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Nickel Accumulation in Saliva and Urine Post Orthodontic Fixed Appliance Treatment in Malay Population

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ABSTRACT

Objective: Prolonged nickel exposure, even in low amounts, is cytotoxic, genotoxic and carcinogenic. The study aims to evaluate the salivary and urinary nickel levels post-orthodontic fixed appliance treatment.

Materials and Methods: Saliva and urine samples of fourteen orthodontic patients were taken at debonding (T0), after one month (T1), and three months post-debonding (T2), and compared with the control group. Samples were analysed using Inductively Coupled Plasma Optical Mass Spectrometry (ICP-MS) and IBM SPSS Version 27 was used for statistical analyses.

Results: The mean (SD) of orthodontic treatment duration was 33.6 (9.6) months. In comparison to the control group, the mean (SD) nickel levels in saliva and urine were highest at debonding (T0) with 12.71 ppb (9.64) and 9.71 ppb (8.27), respectively. In the test group, there was a significant difference in nickel level in saliva between T0 and T1 (MD = 9.75, 95% CI: 3.71, 15.71; $p < 0.05$) and in urine between T0 and T1 (MD = 6.46, 95% CI: 1.38, 11.55; $p = 0.012$).

Conclusion: Our results clearly demonstrated that the nickel level in saliva and urine remained higher at the end of orthodontic treatment compared to the control group. Further studies with a longer duration of observation and larger samples are suggested. Furthermore, our study indicates it is essential to confirm the effects of long-duration of orthodontic treatment and its post-treatment nickel level. This study raised attention about the biocompatibility of mechanical orthodontic treatment vs sustainable general health.

INTRODUCTION

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Nickel is one of the common trace metal elements used in manufacturing industries such as the production of coins, jewellery, nickel-cadmium batteries, and as a catalyst in food and chemical industries (Patra et al., 2019). Nickel in metal alloys was used in dentistry and orthodontics as well due to their exceptional mechanical properties. Orthodontic materials including archwires, brackets and molar bands are made from stainless steel containing approximately 8 to 12% nickel (Givan et al., 2014); (Proffit et al., 2007). The addition of nickel enhances the corrosion resistance and ductility properties of the stainless steel alloy.

Nickel is recognised as a toxic metal element. Therefore, orthodontic fixed appliance treatment warrants in vivo investigations of biocompatibility because it is exposed to the corrosive environment of the oral cavity. Previous studies demonstrated that nickel ion is released as early as the initial phase of orthodontic treatment (Imani et al., 2019);(Elides et al., 2003);(Ousehai et al., 2012); (Petoumenou et al., 2009). Patients were subjected to chronic exposure to nickel due to the long duration of fixed appliance treatment. It was also proven that orthodontic appliances release metal ions in sufficient quantities to induce a genotoxic effect (Buczko et al., 2017); (Natarajan et al., 2011). Previous in vivo or in vitro studies focused on the levels and effects of nickel before and during active orthodontic treatment. However, there is no report yet on the exact level of nickel post-orthodontic treatment. Likewise, the degree of changes in the nickel level post-orthodontic fixed appliance treatment in a time course remains unknown.

Chronic exposure to nickel from environment or occupation will lead to accumulation of nickel in internal organs such as liver, kidneys, stomach and brain (Adamska et al, 2021).

Different types of biological samples were used in the past such as blood and hair. However, these types of samples are invasive, complex sample preparation and technique sensitive (komarova et al., 2021); (Imani et al., 2019). In this study, the use of saliva and urine have several advantages such as non-invasive collection, less discomfort, straightforward sample collection and easy to store and transport. In addition, salivary and urinary mechanisms play an important role in excreting nickel from the body and can reflect the baseline nickel level in human (Daniel et al., 2018); (Holm et al., 2016).

Therefore, the aim of this study was to evaluate the salivary and urinary nickel level post-orthodontic fixed appliance treatment and to assess the relationship between nickel level and the duration of orthodontic treatment.

MATERIALS AND METHODS

This prospective cohort study was conducted at ###. Ethical approval was granted from ### on 28th March 2019 (reference number: 600-IRMI (5/1/6)). The test group consisted of fourteen orthodontic patients who were treated with conventional metal brackets for at least 20 months, no intraoral or extraoral auxiliary appliances soldered or welded to bands, and near completion of treatment. For the control group, subjects must be in a permanent dentition and have no history of orthodontic treatment. Those who have any systemic illness, under long-term drug therapy, presence of amalgam restorations or oral and body prostheses, presence of tongue or lip piercing, smoker or had betel nut chewing habit, were excluded. All sample collection were done in the morning.

Sample Size Calculation

G*Power software (version 3.1.9.3) was used where the study power of 80% and $p < 0.05$ were set. Test and control groups were included in this study with 14 subjects in each group. Samples were collected from each subject at T_0, after one month (T_1), and three months (T_2).

Sample Collection

All subjects were instructed to rinse their mouth thoroughly before saliva collection and to minimise their orofacial movements. Five ml of unstimulated saliva was collected using passive drooling method into a 50 ml sterile plastic centrifuge tube. For urine collection, subjects were provided with a 50 ml sample collection bottle and were asked to collect urine after discarding the first flush. Instructions were given not to contaminate the collection bottle by wiping or rinsing the internal surface of the bottles. Each sample taken was stored immediately at -20 °C freezer until further analysis.

Sample Analysis

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (ELAN 9000 Perkin Elmer) was used to determine the nickel level. For each sample, the test was repeated three times and the mean reading was taken as the final value of nickel level. All results were detected by computer software and presented as mean nickel level.

Statistical Analysis

Results obtained were subjected to statistical analysis using the Statistical Software for Social Science (IBM SPSS Statistics, Version 27). The level of statistical significance was set at $p < 0.05$. Descriptive analysis was used to analyse the socio-demographic data such as age, gender and duration of orthodontic fixed appliance treatment. The repeated measures Analysis of Variance (ANOVA) test was performed to compare the mean salivary and urinary nickel levels between the test and the control group based on three-time points.

Pearson correlation analysis was conducted to identify the relationship between salivary and urinary nickel level post-orthodontic fixed appliance treatment and duration of treatment.

RESULTS

The results of this study showed that the mean (SD) subjects' age was 31.57 (3.74) years old and 71% (n=10) were female. It can be seen from the data in Table 1, the proportion of female subjects were more than two times higher than male. However, there was no significant correlation was observed between genders and nickel ion level at any time points ($p > 0.05$).

Table 1. Overall demographic data of test and control subjects

Variables	Test group	Control group	Total
Number of subjects (n)	14	14	28
Age (years) Mean (SD)	23.07 (3.58)	31.57 (3.74)	27.32 (5.62)
Gender (n / %)			
Male	4 (29)	4 (29)	8 (29)
Female	10 (71)	10 (71)	20 (71)

The duration of orthodontic fixed appliance treatment ranged from 20 to 50 months with a mean (SD) of 33.6 (9.6) months. A Pearson product-moment correlation coefficient was computed to assess the relationship between salivary and urinary nickel level post orthodontic fixed appliance treatment and duration of treatment. There was a moderate positive correlation between the nickel level in saliva and

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duration of treatment ($r = .641$, $n = 14$, $p = 0.014$). Furthermore, there was a moderate positive correlation between the nickel level in urine and duration of treatment as well ($r = 0.575$, $n = 14$, $p = 0.031$). It shows that a longer duration of treatment correlated with a higher salivary and urinary nickel level at the end of orthodontic fixed appliance treatment.

In comparison to the control group, the mean (SD) nickel levels in saliva and urine were highest at debonding (T0) with 12.71 ppb (9.64) and 9.71 ppb (8.27), respectively. In the test group, there was a significant difference of nickel level in saliva between T0 and T1 (MD = 9.75, 95% CI: 3.71, 15.71; $p < 0.05$) and in urine between T0 and T1 (MD = 6.46, 95% CI: 1.38, 11.55; $p = 0.012$). The results are presented in Figures 1 and 2.

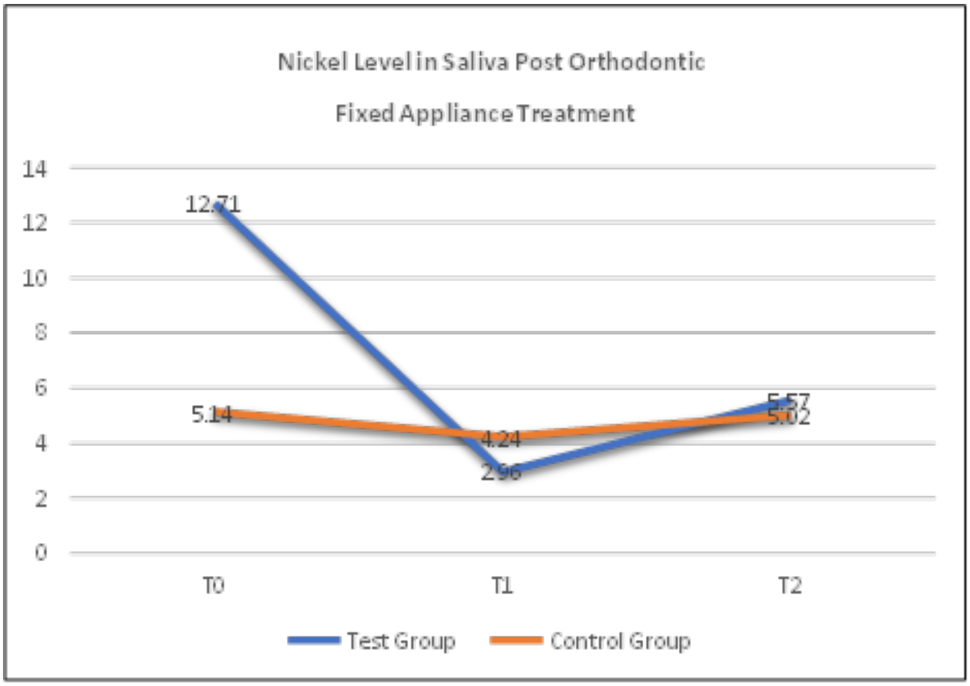

Fig. 1. Graph Showing Nickel Level in Saliva Post Orthodontic Fixed Appliance Treatment (in ppb)

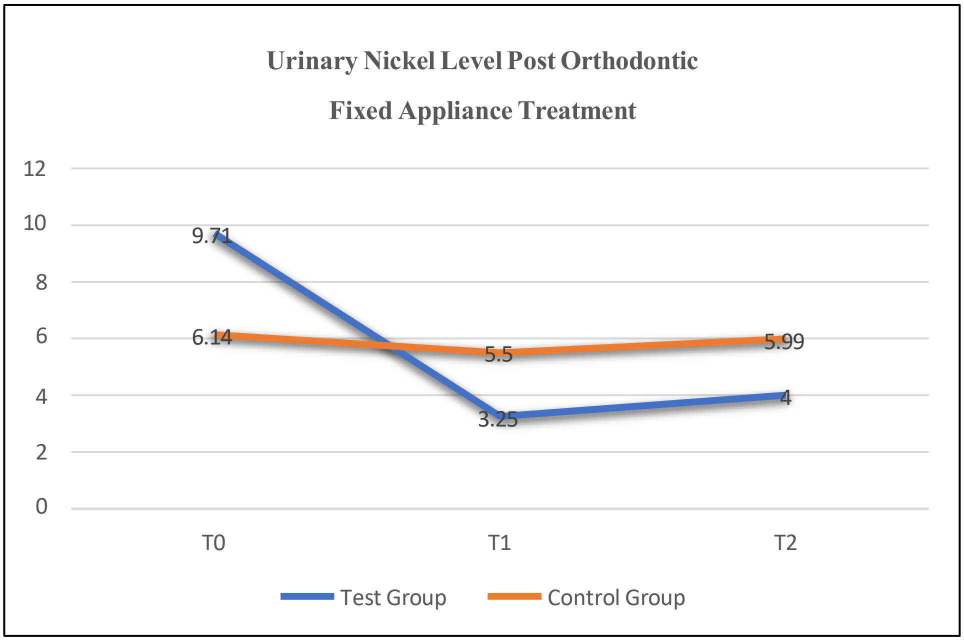

Fig. 2. Graph Showing Nickel Level in Urine Post Orthodontic Fixed Appliance Treatment (in ppb)

DISCUSSION

Nickel allergy is often experienced by all clinicians throughout their careers, nonetheless, the study on the nickel concentration in the body fluids has been rare. In this regard, the study unveils invaluable questions in the dentistry avenue.

The International Agency for Research on Cancer (IARC) has identified nickel and its compounds as human carcinogens, and the toxicity depends on the physico-chemical characteristics, the dosage, route of exposure and solubility of the nickel compounds in humans (Genchi et al., 2020); (IARC 2018). It is well documented that nickel caused change in tumour suppressor p53 gene that leads to cell apoptosis and cancer risks. Other than that, nickel can cause a variety of health effects, such as contact dermatitis, cardiovascular disease, asthma, lung fibrosis, and respiratory tract cancer.

The mean urinary nickel level at the near-end of orthodontic fixed appliance treatment found in this study was 9.71±8.27 ppb. This is higher than previous studies which found the mean nickel level between 4.54 to 5.61 ppb after six months into treatment (Mudjari et al., 2018); (Guo et al., 2014). However, the finding of this study is similar to Amini et al. (2012) where the authors included the test subjects who were undergoing fixed appliance treatment for at least one year. This demonstrates that prolonged exposure to nickel from fixed appliance components may have an effect on nickel release from the components, as indicated by a higher nickel level in the urine.

Salivary samples can also be used to assess the metal ions that leach out from orthodontic appliances. In orthodontics, a lot of emphases has been laid on release of nickel and chromium ions because of the hazardous nature of these elements. Several studies have been conducted to detect nickel ions levels in

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saliva in patients undergoing orthodontic treatment, although, no significant differences have been found in the salivary levels of metals in orthodontic patients and normal population (Dhiman et al., 2014).

Previous reports documented the level of nickel before and during orthodontic treatment. (Dhiman et al., 2014); (Nayak et al., 2015); (Kumar et al., 2016); (Guo et al., 2014). Papers and observation duration into treatment. However, there was no reported studies on nickel level at the end on orthodontic treatment. Our result indicated that, at the end of orthodontic treatment (20-50 months, average 33 months), the nickel level was 2.5 times higher that control group (figure no). Our result strongly indicate this high level of nickel exposure during treatment time, is causing nickel toxicity concern/ health risks to patients. The result of our study showed that even after 3 months debonding, the nickel level in saliva is higher. However, urine did not show the same behaviour. (Figure 1 & 2).

In our experiment, the results show the nickel level in saliva after one month went down below that control group, however after 3 months, the level was higher that control group. In 3 months, the nickel level may be due to subjects' own metabolism and elimination of nickel. However, due to the lockdown situation, the experiment could not be repeated. Therefore, further studies are required to substantiate this decreasing pattern.

In this study, the maximum value of nickel in saliva and urine for test group was 12.71 ppb and 9.71 ppb, respectively. The values were far below the average dietary intake where oral daily intake of nickel by food is estimated to be between 300-600 µg 19. Although fixed orthodontic appliances did not have any effect on the general salivary and urinary nickel level, it cannot be excluded that even in minor amount of nickel released from fixed appliances could elicit hypersensitivity reaction to patients who are allergic to metal ions. Oral clinical sign and symptoms can include gingival hyperplasia, angular cheilitis, burning sensation, gingival hyperplasia and others (Golz et al., 2015);(Nobel et al., 2008). Furthermore, nickel can induce cytotoxic and genotoxic effects even at a low concentration (Hafez et al., 2011). This proves the need for further investigations regarding the biocompatibility and safety of nickel use in patients.

The advantages of using saliva and urine as biological diagnostic media in this study were due to the ease of collection and storage methods. Most importantly, saliva and urine sampling involved non-invasive techniques which were more tolerable and allowed better compliance by the study subjects. However, more research is needed to determine the levels of other metal ions such as chromium, titanium, and aluminium in saliva and urine, as well as other diagnostic media such as serum and hair. This will provide more definitive information on the effects of orthodontic fixed appliances on systemic metal ion changes.

In the present study, saliva and urine were collected as biological samples to evaluate the nickel level. Human saliva composition was proven by numerous past studies that it can provide information to many clinical, experimental and diagnostic protocols (Michalke et al., 2014); (Pfaffe et al., 2011) while urine has the ability to reflect systemic nickel levels in human due to its metabolic route is through the kidneys (Daniel et al., 2018); (Guo et al., 2014). Both saliva and urine are considered more tolerable to subjects because they are non-invasive techniques, accessible, convenient, and require simpler handling and sample preparation(Jayachandran et al., 2020); (Pathiyil et al., 2019); (Wang et al., 2015).

It is noteworthy to mention that different methods of sample preparation and analytical technique used will provide heterogenous result. In this study, ICP-MS was used to analyse the level of nickel in the saliva and urine samples, which was similar to recent studies (Nayak et al., 2015); (Dwivedi et al., 2015); (Moghadam et al., 2019). ICP-MS is a robust and widely used technique, have low detection limit and highly sensitive for nickel analysis (Enamorado et al., 2013).

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Orthodontic practitioners should create an efficient treatment plan to ensure that orthodontic fixed appliance treatment can be completed promptly. This is to reduce the duration of exposure to nickel and to avoid iatrogenic effects. Most importantly, a recent study (Kapadia et al., 2018) documented a significant increase in the Ni concentration detected in cells of buccal mucosa in patients during orthodontic treatment, further this study observed quantitative DNA damage in these patients. In conclusion, the team strongly recommend the timely checking of DNA damage in patients undergoing orthodontic treatment. Further studies are required to determine alternative elements to nickel that are more biocompatible for orthodontic and dentistry use.

The study was aimed to grope the Ni level in the easily-taking body fluid such as saliva and urine along with orthodontic treatment with metallic appliances and also to establish the norms of Ni concentration in the Malaysian population. The time slot of taking the specimen at the beginning of the treatment as the baseline measurement is recommended for the follow-up study.

CONCLUSION

Our present results clearly demonstrated that the nickel level in saliva and urine remained higher at the end of orthodontic treatment compared to the control group. This study raised attention about the biocompatibility of nickel use in orthodontic fixed appliances for the sustainable general health. This warranted a longer observation is needed to provide a clearer and more accurate pattern of nickel ion levels in the body due to orthodontic treatment.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to the contents of this article.

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