Statistical Evaluation Of Antimicrobial Influence Of Medical Ozone As A Part Of Inflammatory Prevention Of Periodontal Diseases.

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ABSTRACT

Treatment and prevention of periodontal diseases is considered to be one of the most significant challenges nowadays; its importance is determined by the high incidence and intensity of the given pathology, critical decrease of functional potentials of the dento-facial system, premature teeth loss, negative impact on the human general health and quality of life. The article describes results of treatment of inflammatory periodontal diseases using an ultrasound device "Vector" with ozonized water in comparison with the conventional treatment methods. Application of the given method of treatment provides increased therapeutic efficiency of inflammatory periodontal diseases, prolonged terms of remission without disease recurrences, arrest of the inflammatory process in periodontal tissues in short terms, and gives an opportunity to use this technique in patients intolerant to pharmacotherapyб having comorbid conditions. This method is reported to be an acute preventive method of inflammatory periodontal disease complications and recurrences, which has been statistically confirmed.

Keywords: periodontium, an ultra-sound device "Vector", medical ozone, inflammatory periodontal diseases, medical statistics, secondary prevention.

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INTRODUCTION

Medical statistics is known to be an independent social science studying quantitative characteristics of mass public phenomena in continuity with their qualitative properties. It allows investigating regularities of these phenomena, important processes in economic and social public life, population health, the system of population healthcare management using rounding factors method. It appears to be an essential part of any research study and takes the first place in validation of benefits and negative outcomes of various therapeutical and preventive methods, application of pharmaceuticals and so on. As it is known, most of biological and medicinal findings do not comply with the law of normal distribution (Feinstein, 1987). A small number of investigated samples in the context of essential number of investigated signs, non-zero values of asymmetry and excess for most variables, lack of normal distribution of variables serve prerequisite for the fact that a median line (which divides a sample into two parts equal in number of elements, quartile coinciding with probability 0.5) is that parameter of descriptive statistics that is applied to represent data structure, as well as lower and upper quartiles (quartiles with probabilities 0.25 and 0.75 respectively). Non-parametric Mann-Whitney U test is used to assess differences between two samples according to their attribute level. This method was offered by F. Wilcoxon and later revised by H.B. Mann and D.R. Whitney.

Inflammatory periodontal diseases is widely known to result from and be supplied by products of life of bacterial microflora; that is why, its treatment starts with mechanical removal of plaque, deposits and biofilms from the tooth surface. Hand tools and ultra-sound devices are used for this procedure. A "Vector" device produced by Durr Dental (Germany) removes biofilm, dental plaque, calculus, endotoxins, granulated tissues allowing performing de-epithelization of an inner wall of the periodontal pocket, polishing root without excessive cement removal; this is important for regeneration of tissue structure and rapid and effective elimination of bacteria causing diseases. However, even considering beneficial effects of treatment, which are evident immediately after the first procedure – decrease of pain, bleedings, and suppuration, - a great number of researchers state its insufficient antimicrobial effect; whereas, depression of vital activity of microorganisms infecting periodontal pockets should be the basics of all therapeutical and preventive methods of inflammatory periodontal diseases [4, 5].

The important fact is that high chemical resistance of anaerobic bacteria essentially restrains efficiency of conventional methods [7]. In this respect performance of ozone-therapy has apparent benefits [1, 8]. Its efficiency is determined by the processes of normalization of oxygen tissue saturation, inhibition of lipid peroxidation and activation of the anti-oxidant system; these facts being crucially significant for the recovery of periodontal tissue homeostasis. Not less important is the fact that ozone demonstrates expressed microbicidal effect, potentiates an antibiotic effect increasing susceptibility of microorganisms to antibiotics. Finally, ozone does not result in selection of antibiotic resistant strains [1]. However, when applied locally, ozone has only surface impact and does not influence bone tissue trophicity and periodontal tissue in general, the fact that is considered to be the drawback of this technique [3, 4, 8]. We believe, that insufficient preventive directedness of therapeutic methods of inflammatory periodontal diseases regarding their recurrences and complications is one of their major drawbacks. In most cases assessment of efficiency of the performed periodontal inflammatory disease therapy is carried out using clinical methods; in rare cases arrest of inflammation and beginning of remission period is supported by cyto-bacterioscopic findings. That is why, it is essential to develop statistical evidence orientation that can objectively assess therapeutical efficiency.

Aim of study was to statistically assess efficiency of medical ozone impact on periodontal tissues for further prevention of inflammatory disease recurrences and complications.

MATERIALS AND METHODS

The study was performed on the basis of Department of Hospital Dentistry, in the dental clinic of Voronezh N.N. Burdenko State Medical University. The study included 101 patients with inflammatory periodontal diseases, aged 23-60, who did not have comorbid conditions of inner organs and general somatic pathologies in the active phase that may either burden their dental status or promote inflammatory periodontal diseases. All patients were further divided into two groups. Group I (main) included 61 patients, 33 females (54.1%) and 28 males (45.9%). Group II (control) included 40 patients, 21 females (52.5%) and 19 males (47.5%).

Professional oral hygiene in patients of both groups traditionally became the first stage of treatment. It was comprised of removal of supra- and subgingival hard and soft dental deposits using ultra-sound and water-abrasive devices; 0.05% chlorhexidine bi-gluconate solution was applied for antiseptic treatment of the oral cavity in the control group, and ozonized water, concentration 2000 mg/l, was applied for antiseptic treatment in the main group. After that, tooth and filling surfaces were polished by abrasive paste with brushes and rubber heads; they were followed by stripping and flossing. Patients were educated on proper oral hygiene rules; they were shown on the model how to properly brush teeth; personal oral hygiene products (a toothbrush, toothpaste, mouthrinse) were chosen individually. This stage is a must in inflammatory periodontal disease therapy.

After professional hygiene and dental health education patients of the main group were administered a vector therapy with ozonized water. Distilled water was ozonized using YOTA-60-01 "Medozon" ozonator. Distilled water was preliminary put into a 200 ml vial with a rubber ground-in lid; after that, time (5 minutes) and concentration (35 mg/l) of ozonation were set according to the instruction for use. Due to permanent ozone decomposition in a vial, it is recommended to ozonize solution immediately before the vector therapy procedure. Next, ozonized distilled water was put in the 120 ml tank placed under the front cover of "Vector" device.

Each tooth was treated for 1-1.5 minutes; this time was enough to polish a root and antiseptically process a periodontal pocket using ozonized distilled water and a proper tool determined by an anatomical structure of a tooth. Dentist's motions were linear, parallel to root surface, removing biofilm and granulation and polishing a root; they were controlled tactually. A linear sounder Paro was applied to treat vestibular, tongue and palatal surfaces of incisors and canines, a lancet Paro was applied to treat similar surfaces of premolars and molars, a curette Paro was the most effective tool kit to apply in the aproximal surface area of all teeth [7].

In the control group vector therapy was performed conventionally: distilled water was used, and after that antiseptic treatment was carried out irrigating a periodontal pocket by ozonized distilled water using a 3.5 ml syringe (per one tooth); they were also administered: antibacterial treatment - metronidazolum, 250 mg one tablet twice a day for 5 days, desensitizing treatment – claritin, 1 tablet in the evening for 5 days, stimulating treatment – ascorutin 1 tablet twice a day for 21 days.

Treatment efficiency of patients with inflammatory periodontal diseases was assessed based on the results of clinical, clinical-laboratory, X-ray methods of investigations before treatment, at the immediate (in 7 days) and at the delayed (in a year) stage after treatment. Dynamics of after-therapeutical findings in comparison groups was assessed by non-parametric methods, i.e. all of them were samples of a small volume (N<50). Since dynamics of reduced inflammation and disease recurrence after the performed treatment was determined on the same groups of patients, criteria for dependent sample were applied.

Quality of treatment regarding terms of manifestation of inflammation signs in periodontal tissues was assessed using the following methods: 1) patients' interviewing and collection of their complaints; 2) inspection of the oral cavity; 3) cyto-bacterioscopic investigations; 4) gingivoscopy; 5) papillar-marginal-alveolar index (PMA index); 6) oral hygiene index (OHI); 7) periodontal index (PI); 8) sulcus bleeding index (SBI) (according to Muhlemann); 9) X-ray evaluation; 10) statistical and mathematic evaluation (Table 1).

Spearman's rank *correlation coefficient (rho) was applied to perform a correlative analysis allowing to determine presence and level of conformity of two or more parameters. Correlation coefficient represented co-variation of two sets of variables on their value. Valid evaluations of correlation coefficient were classified according to the following scale (Dogle N.V., 1984):* $r_s < 0.3$ *– slight correlation,* $0.3 \leq r_s < 0.70$ *– moderate correlation,* $r_s \geq 0.70$ *– severe correlation.* Spearman's *rank* correlation coefficient was used to apply quantitative/ qualitative variables.

A diagnosis was made individually based on clinical, laboratory, X-ray methods of investigations, this was described in our earlier publications [1, 8]. In this study we paid attention at personified curative procedures as a part of secondary prevention of periodontal diseases. During the interview patients complained of dental deposits, gum bleeding and swelling, pain and unpleasant feeling during tooth brushing, bad breath. Hard and soft supra- and subgingival deposits, gum swelling and bleeding were revealed on

examination. An X-ray investigation registered osteoporosis, interdental septum resorption from ¼ to ½ root length (horizontal), periodontal pockets 4-4.5 mm deep.

As Table 1 demonstrated, the initial clinical periodontal state was in general compared in patients of all groups (no significant differences were revealed, p<0.05). PMA index value supported moderate gingival inflammation – 39.9 (32.4;45.1), Russell's periodontal index PI supported moderate periodontal inflammation – 3.1(2.1;3.6), oral hygiene index OHI was high, this characterized unsatisfactory oral hygiene status, sulcus bleeding index constituted 2 (2; 3) and was determined on probing – a gingival crevice was filled with blood (according to Muhlemann). Iodine-positivity of the 3rd degree was determined during gingivoscopy; this characterized expressed inflammation of the periodontal tissues (when staining by 2% water Lugol's solution a gum was painted brown, this corresponded to 3 points). Statistical and mathematic methods are given in tables.

Table 1: Index of findings in patients with inflammatory periodontal diseases at the stage of initial examination(median lines, 25th and 75th quartiles), (n=101)

index	Initial examination	
groups	Main group (N=61)	Control group (N=40)
PMA	39,4(32,8;44,9) *	39,9(35,0;45,1)
OHI	1,8 (1,2;2,1)	2,0 (1,6;2,0)
PI	2,7 (2,1; 3,1)	3,1 (2,8; 3,5)
SBI	2 (2; 3)	2 (2; 3)
Gingivoscopy	3 (2; 3)	3 (2; 3)

*Formed groups of patients, which were differentiated depending on the performed treatment (independent groups), were compared on the majority of studied parameters. Non-parametric Mann-Whitney U test was used to assess them, since groups were small samples. This method determines, whether the zone of crossing values between two ranges (a ranged parameter in the first sample and a similar parameter in the second sample) is sufficiently small. The smaller criterion value is, the more probable the fact that differences between values in the sample are valid. Values with the significance level p<0.05 were considered to be valid. The obtained values were statistically processed using Excel 97, Statistica 6.0 (StatSoft, USA).

RESULTS AND THEIR DISCUSSION

The vector system with ozonized water method demonstrated apparent advantage in comparison with conventional methods (vector therapy and irrigations by ozonized water). Rapid elimination of inflammatory signs and gum bleeding in patients of the main group occurred on the day of their visit to a dentist or on the next day. Immediately on the second day of treatment majority of patients reported about significant improvement of their gingival status: periodontal marginal swelling reduced, periodontal exudate formation activity reduced. Patients reported about decrease of gum itching and comfortable sensations.

We consider that these phenomena occur due to the following: application of antiseptic solution using syringe is not regular, it is difficult to control pressure of the supplied solution while irrigating a periodontal pocket. We have developed an optimal regimen of periodontal pocket irrigation by ozonized water using "Vector" device. Uninterrupted solution supply is controlled by foot pressing on the pedal. 36-38 pressings are necessary when treating 1 tooth, that is 3.3 ml of solution. This provides regular and efficient irrigation of a periodontal pocket with simultaneous root polishing by the "Vector polish fluid" suspension, which results in recovery and stable remission of the condition for a year.

After performed treatment we received expected results in the control group, they were supported by research results of other authors and our own investigations; whereas, novel effects were revealed in the main

group: application of ozonized water using a "Vector" device made it possible to reduce time of treatment to 1 minute, bleeding was not registered during the first visit, remission lasted for a year, allergic and other side reactions were avoided (Table 2).

Table 2: Dynamics of index and cyto-bacterioscopic parameters in patients with inflammatory periodontal diseases at various stages of treatment,M± m ,(n=101)

Parameter	Control group				Main group			
	Acute period	7 days	6 months	1 year	Acute period	7 days	6 months	1 year
OHI	1.9±0.5	0.6±0.1*	0.8±0.1*	1.2±0.8*	1.7±0.6	0.6±0.2*	0.6±0.1*	0.6±0.2*
PMA	38.8±7.5	7.5±3.5*^	21.5±8.9*^	26.6±10.9*^	39.1±7.2	2.2±1.1*^	8.3±4.6*^	12.9±6.8*^
Sulcus bleeding index	1.9±0.6	0*	1.1±0.5*^	1.2±0.5*^	2.2±0.7	0*	0*^	0.8±0.4*
PI	3.2±0.5	2.3 ±0.6*^	2.2±0.7^	2.4±0.7*^	3.1±0.6	1.5±0.5*^	1.4±0.4*^	1.4±0.4*^
gingivoscopy	2.6±0.5	1.5±0.5*	2.0±0.3^	2.1±0.5*	2.6±0.5	1.0±0.2*	1.3±0.5*^	1.7±0.2*^
macrophages	2.3±0.5	0*	1.0±0.4^	1.3±0.5^	2.3±0.5	0*	0*^	0.9±0.4*^
neutrophils	17.9±3.0	7.6±2.5*^	13.6±2.6^	16.6±2.6 ^	17.8±2.8	3.8±1.1*^	5.1±1.7*^	9.8±2.5*^
Lymphocytes	4.8±1.1	1.1±0.1*	3.3±1.4^	3.2±1.4^	4.8±1.5	0.5±0.1*	1.0±0.1*^	2.9±1.4^
Candida	10.7±2.4	6.1±2.1*^	5.5±1.5*^	7.0±1.7^	11.8±2.0	2.9±1.1*^	3.6±1.1*^	4.5±1.7*^
Coccal flora	2.0±0.6	0*	0.9±0.3^	1.1±0.5*	1.9±0.6	0*	0*^	0.5±0.4*^

*parameters statistically differ from acute period parameters (p<0.05)
^parameters statistically differ from parameters between groups (p<0.05)

Evaluating index and cyto-bacterioscopic parameters we can conclude: on the 7th da OHI was compared and conformed to good oral hygiene in patients of all groups; in 6 months OHI increased and conformed to satisfactory oral hygiene in patients of the control group; OHI did not change in patients of the main group. On the 7th day PMA reduced in 5.2 times in the control group and in 17.7 times in the main group; this proved inflammation arrest in the main group. In 6 months PMA was 21.5±8.9 in patients of the control group; PMA was 8.3±4.6 in patients of the main group; this was considered to be a negligible single inflammation having no statistical value. On the 7th day gum bleeding was not registered in patients of both groups; in 6 months bleeding on periodontal pocket probing was registered in patients of the control group; in 6 months sulcus bleeding index was equal 0 in patients of the main group, this fact, the same as PMA, proved lack of inflammation and disease remission. On the 7th day PI reduced in 1.4 times in the control group, in 2.1 in the main group. Thus, level of involvement of periodontal structures in the pathological process reduced more in the main group. These parameters were also stable in 6 months. In a year PI values were the same in patients of the control group, these parameters were similar to those of an acute period in patients of the control group. On examination after the performed advanced gingivoscopy area of inflammation reduced from 90% to 20% on the 7th day in patients of both groups; in 6 months these parameters were the same in patients of the main group, whereas, they were similar to those observed on the initial examination in patients of the control group. Index parameters characterizing inflammatory process in periodontal tissues are supported by cyto-bacterioscopic investigations. Lack of macrophages and coccal flora on the 7th day proved inflammation arrest. In 6 months stable remission was registered in patients of the main group, this was proved cyto-bacterioscopically; inflammation parameters increased and repeated treatment was necessary in patients of the control group in 6 months. This was supported by periodontal pocket investigation and index parameters. On the 7th day lymphocytes were single in patients of both groups; this supported lack of inflammation. In 6 months the number of lymphocytes increased in patients of the control group (3.3±1.4); these parameters did

not statistically change in patients of the main group. In a year a number of lymphocytes was comparable to that of the 6th month stage in patients of both groups. Quantitative lymphocyte assessment had direct correlation with quantitative coccal flora assessment (rxy = 0.624, при P<0.05). On the 7th day fungal flora reduced in 4 times in the main group, in 6 months and in a year the number of Candida fungi was 3 times less in comparison with the acute period. These results characterize peculiarities of treatment in the given groups of patients.

Optimal therapeutic outcome was not obtained in patients of both groups, this was determined by changing eating habits and place of residence of some patients, decrease of their immune status, development of co-morbid pathologies or acute exacerbation of chronic diseases, as well as bad compliance with the dentist's recommendations on proper oral hygiene [13]. Considering these facts, the number of recurrences in the main group constituted 5%, in the control group – 14%. Therefore, in this study we have first validated antimicrobial effect of medical ozone on periodontal tissues depending on the way of its application. As a result, we statistically proved the advantage of the developed method of antiseptic periodontal pocket treatment using a "Vector" device, which was applied as a part of secondary personified prevention of inflammatory periodontal diseases.

CONCLUSIONS

Clinical application of the developed treatment method of inflammatory periodontal diseases using a "Vector" device with ozonized water has demonstrated advantages of antimicrobial effect of medical ozone in comparison with conventional therapeutic methods of its application on periodontal tissues. This helped to conclude the following:

1) When performing personified therapeutic-preventive procedures using a "Vector" device with ozonized water and polishing suspension Fluid Polish we obtained efficient arrest and, further, stable stabilization of the periodontal inflammatory process, this effect preserving for a year. According to literature data, periodontal treatment with application of the conventional vector therapy is characterized by similar results only in 3-6 months; this fact being proved by our investigations in the control group.
2) When assessing microbial status of periodontal pocket in patients who were performed vector therapy with ozonized water, positive dynamics of cytological parameters, decrease of periodontal pocket bacterization, microbiological monitoring proved significant decrease of Candida fungi in comparison with similar parameters in patients of the control group.
3) Moreover, practically painless application of the "Vector" system provided in patients increased motivation to perform supporting therapy as a part of secondary prevention of periodontal diseases using the described technique.
4) Thus, we can conclude that clinical-laboratory findings of the patients involved in the study allow validating antimicrobial effect of medical ozone in complex treatment with application of ultra-sound "Vector" device with ozonized distilled water. It is also reported to be crucial prevention of complications and recurrences of periodontal inflammatory diseases.

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