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EFFECT OF MULTIPLE STAINLESS STEEL CROWNS ON SALIVARY PH, NICKEL, AND CHROMIUM LEVELS

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ABSTRACT

Background: The use of stainless steel crowns (SSCs) in pediatric dentistry is widely used. Recently, there has been an increasing ecological and global public health concern associated with environmental contamination by metallic alloys. **Aim of the study:** To determine the in-vitro and in-vivo effect of multiple stainless steel crowns on the salivary pH, nickel and chromium levels at different periods.

Materials and methods: This study consisted of: **I-In-vitro study:** A total of 200 standard sizes of SSCs were divided into 4 groups (10 samples/each). Each sample/group contained 2, 4, 6, and 8 SSCs, respectively. All SSCs were trimmed 1mm circumferentially, smoothed, re-contoured, fitted, and cemented on standard crowns of plastic teeth models. Each group was placed in a closed container containing artificial saliva with a standard pH. **II-In-vivo study:** A total of 40 patients indicated for SSC restorations were selected, aged 5-9 years. Similar to in-vitro, they were allocated into 4 groups according to the numbers of SSCs restorations used. 5ml of unstimulated saliva was taken for pH, nickel, and chromium analysis at 5-intervals: baseline, and 1day, 1week, 2weeks, 1month, and 2-months post-cementation. **Data analysis:** The mean and standard deviations, one way ANOVA, and the Person correlation coefficient were used. The level of significance was adopted at $p \leq 0.05$.

Results: In both studies: The more the increase in the number of SSCs, the more the increase in acidity and release of ions. **As regards pH value**; within the groups at 5 intervals, there was only a significant difference in groups containing 8 SSCs and 6 SSCs, and between the intervals and the baseline ($p \leq 0.05$). The group that has 8 SSCs recorded the lowest pH value. Among groups: in-vitro: all groups showed a significant difference during 3-time intervals ($p \leq 0.05$) except at 2-months; the difference was not significant ($p > 0.05$), while in-vivo, the difference was not significant ($p > 0.05$). **As regards the released ions:** Within groups, both studies showed a significant difference among all intervals, and between the intervals and the baseline ($p \leq 0.05$). Among groups: In-vitro, all groups showed significant differences during the intervals ($p \leq 0.05$), while the only period showing no significant difference was at 2 months in the chromium group ($p > 0.05$). The group that has 8 SSCs recorded the highest level and the difference was significant ($p \leq 0.05$). The peak level of the released ions and reduced pH value was at 1day and 1week for in-vitro and in-vivo study, respectively.

Conclusion: The released nickel and chromium ions were directly proportional to the number of stainless steel crowns whereas the pH value was inversely proportional. The maximum level recorded was 1day and 1-week post-cementation for in-vitro and in-vivo study, respectively. The peak released of ions was much lower than the toxic level or the level of dietary intake.

KEYWORDS: biodegradation, nickel-chromium levels, pH, stainless steel crowns, children

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INTRODUCTION

The oral cavity is an ecosystem that is known to cause the biodegradation of metals as a result of the process of electrochemical breakdown [1]. It has been reported that wearing different pediatric metal appliances might lead to the alteration of the salivary levels of electrolytes, with an increase in salivary nickel (Ni) or chromium (Cr) concentrations [2].

The main component of fixed orthodontic appliances, space maintaining bands and loops, and stainless steel crowns (SSCs) is the austenitic stainless steel that contains significant amounts of nickel and chromium [3]. Up to100 years, harming potentials like contact allergy and cancer risk of Ni and Cr and their compounds have been documented in the literature [4-6].

The components of SSCs include 65-70% Iron, 17-20% Chromium, 8-13% Nickel, less than 2% of Manganese, Silicon and Carbon. Intra-orally, a little amount of metal ions can be released from SSC, which may act as allergens [7-9].

The metal appliances in pediatric dentistry release metal ions into saliva through electrogalvanic current and saliva acts as a mediator [2,10,11]. The release is increased after teeth brushing with an increase in salivary pH [12]. Electrogalvanic reactions "corrosion" release the metal ions due to metal surface destruction. Intrinsic factors of corrosion are determined by the structure and composition of that metal, while extrinsic factors depend upon the environment such as pH, mediator composition, strain, and temperature [13].

A stainless steel crown is the most effective and durable prefabricated metal restoration for restoring the primary molar after caries removal and/or pulp therapy [14,15]. Based on the evidence, the salivary and urinary release, and the serum level of Ni and Cr from SSCs and different fixed orthodontic appliances are below the toxic level but not up to daily regimen intakes. The maximum amount of Ni in food and water is approximately 0.21 and 0.04 mg/day, respectively, while the Cr is about 0.17 and 0.002 mg/day, respectively [16,17].

Mostly, the preformed SSCs do not have an accurate fit on the treated teeth. Sometimes, the margins' height may interfere with the crown sitting causing pain with gingival blanching. So, the clinician tries to "trim" the excess margins affecting the amount of Ni and Cr release and probably reaching it to the critical level, because of integrity disturbance [18,19].

Nickel is one of the main reasons for allergy and approximately 10-28% of people are allergic to it[20,21]. The nickel and chromium-containing appliance may lead to harmful outcomes such as hypersensitivity and allergic dermatitis, mucosal ulcers, cellular toxicity, lichenoid plaques in buccal mucous adjacent to allergens, asthma and a change in cellular function [22-24]. Furthermore, it has a carcinogenic and mutagenic effect [21,25]. Severe inflammatory hyperplasia around the crowns, stomatitis, alveolar bone loss, and throat edema, palate, and gums may be also another side effects[26-28]. The intra-oral long-term exposure to nickel and chromium-containing appliances may damage the mucosal cells, monocytes, and cultured cells [29-32].

In the past, most of the studies [2,10,11,13,21,23] were conducted to evaluate the salivary release of Ni and Cr elements from metal orthodontic appliances but there were rare clinical and laboratory publications about the salivary pH level, nickel and chromium release from SSCs. Thus, this study was directed to determine, in-vitro and in-vivo, the effect of multiple stainless steel crowns on salivary pH value, nickel, and chromium levels.

MATERIALS AND METHODS

This study consists of two parts.

I- In-vitro study

A total of 200 standard sizes of SSCs (Kids Crown, Shinhung Dental, Seoul, Korea) were used in this part of the study. They were divided into

4 groups (10 samples/each). Each sample/group had contained 2, 4, 6, and 8 SSCs, respectively.

Initially, all margins of SSCs were trimmed 1mm distance from the edge of crowns using a disk under water spray and smoothed by pink mullet and white polish rubber. All crowns were cemented on standard crowns of plastic dental teeth models, using zinc polycarboxylate luting cement (Adhesor Carbofine, Spofa Dental, Prague, CzeCh Republic). Fig 1A.

Each group was placed in a numbered sterilized closed glass container containing 50 ml of artificial saliva with a pH of 6.43 ± 0.26 (fig 1B). The formula of artificial saliva was 0.8 g NaCl, 2.4 g KCl, 1.5 g NaH2PO4, 0.1 g Na2S, and 2 g CO[NH2]2), that stored in an incubator at 37°C for 4 weeks[33]. Any adjustment for the standard pH value is required, an increment of 1N sodium hydroxide or 1 N hydrochloric was added [34]. The artificial saliva used was prepared in the Pharmaceutical Chemistry Department, Faculty of Pharmacy, Kafrelesheikh University.

Another two containers containing only the artificial saliva without crowns were used as a baseline. To avoid saturation of the solution, the same samples were removed from each container at the end of each interval and immersed in new containers with freshly prepared artificial saliva. The solutions were replaced weekly during the study period [34]. The closed containers containing samples were placed in an incubator (Memert GmbH, Büchenbach, Germany) regulated at 37º C. All samples were shaken gently during the immersion, to get a uniform solution and to confirm bathing all samples in the artificial saliva [33].

The pH value of artificial saliva at each interval was measured by a pH Meter (pH Meter 3310, JENWAY, Felsted, Essex, UK) (fig 1C). After each record, the pH electrode was irrigated, using distilled water and was kept in the kit's standard 7-pH solution [35]. It was rinsed and dried before each measurement.

The released Ni and Cr elements were measured at each interval using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Prodigy 7 instrument (Teledyne Leeman Labs, Mason, Ohio, USA) in Geochemistry of Trace and Toxic Elements Lab (accredited according to ISO/IEC (17025/2017), Faculty of Agriculture, Kafrelsheikh University, Egypt (fig1D). Nickel and chromium levels of each sample were measured thrice, and the average was recorded.

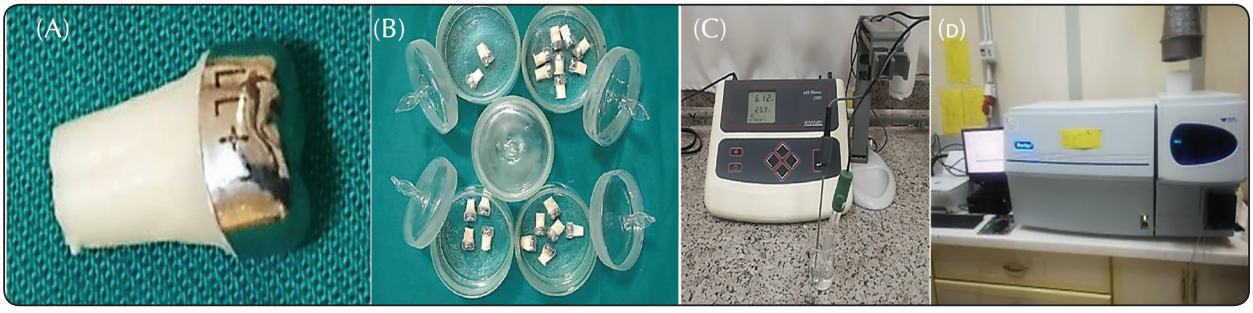

Fig. (1) The cemented SSCs on standard crowns of plastic teeth models after immersion in glass containers contained artificial saliva(A,B), pH Meter 3310 for measurement of pH value (C), and ICP-OES Prodigy 7 for assessment of released Ni and Cr ions (D).

II- In-vivo study

A total of 40 children of both sexes (23 boys and 17 girls), aged 5-9 years (mean ages of 6.5±1.24) participated in this study from patient attending the clinic of Pediatric Department, Faculty of Dentistry, Kafrelsheikh University.

Informed consent was taken from children's parents according to the proposed Guideline of the Research Ethics Committee, Faculty of dentistry, Kafrelsheikh University. All children's parents participating in this study were thoroughly informed of the objective and procedures of the research. Medical and dental histories were taken.

They were allocated into 4 groups according to the number of SSC restorations used, similar to the in-vitro study. The inclusion criteria were: a clinical indication for conventional preformed SSCs, without any further need for more modification within the next 2-months, and cooperative child according to Frankl rating scale (++) or (+). All inclusion criteria had to be fulfilled during the study period [36].

The exclusion criteria were: patients with any oral or systemic diseases, syndromes, illnesses related to genetic damage, allergies, allergies to costume jewelry, watches, or any other sources of Ni or Cr, any amalgam fillings or metal restorations, previous orthodontic treatment, patients who received antibiotics or steroids or under local therapy of fluoride, probiotic, and alcohol-based mouthwashes [37].

To standardize the sampling methods [36], oral hygiene maintenance with the use of one type and brand of toothpaste was instructed to all parents and children before the study. There was a need for placing all indicated SSCs in the same session, and a list of nickel-rich foods/drinks were handed to the parents to exclude them within 48 hrs before sampling. They were also asked not to rinse with fluoridated mouthwashes 24 hrs before the next visit, till after sample collection [38-41]. The children were directed not to use an oral rinse, not to eat, drink or brush their teeth on the morning of the scheduled visit [36,42]. If any patients were dropped out of the study, they would be replaced with new patients.

Preparation of SSCs

The occlusal surface of SSCs was reduced by 1–1.5 mm and the occlusal third of the buccal surface was beveled using a diamond wheel bur and round of all line angles. The proximal contacts were opened at the appropriate depth in a single sweeping motion, using tapered fissure bur. The properly-selected size of SSCs was just trimmed to flat edges, smoothed, fitted, re-contoured, and crimped. All margins were checked for an accurate fit. All SSCs were cemented with zinc polycarboxylate luting cement, the excess was removed by a sharp instrument, and the occlusion was checked. All treated primary molars underneath the SSCs were 70% pulpotomy,

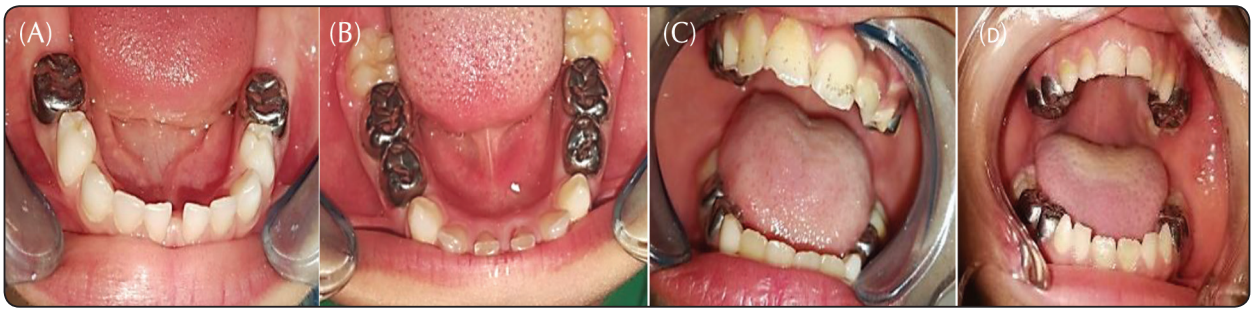

Fig. (2) Photographs represent the 4 groups of the study, respectively (A-D).

20% pulpectomy, and 10% multi-surface caries restorations and developmental defects.

The before-after clinical trial was carried out on 240 observations from 40 pediatric dental patients, recorded at 6-time points: pre-treatment (baseline) and 1 day, 1 week, 2 weeks, 1 month, and 2 months post-treatment.

Saliva Sampling

In the morning before breakfast, each patient was instructed to rinse the mouth thoroughly with distilled and deionized water before sampling. In each session, 5 ml of unstimulated saliva was collected within 5 minutes with the mouth closed, then transferred to sterilize nickel/chromium-free polyethylene test tubes by direct spitting [12]. The sample divided into two equal parts, one part for analysis of pH value and the other for analysis of Ni and Cr ions. The sampling was undertaken from the patients at 6-time points.

Salivary pH level measurement

The pH value was recorded after sampling using pH meter, to avoid any pH/CO2 changes [42]. The average of 3 records for each sample was reported.

Salivary ions Measurement

The samples were transferred to the Geochemistry of Trace and Toxic Elements Lab, Faculty of Agriculture, Kafrelsheikh University, Egypt. At the laboratory, the sample was centrifuged at 5000–8000 rpm for 5 minutes using Centurion Scientific Benchtop centrifuge (Cole-Parmer, Southampton, Hampshire, UK) and its impurities, debris, and proteins were removed. Then, it was diluted ten-fold with 0.1% nitric acid. Afterward, the salivary Nickel/Chromium concentration of each container was measured thrice using ICP Prodigy7. The average per sample was recorded as the main value[34,35,42,43] was recorded in μg/L "micrograms per liter ". Noting that 1ppm=1000 ppb, 1ppm=1mg/L, 1mg=1000 μg, and 1L=1000mL.

Data analysis

The collected data were organized, tabulated and statistically analyzed using SPSS program, (Statistical Package for Social Studies) version 23.0 (IBM, Illinois, Chicago, USA). For numerical values, the mean and standard deviations were calculated. The difference within each group at a different time of intervals and the difference between each group at different specific periods were tested using one way ANOVA. Also, the Person correlation coefficient was calculated. The level of significance was adopted at $p \leq 0.05$.

RESULTS

Four groups were used in each in-vitro and in-vivo study, each group including 10 samples containing 2, 4, 6, and 8 standard SSCs, respectively. Salivary pH value, Ni, and Cr levels were determined at 6-time intervals: pre-treatment (baseline) and 1day, 1week, 2weeks, 1month, and 2 months post-crowns cementation. The age of the patients ranged from 5-9 years with the mean age of 6.5±1.24. All patients were available for assessment till the end of the recall.

In-vitro assessment:

pH/ Ion Measurement

Table 1. The more the increase in the number of SSCs, the more the increase in acidity. According to pH value, within the groups at the 5-time intervals, there was only a significant difference in the pH value in two groups; IV (8 SSCs) and III (6 SSCs), and also between intervals and baseline in both groups ($p \leq 0.05$). Among the groups at the different specific intervals, group IV (8 SSCs) recorded the lowest pH value and the difference was significant among them ($p \leq 0.05$). All groups showed a significant difference at 4 of the 5-intervals ($p \leq 0.05$) except at 2 months,

and the difference was not significant among them ($p>0.05$).

Tables 2&3. As regards the released Ni and Cr ions, the released elements were directly proportional to the number of SSCs in the synthetic saliva. All groups showed a significant increase in the released ions during the first phase post-cementation, thereafter, they decreased gradually during the time intervals but the significance was still higher than the baseline ($p\leq0.05$). The group IV (8 SSCs) recorded the highest level of significance ($p\leq 0.05$). The peak of acidity, nickel, and chromium levels was seen at 1-day post-cementation.

TABLE (1) In-vitro: Comparison of pH value during the follow-up period

Follow-up period	Mean ± standard deviation				F	P
	Group I	Group II	Group III	Group IV		
Baseline	6.43±1.44	6.43±1.70	6.43±0.15	6.43±0.75	0.356	0.073
1 day	5.30+0.95	4.68+0.89	3.56+0.22	3.09+0.49	27.356	0.001*
1 week	5.88±1.46	5.41±1.47	4.34±1.55	3.93±1.13	10.852	0.013*
2 weeks	5.71±0.84	5.22±1.21	4.12±1.04	4.80±0.72	17.081	0.001*
1 month	6.04±0.62	5.80±0.85	4.73±1.31	5.41±1.05	10.792	0.013*
2 months	6.40±1.53	6.29±1.93	6.21±0.96	6.14±1.67	0.222	0.974
F	6.948	9.714	31.600	25.086		
P	0.225	0.084	0.001*	0.001*		

F=F value ****Significant at p≤0.05***

TABLE (2) In-vitro: Comparison of Nickel level during the follow-up period (μg/L)

Follow-up period	Mean ± standard deviation				F	P
	Group I	Group II	Group III	Group IV		
Baseline	0.0	0.0	0.0	0.0	-	-
1 day	1.73+0.61	3.22+1.80	4.98+1.07	6.00+1.61	24.380	0.001*
1 week	1.22+0.39	2.28+0.82	3.58+1.05	5.28+1.53	23.541	0.001*
2 weeks	1.19±0.34	2.22±0.95	3.47±1.31	4.10±0.89	24.264	0.001*
1 month	0.77±0.36	1.43±0.77	2.43±0.75	2.98±0.95	32.963	0.001*
2 months	0.21±0.04	0.28±0.04	0.40±0.08	0.55±0.07	17.103	0.001*
F	43.797	39.543	42.629	44.914		
P	0.001*	0.001*	0.001*	0.001*		

F= F value ****Significant at p≤0.05***

TABLE (3) In-vitro: Comparison of Chromium level during the follow-up period (μg/L)

Follow-up period	Mean ± standard deviation				F	P
	Group I	Group II	Group III	Group IV		
Baseline	0.0	0.0	0.0	0.0	-	-
1 day	0.57±0.11	1.14±0.57	1.62±0.46	1.98±0.44	22.720	0.001*
1 week	1.01±0.14	2.02±0.62	2.61±1.02	3.48±1.29	24.694	0.001*
2 weeks	0.63±0.13	1.25±0.57	1.77±0.45	2.17±0.47	25.738	0.001*
1 month	0.44±0.18	0.87±0.10	1.19±0.11	1.50±0.80	36.316	0.001*
2 months	0.15±0.23	0.18±0.05	0.23±0.03	0.33±0.05	34.134	0.001*
F	43.453	40.702	43.314	43.314		
P	0.001*	0.001*	0.001*	0.001*		

F= F value ****Significant at p<0.0***

In-vivo assessment:

pH/Ion Measurement

Table 4 showed that there was no significant change in pH in group I (2 SSCs) and II (4SSCs) during all intervals and between the intervals and the baseline (p>0.05), but the difference was significant in group IV (8 SSCs) and group III (6 SSCs) (p≤0.05).

The group, that has 8 SSCs (IV), recorded the lowest pH value than the other groups, but the difference was not significant (p>0.05).

The peak reduction level was recorded at 1-week post-treatment for all groups, thereafter; the pH value was gradually increased to be near the baseline at 2 months interval.

TABLE (4) In-vivo: Comparison of pH value during follow-up period

Follow-up period	Mean ± standard deviation				F	P
	Group I	Group II	Group III	Group IV		
Baseline	6.87±1.73	6.79±1.51	6.91±0.97	6.81±1.67	0.329	0.954
1 day	6.01±1.01	5.60±0.98	5.61±1.31	4.99±1.01	5.014	0.171
1 week	6.01±1.01	5.30±1.76	4.51±0.84	4.10±0.94	6.496	0.171
2 weeks	6.73±1.27	6.41±1.13	6.01±0.96	5.50±1.31	4.387	0.223
1 month	6.65±0.64	6.55±1.15	7.13±1.81	6.12±1.40	3.207	0.361
2 months	6.73±1.39	6.73±0.94	6.61±1.47	6.61±0.78	0.298	0.960
F	9.613	0.7600	15.829	24.343		
P	0.087	0.180	0.001*	0.001*		

F= F value ****Significant at p≤.05***

Within groups at the different 5 recalls, there was a significant difference in the level of the released ions among the recall times and between the recalls and the baseline (p≤0.05). Tables 5&6.

Among groups, there was a significant difference in the level of the released ions post-treatment among the 4-recall and between the recalls and the baseline (p≤0.05), however, this difference was not significant at 2 months of recall (p>0.05).

Group IV, that has 8 SSCs, recorded the highest level for both trace elements compared to the other groups, and the difference was significant among them (p≤0.05).

The peak level of released ions was recorded at 1-week post-treatment, which decreased gradually afterward during recalls and the difference was significant (p≤0.05).

TABLE (5) In-vivo: Comparison of Nickel level during follow-up period (μg/L)

Follow-up period	Mean ± standard deviation				F	P
	Group I	Group II	Group III	Group IV		
Baseline	4.90±2.38	4.12±1.97	4.32±1.40	4.90±1.47	0.472	0.705
1 day	11.87±2.88	20.91±6.90	34.87±19.14	43.67±19.30	12.28	0.001*
1 week	12.99+3.05	22.95±3.87	37.09+12.40	51.00+19.30	20.012	0.001*
2 weeks	9.10±1.88	17.68±5.74	25.77±9.00	32.79±15.79	11.411	0.001*
1 month	7.60±2.81	10.40±4.90	16.60±14.44	19.30±6.63	4.110	0.005*
2 months	5.42±1.36	4.89±2.03	4.85±0.85	5.99±2.22	0.990	0.409
F	37.771	40.286	35.714	41.657		
P	0.001*	0.001*	0.001*	0.001*		

F= F value ****Significant at p≤0.05***

TABLE (6) In-vivo: Comparison of Chromium level during follow-up (μg/L)

Follow-up period	Mean ± standard deviation of chromium				F	P
	Group I	Group II	Group III	Group IV		
Baseline	0.32±0.20	0.38±0.07	0.35±0.10	0.39±0.15	0.46	0.710
1 day	1.63±0.77	3.01±1.69	6.72±2.79	8.89±2.08	12.18	0.001*
1 week	1.85±0.80	2.63±1.25	4.19±1.59	7.02±3.66	25.14	0.001*
2 weeks	0.88±0.25	1.76±1.83	3.02±1.64	5.49±1.84	16.93	0.001*
1 month	0.62±0.11	1.57±1.33	2.17±1.28	3.47±1.39	10.60	0.001*
2 months	0.40±0.08	0.51±0.27	0.45±0.12	0.43±0.09	0.83	0.486
F	40.279	28.800	40.000	40.971		
P	0.001*	0.001*	0.001*	0.001*		

F= F value ****Significant at p≤.05***

The correlation coefficient between the in-vitro and in-vivo results is displayed in table 7. The peak value of the correlation between the two parts of the study was observed at 1-week of the time interval for pH value, however, it was found at 1-week for Ni and at 2-month for Cr post-treatment, where rank 1 was recorded for both pH value and Ni level, but rank 2 was recorded for Cr level. The Cr group was strongly correlated during all intervals.

The correlation coefficients at the first phase of treatment (1-week time interval) showed that, in both parts of this study, there was a negative correlation between the pH value and Cr release, and only in-vivo with Ni release. There was a strong correlation between the released Cr in both parts of the study, and between released Ni in both parts of the study. The Cr released in-vitro showed a moderate positive correlation with Ni release in both parts of the study. Table 8.

TABLE (7) Correlation coefficients between in vitro and in-vivo results regarding pH value, Ni, and Cr levels during follow-up period.

pH			
Follow-up period	Correlations	P value	Rank
1day	0.15	0.494	5
1week	0.45	0.004*	1
2 weeks	0.38	0.014*	2
1month	0.14	0.396	4
2 months	0.15	0.369	3
Ni			
Follow-up period	Correlations	P value	Rank
1day	0.57	0.000*	2
1week	0.43	0.000*	1
2weeks	0.40	0.007*	4
1month	0.38	0.016*	4
2 months	0.24	0.141	5
Cr			
Follow-up period	Correlations	P value	Rank
1 day	0.45	0.004*	5
1 week	0.74	0.000*	4
2 weeks	0.64	0.000*	2
1 month	0.65	0.000*	3
2 months	0.80	0.000*	1

pH=Potential of Hydrogen Ni=Nickel Cr= Chromium
****Significant at p≤0.05***

TABLE (8) Correlation coefficients between salivary pH, Ni, and Cr levels at 7-week interval.

		Cr in-vivo	**Cr** in-vitro	**Ni** in-vivo	**Ni** in-vitro	**pH** in-vivo	**pH** in-vitro
Cr in-vivo	Pearson Correlation	1	.639*	.645*	.606*	-.326*	-.326*
	Sig. (2-tailed)		.000	.000	.000	.040	.040
	N	40	40	40	40	40	40
Cr in-vitro	Pearson Correlation	.639*	1	.550*	.421*	-.343*	-.343*
	Sig. (2-tailed)	.000		.000	.007	.030	.030
	N	40	40	40	40	40	40
Ni in-vivo	Pearson Correlation	.645*	.550*	1	.708*	-.309	-.309
	Sig. (2-tailed)	.000	.000		.000	.052	.052
	N	40	40	40	40	40	40

		Cr in-vivo	**Cr** in-vitro	**Ni** in-vivo	**Ni** in-vitro	**pH** in-vivo	**pH** in-vitro
Ni in-vitro	Pearson Correlation	.606*	.421*	.708*	1	.015	.015
	Sig. (2-tailed)	.000	.007	.000		.927	.927
	N	40	40	40	40	40	40
pH in-vivo	Pearson Correlation	-.326*	-.343*	-.309	.015	1	1.000*
	Sig. (2-tailed)	.040	.030	.052	.927		.000
	N	40	40	40	40	40	40
pH in-vitro	Pearson Correlation	-.326*	-.343*	-.309	.015	1.000*	1
	Sig. (2-tailed)	.040	.030	.052	.927	.000	
	N	40	40	40	40	40	40

*. Correlation is significant at the 0.05 level (2-tailed).

pH=Potential of Hydrogen Ni=Nickel Cr= Chromium

DISCUSSION

Some heavy metals such as nickel and chromium are widely distributed toxic industrial ions, posing environmental and occupational exposure risks leading to harmful outcomes. The exposure may be due to direct contact with the contaminated soil, water, air, and food, or by absorption through the skin or mucous membrane as a result of manufacturing, pharmaceutical, or industrial processes or consumer products such as stainless steel, magnets, coins, and alloys [44-46].

In pediatric dentistry, the stainless steel used in the manufacture of SSCs, bands, orthodontic braces, metal appliances, and wires. Intra-orally, most of these metals can be expected to undergo biodegradation by electrochemical breakdown with salivary releasing of Ni and Cr ions. Also, the most significant human exposure to Ni and Cr occurs through dietary intake. So, the sum of ions consumption as a result of both dietary intake and metal-induced appliances are the most common cause of harmful allergic, cytotoxic, genotoxic, and possibly mutagenic effects [47-51] associated with the metallic taste and the change of salivary pH value.

Despite, the presence of advanced technology in quality, composition, biocompatibility, and durability of alloys used in dental appliances, the increase of allergic and toxic reactions relating to this issue has been reported [52] with the remaining of its effects intra-orally for 6-months [53].

According to the previous and present study, the amount of Ni and Cr released from SSC in the in-vitro and in vivo study is not critical, but placing multiple SSCs intraoral in child at the same time or the simultaneous presence of crowns, space maintainers and orthodontic appliances may cause Ni and Cr release exceeding critical limits [15,54]. Thus, this study aimed to evaluate the in-vitro and in-vivo effect of multiple SSCs on the salivary levels of pH, Ni, and Cr at different periods.

Most of the dental general practitioners are using the traditional SSCs which required trimming and contouring to avoid gingival blanching, pain, gingivitis, and periodontitis. This trimming may affect the amount of Ni and Cr release and probably reach a critical level [18,19]. Therefore, in this study, we intentionally removed circumferentially 1mm from all margins of SSCs.

In this in-vitro study, a saliva substitute was prepared chemically with a standard value of pH as a replacement medium for the natural saliva. Besides, all SSCs were cemented on the standard crowns of plastic dental teeth models using conventional cement of adhesor carbofine zinc polycarboxylate. To mimic the intra-oral temperature, all the samples were kept in an incubator at 37°C [4]. Natural saliva may be affected by many physiologic variables such as health conditions and medications.

The salivary analysis is the most significant method for detecting the level of trace metal elements rather than the blood, urine, epithelial cells, and hair analysis. It has several advantages being the first diluents of the human body, allowing a longer period of analysis with no invasive matrix, no risk of infection, and not needing a special handling or preservation condition[53,55,56]. Therefore, in the present study, the saliva sample was used as a method for pH, nickel, and chromium analysis.

In this in-vivo study, the saliva sample was collected by the unstimulated method because the stimulation could change the protein composition of saliva and both Ni and Cr had a rapid tendency to combine with protein, which might affect the salivary level of ions [57]. However, saliva in the stimulated method had different compositions because all salivary glands were stimulated. Thus, the unstimulated salivary samples were preferred to calculate the actual concentration of nickel and chromium in flowing saliva [58].

The ICP-OES Prodigy 7 instrument was used in this study to analyze Ni and Cr release instead of Atomic absorption spectrophotometer, Ultraviolet spectrophotometry, and Calirometric analysis, as it offers all of the advanced capabilities to give high-quality data with a highly sensitive detection method. It measures the trace metal elements by parts per billion (ppb) not by parts per million (ppm) [59].

The main advantage of the in-vitro part of this study is that it can easily standardize all the variables which are difficult to control in-vivo. Meanwhile, the in-vivo part has many advantages as the artificial saliva undergoes precipitation over a period of time and is not allowed to flow through the system as intraoral and the release of any salivary ion is affected by many factors such as pH, quantity and temperature of saliva, microflora, chemical and physical properties of food and drink, and the general oral health condition, which could give a better and reliable result [60]. Therefore, this study had in-vitro and in vivo part to complement each other to obtain guaranteed results.

Because most of the studies reported that the salivary nickel and chromium levels peak at the first months post-treatment [4,23,36,38,52,60], especially at the first one, and might tend to reduce later "back to baseline levels or lower". Thus, the 2 months were selected as a recall end interval in this study.

The relation between salivary pH value and nickel-chromium release from oral metal appliances is still controversial, and it is not quite known which one affects the other. In this in-vitro study, it was found that there was an inverse relationship between the pH value and the ions release. The lower the acidity, the more the release of Ni and Cr ions. This is in agreement with the study of Menek et al. [23] who reported a significant increase in ions discharges when the environment was acidic. This may be explained by the constant disruption of the formed anti-oxide film [23]. Other mechanisms may be factors such as corrosion of Ni and Cr, and the simple redox reaction with the surrounding, which can be strengthened by the acidic pH caused by dietary intake of acidic foods/drinks or by anaerobic biofilm activities [61,62].

The available publications on ions leached from multiple SSCs were highly controversial. The laboratory studies were limited to only Ni-release. In this study, the mean of Ni and Cr levels in artificial saliva was determined. The peak levels of both ions were leached at the first phase of treatment that declined gradually towards the end of the interval.

This is in accordance with the studies of Kulkarni et al. [33] who assessed the Ni release over 4 weeks and asserted that the peaks around the 1day that decrease gradually afterwards, and Ramazani et al. [52] who studied the Ni release over a month and asserted that the peak of ion level is on the 1day which declined to less than the half in the 7 day, and then reached zero in further intervals.

In this study, the level of Ni and Cr released was directly proportional to the number of SSCs. The group, that has 8SSCs, showed a more significant increase in Ni and Cr release compared to 6 SSCs, 4 SSCs, and 2SSCs. This agrees with Ramazani et al. [52] and Menek et al. [23] who reported a steady increase of Ni release over time from SSCs immersed in artificial saliva. Furthermore, the corrosion of the steel crowns may play a role in the release as confirmed by the in-vitro study of Dunlap et al. [54] who reported the corrosion potential of metal appliances.

In-vivo, the salivary pH value became more acidic after SSCs cementation and reached a peak at the first phase of treatment. This may be attributed to the leach of the acid cement which releases peaks around the first week post-treatment and due to cement dissolution resulting from lack of adequate marginal adaptation [52]. This finding agrees with Menek et al. [23] who postulated that when the environment was acidic, the formed anti-oxide film was disrupted constantly, leading to a steady increase of nickel leach. Meanwhile, the slight decrease in pH value was correlated negatively with the increases in Ni-Cr amounts. This agrees with the study of Basir et al. [15]. A possible explanation by Basir et al. [15] who found that the more the intense changes in pH value the more the increase of the redox corrosion of nickel. The observed declines in pH would accompany enhanced leach of nickel and chromium ions that reflect their elevated levels as basic ions in this study.

In this in-vivo study, the levels of both metal ions were significantly higher in children receiving SSCs at the first 4-time intervals than the levels in the baseline. The SSCs friction such as interproximal and intraoral wear during mastication may lead to a greater surface corrosion and increase the release of sizeable amounts of salivary Ni and Cr elements [63]. This interpretation is consistent with the interpretation of Bhaskar and Subba Reddy[64], and Yu et al. [65] who explained that stainless steel is a corrosion resistance family of iron alloys that have a minimum of 10.5% chromium. Chromium in SSCs plays a dominant role in reacting with oxygen and moisture intraoral and air to form anticorrosive passive chromium III oxide film, approximately 1-5 nanometres thick, on the steel surface. This film protects SSCs from further attack by additional corrosion. If this film is repeatedly damaged or disturbed by cutting, abrasion or scratching during functional demand, additional corrosion with more nickel and chromium release will happen.

Furthermore, the maximum level of released ions in this study was found at 1 day for in-vitro and 1-week for in-vivo post-cementation, respectively, and the release was gradually diminished with the time interval. This may be attributed to the quickly surface corrosion of Ni and Cr on SSCs during the first phase of treatment; thereafter, the rate of release drops off as the surface Ni and Cr was depleted[16,64]. The passive layer of oxide formed leached Cr on exposure to potentially damaging chemicals and physical agents intraoral [58]. This finding is in accordance with the studies of Singh et al. [66], Sfondrini et al. [67], and Yassaei et al. [68] who found the maximum release was after 1 weak, in the first phase of treatment, and after 20 days of appliances insertion, respectively. On the contrary, few studies reporting lower salivary Chromium levels [15,69,70].

The present study showed that the maximum release of both ions was found at the group containing 8 SSCs. This may be because the release of Ni and Cr elements is directly proportional to the metal surface area exposed to the intra-oral environment; therefore, the available amount of both elements was increased for corrosion. This agrees with the study of and Mohamed et al. [58] and Bhaskar and

Reddy [64] who reported that the release of Ni and Cr elements was directly proportional to the number of metal appliances used. But the finding of this study disagrees with Basir et al. [15] who reported no significant correlations between the number of steel crowns and both ions.

Furthermore, this study showed a negative correlation between salivary pH, and Ni-Cr levels at 1-week post-cementation in both parts of the study. The more the increase in released ions, the more reduced in pH value. This agrees with Basir et al. [15] who observed a negative correlation between pH with nickel or chromium levels either at the baseline or at 2 months.

In both parts of this study, the differences between the groups were significant. This can be taken as an indication of the biodegradation of Ni and Cr from the trimmed multiple SSCs in the saliva, but it appears that the SSCs do not release significant amounts of these ions over a prolonged period of time.

In this study, the differences in the results of released metal ions with some of the studies may be related to the different methodologies, the proportions of the elements in the alloy, the manufacturing of the metal parts, the materials and the sensitivity of analytical techniques used with different lower detection limits. Finally, in vivo, the metal ion released from any metal appliances is also affected by many factors, such as dietary habits, pH, salivary composition, measuring devices, the age of the patients, the time wearing the appliance and microflora [37].

Although the present in-vivo study recorded high peaks level for salivary Ni and chromium release with these multiple SSCs, those never reached the toxic dose of 10 μg /kg body weight[71] and were still below the levels of daily dietary intake (200–300 μg/day for Ni and 280 μg/day for chromium, respectively). But Vreebrug [72] reported that the lethal oral dose for Ni in humans probably lies between 50 and 500 μg /kg body weight.

RECOMMENDATION

Further investigations with a larger sample size and a longer follow-up are required to confirm the findings of the current study and the possible biological effect of the use of trimming multiple SSCs on tissues.

CONCLUSION

The use of multiple SSCs had a significant effect on salivary pH, Ni, and Cr ions. The more the increase in the number of SSCs, the more the increase in acidity and release of ions. The peak level of released ions and pH value showed itself in the first phase; 1day and 1-week post-cementation for in-vitro and in-vivo study, respectively. But, it was still much lower than the levels that would be considered toxic or even reaches the levels of dietary intake.

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