsulin molecule), glycated hemoglobin (HbA1c, a form of hemoglobin that is measured primarily to identify the 2- to 3-month average plasma glucose concentration), glucose, and lactate (it is known that one mole of glucose during the process of glycolysis produces two moles of lactate) were ordered. Incomplete, difficult-to-interpret results were obtained. In addition, a successful attempt of immunohistochemical (IHC) detection of insulin in samples taken from the injection sites was made (**Figure 4**) [21, 22]. The results of the tests carried out in the abovementioned clinical laboratories confirmed our previous experience with the low usefulness of insulin determinations in the autopsy hemolyzed blood specimens (article in press).

3. Postmortem diagnostics of fatal insulin poisoning

3.1. Medicolegal autopsy

A classic postmortem macroscopic examination of the corpses (forensic autopsy) usually does not explain the cause and mechanism of death [23, 24]. Typically a feature of acute cardiorespiratory failure and nonspecific lesions related to the age of victim (e.g., atherosclerotic changes in vessels) can be found. For this reason, additional laboratory tests are necessary in each case. In addition to routinely collected sections from internal organs and body fluids (blood and urine), it is worth to take at least the sample of vitreous humor (VH) and the samples from potential injection sites for both histopathological and directed toxicological analyses.

3.2. Forensic histopathology and immunohistochemistry

A detailed histological examination of all internal organs, especially of the pancreas and liver, aiming at detection of insulinoma (tumor of the pancreas that is derived from β cells and secretes insulin) and morphological symptoms of hypoglycemia, respectively, should be always performed by experienced pathologist. The content of glycogen (multibranched polysaccharide of glucose that serves as a form of main energy storage) in the liver may be evaluated by the Periodic Acid-Schiff (PAS) or Best's Carmine staining. Its low amounts can indirectly confirm insulin overdose, as it was presented in the first case [15]. Another useful option is to perform IHC staining for the presence of insulin at the injection site. It is not necessary to buy special antibodies. These routinely used in clinical histopathology can be successfully used for this purpose, as we demonstrated in the second case [16].

3.3. Forensic toxicology and thanatobiochemistry (postmortem biochemistry)

In routine forensic practice, usually antemortem blood samples of the victim, who sometimes is hospitalized before the death, are unavailable for forensic toxicologists, so they can analyze only postmortem biological material taken during autopsy and nonbiological specimens revealed at the crime scene, like syringes, ampoules, vials, or remnants of the infusion solution and tubings [24–26].

What is important from the medicolegal point of view is that the interpretation of insulin levels in the postmortem biological material is difficult and still in doubt [27–31]. The number of published papers dealing with this problem is relatively low. The time of survival after insulin injection depends on many different factors: type of insulin (differentiated onset of action and insulin half-life), method of administration (injection or insulin infusion pump), anatomical localization of injection sites on the body (different rate of absorption), etc. [32]. It certainly influences the insulin levels detected in the postmortem biological material. Unfortunately, in the forensic practice, investigators usually do not know that time, because the cadavers not infrequently are found after a long time since death at an advanced stage of late postmortem changes, for example, when the victim lived alone or the killer committed suicide [21].

Additionally, insulin determination in postmortem blood has a low diagnostic and testimonial value, mainly because of ongoing thanatochemical processes of autolysis and putrefaction [8, 24]. The main barrier that prevents receiving correct and trustworthy results of insulin determinations in postmortem blood with radioimmunological methods is the blood hemolysis (rupturing of red blood cells and the release of their contents into surrounding plasma). This fact was confirmed in the literature and by our own studies performed in the Department of Forensic Medicine and Forensic Toxicology in Katowice [32, 33].

Fortunately, insulin crosses the blood-retinal barrier and may be identified in the VH, which is generally very valuable alternative material for many different chemical-toxicological analyses [34, 35]. The advantage of this material is that it is easy to obtain during typical forensic autopsy. Another advantage is anatomical isolation, useful especially in the case of advanced autolytic and putrefactive changes *in corpore*. It has also a very low cell count, so there is a small postmortem metabolism of glucose and other substances by surviving cells.

In 2011 Thevis et al. have published the first successful mass spectrometry-based analysis of postmortem material (VH) related to an insulin poisoning case [17]. The natural levels of insulin in vitreous humor determined by the authors were below the liquid chromatography–tandem mass spectrometry (LC-MS/MS) limit of detection. LC-MS/MS is modern advanced instrumental method widely used in analytical chemistry. This was a significant advance in postmortem biochemistry. Our own experience shows that in cases where suicide by insulin poisoning is suspected, determination of its concentration in the vitreous humor and nonbiological material using the immunoradiometric assay (IRMA) gives the opportunity, similarly as the LC-MS/MS analysis, of objective confirmation of the poisoning, so both methods can be used in forensic practice [32].

In our department, we have analyzed material consisted of 93 samples of vitreous humor taken during forensic autopsies. Analysis revealed that in 86 vitreous humor samples (92.5%), the concentration of insulin, determined with IRMA, was below the limit of detection of this method (below 0.5 μIU/ml). The concentration of insulin in vitreous humor was determined only in seven cases (range of results, 1.42–24.42 μIU/ml). We have described above one of these cases, where insulin was used to commit suicide [15].

The IRMA method is known as sensitive, specific, and relatively cheap in comparison to modern methods, but it requires adequate apparatus for the measurement of radioactivity and some experience in its interpretation. It is worth knowing that the studies on insulin determination using antibody-radiolabeled antigen reaction in the late 1950s were the beginning of a new medical discipline—radioimmunology [36]. Until the introduction of radioimmunoassay (RIA), death caused by insulin overdose was extremely difficult to prove [4]. In turn, the huge advantage of modern chromatographic methods (LC-MS/MS) is the possibility of differentiation between different types of insulins (human or animal insulin and their synthetic derivatives/analogues). In 2015, Palmiere et al. have presented preliminary results of postmortem determination of insulin using chemiluminescence enzyme immunoassay (CLEIA). Their conclusion was that the analysis of vitreous humor with CLEIA may provide suitable data, similar to analysis with LC-MS/MS and immunoradiometric assay, to support the hypothesis of insulin overdose [37].

Regardless of the method used in toxicological investigation, an analytically confirmed higher level of insulin in the vitreous humor plays an important and even a decisive role in structuring the final medicolegal opinion about the cause of death. This is the reason why the vitreous humor should be routinely collected and analyzed during forensic autopsy in every case with an “insulin” background [29, 37].

3.4. Forensic molecular biology

An interesting observation, so far unused in the forensic practice, is an increase in the expression of certain genes stimulated by insulin, especially in hyperinsulinemic conditions. This is a potentially promising area for further research. An example might be the changes of neuropeptide Y (NPY) gene expression and its release during hypoglycemic stress. Han et al. found that subcutaneous insulin injection produced an immediate increase in plasma NPY immunoreactivity and delayed increases in adrenal and neuronal NPY mRNA and adrenal NPY immunoreactivity in rats [38]. They have concluded that these results suggest that NPY may play a role in insulin-induced hypertension. Another example can be increased vascular resistance in the equine digit and overexpression of endothelin-1 (ET-1) in the laminar tissue due to the short-term hyperinsulinemia [39].

4. Clinical toxicology of insulin poisoning

An overdose of insulin is a potential life-threatening condition and requires urgent medical attention [40–46]. The clinical manifestations of hypoglycemia occur when the blood glucose level is less than 2.2–2.8 mmol/l (40–50 mg%). Symptomatology includes two groups of symptoms. The first one is caused by stimulation of the autonomic nervous system and includes profuse sweating, anxiety, tremor, and hunger. The second one is caused by progressive dysfunction of the central nervous system (CNS) due to neuroglycopenia and includes nausea, headache, dizziness, blurred vision, abnormal intellectual processes, behavioral disturbances, and finally loss of consciousness, convulsions, and death.

The most optimal place of the treatment is clinical toxicology ward, but patients who are overdosed with insulin can be also treated in typical intensive care units or in less serious cases—in general internal wards. To differentiate endogenous and exogenous insulin overdose, usually insulin/C-peptide [mol/mol] ratio is used, both in clinical and forensic settings [47]. Physiologically for every molecule of insulin formed, a corresponding molecule of C-peptide is formed. If the above-described ratio is >1, it indicates exogenous origin of insulin (as a result of accident, suicide, or homicide). However, it should be remembered that C-peptide is very unstable in postmortem blood [4].

Treatment of hypoglycemia is initially based on the securing of basic vital functions (breathing and circulation). Subsequently, infusions of glucose solution adjusted to the current blood glucose levels are used. Depending on the clinical situation, other drugs are administered s.c. or i.v. (e.g., glucagon which is a glycogenolysis stimulator), as it was presented in above-cited clinical emergency case reports. In the past such specific methods of treatment and management have been reported as excision of insulin injection site or the use of artificial pancreas [48–50]. Assessment of patient prognosis relies on clinical findings. According to the results of prospective study of Mégarbane et al., the observed plasma insulin EC50 (the concentration which induces a response halfway between the baseline and maximum after a specified exposure time) is 46 mIU/l [51].

Tsujimoto et al. have described rare case of rapid onset reversible glycogen storage hepatomegaly caused by suicidal administration of a massive dose of long-acting insulin glargine and subsequent supplementation with large doses of glucose in a 41-year-old type-2 diabetic patient [52]. Supravital liver biopsy revealed hepatocytic glycogen deposition with edematous degeneration. PAS staining revealed many PAS-positive granules containing glycogen. The hepatic computed tomography (CT) attenuation was 83.7 Hounsfield units (HU), being markedly higher than the splenic attenuation (49.5 HU), which indicated pathology of the liver. Such situation (initially higher level of hepatocytic glycogen deposition) must be considered not only by clinicians but also by forensic histopathologist during examination of the insulin fatal poisoning victim's liver.

5. Insulin abuse

An interesting, still not fully explained phenomenon, connecting toxicology, diabetology, and psychiatry, is abuse of insulin as a psychoactive substance. Intentional abuse of insulin is quite rare, but not exceptional. Pudlo et al. described an insulin abuser—the 58-year-old patient who

http://dx.doi.org/10.5772/intechopen.76691

injected himself with overdoses of insulin or consumed considerable amounts of pure sugar to increase its dose [53]. No other reason for his insulin abuse was found than pleasure seeking. According to the patient, he felt "pleasure" after insulin. Sheehy has counted 55 cases of patients developing hypoglycemic episodes by intentional insulin injecting [54]. Sometimes people suffering from Munchausen syndrome can also apply themselves an excessive dose of the drug to cause factitious hypoglycemia and get to the hospital [7, 55]. In the case of Munchausen syndrome by proxy (per procuram), the victims may be the relatives, most often children [56].

6. Conclusions

Despite the often quite clear circumstances of death and a well-established mechanism of action of insulin and its analogues, it is difficult to analytically confirm its excessive exogenous administration in postmortem biological material [8]. There are no uniform standards of conduct in this type of cases, both at the stage of the material sampling and laboratory analysis and in the interpretation of the obtained results.

If insulin overdose is suspected, it is necessary to take the different biological material during autopsy for further testing and to cautiously interpret its results [7]. It seems necessary to immediately develop a unified international standards/algorithm of conduct, similar to those used in clinical medicine, including the determination of insulin level and other parameters of carbohydrate metabolism in the postmortem biological material, taking into account all above-described possibilities and limitations of laboratory analysis [57–59].

Acknowledgements

The author would like to thank Marcin Tomsia, MSc, PhD, for his review of the manuscript; Joanna Nowicka, MSc, PhD; and Ewa Czech, MSc, PhD, for their valuable comments during the preparation of previous articles and presentations and Czesław Chowaniec, MD, PhD, and Christian Jabłoński, MD, PhD, for creating optimal conditions for scientific work.

Conflict of interest

The author has no conflict of interest to declare.

Author details

Rafał Skowronek

Address all correspondence to: rafal-skowronek@wp.pl

Department of Forensic Medicine and Forensic Toxicology, School of Medicine in Katowice, Medical University of Silesia in Katowice, Katowice, Poland

References

[1] Thevis M, Thomas A, Schänzer W. Insulin. In: Thieme D, Hemmersbach P, editors. Doping in Sports, Handbook of Experimental Pharmacology 195. Springer-Verlag: Berlin Heidelberg; 2010. pp. 209-226. DOI: 10.1007/978-3-540-79088-4_10

[2] Holt RI, Sönksen PH. Growth hormone, IGF-I and insulin and their abuse in sport. British Journal of Pharmacology. 2008;**154**(3):542-556. DOI: 10.1038/bjp.2008.99

[3] Thomas A, Brinkkötter P, Schänzer W, Thevis M. Metabolism of human insulin after subcutaneous administration: A possible means to uncover insulin misuse. Analytica Chimica Acta. 2015;**897**:53-61. DOI: 10.1016/j.aca.2015.09.036

[4] DiMaio D, DiMaio VJM. Forensic Pathology. 2nd ed. CRC Press; 2001. 592 p

[5] Marks V. Hypoglycaemia: Accidents, violence and murder. Part 1. Practical Diabetes International. 2005;**22**(8):303-306. DOI: 10.1002/pdi.854

[6] Marks V. Hypoglycaemia: Accidents, violence and murder. Part 2. Practical Diabetes International. 2005;**22**(9):352-358-54. DOI: 10.1002/pdi.875

[7] Marks V. Murder by insulin: Suspected, purported and proven—A review. Drug Testing and Analysis. 2009;**1**(4):162-176. DOI: 10.1002/dta.38

[8] Marks V, Wark G. Forensic aspects of insulin. Diabetes Research and Clinical Practice. 2013;**101**(3):248-254. DOI: 10.1016/j.diabres.2013.05.002

[9] Marks V, Richmond C. Kenneth Barlow: The first documented case of murder by insulin. Journal of the Royal Society of Medicine. 2008;**101**(1):19-21. DOI: 10.1258/jrsm.2007.071002

[10] Batalis NI, Prahlow JA. Accidental insulin overdose. Journal of Forensic Sciences. 2004;**49**(5):1117-1120

[11] von Mach MA, Meyer S, Omogbehin B, Kann PH, Weilemann LS. Epidemiological assessment of 160 cases of insulin overdose recorded in a regional poisons unit. International Journal of Clinical Pharmacology and Therapeutics. 2004;**42**(5):277-280

[12] Löfman S, Hakko H, Mainio A, Timonen M, Räsänen P. Characteristics of suicide among diabetes patients: A population based study of suicide victims in Northern Finland. Journal of Psychosomatic Research. 2012;**73**(4):268-271. DOI: 10.1016/j.jpsychores.2012.08.002

[13] Sarkar S, Balhara YS. Diabetes mellitus and suicide. Indian Journal of Endocrinology and Metabolism. 2014;**18**(4):468-474. DOI: 10.4103/2230-8210.137487

[14] Russell KS, Stevens JR, Stern TA. Insulin overdose among patients with diabetes: A readily available means of suicide. Primary Care Companion to the Journal of Clinical Psychiatry. 2009;**11**(5):258-262. DOI: 10.4088/PCC.09r00802

[15] Skowronek R, Nowicka J, Czech E, Chowaniec M, Rygol K. A case of suicidal insulin overdose—Significance of directed toxicological and histopathological studies for medico-legal opinion. In: Abstracts of the 22nd International Meeting of Forensic Medicine Alpe-Adria-Pannonia; 5-8 June 2013; Kraków, Poland; Archiwum Medycyny Sadowej I Kryminologii. 2013;1:44-45

[16] Skowronek R, Nowicka J, Czech E, Kulikowska J, Pieprzyca E, Rygol K, Kabiesz-Nenic zka S. Analysis of another case of extended suicide with insulin—Dilemmas and controversies in forensic-medical opinioning. In: Abstracts of the XXI Annually Conference of Polish Forensic Toxicologists; 7-9 May 2014; Ciechocinek, Poland; 2013. pp. 63-64

[17] Thevis M, Thomas A, Schänzer W, Ostman P, Ojanperä I. Measuring insulin in human vitreous humour using LC-MS/MS. Drug Testing and Analysis. 2012;**4**(1):53-56. DOI: 10.1002/dta.368

[18] Ojanpera I, Sajantila A, Vinogradova L, Thomas A, Schanzer W, Thevis M. Post-mortem vitreous humour as potential specimen for detection of insulin analogues by LC-MS/MS. Forensic Science International. 2013;(1-3):328-332. DOI: 10.1016/j.forsciint.2013.10.009

[19] Hess C, Madea B, Daldrup T, Musshoff F. Determination of hypoglycaemia induced by insulin or its synthetic analogues post mortem. Drug Testing and Analysis. 2013;**5**: 802-807. DOI: 10.1002/dta.1500

[20] Hess C, Thomas A, Thevis M, Stratmann B, Quester W, Tschoepe D, Madea B, Musshoff F. Simultaneous determination and validated quantification of human insulin and its synthetic analogues in human blood serum by immunoaffinity purification and liquid chromatography-mass spectrometry. Analytical and Bio analytical Chemistry. 2012;**404**(6-7):1813-1822. DOI: 10.1007/s00216-012-6271-5

[21] Lutz R, Pedal I, Wetzel C, Mattern R. Insulin injection sites: Morphology and immunohistochemistry. Forensic Science International. 1997;**90**(1-2):93-101

[22] Wehner F, Mittmeyer HJ, Wehner HD, Schieffer MC. Insulin- or morphine-injection? Immunohistochemical contribution to the elucidation of a case. Forensic Science International. 1998;**95**(3):241-246. DOI: 10.1016/S0379-0738(98)00099-1

[23] Kernbach-Wighton G, Püschel K. On the phenomenology of lethal applications of insulin. Forensic Science International. 1998;**93**(1):61-73. DOI: 10.1016/S0379-0738(98)00032-2

[24] Labay LM, Bitting CP, Legg KM, Logan BK. The determination of insulin overdose in postmortem investigations. Academic Forensic Pathology. 2016;**6**(2):174-183. DOI: 10.23907/2016.019

[25] Koskinen PJ, Nuutinen HM, Laaksonen H, Klossner JA, Irjala KM, Kalimo H, Viikari JS. Importance of storing emergency serum samples for uncovering murder with insulin. Forensic Science International. 1999;**105**(1):61-66. DOI: 10.1016/S0379-0738(99)00111-5

[26] Junge M, Tsokos M, Püschel K. Suicide by insulin injection in combination with betablocker application. Forensic Science International. 2000;**113**(1-3):457-460. DOI: 10.1016/S0379-0738(00)00283-8

[27] Hess C, Musshoff F, Madea B. Disorders of glucose metabolism-post mortem analyses in forensic cases: Part I. International Journal of Legal Medicine. 2011;**125**(2):163-170. DOI: 10.1007/s00414-010-0509-6

[28] Musshoff F, Hess C, Madea B. Disorders of glucose metabolism: Post mortem analyses in forensic cases: Part II. International Journal of Legal Medicine. 2011;**125**(2):171-180. DOI: 10.1007/s00414-010-0510-0

[29] Hess C, Madea B. Insulin and hypoglycemia. Forensic considerations. Rechtsmedizin. 2014;**24**(5):429-443. DOI: 10.1007/s00194-014-0965-2

[30] Palmiere C, Mangin P. Postmortem chemistry update part I. International Journal of Legal Medicine. 2012;**126**(2):187-198. DOI: 10.1007/s00414-011-0625-y

[31] Belsey SL, Flanagan RJ. Postmortem biochemistry: Current applications. Journal of Forensic and Legal Medicine. 2016;**41**:49-57. DOI: 10.1016/j.jflm.2016.04.011

[32] Nowicka J, Skowronek R, Czech E, Kulikowska J, Olszowy Z. Comments on 'Measuring insulin in human vitreous humour using LC-MS/MS' by Thevis et al. Drug Testing and Analysis. 2013;**5**:1-17. DOI: 10.1002/dta.1392

[33] Chevenne D, Letailleur A, Trivin F, Porquet D. Effect of hemolysis on the concentration of insulin in serum determined by RIA and IRMA. Clinical Chemistry. 1998;**44**(2):354-356

[34] Kubo Y, Hosoya K. Inner blood-retinal barrier transporters: Relevance to diabetic retinopathy. In: Shamsul Ola M, editor. Diabetic Retinopathy. InTech; 2012. pp. 91-108. DOI: 10.5772/33992

[35] Bévalot F, Cartiser N, Bottinelli C, Fanton L, Guitton J. Vitreous humor analysis for the detection of xenobiotics in forensic toxicology: A review. Forensic Toxicology. 2016;**34**:12-40. DOI: 10.1007/s11419-015-0294-5

[36] Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. The Journal of Clinical Investigation. 1960;**39**:1157-1175. DOI: 10.1172/JCI104130

[37] Palmiere C, Sabatasso S, Torrent C, Rey F, Werner D, Bardy D. Post-mortem determination of insulin using chemiluminescence enzyme immunoassay: Preliminary results. Drug Testing and Analysis. 2015;**7**(9):797-803. DOI: 10.1002/dta.1775

[38] Han S, Chen X, Wu YM, Naes L, Westfall T. Elevated neuropeptide Y gene expression and release during hypoglycemic stress. Peptides. 1997;**18**(9):1335-1340. DOI: 10.1016/S0196-9781(97)00212-X

[39] Gauff F, Patan-Zugaj B, Licka TF. Hyperinsulinaemia increases vascular resistance and endothelin-1 expression in the equine digit. Equine Veterinary Journal. 2013;**45**(5):613-618. DOI: 10.1111/evj.12040

[40] Fuller ET, Miller MA, Kaylor DW, Janke C. Lantus overdose: Case presentation and management options. The Journal of Emergency Medicine. 2009;**36**(1):26-29. DOI: 10.1016/j.jemermed.2007.02.038

[41] Lu M, Inboriboon PC. Lantus insulin overdose: A case report. The Journal of Emergency Medicine. 2011;**41**(4):374-377. DOI: 10.1016/j.jemermed.2010.04.007

[42] Matsumura M, Nakashima A, Tofuku Y. Electrolyte disorders following massive insulin overdose in a patient with type 2 diabetes. Internal Medicine. 2000;**39**(1):55-57. DOI: 10.2169/internalmedicine.39.55

[43] Shibutani Y, Ogawa C. Suicidal insulin overdose in a type 1 diabetic patient: Relation of serum insulin concentrations to the duration of hypoglycemia. Journal of Diabetes and its Complications. 2000;**14**(1):60-62. DOI: 10.1016/S1056-8727(00)00057-X

[44] Thewjitcharoen Y, Lekpittaya N, Himathongkam T. Attempted suicide by massive insulin injection: A case report and review of the literature. Journal of the Medical Association of Thailand. 2008;**91**(12):1920-1924

[45] Tofade TS, Liles EA. Intentional overdose with insulin glargine and insulin aspart. Pharmacotherapy. 2004;**24**(10):1412-1418. DOI: 10.1592/phco.24.14.1412.43147

[46] Wong OF, Tsui KL, Kam CK. A case of acute insulin poisoning. Hong Kong Journal of Emergency Medicine. 2006;**13**(4):232-234

[47] Iwase H, Kobayashi M, Nakajima M, Takatori T. The ratio of insulin to C-peptide can be used to make a forensic diagnosis of exogenous insulin overdosage. Forensic Science International. 2001;**115**(1-2):123-127. DOI: 10.1016/S0379-0738(00)00298-X

[48] Campbell IW, Ratcliffe JG. Suicidal insulin overdose managed by excision of insulin injection site. British Medical Journal (Clinical Research Ed.). 1982;**285**(6339):408-409

[49] Levine DF, Bulstrode C. Managing suicidal insulin overdose. British Medical Journal (Clinical Research Ed.). 1982;**285**(6346):974-975

[50] Gin H, Larnaudie B, Aubertin J. Attempted suicide by insulin injection treated with artificial pancreas. British Medical Journal (Clinical Research Ed.). 1983;**287**(6387):249-250

[51] Mégarbane B, Deye N, Bloch V, Sonneville R, Collet C, Launay JM, Baud FJ. Intentional overdose with insulin: Prognostic factors and toxicokinetic/toxicodynamic profiles. Critical Care. 2007;**11**:R115. DOI: 10.1186/cc6168

[52] Tsujimoto T, Takano M, Nishiofuku M, Yoshiji H, Matsumura Y, Kuriyama S, Uemura M, Okamoto S, Fukui H. Rapid onset of glycogen storage hepatomegaly in a type-2 diabetic patient after a massive dose of long-acting insulin and large doses of glucose. Internal Medicine. 2006;**45**(7):469-473. DOI: 10.2169/internalmedicine.45.1548

[53] Pudlo R, Pudlo M, Matysiakiewicz JA. Abuse of insulin as psychoactive substance. Postępy Psychiatrii i Neurologii. 2000;**9**(suppl. 3):47-50

[54] Sheehy TW. Case report: Factitious hypoglycemia in diabetic patients. The American Journal of the Medical Sciences. 1992;**304**(5):298-302

[55] Jermendy G. Hypoglycemia factitia: Munchhausen syndrome in diabetes mellitus. Journal of Diabetes and its Complications. 1996;**10**(4):223-225

[56] Kucuker H, Demir T, Oral R. Pediatric condition falsification (Munchausen syndrome by proxy) as a continuum of maternal factitious disorder (Munchausen syndrome). Pediatric Diabetes. 2010;**11**(8):572-578. DOI: 10.1111/j.1399-5448.2009.00631.x

[57] Wunder C, Kauert GF, Toennes SW. Factors leading to the degradation/loss of insulin in postmortem blood samples. Forensic Science International. 2014;**241**:173-177. DOI: 10.1016/j.forsciint.2014.06.003

[58] Madea B, Musshoff F. Postmortem biochemistry. Forensic Science International. 2007;**165**(2-3):165-1671. DOI: 10.1016/j.forsciint.2006.05.023

[59] Harmonisation of Medico-Legal Autopsy Rules. ECLM update of the principles and rules relating to medico-legal autopsy procedures. European Council of Legal Medicine. London, Fall/Winter 1994-1995. Updated, Dubai, January 2014. Available from: http://eclm.info/docs/Documents/ECLM_Harmonisation_of_Autopsy_Rules_2014.pdf

IntechOpen