Correspondence letter

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Comments on 'Measuring insulin in human vitreous humour using LC-MS/MS' by Thevis *et al.*

Joanna Nowicka,[a] Rafał Skowronek,[a]* Ewa Czech,[b] Joanna Kulikowska[a] and Zofia Olszowy[c]

To the Editor

We have read the article by Prof. Mario Thevis and his team titled 'Measuring insulin in human vitreous humour using LC-MS/MS' with great interest.[1] Congratulations to the authors on the first successful mass-spectrometry-based analysis of post-mortem material related to an insulin poisoning case. It is another step forward in the post-mortem diagnostics of insulin overdose.

The vitreous humour (also called a 'vitreous body') is a very valuable material for chemical-toxicological analyses and easy to obtain during autopsy. Its main advantage is anatomical isolation, useful especially in the case of advanced autolytic and putrefactive changes. Insulin is a very important peptide hormone in forensic toxicology for two reasons: (1) its casual or intentional overdose may be lethal and (2) it is also one of the most frequently used anabolic doping agents in sport.[2]

The case presented of a 55-year-old non-diabetic victim who died from an insulin overdose is quite interesting, especially because of the availability of ante-mortem blood samples. We have to remember that such a situation is very rare in routine forensic practice, however, where we may analyze only post-mortem biological material and non-biological specimens, like syringes, ampoules, vials, or remnants of the infusion solution and tubings, as in the commented article.

The interpretation of insulin levels in the post-mortem biological material is very difficult. 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), localization of injection sites on the body (different rate of absorption), etc. The time of survival certainly influences the insulin levels detected in the post-mortem material. Unfortunately, in forensic practice, we do not usually know that time, because the cadavers are frequently found a long time after death, for example when the victim is living alone. The processes of ante- and post-mortem elimination, distribution, and insulin transport across the blood-ocular barrier need to be studied more.

Further to the Thevis *et al.*' article, we want to shortly present our own, so far unpublished experiences in insulin determination in autopsy blood and vitreous humour.[3] So far we have only presented this work during the 9th National Congress of the Polish Society of Toxicology in Szczyrk in 2008. In contrast to the authors of the commented article we used immunoradiometric assay (IRMA KIT IMMUNOTECH), routinely used for *in vitro* determination of insulin in human serum and plasma. This method is very sensitive, specific and – what is important – relatively cheap, but requires adequate apparatus for measurement of the radioactivity (measuring range: 0.5–300 μIU/ml; the norm for serum: < 22 μIU/ml). It is worth remembering that the studies on insulin determination using antibody-radiolabelled antigen reaction in the late 1950s were the beginning of a new discipline – radioimmunology – which today is a part of radiochemical research.[4]

Insulin determination in post-mortem blood, mainly because of running thanatochemical processes, has little diagnostic and testimonial value. The barrier that prevents receiving correct and trustworthy results of insulin determinations in the post-mortem blood with radioimmunological methods is also the blood hemolysis. This fact was confirmed in the literature and by the results of our own comparative studies.[3,5]

In our research, the analysed material consisted of 93 samples of vitreous humour taken during medico-legal autopsies done in the Chair and Department of Forensic Medicine and Forensic Toxicology, Medical University of Silesia in Katowice. Analysis revealed that in 86 vitreous humour samples (92.5%), the concentration of insulin, determined with IRMA assay, was below the limit of detection of this method (below 0.5 μIU/ml). These are similar to the results of Mario Thevis' team (the natural levels of insulin in vitreous humour determined by the authors were below the liquid chromatography-tandem mass spectrometry (LC-MS/MS) limit of detection). The concentration of insulin in vitreous humour was determined in only seven cases (range of results: 1.42–24.42 μIU/ml). Special attention should be paid to the case of a non-diabetic man, where the highest insulin concentration in vitreous humour (24.42 μIU/ml) and the presence of insulin in a secured syringe found near the body at the crime scene were detected. All of these analytical results, in addition to low amounts of glycogen in the liver (evaluated by the Periodic Acid-Schiff (PAS) staining) have confirmed the Prosecutor's information about possible suicidal poisoning.

In cases where insulin overdose is suspected determination of its concentration in the vitreous humour and non-biological

* *Correspondence to: Rafał Skowronek MD, Department of Forensic Medicine and Forensic Toxicology, Medical University of Silesia, Medyków 18 Street, 40–752 Katowice, Poland. E-mail: rafal-skowronek@wp.pl*

a *Department of Forensic Medicine and Forensic Toxicology, Medical University of Silesia, Medyków 18 Street, 40-752 Katowice, Poland*

b *Department of Histology and Embryology, Medical University of Silesia, Medyków 18 Street, 40-752 Katowice, Poland*

c *Department of Laboratory Diagnostics, Institute of Occupational Medicine and Environmental Health, Kościelna 13 Street, 41-200 Sosnowiec, Poland*

material with the IRMA method gives the opportunity, similar to analysis with LC-MS/MS, to objectively confirm poisoning. The advantage of LC-MS/MS analysis, presented by Mario Thevis' team, is the possibility of differentiation between the insulin species (human or animal insulin and their synthetic derivatives).

An analytically confirmed higher level of insulin in vitreous humour plays an important and even decisive role in structuring the final medico-legal opinion about the cause of death. Besides detailed examination of the pancreas to exclude the presence of insulinoma (a tumour of the pancreas that is derived from beta cells of the islets of Langerhans and secretes insulin), detailed histological analysis of the subcutanous insulin application sites, and searches of morphological symptoms of hypoglycaemia, the vitreous humour should be routinely collected and analysed during autopsy in every case with an 'insulin' background.

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