STRUCTURAL AND MOLECULAR ANALYSIS FROM SUBMERGED HUMAN BONE SAMPLES USING SPECTROSCOPIC TECHNIQUES AND PCR MULTIPLEX ASSAY FOR TYPING THE MITOCHONDRIAL CONTROL REGION

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DNA has been a valuable tool for human identification. Neverthless, due to the fast degradation rate in soft tissues, often bones are the only available source of DNA from post-mortem individuals. This is a remarkable issue when human remains are recovered from aquatic enviroments, once their structure use to be deeply damaged. In such cases, commonly, the genetic material is recovered in small quantity and low quality. Thus, alternative methods to already standardized STRs should be considered and mitochondrial DNA (mtDNA) has proven to be an eligible source under such circumstances. Additionaly, morphological and structural data from human bones are scarce, particularly data that have been correlated with information on the preservation of molecules such as DNA. Here we examine the structure of fragments of human skeletal remains submerged in water up to 90 days by Scanning electron microscopy with energy dispersive X-ray (SEM-EDX) and Raman spectroscopy, and the quantity and quality of DNA extracted from these bone fragments using quantitative polimerase chain reaction (qPCR) and PCR multiplex assay with primers to amplify the entire mtDNA Control Region (CR). Eight exhumed human femurs of cold cases were analyzed. Samples were cut into small sections with a handdrill and submerged in water for 30, 60 and 90 days. After this period, the samples were cleaned and analyzed by SEM-EDX and Raman. Then, the samples were pulverized and the DNA extracted from the bone powder according to the protocol previously described. A qPCR with Quantifiler Duo® was performed according to the manufacturer's instructions. Ten primer pairs were selected and combined to generate five overlapping midi-amplicons, divided in two non overlapping PCR multiplexes, covering the entire CR. Subsequent electrophoresis was performed on polyacrylamide gel. The analysis of SEM and Raman showed morphological changes in the samples submerged as well as the absortion of elements as Aluminium, Manganese and Silicon. The quantification results by real-time PCR with Quantifiler Duo showed no significant difference between the amounts of DNA as a function of the environmental exposure up to 90 days. Similarly, the analysis of polyacrylamide gels allowed to infer no significant decrease in DNA quality due to the environmental exposure nor the time studied. We concluded that the set of mini primers for multiplex PCR assay showed promising results when tested in DNA recovered from skeletal remains submerged in water for periods of up to 90 days even under morphological and structural changes.