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ORIGINAL PAPER

AGE-DEPENDENT CHANGES IN THE CALCIUM CONCENTRATION IN WOMEN'S BONES AND TEETH*

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Abstract

The inorganic part of bone tissue is mainly composed of Ca. In adult people, the resorption processes lead to the removal of minerals from calcified tissues. Women's bodies are particularly vulnerable to a decrease in the concentration of Ca because the osteolysis processes that occur with age are compounded by hormonal changes during the menopause. The aim of the study was to determine the age-dependent concentration of Ca in the bones and teeth of adult women. The object of the research was calcified tissues bones and teeth (n=323). Samples were taken from women aged 26 - 85 years (mean: 55.7 years), living in Poland, in the Silesian Province. Samples were submitted to wet microwave mineralization (spectrally pure nitric acid). The Ca concentration in teeth and bones was determined with the AAS method. The concentration of Ca in the tested samples of calcified tissues from women aged 26 - 85 years was 10.1-32.0%, and the average concentration was 16.7±3.5% in bones and 22.2±4.7% in teeth. The calcified tissues, from both bones and teeth, show a progressive decrease in the Ca concentration that occurs as women age. The decreasing level of Ca in bone and teeth was statistically significant (p<0.05). There was a difference in what age calcium is released from bones and teeth. The research showed that a decrease in the Ca concentration in bone tissue occurs earlier than it happens in teeth (about 10 years). The decrease in the Ca concentration in calcified tissues of teeth was slight greater than in bones, indicating slightly greater mobilization of Ca from the tissues of teeth.

Keywords: calcium, bones, teeth, AAS.

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INTRODUCTION

The inorganic part of bone tissue is mainly composed of hydroxyapatite ($Ca_{10}(OH)_2(PO_4)_6$), whose structure is 99% formed by the systemic amount of Ca (Bonjour 2011). Bone tissue is a dynamic system, in which processes of creation and resorption occur simultaneously (Reynard, Balter 2014). In adults, the resorption processes are intensified, thus minerals including Ca are removed from bone tissue (Hill 1998). An intensive decrease in the concentration of Ca in bone tissue can result in osteoporosis (Cosman et al. 2014). Women's bodies are particularly vulnerable to a decrease in the concentration of Ca because the osteolysis processes that occur with age are compounded by hormonal changes during the menopause (Makker et al. 2012, Pavel et al. 2016).

The menopause is caused by hormonal changes which occur physiologically in women's bodies. This process is defined as a naturally inhibited activity of female gonads and the loss of the ovarian follicular function. The disappearing endocrine function of the gonads has an effect on the functioning of the entire organism and can lead to dysfunction and disorders (Freeman et al. 2014, Jehan et al. 2015, Karim et al. 2015, Salerni et al. 2015, Goh, Hart 2016, Lohana, Samir 2016). Hormonal changes can contribute to disorders in the elemental balance of the body (Prokopowicz et al. 2014, Ansar et al. 2015, Schmidt 2017). One of its effects is the decreasing amount of calcium in women's bodies during the post-menopausal period (Woźniak-Holecka, Sobczyk 2014).

A variety of physiological and behavioural factors can influence the onset and course of the menopause (Poluzzi et al. 2014, Schmidt 2017). Geographical and cultural diversity is indicated as well (Shobeiri, Nazari 2014, Schmidt 2017). Hispanic and Afro-American women reach the menopause earlier, while Chinese and Japanese women enter into the menopause later than the average Caucasian women, who experience the menopause at about 51.5 years of age (Shobeiri, Nazari 2014). In the United States, 52.54 years is the median age for natural menopause (Gold et al. 2013, Schmidt 2017). However, the average age at which the menopause is diagnosed in women in Europe is 51.25 years (25^{th} percentile 49, 10^{th} percentile 43, 75^{th} percentile 54, 90^{th} percentile 56 years) (Parazzini 2007, Bjelland et al. 2018). Based on the probability of the occurrence of the menopause as defined by the 10^{th} and 90^{th} percentile, it can be assumed that the menopause occurs between 43-56 years of age (Bjelland et al. 2018).

In the research, the Ca concentration in mineralized tissues of teeth and bones of adult women of all ages was determined. We also studied changes in the concentration of Ca in calcified tissues during the perimenopausal period. The common inorganic structure of both bones and calcified tissues of teeth, based on the hydroxyapatite structure, prompted us to investigate both types of tissues in the research.

The literature shows a difference in the possibility of restructuring hydroxyapatite between bone tissue and tooth tissue. In terms of human bones, hydroxyapatite crystals as a bioactive ceramic cover 65 up to 70% of the bone by weight. Furthermore, the architecture of the bone comprises type-I collagen as an organic component and hydroxyapatite as an inorganic component. With regard to the dental role of a hydroxyapatite crystal, it makes up 70 up to 80% of dentin and enamel by weight (Habibah, Salisbury 2018). Within the human body, enamel is the hardest substance, consisting of relatively large hydroxyapatite crystals (Mate-Sanches de val et al. 2016). Contrary to bone, enamel does not contain collagen. Amelogenins and enamelins replace the function of collagen by providing a framework for mineralization (Habibah, Salisbury 2018). The mineral structure of teeth is considered more stable, subject to processes of elemental accumulation (Leventouri et al. 2009, Sehrawat, Singh 2019).

In the light of the above information, the inclusion of both teeth and bones in the research aimed at demonstrating whether these calcified tissues show differences in changes of the Ca concentration taking place in women's bodies with age.

MATERIAL AND METHODS

The material for the research consisted of samples of permanent teeth and bone (femoral head) taken from women residing in Poland, in the Silesian Province (cities: Katowice DMS: 50°15′53.611″ N 19°1′25.613″ E, Sosnowiec DMS: 50°17′10.55″ N 19°6′14.685″ E, Mysłowice DMS: 50°12′28.968″ N 19°9′57.785″ E, Bytom DMS: 50°20′54.174″ N 18°54′56.583″ E, Chorzów DMS: 50°17′50.958″ N 18°57′16.462″ E, Zabrze DMS: 50°19′29.74″ N 18°47′8.587″ E).

The study group was composed of 323 women aged 26-85 years (Table 1). The average age of women from whom teeth were taken was 45.2 years. For bone samples, the average age of women was 64.8 years. The differences in the age of women from whom bone and tooth samples were taken for the research arose from the specificity of medical indications for medical procedures during which samples were collected for research.

Teeth and bone sampling

Teeth were taken in a dentist's office during an extraction surgery carried out for medical indications. Teeth taken for the research were considered postsurgical debris. Indications for tooth extraction were caries and its complications, as well as prosthetic recommendations. The research did not include teeth with dental fillings and teeth that had undergone a root canal treatment.

Table 1

Characteristics of the study group

Specification	Age group	*N*	Years	
			min.-max.	mean
Teeth	**All**	223	26 - 75	45.2
	1	59	26 - 35	30.9
	2	62	36 - 45	40.5
	3	57	46 - 55	50.1
	4	30	56 - 65	59.7
	5	15	66 - 75	71.2
Bones	**All**	100	26 - 85	64.8
	1	6	26 - 35	30.5
	2	10	36 - 45	40.0
	3	8	46 - 55	50.5
	4	21	56 - 65	60.4
	5	37	66 - 75	69.9
	6	18	76 - 85	79.0

Samples of the femoral head taken for the research were obtained intraoperatively during a hip replacement procedure performed in treatment of degenerative and deforming changes of the hip joint. Before the research, the samples were stored at a temp. of -20ºC. The research was approved by the Bioethics Committee (SUM No. NN 6501-109/06).

The data on the condition of the women's health were collected through questionnaires. The research did not include samples of bones and teeth from women undergoing a hormone replacement therapy during the menopause or from women who took dietary supplements containing minerals.

Sample preparation

After preliminary cleaning of soft tissue, samples of teeth and bones were rinsed with running water followed by rinsing with distilled water, after which they were dried at a temperature of 60-80ºC to constant weight and ground in a porcelain mortar. From each crushed sample, an average weighted portion of approx. 0.3 g was taken for analysis. Samples were subject to microwave wet mineralization (spectrally pure nitric acid).

Determination of calcium concentration

The calcium concentration in samples was determined using the AAS method. The accuracy of measurement in bone and teeth was tested with certified reference material NIST SRM 1486 (Bone Meal). The results were

as follows: certified concentration – Ca 26.58%, marked concentration – 27.80%, recover – 104.60%.

The statistical analysis

Statistical processing of data was supported by statistical software. To determine the statistical significance, the Kruskal-Wallis ANOVA non-parametric tests by ranks were carried out. The level of significance of $p<0.05$ was considered significant statistically.

RESULTS AND DISCUSSION

The marked range of Ca concentrations in the tested samples of teeth and bones from women aged 26-85 years was 10.1-32.0%. The average Ca concentration in bones was 16.7±3.5%, and in teeth 22.2±4.7%. Figure 1 illustrates changes in Ca concentrations occurring in the tested tissues along with the age of women.

There is a gradual decrease in the Ca concentration in both teeth and bones as people age. Changes in Ca concentrations that progress with age

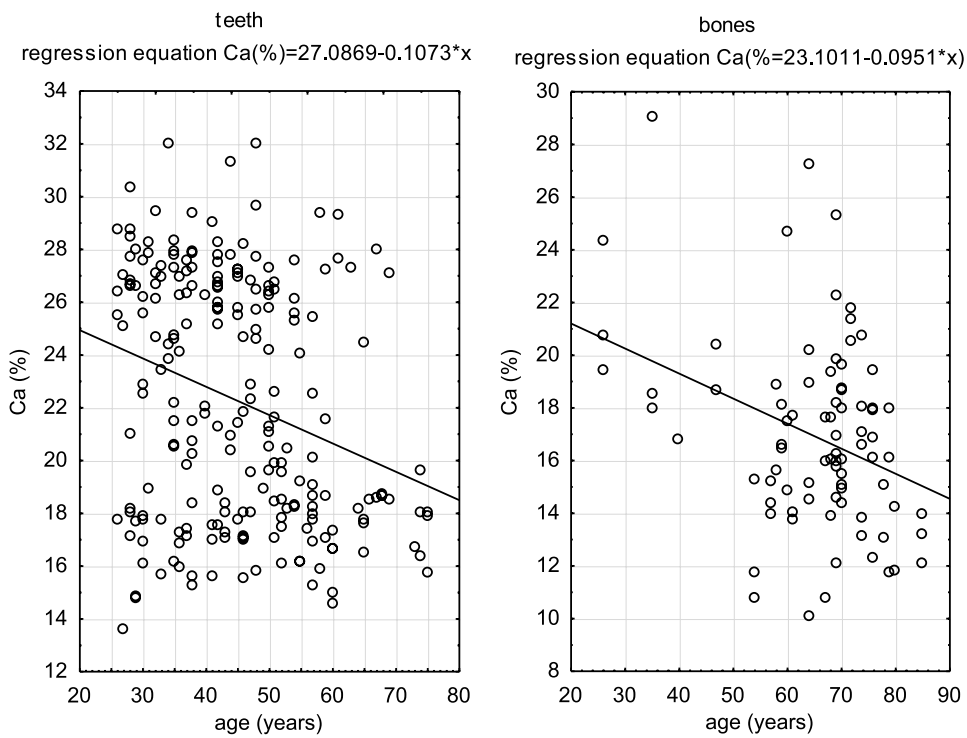

Fig. 1. Change in the concentration of Ca in bones and teeth of women dependent on age (years)

were statistically significant in both types of the studied tissues ($p<0.05$). Based on the determined regression equations, it was estimated that an annual decrease in the Ca concentration in these tissues was approximately 0.1% per year.

The analysis of changes in the Ca concentration in bones and teeth obtained from the distinguished age groups (Figure 2) indicated that the average concentration of this element was the highest in the youngest group (26-35 years), both in bones and teeth (the median values were 20.1% and 25.1%, respectively). The concentration of Ca in the subsequent age groups decreased, falling the lowest in tissues taken from the oldest women. The lowest average Ca concentration in teeth was found in samples taken from women over the age of 56 years, while in bones it was the lowest in women over 76 years of age (6th distinguished age group) – Figure 2.

The average concentration of Ca decreased in both teeth and bones of women within the determined age groups in a statistically significant way (p=0.00 for teeth, p=0.01 for bones). The greatest reduction in the average Ca concentration occurred in teeth of women aged 46-55 years (between the second and the fourth distinguished age group). For the tested bones, a decrease in the Ca concentration was most notable in women aged 36-45 years (between the first and the second distinguished age group) – Figure 2. Figure 3 shows the change in the Ca concentration in the examined women's tissues, taking into account the age at which the menopause is most often diagnosed.

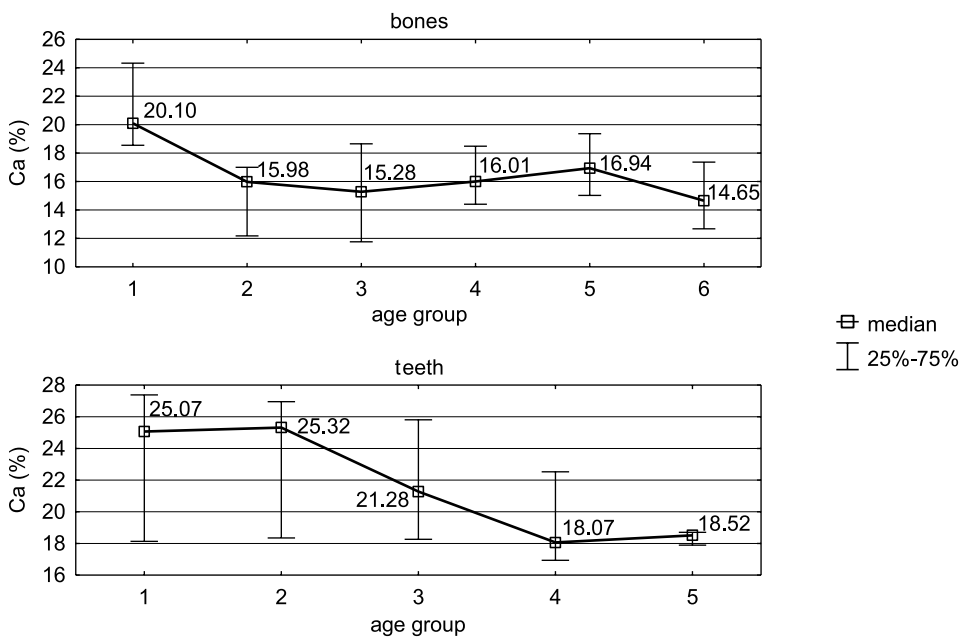

Fig. 2. Average concentration of Ca in bones and teeth of women in the distinguished age groups (1st: 26-35, 2th: 36-45, 3th: 46-55, 4th: 56-65, 5th: 66-75 6th: 76-85 years)

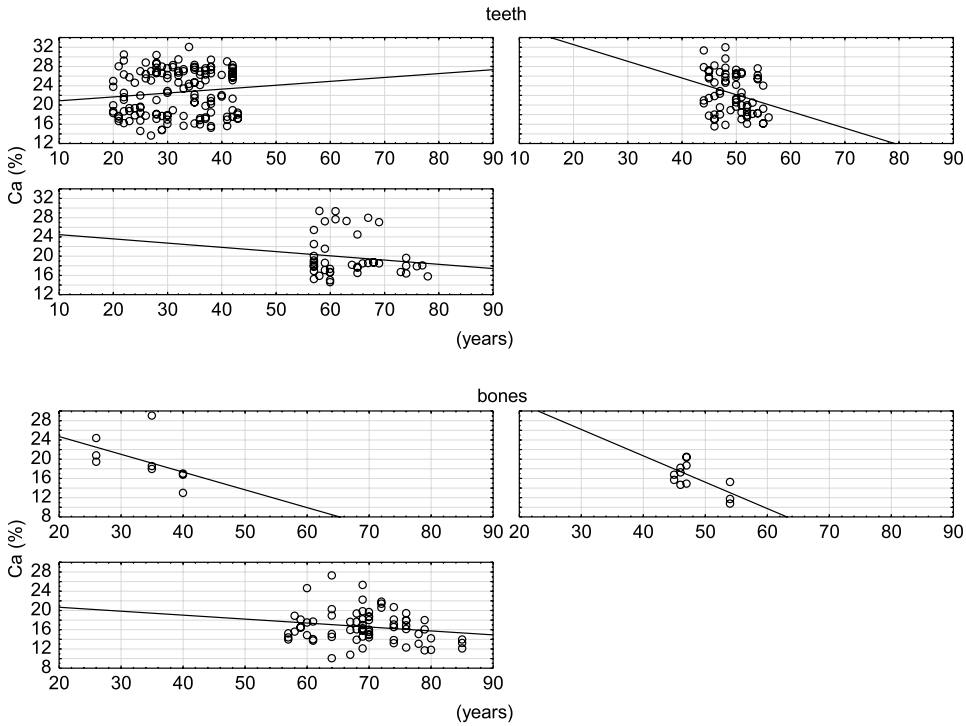

Fig. 3. Change in the concentration of Ca in bones and teeth of women in aged <42, 43-56 and >56 (years)

Assuming that the menopause in the majority of women occurs between 43 and 56 years of age, changes in the concentrations of Ca in that period of life of women as well as before and after the menopause were presented. The concentration of this element in teeth and bones of younger women, i.e. 26-42 years, served as an indicator of changes in the concentration of Ca in bone tissue in response to changes during the menopause. Due to the specific nature of the bone sampling for research (intraoperative sampling), and consequently the limited amount of material, tooth tissues were the main indicator of the level of Ca in women's bodies in the pre-menopausal period. The results showed that a significant decrease in the concentration of Ca in mineralized tissues of both teeth and bones is observed during the menopause (43-56 years).

In response to the changing conditions of life, elemental balance disorders within the body may occur. As a result of the exposure to external factors, chemical elements are introduced into the hydroxyapatite structure. Mineral substances that form the structure of hard tissues may also be subject to processes of release (Reynard, Balter 2014, Sehrawat, Singh 2019).

A variety of factors can cause the dynamic restructuring of tissues. There are many more factors which affect the mineral level of teeth than

that of bones. These effects manifest as the overall mineral content of enamel (Rizel at al. 2010, Rythén et al. 2010). Diseases, for example diabetes, have an effect on the formation of enamel and dentine (Abbasy et al. 2015). In teeth, enamel mineralization is also affected by factors such as poor oral hygiene, alcohol consumption, high intake of dietary carbohydrates. For example, lower mineralization scores for the cervical region may be associated with the hygiene index. Dental plaque can easily deactivate the enamel buffering capacity. This is a process when enamel releases calcium to normalize the saliva pH again, decreasing its mineral content (Akkus et al. 2016).

During the growth of an organism, bone formation processes dominate, which over time are replaced to a certain degree by osteolysis. Bone tissue of women is especially vulnerable to a decrease in the Ca concentration due to age-dependent hormonal changes (Goh, Hart 2016). This research focused on changes in the Ca concentration in mineralized tissues of teeth and bones of adult women of different ages. It has been found that the average Ca concentration was the highest in the examined tissues taken from the youngest women. Then, with the increasing age of women, there was a gradual decrease in the Ca concentration in samples of both bones and teeth. The research results confirm literature reports on the declining concentration of Ca in the body with time, evidenced for example by increased urinary excretion of this element in the elderly (Gallagher, Smith 2014). The decrease in the Ca concentration advancing with age in the samples of calcified tissues was statistically significant ($p<0.05$). The regression equation can be estimated at about 0.1% per year.

Literature reports (Leventouri et al. 2007) show that calcified tooth tissue is considered to be more stable in terms of its elemental construction than bone tissue. This research showed that the decrease in the Ca concentration in calcified tissues of teeth was slightly greater than in bones, indicating slightly higher mobilization of Ca from the tissues of teeth (Figure 1). It has been shown that the average Ca concentration decreased in teeth of the examined women from 25.1% (1st study group: 26-35 years) to 18.5% (5th study group: 66-75 years) and corresponded to 6% of the Ca concentration. In a similar period of life of women (26-75 years), the decrease in the average Ca concentration in bones was smaller and amounted to approximately 3% of the Ca concentration. It has been found that the decrease in the Ca concentration in bone tissue occurs earlier than in teeth. The biggest decrease in Ca in bones occurred between the first and the second distinguished age group, i.e. at the age of 36-45 years (Figure 2). The analysis of changes in Ca concentrations in calcified tissue of teeth indicated that the decrease in the concentration of this element takes place at a similar rate in women aged 46-65 years. Above 65 years of age, the Ca concentration remains constant, at the lowest level determined. In women older than 75 years, there was another decrease in the Ca concentration in bone tissue.

Observations of changes in the concentration of Ca in women over the age of 76 years concerned bone tissue only, and this was caused by a lack of teeth taken for research from women in this age group.

The average age at which the hormonal activity of female gonads is inhibited (51 years) (Woźniak-Holecka, Sobczyk 2014, Bjelland et al. 2018) reflects the research results obtained for women from the third age group (46-55 years). An approximate 15% concentration of Ca was found in bones taken from women in this age group. The concentration of this element in the analysed bone samples was comparable to the lowest Ca concentration recorded among the oldest women. An illustration of the changing concentration of Ca during the menopause, defined by the value of the 10^{th} and 90^{th} percentiles for the range 43-56 years (Kaczmarek 2007), showed an evident decrease in the Ca concentration in both bones and teeth of women in this age group (Figure 3). According to the regression equations, the average annual decrease in Ca from calcified tissues of teeth in postmenopausal women was determined to be approx. 0.3% per year. It is three-fold higher than the average decrease in Ca from the examined tooth tissue throughout the whole studied life of women. Conversely, the Ca concentration in postmenopausal women, i.e. above 56 years of age, changes at the same level of intensity and is independent of the type of the examined material (comparable decrease in calcium concentrations in bones and teeth).

In the light of the results presented above, the most interesting finding is the fact that tooth tissue is considered more stable and demonstrates increased mobilization of Ca in its structure compared to bone tissue. Unlike bones, mineralized tissue which forms teeth is in contact with the external environment. Therefore, a partial effect of exogenous factors on the maintained stability of the hydroxyapatite structure is possible.

One of the factors that may affect the mineral concentration in tooth tissue is oral hygiene agents. It has been shown that fluoride present in toothpastes, which has an anti-carries action, interacts with the hydroxyapatite structure. As a result, hydroxyapatite is converted into fluoroapatite (Rosin-Graget et al. 2013, Souza et al. 2015, Coceska et al. 2016). The production of flouroapatite leads to greater stability of the structure of hard tissues in an acidic environment (Shellis, Wilson 2004). On the other hand, literature research suggests that using toothpastes containing the fluoride compound may result in an increase in the Ca concentration in saliva (Shellis, Wilson 2004). Thus, substances that act by contacting the surface of teeth can change the concentration of mineral components in tooth tissues. In this research, it has been observed that the Ca concentration in calcified tissues of teeth is higher in the youngest female subjects (below 43 years of age). The increase in the Ca concentration in teeth of younger women (up to 42 years of age) points to the cumulative properties of tooth tissues and may indicate the greater stability of their structure. The decrease in the concentration of Ca in tooth samples is observed in women over the age of 45, mainly aged

43-56 years (Figure 3), which means that hormonal changes of the menopausal period may affect the Ca concentration in teeth. Furthermore, the higher percentage decrease of Ca in teeth compared to bones can substantiate the conclusion that the modified hydroxyapatite structure in the former tissues seems to be more unstable in its response to physiological changes. In the light of the results, the inhibition of endocrine hormone activity during menopause is manifested by greater mobilization of Ca from the structure of calcified tooth tissues compared to bone tissue.

SUMMARY

Mineral substances may be released form the structure of hard tissues. Tissues of women are especially vulnerable to a decrease in the Ca concentration due to age-dependent hormonal changes (the menopausal period). The calcified tissues analysed, both from bones and teeth, show a progressive, statistically significant decrease in the Ca concentration which occurs as women age. The decrease in the concentration of Ca was particularly intensive in the bones and teeth of women aged 43-56 years old. There was a difference in what age the calcium is released from bones and teeth. Calcium reduction occurs in the bones of younger women than in the case of teeth. The decrease in the concentration of Ca occurs in bones of women older than 35 years, and it takes place earlier than in teeth. In younger women, the calcified structure in teeth has greater elemental stability than in bones, and a decrease in the Ca concentration appears in those tissues only after 45 years of age.

CONCLUSIONS

1. Changes in the Ca concentration that progress with women's age were statistically significant ($p<0.05$) in bones and teeth.

2. The decrease in the Ca concentration in bones was observed mainly in women aged 36-45 years.

3. The greatest reduction in the average Ca concentration occurred in teeth of women aged 46-55 years.

4. It would be advisable for women to supplement calcium according to the recommended doses.

REFERENCES

Abbassy M.A., Watari I., Bakry A.S., Hamba H., Hassan A.H., Tagami J., Ono T. 2015. *Diabetes detrimental effects on enamel and dentine formation.* J Dent, 43(5): 589-596. DOI: 10.1016/ /j.jdent.2015.01.005

Akkus A., Akkus A., Roperto R., Akkus O., Porto T., Teich S., Lang L. 2016. *Evaluation of mineral content in healthy permanent human enamel by Raman spectroscopy*. J Clin Exp Dent, 8(5): 546-549. DOI: 10.4317/jced.53057

Ansar S., Alhefdhi T., Aleem A.M. 2015. *Status of trace elements and antioxidants in premenopausal and postmenopausal phase of life: a comparative study*. Int J Clin Exp Med., 15(8)10: 19486-19490.

Bjelland E.K., hofvind S., byberg L., eskida A. 2018. *The relation of age at menarche with age at natural menopause: a population study of 336 788 women in Norway*. Hum Reprod., 33(6): 1149-1157. DOI: 10.1093/humrep/dey078

Bonjour J.P. 2011. *Calcium and phosphate: a duet of ions playing for bone health*, J Am Coll Nutr., 30(5): 438-448.

Cosman F., de Beur S.J., Le Boff M.S., Lewiecki E.M., Tanner B., Randall S., Lindsay R. 2014. *Clinician's guide to prevention and treatment of osteoporosis,* Osteoporos Int., 25: 2359-2381. DOI: 10.1007/s00198-014-2794-2

Coceska E., Gjorgievska E., Coleman N.J., Gabric D., Slipper I.J., Stevanovic M., Nicholson J.W. 2016. *Enamel alteration following tooth bleaching and remineralization*. J Microsc., 626(3): 232-244. DOI: 10.1111/jmi. 12357

Freeman E.W., Sammel M.D., Sanders R.J. 2014. *Risk of long-term hot flashes after natural menopause: evidence from the penn ovarian aging study cohort*. Menopause, 21(9): 924-932. DOI: 10.1097/GME.00000000000000196

Gallagher J.C, Smith L.M., Yalamanchili V. 2014. *Incidence of hypercalciuria and hypercalcemia during vitamin D and calcium supplementation in older women*. Menopause**,** 21(11): 1173-1180. DOI: 10.1097/GME.0000000000000270

Goh V.H., Hart W.G., 2016. *Aging and bone health in Singaporean Chinese pre-menopausal and postmenopausal women*. Maturitas, 89: 16-21. DOI: 10.1016/j.maturitas.2016.04.004

Gold E.B., Crawford S.L., Avis N.E., Crandall C.J., Matthews K.A., Waetjen L.E., Lee J.S., Thurston R., Vuga M., Harlow S.D. 2013. *Factors related to age at natural menopause: longitudinal analyses from SWAN*. Am J Epidemiol., 178(1): 70-83. PMID: 23788671, https://doi.org/10.1093/ aje/kws421

Habibah T.U., Salisbury H.G. 2018. *Hydroxyapatite dental materials*. Stat Perarls Publishing, Treasure Island (FL). PMID: 30020686.

Hill P.A. 1998. *Bone remodelling*. Br J Orthod., 25: 101-107.

Jehan S., Masters-Isarilov A., Salifu I., Zizi F., Jean-Louis G., Pandi-Perumal S.R., Gupta R., Brzezinski A., McFarlane S.I. 2015. *Sleep disorders in postmenopausal women*. J Sleep Disord Ther*.,* 4(5): 2-18. DOI: 10.4172/2167-0277.1000212

Kaczmarek M. 2007. *The timing of natural menopause in Poland and associated factors*. Maturitas, 57(2): 139-153.

Karim R., Stanczyk F.Z., Brinton R.D., Rettberg, J., Hodis H.N., Mack W.J. 2015. *Association of endogenous sex hormones with adipokines and ghrelin in postmenopausal women*. J Clin Endocrinol Metab., 100(2): 508-515. DOI: 10.1210/jc.2014-1839

Kondo K.Y., Buzalaf M.A., Manarelli M.M., Delbem A.C., Pessan J.P. 2016. *Effects of pH and fluoride concentration of dentifrices on fluoride levels in saliva, biofilm, and biofilm fluid in vivo*. Clin Oral Invest., 20(5): 983-989. DOI: 10.1007/s00784-015-1583-4

Leventouri T., Antonakos A., Kyriacou A., Venturelli R., Liarokapis E., Perdikatsis V. 2009. *Crystal structure studies of human dental apatite as function of age*. Int J Biomater., 2009: 698547. DOI:10.1155/2009/698547

Lohana C.K., Samir N. 2016. *Risk management of osteoporosis in postmenopausal women; A study of women in a teaching hospital*. Glob J Health Sci., 1,8(11): 36-44. DOI: 10.5539/ /gjhs.v8n11p36

Makker A., Singh M.M., Mishra G., Singh B.P., Jain G.K., Jadhav S. 2012. *Relationship between*

bone turnover biomarkers, mandibular bone mineral density, and systemic skeletal bone mineral density in premenopausal and postmenopausal Indian women. Menopause, 19(6): 642-649. DOI: 10.1097/gme.b013e31823dbbf7

Mate-Sanches de Val J.E., Calvo-Guirado J.L., Gomez-Moreno G., Perez-Albacete Martinez C., Mazon P., de Aza P.N. 2016. *influence of hydroxyapatite granule size, porosity, and crystallinity on tissue reaction in vivo. part a: synthesis, characterization of the materials, and SEM analysis*. Clin Oral Implants Res., 27(11): 1331-1338.

Morabia A., Constanza M.C. 1998. *International variability in ages at menarche, first livebirth, and menopause*. Am J Epidemiol., 148(12): 1195-1205.

Parazzini F. 2007. *Determination of age at menopause in women attending menopause clinics in Italy*. Maturitas, 56: 280-287.

Pavel O.R., Popescu M., Nonovac L., Mogoantă L., Pavel L.P., Vicaș R.M., Trăistaru M.R., 2016. *Postmenopausal osteoporosis – clinical, biological and histopathological aspects*. Rom J Morphol Embryol, 57(1): 121-130.

Poluzzi E., Piccinni C., Raschi E., Rampa A., Recanatini M., Fabrizio De Ponti F. 2014. *Phytoestrogens in postmenopause: the state of the art from a chemical, pharmacological and regulatory perspective*. Cur Med Chem, 21: 417-436.

Prokopowicz A., Pawlas N., Ochota P., Szuła M., Sobczak A., Pawlas K. 2014. *Blood levels of lead, cadmium and mercury in health women in their 50s in a urban area of Poland: a pilot study*. Pol J Environ Stud., 23(1): 167-175.

Reynard B., Balter V. 2014. *Trace elements and their isotopes in bones and teeth: Diet, environments, diagenesis, and dating of archeological and paleontological*. Palaeoecology, 416: 4-16. DOI:10.1016/j.palaeo.2014.07.038

Rizell S., Kjellberg H., Dietz W., Norén J.G., Lundgren T. 2010. *Altered inorganic composition of dental enamel and dentin in primary teeth from girls with Turner syndrome*. Eur J Oral Sci, 118: 183-190.

Rosin-Graget K., Pedros K., Sutej I., Bašić K. 2013. *The cariostatic mechanisms of fluoride*. Acta Med Acad., 42(2): 179-188. DOI: 10.5644/ama2006-124.85

Rythén M., Sabel N., Dietz W., Robertson A., Norén J.G. 2010. *Chemical aspects on dental hard tissues in primary teeth from preterm infants*. Eur J Oral Sci., 118(4): 389-395. DOI: 10.1111/j.1600-0722.2010.00755.x

Salerni S., Di Francescomarino S., Cadeddu C., Acquistapace F., Maffei S., Gallina S. 2015. *The different role of sex hormones on female cardiovascular physiology and function: not only oestrogens*. Eur J Clin Invest., 45(6): 634-645. DOI: 10.1111/eci.12447

Shen C., Wen J., Pan X., Su J., Du W., Pan E., Zhang Q., Zhang N., Sheng H., Liu C., Wang H., Wu M., Qin Y. 2018. *Age at menarche and age at natural menopause as predictors of glycemic control in type 2 diabetic patients*. J Diabetes Complications, 32(7): 623-629. DOI: 10.1016/j.jdiacomp.2018.04.012

Shobeiri F., Nazari M. 2014. *Age at menopause and its main predictors among Iranian women*. Int J Fertil Steril., 8(3): 267-72.

Souza B.M., Comar L.P., Vertuan M., Fernandes Neto C., Buzalaf M.A., Magalhães A.C. 2015. *Effect of an experimental paste with hydroxyapatite nanoparticles and fluoride on dental demineralisation and remineralisation in situ*. Caries Res., 49(5): 499-507. DOI: 10.1159/000438466

Schmidt C.W. 2017. *Age at menopause: do chemical exposures play a role?* Environ Health Perspect, 125(6): 062001. DOI: 10.1289/EHP2093

Shellis R.P., Wilson R.M. 2004. *Apparent solubility distributions of hydroxyapatite and enamel apatite*. J Coll. Interf. Sci., 278(2): 325-332.

Sehrawat J.S., Singh M. 2019. *application of trace elemental profile of known teeth for sex and age estimation of Ajnala skeletal remains: a forensic anthropological cross-validation stud.* Biol Trace Elem Res., DOI: 10.1007/s12011-019-01712-8

Thomas F., Renaud F., Benefice E., de Meeüs T., Guegan JF. 2001. *International variability et menarche and menopause: patterns and main determinants.* Hum Biol., 73(2): 271-290.

Woźniak-Holecka J., Sobczyk K. 2014. *Nutritional education in the primary prevention of osteoporosis in perimenopausal and postmenopausal women.* Prz Menopauz., 18(1): 56-63. DOI: 10.5114/pm.2014.41087 2014