Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.1124

Assessment of the Association between Oral Lichenoid Reactions and Amalgam Restorations and to determine the Salivary Concentrations of Interleukin-6 (IL-6) and IL-8 before and after Replacement of the Amalgam Restorations

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Submitted: 20 January 2023; Accepted: 22 February 2023; Published: 17 March 2023

ABSTRACT

Background: The oral mucosa is affected by chronic autoimmune lesions known as oral lichen planus (OLP) and oral lichenoid reactions (OLR). OLP resembles OLR in terms of histology and clinical features, however, it is a possibly premalignant condition that affects 2% of people. Some experts believe that an allergic reaction on contact with amalgam restoration or the other above-mentioned elements is what causes OLP, whilst others assert that OLP and OLR are two distinct disorders.

Aim: This study sought to determine the efficacy of amalgam restoration, which is thought to be the etiology of OLR, and to measure IL-6 concentration and IL-8 concentrations in saliva prior to and following the replacement of amalgam restoration.

Methods and materials: In this study, amalgam restorations were changed in 40 patients by composite restoration, gold restoration, porcelain restoration, or assembly of these materials. These individuals were assessed in the hospital following a follow-up time of between two months and three and a half years. The severity of OLR and extent of OLR were rated as 1 when there was complete healing of all lesions and there was no presence of lesions. The severity of OLR and extent of OLR were rated as 2 when significant improvement (above 80%) was noticed and it was rated as 3 when there was no change in symptoms. ELISA was carried out in accordance with the package recommendations to measure the concentrations of IL-6 and the concentration of IL- 8 in saliva. The outcomes were reported in pg/mL. The experiment was run twice and then three times. In pg/mL, protein concentration was expressed.

J Popul Ther Clin Pharmacol Vol 30(2):e408–e414; 15 January 2023.

Results: On amalgam removal, there was complete healing of lesions in 32 study participants, while there was more than 80% healing of lesions in 6 study participants. On the other hand, there was no improvement observed and no deterioration of symptoms in the 2 patients. Statistics revealed that there is a significant reduction in the number of lesions on the removal of amalgam restoration giving support to the association between amalgam restoration and oral lichenoid reactions. ($p<0.05$). . Before the fillings were replaced, IL-6 levels were found to be substantially higher than afterward ($P< 0.05$). The levels of IL-8 that were found prior to replacement were similarly considerably higher than the levels that followed ($P<0.05$). In control subjects, the levels of IL-6 tested before and after the intervention did not differ substantially ($P >0.05$). The same holds true for the IL-8 readings ($P<0.05$). Conclusion: Clinical observations showed that tissue recovery from oral lichenoid reaction followed the restorative replacement of amalgam restorations. When amalgam restorations were changed for restorations made of other dental restorations, concentrations of both IL-6 cytokines and IL-8 cytokines in healthy participants returned to normal.

Keywords: *Lichenoid reaction, amalgam, IL-6, IL-8*

INTRODUCTION

The oral mucosa is affected by chronic autoimmune lesions known as oral lichen planus (OLP) and oral lichenoid reactions (OLR).1 OLP resembles OLR in terms of histology and clinical features, however, it is a possibly premalignant condition that affects 2% of people.2 Some experts believe that an allergic reaction on contact with amalgam restoration or the other above-mentioned elements is what causes OLP, whilst others assert that OLP and OLR are two distinct disorders.3Clinically, OLR is typically unilateral while OLP is typically bilateral. OLP is most frequently found on the tongue region, gingival region, and buccal mucosal region. It may appear as reticular pattern, erosive pattern, atrophic pattern, plaque-type pattern, papular pattern, or bullous pattern in addition to other types. OLR demonstrate more extensive and more widespread inflammation.

It is asserted that the involvement of plasma cells inflammatory mediators and sporadic eosinophils serve as diagnostic indicators for OLR.5 Both diseases exhibit increased keratinization of the epithelium layer of oral mucosa and liquefaction disintegration of the basal cell layer. There have been several medications linked to lichenoid reactions, including beta blockers pharmacological agents, dapsone pharmacological agents, oral hypoglycemics pharmacological agents, non- steroidal anti-inflammatory pharmacological agents, penicillamine pharmacological agents, phenothiazines pharmacological agents, sulphonylureas pharmacological agents, and gold salts.4 Through the use of dermatological patch testing on patients, correlations involving OLR and contact with diverse dental materials, notably dental elements, have been studied.5 The majority of studies have discovered that individuals with OLP had a higher prevalence of allergic reactions to frequently used dental products than patients lacking mucosal illness.

By eliminating dental restorations and tracking the development of the lesion after removal, some researchers have tried to investigate the role of restorative materials in oral lichenoid lesions.6 Despite the advantages of amalgam restorations, there are emerging worries about the potential health dangers posed by the introduction of mercury discharged during amalgam setting. It has been demonstrated that mercury can build up in the mucosa of the oral cavity, which in certain people can result in chronic lichenoid inflammation of the mucosal surface of the oral cavity in proximity to an amalgam restoration. Many individuals with amalgam-associated OLR have high sensitivity to mercury-containing substances.

A characteristic of amalgam-associated OLP and OLR is the buildup of a band-like infiltration of polymorphonuclear leukocytes in the zone of

lamina propria next to the zone of basal keratinocyte together with the breakdown of the zone of basement membrane and breakdown of basal keratinocytes.7 Tumor necrosis factor-alpha inflammatory mediator (TNF-alpha) levels were considerably greater in all salivary specimens collected taken from individuals with current OLP lesions compared to healthy controls, according to Pezelj-Ribaric et al.8 Furthermore, these data demonstrated that salivary TNF- concentrations differed between clinical kinds of OLP, being especially high in situations of the disease's erosive pattern and atrophic pattern. Evidently, increased TNF- alpha production in saliva represents clinical modifications and is correlated with the intensity of OLP.9 This study sought to determine the efficacy of amalgam restoration, which is thought to be the etiology of OLR, and to measure IL-6 concentration and IL-8 concentrations in saliva prior to and following the reinstitution of amalgam restoration.

MATERIALS AND METHODS

There were 40 OLR patients in the study. Patients who were taking medications that could result in a lichenoid response and those who had lesions on their skin or in places other than their oral mucosa were excluded. A total of 40 healthy, normal subjects without amalgam restorations of the same gender and age distribution were also recruited. All participants were made aware of the objectives and methods of the study, as well as the idea that their medical information will be analysed subsequently. They were promised that their fundamental ethical as well as bioethical values, such as character and integrity (morality, independence, well-being, and protection), as governed by the Nûrnberg Codex along with the most recent Helsinki Declaration, would be respected throughout the research.

Only those participants have been included who provided written consent in the form of voluntary participation. Each participant answered a questionnaire on their health and demographics. According to the WHO's guidelines, the individuals received a clinical diagnosis and a biopsy confirmed their OLR status.

Lesions

All assessments and restoration evaluations were performed by two examiners. All participants' OLR abnormalities were found close to amalgam restoration. Buccal mucosa involvement was most frequent (28 cases), next to the tongue (8 cases), and then gingiva (4 cases). The conventional clinical parameters were followed when doing the clinical assessment. Lesions were classified as reticular (24 instances) if they primarily displayed lace-like hyperkeratotic designs and as erosive or atrophic (some aggressive ulcers) if they showed erythematous alteration (16 cases).

Histopathological examination

Haematoxylin and eosin staining (HE staining) was used to analyse samples of OLR tissues garnered through biopsy in order to assess the distinctive histopathological characteristics of OLP. Each time, the histological findings of a band-like, mostly lymphocytic intrusion in the zone of connective tissue next to the zone of the basement membrane of the epithelium of oral mucosa, liquefaction degradation of the zone of the basement membrane, and disintegration of the zone of basal keratinocyte supported the clinical diagnosis.

Patch testing

A doctor with experience in skin patch screening performed skin patch tests on all of the patients. Five percent amalgam, one percent mercury ammonium chloride, 0.5 percent mercury, 0.1 percent thimerosal, 0.05 percent phenylmercuric borate, 0.05 percent phenylmercuric acetate, and

0.05 percent phenylmercuric nitrate were tested (Hermal, Reinbek, Germany). According to the International recommendations, readings were taken after 1 day, 2 days, and 3 days.10

Saliva collection and cytokine assay

The entire unstimulated saliva was gathered between 9:00 am and 11:00 a.m. following the methods Navazesh describes following informed consent and the medical history, dental history, and social histories.11 Participants went at least

1.5 hours without eating, drinking, or using chewing gum before the evaluation. Each participant was asked to sit while a saliva sample was taken. The individuals were asked to swallow, lean their heads forward, and secrete all saliva generated during a 5-minute period into 50-mL tubes before samples were collected. Saliva flow rate and ultimate volume were calculated gravimetrically using Analytical Balance (Model WTS- 6001, NY).12 Three months after replacing the amalgam restorations with composite restoration or other substances, the overall process was redone. Saliva samples were kept cold, at -80 °C, unless analysis began. ELISA was carried out in accordance with the package recommendations to measure the concentrations of IL-6 and the concentration of IL-8 in saliva. The outcomes were reported in pg/mL. The experiment was run twice and then three times. In pg/mL, protein concentration was expressed.

Amalgam replacement

In this study, amalgam restorations were changed in 40 patients by composite restoration, gold restoration, porcelain restoration, or assembly of these materials. These individuals were assessed in the hospital following a follow-up time of between two months and three and a half years. The severity of OLR and extent of OLR were rated as 1 when there was complete healing of all lesions and there was no presence of lesions. The severity of OLR and extent of OLR were rated as 2 when significant improvement (above 80%) was noticed and it was rated as 3 when there was no change in symptoms.

Statistical analysis

The ages of the patients in both subgroups are shown as mean value + SD. One-way ANOVA was used to evaluate patient variability in age. The mean value with SD for the IL-6 and IL8 measurements are provided. Non-parametric Wilcoxon statistical test for dependent variables and Mann-Whitney U- statistical test for independent variables were used to compare the results. Pearson's 2 test was used to analyse the presence of different histopathological characteristics. When differences reached the P threshold of < 0.005, it was deemed significant. SPSS software 2021, was used to statistically analyse the data (IBM, USA).

RESULTS

In this study 40 study participants with the histopathologically proven oral lichenoid reaction were included. The Mean age ± SD of study participants was 39.5 ± 4.396 years and M:F was 1:3. (table1). On amalgam removal, there was complete healing of lesions in 32 study participants, while there was more than 80% healing of lesions in 6 study participants. On the other hand, there was no improvement observed and no deterioration of symptoms in the 2 patients. Statistics revealed that there is a significant reduction in the number of lesions on the removal of amalgam restoration giving support to the association between amalgam restoration and oral lichenoid reactions. ($p<0.05$) (table 2). The patch test was positive in 32 study participants while it was negative in 8 study participants. The findings suggest that majority of the study participants with oral lichenoid reactions are hypersensitive to either amalgam or mercury. ($p<0.05$) (table 3).

All patients had saliva samples collected both before and after amalgam filling removal. Using ELISA, the amounts of IL-6, as well as IL-8 in entire saliva samples, were quantified. Before the fillings were replaced, IL-6 levels were found to be substantially higher than afterwards ($P< 0.05$). The levels of IL-8 that were found prior to replacement were similarly considerably higher than the levels that followed ($P<0.05$). In control subjects, the levels of IL-6 tested before and after the intervention did not differ substantially ($P >0.05$). The same holds true for the IL-8 readings ($P<0.05$). According to filling replacement results, pro-inflammatory cytokine readings have

reverted to levels that are consistent with those in the control group. Remedial therapy brought the values closer to normal (table 4).

TABLE 1: Demographic details

Parameter	Values
Mean age ± SD (years)	39.5 ± 4.396
M:F	1:3

TABLE 2: Classification of lesion improvement when amalgam restorations are removed

Status	Healed lesion (no lesions left)	Significant improvement (More than 80% improvement)	No improvement observed or deterioration of the symptoms noticed
Frequency	32	6	2
Statistic	$p<0.05$		

TABLE 3: Outcomes of a patch test for amalgam hypersensitivity or mercury hypersensitivity

Result	Frequency	Statistics
Negative	08	$p<0.05$
Positive	32	

TABLE 4: Concentration of IL-6 and IL-8 before and after removal of amalgam restorations.

	Concentration (10-2 pg/mL)	Mean ± SD
Before removal of amalgam	IL-6	42.875 ±1.886
	IL-8	60.15 ±1.498
After removal of amalgam	IL-6	0.50 ±0.071
	IL-8	0.375 ± 0.083
Control	IL-6	0.335±0.135
	IL-8	0.68 ±0.126

DISCUSSION

There have been numerous reports of lichenoid episodes as hypersensitivity to dental materials. Touch hypersensitivity reaction to dental products has been proven in numerous investigations. such as lichenoid responses caused by dental acrylics materials, composite materials, and amalgam materials. Clinically and histologically, OLR is typically not distinct from idiopathic OLP.13 Previous research has demonstrated that individuals with OLP had considerably greater concentrations of nuclear factor-B linked cytokines, such as TNF- alpha, cytokine IL-1, and cytokine IL-6 in oral keratinocytes, serum and tissue-infiltrating.12 Since 1831, amalgam has already been utilised as a dental restoration material. Resilience, durability, high marginal adaptability, ease of handling, and cost are some of its advantageous traits. There is an ongoing debate over the pathogenic connection between OLR and amalgam restorations in teeth.

IL-6 levels in our research were found to be significantly greater before the fillings were changed than after. Similar to how the levels detected before replacement was significantly greater than the levels found after, so were the levels of IL-8. The levels of IL-6 measured in control subjects before and after the intervention were insignificantly different. The IL-8 readings show a similar pattern. Pro-inflammatory

cytokine measurements have returned to levels that are consistent with those in the control group, according to filling replacement data. Remedial therapy helped the values get more in line with normal.

According to several studies, these restorations may cause lichenoid sensitivity in oral mucosal surfaces in vulnerable patients.14 A large number of sores improved after amalgam restoration removal, however not all patients responded in the same way.15 In their investigation, Little et al. discovered that cells of mucosa of the oral cavity did not express cytokine IL-8 in amalgam-associated OLR or amalgam-associated OLP. They examined the presence of this biomolecule in frozen biopsy samples using conventional immunoperoxidase assays.7

The quantities of IL-6 and IL-8 in saliva were measured using ELISA in accordance with the instructions on the test kit. The results were given in pg/mL. Two times, then three times, the experiment was conducted. Protein concentration was given in pg/mL. In this study, all of the unstimulated saliva was collected in the morning. Before the test, participants lasted at least 1.5 hours without eating, drinking, or using chewing gum. A saliva sample was obtained from each subject, who was then invited to sit. Before samples were taken, the participants were instructed to swallow, incline their heads forward, and secrete all saliva produced during a 5-minute period into 50-mL tubes. Utilizing analytical Balance, saliva flow rate and ultimate volume were estimated gravimetrically. The entire procedure was redone three months after composite restorations or other materials were used to replace the amalgam restorations. Before analysis began, saliva samples were stored at a low temperature of -80 °C.

However, Sun et al. demonstrated in their study that serum IL-8 concentrations can serve as an independent marker in observing various phases of OLP.17 Additionally, Maie et al demonstrated that salivary cytokine IL-8 can act as an indicator in the diagnosis of squamous cell carcinoma of the oropharynx.18 In this study, we observed that patients who had amalgam fillings replaced with fillings made of composite resin, gold, porcelain, or a mix of these materials experienced statistically significant decreases in salivary concentrations of the cytokines IL-6 and cytokines IL-

Clinical modifications supported these findings

In 32 study participants, lesions completely healed after amalgam removal, while in 6 study participants, lesions healed to more than 80% of their original size. On the other hand, no symptoms worsening or improvement were seen in the 2 patients. Statistics showed a considerable decrease in lesions after the removal of the amalgam restoration, supporting the link between amalgam restoration and lichenoid reactions in the mouth. 32 study participants had good results from the patch test, whereas only 8 had negative results. The results imply that the majority of research subjects who experienced oral lichenoid reactions are extremely susceptible to either mercury or amalgam.

These findings are consistent with earlier research indicating that 16 percentage to 64 percentage of OLR patients had a mercury allergy. 16 In three different types of liquids from OLP patient populations, cytokines IL-6 and cytokines IL-8 were effectively identified at markedly higher levels, and Rhodus et al investigation .'s found that the shift in NF-B associated cytokines in saliva partially represented the progress of the cancerous transformation of OLP.12 Their observations, which are congruent with ours, showed that all of the cytokines described above had higher amounts in individuals having OLP in all tested oral fluids. Rhodus et al. conducted similar research, but their samples came from tissue transudate instead of tissue, and their findings revealed elevated levels of TNF inflammatory mediator, IL-1 inflammatory mediator, IL-6 inflammatory mediator, and IL-8 cytokines.19

It should be noted that numerous authors have demonstrated that IL-6 and IL-8 are viable biomarkers for OLP monitoring utilising various oral fluids. According to clinical characteristics in our analysis, restorative therapy produced tissue repair. Salivary IL-6 and IL-8, which

demonstrated a statistically significant distinction between prior to and after filling replacement, were additional noteworthy markers. When amalgam restorations were replaced with fillings made of alternative materials, cytokine levels shifted toward normal ranges.

CONCLUSION

Clinical observations showed that tissue recovery from oral lichenoid reaction followed the restorative replacement of amalgam restorations. When amalgam restorations were changed for restorations made of other dental restorations, concentrations of both IL-6 cytokines and IL-8 cytokines in healthy participants returned to normal.

ACKNOWLEDGE

Deanship of Scientific Research at Najran University

REFERENCES

1. Nissalo S, Hietanen J, Malmstrom M, Hukkanen M, Polak J, Kontinnen YT. Disorder- specific changes in innervation in oral lichen planus and lichenoid reactions. J Oral Pathol Med 2000; 29: 361–369.
2. Axell T, Rundquist L. Oral lichen planus – a demographic study. Commun Dent Oral Epidemiol 1987; 36: 141–146.
3. López-Jornet P, Camacho-Alonso F, Gomez-Garcia F, Bermejo Fenoll A. The clinicopathological characteristics of oral lichen planus and its relationship with dental materials. Contact Dermatitis 2004; 51: 210.
4. Rice PJ, Hamburger J. Oral lichenoid drug eruptions: their recognition and managmnet. Dent Update 2002; 29: 442–447.
5. Dunsche A, Kastel I, Terheyden H, Springer ING, Chritophers E, Brasch J. Oral lichenoid reactions associated with amalgam: improvement after amalgam removal. Br J Dermatol 2003; 148: 70–76.
6. Wong L, Feeman S. Oral lichenoid lesions (OLL) and mercury in amalgam fillings. Contact Dermatitis 2003; 48: 74–79.
7. Little MC, Griffiths CEM, Watson REB, Pemberton MN, Thornhill MH. Oral mucosal keratinocytes express RANTES and ICAM-1, but not interleukin-8, in oral lichen planus and oral lichenoid reactions induced by amalgam fillings. Clin Exp Dermatol 2003; 28: 64–69.
8. Pezelj-Ribaric S, Brekalo Prßo I, Abram M, Glazar I, Brumini G, Simunovic-Soskic M. Salivary levels of tumor necrosis factor-alpha in oral lichen planus. Med Inflamm 2004; 13: 131–133.
9. Greenberg MS, Glick M. Burket's Oral Medicine: Diagnosis and Treatment, 10th edn. Lippincot-Raven, Hamilton, Canada, 2006: 108.
10. Richter G. Dental materials-problem substances in allergologic diagnosis? II. Patch test diagnosis and relevance evaluation of selected dental material groups. Hautartz 1996; 47: 844–849.
11. Navazesh M. Methods for collecting saliva. ANN NY Acad Sci 1993; 694: 72–77.
12. Rhodus NL, Cheng B, Myers S, Bowles W, Ho V, Ondrey F. A comparison of the pro-inflammatory, NF-kappaB-dependent cytokines: TNF-alpha, IL-1-alpha, IL-6, and IL-8 in different oral fluids from oral lichen planus patients. Clin Immmunol 2005; 114: 278–283.
13. Sumairi BI, Satish KS, Rosnah BZ. Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis, management and malignant transformation. J Oral Sci 2007; 49: 89–106.
14. Laine J, Kalimo K, Happonen RP. Contact allergy to dental restorative materials in patients with oral lichenoid lesions. Contact Dermatitis 1997; 36: 141–146. Staines KS, Wray D. Amalgam–tattoo-associated oral lichenoid lesion. Contact Dermatitis 2007; 56: 240–241.
15. Thornhill MH, Pemberton MN, Simmons RK, Theaker ED. Amalgam-contact hypersensitivity lesions and oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95: 291–299.
16. Laine J, Kalimo K, Happonen RP. Contact allergy to dental restorative materials in patients with oral lichenoid lesions. Contact Dermatitis 1997; 36: 141–146.
17. Sun A, Wang JT, Chia JS, Chiang CO. Serum interleukin-8 level is a more sensitive marker than serum interleukin-6 level in monitoring the disease activity of oral lichen planus. Br J Dermatol 2005; 152: 1187–1192.
18. Maie AR, Li Y, Zhou XF et al. Interleukin 6 and Interleukin 8 as potential biomarkers for oral cavity and oral squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2004; 130: 929–935.
19. Rhodus NL, Cheng B, Ondrey F. Th1/Th2 cytokine ratio in tissue transudate from patients with oral lichen planus. Med Inflammat 2007; 28: 19854