MARIJA B. RADOVIĆ, University of Belgrade,
Faculty of Philosophy, Department of Archaeology, Laboratory for Bioarchaeology, Belgrade

AGEING IN THE DANUBE GORGES POPULATION (9500–5500 BC) – TOOTH CEMENTUM ANNULATION METHOD

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e-mail: mradovic@f.bg.ac.rs

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Abstract. – Tooth cementum annulation, the microscopic method for the determination of an individual's age, gives results that are highly correlated with the chronological age of an individual. Nevertheless, this method is still rarely used for age estimation in archaeological populations. In this study, using the tooth cementum annulation method, teeth of 21 individuals of the Djerdap anthropological series, dated to a period from the 10th to the 6th millennium BC, were analysed. The obtained data are important for overcoming some methodological issues in anthropology of the Danube Gorges, as well as with the precise age estimation of old individuals and with the assessment of age in cases where the skeletal material has been very poorly preserved. The only obstacle to the full application of the tooth cementum annulation method is the taphonomy changes of tooth cementum which were detected on several teeth in this study.

Key words. – Individual's age, dental anthropology, tooth cementum annulation, the Đerdap anthropological series, taphonomy.

Dental anthropology is a sub discipline of physical anthropology, and dealing with dental anthropology involves the analysis of teeth and jaws. In 1542, Andreas Vezalius noted that the teeth are very different from the bones in their biological properties and function, and today it is clear that they cannot be generally viewed as parts of the skeleton in a narrow sense[1]. The teeth, as a separate component of the skeleton and body, possess certain anatomical and physiological characteristics that are very important for researchers. Hard dental tissues (cementum, dentine and enamel) are compact and could be well preserved in the soil, while the enamel itself has a feature that remains unchanged over time and it is resistant to taphonomic changes. Teeth are the only part of the skeleton which is influenced by the physiological and metabolic processes in the body, while at the same time are also under the influence of the environment. We should not underestimate the importance of the genetic component and its impact on the teeth, or the fact that teeth evolve slowly, allowing us to easily compare archaeological data with recent dental materials[2]. All these facts make teeth an important source of information, and dental anthropology, a discipline significant in the study of ancient populations.

[1] Hillson 2005.
[2] Irish and Nelson 2008.

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An important issue when it comes to palaeodemographic analysis is the reconstruction of patterns of aging and mortality in the past. The reliability of the reconstruction of these patterns depends on the accuracy of individual age estimations. For decades, physical anthropologists and palaeodemographers have tried to improve this method. The importance of such studies is emphasised in the new approach to palaeodemographic analysis described by Hoppa and Vaupel [3]. The main problem in almost all macroscopic methods for age estimation is the accuracy of the method itself [4]. Age estimation errors occur when the skeleton is observed macroscopically and only biological changes are visible which often do not correspond to individual's actual chronological age. Due to the high variability in the correlation of the biological and chronological age in one individual, the age estimation error can be up to 7 years[5]. This error increases in older individuals, as do our methodological problems. It is clear that there is a need for a method for age estimation that is less susceptible to continuous changes in the skeletons that can not be quantified and that increase with the age of the individual.

Recent research has shown that a solution could be found in an alternative method for the determination of an individual's age based on counting the incremental lines in tooth cementum. These lines (lines of annual layering of dental cementum) may be a more stable indicator of an individual's age in comparison with any morphological or histological characteristics of the skeleton and offer a microscopic method for the determination of tooth cementum annulation (TCA). The method relies on the counting of incremental lines on cross sections of the root, about – 80 μm thick, under a microscope with a magnification of 400 x.

The accurate age estimation of individuals from ancient populations is of great importance for palaeodemographic research in archaeology and anthropology. The goal of this paper is to demonstrate the advantages of the TCA method for such purposes. In addition, the further aim of this paper is to test the "readability" of incremental lines and the consistency of this method for the age determination of a sample of teeth from 21 individuals from the Djerdap anthropological series. The Đerdap anthropological series includes over 500 funerals, with about 600 individuals excavated at the 12 locations of the Lepenski Vir culture[6]. Sites which were inhabited from the 10th to the 6th millennium BC, were found and investigated on both sides of the Danube.

APPLICATION OF TOOTH CEMENTUM ANNULATION IN AGE ESTIMATION

Tooth cementum annulation is a microscopic method for the determination of an individual's age based on the analysis of the acellular extrinsic fibrous cementum (AEFC). This type of dental cementum, as compared to the other four types, is located at the cervical third of each root of deciduous or permanent teeth and allows the tooth to be anchored into the alveolar cavity. All the while surrounded and provided with nutritionally intact desmodontium, AEFC is subject to the additional growth that is reflected in histological samples by equal, alternating light and dark rings. Depending on the individual's age, the thickness of AEFC ranges from 20 to 250 μm. Alternating light and dark rings are subject to seasonal rhythms, which most likely occur under the influence of several factors such as a dose of UV-radiation, climatic conditions, different qualities of diet and the hormonal status of the individual. A pair of one dark and one light ring constitutes an incremental line. Seasonality in the rhythm of layering cementum lines has been seen in more than 50 different mammalian species worldwide, and it was concluded that it occurs as a result of a natural metabolic rhythm induced by seasonal changes[7]. These research has shown that the metabolism of the parat hormone has a major role in the formation of incremental lines, and is responsible for regulating levels of calcium in the blood's interaction with vitamin D, which in turn regulates calcium absorption. The interaction of hormones and vitamins, driven by a complex mechanism of external physical and chemical factors, leads to the formation of this circular annual rate in the root of a tooth[8].

The use of cementum in the determination of age in humans begins with the measuring of the total width of the layer of cementum before the lines are counted[9]. In the early eighties, studies conducted on three human teeth[10] showed that the method of tooth cementum

[3] Hoppa and Vaupel 2002

[4] Buikstra and Ubelaker 1994; Jackes 2001.

[5] Buikstra and Ubelaker 1994; Jackes 2001.

[6] Борић, Димитријевић 2007.

[7] Laws 1952; Geiger 1993; Grue and Jensen 1979; Kay et al. 1984.

[8] Gustafson 1950.

[9] Stott et al. 1982.

[10] Naylor et al. 1985.

annulation could be applied to the determination of age in humans, as previously applied in other mammals[11]. Further technical improvements have led to the method of tooth cementum annulation being accepted as a more advanced method compared to others which have used teeth to determine an individual's age. So today, the method is based on counting the incremental lines, this number is then added to the number of years in which the observed tooth erupts, and the result is a calendar age of the observed individual.

Initially, the method was applied only to freshly extracted teeth, but since the late eighties it has been implemented to determine the age of individuals from historical and archaeological skeletal series, with equal success in both the inhumed and the cremated[12]. This research has also clarified the fact that the number of incremental lines remains fixed, even in circumstances when other characteristics of incremental lines change (eg, width and degree of mineralization[13]) due to the impact of environmental or physiological disorders. For these reasons, the method of tooth cementum annulation has recently been considered one of the most accurate techniques for the determination of age in the skeletal material of adult individuals[14]. In younger individuals, analyses of the microstructure of enamel and dentin have achieved even greater precision in estimating the chronological age, sometimes with accuracy to within days[15]. However, there are still problems that prevent the full implementation of the tooth cementum annulation method. One such problem is that previous studies were done on small samples, which limited the establishment of a good statistical method for processing results obtained by the tooth cementum annulation method in palaeodemographic and forensic studies. In addition, it has still not clarified the issue of the impact of dental, especially periodontal, diseases on the accuracy of the method. Some researchers claim that periodontal diseases do not affect the number of lines of cementum[16], while others say that pathology reduce, or completely prevent the formation of incremental lines in tooth cementum[17].

MATERIALS AND METHODS

Dental samples in this study come from the Mesolithic and the Neolithic sites in the Danube Gorges (Lepenski Vir, Vlasac, Padina, Hajdučka vodenica) and date back to the period from the 10th to the 6th millennium BC. In a survey conducted by the author at the Faculty of Biology, in the Ludwig Maksimilianus University in Munich, 24 tooth roots were analysed from 21 individuals from the Danube Gorges' anthropological series (Table 1). In this sample, three individuals came from new excavations at the site of Vlasac. For most individuals one tooth was extracted (except in the case of individuals from Vlascac burials 17 and 29 and burial number 88 from Lepenski Vir where two teeth were extracted). After extraction, a selection was made

Site	Grave	Tooth
Lepenski Vir	20	44
Lepenski Vir	60	14
Lepenski Vir	64	34
Lepenski Vir	88	14, 15
Vlasac	2	15
Vlasac	9	25
Vlasac	17	24, 25
Vlasac	29	24, 44
Vlasac	41	34
Vlasac	55	45
Vlasac	67	15
Vlasac	79	34
Vlasac	U–53	PM
Vlasac	U–232	34
Vlasac	U–267	23
Padina	6	14
Padina	15	35
Padina	16	35
Padina	30	25
Hajdučka Vodenica	13	14
Hajdučka Vodenica	33	PM

Table 1. The structure of the sample

Табела 1. Структура узорка

[11] Gustafson 1950, 1955; Azaz et al. 1974; Philipsen and Jablonski 1992.

[12] Großkopf 1989, 1990.

[13] Karger, Grupe 2001.

[14] Wittwer-Backofen, Buba 2002.

[15] Antoine et al. 2000.

[16] Großkopf et al. 1996

[17] Kagerer, Grupe 2001.

based on the type of tooth, and the sample examined in this study consisted only of permanent premolars from the upper and lower jaws.

All teeth are completely submerged in biodur pitch (compared to the protocol of making a resin mixture of 100 ml biodur: 28 ml E7 hardener resin for 20 doses, Gunther von Hagens). Depending on the preservation of the roots, between 4 and 10 cross-cut sections of each root were prepared for analysis. Each of them was between 70 µm and 80 µm thick. The teeth were cut in a direction towards the apex of the root by a rotating diamond blade (Leitz 1600). Each cross section was analysed under a transmitted light microscope (Zeiss Axioskop 2 plus, Zeiss / Jena, equipped with a CCD camera AxioCam MRC colour and Axio Vision Release 4.3 software) with a 400 x magnification. Incremental lines were counted on digital photos, which were further processed with Adobe Photoshop 8.0.1. Up to 10 shots were taken for each root cross section.

In the next stage of analysis, incremental lines were counted three times on each photo by the observer. The individual's age was determined on the basis of the following three criteria: the mean value of the number of incremental lines, the maximum number and the most frequent number of incremental lines. The criterion for counting incremental lines is to count only those parts of the sections where the lines are equal, with a clear contrast between light and dark rings, which have as little micro bacterial decomposition and erosion as possible and avoiding those parts of cross sections with visible traces of the cuts of the diamond blade, etc.

RESULTS

Out of the 24 analysed teeth, at least 17 had one readable segment from any of the ten transverse slices (Table 2, Fig. 1). For the remaining 7 teeth, it was not

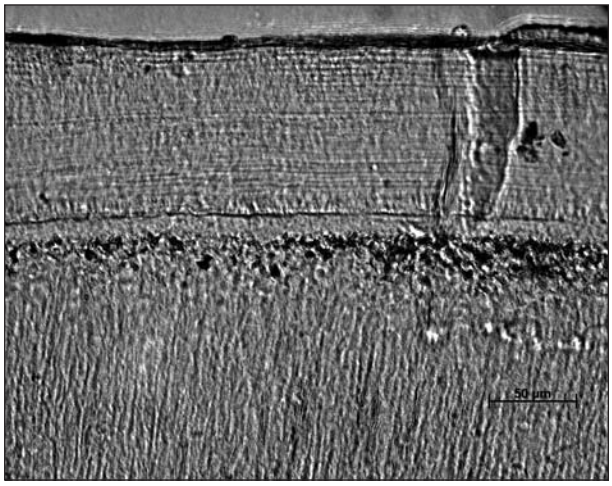
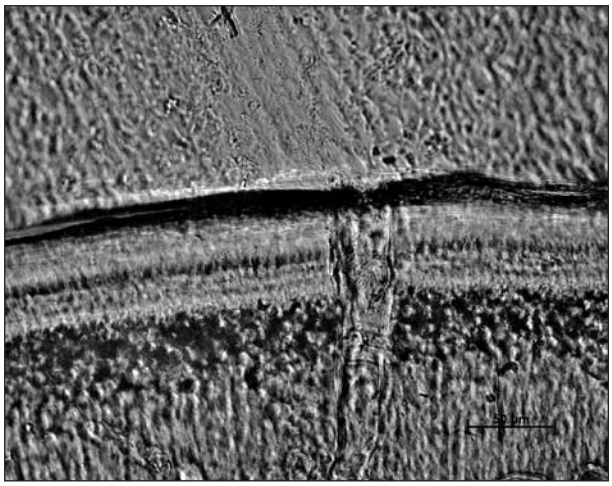
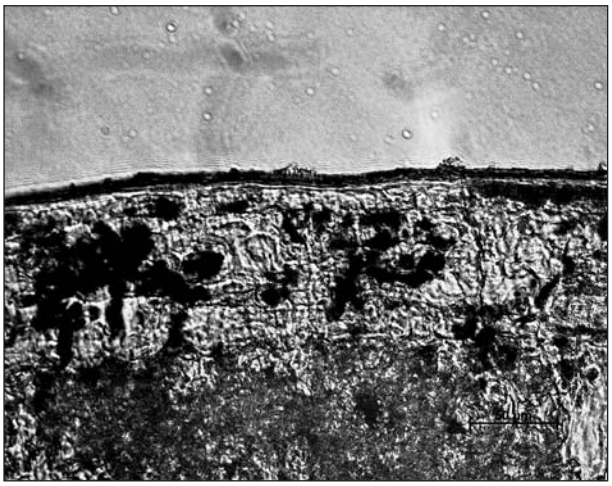


Fig. 1. A segment with clear incremental lines, Padina 16
Fig. 2. Pits in the dental cementum, Hajdučka Vodenica 33
Fig. 3. Longitudinal cracks dental cementum, Vlasac 2

Сл. 1. Сеīмент са јасним инкрементним линијама, Падина 16
Сл. 2. Шупљине у зубном цементу, Хајдучка воденица 33
Сл. 3. Уздужна пукотина зубноī цемента, Власац 2





Grave	Maximum No. of incremental lines	Mean No. of incremental lines	Most frequent No. of incremental lines
LV20	43	32	40
LV60	14	13	14
LV64	42	31	36
LV88	45	41	43
VL 2	22	21	22
VL29	17	16	17
VL41	42	37	40
VL55	40	37	40
VL67	19	18	19
VL79	61	50	42
VLU53	45	40	42
VLU232	18	15	12
VLU267	58	50	46
P16	40	34	34
P15	13	12	13
P30	27	24	24
HV13	35	31	34

Table 2. Number of readable incremental lines from 17 teeth represented by 10 cross sections

Табела 2. Број прочитаних инкрементних линија са 17 зуба представљених са по 10 исечака

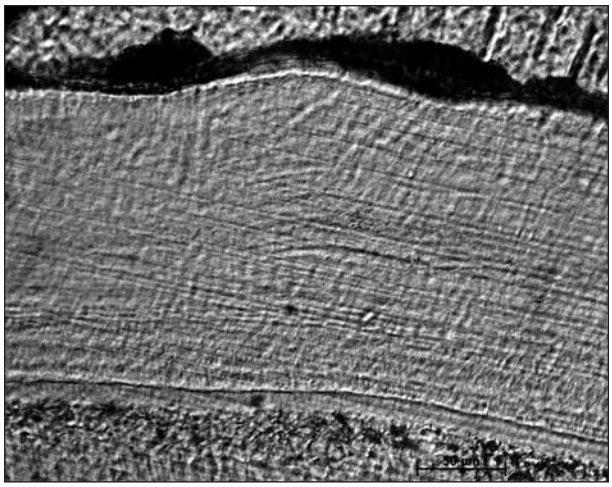
possible to see a segment that showed continuously visible incremental lines from the cementum and dentin circuit to the edge of the root. For each of these seven tooth roots, cross sections displayed very weak lines, outlines or segments where lines were intersected by pits (Fig. 2) and vertical or horizontal cracks (Fig. 3). In two cases the incision edges were parallel to the incremental lines so that the lines appeared to be "unreadable" (Fig. 4). In addition to the described phenomenon, and due to the secondary mineralisation that affected a portion of the sample, the clarity of incremental lines in some slices of teeth made analysis difficult. The appearance of secondary mineralisation was expected in the case of skeletal material dating from the Mesolithic period. The consequences of this process on the readability of the number of incremental lines are reflected in the fact that it reduces the number of visible lines, i.e. it seems that two to three lines were grouped into one (Fig. 5). However, unresolved factors that influence the development process of secondary mineralisation are yet to be discovered.

The presence of hypercementosis, periodontal disease or the degree of wear of the tooth crowns were not indicators upon which we decided to exclude some teeth from the analysis. The results showed that the presence of dental pathology and crown wear did not affect the readability of the incremental lines in this sample.

More than one readable segment was available for 13 out of 17 teeth. The comparison of the variation in the number of incremental lines in the 13 samples showed that in only two cases did samples provide an age range of more than 20 years (the level of uncertainty in the process of estimating the age was similar to that obtained using the standard macroscopic method).

DISCUSSION

In this research, a number of teeth were excluded from further analysis (7 teeth representing a total of 29.1%) because the incremental lines in the cross sections had been affected by diagenetic processes in more developed stages. Some sections were lacking visible lines, on others, the lines were wavy and intersected by pits and cracks (Fig. 2, Fig. 3) and moreover, there were a number of sections with lines which were shaded,



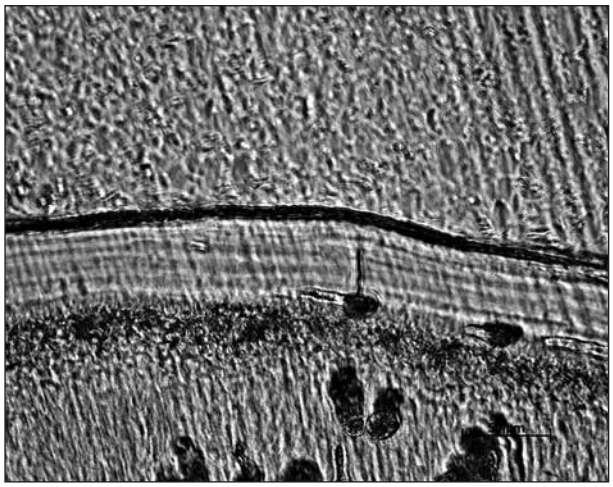


Fig. 4. Diamond blade cutmarks, Vlasac U267
Fig. 5. Secondary mineralization of dental cementum, Vlasac U232

Сл. 4. Урези сечива, Власац U267
Сл. 5. Секундарна минерализација зубноī цемениī, Власац U232

or where the lines were affected by secondary mineralisation (Fig. 5). Research in this area on the remains from archaeological material also presents problems relating to unreadable segments[18]. However, the decision as to whether a segment is "readable" or not, is certainly subjective[19]. Thus, in the case of multiple observers in the study conducted by Vitver-Bakofen et al., a senior author excluded a total of 14.1% of the teeth from their study, while another observer designated 22.3% of the teeth in the sample as unreadable. In the study conducted by Roksandić et al., the decision as to which teeth were to be excluded from further analysis was made by the senior author on the basis of the clarity and continuity of incremental lines at each cross section[20]. In the aforementioned study, counting was carried out only by the author and only on the sections where the lines were equal, with a clear contrast between light and dark rings, and with the least micro bacterial decomposition, erosion, or traces of the blade.

The poor microscopic preservation of nearly a third of the sample in this study may be explained by the influence of several factors such as the chemical conditions in the soil (ion exchange with ground water and the precipitation of minerals) and/or post-mortem biological activity (bacteria and fungi). All these factors may contribute to the change in the tissue at the microscopic level, leaving a vague or structurally altered histological picture[21]. The organic components of bones stored in the soil break down due to the impact of water and microorganisms[22], while inorganic chemical subjects contribute to the intense impact and degradation caused by microorganisms which facilitate the impregnation of soil minerals such as CaCO, Fe_2O_3 and SiO_2. Studies have shown that the effect of chemical agents in the soil can lead to the complete or partial replacement of the component building blocks of tissue at the microscopic level, leaving no macroscopically visible changes in morphology of the bone[23]. Therefore, tooth cementum in these conditions acts as a bone and suffers diagenetic changes. In this study, incremental lines in the cementum could not be read from one third of the sample. It is this "illegibility", caused by diagenetic changes, that represents the biggest limitation of the application, and precise method, of the annulation of dental cementum in determining the age of individuals in ancient populations. Establishing the standards of line clarity between researchers in the future, must be imposed as necessary to maximise the safety of this method.

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[18] Cipriano-Bechtle et al. 1996; Roksandić et al. 2009; Wittwer-Backofen et al. 2008.

[19] Wittwer-Backofen et al. 2008

[20] Roksandić et al. 2009.

[21] Pfeiffer 2000; Nonato do Rosario Marinho et al. 2006.

[22] Henderson 1987; Heuck 1993.

[23] Lambert et al. 1979; Francillon-Veilliellot et al. 1990; Gilber 1997; Gill-King 1997.

THE IMPORTANCE OF THE DENTAL CEMENTUM ANNULATION METHOD FOR RESEARCH IN THE DANUBE GORGES PALAEODEMOGRAPHY

The results of the chronological age of individuals obtained by the annulation method may indirectly contribute to a clearer palaeodemographic picture of the past. In the case of the Djerdap anthropological series, questions about the more precise age of the old individuals in the population and the length of the reproductive capacity in women have remained open for decades. This study gave us information of great importance for solving these problems in the Iron Gate palaeodemography.

Based on the macroscopic examination of human remains from the Djerdap series, it was known that there were individuals older than 40 years. Given the bias towards a short life expectancy of people in prehistoric times and the fact that due to the living habits and conditions, individual skeletal morphology may show a greater biological age, many individuals from the Djerdap anthropological series were placed in a category of between 40–60 years[24]. In the sample taken for this study, 12 individuals were designated older than 40 years on the basis of the morphology of the skeleton. Results from the annulation method showed that 3 out of these 12 individuals were approximately 50 years old (between 46 and 52 years of age) and 2 individuals were approximately 55 years old (between 52–57 years of age). Finally, the results showed an advanced age in two individuals from the Vlasac site (VL–VL79 and U267), which were determined to be between 60–70 years of age. In conclusion, it is now legitimate to say that people in prehistoric times in the Danube Gorges could have reached ages of up to 70 years.

As for the length of the reproductive period in women, it is crucial to point out to the case of a pregnant woman from the grave VL67 at Vlasac, dating from the Mesolithic period. The annulation of dental cementum method showed that her age at the time of death was between 30–32 years.

CONCLUSION

This research contributes to the identification of an individual's age. More precisely, the estimated age of the individual is given within a narrow range. In the case of the Djerdap anthropological series, we are now able to obtain, with greater certainty, the precise age of the adults, especially the oldest individuals. In addition, the dental cementum annulation method can be used to determine age even in cases of the very poor preservation of skeletal material, as the research is performed only at the root of the tooth. This is of great importance for the anthropological study of ancient populations. Moreover, using this method we could provide data essential for the future research of palaeodemography. For palaeodemographic studies, it is important to access data on the precise age of individuals, especially the oldest individuals in the population. The maximum age reached in a population as well as the number of individuals who attained it, significantly affects the calculation of several parameters of mortality[25]. The fact that the chronological age of the eldest individuals is determined helps to better estimate the length of life of individuals in this population. It can also be determined how the life length is distributed among the individuals from the Djerdap anthropological series. Possessing this type of information, we will be able to make palaeodemographic comparisons among different populations, taking into account the proportion of the oldest individuals in them.

In addition to the presented anthropological results for the Danube Gorges, the study pointed to several obstacles to the full implementation of the tooth cementum annulation method. These barriers are reflected in changes in dental tissues at the microscopic level, as the result of taphonomic changes. The fact that diagenetic processes that affect changes in incremental lines of dental cementum are not yet fully understood, needs to be stressed too. A recommendation for future analysis is the research of taphonomic effects on hard dental tissue histology.

Translated by Marija Radović

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[24] Roksandić 1999.

[25] Wood et al. 2002.

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Резиме: МАРИЈА РАДОВИЋ, Универзитет у Београду, Филозофски факултет, Одељење за археологију

ИНДИВИДУАЛНА СТАРОСТ СТАНОВНИКА ЂЕРДАПА (9500–5500 година пре н. е.) – ПРИМЕНА МЕТОДА АНУЛАЦИЈЕ ЗУБНОГ ЦЕМЕНТА

Кључне речи. – индивидуална старост, дентална антропологија, анулација зубног цемента, популација Ђердапа, тафономија.

Анулација зубног цемента је микроскопски метод за утврђивање индивидуалне старости. Резултати добијени применом ове методе најприближнији су стварној (хронолошкој) старости индивидуе. И поред тога, ова метода се још увек ретко користи у одређивању старости у оквиру археолошких популација. У овом истраживању методом анулације зубног цемента анализирана су 24 зуба која су припадала 21 индивидуи ђердапске антрополошке серије. Од свих анализираних зуба, укупно 17 је имало бар један читљив сегмент на попречним пресецима. Код преосталих 7 зуба није било сегмената са континуирано видљивим инкрементним линијама. Присуство хиперцементозе и пародонтопатије или пак истрошености круница зуба није било критеријум за искључивање узорака из анализе. Резултати су показали да присуство патолошких промена и истрошености круница није утицало на читљивост инкрементних линија у овом узорку. Поређење варијација у броју линија у овом узорку показало је процењену старост у распону од преко 20 година само код две особе (у том распону се процењује индивидуална старост применом макроскопских метода). Добијени подаци су од значаја не само за превазилажење неких методолошких проблема у антропологији Ђердапа већ, такође, и зато што на овај начин имамо и прецизне податке о старости праисторијских становника Ђердапа. Примена методе анулације зубног цемента омогућава утврђивање старости и у случајевима веома лоше очуваног скелетног материјала. Једину препреку за примену методе анулације у овом истраживању, али само у неколико случајева, представљала су стања измењене структуре зубног цемента под утицајем тафономских процеса.