Application of analytical chemical methods in the examination of human bone residues

Doctoral (PhD) thesis

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Introduction

The identification of living and deceased persons is one of the scopes of scrutiny of forensic medicine in the present. While historical anthropology studies the bone finds of excavation sites, the examination of recent bone remains are done by forensic experts in Hungary.

Separation sciences began their rapid development in the middle of the 20ieth century hence new methods were available for scholars of forensic medicine. We were able to witness several changes because of this development.

It is possible to conduct the complex analysis of bone residues by chemical methods that require only small samples in order to determine genetic gender, age, and the duration of stay in the ground and the presence of several illnesses.

Precursors

Our team began the examinations in bone chemistry at the Department of Analytical Biochemistry of the Institute of Biochemistry and Medical Chemistry of the Faculty of General Medicine of the University of Pécs in 2003. We

began to conduct the chemical analysis of historical bone samples at the Department of Anthropology of the Faculty of Science of the University of Szeged.

We were requested by the Institute of Forensic Medicine to conduct the identification process of several human body residues a few months after we had started our endeavor.

In order to determine the personal identity of the deceased person we had to determine the gender of the person, as well. Our question was the following: Is it possible to apply the method of bone chemistry that had proved to be effective in the analysis of historical bone samples in forensic medicine in practice?

We applied infrared spectroscopy to examine bone samples of healthy individuals excavated from archaeological sites. These bone samples showed signs of several infectious diseases and did not show any morphological anomalies. The measurement results proved to be promising for developing a method of the determination of time of stay in the soil.

It is important to determine the time of stay of human residues for practical reasons.

Aims

I was looking for the solution of the following problems during my research:

- *Establishing a test documentation procedure that meets international professional requirements.*
- *Examination of the chemical method of determining biological gender and integrating it into the protocol of forensic medicine.*
- *Finding, developing and testing the practical application of a chemical method for determining the duration of stay of bones in the ground.*
- *Developing an optimal sampling procedure.*

The theory of the applied methods

Anthropometry

We begin the analysis of bone residues by an anthropological examination. Metric methods are only effective under optimal circumstances. In the case of a nearly complete human skeleton we are able to make a valid statement about the gender, the age, the height, and the bone injuries of the person and also about the duration of the bones that stayed in the ground.

MALDI-TOF Mass Spectrometry

By using MALDI (Matrix Assisted Laser Desorption Ionization) we can effectively measure the decomposition of massive molecules (e.g.: proteins, carbohydrates, oligonucleotides, polymers). An ideal analyzer of MALDI is the so-called: time of flight (TOF). By applying TOF one may access great resolution and sensitivity. This method is quite widespread in biological and medical-chemical researches.

Infrared Spectroscopy

The advantage of infrared spectroscopy is that by using it we can examine sample either in gas or liquid state without the change of its structure. We conduct the analytic measurements in medium infrared range. When using the appropriate measurement setting the result can be considered as the "chemical fingerprint" of the particular chemical compound.

Crystallization index and the Carbonate-Phosphate ratio

After death, the structure of the hydroxyapatite crystals in the bones is slowly changing, these crystals become more orderly, so larger crystals are created. The structure and

order of the crystals can be characterized by the crystallization index. The carbonate-phosphate ratio decreases with the passage of time after death as a result of decomposition processes.

Measuring the time-dependent change of the crystallization index and the C/P ratio may be appropriate to determine the stay time of the bones.

Materials and Methods

Material of anthropological examinations

I conducted the anthropological examinations for the personal identification of several cases at the Institute of Forensic Medicine of PTE University.

Materials that were examined by the MALDI-TOF/TOF mass spectrometer

Recent bone samples come from the material of my anthropological studies. I did all the anthropometric measurements of the bones where the conditions of the bones allowed the procedure. The results of the chemical tests were compared with the results of metric determination of gender and of the DNA analysis (amelogenin).

I conducted the bone chemistry measurements at the

Analytical Biochemical Institute of the Institute of Biochemistry and Medical Chemistry of the Faculty of General Medicine of the University of Pécs.

Materials of the examination of FT-IR spectroscopy

I received historical bone samples from the Department of Anthropology of the Faculty of Science of the University of Szeged. The recent bone samples were from those samples that I received from the Institute of Forensic Medicine of the Faculty of General Medicine of the University of Pécs, where I conducted anthropological examinations and from other bone samples that I received and I was asked to conduct DNA analysis.

During my research, I performed measurements of healthy *teeth* removed for dental treatment, as well.

I received the teeth samples from the Clinic of Dentistry and Oral Surgery of the Clinical Center of the University of Pécs and the Department of Oral Surgery of Kaposi Mór Hospital of Medical Education.

I conducted the measurements at the Department of Analytical Biochemistry of the Institute of Medical Chemistry of the University of Pécs.

Preparation of the samples for MALDI-TOF/TOF mass spectrometry

I ground the bone sample in an agate mortar and I weighed 0.50 grams of bone powder. I added a 1.00 cc water-acetonitrile mixture to the bone powder. Extraction was performed in an ultrasonic bath. I dropped 1 micro liter of the extraction on an Mtp 384 stainless steel plate and I conducted the measurement after the drying of extraction. I used alpha-cyano-4-hydroxy-cinnamic acid (CHCA) as matrix and I also applied saturated toluene solution of C60 and C70 fullerene.

FT-IR Spectroscopy

The preparation of the samples varied depending on the surface of the bone covered by soft residues. In the case of historical bone samples, soil contamination was removed from the surface of the bones by washing them (with distilled water and methanol). I obtained an average particle size of 2 microns of from the bone granules that weighed 0.3-0.5 grams. I removed the soft remnants from the recent bone samples (bone marrow, muscle tissues) by mechanic cleaning. Then the bone samples were ground to a powder. The average size of the bone particles was 2

micrometers. Then the bone samples were degreased and the germs were removed. I cleaned the *teeth* by removing the soft parts and blood residue by mechanical cleaning. I removed the germs and proteins from the bone samples.

The archeological, judicial and dental samples were dried in a desiccator for 3-4 weeks. I added potassium bromide to the dried bone powder and made a pastel out of it at a pressure of 80-90 atm.

Results

Results of anthropological examinations

In the course of my work I identified 12 bone fragments of and 10 unknown corpses. I used the anthropometric methods standardized by Rudolf Martin in order to examine the bones. The residues were examined in detail and the bones of human and of doubtful origin were recorded. I focused on the problems that are usually encountered during examinations that are done by anthropological methods. Differentiation of genetic gender and the determination of age were done by several methods. I had a reliable result in an ideal situation.

Isolation of Genetic Gender by MALDI TOF Mass Spectrometry

I conducted the examinations by a MALDI TOF/TOF mass spectrometer that applied laser desorption ionization technique and a tandem flight time analyzer with the assistance of the 2nd type of Bruker Autoflex matrix. I summarized a mass spectrum of 1100-1200 shots on average. Mass spectra were registered at the range of 50 and 1200 m/z. I examined the standard solution of sex hormones (estrone, estradiol, estriol, progesterone and testosterone) in the first phase and I processed the recurrent bone samples in the second phase. These examinations were done alongside with the genetic examinations that were required by the forensic medical institute. I finally compared the results.

Determination of stay time by FT-IR spectrometry

Measurements were performed with Impact 400 (Nicolet) FT-IR spectrometer in the analytical domain. The measured values were compared with the data that were published in other studies.

The number of archaeological specimens is 39. Anthropologists and archeologists determined the gender

and age of the individuals by using metric methods. The bone samples were collected from deceased individuals, some of whom had died at adult age and the others had died at an old or a very old age. The distribution of gender of the individuals is as follows: 20 of them were men, and 19 of them were women. The bones stayed in the soil between 4900 B.C.E and 1500 A.D. based on the results of radiocarbon examinations. The average of the crystallization index is 3,25. The ratio of Carbonate to Phosphate is 0.24.

I examined a total of 52 roots of teeth. The distribution of the types of teeth are as follows: There were 5 molars, 5 premolars, 5 incisors and 36 wisdom teeth. The distribution of the gender of the humans are as follows: there were 31 women and 21 men. There was only a short time that was spent between the removal of the teeth and the time of the examinations.

The crystallization indices have nearly the same values. The lowest one was 2.52 and the highest one was 2.99. The Carbonate/Phosphate ratios showed mild fluctuations. The lowest measured value was 0.26 and the highest value was 0.48. The C.I. and C/P values of the roots of teeth, the

recent and archaeological bone samples are represented on a chart. Teeth and recent bone samples formed separate sets from the archaeological samples.

Discussion

Morphological analyses are based on the right amount and quality of bone samples. The complex analysis of bone remains is a prerequisite for a successful identification.

In case of ineffectiveness a unified documentation can lead to a successful identification in the future if we acquire new data to be investigated.

I did not have the adequate quality and quantity of bone samples to be examined during my examinations therefore my anthropological examinations were of limited value.

The analyses of *MALDI TOF mass spectrometry* are made by using so-called "fingerprint technology", so we compare mass spectra of standards with mass spectra of various bone fragments. Its disadvantage is that it is not suitable for quantitative determination. Its advantage is that you the examiner may receive reliable results in a short time.

The results of the control examinations show that the analytical chemical test method is perfectly suitable for the

separation of the biological gender of bone residues in a forensic case. The inaccuracy resulting from the bone measurement method can be eliminated by chemical analysis.

FT-IR spectroscopy is a method that requires a low amount of samples, and it can be performed very fast. However the preparation of the samples (desiccation) takes a long time.

The C.I. and the C/P ratio did not show significant differences between the genetic genders or age groups in the case of the historical samples that I examined. I also experienced a similar pattern when I examined recent samples.

When the crystallization index is shown depending on the C/P ratio we can make a difference between the recent and the archaeological samples. On the basis of FT-IR spectroscopy, we can only give a probabilistic opinion, such as: the examined sample is more likely to belong to recent or to historical samples.

In the examination of the teeth, I received values corresponding to the data of similar previously published studies. I found no significant differences between the

ration of C.I. and C/P between the tooth cement and the bones. The measured values of the teeth, similar to the bones, were clearly different from the archaeological samples. In conclusion I may state that the measurement of teeth can be applied similarly to tubular bones.

The data of C.I. and C/P values that were measured by FT-IR spectroscopy meets the requirements of modern analytical chemical methods, i.e. it can be routinely applied, reproduced, and used for processing a large number of samples.

Summary of New Results

- I have developed a uniform documentation (anthropological record) and sampling procedure.
- The analysis of bone residues was done in a complex way (anthropometry, analytical chemistry and genetics).
- I modified the preparation of the archaeological samples and applied them to the examination of the recent bones.
- I tried different analytical chemical methods (HPLC, MALDI-TOF, FT-IR) and made the necessary adjustments for their practical application.

- I examined the question whether certain chemical methods can be used to determine personal identity.
- I was the first researcher who used MALDI-TOF mass spectrometry in identity determination cases.
- I adapted bone structure measurements by using FT-IR spectrometry for the examination of recent samples.
- I made a protocol for the preparation of dental and recent bone samples.
- I was the first researcher who used FT-IR spectrometry to determine identification in a forensic case.

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