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THE EFFECT OF OZONATED WATER VERSUS ALKALINE PEROXIDE CLEANERS ON COLOR STABILITY, SURFACE ROUGHNESS AND HARDNESS OF TWO DIFFERENT DENTURE BASE MATERIALS

Marwa El Saied Hamada*, Ahmed Mohamed Alam-Eldein** and Zeinab Ahmed El Shorbagy***

ABSTRACT

Purpose: Evaluating the effect of ozonized water versus alkaline peroxide (Corega) denture cleaner on color stability, hardness and surface roughness of materials of two denture base.

Materials and methods:180 samples were made from two commercially available denture base materials, conventional heat cure resin and polyamide resin. Denture cleansers were divided into three equal groups; ozonized water using Sota Woz-5 home ozone generator, Corega tablets and distilled water. The effect of three cleansing agents on the two different denture was evaluated and compared to color stability using spectrophotometer, surface roughness using 3D Optical profilometer and hardness using Vickers Hardness Tester.

Results: Regarding to color change (ΔE); it was found that alkaline peroxide recorded statistically significant change higher than ozonized water on both denture materials, while distilled water showed no significant difference. According to the surface roughness, denture cleaners recorded statistically non-significant change on both denture materials. While in hardness, Ozonized water and alkaline peroxide recorded statistically significant on both denture materials, but alkaline peroxide recorded statistically significant higher change mean value on Polyamide than PMMA, while distilled water showed non-significant difference on both denture materials.

Conclusion: Ozonized water regenerated by ozone generators is useful cost-effective means of denture cleaning. Ozonized water showed less color changes to both PMMA and polyamide materials than alkaline peroxide cleanser. Both ozonized water and alkaline peroxide had no negative effect on surface roughness and slight deteriorating effect in hardness with clinically accepted degree of change.

KEYWORDS: Ozonized Water, PMMA, Polyamide, Color Stability, Surface Roughness.

* Dentist at Kafr Essam Family Hospital, Ministry of Health, Elgharbya, Tanta, Egypt, Tanta, Egypt.
** Associated Professor of Prosthodontics, Faculty of Dentistry, Tanta University, Tanta, Egypt.
*** Professor of Prosthodontics, Faculty of Dentistry, Tanta University, Tanta, Egypt.

INTRODUCTION

Denture base materials are many. Polymethyl methacrylate (PMMA) and Polyamide materials are the most common used denture base materials for rigid and flexible dentures representatively [(1)]. Regardless of the type of denture base material used, denture care is critical for oral health; otherwise, the denture will become unsanitary and undesirable effects such as bad breath, unpleasant staining and biofilm, and calculus accumulation on the denture will occur, which can result in denture stomatitis, angular cheilitis, and poor oral health. [(2).]

There are two methods for cleaning dentures: mechanical and chemical. Brushing with or without paste and ultrasonic agitation are two mechanical cleaning procedures. However, it has been observed that mechanical techniques alone are unable to eliminate germs from the denture completely. As a result, many authors favour chemical cleaning procedures. Dentures can be cleaned chemically using alcohol-based disinfectants and denture cleaners. [(2)].

Denture cleaners sold commercially are classified as alkaline peroxides, alkaline hypochlorites, dilute organic or inorganic acids, and enzymes. Solution of alkaline peroxide made by dissolving sodium perborate in water. However, studies indicate that when peroxide cleaners are used for frequent denture washing, they may change some physical features of denture bases. [(3)].

Three oxygen atoms make up ozone, a naturally occurring chemical (triatomic oxygen or tri-oxygen). It is present in the stratosphere as a gas at a concentration of 1-10 PPM or is generated by ozone generators. Ozone has been increasingly utilised in dentistry in recent years because to its unique qualities, which include antibacterial, immunological stimulant, analgesic, and detoxifying capabilities. Ozone has high oxidizing potential which acts on microorganisms to inhibit or kill their growth, necessary for plaque maintenance [(4)].

Ozone is utilised in dentistry in the form of gaseous ozone, ozonized water, and ozonized oils and has been shown to be effective against bacteria, fungus, and viruses. The benefits of ozone in the aqueous phase are its potency, simplicity of handling, fast microbicidal action, and appropriateness as a soaking solution for medical and dental tools. In addition, its ability to destroy Candida Albicans and prevent denture stomatitis [(5)].

Ozone's clinical efficiency as denture cleanser was proved by more than one evidence. However, few research have examined its influence on the denture base's physical and mechanical characteristics. The objective of this study is to compare the color stability, surface roughness, and hardness of two denture base materials when cleaned with ozonized water vs alkaline peroxide cleansers[(5, 6)].

The aim of this in vitro study was to evaluate the effect of ozonized water versus alkaline peroxide (Corega) denture cleaner on color stability, surface roughness and hardness of two denture base materials (acrylic and polyamide denture base).

MATERIALS AND METHODS

180 samples of two commercially available denture base materials were prepared: conventional heat cure acrylic resin (Acrostone, Cairo, Egypt) and polyamide resin (Vertex Thermosens: Vertex-Dental B.V. 3705 HJ Zeist, Netherlands).

The Research Ethics Committee approved this study. The present study was designed and conducted in accordance with the research standards established by the research and ethical council.

Three denture cleansers were tested in the study; Distilled water was used as a control. Ozonized water specially prepared for the study by using home ozone generator (Sota Instruments Inc., Penticton, BC, Canada). Alkaline peroxide solution prepared by effervescence tabs (Corega Denture Cleanser GlaxoSmithKline, Dublin, Ireland).

Three sets of samples were created. Each group consisted of 60 samples, 30 from each of the denture base materials. Ten samples were randomly chosen for group 1 and exposed to distilled water as a control, ten samples for group 2 were exposed to ozonized water as the evaluated cleansing agent, and ten samples for group 3 were exposed to Corega cleanser. Three cleansing agents were examined and compared in terms of color stability, surface roughness, and hardness on two distinct denture base materials.

Preparation of template metal disks: A Metal disk shape of dimension 3 mm thickness and 20 mm diameter was fabricated for testing color stability, surface roughness and hardness. 192,193 The design of the metallic disk was carried out via 3D electronic drawing by 'Solidworks' software (Solidworks 2018, corporation, Dassault Systemes, USA) and the design was transferred to the milling machine. The milling was done by computer numerical control machine 'CNC' (ED5X, Dental Series, Emar, Egypt) These metal disks were used to prepare the samples for the two denture base materials.

Preparation of heat-cure acrylic resin samples: Using dental stone, metal discs were inserted into dental flasks. The acquired mould spaces were utilised to prepare test samples. On the dental stone mould, a separating medium was placed. The powder and liquid have been combined according to the manufacturer's recommendations. When the mixture reached the dough stage, it was annealed and packed into the mould cavity, followed by final closure using a bench press. The flasks were allowed to polymerize for 1.5 hours in a water bath set at 72°C, followed by 30 minutes boiling in 100°C water. After the curing cycle was completed, the flasks were cooled on the bench until they reached room temperature (7). The samples were retrieved. Following polymerization, all samples were polished with burs, abrasive paper, and pumice, as is customary in traditional denture fabrication. All samples obtained in this manner were submerged in distilled water at 371°C for 24 hours to eliminate residual monomers[8].

Preparation of polyamide resin samples

Polyamide denture base material samples were manufactured in accordance with the manufacturer's instructions by injecting thermoplastic materials into special flasks equipped with holes in the capsule containing the thermoplastic powder. After filling the flask with stone, the metal discs were added to form the mould. The soft wax sprue was inserted into the injection channel of the flask containing the sample of metal discs embedded in the stone.

The flask's two sides were assembled and secured with screws. Allowing the stone to solidify was permitted. For five minutes, the flasks were put in 70^{o} C water to soften the wax. The flasks were opened, the wax was removed, and the flasks were cleaned with hot water (IV-15). Thermo-Flow insulation was used to separate the stones. The flasks were warmed in 90 °C water for 15 minutes before being placed in the injection moulding machine (Thermopress 400® – Bredent, UK). Following 16 minutes of preheating the cartridge to 250^{o}C, 6.5 bar of pressure was injected. For 30 minutes, full flasks were put in an oven (> 100^{o}C) or in boiling water. The flasks are designed to achieve the optimum quality of the material. The samples were carefully collected, and the injection channel was closed. The cross-standard bur was used to smooth the edges. Silicone polishers were used to polish the surface. To finish, Thermo-Gloss and a microfiber polishing brush were utilised. The finishing touch was provided by the brush felt cloth to get smooth samples (**Figure 1**) [8].

Measured parameters

Color stability testing: This test was conducted using a spectrophotometer (Figure 2) and the International Commission on Illumination (CIE)

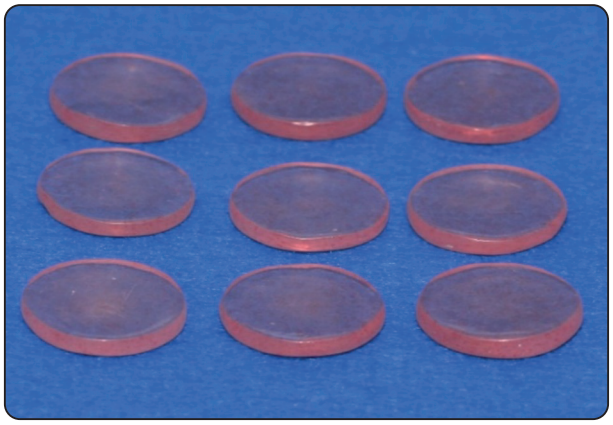

Fig. (1): Polyamide samples after finishing and polishing

Fig. (2): Sample (Pink Circle) was fixed to the spectrophotometer holder.

methodology of measuring the light absorption of each specimen before and after immersion[9]. The device used for the measurements was Agilent Cary 5000 spectrophotometers. It was developed in accordance with a quality control system certified to ISO 9001. The basic principle of CIE is that all colors can be matched by combining the relative proportions of the three light primaries: red (X), green (Y) and blue (Z).

These values can then be transferred to [L], [a], and [b]; [L] is a measure of the degree of lightness, [a] is the position on the red-green axis. As the value becomes more positive, the color becomes more red; as the value is more negative, the color is more green and [b] is the position on the yellow-blue axis. As [b] becomes more positive in value, the color becomes more yellow; as [b] becomes more negative in value, the color becomes more blue.

ΔE (Color Changes Value) = $[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$

Where ΔL, Δa and Δb are the variations in the values L, a and b before and after immersion at each time interval. Only the ΔE result was used for analysis in this study. The National Bureau of Standards (NBS) is used to measure the difference of color (10). Critical color difference points according to the NBS are seen in table (IV-2). The following formula is used to indicate the NBS units.

NBS unit=ΔE x 0.92

Where ΔE is the color change, The samples were secured in the spectrophotometer's holder, and the instrument was switched on before testing. To determine the effect of cleansing agents, all samples were tested for color stability after and before immersion in cleansing agents and water.

ii. Surface roughness testing:

The optical profilometry aims to fulfil the need for quantitative surface topography characterisation without contact (11). Quantitative examination of specimens and their antagonists' two-body wear was performed prior to and following loading into a 3D-surface analyser system. The samples were photographed with a USB digital microscope equipped with a built-in camera (U500X, Digital Microscope, Guangdong, China) linked to an IBM compatible personal computer at a fixed magnification of 120X (Figure 3). Each image was captured at a resolution of 1280 1024 pixels. Cropping digital microscope pictures to 350 x 400 pixels using the Microsoft Office picture manager allows you to select / standardise the roughness of the measuring region.

Cropped images were analysed using WSxM software [12]. All limitations, sizes, frames, and characteristics monitored inside the WSxM software are represented in pixels. As a result, the system was calibrated to convert the pixels to their absolute real-world units. Calibration was performed by comparing a known-size object (in this case, a ruler) to a scale created by the programme. The average height (Ra) in m was calculated using the WSxM programme, which may be regarded to represent valid surface roughness indices [13]. Following that, a 3D image of the specimens' surface profile was produced using the Digital Image Analysis System (Image J 1.43U, National Institute of Health, USA). The untouched surface provided as a point of comparison. This approach created a three-dimensional geometry of the worn surface.

iii- Hardness testing: The materials' surface microhardness was measured using a Digital Vickers Micro-Hardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd., China) equipped with a Vickers Diamond Indenter and a 20X objective lens1 (Figure 4). For 20 seconds, a force of 200 g was applied to the specimen's surface. Three indentations were created on the surface of each specimen, evenly spaced around a circle and not closer than 0.5 mm to neighbouring indentations. Using a scaled microscope, the diagonal lengths of the indentations were measured, and the Vickers values were translated to micro-hardness values (14).

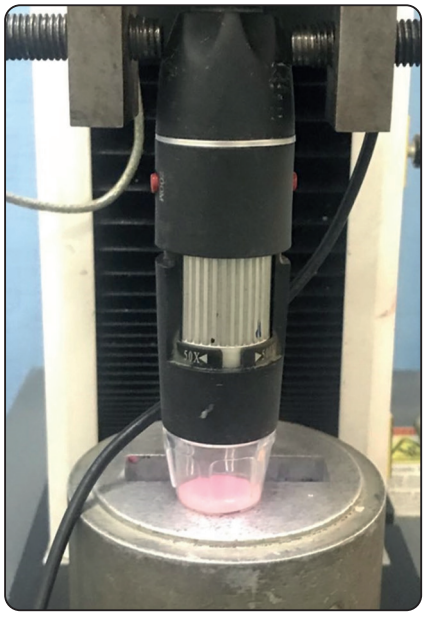

Fig. (3): Scanning of a sample placed in the platform under digital microscope

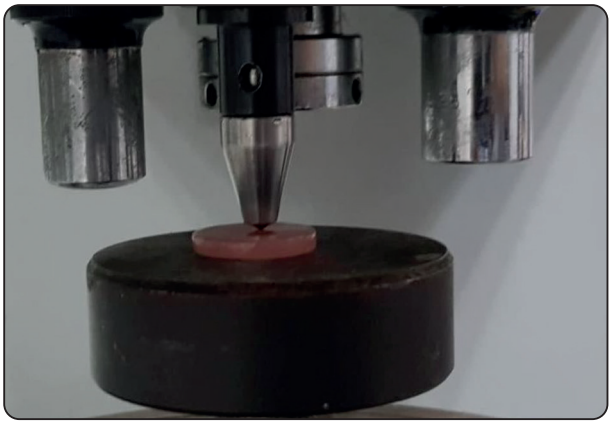

Fig. (4): A sample under Vickers diamond indenter

Micro-hardness calculation: Micro-hardness was obtained using the following equation:

$HV=1.854\ P/d^2$

HV denotes Vickers hardness in Kgf/mm2, P denotes the load in Kg, and d denotes the diagonal length in mm. Samples were immersed in the cleaning agent according to the cleaning protocol in items of concentrations and amount of cleansers have been used.

Immersion in the denture cleaners

For each of cleansing solution, 30 sample of acrylic and 30 sample of polyamide were immersed in beakers contain 200ml of each cleansing solution according to the manufacturer instruction for time equivalent of 180 days. The solutions were changed on daily basis as follow:

For the water control, the specimens were maintained at room temperature in distilled water for the entire 180 days [15].

For the ozonized water: the ozonized water was freshly prepared using Sota Woz-5 ozone gas generator machine. The preparation was done by placing the ceramic stone connected to the silicone tube getting out of the ozone generator to start bubbling of the ozone into the distilled water allowing its ozonisation. The concentration of ozonized water should range from 2 – 4.0 mg/l according to **Nagayoshi et al.** [16] and **Arita et al. (6)** and according to the manufacturer instructions for the 150 ml water amount, 5minute ozonisation will be sufficient (Figure 5). The ozonized water will be used within 20 minutes after its preparation. After each cycle, test specimens were washed under running water and kept in distilled water until the following round. Procedures for immersion were performed 180 times to replicate a 180-day period of usage [17].

For Alkaline peroxide solution (Corega tabs), were be prepared according to the manufacturer's instructions, by adding one tablet to 200 ml of water (37°C). Following each 5-minute cycle. After discarding the soaking solution, the specimens were thoroughly washed under running water. Between soaking operations, the specimens were maintained at room temperature in distilled water [11, 17, 18].

Procedures for immersion were performed 180 times to replicate a 180-day period of usage [17, 18].

After 180 repetitions of the immersion technique, final measurements of color, surface roughness, and hardness were taken, the difference in color, surface roughness and hardness were calculated and the data were tabulated and statistically analysed using SPSS software version 18.

RESULTS

Color Stability

Both PMMA (subgroup A) and Polyamide (subgroup B) showed higher values of color changes for alkaline peroxide (1.847± 0.134) in PMMA and (2.226± 0.154) for PA followed by ozonized water (1.145±0.091) for PMMA and (1.379±0.089) for PA, while the least color change was reported for distilled water (1.032±0.053) for PMMA and (1.006±0.086) for PA. According to ANOVA test for comparison between the value of color changes after immersion in different cleaners showed significant difference with (P-value = <.001). With pair-wise Tukey's post-hoc tests, it was recorded that there were statistically significant changes between group I&II, group I&III and group II&III as (P-value = <.001). Comparison between PMMA and PA with both ozonized water solution and alkaline peroxidase, it was found that polyamide recorded statistically significant difference (P = <.001 < 0.05) higher than PMMA as proved by student t-test.

According to NBS system the following formula is used to indicate the NBS units. NBS unit=ΔE x 0.92. It was found that the color changes in distilled water and ozonized water groups were classified as "slight" (<1.5). However, for effervescent tablets (alkaline peroxide Tabs) group, the color changes were classified as "noticeable": (1.5-3.0; 4). (Table 1)

Table (1): Shows the results in NBS units.

		Groups		
		Distilled Water	ozonized Water	Alkaline Peroxide Tablet
Color stability	PMMA	0.949	1.053	1.6992
	Polyamide	0.925	1.2686	2.0479
NBS unite		Slight	Slight	Noticeable

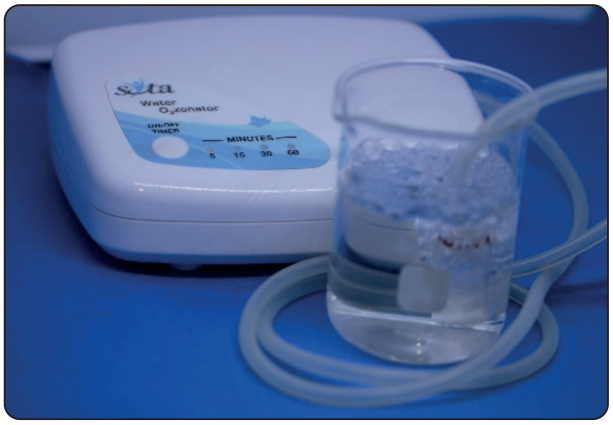

Fig. (5): Preparation of ozonated water

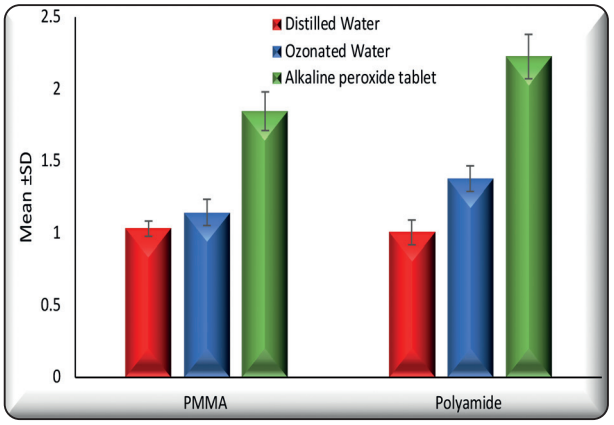

Fig. (6): comparison between different subgroups after immersion in different denture cleansers

Surface Roughness

According to ANOVA test for comparison between the value of roughness changes after immersion in different cleaners showed no significant difference with (P- value =0.069). Comparison between PMMA versus PA using both ozonized water solution and distilled showed no significant difference (p value =0.292), (p value =1.000). with distilled and ozonized water solution respectively, while with alkaline peroxide solution; both PMMA and PA showed significant difference (p value =0.018*). (Figure 19; Figure 20 Figure 19: 3D profiler photographs of PMMA denture base resin at baseline and after immersion in a) distilled water; b) ozonated water; c) alkaline peroxide.).

Hardness test

Comparison between PMMA and PA, both distilled water and ozonized water showed non-significant higher values for PMMA than PA (P-value =0.465), (p=0.109) in distilled water and ozonized water respectively, while with alkaline peroxide solution; It was found that polyamide recorded statistically significant (p=<.0001 < 0.05) higher change mean value (p=0.046*) than PMMA. (Figure 23 and Figure 24)

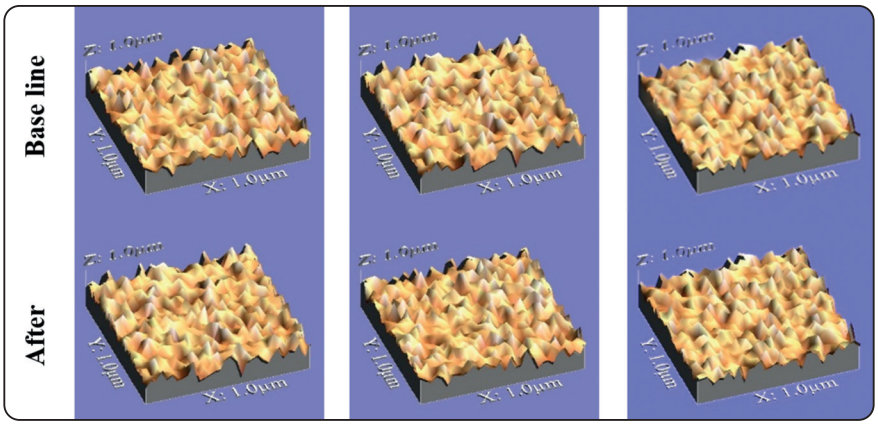

Fig. (7): 3D profiler photographs of PMMA denture base resin at baseline and after immersion in a) distilled water; b) ozonated water; c) alkaline peroxide.

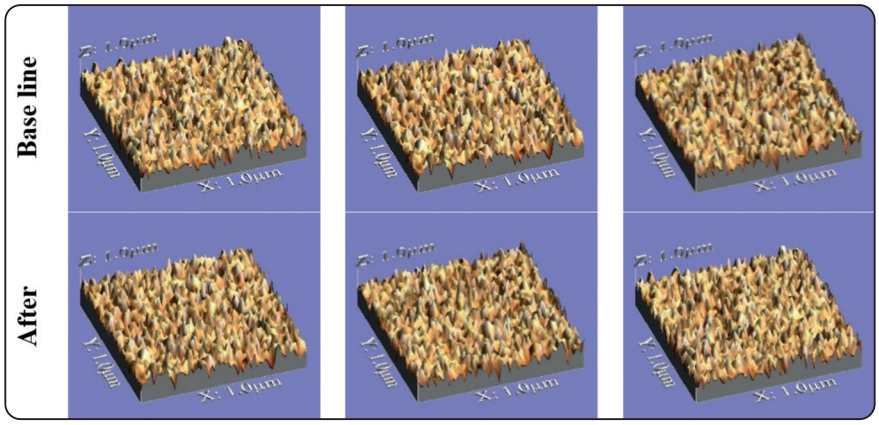

Fig. (8): 3D profiler photographs of Polyamide denture base resin at baseline and after immersion in a) distilled water; b) ozonated water; c) alkaline peroxide.

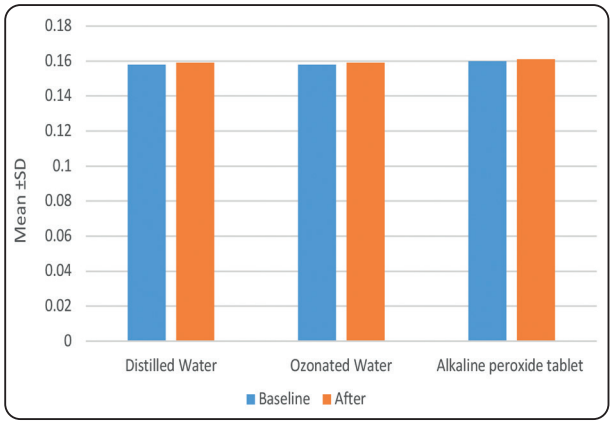

Fig. (9): Bar chart showing roughness mean values for PMMA at baseline and after immersion in different denture cleansers.

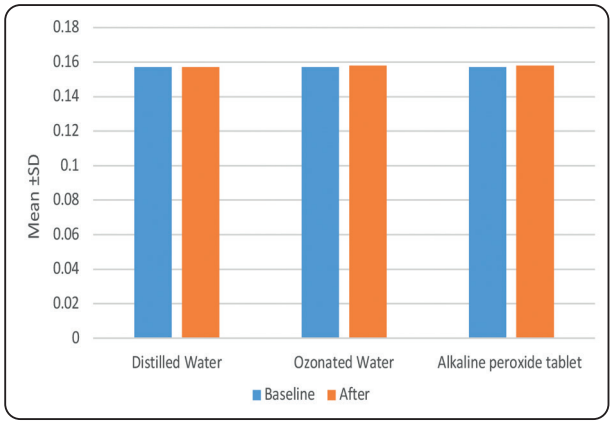

Fig. (10): Bar chart showing roughness mean values for Polyamide at baseline and after immersion in different denture cleansers.

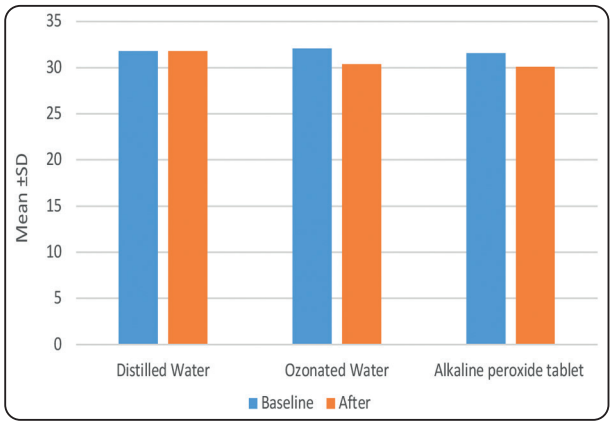

Fig. (11): Bar chart showing hardness mean values for PMMA at baseline and after immersion in different denture cleansers

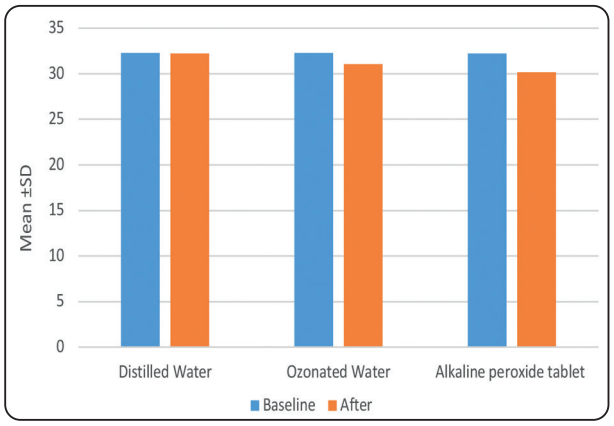

Fig. (12): Bar chart showing hardness mean values for Polyamide at baseline and after immersion in different denture cleansers.

DISCUSSION

Corega tabs recorded statistically significant high (ΔE) mean values when compared to control group. These results were in accordance with **Peracini et al.**[12] who studies the color stability and surface roughness in response to two different types of alkaline peroxide cleaners on PMMA denture base material. The agreement was in finding Corega specifically showed higher statistically significant color changes measured by NBS system among other alkaline peroxide cleaners.

Moreover, in agreement of the study results, Porwal et al.[18] who studied the color stability of three different denture base materials prior and after immersion of alkaline peroxide and sodium hypochlorite for 180 days. They used NBS system of color judgment. Authors recorded higher color changes in alkaline peroxide on both heat cured PMMA and polyamide denture base materials.

This may be related to the deleterious combination of oxidation and strong alkaline solution. Peroxide denture cleansers include an effervescent component such as sodium perborate or sodium bicarbonate, when sodium perborate tablets are dissolved in water, they breakdown to create an alkaline peroxide solution. Following that, this peroxide solution will release oxygen and remove dirt by mechanical mechanisms[19].

As a result, the usage of these denture cleaners may result in the hydrolysis and breakdown of the polymerized acrylic resin itself, which may explain why these cleansers had a higher effect on the color stability of denture foundation[20].

However, **Sato et al.**[21] who studied the effect of color change of different types of alkaline peroxides on acrylic resins and failed to detect any color changes in the 105 specimens of the study. The disagreement of these outcomes in relation to this study results may come from the way of assessment of the two researches, where photographic and visual means of judgment is not as accurate as mathematical way of NBS system and spectrophotometer.

In addition, **Durkan et al, 2013**[17] were in disagreement with the color changes results of the present study. The study recorded no change in color in polyamide denture base material. This may be a result of less time of the immersion period as it was just 20 days in comparison to the 180 days in this study. That 20 days may not be sufficient to initiate color changes from the alkaline peroxide cleaner. The number of specimens was only 35 in comparison to 90 polyamide specimens which was may not enough to get an accurate outcome about the reality of the effect of the cleaner effect, as well.

In the present study, ozonized water recorded statistically significant high (ΔE) mean values in both PMMA and polyamide when compared to control group. However, these changes were considered clinically not detectable and recorded according to NBS as 'slight'. In disagreement of the study results, **Suzuki et al. 2002** (22) revealed no statistically significant color changes neither with commercially used denture cleaners (Steradent and Polident) which are alkaline peroxide-based denture cleaners, nor ozonized cleaner. The only resultant changes in color caused by higher doses ozonized cleaner group. However, the mentioned change caused was by external effect on the stained specimens not on the native color of the acrylic resin.

However, **Klonica et al, 2016** (23) were in agreement with the present study outcomes. The authors studied the physical and chemical state of industry polyamide material following ozone treatment. They found that after ozone treatment, the spectrometer recorded higher increase in the color changes after ozone treatment. These observations might be explained by Ozone solution's strong oxidising capacity.

The current study showed that the color changes in the control and ozonized water subgroups

were classified as "slight" (<1.5). However, for effervescent tablets (Corega Tabs) subgroup, the color changes were classified as "noticeable" (1.5-3.0). The National Bureau of Standards (NBS) has measured the crucial remark of color change (E) as NBS units of color difference. The following formula is used to express NBS units: NBS unit = $\Delta E \times 0.92$(10).

Durkan et al [17] investigated the effects of three sodium perborate-containing denture cleaners (Corega, Protefix, and Valclean) on the surface roughness, hardness, and color stability of two polyamides and a PMMA polymer used as a control. After 20 days of repeated immersion, the surface roughness of the polyamide increased independently of the solution utilised. One possible reason for the observed increase in roughness for resin specimens is that the investigation utilised a high water temperature (50°C).

ozonized water which's an environment friendly disinfectant solution had been tested for the effect on surface loss and hence surface roughness of PMMA, it was found no effect of ozonized water on surface roughness.

That outcomes matched that of Matsuura et al. (24) who studied the effect of ozonized water with or without microbubbles which supposed to have more effect on the surface of PMMA versus other polymers. They found that although ozonized water has the ability to degrade organic compounds with its high oxidizing ability and ability to produce reactive oxygen species, it didn't affect PMMA with and without microbubbles. That may be explained by lacking the PMMA of neither C-C bond nor benzene ring which sensitive to ozone itself.

However, Klonica et al. (23) found that ozone treatment for polyamide resulted in softness of the surface layer. This softness yielded to decreased surface topography and reduced reading for the 3D optical profilometer used in the study. These outcomes were in disagreement with our study.

In this study, the hardness of PMMA resin and the polyamide resins decreased after repeated immersion, regardless of the type of the cleansing solution used. In agreement of the present study, **Durkan et al**. [17] evaluated the influence of three sodium perborate-containing denture cleaners (Corega, Protefix, and Valclean) on the surface roughness, hardness, and color stability of two polyamides, a butadien styrene copolymer PMMA, and a PMMA polymer used as a control group. They revealed that the hardness values of both PMMA resin and polyamide was decreased where Polyamide resins demonstrated lower Vickers hardness values before and after immersion in the denture cleansers. In addition, **Neppelenbroek et al.** [25] and **Machado et al.** [26] reported acrylate resins treated with sodium perborate solution showed a substantial reduction in hardness. Likewise, in the current study, the hardness of the PMMA resin reduced.

Hardness levels may decrease as a result of the polymerization activity continuing, monomer release, or leftover monomers interacting with active free radicals through oxygen bonding. Different alkaline peroxide-based denture cleaners were used in the hope that slight structural variations might result in a different effect; however, no difference in terms of hardness reduction was seen. This might be explained by the fact that resins cleansed with alkaline peroxide were damaged by the peroxide's breakdown into free radicals. By reduction and oxidation reactions, these free radicals transform big molecules into tiny ones. When resins are submerged in hot water, hydrogen peroxide is generated, which decomposes the oxygen in their structure into free oxygen radicals, therefore removing water molecules. Oxygen may induce a chemical softening of the resin surface by interfering with the polymer's inter-chain tensions.

The results of the Vickers hardness values of the polyamide specimens after ozonized water immersion was found to be decreased. These results were in accordance with **Klonica et al.** [23] who studied the physical changes of the surface

of polyamide. The authors revealed increase of the surface softness and reduction in modulus of elasticity. These outcomes significantly affect the resistance of polyamide to indentation which gives rise to reduced hardness of the matter.

CONCLUSION

Based on the results of this study, we can conclude that ozonized water showed less color changes to both PMMA and polyamide materials than Corega cleaner. In addition, both ozonized water and Corega had no negative effect on surface roughness. Moreover, both ozonized water and Corega cleaner had slight deteriorating effect in items of hardness with clinically non-recognisable and accepted degree of change. PAs was affected more than PMMA which always showed less readings in all denture properties. Finally, ozonized water regenerated by home ozone generators can be a useful and easily used and everlasting means of denture cleaning. Ozonized water can be used also with superiority to Corega as it is readily available in homes rather than buying them routinely which is considered cost-effective means.

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