***Original article*:**

Type ii osteoporosis pathogenesis as a result of secondary edentulous

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Abstract

Purpose: The main sign of osteoporosis is a significant decrease of bone mass that is caused by imbalance between resorption and osteogenesis. There are two main factors that contribute to the genesis of this illness. All these factors bring todecrease of thickness of bone and its destruction doesn't depend on their combination. Therefore, our objective was to study the changes in the alveolar bones of elderly people in secondary edentulous. ***Material and Methods:*** For deeper study of morphological changes in segments of teeth we have conducted pathological researches of biopsy material of dead patients at the Center of Pathological Studies of the Sumy Medical University. The first group of patients included n=7 who died of various somatic abnormalities and had no significant atherosclerotic lesions. The second group includes segments of teeth and jaws of patients n=7 who died from complications of atherosclerosis In our studies, we used: Histological study; Fluorescence microscopy; Immunohistochemistry; Microphotography and image analysis; Mathematical calculations. ***Results*:** Based on the statistical analysis we can see a downward trend in the number of osteocytes in trabeculae of the periapical third part of the tooth at atherosclerosis 26.85±7.44; P=0.05. We can also see the dependence of trabecular thinning of toothless area of the alveolar bone 226.57±70.53; P=0.02 from losing of teeth against the background of atherosclerosis with hypertension. The osteopontin expression and fluorescencetion of toothless bone area also tend to decrease in atherosclerosis and hypertension 42.81±16.24; P=0.048. ***Conclusion:*** The cause of tooth loss is difficult to determine, since it is not clear whether it is due to osteoporosis or some forms of periodontitis in patients. The bone resorption has actived by reactive oxygen species both during atherosclerosis. Through our research found out that in order to reduce changes in the toothless bone we should reduce the pressure on chewing toothless alveolar sprout by dental implants.

Key words: bone resorption; osteolysis; osteoporosis of type II.

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Introduction

The main problem of women and elderly people of both sexes nowadays is osteoporosis. The widespread consequence of osteoporosis is cervical hip fracture that brings down quality of life and is difficult to treat especially if old people are affected. According to statistics 10-20% of people with cervical hip fracture die within half a year, 50% never regain full activity of the joint and they cannot walk without help, and 25% demand constant care[1].

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The main sign of osteoporosis is a significant decrease of bone mass that is caused by imbalance between resorption and osteogenesis. There are two main factors that contribute to the genesis of this illness. The first is dysfunction of gonads and the second is aging. After the age of 40-50 years people start to loose from 0.3 to 0.5% of bone mass per year. These numbers grow as much as 10 times after menopause (women) or castration (men) [2].

In the process of bone involution a significant loss of bone stock occurs that brings to senile osteoporosis. The bone mass decreases with the age. This fact is proved by radiologic, X-ray, histologic and other methods [3].

Bones of old people are more fragile especially in the places where there is more of spongy substance. With aging the ratio of soluble and non-soluble collagen significantly changes in favour of the last one. Elasticity of a bone depends on organic substances and its hardness depends on mineral substances. Combination of organic and non-organic components gives sturdiness and elasticity to bones. Bones of small kids that contain more organic substances differ in big elasticity and seldom break. In the old age when non-organic components prevail bones on the contrary become less elastic and more fragile. As a result old people get fractures more often [3].

Many definitions of osteoporosis that described different results like fractions and fragility of bones were offered during many years. More precise and coherent definition of this disease that covers the whole spectrum of its symptoms and after-effects of bone mass loss is the definition of osteoporosis as a disease that is characterized by low bone mass and disturbance of microarchitecture of bone tissue that brings to an increase in fragility of bones and successive increase in fracture risks[4].

Fractures are clinical symptoms of osteoporosis. They can be vertebral fractures, such as "Colles" fracture of the distal forearm, and hip fractures. But if thickness of bone tissues decreases fractures may happen in other places[5].

Depending on the origin there are two types of osteoporosis. Primary osteoporosis appears in postmenopause or as a result of body's aging (senile osteoporosis). Secondary osteoporosis is characterized by the loss of bone tissue as the result of thyrotoxicosis or hyperadrenocorticism[6]. Still it remains unexplored what kind of changes may occur in alveolar bone after the loss of teethIn 1948 Albright and Reifenstein[7] offered to divide primary osteoporosis into two types because on the one hand it is connected with loss of estrogen in menopause and on the other hand with body aging. This conception was representedby Riggs and associates [8] who offered to define loss of sponge tissue of bone after menopause by term "osteoporosis of type I" and loss of sponge and cortical substance by men and women in the result of aging as "osteoporosis of type II".That means that type I is only the result of absence of endogenous estrogen in woman's body; type II reflects combined influence of effectiveness of bone resorption, secretion of parathyroid hormones, sufficient amount of calcium and vitamin D and digestion of mineral components by a human body. Recent studies have indicated that there is coexistence of nutritional deficiencies and appreciable over nutrition in the form of central obesity and overweight. In developing countries one quarter of the population is obese, which may be risk factors for cardiovascular disease and osteoporosis[9,10].

There are certain diseases that influence the process of bones mineralization, cause their dysfunction and increase the risk of osteoporosis. They can be genetic and acquired. One of them is leucosis[11-15]. We should understand that bones in the adult age reach the peak of bone mass that starts to decrease with age. All these factors bring todecrease of thickness of bone and its destruction doesn't depend on their combination. Therefore, our objective was to study the changes in the alveolar bones of elderly people in secondary edentulous.

Materials and methods

*Pathomorphologicalresearches.*Segments of jaws from live patients don't meet the standards of orthopedic treatment, so segments of teeth and jaws for histopathological researches were taken from dead patients. Patients under the study have died while being hospitalized in the departments of Sumy Regional Hospital.

For deeper study of morphological changes in segments of teeth we have conducted pathological researches of biopsy material of dead patients at the Center of Pathological Studies of the Sumy Medical University.

All patients were divided into two groups. The first group (test group) of patients included n=7 who died of various somatic abnormalities and had no significant atherosclerotic lesions. The second group (study group) includes segments of teeth and jaws of patients n=7 who died from complications of atherosclerosis (myocardial infarction, hemorrhagic

stroke, mesenteric thrombosis).

Criteria to include the patients into the researches were as follows:

- the patient had defected jaws;
- male 45-56 years;
- the patient's voluntary consent to treatment, signed while he was alive;
- examination and referral to autopsy signed by the head of the medical unit;

Taking autopsy material (toothless alveolar process and 2 alveolar bones with a tooth) for histological examination carried out by Luer nippers. Luer nippers have the round shape working part with a cavity inside, where cut fragments of investigated alveolar process were located.

Histological study. Preparations stained with hematoxylin-eosin to study the morphological structure. Methods of dyeing with hematoxylin-eosin of our modifications included the following steps: (a) fixing material in the liquid Karnua; (b) decalcification in 17% EDTA and putting material in paraffin; (c) washing de-paraffined sections in two changes of absolute ethanol; (d) processingsections with hematoxylin for 5 hours at 37 ° C; (e) washing with running water for 2-5 minutes; (f) coloration sections with eosin 5 seconds at room temperature; (g) dehydration in alcohol, xylene and washing in 2 changes karbol-xylene; (h) placing in Canadian balsam.

Fluorescence microscopy. Sections for coloring were applied to a glass slide and got dried in an incubator at 37°C for 12 hours. De-paraffining was performed according to the standard method by xylene and ethanol. Washed with 0.9% sodium chloride solution. 0.5 ml 0.01% solution of acridine hydrochloride in 0.5M acetate buffer was applied on materials. Incubated for 15 minutes at 37°C. Washed thoroughly with tap water and allowed to dry. The structure of teeth segments was studied with the help of fluorescent microscope МБИ 15 using immersion lenses.

The results of fluorescence microscopy were assessed by fluorescence intensity of bone by percentage of evaluation:

- lack of fluorescence - 0%;
- low positive fluorescence + = 33,3%;
- medium positive fluorescence ++ = 66,6%;
- highly positive fluorescence +++ = 100%.

Immunohistochemistry. Paraffin sections have been produced with thick 3 - 5 microns, de-paraffined by the standard method followed by washing in PBS with the pH 7,4 rate.

De-masking was continued for 30 minutes in a citrate buffer. To block endogenous peroxidase sections were incubated for 10 minutes in 1% H_2O_2 solution and washed in phosphate buffer (PBS). Then they were incubated in a humid chamber at temperature of +37°C for 30 minutes with serum to block nonspecific antibody binding. Primary antibodies against OPNT were used. Then sections were incubated in a humid chamber at temperature of +37°C for 30 minutes with secondary species-specific, conjugated with horseradish peroxidase antibody (1:200) produced by «Jackson Immuno Research» (USA). Sections were washed from primary and secondary antibodies three times in PBS with addition of 0,1% Tween-20. Visualization of antigen-antibody complex was carried out using 3,3'-diaminobenzidine activator peroxidase (DAB), where sections were incubated for 2-3 minutes. The nuclei with negative reaction were extra painted by Mayer hematoxylin.

The results of reaction of antigens with bone localization (OPN) were assessed by the percentage of evaluation:

- no reaction - 0%;
- low positive reaction + = 33,3%;
- medium positive reaction ++ = 66,6%;
- highly positive reaction +++ = 100%.

Microphotography and image analysis. Photographing performed with the microscope «Carl Zeiss» with lenses of 10 ×, 40 ×, 60 × and 100 ×. Images have been shot with a digital camera «DCM310» with a resolution of 5.0 M pixels. Image specimens have been analyzed in a computer environment morphometric program «Digimizer» and determined the average values of morphometric parameters: the number of osteoblasts and osteoclasts, the thickness of the cortical layer of bone, trabecular number and area.

Mathematical calculations have been performed in the program STATISTICA 8 (Serial number 31415926535898) using cluster analysis and Ansar Bradley criteria.

Results

Jaw bone serves as a support organ of the mouth, is a reserve of macro- and micronutrients, performs exchange function, and forms a cavity for bone marrow. Jaw bone is sensitive to different regulatory mechanisms, as well as exogenous and endogenous

influences. Adentia enhances endogenous and exogenous factors on the alveolar bone. According to the Wolf's law of transformation any change of function entails anatomical and structural transformation. So adentia causes changes in the bone tissue.

Toothless jaw bone areas are all covered by periosteum. Periosteum of the alveolar process increases the elasticity, firmness and resistance of the bone to mechanical stress during the act of chewing. We observed two layers of outer periosteum (fibrous) (***Figure 1***) and internal (***Figure 2***) osteogenic of the dead patients in the control group.

The cell structure of the fibrous layer is represented by a small number of immune cells and fibroblasts, which are located along the collagen fibers.

The inner osteogenic layer of the periosteum is formed by osteocytes and osteoblasts, which gradually form a small number of osten (***Figure 2-B***).

Toothless jaw portion of patients with hypertension and atherosclerosis has significant morphological changes in the periosteum (***Figure 3***). There is a significant swelling between collagen fibers of the fibrous layer of the periosteum (***Figure 3-C***). There is a significant lacunar resorption in the osteogenous layer (***Figure 3-A***), which reaches osten. The number of osten rows decreases to 6±3.

Sponge filling toothless alveolar ridge forms a lamellar bone, which in its turn forms trabeculae (***Figure 4***). Trabeculae are composed of several layers of bony plates 8±2. Trabecular thickness varies and ranges from 0.3-0.8 mm. Trabeculae are covered by endoost (***Figure 4-V***). Trabeculae space is formed by loose connective tissue with blood vessels (***Figure 4-C***).

A common contraindication for dental implants is cardiovascular disease, which in most cases is caused by hypertension, atherosclerosis and arteriosclerosis. Atherosclerosis and arteriosclerosis lead to poor blood supply to the bone and as a result to ischemia. On the one hand changes in the spongy bone of the toothless alveolar process are caused by atrophy under the influence of masticatory load. On the other hand they are cumulated by atherosclerosis and arteriosclerosis violations in average diameter arteries and potentiate changes in arterioles on the hypertension background.

Patients of experimental group had macroscopic changes in all parts of the aorta (***Figure 5***).

Figure 6 demonstrates changes of arterioles spongy alveolar process of the A3 patient on a background of significant atherosclerotic lesions of the abdominal aorta and hypertension of III stage rate 3.

Combination of hyalinosis, hypertrophy and necrosis of the vascular wall was observed in all the investigated samples of the patients with hypertension and atherosclerotic lesions in the aorta.

Sponge filling toothless alveolar process has changed in most patients of the experimental group. We observed the absence of endoost in a large space of trabeculae (***Figure 7-B***). Lacunar resorption is progressed in trabeculae (***Figure 7-B***). Bone plates were partially resorptedand had scalloped look. Out of trabekulae space was characterized by the growth of connective tissue (***Figure 7-C***). Osteoclasts, which formed gaps, were seen on the surface of trabeculae.

These changes indicated a strengthening of atrophy of alveolar process against the background of somatic disorders.

Fibers of collagen-like proteins, collagen and elastin, which surrounded connective tissue cells, as well as osteoblasts and osteocytes have the ability to fluorescent under the influence of ultraviolet radiation. We have examined the distribution of collagen-like bone proteins using auto-fluorescent.

So the spatial orientation of the collagen-like proteins in a bone (proteoglycans, which are related to the 1st type of collagen and glycoprotein) could be localized.

Based on our observations it could be argued that unstructured placing of collagen-like proteins in trabeculae was observed in patients with hypertension and aortic atherosclerotic (***Figure 8-A***). Trabeculae of patients without atherosclerosis had a linear distribution of collagen-like proteins. Fluorescence of the trabeculae was little more intense, and it corresponded to the number of amino which were composed of a ring chemical structure (tryptophan, proline, tyrosine, histidine, phenylalanine) (***Figure 8-C***).

During the study of the periapical part of the alveolar bone, bone tissue was composed of external and internal cortical plates. The direction of trabecular bone responded the vector of mechanical stress on the teeth. The control group of patients (***Figure 9-I***) had a uniform periodontal slit with a properly arranged collagen fibers without pathological changes.

Patients with atherosclerosis and hypertension (***Figure 9-II***) were characterized by the resorptive changes in external and internal cortical plates to form resorption lacunae (***Figure 9-D***). Resorption lacunae were filled with fibrous tissue with randomly arranged collagen and elastic fibers. Changes in the cement of the tooth root were not observed in the

studied groups. Changes in the periapical part of the alveolar bone in patients with atherosclerosis and hypertension were more in the toothless area of alveolar process. We also noted the presence of hyalinosis arterioles in periapical arteries, indicating a complicated course of hypertension, ischemia and resultant periodontal tissues.

Epithelium of toothless jaws area (control group), after the tooth had been extracted, was characterized by changes due to the influence of masticatory load. Outgrowths in lamina propria were small in size. Stratification of epithelium was preserved. The surface was capable to keratinization (***Figure 10-I***). Epithelium looked like "chewing-type epithelium".

Patients with atherosclerosis and hypertension had changes in the epithelium of toothless jaw area compared with the control group. Outgrowths in lamina propria were significant in the basal layer (***Figure 10-II***). Stratification of epithelium had no changes. The surface of epithelial had the signs of desquamation.

The patients' osteocytes from control group had a strong and high expression of osteopontin (***Figure 11-A***). Osteopontin had an ability to bind quite firmly to hydroxyapatite, and connect with mineralized bone tissue. Bone tissue of toothless alveolar bone had a significant decrease in the expression of osteopontin of the patients with arteriosclerosis. We observed the absent of osteopontin expression in osteocytes on the surface of trabeculae (***Figure 11-B***), but in the middle of trabecular the weak expression of osteopontin was preserved (***Figure 11-C***).

In our studies we have analyzed a number of morphological parameters in study groups:

- the thickness of the cortical bone;
- number of cortical bone osteocytes and osteoclasts;
- the thickness of the trabecular toothless area of alveolar bone and periapical third part of the tooth;
- number of osteoclasts and osteocytes in trabecular periapical third part of the tooth and toothless alveolar bone.

We have also statistically processed a degree of bone fluorescencetion and volume of osteopontin expression.

In our studies we have used xi-squared test, implemented in XI2TECT function in Excel to test the normality. The arguments were the range of the control group and the range of the experimental group for the appropriate intervals.

XI2TECT function in Excel calculates the probability of the observed convergence of control and experimental values. If the calculated probability was lower than the significance level (0.05), the null hypothesis would be rejected and confirmed that the observed values did not meet the normal distribution. If the calculated probability was close to 1, then a high degree of compliance of experimental data to normal distribution would be confirmed. The obtained data are presented in ***Table 1***.

Analysis of the data presented in Table 1 shows that five out of twelve indicators correspond to normal distribution, and the rest of them do not meet the normal distribution. On the basis of used xi-square test we can test the hypothesis of equality of samples Ansari-Bradley criterion. The results of samples analysis are given in ***Table 2***.

Based on the statistical analysis we can see a downward trend in the number of osteocytes in trabeculae of the periapical third part of the tooth at atherosclerosis P=0.05. We can also see the dependence of trabecular thinning of toothless area of the alveolar bone P=0.02 from losing of teeth against the background of atherosclerosis with hypertension. The osteopontin expression and fluorescencetion of toothless bone area also tend to decrease in atherosclerosis and hypertension P=0.48 and P=0.49.

In order to clarify the pathogenic links between statistically significant indicators (fluorescencetion of the toothless bone area of the alveolar process, %; expression of osteopontin, %; the thickness of the trabecular toothless area of the alveolar bone, microns; number of osteocytes in the trabecular periapical third part of the tooth with increasing) we have conducted cluster analysis using the method of k-means. Based on cluster analysis we have created dendrohrama (***Figure 12***).

Indicators of the first group have been merged into a cluster on the first stage of clustering (***red line Figure 12***). The first cluster describes the changes of organic component of the bone. It can be argued with certainty that reducing expression of osteopontin causes changes in the organic part of the bone in hypertension with arteriosclerosis.

Ateriosclerosis in combination with hypertension leads to changes of the number of osteocytes in trabeculae of the periapical third part of the tooth, which causes changes of thickness in trabeculae of the toothless alveolar bone. These figures are included into the second cluster (***Figure 12 green line***) and determine the progression of secondary osteoporosis.

Discussion:

Osteoporosis may affect the jawbones [16]. In addition, these modifications might potentially speed up the periodontal tissues breakdown caused by periodontitis [17].

The second disease that increases possibility of osteoporosis is pancreatic diabetes of type II. These patients have heightened BMD. It is confirmed by the results of meta-analysis [18] but the risk of osteoporosis also increases. Our studies have demonstrated the existence of some other potentially important periodontal risk factors of osteoporosis in the jaw. Atherosclerosis and toothless jaw increase the risk of the second type of osteoporosis in the jaw. According to some studies, osteoporosis may be a very important factor of adentia [19-21].

Kribbs et al. [22] Ana Pejčićet al.[23] compared patients with osteoporosis and without it and found out that the osteoporoticgroup comprised more subjects with no teeth or with agreater number of lost teeth. Our studies showed morphological changes that confirmed the clinical opinions of Kribbs et al., Ana Pejčićet al. Diabetes, atherosclerosis and adentia have formatted bone tissue disorders and its strength decreases. It is confirmed by the investigations that point out at decrease of osteoblasts activity [24] that slows down the curation process after fractures[25]. Oxidative stress has played a part in the development of osteoporosis [26]. Reactive oxygen species has oxygen-containing molecules that are produced during normal metabolism.

When the production of the damaging reactive oxygen species exceeds the capacity of the body's antioxidant defenses to detoxify them, a condition known as oxidative stress occurs [27]. Reactive oxygen species can cause bone tissue damage, particularly in the endothelial tissue [28]. Lipids and lipoproteins are also affected by reactive oxygen species. The oxidative modification hypothesis suggests that lipids and proteins oxidation in the vascular wall may cause atherosclerosis.

Our studies have showed that the fluorescence decrease of bone toothless alveolar process in patients with hypertension and atherosclerosis is lower compared with the patients of the test group. Hydroxyproline and pyridine promote bone strength by the covalent bonds between amino acids comprising the collagen polypeptide chains. Reduced hydroxyproline and pyridine lead to the decrease of fluorescence and to a consequent decrease of the number of bonds between molecules of collagen within the bone. We observed osteopontin reduction in patients with atherosclerosis and hypertension. We believe that reducing the synthetic activity of cells associated with cells hypoxia is caused by arteriosclerosis and reactive oxygen species. Hypoxia promotes osteolysis with the acid metabolic products in osteocytes. Metabolic acidosis forms a large amount of heavy acids as pyruvates and lactates.

The results of our studies have shown the association of osteopontin expression and the level of the bone fluorescence in the cluster analysis, that decrease the expression of osteopontin which leads to acidosis due to decrease in production of the bone matrix and consequently reducing the thickness of trabeculae. Osteolysis by osteocytes causes the expansion of bone tubules. Compact layer and spongy substance of the toothless alveolar bone in the control and experimental group are associated with the synthesis and secretion of proteolytic enzymes and acid metabolic products in osteocytes under the influence of chewing pressure. Under the influence of arteriosclerosis this process is more intense. These causes decreased the number of osteocytes around tooth root, as a result of the bone thinness. Spongy bone regeneration is an area of the alveolar ridge. It is possible to assume that changes in bone resorptive in the adjacent area of the defect of teeth contribute to the development of secondary osteoporosis. The number of osteocytes and trabecular thickness of toothless alveolar process indicate in favor of this. Osteocytes and trabecular thickness have merged into one cluster in the statistical analysis.

Conclusion

The cause of tooth loss is difficult to determine, since it is not clear whether it is due to osteoporosis or some forms of periodontitis in patients. The bone resorption has actived by reactive oxygen species both during atherosclerosis. Through our research found out that in order to reduce changes in the toothless bone we should reduce the pressure on chewing toothless alveolar sprout by dental implants.

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Authors' contributions: OB and VB responsible for the study design. KY analyzed and interpreted the data. DO wrote the report. LM and GO did the laboratory work. All authors read, commented and approved the final article.

Ethics: All studies were conducted in accordance with ethical standards of Ukraine Health Ministry and expertise in bioethics commission from the Sumy State University protocol No. 013U003379.

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Conflict of interest: The authors declare that they have no conflict of interest.

Table 1:Probability of matching of controled and obsered values of studied groups

Name intervals												
	Fluorescencetion of toothless bone area of alveolar process	Fluorescencetion of bone of periapical third part	Expressio of osteopontin	The thickness of the cortical bone, microns	Number of cortical bone osteocytes	Number of cortical bone osteoclasts	The thickness of the trabecular periapical third part of the tooth, microns	The thickness of the trabecular toothless area of alveolar bone, microns	Number of osteoclasts in trabecular periapical third prt of the tooth	Number of osteocytes in trabecular periapical third prt of the tooth	Number of osteoclasts in trabecular toothless alveolar bone	Number of osteocytes in trabecular toothless alveolar bone
Value	1.17554E-72	2.03199E-12	2.7296E-58	0	0.001	0.533	1.30676E-35	4.7207E-304	0.868	0.326	0.829	0.768

Table 2: Investigated indicators of patients groups and Ansari-Bradley's criteria values

Sample name	m±SDat n=7, Group 1	m±SDat n=7, Group 2	P value of Ansari-Bradley criterion
Fluorescencetion of toothless bone area of alveolar process, %	76.15±25.21	42.81±16.24	0.049*
Fluorescencetion of bone of periapical third part, %	61.84±12.58	52.32±17.79	0.58
Expressio of osteopontin, %	76.14±16.29	42.81±16.24	0.048*
The thickness of the cortical bone, microns	4230±622.76	2357.71±554.32	1
Number of cortical bone osteocytes, increased × 150	36.42±4.75	28.71±4.57	0.43
Number of cortical bone osteoclasts, increased × 150	2±1.15	3±0.81	1
The thickness of the trabecular periapical third part of the tooth, microns	254.28±52.27	201.85±37.42	0.68
The thickness of the trabecular toothless area of alveolar bone, microns	376.28±204.38	226.57±70.53	0.02*
Number of osteoclasts in trabecular periapical third prt of the tooth, increased × 150	2.28±0.75	3.14±0.69	0.9
Number of osteocytes in trabecular periapical third prt of the tooth, increased × 150	27±3.69	26.85±7.44	0.05*
Number of osteoclasts in trabecular toothless alveolar bone, increased × 150	2.57±0.53	3.14±0.69	0.7
Number of osteocytes in trabecular toothless alveolar bone, increased × 150	27.57±4.89	26.57±4.07	0.69
P = 0.05 - a trend at f = n-1			

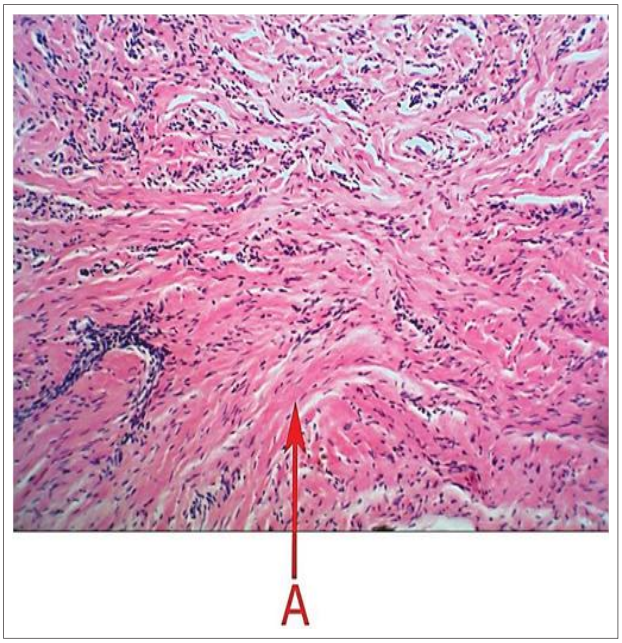

Figure 1: Fibrous bone layer of control group.Increased×150. The color is hematoxylin-eosin: A - fibrous fiber.

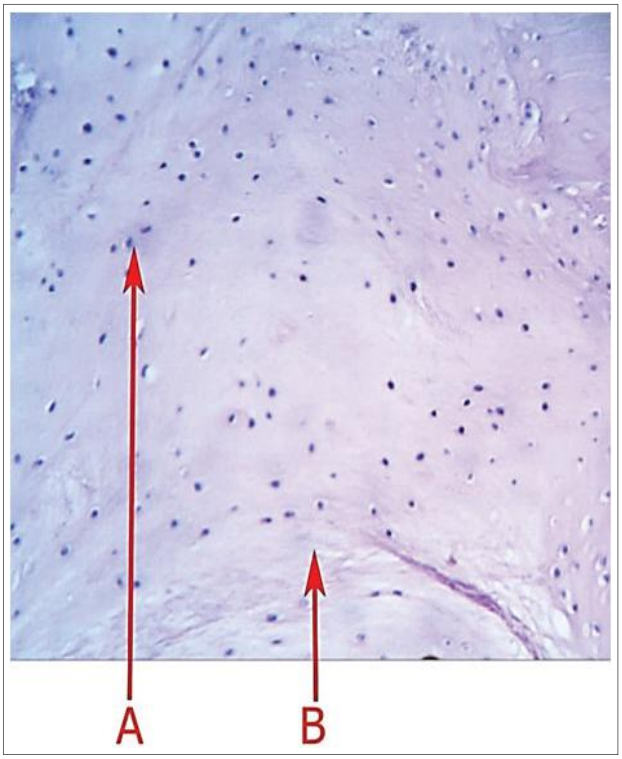

Figure 2. Osteogenic bone layer of control group.Increased×150. The color of hematoxylin-eosin: A - endless bone layer (general plate); B - lines of cementing surface osteon

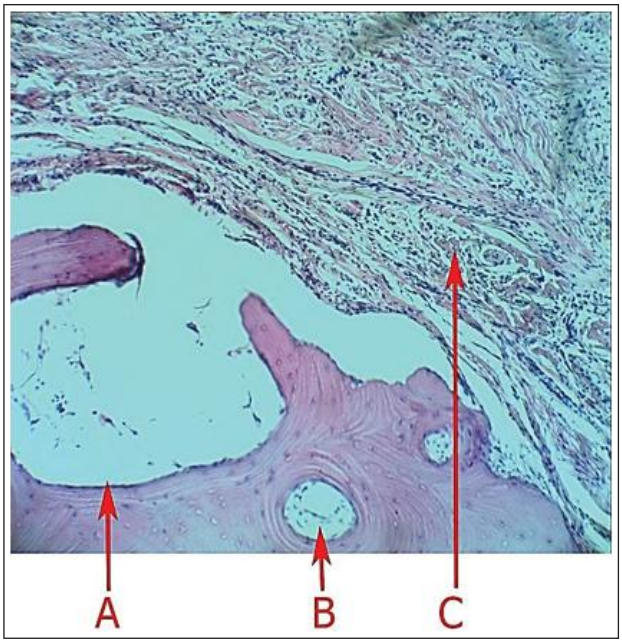

Figure 3. Osteogenic bone layer control group.Increased ×50. The color of hematoxylin-eosin: A - lacuna resorption; B - osteon; C - fibrous layer of the periosteum with significant changes of edema

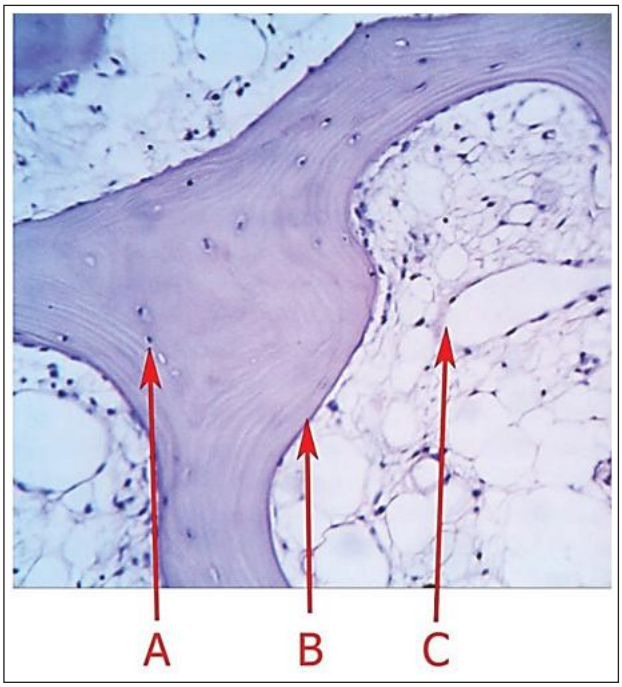

Figure 4. Sponge filling of toothless alveolar process of control group.Increased ×150. The color of hematoxylin-eosin: A - layers of bony plates; B - endoost with no signs of resorption; C – out of trabeculae space formed with vessels and connective tissue

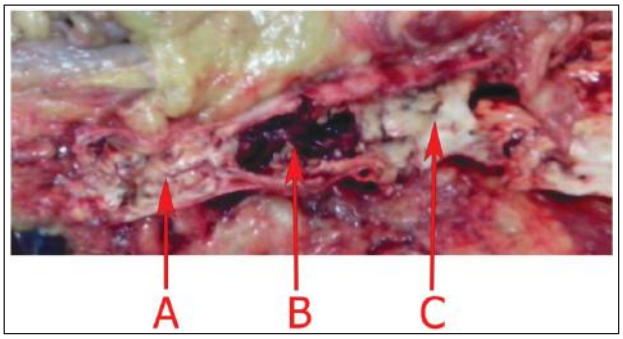

Figure 5. Macroscopic changes in the patient's aorta A3 with atherosclerosis and hypertension stage III: A - calcification of the aorta; B - blood clots; C - intima lipid spots.

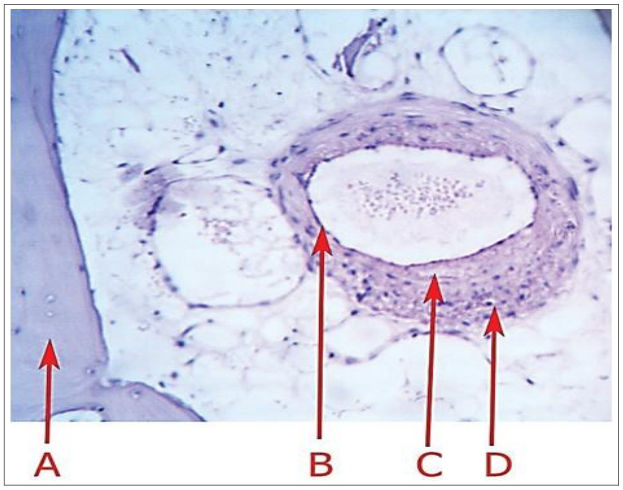

Figure 6. Spongy bone of the patient A3 with atherosclerosis and hypertension III stage, rate 3. Increased ×150. The color of hematoxylin-eosin: A - bone trabeculae; B - endothelium;C - necrotic changes; D - macrophagal infiltration

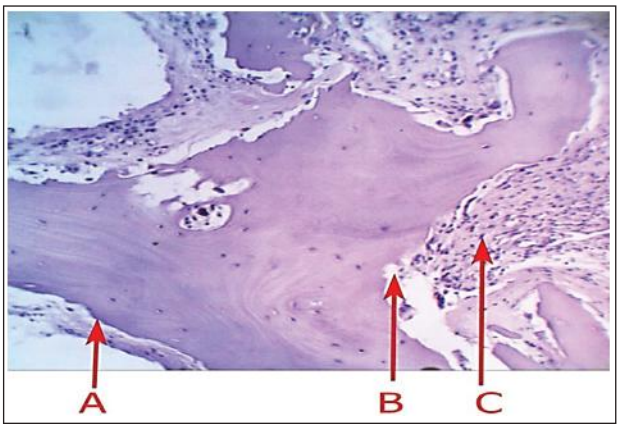

Figure 7. Spongy bone of the patients with atherosclerosis and hypertension.Increased ×150. The color of hematoxylin-eosin: A - preserved part of endoosta with no signs of resorption; B - resorption lacunae trabeculae, endoost is absent; C - proliferation of connective tissue in layers of trabekulae.

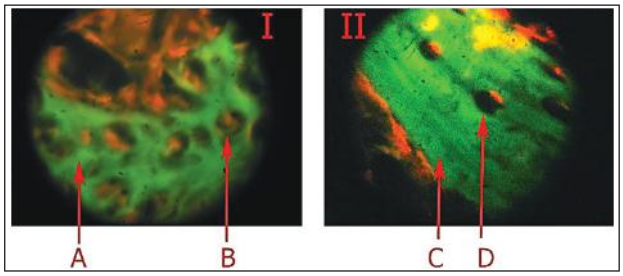

Figure 8. Fluorescence microscopy of spongy bone of alveolar process of studied dead patients. Increased ×400: I - patients with atherosclerosis and hypertension; II - patients of control group; A - randomly arranged collagen-like fibers; B - patients' osteocyte with hypertension and atherosclerotic lesions in the aorta; C - linear arrangement of collagen-like proteins; D - patients' osteocyte of the control group.

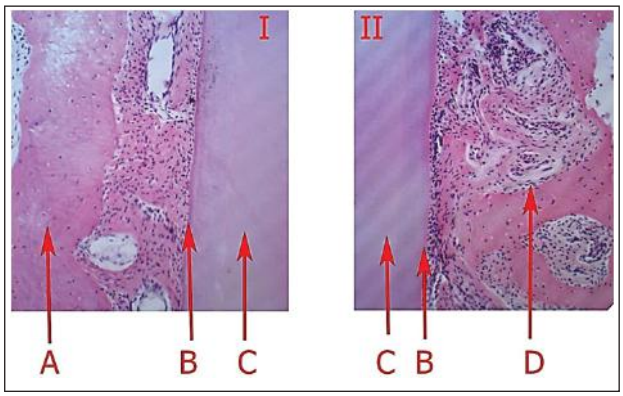

Figure 9. Third part of periapical of periodontal slit of studied dead patients. The color of hematoxylin-eosin. Increased × 150: I - control group of patients; II - patients with atherosclerosis and hypertension; A - bone tissue of alveolar bone; B - cement; C - dentine; D - lacunar resorption of alveoli.

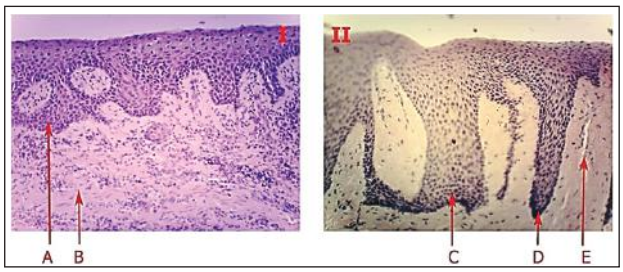

Figure 10. Epithelium of toothless jaw area of dead studied patients.The color of hematoxylin-eosin. Increased × 150: I – a control group of patients; II - patients with atherosclerosis and hypertension; A – epithelium without signs of proliferative activity; B – own plate; C – outgrowths in the thickness of the lamina propria (rete ridges); D – actively proliferating basal layer of the epithelium; E - minor swelling changes in the lamina propria.

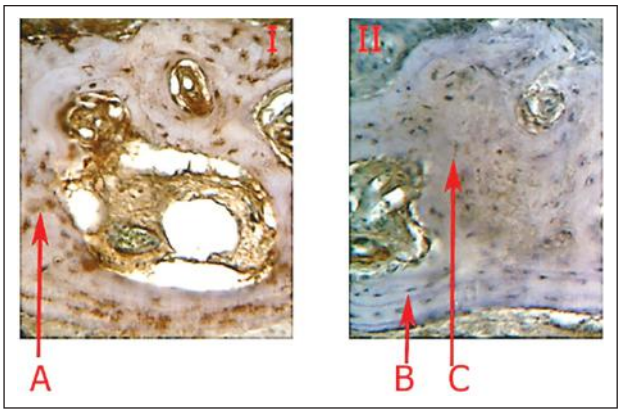

Figure: 11. Immunohistochemical study (Osteopontin) of spongy bone of the alveolar bone of dead studied patients. Increased × 400: I – control group of patients; II - patients with atherosclerosis and hypertension; A - osteocytes with strongly positive osteopontin expression; B - osteocytes without osteopontin expression; C - osteocytes with weak osteopontin expression.

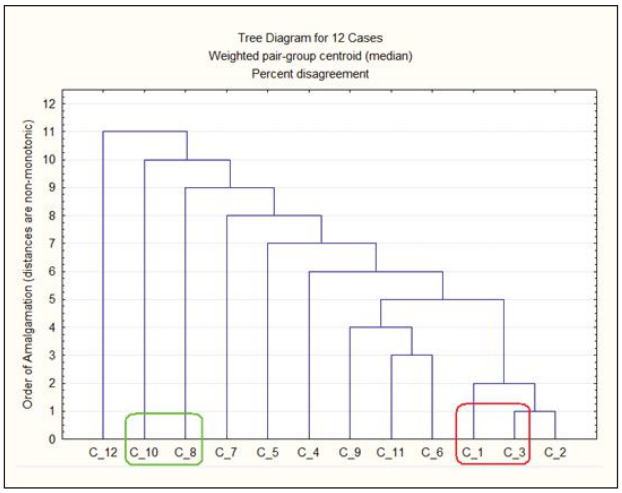

Figure: 12. Dendrohram of clustering of studied parameters.C_1 - fluorescencetion of toothless bone area of alveolar process, %; C_2 - fluorescencetion of bone of periapical third part, %; C_3 - expressio of osteopontin, %; C_4 - the thickness of the cortical bone, microns; C_5 - number of cortical bone osteocytes, increased × 150; C_6 - number of cortical bone osteoclasts, increased × 150; C_7 - the thickness of the trabecular periapical third part of the tooth, microns; C_8 - the thickness of the trabecular toothless area of alveolar bone, microns; C_9 - number of osteoclasts in trabecular periapical third prt of the tooth, increased × 150; C_10 - Number of osteocytes in trabecular periapical third prt of the tooth, increased × 150; C_11 - Number of osteoclasts in trabecular toothless alveolar bone, increased × 150; C_12 - Number of osteocytes in trabecular toothless alveolar bone, increased × 150.

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