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Posted Date: 14 November 2023

doi: 10.20944/preprints202311.0866.v1

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Effect of Teeth Whitening Procedure on the Mineral Composition of Oral Fluid

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Abstract: The basis of modern teeth whitening systems is the use of a whitening gel, usually containing hydrogen peroxide or carbamide peroxide. The purpose of our study was to evaluate the effect of chemical and modified office teeth whitening systems on the mineral metabolism of oral fluid and to determine a safer method. The study covered 81 patients aged 22 to 35 years of both sexes with teeth color A2 and darker on the Vita Classic scale. Determination of the concentration of calcium and phosphates in the oral fluid was carried out on a spectrophotometer using ready-made Human kits. Chemical tooth whitening was performed using a gel based on 40% hydrogen peroxide. After the procedure, remineralization therapy with enamel-sealing liquid was performed for 14 days. The obtained results indicate that the most pronounced changes in the content of calcium (+74.4%, $p < 0.001$) and phosphates (phosphorus) (-23.07%, $p < 0.001$) were observed when using a chemical bleaching system. After the teeth whitening procedure using a modified technique, less pronounced changes in the concentrations of calcium (+29.07, $p < 0.001$) and phosphorus (-14%, $p < 0.001$) were observed. The use of remineralizing agents led to a faster recovery of the initial levels of calcium and phosphates in the oral fluid. Both bleaching systems caused similar changes in the chemical composition of the oral fluid.

Keywords: oral fluid; professional whitening systems; chemical teeth whitening system; modified office teeth whitening system; hydrogen peroxide; calcium; phosphorus

1. Introduction

Oral homeostasis is contingent upon not only the functionality of oral tissues, anatomical structures within the oral cavity, and the unique characteristics of blood composition but also upon the composition and inherent properties of oral fluid, commonly referred to as mixed saliva. Oral fluid is the least studied and most underestimated of all the body's biological media. Nevertheless, this small volume secretion plays a vital role in keeping the oral tissues integrated. A comparative analysis of salivary gland metabolism reveals that its intensity is only marginally lower than that observed in the kidneys and notably higher than that observed in the liver. [1–3].

Oral fluid comprises a diverse array of well-established and prospective disease biomarkers, leading to its growing utility as a biological fluid for disease diagnosis, the ongoing assessment of dental health conditions, and the prediction of disease advancement [4–6].

Despite the extensive body of scientific research dedicated to the issue of teeth whitening, numerous questions remain unanswered. The currently employed teeth whitening systems and methods for safeguarding whitened teeth lack adequate theoretical substantiation. There is conflicting evidence on the effects of whitening agents on the biochemical composition of oral fluid.

Several studies have demonstrated alterations in mineral metabolism markers due to the influence of various teeth whitening agents [7,8]. Some literature sources propose that whitening systems do not exert an influence on mineral metabolism parameters in mixed saliva and dental hard tissues [9]. The research results obtained are contradictory.

In light of the aforementioned, there arises a necessity to investigate the dynamics of biochemical parameters within oral fluid at various observation intervals during professional teeth whitening procedures, with the goal of formulating the most secure approach to mitigating the resultant alterations.

Contemporary teeth whitening systems rely on the application of a whitening gel, typically formulated with hydrogen peroxide or carbamide peroxide. When exposed to light, heat, or an alkaline environment, hydrogen peroxide reacts and breaks down, readily forming hydroxyl radicals (-OH), hydrogen (-H), and perhydroxyl (HOO-) radicals. Peroxides can affect not only the surface but also the inter- and intraprismatic regions of enamel, providing moderate deproteinization. Whitening gels operate by cleaving the unsaturated carbon bonds within pigment molecules, thereby reducing their size and complexity, which in turn diminishes absorption and enhances both light reflection and transmission. This process has the potential to remove not only pigment molecules but also mineral elements. This may account for the depletion of calcium and phosphate in these regions and, consequently, the release of minerals into the external environment. This phenomenon is attributed to the nonspecific action of oxygen radicals generated by the whitening agent.

2. Materials and Methods

2.1. Patients and whitening systems

Eighty-one patients of both sexes, approximately equal in number from 22 to 35 years old, with tooth color A2 and darker according to the Vita Classic scale, took part in the experiment. The Ethics Committee of Samara Medical University approved this study (protocol 213, 23-04-2020). This protocol was compliant with the Helsinki Declaration of 1975, as revised in 2000. The protocol was explained to all participants, and after answering every question, patients signed informed voluntary consent to participate in the study and process personal data. Patients with a sanitized oral cavity without general somatic pathology were under observation. The objective of this study is to assess the impact of two office teeth whitening systems (chemical and modified) on the mineral metabolism markers within oral fluid, specifically calcium and phosphorus levels, and to identify a safer approach to teeth whitening.

All patients had professional oral hygiene 2 weeks before the office whitening procedure using an ultrasonic device DTE-D7 LED for removal of mineralized dental deposits, an air flow device for removal of pigmented plaque by the air-jet method and polishing with a nylon brush and paste.

Depending on the method of medical teeth whitening, patients were divided into 2 groups: for chemical teeth whitening and for teeth whitening using a modified technique. Chemical whitening of teeth of the patients in the first group was performed using a gel based on 40% hydrogen peroxide with the Opalescense Boost system (Ultradent Products, Inc. USA). Taking into account the clinical and experimental results obtained over time, we modified the chemical method of teeth whitening. When carrying out the teeth whitening procedure using a modified technique, patients in group 2 used a mouth opener, a protective film-forming aerosol and an instrument for aspiration of the whitening gel. Following the procedure, remineralization therapy was administered using enamel-sealing liquid over the course of 14 days. To prevent discomfort and pain, all patients using the modified technique received Relief ACP gel in an individual mouthguard for 10 minutes immediately before the procedure. This gel contains 5% potassium nitrate, which penetrates the dentinal tubules and has a membrane-depolarizing potential, and 0.22% sodium fluoride, which obstructs the dentinal tubules. Before collecting oral fluid, patients rinsed their mouths with water, and the test tube with the resulting sample was frozen at -20ºC. To determine the indicators of oral fluid, the test tube with the sample was thawed at t +3ºC for ten hours, the sample temperature was brought to room temperature.

2.2. Collection of saliva and measurement of its mineral composition

The determination of the mineral composition of oral fluid was carried out at the Institute of Biomedical Chemistry. For this purpose, tubes with oral fluid samples were centrifuged at a speed of 5000 g for 5 minutes on an Eppendorf MiniSpin centrifuge. To assess the effect of the teeth whitening procedure, the mineral composition of the oral fluid was determined before the procedure, immediately after, 14 days and 30 days after the procedure on an Aquarius CE 7200 spectrophotometer (Cecil Instruments Ltd, UK). The concentrations of calcium (photometric method with o-cresolphthalein complexone) [10] and the phosphorus content was measured (photometric method with antilipid factor) [11] were measured using Human kits (Human, Germany) and control serum Humatrol (Human, Germany).

2.3. Statistical analysis

Statistical data analysis was performed using SPSS 25 software (IBM SPSS Statistics, Armonk, NY, USA). Descriptive statistics used M - mean value, SD - standard deviation, Δ% - difference in percentage of sample mean values (Mtest/Mcontrol) x 100%). Statistical analysis of the results was performed on a personal computer using the SPSS 25 software package (IBM SPSS Statistics, USA). Descriptive statistics in the tables are presented in the form M±SD, where M is the arithmetic mean, SD is the standard deviation, Δ% - difference in percentage, calculated using the formula a/b*100%.

a = value before teeth whitening procedure

b = value of the indicator after the teeth whitening procedure, after 14, after 30 days.

+ in the direction of increase in relation to the value of the indicator before the teeth whitening procedure

- towards a decrease in relation to the value of the indicator before the teeth whitening procedure.

Comparisons of independent groups were performed using Kruskal-Walli's analysis (Pk – W) followed by pairwise comparisons using the Mann–Whitney test. Differences were considered statistically significant at values of p <0.05.

3. Results

The calcium ion content in saliva ranges from 0.75-3.0 mM (same as in plasma). Calcium can be in ionized (Ca2+) or protein-bound forms. After the in-office teeth whitening procedure, both group 1 and group 2 patients had increased calcium ion concentrations in oral fluid (Table 1).

Table 1. Effect of the tooth whitening procedure on the concentration of calcium ions in oral fluid (mM).

Calcium ion concentration in oral fluid	Before the procedure	After the procedure	After 14 days	After 30 days
Chemical office teeth whitening (group 1)				
M±SD	2.19±0.16	3.82±0.24	2.95±0.18	2.14±0.13
Δ%	-	+ 74.4%	+34.7%	- 2.3%
p	-	<0.001	<0.01	0.553
Modified office teeth whitening (group 2)				
M±SD	2.30±0.09	2.97±0.11	2.44±0.11	2.20±0.09
Δ%	-	+29.1%	+11.4%	-4.3%

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p	-	<0.001	<0.01	0.237

M – mean value, SD - standard deviation, Δ% - difference in percentage of sample mean values – Mtest/Mcontrol x 100% ("+" indicates the increase in % relative to the value in "Before whitening" group; "-"indicates the decrease in % relative to the value in "Before whitening" group).

When comparing the postwhitening calcium ion concentration between the two office systems, a statistically significant difference in the macroelement level within the oral fluid was observed (Table 2).

Table 2. Comparison of calcium ion concentration in oral fluid after the tooth whitening procedure with two different office systems (mM).

Calcium ion concentration in oral fluid	Chemical office teeth whitening		Modified office teeth whitening technique		Comparison of two systems p-level
	Before	After	Before	After	
M±SD	2.19±0.16	3.82±0.24	2.30±0.09	2.97±0.11	
Δ%	-	+ 74.4%	-	+29.1%	
p	-	<0.001	-	<0.001	0.012

M – mean value, SD - standard deviation, Δ% - difference in percentage of sample mean values –– Mtest/Mcontrol x 100% ("+" indicates the increase in % relative to the value in "Before whitening" group; "-"indicates the decrease in % relative to the value in "Before whitening" group).

The results obtained show a decrease in the phosphate content in the oral fluid after the teeth whitening procedure in patients of both groups (Table 3).

Table 3. Effect of the tooth whitening procedure on the concentration of phosphate in oral fluid (mM).

Phosphate concentration in oral fluid	Before the procedure	After the procedure	After 14 days	After 30 days
Chemical office teeth whitening (group 1)				
M±SD	4.45±0.22	3.43±0.18	3.88±0.17	4.44±0.22
Δ%	-	- 23.07%	- 13%	- 0.3%
p	-	<0.001	<0.01	0.674
Modified office teeth whitening (group 2)				
M±SD	4.45±0.21	3.83±0.21	4.46±0.21	4.41±0.20
Δ%	-	- 14%	+ 0.22%	- 0.9%
p	-	<0.001	p>0.05	0.221

M – mean value, SD - standard deviation, Δ% - difference in percentage of sample mean values –– Mtest/Mcontrol x 100% ("+" indicates the increase in % relative to the value in "Before whitening" group; "-"indicates the decrease in % relative to the value in "Before whitening" group).

In contrast to the statistically significant changes in calcium ion concentration observed separately in the two office systems, there was no statistically significant alteration (p=0.238) in phosphate content when the teeth of patients in the first and second groups were subjected to whitening.

 doi:10.20944/preprints202311.0866.v1

4. Discussion

It is scientifically proven that bone tissue is involved in the systemic regulation of calcium metabolism. There are two fractions of calcium in bone tissue, labile and stable. The labile calcium fraction of bone actively exchanges calcium with oral fluid. Furthermore, there exists a bidirectional exchange of calcium between the labile and stable fractions of bone tissue, a process under the control of factors governing bone remodeling. Thus, the chemical composition of the dental hard tissue is completely dependent on the composition of the surrounding oral fluid.

The findings indicate a substantial rise in calcium ion concentration within the oral fluid following the teeth whitening procedure in group one (+74.4%, $p < 0.001$) and group two (-23.07%, $p < 0.001$) compared to the values recorded prior to the teeth whitening procedure. Simultaneously, the highest calcium ion content was observed when the chemical whitening system was employed in the first group. The use of a modified tooth whitening system resulted in a less significant increase in calcium ion levels. The results suggest the release of calcium ions from the enamel crystal lattice, leading to an elevation in the concentration of calcium ions in the oral fluid. The calcium concentration reverted to baseline levels after one month in both groups, a phenomenon attributed to the processes of enamel remineralization.

According to the data of O.A. Uspenskaya et al., during professional tooth whitening, there is an increase in the level of calcium ions in the oral fluid, which, according to the authors, indicates the release of calcium ions from the enamel layers during the procedure [12]. A decrease in the level of phosphorus and an increase in the concentration of calcium in the oral fluid was established immediately after the teeth whitening procedure separately using three different whitening systems, including chemical ones [13]. Some studies by various authors indicate that tooth whitening is correlated with a reduction in the calcium and fluoride content within the hard tissues of teeth [14,15]. Several studies have reported that there were no observed changes in dental hard tissues following either professional or at-home tooth whitening procedures [16,17]. In the studies of F.Y. Cakir, it was established that teeth whitening systems decreased calcium and potassium levels in tooth enamel, while F and O levels in enamel increased [18].

A decrease in phosphate concentration within the oral fluid was observed immediately after the tooth whitening procedure in both the first group (-23.07%, $p < 0.001$) and the second group (-14%, $p < 0.001$) of patients. The chemical tooth whitening system exhibited a more significant change in phosphate concentration, which persisted for 14 days and returned to baseline levels after 30 days. No significant changes were observed in phosphate concentrations after 14 days with the modified tooth whitening system. The use of a combination of prophylactic agents in the second group resulted in a slight increase in phosphate levels and a swift restoration of the levels of this trace element in the oral fluid.

Some publications report an increase in phosphate content in oral fluid, while other studies have found that the content of this trace element remained unchanged. In the work of Carmen Llena, the calcium and phosphate contents of enamel and dentin were evaluated after application of 37.5% hydrogen peroxide and 35% carbamide peroxide. Calcium and phosphate contents decreased in dental hard tissues with no significant differences between them and with respect to control samples ($p > 0.05$) [19]. The decrease in phosphate levels in the oral fluid immediately after the tooth whitening procedure in our study can be attributed to the uptake of phosphate from the oral fluid and its utilization in the process of tooth enamel remineralization.

A statistically significant difference was found in the calcium concentration in the oral fluid after the whitening procedure with the two teeth whitening systems tested ($p=0.012$). There was no significant difference in the concentration of phosphate in the oral fluid during tooth whitening between the first and second groups of patients ($p=0.238$).

5. Conclusions

Whitening agents have an impact on mineral metabolism indicators, specifically calcium and phosphate levels, which should be considered when undergoing tooth whitening procedures. The whitening systems tested caused similar changes in oral fluid mineral. The chemical tooth whitening method results in more significant alterations in the concentrations of calcium and phosphate ions in oral fluid compared to the modified office whitening technique. The assessment of calcium and phosphate ion fluctuations in oral fluid has been suggested as a means to assess the safety of various tooth whitening systems. During the procedure of teeth whitening, it is necessary to apply a set of preventive measures. The utilization of a rotoraspirator, protective film-forming aerosol, an aspiration tool for the whitening gel, and a 14-day remineralizing therapy with enamel-sealing liquid led to minor alterations in calcium and phosphate ion concentrations and facilitated a swift return to their baseline levels in the oral fluid. The chemical whitening technique has been shown to be more aggressive, while the modified technique is safer and gentler. In dentistry, employing biochemical analysis of oral fluid for diagnosing the mineralization properties of oral fluid will enable the early assessment of the impact of various whitening agents on the oral fluid composition. Additionally, it will facilitate timely metabolic correction of detected disorders. Utilizing a systematic approach with interventions and tools that influence calcium-phosphorus metabolism in the oral cavity will enhance the effectiveness of therapeutic, preventive, and rehabilitative procedures during tooth whitening.

Author Contributions: Conceptualization, E.A.R., D.D.Z.; methodology, D.D.Z., E.A.R., O.A.M., saliva sampling O.A.M., F.N.G.; statistics, M.A.P., validation, D.D.Z.; formal analysis, E.A.R., D.D.Z.; data curation, O.A.M., T.A.L., A.N.S.; resources, E.A.R., D.D.Z.; writing—original draft preparation, review and editing, E.A.R., D.D.Z.; supervision, E.A.R., D.D.Z.; project administration and funding acquisition, D.D.Z. All authors have read and agreed to the published version of the manuscript.

Funding: The work was done in the framework of the Russian Federation fundamental research program for the long-term period (2021-2030) (№ 122022800499-5).

Institutional Review Board Statement: All procedures performed in this clinical study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Ethical Committee of Samara State Medical University approved the protocol 213 23-04-2020.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Informed consent for this study is available on request from the corresponding author.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to ethical restrictions.

Conflicts of Interest: The authors declare no conflicts of interest.

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