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Variability of trace element content in human tooth sequences – a multivariate analysis

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Abstract

Analyses of human bone material expand our knowledge of aspects of modern and historical population ecology, the etiology of diseases, reconstruction of historical diets, and the social and economic status of human groups. 35 adult lower jaw tooth sequences from the 17th-century Cracow population were analysed. The skeletons were found in crypts of the medieval St. Mark's church, following international standards. Levels of Pb, Zn, Cu and Cd were determined in undamaged permanent teeth P1, P2, M1, M2 and M3, using anodic stripping voltammetry (ASV), while strontium concentrations were determined using AAS method.

There were statistically significant differences in the levels of the analysed trace elements within the investigated tooth sequences. High interspecimen variability in the amount of accumulated microelements, probably resulting from nutritional, developmental and physiological stress, was also observed. The accumulation of Pb, Cd and Zn was the highest in M3 teeth and the lowest in M1.

The results indicate that only one type of teeth should be used for intergroup and intragroup comparison of trace element content.

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Introduction

Studies of both historical and modern human populations increasingly often apply chemical and physico-chemical methods aimed at extending the body of knowledge about the biological status of human groups, diet and paleodiet and etiology of various diseases [KEEGAN, 1989, STUART-MACADAM 1989, KATZENBERG 1992, ROTHSCHILD 1992, SANDFORD 1992, AMBROSE 1993, KLEPINGER 1993]. In many cases it is no longer merely determining the mean concentrations of toxic trace elements and biogenic elements in various materials (blood, hair, nails, bones, teeth) characteristic of the studied group. Applications have been developed with the objective of finding the best indicators of the environment's impact on the body [BROCKHAUS 1988, SZOSTEK 1992, BERCOVITZ & LAUFER 1993, GIL *ET AL.* 1994, GŁĄB & SZOSTEK 1995, EVANS *ET AL.* 1995] and specific indicators of various diseases [FORNACIARI *ET AL.* 1981, STUART-MACADAM 1985, KLEPINGER 1993, GRUPE 1995]. In many cases, chemical analyses are used to develop precise models to determine nutritional status and social status, which is often associated with the diet [AUFDERHEIDE *ET AL.* 1981, SILLEN

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1981, ELIAS 1985, FULLMER 1991, WOLSPERGER 1992, BURTON, WRIGHT 1995].

A direct application of trace element analyses in the paleoanthropological studies will not provide satisfactory results in most cases. In the analysis of skeletal material, one cannot use the best indicators of the biological status, such as blood or hair. Bones and teeth, on the other hand, are to a greater or lesser degree structurally and chemically altered by diagenetic absorption or post mortem leaching of chemical elements [SANDFORD 1992, EDWARD & BENFER, 1993, SANDFORD 1993]. Selecting material (bones or teeth) and attempting to eliminate (if possible) the diagenetic alteration factor is of paramount importance in the interpretation of results and possible comparisons among different populations.

Teeth accumulate micro- and macroelements during mineralisation and incorporate them into the enamel and dentine [PURCHASE & FERRGUSSON 1986, APPLETON 1991]. In contrast to the postcranial skeleton, teeth are of very stable material which does not undergo the continuous process of elemental exchange ("remodelling" and "turnover") so intensively [BERCOVITZ & LAUFER 1990]. Because of this, teeth have become a universal indicator used in monitoring the environmental and nutritional impact on an organism.

During mineralisation, and particularly during the tooth eruption, the development of a tooth depends on environmental factors including dietary and pathological factors. It also depends on the rate of growth of the upper jaw and the mandible. It has been found that in humans there are great individual differences both in the order and dynamics of the eruption of permanent teeth. It is also known that permanent teeth originate from two different dental ledges, deciduous and permanent, and that the formation of the full permanent tooth sequence spans a significant period of time. Depending on the type of tooth, there are two phases of eruption of the permanent teeth and the third one for the eruption of the M3 tooth.

Few reports [PURCHASE & FERRGUSSON 1986, BERCOVITZ & LAUFER, 1990] suggested certain variability and differences in the levels of trace element accumulation among various types of teeth. Most of the studies, however, were conducted using permanent or deciduous teeth obtained in a random manner, without regard to the tooth type. Moreover, these studies were a part of comprehensive or overview studies. Only in exceptional cases do the studies regard the individual tooth sequences. Cross-sectional population characteristics regarding concentrations of trace elements, although very important for overall knowledge, can impose major limitations in terms of methodology and interpretation. Thus, they not always fulfil their function of providing a complete general description.

An approach to the study of accumulation of trace elements in human teeth following the accumulation trend model, in the context of individual variability and individual accumulation patterns, may permit reliable verification of the results of previous studies, conducted using non-selective, cross-sectional and more or less randomly selected material.

Taking into consideration:

1. great variability of development within individual tooth sequences,

2. effects of environmental factors on the formation and eruption of a tooth

3. high stability of the material (low "remodelling" and "turnover"),

an attempt was made to determine individual variability of trace element levels from tooth sequences.

Because of the practical impossibility of obtaining a full sequence of permanent teeth from a live individual, the present study employed archaeological material providing such sequences. The aims of the study were:

1. to determine individual variability of lead, cadmium, copper, zinc and strontium concentrations in sequences of permanent human teeth,

2. to show possible variability in the accumulation of trace elements and the ultrastructural differences depending on the tooth type, and hence to create a model and universal pattern useful in studies of trace element concentrations in human teeth, and

3. to determine sexual dimorphism and age-related differences in the above parameters in sequences of permanent human teeth.

Materials and methods

Historical and archaeological description of the site

In the course of systematic and comprehensive restoration of St. Mark's Church in Cracow, a number of previously unknown archaeological artifacts and strata were found with skeletal graves. The burial sites found in the church occurred at various levels and were dated, using a number of movable artifacts (ceramics and artifacts made of other materials), to three chronological phases: early medieval, late medieval and modern. In medieval times, the dead were buried both in cemeteries and within the churches. The place of burial depended on the social status of the deceased and on the material resources of his family. The most desirable places for burial were, of course, the interiors of the places of worship, where bishops, abbots and members of princely families and founders who endowed the church were buried [MYSZKA 1996]. Till the beginning of the 17th century, the synodic rules and church regulations prohibited lay people from being buried within the houses of worship. At the end of the 16th and the beginning of the 17th century, the new prevailing features of funeral ceremonies aimed at consolidating and securing the place of burial, rare in the Middle Ages. These were brought about both by changes occurring after the Council of Trent and by a great accumulation of graves in churches, quite often leading to the deformation of tiled church floors. Church crypts and chapels with underground crypts began to be built, funded by lay and church notables, and in churches vaulted burial crypts gradually replaced graves dug in the ground. Church crypts were initially used to bury the clergy, monks, friars, and later lay people.

The bone material used in the present analyses was obtained from one of such crypts built at the same time as the church vestibule and tower in 1617 [MYSZKA 1996]. During exploration of the crypt it was found to be full of mixed skeletal remains. The graves occurred at depths of 40–80 centimeters in the ground made of loose sandy soil mixed with grey and black soil. There were also remnants of wooden caskets.

Material

Before the bone remains were explored, the whole crypt was investigated according to international rules and standards regarding trace element analyses in bones [SANDFORD 1992]. Teeth whose enamel was visibly cracked, or those with large pieces of enamel missing were excluded from further analysis.

The material for the analyses consisted of permanent tooth sequences obtained from 35 individuals. The tooth sequences were obtained following earlier determination of the sex of the individual [BUIKSTRA & UBELAKER 1994] and age on the basis of tooth wear [BROTHWELL 1981]. In the cases of 10 individuals, only two teeth were obtained from the molar sequences: M1 M2, M1 M3, and M2 M3. In 25 individuals, full molar sequences, M1, M2 and M3, were analysed, supplemented by various combinations of P1 and P2. In all, 106 teeth were analysed (30 M1, 33 M2, 32 M3, 6 P1, 5 P2). Due to the small number of P1 and P2 teeth and incomplete sequences of these teeth, they were not considered as a separate group.

Laboratory methods

Each tooth was carefully washed in spectrally pure water obtained from a Milipore Water Purification System and wiped with acetone of analytical purity grade in order to remove superficially absorbed organic and inorganic contaminants. An ultrasound washing device was used to remove contaminants from microcracks in the enamel and from worn surfaces of the teeth where the dentine was exposed. These are the locations where postmortem penetration of exogenic compounds is most intensive [MARCSIK *ET AL.* 1992]. After washing, each tooth was dried in an oven at 80 °C for 120 minutes. Teeth thus prepared were then wet-digested on graphite plates in a 4:1 mixture of spectrally pure perchloric (65% Suprapur, Merck) and nitric (70% Suprapur, Merck) acids. Wet digestion was carried out in quartz crucibles for 48 hours. At the same time, control samples were prepared using the same reagents as those used for analysis. Standards used in all analyses were made by Merck Tritest. After complete evaporation of the acids, the samples were carefully quantitatively transferred to 25 ml calibrated flasks, and then diluted with spectrally pure water.

The strontium levels were determined in a Varian atomic absorption spectrophotometer using a graphite vessel. The strontium content in each sample was determined as the average of three consecutive measurements. The standard error did not exceed 1%, and the lowest detectability level was 0.1 mg/ml.

The lead, copper, cadmium and zinc concentrations were determined by the anodic stripping voltammetry (ASV) [KARAI *ET AL.* 1980, WANG 1985] using the MAV-Radius apparatus. The measurements were made with impregnated main graphite electrodes and calomel and platinum reference electrodes. Three consecutive measurements provided the average value. The standard error did not exceed 0.5%, and the lowest detectability level for all these elements was 0.0001 mg/ml.

The parallel control samples were analysed for Sr and Pb, Cu, Cd and Zn at the detectability levels for each of these elements, and the results subtracted from each concentration obtained. All concentrations were then calculated per weight of sample and expressed in micrograms per gram of dry mass.

Photographs showing the ultrastructure of various types of teeth (Figs. 1 and 2) obtained from the same individual

were made under a scanning electron microscope by using JOEL JSM-541 apparatus with accelerating voltage of 20 kV and x1000 magnification. In order to avoid possible errors resulting from differences in the section planes, the photographs were taken at the bottom of the dental chamber where the lumen was reached by a natural fracture at the enamel-dentine boundary.

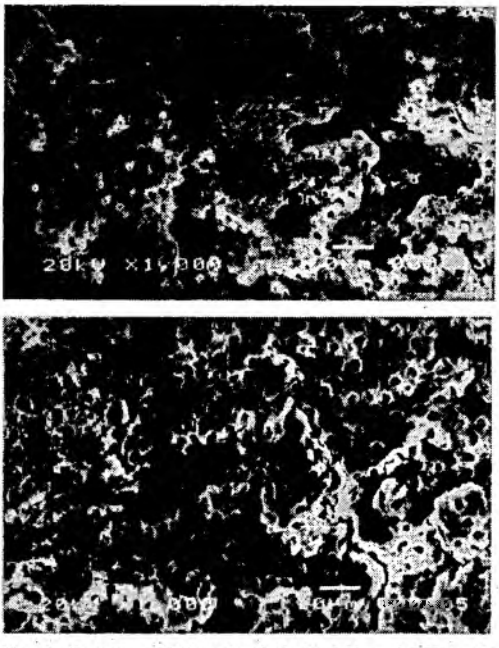

Fig. 1. Structure of dental tubulesin M1 and M3 teeth obtained from the sample individual

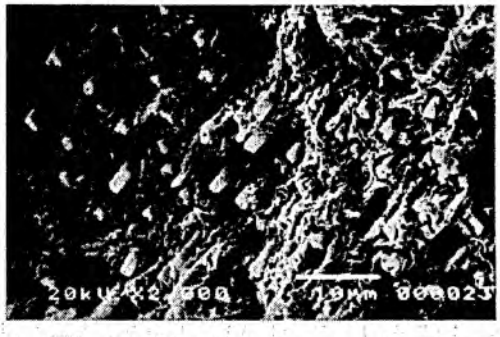

Fig. 2. Structure of filled dental tubules

Statistical methods

To determine if the material meets the necessary requirements to use multivariate statistical methods, normal distribution analysis was conducted using the statistical software package STATGRAF 6.0 Plus. Three elements (Pb, Cd, Cu) among those studied did not meet the normal distribution requirements and thus logarithmic transformations were applied to them, following which normal distributions were obtained. Then statistical methods requiring such a distribution could be used. Further statistical analysis involved a factor analysis of variance method using the Bonferroni test as the range test. The analysis examined three factors (type of teeth, age, and sex) and the concentrations of Sr, Pb, Cu, Cd and Zn.

In order to comprehensively analyse complex configurations and intricate relationships between elements resulting from their influence on each other, a multivariate analysis was used. This analysis was applied to identify and interpret interrelationships among elements in the studied types of teeth. In doing this, log Pb, log Cu, log Cd, Zn and Sr variables were used applying a varimax rotation of correlation matrices.

The factor analysis utilizes linear combinations of logarithmically transformed variables and non-logarithmic ones. All the variables involved are weighted for their loadings and are grouped according to the most similar weights. This grouping is made within a range of specific factors. Each factor, in turn, explains particular fractional variances that are summed up to give the total variance of the analysed material [SANDFORD & KISSLING 1994].

Results

The sex and age structures are presented in Table 1. Table 2 presents the

Table 1. Frequency of investigated individuals by age and sex

Sex/Age	Males	Females	Total
Adultus	8	8	16
Maturus	14	5	19
Total	22	13	35

Table 2. Numbers of analysed particular tooth sequences of investigated individuals

Tooth sequences	Number of individuals
M1 M2 M3	25
M2 M3	5
M1 M2	3
M1 M3	2
Total	35

Table 3. Average elemental concentrations in the studied material

Element	Elemental concentration μg/g		
	No. of teeth	Mean	SD
Pb	106	22.30	26.0
Cu	106	8.18	5.97
Cd	106	0.86	0.87
Zn	106	119.66	69.49
Sr	99	61.67	13.49

numbers of particular tooth sequences obtained from the same individuals. The average concentrations of the chemical elements: Pb, Cu, Cd, Zn and Sr as well as their proportions in the studied material, without breaking them down by age, sex or type of tooth, are presented in Table 3. Large standard deviations, high coefficients of variability and the absence of normal distribution suggest very high variance in the case of Pb and Cd, and high variance in the case of Cu. The results of the chemical analysis of particular type of teeth from the investigated individuals are listed in Table 4.

The concentration levels of trace elements taking into account sexual dimorphism, type of teeth and age in the studied material are presented in Table 5. Significant sex difference was found for strontium (a higher level in females). Pb, Cd and Zn concentrations, based on analysis of variance, were significantly different for the sequences of M1, M2 and M3 teeth. The relationships obtained result from differences between M1 and M3. No significant differences were found, however, between Cu and Sr levels among M1, M2 and M3, but the direction of the relationship was the same as in the case of the Pb, Cd and Zn levels.

In order to determine whether the differences in Pb, Cd and Zn were not an accidental effect of the presence of individuals with uncertain M1, M2 and M3 sequences, a new homogeneous group was distinguished. This new group consisted of 25 individuals with complete sequences of all teeth (M1, M2 and M3)

Table 4. Trace element concentrations in different tooth types (ANOVA)

Element	Type of tooth								
	M1			M2			M3		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Pb (μg/g)	30	8.94*	8.78	33	10.38	11.37	32	19.20*	20.66
Cu (μg/g)	30	5.93	3.02	33	6.17	3.06	32	7.96	3.99
Cd (μg/g)	30	0.59*	0.48	33	0.64	0.54	32	1.20*	1.16
Zn (μg/g)	30	105.38*	52.98	33	123.50	84.79	32	151.48*	75.17
Sr (μg/g)	29	59.38	13.58	21	57.09	9.87	25	61.21	8.90

* Differences statistically significant ($p < 0.05$)

Table 5. Analysis of variance; results with Bonferroni correction

	Pb (μg/g)		Cd (μg/g)		Cu (μg/g)		Zn (μg/g)		Sr (μg/g)	
	F	P	F	P	F	P	F	P	F	P
Type of tooth	2.87	0.03*	3.30	0.02*	1.22	0.30	3.64	0.01*	1.95	0.13
Age	1.54	0.15	1.26	0.28	0.73	0.41	1.75	0.14	0.18	0.66
Sex	2.14	0.08	1.38	0.26	0.93	0.34	2.2	0.07	3.85	0.01*

* Statistically significant differences ($p < 0.05$)

in the mandible. The differences in the Pb, Cd and Zn levels among the three teeth were the same as previously but the coefficients of variation were lower. In order to explain this phenomenon found both in males and females, ultrastructure study and multivariate statistical analyses were employed.

The elevated levels of the studied trace elements in the M3 tooth, irrespective of sex of individuals, do not seem to be an accidental occurrence and involve at least two factors.

The first, structural factor that may contribute to the above phenomenon is the effect of the dynamics of tooth development associated with changes in its structure during ontogenetic development. Figure 1 shows the dental tubules obtained from the same individual M1, M3 tooth sequences. Worth noting are smaller diameters and numbers of dental tubules.

The differences in number and diameter of the dental tubules may result from structural differences between teeth or may be an effect of occlusion of cavities and their filling with mineral salts binding all microelements supplied to the body during the formation and development of the tooth till its complete mineralisation. Complete mineralisation most probably involves filling all tubules entirely and closing the lumen of the opening (Fig. 2). It is known that the full mineralisation and formation of the M1 tooth occurs up until the 14th year of life, and any further accumulation within this tooth is slight or nonexistent. By contrast, the M3 tooth is formed many years later, sometimes even after the 25th year. Thus, the relative duration of active accumulation is longer. Most importantly, however, it falls into a different phase of the human life, associated with different feeding habits and a new social and economic environment.

The second factor is morphological and chemical. Multivariate statistical analysis explains differences between elements within particular types of teeth. Table 6 presents the factors analysis of the M1 and M3 teeth. In the M1 tooth samples, one should note that the factor explaining 45.4% of the total variance includes Sr and Zn variables. The configuration of components indicates antagonism between Zn and Sr, giving them weights with opposite signs. The factors 2 and 3 include the variables log Pb and log Cd. It shows a simultaneous relationship involving log Cu that is present in both factor 1 and factor 2. In the case of the M3 tooth, the factors 1 and 2 are explained by log Pb and log Cd components with an admixture of the Sr component. In the case of M3, however, the Zn variable is explained only by factor 3. As in the case of the M1 tooth, a remarkable relationship holds between log Cu and factors 1 and 2. Such a great disproportion between variables explain-

Table 6. Factor analysis results of first and third molar

	First molar			Third molar		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
log Pb	0.126	0.030	**0.990**	**0.945**	0.195	0.055
log Cu	**0.721**	**0.523**	0.044	**0.567**	**0.701**	0.191
log Cd	0.102	**0.957**	0.025	**0.511**	**0.931**	−0.042
Zn	**−0.488**	0.188	0.085	0.089	0.045	**0.898**
Sr	**0.699**	0.267	0.131	**0.946**	0.121	0.085
% variance	45.4	20.1	16.7	50.2	19.9	16.4
Cumulative % variance	45.4	65.5	82.2	50.2	70.1	86.5

ing specific factors suggests qualitative differences among various configurations of Zn log Pb and log Cd in M1 and M3 teeth.

Discussion

The factor that affects the postmortem diagenetic changes in the chemical composition of bones and teeth most crucially is the pH of the soil. At low and extremely high pH values, the leaching of the trace elements firmly bound to the mineral salt structures (hydroxyapatites and dihydroxyapatites) in teeth is most intensive. The material obtained from such sites should not be analysed because the rates, quantities and qualitative composition of leached compounds are not known. A neutral soil pH is prerequisite to any further analyses.

In the whole crypt, irrespective of depth, the pH of the soil ranges from 6.2 to 6.8. The nearly neutral reaction found suggests that possible postmortem changes in bone chemistry were probably slight. Certainly the present acidity of the soil does not rule out the possibility that it had a different reaction in the past, but the stable microclimate in crypts, lack of any direct effects of the environment on skeletons and little change in soil properties suggest that the present conditions are close to the initial conditions that obtained 300 years earlier. An even smaller but significant effect on the diagenetic process is exerted by the soil type. The mixture of permeable sandy soils with black and gray soils has great absorption capacity in its surface layer in which chemical elements show little mobility and low activity [KABATA-PENDIAS & PENDIAS 1979]. The neutral or slightly alkaline soil pH, the low activity of chemical elements and good or very good preservation of teeth exclude, to much extent, a strong effect of external factors on skeletons, thus minimizing the process of diagenesis.

The complexity of relationships between chemical elements and their correlations result from intricate and elusive physiological processes, environment-related variability and diagenesis. In many cases it is possible to separate factors affecting the level of microelements in the body which are, for the purpose of this study, called ante- and post-mortem factors. This is, of course, in regard to archaeological material, provided that exact compliance with the standard methods of investigating skeletons is observed with a view to the requirements of chemical analyses [SANDFORD 1992].

A much more difficult problem, which has not been fully solved yet, is the isolation of environmental factors from widely defined biogenic factors. Both these groups of factors contribute to the overall population diversity and are the chief source of variability in the levels of chemical elements. The levels of trace elements investigated in this study show great variability and high coefficients of variation (Table 5). A fact which is worth noting here is that, as confirmed in earlier studies of material obtained from modern populations [YAMAMOTO *ET AL.* 1987, SZOSTEK 1992], the proportions between Pb, Cd, Cu and Zn are identical to those in modern studies. The relationship between the elements can be presented as Zn > Pb > Cu > Cd. This result, combined with the analysis of the church crypt environment, indicates antemortem levels of these elements in the historical material analysed.

In many studies in the field of trace element analysis, contradictory results are obtained regarding sexual dimorphism. Our studies confirmed earlier reports [DRASH 1982, YAMAMOTO *ET AL.* 1987, BROCKHAUS *ET AL.* 1988, GIL *ET AL.* 1994] of a principal absence of sex differences in the levels of microelements. There is, however, a puzzling difference in the strontium levels (Table 4) which is higher in females than in males, and a nearly statistically significant difference in the concentrations of zinc, which are higher in males. This may indicate dimorphic differences in diet, strontium and zinc are regarded as good indicators of a meat and vegetable diet, respectively.

The different timing of budding and eruption of specific types of teeth could suggest that in those remaining longer in the upper jaw or mandible, i.e. in those whose buds form the earliest (M1) and whose eruption occurs in the first phase, the concentration levels of trace elements should be higher than in the M3 tooth. Such reasoning assumes that the trace elements accumulate in teeth throughout life from the time of tooth formation and thus assumes the existence of an accumulation trend. When verifying this hypothesis, differences were found within the M1, M2 and M3 molar sequence in a comprehensive cross-sectional analysis and in a specific case of individuals selected for identical individual sequences.

The statistical analysis revealed significant differences between types of teeth. The most surprising fact was, however, that the direction of the relationship was the reverse of what had been previously assumed: The differences between M1, M2 and M3 were associated with higher Pb, Cd and Zn levels in the M3 tooth (it is known that this tooth is the youngest in the course of ontogenesis and has the shortest period of remaining in the occlusion line).

All of these observations provide some grounds for the assumption that the differences in relative contents of Pb, Cd and Zn and the lack of differences in Sr and Cu levels result from complex processes based on structural and developmental differences between M1 and M3 teeth. There is also interference by intricate physiological processes and nutritional stresses, probably occurring at different rates and intensities, depending on the type of tooth, still likely to enhance the effect of changes found within the types of teeth under study.

Unfortunately, having no Infans II or Juvenis individuals, it is not possible to precisely identify the kinetics and the rate at which chemical elements saturate particular teeth in the M1, M2, M3 sequence. It seems, however, that the differences between age classes, if any, may appear between juvenile individuals of Infans II or Juvenis groups and those of the Adultus and Maturus groups.

Conclusions

1. There are differences in lead, cadmium and zinc concentrations between the types of teeth obtained from M1, M2, M3 tooth sequences. The observed differences probably result from complex processes connected with structural and developmental distinctions between the studied teeth and changing external conditions (diet, socio-economic status) during ontogenesis.

2. No sexual dimorphism was found in the concentration levels of the studied elements except strontium whose level

was higher in females than in males. This probably results from differences in diets.

3. No differences were found in the levels of any of the studied elements between the age groups.

In order to carry out an accurate comparative analysis of the chemical studies of human teeth for various human populations, the concentrations of elements should be compared using specific, homogeneous types of teeth. Using various types of teeth may result in a distorted interpretation of the general biological trends owing to overlapping factors affecting the human body during its ontogenetic development in different ways.

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Streszczenie

Badania historycznych oraz współczesnych populacji ludzkich coraz częściej wykorzystują metody chemiczne i fizykochemiczne w celu powiększenia wiedzy dotyczącej stanu biologicznego grup ludzkich, diety i paleodiety oraz etiologii różnorodnych schorzeń. W wielu przypadkach nie jest to jedynie określanie średnich zawartości toksycznych pierwiastków śladowych i biogenów w różnorodnym materiale (krew, włosy, paznokcie, kości, zęby) charakterystycznym dla badanych grup. Aplikacje bowiem idą w kierunku poszukiwania najlepszych wskaźników określających wpływ środowiska na organizm oraz specyficznych wyznaczników różnorodnych. Analizy chemiczne wykorzystuje się w celu stworzenia precyzyjnych modeli, określających status żywieniowy oraz, w wielu przypadkach związany z dietą, status społeczny. Zęby akumulują mikro- i makroelementy podczas mineralizacji i formowania, wbudowując je w szkliwo i zębinę. Są one, w przeciwieństwie do kości szkieletu postkranialnego, materiałem bardzo stabilnym, nie podlegającym procesom ciągłej wymiany pierwiastków. W związku z tym są uniwersalnym wskaźnikiem stosowanym w celu śledzenia presji środowiskowej i żywieniowej na organizm.

Przekrojowe charakterystyki populacyjne dotyczące średnich koncentracji pierwiastków śladowych, aczkolwiek poznawczo bardzo ważne, mogą nieść duże ograniczenia metodyczno-interpretacyjne, nie zawsze spełniając funkcję pełnego opisu ogólnego. Ujęcie problematyki badań nad akumulacją pierwiastków śladowych w zębach ludzkich

zgodnie z modelem trendu akumulacyjnego, w kontekście indywidualnej zmienności i osobniczych wzorców akumulacji, może umożliwić weryfikację wyników badań prowadzonych na materiale nie selekcjonowanym, przekrojowym i dobieranym bardziej lub mniej losowo.

Celem pracy było: 1) zbadanie indywidualnej zmienności koncentracji ołowiu, kadmu, miedzi, cynku i strontu w sekwencjach zębów stałych człowieka; 2) wykazanie ewentualnego zróżnicowania w akumulacji pierwiastków śladowych oraz ultrastrukturalnych różnic w zależności od typu zęba, a przez to stworzenie modelowego i uniwersalnego wzorca, przydatnego w badaniach zawartości pierwiastków śladowych w zębach człowieka; 3) określenie dymorfizmu płciowego oraz zróżnicowania wiekowego w koncentracjach wymienionych wyżej pierwiastków.

W efekcie przeprowadzonych badań wykazano istnienie różnic pomiędzy typami zębów pochodzących z sekwencji M1, M2, M3, w poziomie zakumulowanego ołowiu, kadmu i cynku. Zaobserwowane różnice są prawdopodobnie wynikiem złożonych procesów związanych ze strukturalną i rozwojową odrębnością badanych typów zębów oraz zmieniającymi się warunkami środowiskowymi (dieta, status społeczno-ekonomiczny) w trakcie rozwoju osobniczego. Nie stwierdzono dymorfizmu płciowego w poziomach badanych elementów, z wyjątkiem strontu, którego poziom był wyższy u kobiet niż u mężczyzn. Różnice te wynikają prawdopodobnie ze zróżnicowanej diety. Nie potwierdzono istnienia trendu akumulacyjnego pierwiastków z wiekiem, z wyjątkiem poziomu ołowiu w zębie M3.

W celu dokonania porównań pomiędzy populacjami, należy określać koncentracje elementów używając poszczególnych, jednorodnych typów zębów: M1 w celu określenia historii życia do wieku ok. 20 lat, natomiast M3 w celu śledzenia fazy dojrzałości i dorosłości. Analiza różnych typów zębów może przyczynić się do interpretacyjnego zniekształcenia ogólnych prawidłowości biologicznych poprzez nakładanie się na siebie czynników odmiennie działających na organizm w trakcie rozwoju ontogenetycznego.