Volume 8, Issue 4, 2019, 754 - 761 **ISSN 2284-6808**

Letters in Applied NanoBioScience

https://nanobioletters.com/

https://doi.org/10.33263/LIANBS84.754761

Original Research Article **Open Access Journal**

Received: 09.12.2019 / Revised: 20.12.2019 / Accepted: 22.12.2019 / Published on-line: 27.12.2019

Effectiveness of nanocrystalline cerium dioxide for secondary prevention of inflammatory periodontal diseases in young individuals with obesity

Maksym Skrypnyk [1], Tetiana Petrushanko [1], Karine Neporada [1], Rostyslav Bubnov *[2], Oleksandr Shcherbakov [2], Mykola Spivak [2, 3]

[1]Poltava Ukrainian Medical and Stomatological Academy, Poltava, Ukraine
[2]Zabolotny Institute of Microbiology and VirologyNational Academy of Sciences of Ukraine, Kyiv, Ukraine
[3]LCL 'DIAPROF', Kyiv, Ukraine

*corresponding author e-mail address: ***dr.rbubnov@gmail.com*** | Scopus ID 38360919800

ABSTRACT

Obesity is a non-infectious pandemic, largely associated with oral diseases development. Oxidative stress is one of the crucial mechanisms in the development of inflammatory periodontal diseases in obesity. Nanomedicine is a highly promising to alleviate pbesity and associated conditions. The aim was to study the influence of cerium dioxide nanoparticles on periodontal tissues of patients with diverse values of BMI and assess changes of periodontal tissues. We included 125 young Ukrainian individuals of both sexes (18-22 years old), divided into four groups according to Body mass index (BMI). Initial oral status was detected with index assessment that include the determination of caries intensity by DMFT index, oral hygiene determination (Green-Vermillion index), periodontal tissues (Rateitchak index), PMA (Parma), CPI (Leus), PBI (Saxer and Muhlemann). The periodontograma was completed for all patients. After the initial checkup all examined patients with generalized catarrhal gingivitis were randomly divided into two groups: treated with «Cerera» (the active substance is nanoparticles of cerium dioxide 2-7 nm in concentration of 140 mcg/ml that were stabilized with sodium citrate) per os for 10 drops every day in the morning diluted in 50 ml of water for 10 days and controls. Patients with 1st and 2nd degree obesity have a significantly higher grade of periodontal alteration compared with normal BMI and overweight (values of CPI 1.7±0.9 and 1.66±0.12 vs 1.33±0.11 and 1.42±0.11 respectively). The intensity and the prevalence of gingiva inflammation assessed by PMA and PBI index were significantly higher in obese individuals. In all overweight patients the level of catalase activity was significantly lower than in patients with normal values of BMI. The administration of «Cerera» significantly increased the activity of catalase regardless of BMI, significantly changed the values of gingival inflammation indexes; the severity of inflammatory process became less intensive vs baseline (determined by PBI values before 17.6%±2.1 and 7.8%±2.1 after treatment; in the 4th group the values of PBI had declined significantly by 1.6 times). After the 10 days administration of «Cerera» the content of GAG and fucose in oral liquid of all obese individuals significantly decreased; lipid oxidation parameters decreased in excessive obesity: TBA-active products levels in patients with BMI 35.00-39.99 kg/cm^2 dropped down from 53.9±2.11 μmol/L to 47.43±2.29 μmol/L. Cerium dioxide nanoparticles demonstrate high effectiveness against inflammatory changes in periodontal tissues in obese patients. The mechanisms of action of cerium dioxide nanoparticles in the periodontal structures are needed to be precisely determined in further studies.

Keywords: *Nanocrystalline cerium dioxide, Nanoceria, Obesity, Oral cavity, Periodontal tissues, Inflammatory periodontal diseases, Clinical trial, Young individuals, Oxidative stress*

1. INTRODUCTION

Obesity was named a non-infectious pandemic because is not just limited to one part of the world. The prevalence of obesity around the globe is approximately 2.1 billion near 30% of the total population. All nations have obese citizens and their number has a tendency to grow. Some, however, have a far higher proportion than others. Many countries with high economy income like the United States and the United Kingdom are ranked 12th and 36th out of other 197 countries due to the latest epidemiological research [1]. More than 3 million people each year die from complications of obesity. The worldwide obesity rate has tripled since 1975. In addition to a dramatic decrease in lifespan, the quality of life of an obese person is affected. There are many comorbid conditions related to obesity such as health risks, higher risks of diabetes mellitus type 2, heart disease and even certain types of cancer. It is known that students with extra weight predispose to periodontal diseases and some mucosal lesions. The prevalence of recurrent aphthous stomatitis was significantly higher in obese individuals than in their peers with normal weight [2].

People with obesity require special medical care especially during dental treatment due to the high frequency of medical emergency and non-typical pathway of periodontal diseases. Special guidelines on medical emergency during dental treatment are recommended for children with obesity due to high frequency of hypertension even in child age [3]. Even the duration of orthodontic treatment significantly depends on body mass index because of high levels of the adipokines, leptin and resistin, the inflammatory marker myeloperoxidase and the cytokine receptor

for nuclear factor kappa-B ligand is significantly different between obese- and normal-weight patients and associated with observed rates of tooth movement [4]. However, the result of many studies are quite controversial and need further in depth investigation [5]. It is clear that overweight and obesity have an influence on the pathway of periodontal and mucosal diseases and on their treatment. That is why a further investigation into influence of overweight and obesity on oral structures is necessary for highly effective management of oral diseases.

Obesity is characterized by a chronic subclinical inflammation that could maintain and exacerbate other mild inflammatory diseases of oral cavity such as periodontitis, gingivitis. Periodontitis is in 10 most widespread chronic diseases affecting the world population [6]. Extremely dangerous is visceral type of obesity because of the more intensive production of proinflammatory adipo cytokines into blood stream. The results of up to day research are very ambivalent. Even the influence of gender on periodontal disease course is described differently by diverse studies. Some of them indicate males as a greater risk of developing periodontitis while other one female due to hormonal fluctuation that increases inflammatory processes [7]. That is why it is necessary to investigate pathophysiological ways in development and maintenance of inflammatory periodontal diseases in patients with extra weight. For that matter to create highly effective drugs that will influence on etiological component and pathological ways in development of periodontal diseases.

The traditional clinical procedures of scaling, root planning and periodontal flap surgery, if followed by adequate postoperative supportive periodontal care, results, in most cases, in successful management of progressive periodontal diseases. Nonetheless, there has been significant progress made in recent years with the development and introduction of various metallic and polymeric materials structured in nanoscales. Tissue engineering of such complex interfaces requires a contiguous scaffold system with at least two cell types associated with the engineering of both hard and soft connective tissues [8].

Nanomedicine is a highly promising direction in the modern medicine and pharmacy. Usage of nanotechnology in regenerative medicine has revolutionized the designing of grafts and scaffolds which has resulted in new grafts/scaffold systems having significantly enhanced cellular and tissue regenerative properties. Since the cell-cell and cell-matrix interaction in biological systems takes place at the nanoscale level, the application of nanotechnology gives an edge in modifying the cellular function and/or matrix function in a more desired way to mimic the native tissue/organ [9]. The latest studies of nanoparticles properties demonstrate that most of this materials biocompatible and are highly efficient in supporting the damaged tissues. Effects of nanoparticles of diverse metal and non-metal elements are widely studying in enhancement of dental implants integration, in periodontology as supplemental material in guided tissue regeneration etc. Nanoparticles are highly interesting material for further research due to their properties that are depended on their shapes, size, ways of synthesis, solvents etc [10].

Oxidative stress is one of the crucial mechanisms in the development of inflammatory periodontal diseases of periodontal tissues alteration. Due to the intense vascularization and innervation periodontal tissues are extremely sensitive to different inner and external triggers such as emotional stress, inner organs diseases, obesity, sub- and supragingival dental plaque etc. that provoke development of oxidative stress alteration processes in the whole organism. Soft periodontal tissues are rapidly reacted to pathogenic factors that clinically manifested with bleeding of the gingiva margin, itching in gingiva, recession of the gingival margin etc. Oxidative stress alteration was detected as one of the main factors of inflammatory changes development in periodontal tissues in rats with obesity. Due to the following research administration of cerium oxide nanoparticles (nanoceria) as a nanozyme significantly depressed activation of oxidative stress alteration in soft periodontal tissues of rats with obesity and overweight [11] and demonstrtsed prebiotic properties [12].

Nanoceria is referred to nanozymes and compared to naturals enzymes nanoparticles of cerium do not require strict physiological conditions for doing their catalytic functions. They are less active than native enzymes but their synthesis, isolation and purification are significantly cheaper than for natural one. All nanozymes can be divided into two big groups those who have pro- and antioxidant activity. Presence of powerful antioxidant properties (catalase, superoxide dismutase) in cerium dioxide nanoparticles was detected by a few researchers [11-14]. Furthermore, cerium dioxide nanoparticles are prone to auto-regeneration process within a few days, where they regenerate the Ce^{+3} oxidation state atoms from oxidized Ce^{+4} atoms (during the superoxide radical dismutation process) [15]. Although cerium dioxide nanoparticles have antigenotoxic properties against oxidative stress alteration against buthionine sulfoximine in keratinocytes.

All nanozymes have enormous potential in treatment of most oxidative stress related diseases. Also it requires further clinical trials for further investigation of their mechanisms of action in human body. Our research is among the very few *in clinico* studies dedicated to the influence of cerium dioxide nanoparticles (nanoceria, «Cerera») on human organism.

The aim was to study the influence of cerium dioxide nanoparticles on periodontal tissues of patients with diverse values of BMI and assess it objectively by changes of some biomarkers content in oral liquid and clinically by determination of dental indices.

2. MATERIALS AND METHODS

The research was performed on 125 young Ukrainian individuals of both sexes (18-22 years old). All of them were first and second year's students of Ukrainian Medical Stomatological Academy (UMSA). Pregnant and lactating women, patients with endocrine forms of obesity, patients with non-Ukrainian origin, presence of non-removable orthodontic appliances fel under the exclusion creteria. All participants were informed about using their personal information in the material of research and signed a written agreement for further examination. This study was carried out at the end of the second term of 2018 – 2019 academic year (April-June). This research is the part of research direction of the department of the therapeutic dentistry and biological chemistry of

UMSA. The clinical part of research was done on the basis of clinic in the department of therapeutic dentistry public utility «Poltava State Dental Clinic». Detection of biological substations in oral liquid was done in the research laboratory in the department of biological chemistry of UMSA.

All personal, medical and dental data's were noted into the specially designed «Health history form». Height, hips-waist ratio and weight measurements were done by specially training stuff on the same measuring device in order to unify obtained data's. Body mass index (BMI) was calculated and in order to divide patients into four groups. To the first group belong the patients with normal weight (BMI – 18,5 – 24.99 kg/cm^2) n=31; 2) overweight patients (BMI - 25 – 29,99 kg/cm^2) n=49; 3) patients with the first degree obesity (BMI - 30 – 34.99 kg/cm^2) n=20 ; 4) patients with the second degree obesity (BMI - 35 – 39.99 kg/cm^2) n=25.

Initial oral status was detected with index assessment that include the determination of caries intensity by DMFT index [16], oral hygiene determination (Green-Vermillion index) [17], periodontal tissues (Rateitchak index) [18], PMA (Parma) [19], CPI (Leus) [20], PBI (Saxer and Muhlemann)[18]. The periodontograma was completed for all patients. Periodontal diagnoses was determined due to M.F. Danilevsky classification [20], alteration in oral mucosa and lips diseases were classified due to P.T. Maksymenko [21].

After the initial checkup all examined patients with the presence of generalized catarrhal gingivitis were divided into two groups. The first one called the control group (does not receive any medication) and the second one consisted of the patients who were treated with «Cerera» (the active substance is nanoparticles of cerium dioxide 2-7 nm in concentration of 140 mcg/ml that were stabilized with sodium citrate) per os for 10 drops every day in the morning diluted in 50 ml of water for 10 days. Any dental treatment was not provided to patients from the following groups at least 3 months before the research had done. Cerium dioxide nanoparticles («Cerera») was synthesized and kindly provided by Prof. Mykola Spivak (Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine). «Cerera» is registered in Ukraine as biological active supplement (the registered number is TYY 10.8-2960512097-004:2015).

Before individuals had started treatment with «Cerera» samples of oral liquid were collected. As well as after the 10 days of following medication consumption. All samples of oral liquid in volume of 15 ml were collected twice (initially and on 11-12 day) into special container for biological liquids URI-BOX (F.L. MEDICAL Italy) and immediately stored into the freezer. All samples were collected in the morning time between 8 AM to 10 AM on an empty stomach. On the second appointment the complex clinical examination and index assessment were performed.

In the samples of oral liquid we determined the total proteolytic activity, the total antitrypsin activity, activity of ornitinedecarboxilase (ODK), arginase, and amylase activity. The metabolism of connective tissues was determined by the content-free fucose and glycosaminoglycanes (GAG). Development of oxidative stress was evaluated due to the content of oxidation-modified proteins (OMB), content of TBA-active products and catalase activity. Activity of nitrosative stress was assessed by general activity of NO-synthase, content of nitrites and by activity of nitrate and nitrite reductase.

Statistical processing was performed by using the Statistica (StatSoft,http://www.statsoft.com/Products/STATISTICAFeature) . All results were described as average and standard errors. For data analysis, we used one-factor analysis of variance (one-way ANOVA) for unrelated sampled and corrections Bonferroni for multiple comparisons was done. Pairwise comparisons were performed by using the Student's two-sample t-test for independent samples from unequal variances. The difference between groups was considered statistically significant at $p < 0.05$.

3. RESULTS

One of the key factor of metabolic syndrome is a waist parameter more than 102 cm for men and 88 cm for women. Up to 75% of individuals in the 3th group (BMI = 30 -34.9 kg/cm^2) had excessive values of this data and all of the patients in the 4th group had increased meaning of waist values. However, the presence of abdominal obesity is detected by a waist-hip ratio. It is a coefficient ≤1.0 and for women ≤0.85 and for men. Therefore, the presence of abdominal obesity was detected in 15% of cases in the 3th group and in 35% of individuals in the 4th group.

Due to obtaining results, there were not any significant differences between the prevalence and intension of carries and BMI as well as prevalence of non-carious lesions.

The prevalence of periodontal diseases was significantly higher in patients with the 2nd degree obesity up to 88% in the group of 1st degree obesity up to 80% in the group with overweight the prevalence of periodontal diseases was up to 75,5% and the smallest was in group with normal BMI up to 48,4%. The prevalence of generalized form of periodontitis and gingivitis out of all periodontal pathology was the following: 20% in the 1st group, 43.2% - 2nd, 62.5% - 3th, 77.3% - in the 4th. The most frequently chronic generalized catarrhal gingivitis was diagnosed among all examined individuals.

In patients with overweight and obesity poor oral hygiene was observed compared with better values in students with normal BMI the results of hygienic index assessment are presented in the Table 1. Also, patients with 1st and 2nd degree obesity have a significantly higher grade of periodontal alteration as evidenced by higher values of CPI index in those groups - 1.7±0.9 and 1.66±0.12 compared with students with normal BMI and overweight 1.33±0.11 and 1.42±0.11. Furthermore, the intensity and the prevalence of gingiva inflammation assessed by PMA and PBI index were significantly higher in obese individuals and correlated directly with their BMI. Also there were close values of oral hygiene indexes (OHI, API) in patients with overweight and 1st and 2nd degree obesity but inflammatory respond to dental plaque was significantly higher in individuals with BMI > than 30 kg/cm^2 that assessed by values of PBI index 24.9%±2,7 in the 4th group compared with 15.6%±2.4 in the 2nd and 16.7%±1. 9 in the 3th group. We suggest that the reason for such inflammation respond is chronicle systemic inflammation that is observed in obese individuals.

Well known are functional disturbances and biochemical changes associated with the clinical manifestation of periodontal diseases. Therefore, we considered to explore the influence of

extra weight on the content of biomarkers of periodontal soft tissue alteration in the oral liquid of patients with diverse BMI and explore the effect of proposed treatment.

The levels of GAG and fucose in oral liquid of all individuals with BMI < 30 kg/cm^2 (the 1st and the 2nd groups) was within normal limits and do not change significantly with administration of «Cerera» (Table 2). Otherwise, the content of fucose in the oral liquid of patients with 1st and 2nd degree obesity was significantly higher than in patient with BMI < 30 kg/cm^2. After the 10 days administration of «Cerera» the content of fucose in the oral liquid decreased significantly from 2.15±0.28 μmol/l to 1.52±0.13 μmol/l in the 3th group and in 1,2 times in the 4th group.

The content of GAG was significantly higher in the 4th group compared with others up to 2.1 μmol/l. After the course of cerium dioxide nanoparticles administration values of connective soft tissues metabolites' in the 3th and 4th groups were up to the individuals with normal BMI. Such results attest for protective properties of cerium dioxide nanoparticles against depolymerization of main components of intercellular matrix in periodontal soft tissues that observed during inflammatory periodontal diseases. We hypothesise that enzymatic antioxidant properties are the main reason for beneficial effects of cerium dioxide nanoparticles.

Table 1. Index assessment of oral hygiene in patients with diverse BMI, (M±m).

Index \ Group (BMI)	18.5-24.99 kg/cm^2 (n=31)	25-29.99 kg/cm^2 (n=49)	30-34.99 kg/cm^2 (n=20)	35-39.99 kg/cm^2 (n=25)
OHI	0.5±0.09	1.3±0.07	1.62±0.14	1,6±0,13
p_{1-2} < 0.05 p_{1-3} <0.05 p_{1-4} <0.05 p_{2-3} < 0.05 p_{2-4} >0.05 p_{3-4}>0.05				
API, % (Rateitchak)	16.53±2.3	11.4±3.2	5.67±0.9	8.7±2.1
p_{1-2} > 0.05 p_{1-3} <0.05 p_{1-4} >0.05 p_{2-3} < 0.05 p_{2-4} >0.05 p_{3-4}>0.05				
PMA, %	6,1±1,5	10,3±1,3	16,2±1,53	18,9±1,2
p_{1-2} < 0.05 p_{1-3} <0.05 p_{1-4} <0.001 p_{2-3} <0.05 p_{2-4} <0.05 p_{3-4}>0.05				
PBI, % (Saxer, Muhlemann)	6.4±0.9	15.6±2.4	16.7±1.9	24.9±2.7
p_{1-2} < 0.001 p_{1-3} <0.05 p_{1-4} <0.05 p_{2-3} >0.05 p_{2-4} <0.05 p_{3-4}>0.05				
CPI (Leus)	1.33±0.11	1.42±0.11	1.7±0.09	1,66±0,12
p_{1-2} > 0.05 p_{1-3} <0.05 p_{1-4} >0.05 p_{2-3} <0.05 p_{2-4} >0.05 p_{3-4}>0.05				

Note:
p1-2 - the level of significance is obtained when comparing groups of patients with normal weight and overweight patients;
p1-3 - the level of significance is obtained when comparing groups patients with normal weight and patients with the first degree obesity;
p1-4 - the level of significance is obtained when comparing groups patients with normal weight and patients with the second degree obesity;
p2-3 - the level of significance is obtained when comparing groups of overweight patients and patients with the first degree obesity;
p2-4 - the level of significance is obtained when comparing groups of overweight patients and patients with the second degree obesity;
p3-4 - the level of significance is obtained when comparing groups patients with the first degree obesity and patients with the second degree obesity.

Table 2. Content of soft connective tissues biopolymers in oral liquid of patients with diverse BMI with and without treatment with Cerera for 10 days, (M±m).

	Groups of patients (BMI, kg/cm^2)	Number of patients in each group	Content of fucose μmol/l	Content of GAG μmol/l
	Ist group (18,5-24,99)	15		
1	Initial control	7	0.91±0.25	1.24±0.07
2	Initial Cerera	8	0.87±0.13	1.31±0.04
3	10 days control	7	0.93±0.22	1.11±0.06
4	10 days Cerera	8	0.8±0.09	1.02±0.05
	Level of significance		p_{1-2} > 0.05, p_{2-3} >0.050.05 p_{1-3} >0.05, p_{2-4} >0.050.05 p_{1-4} >0.05, p_{3-4}>0.050.05	p1-2 > 0.05. p2-3 >0.05 p_{1-3}> 0.05. p_{2-4} < 0.05 p_{1-4}> 0.05. p_{3-4}>0.05
	IInd group (25.00-29.99)	20		
1	Initial control	10	1.14±0.07	1.54±0.07
2	Initial Cerera	10	1.2±0.07	1.57±0.09
3	10 days control	10	1.16±0.11	1.6±0.09
4	10 days Cerera	10	1.02±0.09	1.29±0.1
	Level of significance		p_{1-2} > 0.05, p_{2-3} >0.05 p_{1-3}> 0.05, p_{2-4} > 0.05 p_{1-4}> 0.05, p_{3-4} > 0.05 …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) > 0.05	p_{1-2} > 0.05, p_{2-3} >0.05 p_{1-3}> 0.05, p_{2-4} > 0.05 p_{1-4} > 0.05, p_{3-4} > 0.05 …….. pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) < 0.05
	IIIth group (30.00-34.99)	18		
1	Initial control	8	2.02±0.3	1.33±0.2
2	Initial Cerera	10	2.15±0.28	1.56±0.21
3	10 days control	8	2.1±0.27	1.39±0.16
4	10 days Cerera	10	1.52±0.13	1.11±0.13
	Level of significance		p1-2 > 0.05, p2-3 >0.05 p_{1-3}> 0.05, p_{2-4} < 0.05	p1-2 > 0.05. p2-3 >0.05 p_{1-3}> 0.05. p_{2-4} < 0.05

	Groups of patients (BMI, kg/cm^2)	Number of patients in each group	Content of fucose µmol/l	Content of GAG µmol/l
			$p_{1-4} < 0.05$, $p_{3-4} > 0.05$ …….. pII-III (1) < 0.05 pII-III (2) < 0.05 pII-III (3) < 0.05 pII-III (4) < 0.05	$p_{1-4} > 0.05$. $p_{3-4} > 0.05$ ……. pII-III (1) > 0.05 pII-III (2) > 0.05 pII-III (3) < 0.05 pII-III (4) > 0.05
	IVth group (35.00-39.99)	20		
1	Initial control	8	2.0±0.19	2.08±0.12
2	Initial Cerera	12	2.14±0.17	2.1±0.11
3	10 days control	8	2.02±0.12	2.03±0.09
4	10 days Cerera	12	1.74±0.1	1.43±0.09
	Level of significance		p1-2 > 0.05, p2-3 >0.05 $p_{1-3} > 0.05$, $p_{2-4} < 0.05$ $p_{1-4} < 0.05$, $p_{3-4} < 0.05$ …… pIII-IV (1) > 0.05 pIII-IV (2) > 0.05 pIII-IV (3) > 0.05 pIII-IV (4) > 0.05	p1-2 > 0.05. p2-3 >0.05 $p_{1-3} > 0.05$. $p_{2-4} < 0.05$ $p_{1-4} < 0.05$. $p_{3-4} < 0.05$ …….. pIII-IV (1) < 0.05 pIII-IV (2) > 0.05 pIII-IV (3) < 0.05 pIII-IV (4) > 0.05

Note: in this tab. and next tab. 3, 4:
p1-2 - the level of significance is obtained when comparing groups of patients from control group and those who were administered with Cerera both of groups before 10 days period;
p1-3 - the level of significance is obtained when comparing groups patients of control group before 10 days period and after 10 days period;
p1-4 - the level of significance is obtained when comparing groups patients of control group before 10 days period and patients who were treated with Cerera for 10 days;
p2-3 - the level of significance is obtained when comparing groups of patients who were treated with Cerera before administration and patients of control group after 10 days period;
p2-4 - the level of significance is obtained when comparing groups of patients who were treated with Cerera before and after 10 days period;
p3-4 - the level of significance is obtained when comparing groups patients who were treated with Cerera for 10 days and another group who do not underwent any treatment after 10 days observation.

After the dot line the level of significance between diverse groups was presented where the Romans numbers mean the group name and Arabic number in bolds means an intragroup name where (initial control – 1, initial Cerera – 2, 10 days control -3, 10 days Cerera -4).

TBA-active products and oxidation-modified proteins are the products of oxidative stress alteration. By the quantity of TBA-active product intensity of cells lipids oxidation is assessed and by the content of oxidation-modified proteins oxidative stress alteration of proteins can be detected. Due to our studies the content of TBA-active products and OMB in patients with normal BMI values (18.5-24.99 kg/cm^2) was minimal compared with other groups and do not change significantly with treatment with «Cerera». There was lack of differences in the content of OMB between patients with overweight and 1st and 2nd degree obesity, but the content of OMB was significantly higher than in patients with normal BMI. Therefore, administration of «Cerera» does not decrease significantly the content of OMB in oral liquid in all groups of patients.

However, 10 days administration of «Cerera» significantly decreased lipid oxidation in the 4th group the content of TBA-active products in patients with BMI 35.00-39.99 kg/cm^2 dropped down from 53.9±2.11 µmol/L to 47.43±2.29 µmol/L. In other groups administration of cerium dioxide nanoparticles does not change the content of TBA-active products in saliva. Yet the content of TBA-active products in oral liquid correlated directly with values of BMI (Table 3).

Table 3. Determination of oxidative stress alteration markers in the oral liquid of patients with diverse BMI before and after treatment with Cerera (M±m).

	Groups of patients (BMI, kg/cm^2)	Number of patients in each group	Oxidation-modified proteins (OMB)	Content of TBA-active products µmol/L	Catalase activity, nkat/l
	Ist group (18.5-24.99)	15			
1	Initial control	7	1,46±0,1	36,62±4,55	8,43±0,32
2	Initial Cerera	8	1,5±0,13	39,31±4,11	8,13±0,23
3	10 days control	7	1,39±0,12	37,11±4,6	8,6±0,21
4	10 days Cerera	8	1,47±0,09	37,02±3,25	8,8±0,09
	Level of significance		$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} < 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$
	IInd group (25.00-29.99)	20			
1	Initial control	10	1.81±0.07	46.78±1.47	7,1±0,27
2	Initial Cerera	10	1.74±0.07	44.57±2.19	7,57±0,19

Groups of patients (BMI, kg/cm^2)		Number of patients in each group	Oxidation-modified proteins (OMB)	Content of TBA-active products µmol/L	Catalase activity, nkat/l
3	10 days control	10	1.86±0.11	43.6±1.39	7,6±0,13
4	10 days Cerera	10	1.72±0.09	42.29±1.22	8,89±0,1
Level of significance			$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) > 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …….. pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) < 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} < 0.05$ $p_{1-4} < 0.05$, $p_{3-4} < 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) < 0.05 pI-II (4) < 0.05
IIIth group (30.00-34.99)		18			
1	Initial control	8	1.8±0.12	52.33±2.82	7,33±0,29
2	Initial Cerera	10	1.85±0.18	52.56±3.1	7,28±0,21
3	10 days control	8	1.77±0.27	53.39±3.16	7,39±0,16
4	10 days Cerera	10	1.8±0.13	50.11±2.13	8,31±0,13
Level of significance			$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …… pII-III (1) >0.05 pII-III (2) >0.05 pII-III (3) > 0.05 pII-III (4) >0.05	$p_{1-2} > 0.05$. $p_{2-3} > 0.05$ $p_{1-3} > 0.05$. $p_{2-4} > 0.05$ $p_{1-4} > 0.05$. $p_{3-4} > 0.05$ …… pII-III (1) > 0.05 pII-III (2) < 0.05 pII-III (3) < 0.05 pII-III (4) < 0.05	$p_{1-2} > 0.05$. $p_{2-3} > 0.05$ $p_{1-3} > 0.05$. $p_{2-4} < 0.05$ $p_{1-4} < 0.05$. $p_{3-4} < 0.05$ …… pII-III (1) > 0.05 pII-III (2) > 0.05 pII-III (3) > 0.05 pII-III (4) > 0.05
IVth group (35.00-39.99)		20			
1	Initial control	8	1.86±0.19	52.28±2.62	6..8±0.12
2	Initial Cerera	12	1.84±0.17	53.9±2.11	6.6±0.21
3	10 days control	8	1.76±0.12	49.03±2.09	6.3±0.19
4	10 days Cerera	12	1.74±0.1	47.43±2.29	8.43±0.23
Level of significance			$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) > 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …….. pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) < 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} < 0.05$ $p_{1-4} < 0.05$, $p_{3-4} < 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) < 0.05 pI-II (4) < 0.05

Table 4. Values of oral hygiene indexes in the oral liquid of patients with diverse BMI before and after treatment with Cerera (M±m).

Groups of patients (BMI, kg/cm^2)		Number of patients in each group	PMA, %	PBI, % (Saxer, Muhlemann)	CPI (Leus)
Ist group (18.5-24.99)		15			
1	Initial control	7	5.8±0.32	6.45±0.55	1,23±0,12
2	Initial Cerera	8	6.4±0.43	5.91±0.4	1,33±0,2
3	10 days control	7	6.5±0.37	6.1±0.6	1,4±0,18
4	10 days Cerera	8	6.17±0.29	4.62±0.33	1,25±0,24
Level of significance			$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} < 0.05$ $p_{1-4} < 0.05$, $p_{3-4} < 0.05$
IInd group (25.00-29.99)		20			
1	Initial control	10	10.1±1.4	16.8±1.73	1,32±0,17
2	Initial Cerera	10	11.4±1.7	14.5±1.34	1,22±0,11
3	10 days control	10	11.6±1.2	17.2±1.89	1,3±0,14
4	10 days Cerera	10	10.2±0.9	14.2±1.2	1,19±0,1
Level of significance			$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) > 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …….. pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) < 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} < 0.05$ $p_{1-4} < 0.05$, $p_{3-4} < 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) < 0.05 pI-II (4) < 0.05
IIIth group		18			

Groups of patients (BMI, kg/cm^2)		Number of patients in each group	PMA, %	PBI, % (Saxer, Muhlemann)	CPI (Leus)
(30.00-34.99)					
1	Initial control	8	16.6±1.42	16.7±1.9	1,7±0,09
2	Initial Cerera	10	16.5±1.58	17.6±2.1	1,8±0,11
3	10 days control	8	17.7±1.47	17.9±1.86	1,75±0,16
4	10 days Cerera	10	9.8±1.32	7.8±2.1	1,42±0,15
Level of significance			$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) > 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …….. pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) < 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} < 0.05$ $p_{1-4} < 0.05$, $p_{3-4} < 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) < 0.05 pI-II (4) < 0.05
IVth group (35.00-39.99)		20			
1	Initial control	8	18.6±1.2	24.9±2.7	1,6±0,12
2	Initial Cerera	12	19.4±1.4	23.4±2.6	1,6±0,14
3	10 days control	8	20.6±1.9	23.6±2.5	1,46±0,13
4	10 days Cerera	12	15.0±1.9	14.4±1.7	1,43±0,14
Level of significance			$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) > 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} > 0.05$ $p_{1-4} > 0.05$, $p_{3-4} > 0.05$ …….. pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) > 0.05 pI-II (4) < 0.05	$p_{1-2} > 0.05$, $p_{2-3} > 0.05$ $p_{1-3} > 0.05$, $p_{2-4} < 0.05$ $p_{1-4} < 0.05$, $p_{3-4} < 0.05$ …… pI-II (1) < 0.05 pI-II (2) < 0.05 pI-II (3) < 0.05 pI-II (4) < 0.05

In all patients with BMI > 25 kg/cm^2 the level of catalase activity was significantly lower than in patients with normal values of BMI. The administration of «Cerera» significantly increase the activity of catalase in all 4 groups regardless of BMI values (Table 3). That was possible due to catalase mimetic activity of cerium dioxide nanoparticles [15].

The values of plaque and dental calculus (OHI and API) did not change significantly in all individual because they do not underwent any dental treatment during the period of the research. Otherwise, the values of indexes that are determined gingival inflammation had changed significantly especially in patients with BMI > 30 kg/cm^2 (Table 4). After 10 days of «Cerera» administration in patients of the 3th group the extension of inflammatory area in gingiva was shortened by 1.6 times that was determined by PMA index, the same result were in the 4th group. In the 3th group after 10 days treatment with «Cerera» the severity of inflammatory process became less intensive compared with initial state that was determined by PBI values before 17.6%±2.1 and 7.8%±2.1 after treatment. In the 4th group the values of PBI had declined significantly by 1,6 times. The values of CPI did not change dramatically with «Cerera» administration.

We can explain such changes in PMA and PBI values after 10 days administration of «Cerera» by vasoprotective properties of cerium dioxide nanoparticles [22]. But this statement is needed to be confirmed by further laboratory and clinical studies.

In this context Stratification patients for hypoxic signaling, for Flammer syndrome phenotype, dry mouth, eating disorders impaired wound healing [23,24] would help to develop personalized treatments and preventive measures. Future research considering aspects of oral microbiome involved in the pathology associated with gastrointestinal microbiology [25] and leading to obesity is needed, validated in a large-scale clinical studies including simultaneous administration of cerium nanoparticles as a tailored prebiotic [12] with probiotics.

4. CONCLUSIONS

Cerium dioxide nanoparticles demonstrate high effectiveness against inflammatory changes in periodontal tissues in obese patients. The nanoceria administration improve of a number of clinical indexes and laboratory parameters of the oral liquid. The mechanisms of action of cerium dioxide nanoparticles in the periodontal structures are needed to be precisely determined in further studies.

5. REFERENCES

1. Most Obese Countries Population. (2019-08-24). Retrieved 2019-09-03, from http://worldpopulationreview.com/countries/most-obese-countries/.

2. Skrypnyk, M.; Petrushanko, T.; Kryvoruchko, T.; Neporada, K. Conditions of the oral cavity status in youth with alimentary-constitutional form of obesity. *The Medical and ecological problems* **2019**, *23*,17-21, https://doi.org/10.31718/mep.2019.23.1-2.04.

3. Frieda, A.P. Preventing Medical Emergencies: *Use of the Medical History in Dental Practice.* 3rd Edition, Kindle Edition. Wolters Kluwer Health; 3 edition 2014; pp. 352.

4. Saloom, H.F.; Papageorgiou, S.N.; Carpenter, G.H.; Cobourne, M.T. Impact of obesity on orthodontic tooth movement in adolescents: a prospective clinical cohort study. *J Dent Res.* **2017**, *96*, 547-554, https://doi.org/10.1177/0022034516688448.

5. Consolaro, A. Obesity and orthodontic treatment: is there any direct relationship? *Dental Press J Orthod*. **2017**, *22*, 21–25, https://doi.org/10.1590/2177-6709.22.3.021-025.oin.
6. Nascimento, G.G.; Leite, F.R.; Correa, M.B.; Horta, M.B. Relationship Between Periodontal Disease and Obesity: The Role of Life-Course Events. *Braz. Dent. J. Ribeirão Preto* **2014**, *25,* 87-89, http://dx.doi.org/10.1590/0103-6440201300019.
7. Martinez-Herrera, M.; Silvestre-Rangil, J.; Silvestre, F.J. Association between obesity and periodontal disease. A systematic review of epidemiological studies and controlled clinical trials. *Med Oral Patol Oral Cir Bucal*. **2017**, *22,* e708–e715, https://doi.org/10.4317/medoral.21786.
8. Kong, L.X.; Peng, Z.; Li, S.D.; Bartold, P.M. Nanotechnology and its Role in the Management of Periodontal Diseases. *Periodontology* **2006**, *40,* 184-96, https://doi.org/10.1111/j.1600-0757.2005.00143.x.
9. Chaudhury, K.; Kumar, V.; Kandasamy, J.; Roy, C.S. Regenerative nanomedicine: current perspectives and future directions. *Int J Nanomedicine* **2014**, *9*, 4153–4167, https://doi.org/10.2147/IJN.S45332
10. Gatoo, M.A.; Naseem, S.; Arfat, M.Y.; Dar, A.M.; Qasim, K.; Zubair, S. Physicochemical Properties of Nanomaterials: Implication in Associated Toxic Manifestations. *BioMed Research International* **2014**, *2014*, 8, https://doi.org/10.1155/2014/498420.
11. Beregova, T.V.; Neporada, K.S.; Skrypnyk, M.; Falalyeyeva, T.M.; Zholobak, N.M.; Shcherbakov, O.B.; Spivak. M.Y.; Bubnov, R.V. Efficacy of nanoceria for periodontal tissues alteration in glutamate-induced obese rats-multidisciplinary considerations for personalized dentistry and prevention. *EPMA J.* **2017**, *8,* 43–9, https://doi.org/10.1007/s13167-017-0085-7.
12. Bubnov, R.; Babenko, L.; Lazarenko, L.; Kryvtsova, M.; Shcherbakov, O.; Zholobak, N.; Golubnitschaja, O.; Spivak, M. Can tailored nanoceria act as a prebiotic? Report on improved lipid profile and gut microbiota in obese mice. *EPMA Journal* **2019**, *10,* 317-335, https://doi.org/10.1007/s13167-019-00190-1.
13. Yefimenko OY, Savchenko YO, Falalyeyeva TM, Beregova TV, Zholobak NM, Spivak MY, et al. Nanocrystalline cerium dioxide efficacy for gastrointestinal motility: potential for prokinetic treatment and prevention in elderly. *EPMA J.* **2015**;*6*:6.
14. Kobyliak NM, Falalyeyeva TM, Kuryk OG, Beregova TV, Bodnar PM, Zholobak NM, et al. Antioxidative effects of cerium dioxide nanoparticles ameliorate age-related male infertility: optimistic results in rats and the review of clinical clues for integrative concept of men health and fertility. *EPMA J.* **2015**; *6*:12. https://doi.org/10.1186/s13167-015-0034-2.
15. Singh, S. Nanomaterials Exhibiting Enzyme-Like Properties (Nanozymes): Current Advances and Future Perspectives. *Front. Chem.* **2019**, *7*, https://doi.org/10.3389/fchem.2019.00046.
16. Petersen, P.E.; Baez, R.J. *Oral health surveys-basic methods*. World Health Organization.Geneva. **2013**; ed. 5th, pp. 125.
17. Green, J.C. The simplified oral hygiene index: A method for classifying oral hygiene status. *J.Am.Dent.Assoc.* **1960,** *61*, 172–175, https://doi.org/10.14219/jada.archive.1964.0034.
18. Volf, G.F. *Periodontology second edition.* Moscow Medpress-inform, 2014; pp. 548.
19. Schour, I. Survey of gingival disease using the PMA Index. *J. Dent. Res.* **1948**, *27*, 733–735.
20. Danilevskij, M.F.; Borisenko, A.V. Therapeutic dentistry Periodontal diseases. *Kyiv: Medicina* **2008**, *3*, 616.
21. Nikolishin, A.K. Therapeutic dentistry. *Poltava: Divosvit* **2005**, *1*, 392.
22. Hood, E.D.; Chorny, M.; Greineder, C.F.; Alferiev, S.I.; Levy, R.J.; Muzykantov, V.R. Endothelial targeting of nanocarriers loaded with antioxidant enzymes for protection against vascular oxidative stress and inflammation. *Biomaterials* **2014**, *35*, 3708–3715, https://doi.org/10.1016/j.biomaterials.2014.01.023.
23. Avishai E, Yeghiazaryan K, Golubnitschaja O. Impaired wound healing: facts and hypotheses for multi-professional considerations in predictive, preventive and personalised medicine. *EPMA J.* **2017**; 8(1):23–33. https://doi.org/10.1007/s13167-017-0081-y.
24. Bubnov R and Golubnitschaja O. Flammer syndrome, disordered eating and microbiome: interrlations, complexity of risks and individual outcomes. In book. *Flammer syndrome – from phenotype to associated pathologies, prediction, prevention and personalisation.* Springer **2019**, ISBN 978-3-030-13549-2 ISBN 978-3-030-13550-8 (eBook), https://doi.org/10.1007/978-3-030-13550-8.
25. Reid G, Abrahamsson T, Bailey M, Bindels LB, Bubnov R, Ganguli K, et al. How do probiotics and prebiotics function at distant sites? *Benefic Microbes.* **2017**; 20:1–14. https://doi.org/10.3920/BM2016.0222.