Determination of Sex Based on Sexually Dimorphic Amelogenin Peptides in Human Tooth Enamel

Marko Fonović, Ph.D., Associate Professor of Biochemistry, Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute, Slovenia. E-mail: marko.fonovic@ijs.si

Tamara Leskovar, Ph.D., Assistant Professor of Archaeological Science and Archaeometry, Faculty of Arts, University of Ljubljana, Slovenia. E-mail: tamara.leskovar@ff.uni-lj.si

Iztok Štamfelj, Ph.D., Assistant Professor of Dental Diseases and Normal Morphology of the Dental Organ, Faculty of Medicine, University of Ljubljana, Slovenia. E-mail: iztok.stamfelj@mf.uni-lj.si

The determination of sex in forensic and anthropological analyses of human skeletal remains depends mainly on the sexually dimorphic morphological characteristics of the skeleton and on the analysis of molecular markers on the sex chromosomes. Recent advances in proteome analysis of the enamel using mass spectrometry have made it possible to determine the sex based on the identification of sexually dimorphic peptides of amelogenin. Amelogenin proteins are encoded by the AMELX gene on the X chromosome and AMELY on the Y chromosome. In female enamel, there are amelogenin peptides specific for the X chromosome (APX), and in male enamel, there are also amelogenin peptides specific for the Y chromosome (APY).

For this research, we used teeth extracted during dental treatment in three female and three male patients. The peptides were extracted by etching the enamel surface with hydrochloric acid for 2 minutes, which did not observably change the size and shape of the tooth crowns. The etched products were analysed using liquid chromatography and tandem mass spectrometry (LC-MS/MS). The PEAKS software package and the nrNCBI human protein database were used to identify the peptides. Based on the identification of the peptide pairs AMELX-(44-50) (SIRPPYP) and AMELY-(58-65) (SMIRPPYS), we were able to determine the sex of all individuals correctly. In males, fewer than 5 APY, which are crucial for the determination of male sex, were identified in the samples. Therefore, we recommend the use of extraction protocols with prolonged enamel etching or protocols with the removal of a piece of enamel.

Previous research, conducted mostly on teeth from archaeological collections, has shown that the proteomic method is the most sensitive and allows sex determination, even in cases where osteological and DNA analysis fail. This new method represents a proteomic version of the amelogenin test, with which it shares a common disadvantage - incorrect sex determination in males with AMELY deletion because they have only APX in their enamel. Although the percentage of AMELY negative men is greater than 1% only in the populations of the Indian subcontinent, the problem cannot be neglected, as incorrect sex determination can have serious consequences for the course of a criminal

investigation. By validating the method on a large number of differently preserved recent teeth and determining the most optimal laboratory protocol, the proteomic method could be established as a complementary method for sex determination in combination with established forensic DNA analyses.

Keywords: sex determination, tooth enamel, peptides, amelogenin, LC-MS/MS

UDC: 343.983.2