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Evaluation of antiseptic mouthwashes protocol against SARS-CoV-2 on orthodontic appliances (an *in vitro* study)

Avaliação do protocolo de enxaguantes bucais antissépticos contra SARS-CoV-2 em aparelhos ortodônticos (estudo *in vitro*)

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ABSTRACT

Objective: The objective of this study was to evaluate the impact of daily mouthwash rinsing protocols recommended against SARS-CoV-2 on metal ions discharged from fixed orthodontic appliances, specifically Nickel (Ni) and Chromium (Cr). **Material and Methods:** Total of 400 hemi-arch fixed appliances were segregated into two groups, namely Nickel Titanium (NiTi) and Stainless Steel (SS), based on the type of archwire employed. Each set was submerged in 2% povidone-iodine, 1% hydrogen peroxide, 0.2% chlorhexidine and cetylpyridinium chloride mouthwashes. Distilled water was used for comparative measurements of the ions released as a control group (n=10/group). They were incubated for four periods at 37°C (one hour, twenty-four hours, one, and 3 weeks). Nil and Cr ions released from the fixed appliance were evaluated by atomic absorption spectroscopy. The data were analyzed by the Kruskal-Wallis test and paired comparison analysis. **Results**: The worst levels of Ni and Cr liberated from the SS group observed in povidone-iodine mouthwash at 1.173 and 1.701 ppm, respectively, while the chlorhexidine mouthwash released accepted level of Ni and Cr at 0.033 and 0.056 ppm, respectively. The NiTi group displayed the appalling ions released of Ni and Cr in povidone-iodine mouthwash at 1.87 and 2.4 ppm, respectively. Whereas the released levels of Ni and Cr ions from the chlorhexidine group and cetylpyridinium chloride were 0.048 and 0.127 ppm, respectively, with significant differences between the tested groups and intervals. **Conclusion**: Chlorhexidine and Cetylpyridinium chloride mouthwashes were the appropriate options for orthodontic patients to minimized ions released according to this study protocol.

KEYWORDS

Metal release; Mouthwash; Nickle Ions released; SARS-CoV-2; WHO.

RESUMO

Objetivo: O objetivo deste estudo é avaliar o impacto dos protocolos diários de enxaguatório bucal recomendados contra SARS-CoV-2 em íons metálicos liberados de aparelhos ortodônticos fixos, especificamente Níquel (Ni) e Cromo (Cr). **Material e Métodos:** Um total de 400 aparelhos fixos de hemiarcada foram segregados em dois grupos, Níquel Titânio (NiTi) e Aço Inoxidável (SS), com base no tipo de fio utilizado. Cada conjunto foi submerso em enxaguantes bucais com iodopovidona a 2%, peróxido de hidrogênio a 1%, clorexidina a 0,2% e cloreto de cetilpiridínio. Água destilada foi utilizada para medições comparativas dos íons liberados como grupo controle (n=10/grupo). Eles foram incubados por cinco períodos a 37°C (uma hora, vinte e quatro horas, uma e 3 semanas). Os íons Nil e Cr liberados do aparelho fixo foram avaliados por espectroscopia de absorção atômica. Os dados foram analisados pelo teste de Kruskal-Wallis e análise de comparação pareada. **Resultados:** Os piores níveis de Ni e Cr liberados no grupo SS foram observados no enxaguatório bucal com iodopovidona em 1,173 e 1,701 ppm, respectivamente, enquanto o enxaguatório bucal com clorexidina liberou níveis aceitos de Ni e Cr em 0,033 e 0,056 ppm, respectivamente. O grupo NiTi exibiu os terríveis íons liberados de Ni e Cr no enxaguatório bucal com iodopovidona a 1,87 e 2,4 ppm, respectivamente. Já os níveis liberados de íons Ni e Cr do grupo clorexidina e cloreto de cetilpiridínio foram 0,048 e 0,127 ppm, respectivamente, com diferenças significativas entre os grupos e intervalos testados. **Conclusão:** Enxaguatórios bucais com clorexidina e cloreto de cetilpiridínio foram as opções adequadas para pacientes ortodônticos para minimizar a liberação de íons de acordo com o protocolo deste estudo.

PALAVRAS-CHAVE

Liberação de metal; Enxaguante bucal; Liberação de íons de níquel; SARS-CoV-2; OMS.

INTRODUCTION

Globally, the World Health Organization (WHO) has received reports of 650,332,899 confirmed COVID-19 cases worldwide, including 6,649,874 fatalities. The disease can be asymptomatic but is nevertheless contagious [1]. SARS-CoV-2 is extremely spreadable because it can be disseminated by aerosol droplets [2]. Additionally, cross-infection can be the result of objects with saliva contamination [3]. Therefore, it is essential to regulate the viral burden in the saliva and respiratory secretions [4-6]. Consequently, numerous entities recommend a daily mouthwash protocol [2,3].

The New Zealand Dental Association prescribed gargling either with 0.2% povidone-iodine (PVP-I), 1% hydrogen peroxide (H_2O_2), 0.2% chlorhexidine (CHX), or Listerine containing cetylpyridinium chloride (CPC) for 30 seconds. In addition, the American Dental Association has stated that gargling with 0.2% PVP-I twice daily for 30 seconds could reduce the infectiousness of SARS-CoV-2 to undetectable levels. In addition, it was determined that PVP-I mouthwash could reduce the coronavirus in saliva and prevent virus attachment to oral and nasopharyngeal mucosa [2,3,7]. The International Federation of Endodontic Association and Scottish Dental Clinical Effectiveness Programs recommended H_2O_2 and PVP-I mouthwashes [1,2].

In recent studies, 30 seconds of gargling with Listerine antiseptic mouthwash that contains CPC was enough to decrease the coronavirus saliva load by up to 99.9% [3,8]. CHX is a well-known, broad-spectrum antiseptic mouthwash that dentists recommend daily for maintaining oral health [9]. CHX can increase cell wall permeability, disrupting the microbial membranes, and making it effective against anaerobes, aerobes, Gram- positive and negative bacteria, and fungi [3,8]. CHX's effects against the SARS-CoV-2 were controversial, in comparison to other mouthwashes [1]. However, Chopra et al. [3] suggested it as a simple and safe choice to inhibit the spread of coronavirus. Additionally, mouthwashes influence improving the buffering capacity of the saliva [10].

WHO deliberates that teeth malocclusion is the most significant issue regarding oral health, following caries and periodontal diseases. Its incidence among children and adolescents ranges from 39 to 93 percent. Crowding comes first with a prevalence of up to 84%, while spacing is second, which reached up to 60% [4,11,12].

The effect of these daily mouthwash protocols on the shear bond strength was previously evaluated [13]. However, their impact on the orthodontic appliance has not been clarified yet, as it was stated that the oral fluids and mouthwashes significantly affect ions liberated from the orthodontic appliance [6].

Thus, this study aimed to estimate the effects of this daily mouthwash rinsing protocol on the amount of corrosion in fixed appliances as metal ions are released, especially the Ni and Cr ions from orthodontic appliances incorporating SS, NiTi archwires exposed to different time expositions, and its possibility to reach toxic levels.

METHODS

Study design and setting

This experimental research was conducted *in vitro*, in the lab of the College of Dentistry of the University of Mosul - Iraq, from July 2023 to September 2023. The ethical committee approved the protocol of this study (no. PO 22O265UoM).

- Each set comprised:
  - Five edgewise standard SS brackets (anterior teeth, canine, and bicuspid).
  - One molar band.

  - Either a hemi-arch SS archwire (Gauge 1722") or a NiTi archwire (Gauge 1722") manufactured by Dentarum, Isinberge, Germany.
- The 400 sets were divided into two groups based on the archwire used, with 200 sets each.
- Each group of 200 sets was further divided into five subgroups based on the mouthwashes used in the study.
- Distilled water was used as a control group for comparative measurements (40 sets each).
- Each subgroup was divided into four clusters based on the testing intervals (10 sets each).

Exposure protocol

Each sample was exposed to the mouthwashes for 30 seconds twice daily, simulating a gargling protocol. The mouthwashes used in the study and their chemical compositions are listed in Table I.

Incubation and measurements

For each mouthwash, 10 samples were tested, and the reassessment was performed at four time points. Thus, the vials for each mouthwash were divided into four subgroups according to the four incubation periods.

All the samples were incubated for one hour, twenty-four hours, one week, and three weeks intervals at 37C° using a Memmert Incubator (model IN 55, USA) with unceasing shaking. Ten vials of each mouthwash were randomly selected for analysis at each time interval. After getting the appliance out of the solution, a drop of nitric acid (65%) was used to stabilize the liberated ions in the solution before they were sent for analysis. A new plastic disposable syringe with a plastic tip aspirated this solution.

The Atomic Absorption Spectroscopy (Buck 230ATS, USA) was used to compute the Ni and Cr ions released from each tested mouthwash at different intervals.

Statistical analysis

SPSS software (version 26) was employed for statistical data analysis. Descriptive statistics (mean, standard deviation) were performed to analyze the Ni and Cr Ions data released from the hemi-sets of orthodontic appliances. The Kolmogorov-Smirnov test was used to inspect the normal distribution of the data. Kruskal-Wallis statistical test was performed to analyze the final data with pairwise comparisons analysis. The significant level was adjusted to 5%.

RESULTS

The descriptive statistics (mean, standard deviation) of the Ni ions released from the SS wire group for different intervals are shown in Table II. Table III shows the mean and standard deviation of the Cr ions released from the SS wire group for the four tested intervals. Tables IV and V display the mean of the Ni and Cr ions released from the tested mouthwashes at different intervals.

This experimental study demonstrated an increase in the concentration of nickel and chromium ions emitted from appliances containing SS and NiTi archwires, with significant differences between them (Tables II, III, IV, and V).

The maximum release of Ni and Cr ions from the SS appliance group was 1.173 and

Table I - Mouthwashes used in this study and their pH

Trade name	Company\ manufacturer	Chemical composition	pH
Klorhex Chlorhexidine	Drogsan, Ankara - Turkey	2% chlorhexidine solution	6.5
Naturel Hydrogen Peroxide	Naturel Medical Pharma, Istanbul - Turkey	3% hydrogen peroxide, 0.03% stabilizer, 96.97% solvent	7.3
Colgate Plax Cool Mint	Colgate, London - UK	aqua, glycerin, propylene glycol, poloxamer, aroma, cetylpyridinium chloride, potassium sorbate, sodium fluoride, sodium saccharin, menthol	7.1
Batticon	ADEKA, Istanbul -Turkey	10% povidone-iodine solution, 1.5% emulsifier, 0.5% stabilizer, 0.5% pH adjuster, 87.829% solvent	6.1
Distilled water			7

Table II - Mean and the standard deviation of Ni ion released from SS appliance at four intervals

Time\ Mouthwash	DW	H_2O_2	PVP-I	CHX	CPC	*p***
1h	0.01[a] (0.0)	0.011[a] (0.001)	0.255[b] (0.005)	0.0065[a] (0.0005)	0.007[a] (0.0)	0.01
24h	0.014[a] (0.001)	0.033[b] (0.001)	0.263[c] (0.0015)	0.01[d] (0.0011	0.022[e] (0.0005)	0.009
1 week	0.0175[a] (0.0025)	0.011[a] (0.0015)	0.345[b] (0.035)	0.01[a] (0.0)	0.012[a] (0.0)	0.013
3 weeks	0.009[a] (0.0)	0.009[a] (0.001)	0.31[b] (0.03)	0.0065[a] (0.0005)	0.0285[a] (0.0025)	0.011
*p**	0.00	0.00	0.000	0.000	0.00	
Total	0.0505	0.065	1.173	0.033	0.0695	

**p* Kruskal Wallis test value for the four different intervals; ***p* Kruskal Wallis test value among different mouthwashes used in this study; DW: Distilled water; H2O2: Hydrogen peroxide: PVP-I: Povidon-eiodine; CHX: Chlorhexidine; CPC: Cetylpyridinium chloride. The same letters at the same row expressed non-significant differences regarding the tested mouth washes diagnosed by pairs comparison test.

Table III - Means and standard deviation of Cr ion released from SS appliance at four intervals

Time\ Mouthwash	DW	H_2O_2	PVP-I	CHX	CPC	*p***
1h	0.01475[b] (0.025)	0.005[a] (0.0000)	0.1885[d] (0.0035)	0.0085[a] (0.0005)	0.0235[c] (0.0015)	0.009
24h	0.004[a] (0.001)	0.004[a] (0.001)	0.08[b] (0.01)	0.004[a] (0.001)	0.0087[a] (0.0005)	0.026
1 week	0.0055[ab] (0.0005)	0.0075[b] (0.0005)	0.129[d] (0.001)	0.005[a] (0.0)	0.0685[c] (0.0015)	0.010
3 weeks	0.0065[a] (0.0005)	0.005[a] (0.0)	0.1305[c] (0.0035)	0.0055[a] (0.0005)	0.045[b] (0.002)	0.010
*p**	0.000	0.000	0.000	0.000	0.000	
Total	0.03075	0.0215	0.528	0.023	0.146	

**p* Kruskal Wallis test value for the four different intervals; ***p* Kruskal Wallis test value between different mouth washes used in this study; DW: Distilled water; H2O2: Hydrogen peroxide: PVP-I: Povidon-eiodine; CHX: Chlorhexidine; CPC: Cetylpyridinium chloride. The same letters at the same raw expressed non-significant differences regarding the tested mouth washes diagnosed by pairs comparison test.

Table IV - Means and standard deviation of Ni ion released from NiTi appliance at four intervals

Time\ Mouthwash	DW	H_2O_2	PVP-I	CHX	CPC	*p***
1h	0.011[c] (0.0006)	0.005[a] (0.00029)	0.162[d] (0.002)	0.008[b] (0.0005)	0.007[ab] (0.0005)	0.009
24h	0.012[a] (0.001)	0.034[b] (0.001)	0.299[c] (0.0083)	0.008[a] (0.001)	0.015[a] (0.001)	0.010
1 week	0.55[c] (0.01)	0.225[b] (0.015)	0.54[c] (0.06)	0.0185[a] (0.0005)	0.014[a] (0.001)	0.012
3 weeks	0.0105[a] (0.0005)	0.215[b] (0.005)	0.875[c] (0.035)	0.014[a] (0)	0.0175[a] (0.0025)	0.009
*p**	0.00	0.00	0.00	0.00	0.00	
Total	0.5835	0.479	1.876	0.0485	0.0535	

**p* Kruskal Wallis test value for the four different intervals; ***p* Kruskal Wallis test value among different mouthwashes used in this study; DW: Distilled water; H2O2: Hydrogen peroxide: PVP-I: Povidon-eiodine; CHX: Chlorhexidine; CPC: Cetylpyridinium chloride. The same letters at the same row expressed non-significant differences regarding the tested mouth washes diagnosed by pairs comparison test.

Table V - Means and standard deviation of Cr ion released from NiTi appliance at four intervals

Time\ Mouthwashes	DW	H_2O_2	PVP-I	CHX	CPC	*p***
1h	0.0055[a] (0.0005)	0.004[a] (0.001)	0.2145[b] (0.021)	0.007[a] (0)	0.0275[a] (0.0025)	0.009
24h	0.002[a] (0.00006)	0.005[b] (0.00021)	0.062[d] (0.00153)	0.003[ab] (0.001)	0.008[c] (0.001)	0.010
1 week	0.004[a] (0)	0.0035[a] (0.0005)	0.11[b] (0.01)	0.072[ab] (0.058)	0.0165[a] (0.0005)	0.019
3 weeks	0.006[a] (0.0)	0.005[a] (0.001)	0.1385[c] (0.0055)	0.0055[a] (0.0005)	0.022[b] (0.002)	0.016
*p**	0.00	0.00	0.00	0.00	0.00	
Total	0.0175	0.0175	0.525	0.0875	0.074	

**p* Kruskal Wallis test value for the four different intervals; ***p* Kruskal Wallis test value among different mouthwashes used in this study; DW: Distilled water; H2O2: Hydrogen peroxide: PVP-I: Povidon-eiodine; CHX: Chlorhexidine; CPC: Cetylpyridinium chloride. The same letters at the same row expressed non-significant differences regarding the tested mouth washes diagnosed by pairs comparison test.

1.701 ppm, respectively, from the PVP-I mouthwash. Meanwhile, the CHX group released the least amount of Ni and Cr at 0.033 and 0.056 ppm, respectively. The NiTi group displayed the highest percentage of Ni and Cr ions emitted in the PVP-I mouthwash, at 1.87 and 2.4 ppm, respectively. Meanwhile, the least released amount of the Ni and Cr ions was observed from the CHX group, and the CPC was 0.048 and 0.127 ppm, respectively.

However, the total Ni ion released from the SS group ranged between 0.05 to 1.173 ppm, as shown in Table II. While the total Cr ion released from the SS group ranged between 0.023 to 0.528 ppm as shown in Table III. The last row in Tables IV and V displayed the total Ni and Cr ions released from the NiTi group, which range between 0.0535 and 1.876 and 0.0175 and 0.525 ppm, respectively.

Kolmogorov-Smirnov test results displayed the non-normal distribution of the data ($p = 0.000$). The Kruskal-Wallis's test shows significant differences between the number of ions released from the mouthwashes used at different intervals. Pairwise comparison analysis diagnosed the non-significant difference between the mouthwashes, expressed as the same letters in the Tables (II, III, IV, V).

DISCUSSION

Daily gargling with mouthwash is a recommended procedure by WHO due to its suitability, safety, and simplicity [1,2]. Clinical studies have shown that mouthwashes can reduce the activity of the SARS-CoV-2 in the mouth [2,3]. However, there is limited academic research on the impact of certain newer mouthwashes on metal ions released by fixed orthodontic appliances, such as PVP-I, H_2O_2 and CPC.

This study aimed to assess the efficacy of different mouthwashes against distilled water in minimizing the release of metal ions from fixed orthodontic appliances over varying time intervals. The goal was determining the most appropriate mouthwash for individuals undergoing orthodontic treatment. The investigation specifically targeted metal ions with the greatest potential to affect human health and cause sensitivity. Prior research has established that metal ion release from fixed orthodontic appliances commences immediately upon immersion and reaches its highest levels on the seventh day [14-16].

This research delves into the potential release of Ni and Cr ions from fixed orthodontic appliances. These ions have been known to trigger allergies and toxicity, leading to symptoms that can range from short-lived and intense to long-lasting and mild [17]. While nickel toxicity is a concern, the body's natural ability to eliminate nickel is more significant than the accumulation of nickel, rendering the risks negligible [18,19]. Healthcare professionals should be aware that metal ion release may cause hypersensitivity of the buccal soft tissues, such as moderate erythema and redness with or without oedema [14,20-23]. Additionally, research suggests that an allergic reaction to Ni or Cr ions released from a fixed orthodontic appliance, rather than poor dental hygiene, may be linked to severe gingivitis [24].

The quantity of chromium (Cr) and nickel (Ni) consumed through food varies between 5 to 100 mg and 300 to 500 mg daily, respectively [15,16]. The difference was minimal when comparing the present research results to the amount of Ni and Cr ions congested daily from food and water. Prior research has established that metal ion release from fixed orthodontic appliances commences immediately upon immersion and reaches its highest levels on the seventh day [14-16].

The study found that H_2O_2 mouthwashes resulted in the least amount of Cr release compared to other mouthwashes evaluated. CPC came in second regarding the maximum release of Cr, followed by CHX. However, the lack of a significant difference in acidity between these three mouthwashes may be attributed to the corrosiveness of CPC compared to the other mouthwashes, which demonstrated a reduction in the efficiency of stainless steel corrosion [25].

According to this study, using H_2O_2 mouthwash on NiTi archwires can release high Ni ions over time. This is due to H_2O_2's ability to break down the protective layer on the surface of the wire, making it easier for the ions to escape. In addition, certain properties can also be released by H_2O_2. Several factors, such as fluid pH, immersion duration, oxygen content and temperature, can influence the rate of metal corrosion in a fluid. It should be noted that aeration of distilled water can increase steel corrosion rates, as oxygen dissolving in water can be up to five to ten times more aggressive than carbonic acid [26-28]. It is worth noting that the quantity of ions that a hemi-fixed appliance releases is not exclusively dependent on CHX's corrosive capability. This is because corrosion can happen in an acidic atmosphere. Furthermore, research has demonstrated that H_2O_2 cannot release Cr ions. From a clinical standpoint, the corrosion of the hemi-fixed appliance can impact the sliders' movement over the archwire, which may jeopardize the efficacy of orthodontic treatment [29,30].

The corrosion mechanism and consequent liberation of metal ions from fixed orthodontic devices entail the depletion of the chromium hydroxide and chromium oxide passive layer that develops on the stainless steel surface upon contact with oxygen. Crevice corrosion, characterized by highly concentrated and localized corrosion that occurs at shielded regions of a metal surface, is the underlying mechanism behind the corrosion of orthodontic brackets [27,31].

The levels of nickel and chromium released throughout three weeks in distilled water in the current investigation were higher than those reported in a previous study by Barrett et al. [15]. Differences in study design, tested mouthwash solutions, measurement methods, and duration could account for this discrepancy. Barrett et al. used atomic absorption spectrophotometry to measure the ion releases in artificial saliva from bands and brackets over four weeks. Additionally, discrepancies in the metal release have been discovered between equivalent items from various manufacturers [31,32]. This investigation did not include the determination of the surface area and geometry of orthodontic bands, archwires, and brackets despite the significance of the surface area in metal corrosion [14,32,33]. The concentrations of chromium (Cr) and nickel (Ni) ions that were released in both CHX and distilled water over three weeks were found to be higher than the levels obtained through other means [33]. Comparing research studies can be challenging due to variations in study designs and diverse electrochemical variables. Difficulties in measuring surface areas with intricate geometries must be considered when comparing studies [31,32]. Based on our findings, we concluded that the corrosiveness of mouthwash, which is determined by its chemical structure, is the primary cause of fixed appliance corrosion.

The limitations of this study include the full surface area of brackets exposed to the solution, which is not representative of the clinical situation where the bases of the brackets were fixed to the tooth surface via an adhesive. Furthermore, the study did not consider the effect of thermocycling [27].

Future research could explore the effects of different concentrations of mouthwashes on the release of Ni and Cr ions, as well as the impact of saliva on these mouthwashes and their relationship to metal release.

CONCLUSION

According to this study protocol, the following points could be concluded:

1. CHX and CPC mouthwashes are the appropriate options for orthodontic patients to mitigate the quantity of metal discharge amid the ongoing pandemic;
2. It is recommended to refrain from using PVP-I mouthwashes in orthodontic patients due to their potential to induce a significant release of Ni and Cr.

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Author's Contributions

SS, LHQ: Conceptualization. SSS, AAQ: Data Curation. SSS, LHQ, AAQ: Formal Analysis. SSS, LHQ, AAQ, HMD: Funding Acquisition. SSS, LHQ, AAQ, HMD: Investigation. SSS, LHQ: Methodology. SSS, AAQ: Project Administration. SSS, LHQ, AAQ: Resources. SSS: Software. AAQ: Supervision. SSS, LHQ, AAQ: Validation. SSS, LHQ: Visualization. SSS, HMD: Writing - Original Draft Preparation. SSS, HMS: Writing - Review & Editing.

Conflicts of Interest

There are no conflicts of interest.

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Regulatory Statement

This study was conducted in accordance with all the provisions of University of Mosul Ethical Committee Agency. The approval code for this study is: no. PO 22O265UoM.

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